# ACTA PHYSIOLOGICA

## ACADEMIAE SCIENTIARUM HUNGARICAE

ADIUVANTIBUS

P. BÁLINT, SZ. DONHOFFER, E. ERNST, B. ISSEKUTZ SEN., L. KESZTYŰS, J. KNOLL, F. OBÁL

REDIGIT

K. LISSÁK

TOMUS XXXV FASCICULUS 1

AKADÉMIAI KIADÓ, BUDAPEST

1969

# ACTA PHYSIOLOGICA

## A MAGYAR TUDOMÁNYOS AKADÉMIA KÍSÉRLETES ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21

Főszerkesztő:
LISSÁK KÁLMÁN
akadémikus

Technikai szerkesztő:
KORÁNYI LAJOS

Az *Acta Physiologica* német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudományok köréből.

Az *Acta Physiologica* változó terjedelmű füzetekben jelenik meg: több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az *Acta Physiologica* előfizetési ára kötetenként belföldre 120 forint, külföldre 165 forint. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv és Hírlap Külkereskedelmi vállalatnál (Budapest I., Fő utca 32. Bankszámla 43-790-057-181) vagy annak külföldi képviseleteinél és bizományosainál.

---

The *Acta Physiologica* publish papers on experimental medical science in English, French, German or Russian.

The *Acta Physiologica* appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the *Acta Physiologica* is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. — Account No. 43-790-057-181) or with representatives abroad.

# ACTA PHYSIOLOGICA

## ACADEMIAE SCIENTIARUM HUNGARICAE

ADIUVANTIBUS

P. BÁLINT, SZ. DONHOFFER, E. ERNST, B. ISSEKUTZ SEN., L. KESZTYŰS, J. KNOLL, F. OBÁL

REDIGIT

K. LISSÁK

TOMUS XXXV

AKADÉMIAI KIADÓ, BUDAPEST

1969

ACTA PHYSIOLOGICA

Tomus XXXV

# INDEX

## PHYSIOLOGIA

*Airapetianz E. Sh., Konstantinov A. I., Matjushkin D. P.:* Brain Echolocation Mechanisms and Bionics ..... 1

*Avar Z., Monos E.:* Biological Role of Lateral Hypothalamic Structures Participating in the Control of Maternal Behaviour in the Rat ..... 285

*Avar Z., Monos E.:* Behavioural Changes in Pregnant Rats Following Far-Lateral Hypothalamic Lesions ..... 295

*Ángyán L., Rózsavölgyi Margit, Kolta P.:* Stopping Elicited by Conditioned Stimuli as Compared with the Thalamic Induced Arrest Reaction ..... 271

*Banga Ilona:* The Structure of Protein-Polysaccharides and Their Role in the Function of Connective Tissue Fibres ..... 355

*Bohus B.:* Evaluation of the Role of Feedback Effect of Corticosteriods in the Control of Pituitary ACTH release ..... 141

*Dauda G., Dévényi I., Szokoly V.:* Converting Enzyme Activity in Experimental Cirrhosis of the Rat ..... 365

*Endrőczi E.:* Effect of Sex Hormones on the Electrical Activity of Brain Stem and Diencephalon in Castrated Female Rats ..... 31

*Forgács I., Châtel R., Visy Mária:* The Effect of Hypertonic Sodium Chloride Infused into the Renal Artery. ..... 219

*Gergely M., Nagy S.:* Effect of Exclusion of Small Intestine in Haemorrhagic Shock ..... 97

*Gömöri P., Hársing L., Kállay K., Bartha J., Debreczeni L.:* Arterio-Venous Anastomoses in the Kidney. I. Renal Blood Flow Following the Infusion of Serum Albumin ..... 201

*Grubich V., Malomsoki J., Nemessuri M.:* Cardiorespiratory Responses to Graded Positive-Pressure Breath Holding I. ..... 117

*Ismail A., A., Abdel Hay A., Madwar K. R., Talaat M.:* Hormones in Lymph. (E) Capillary Permeability to $I^{131}$, Risaand $N^{131}I$ in Nephritis ..... 345

*Jancsó T., Galgóczy G.:* A Method for the Continuous Recording of Peripheral Vascular Resistance ..... 173

*Kausz I.:* Melanocyte Stimulating Hormone ..... 351

*Korányi L., Endrőczi E., Tamásy Veronica:* Pituitary Adrenocorticotrophic Hormone (ACTH) Induced Hypoglycaemia ..... 41

*Kovách A. G. B., Szász Erzsébet, Pilmayer N.:* Mortality of Various Avian and Mammalian Species Following Blood Loss ..... 109

*Kovách A. G. B., Bálint Terézia:* Comparative Study on Haemodilution after Haemorrhage in the Pigeon and the Rat ..... 231

*Kurcz M., Nagy I., Kiss Cs., Halmy L.:* Estimation of the Blood Prolactin Level: A critical Analysis of Literary Data and of the Sulman-Simkin Method of Extraction ..... 153

*Li Bok Nam, Szelényi I., Zelles T.:* Comparison of Liver Glycoger Assay Methods ..... 75

*Lőzsa A., Kereszti Zita, Berencsi G.:* Effect of Heat and Cold Exposure on Serum Proteins, Glycoproteins and Lipoproteins in the Rabbit ..... 63

*Makara G. B., Stark E., Mihály Katalin:* Corticotrophin Release Induced by Injection of Formalin in Rats with Hemisection of the Spinal Cord ..... 331

*Mészáros I.:* The Effect of Hypnotic Suggestion on Conditioned Evoked Potentials ..... 101

*Molnár L., Benedek Erika, Taraba I.:* Glutaminase Activity of the Kidney in Experimental Renal Failure of Rats ..... 81

*Molnár L., Czopf J.:* Études des problèmes de la régulation du débit sanguin cérébral. I. Sur le rôle de l'hypothalamus dans la régulation du débit sanguin du tronc cérébral ..... 245

*Monos E., Koltay Edit, Sulyok A., Venkey T., Kovách A. G. B.:* Effect of Local Venous Congestion in Adrenocortical Function. I. Changes in Adrenal Corticoid Secretion after Stepwise Reduction of Venous Outflow ..... 305

*Monos E., Biró Zsuzsanna Sulyok A., Kovách A. G. B.:* Effect of Venous Congestion on Adrenocortical Function. III. Adrenal Medullary and Cortical Blood Flow ..... 321

*Nagy S., Barankay T., Tárnoky Klára:* Effect of Haemorrhagic Shock on Oxygen Tension of Thoracic Duct Lymph ..... 87

*Pavel J.:* Induktionsveränderungen der Glutamat-Oxalazetat-Transaminase in der Henne unter Wirkung von 11-Dehydrokortikosteron ..... 133

*Péczely P.:* Effect of the Median Eminence of the Pigeon (Columba Livia Domestica L.) on the Regulation of Adenohypophysial Corticotropin Secretion ..... 47

*Reviczky L. Alice, Szántó L.:* A Simple Method for Thyrotropic Hormone Assay ..... 167

*Sacharov D. A., Salánky J.:* Physiological and Pharmacological Identification of Neurons in the Central Nervous System of Helix Pomatia L. ..... 19

*Spät A., Sólyom J., Sturcz J., Mészáros Ildikó, Ludwig E.:* Effect of Angiotensin Superfusion on the Rate of Aldosterone Production by Incubated Rat Adrenals ..... 149

*Strashimirov D., Bohus B., Kovács S.:* Pituitary-Thyroid Function of the Rat after Suppression of ACTH Release by Dexamethasone Implants in the Median Eminence ..... 335

*Such G., Obál F.:* Bilateral Interaction of Vagal Vasomotor Reflexes ..... 257

*Sulyok A., Monos E., Koltay Edit, Bede Andrea, Kovács A. G. B.:* Effect of Local Venous Congestion on Adrenocortical Function. II. Changes in Corticoid Release into Venous Blood and Lymph and in Oxygen Consumption ..... 313

*Szilágyi T., Tóth L., Miltényi L., Jóna G.†:* Oxygen Poisoning and Thyroid Function ... 59

*Takács L., Debreczeni L., Lulity I.:* Pressor Activity of Renal Venous Blood in Anaesthetized Dogs ..... 211

PHARMACOLOGIA

*Hetényi E., Vályi-Nagy T.†:* Pharmacology of Cherry (Prunus avium) Stalk Extract I. Effect on Smooth Muscle ..... 183

*Hetényi E., Vályi-Nagy T.†:* Pharmacology of Cherry (Prunus avium) Stalk Extract II. Cardiovascular Effects ..... 189

*Molnár J., György L., Unyi Gabriella, Kenyeres Judit:* Effect of Capsaicin on the Isolated Ileum and Auricle of the Guinea Pig ..... 369

RECENSIONES

*Assali N-S.:* (Ed). Biology of Gestation. Vol. I. Academic Press, New York and London, 1968. (K. Lissák) ..... 376

*Bresler J. B.:* (Ed). Environments of Man. Addison-Wesley Publishing Company, Reading, Massachusetts, 1968). (K. Lissák) ..... 199

*Caianiello E. R.:* (Ed). Neural Networks. springer V. Berlin—Heidelberg, New York, 1968. (K. Lissák) ..... 375

*Eickhoff W., Herberhold C.:* Die Lymphbahnen der menschlichen Schilddrüse. Springer V. Berlin—Heidelberg—New York, 1968. (M. Földi) ..... 379

*Erblsöh F., Oksche A., Seitelberger F.:* (Ed). Symposium on Neurologia—Symposium concernant la neurologie—Symposium über die Neurologie. Springer V. Berlin—Heidelberg—New York, 1966. (St. Környei) ..... 378

*C. von Euler, Skoglund A. Söderberg U.:* Structure and Function on Inhibitory Neural Mechanism. Pergamon Press, Oxford, 1968. (K. Lissák) ..... 376

*Gerhard L.:* Atlas des Mittel- und Zwischenhirns des Kaninschens. Springer V. Berlin—Heidelberg—New York, 1968. (E. Endrőczi) ..... 385

*Harders H.:* (Ed). Advances in Microcirculation. Vol. 1. Karger, Basel 1968. (A. Erdélyi and K. Thuránszky) ..... 381

*Hudlicka O.:* (Ed). Circulation in Skeletal Muscle. Pergamon Press, Oxford, 1968. (K. Lissák) ..... 199

*Janowski H.:* (Ed). Bulletin of the Veterinary Institute, Pulaway, Poland. Vol. 11. (A. Forrai) ..... 385

*Kerkut G. A.:* Experiments in Physiology and Biochemistry, Vol. I. Academic Press, London and New York. 1968. (K. Lissák) ..... 377

New Aspects of Human Genetics. British Medical Bulletin. 1969. Vol. 25. No. 1. (A. Tigyi) ..... 384

*Lasarev N. I.:* (Ed). Hormone Therapy of Malignant Neoplasma. Medicina, Moscow, 1968. (M. Kurcz) ..... 380

*Miller P. L.:* (Ed). Aspects of Cell Motility. No. 22. Cambridge University Press, Cambridge, 1968. (N. A. Biró) ..... 379

*Pfister Cl.:* Probleme der molekularen und allgemeinen Biologie der Zelle. VEB Verlag, Volk Und Gesundheit, Berlin, 1967. (A. Tigyi) ..... 382

Polymorphismes Biochimiques des Animaux. X$^{e}$ Congrès Européen sur les Groupes Sanguins et le Polymorphisme Biochimique des Animaux, Imprimerie Firmin-Didot, Paris—Mesnil—Ivry, 1967. (L. Kesztyüs) ..... 383

*Quarton G. C., Melnechuk T., Schmitt F. O.:* (Eds). The Neurosciences. The Rockefeller University Press, New York, 1967. (K. Lissák) ..... 375

*Schreiner G. E.:* (Ed). Proceedings of the Third International Congress of Nephrology, Washington, D. C. 1966. (P. Bálint) ..... 99

*Spicer C. C.:* (Ed). Computing in Medicine. The British Council, London. (K. Lissák) ... 378

*Straub F. B., Friedrich P.:* Symposium on Modern Methods in the Investigation of of Protein Structure. Budapest, 1965. (I. Alkonyi) ..... 200

*Wetterer E., Kenner Th.:* Grundlagen der Dynamik des Arterienpulses. Springer V. Berlin—Heidelberg—New York, 1968. (K. Lissák) ..... 199

## INDEX AUTORUM


### A

*Abdel Hay A.* vide *Ismail A. A.* 345
*Airapetianz E. Sh., Konstantinov A. I., Matjushkin D. P.:* Brain Echolocation Mechanisms and Bionics 1
*Avar Z., Monos E.:* Biological Role of Lateral Hypothalamic Structures Participating in the Control of Maternal Behaviour in the Rat 285
*Avar Z., Monos E.:* Behavioural Changes in Pregnant Rats Following Far-Lateral Hypothalamic Lesions 295
*Ángyán L., Rózsavölgyi M., Kolta P.:* Stopping Elicited by Conditioned Stimuli as Compared with the Thalamic Induced Arrest Reaction 271

### B

*Banga I.:* The Structure of Protein-Polysaccharides and Their Role in the Function of Connective Tissue Fibres 355
*Barankay T.* vide *Nagy S.* 87
*Bartha J.* vide *Gömöri P.* 201
*Bálint T.* vide *Kovách A. G. B.* 231
*Bede A.* vide *Sulyok A.* 313
*Benedek E.* vide *Molnár L.* 81
*Berencsi G.* vide *Lózsa A.* 63
*Biró Zs.* vide *Monos E.* 321
*Bohus B.;* Evaluation of the Role of Feedback Effect of Corticosteroids the Control of Pituitary ACTH Release 141
— vide *Strashimirov D.* 335

### C

*Châtel R.* vide *Forgács I.* 219
*Czopf J.* vide *Molnár L.* 245

### D

*Dauda G., Dévényi I., Szokoly V.:* Converting Enzyme Activity in Experimental Cirrhosis of the Rat 365
*Debreczeni L.* vide *Gömöri P.* 201
— vide *Takács L.* 211
*Dévényi I.* vide *Dauda G.* 365

### E

*Endrőczi E.:* Effect of Sex Hormones on the Electrical Activity of Brain Stem and Diencephalon in Castrated Female Rats 31
— vide *Korányi L.* 41

### F

*Forgács I., Châtel R., Visy M.:* The Effect of Hypertonic Sodium Chloride Infused into the Renal Artery 219

### G

*Galgóczy G.* vide *Jancsó T.* 173
*Gergely M., Nagy S.:* Effect of Exclusion of Small intestine in Haemorrhagic Shock 97
*Gömöri P., Hársing L., Kállay K., Bartha J., Debreczeni L.;* Arterio-Venous Anastomoses in the Kidney. I. Renal Blood Flow Following the Infusion of Serum Albumin 201
*Grubich V., Malomsoki J., Nemessuri M.:* Cardiorespiratory Responses to Graded Positive-Pressure Breath Holding I. 117
*György L.* vide *Molnár J.* 369

### H

*Hársing L.* vide *Gömöri P.* 201
*Hetényi E., Vályi-Nagy T.:* Pharmacology of Cherry (Prunus avium) Stalk Extract. I. Effect of Smooth Muscle 183
*Hetényi E., Vályi-Nagy T.:* Pharmacology of Cherry (Prunus avium) Stalk Extract II. Cardiovascular Effects 189

### I

*Ismail A. A., Abdel Hay A. Madwar K. R., Talaat M.:* Hormones in Lymph (E) Capillary Permeability to $^{131}$I, Risa-and N$^{131}$I in Nephritis 345

### J

*Jancsó T., Galgóczy G.:* A Method for Continuous Recording of Peripheral Vascular Resistance 173
*Jóna G.* vide *Szilágyi T.* 59

## K

*Kausz I.:* Melanocyte Stimulating Hormone 351
*Kállay K.* vide *Gömöri P.* 201
*Kenyeres J.* vide *Molnár J.* 369
*Kereszti Z.* vide *Lózsa A.* 63
*Kiss Cs.* vide *Kurcz M.* 153
*Kolta P.* vide *Ángyán L.* 271
*Koltay E.* vide *Monos E.* 305
— vide *Sulyok A.* 313
*Konstantinov A. I.* vide *Airapetianz E. Sh.* 1
*Korányi L., Endrőczi E., Tamási V.:* Pituitary Adrenocorticotrophic Hormone (ACTH) Indiced Hypoglycaemia 41
*Kovách A. G. B., Szász E, Pilmayer N.:* Mortality of Various Avian and Mammalian Species Following Blood Loss 109
*Kovách A. G. B., Bálint T.:* Comparative Study on Haemodilution after Haemorrhage in the Pigeon and the Rat 231
— vide *Monos E.* 305, 321
— vide *Sulyok A.* 313
*Kovács S.* vide *Strashimirov D.* 335
*Kurcz M., Nagy I., Kiss Cs., Halmy L.:* Estimation of the Blood Prolactin Level: A Critical Analysis of Literary Data and of the Sulman-Simkin Method of Extraction 153

## L

*Li Bok Nam, Szelényi I., Zelles T.:* Comparison of Liver Glycogen Assay Methods 75
*Lózsa A., Kereszti Zs, Berencsi G.:* Effect of Heat and Cold Exposure on Serum proteins and Lipoproteins in the Rabbit 63
*Ludwig E.* vide *Spät A.* 149
*Lulity I.* vide *Takács L.* 211

## M

*Madwar K. R.* vide *Ismail A. A.* 345
*Makara G. B., Stark E., Mihály K.:* Corticotrophin Release Indiced by Injection of Formalin in Rats weith Hemisection of the Spinal Cord 331
*Malomsoki J.* vide *Grubich V.* 117
*Matjushkin D. P.* vide *Airapetianz E. Sh.* 1
*Mészáros I.:* The Effect of Hypnotic Suggestion on Conditioned Evoked Potentials 101
— vide *Spät A.* 149
*Miltényi L.* vide *Szilágyi T.* 59
*Molnár J., György L., Unyi G, Kenyeres J:* Effect of Capsaicin on the Isolated Ileum and Auricle of the Guinea Pig 369
*Molnár L., Benedek E, Taraba I.;* Glutaminase Activity of the Kidney in Experimental Renal Failure of Rats 81
*Molnár L., Czopf J.: Études des problèmes de la régulation du débit sanguin cérébral. I. Sur le rôle de l'hypothalamus dans la régulation du débit sanguin du tronc cérébral 245*
*Monos E., Koltay E., Sulyok A., Venkey T., Kovách A. G. B.;* Effect of Local Venous Congestion in Adrenocortical Function. I. Changes in Adrenal Corticoid Secretion after Stepwise Reduction of Venous Outflow 305
*Monos E., Biró Zs., Sulyok A., Kovách A. G. B.:* Effect of Venous Congestion on Adrenocortical Function. III. Adrenal Medullary and Cortical Blood Flow 321
— vide *Avar Z.* 285, 295
— vide *Sulyok A.* 313

## N

*Nagy S., Barankay T., Tárnoky K.;* Effect of Haemorrhagic Shock on Oxygen Tension of Thoracic Duct Lymph 87
— vide *Gergely M.* 97
*Nagy I.* vide *Kurcz M.* 153
*Nemessuri M.* vide *Grubich V.* 117

## O

*Obál F.* vide *Such G.* 257

## P

*Pavel J.: Induktionsveränderungen der Glutamat-Oxalazetat-Transaminase in der Henne unter Wirkung von 11-Dehydrokortikosteron* 133
*Péczely P.:* Effect of the Median Eminence of the Pigeon (Columba Livia Domestica L.) on the Regulation of Adenohypophysial Corticotropin Secretion 47
*Pilmayer N.* vide *Kovách A. G. B.* 109

## R

*Reviczky L. A., Szántó L.:* A Simple Method for Thyrotropic Hormone Assay 167
*Rózsavölgyi M.* vide *Ángyán L.* 271

## S

*Sacharov D. A., Salánky J.;* Physiological and Pharmacological Identification of Neurons in the Central Nervous System of Helix Pomatia L. 19
*Salánki J.* vide *Sacharov D. A.* 19
*Sólyom J.* vide *Spät A.* 149
*Spät A., Sólyom J., Sturcz J., Mészáros I, Ludwig E.:* Effect of Angiotensin Superfusion on the Rate of Aldosterone Production by Incubated Rat Adrenals 149
*Stark E.* vide *Makara G.* 331
*Strashimirov D., Bohus B., Kovács S.:* Pituitary-Thyroid Function of the Rat after Suppression of ACTH Release by Dexamethasone Implants in the Median Eminence 335

*Sturcz J.* vide *Spät A.* 149
*Such G., Obál F.:* Bilateral Interaction of Vagal Vasomotor Reflexes 257
*Sulyok A., Monos E., Koltay E., Bede A., Kovách A. G. B.:* Effect of Local Venous Congestion on Adrenocortical Function. II. Changes in Corticoid Release into Venous Blood and Lymph and in Oxygen Consumption 313
— vide *Monos E.* 305, 321

**Sz**

*Szántó L.* vide *Reviczky L. A.* 167
*Szász E.* vide *Kovách A. G. B.* 109
*Szelényi I.* vide *Li Bok Nam* 75
*Szilágyi T., Tóth L., Miltényi L., Jóna G.:* Oxygen Poisoning and Thyroid Function 59
*Szokoly V.* vide *Dauda G.* 365

**T**

*Takács L., Debreczeni L., Lulity I.:* Pressor Activity of Renal Venous Blood in Anaesthetized Dogs 211
*Talaat M.* vide *Ismail A. A.* 345
*Tamásy V.* vide *Korányi L.* 41
*Taraba I.* vide *Molnár L.* 81
*Tárnoky K.* vide *Nagy S.* 87
*Tóth L.* vide *Szilágyi T.* 59

**U**

*Unyi G.* vide *Molnár J.* 369

**V**

*Vályi-Nagy T.* vide *Hetényi E.* 183, 189
*Venkey T.* vide *Monos E.* 305
*Visy M.* vide *Forgács I.* 219

**Z**

*Zelles T.* vide *Li Bok Nam* 75

## INDEX RERUM


Achilles tendon-mucoid$_1$, AT-mucoid$_1$ 355
acoustic system of bats 9, 11
—, electrical reactions 10
adenohypophysis 5
—, ACTH content of 51
— —, influence of insuline treatment 51
— — —, prednisolon treatment 51
adrenalectomy 42
—, psychic stress 42
—, systemic stress 43
— —, hypoglycaemia 43
adrenal medullary blood flow 321
—, effect of venous congestion 321, 323
—, under basal conditions 323
adrenal venous blood flow 315
—, effect of congestion on pressure 315
— —, on corticod oupot 315
—, under basal conditions 323
adrenocorticotrophic hormone 41, 47
—, effect on blood sugar level 41
—, induced insulin secretion 44
adrenocortical function 305, 314, 321
—, effect of venous congestion 305, 314, 321
aldosterone production 149
—, effect of angiotensin 150
—, in vitro 149
alkaline phosphatase activity 82, 84
angiotensin 149
approach type conditioned reflex 274
—, stopping of 273
arrest reaction 271
—, thalamic induced 271
arterio-venous anastomoses 201
—, in the kidney 201
attenuator-compensator unit 322
auricle 372
—, effect of capsaicin on 372
automatic feeding device 272
avoidance conditioned reflex 274
—, stopping of 273

bionics 1
bilateral antagonism 263
blood loss 109
—, mortality of various species 109
blood in pigeon and rat 231
—, haemoglobin content 233
—, plasma albumin and globulin 237
—, plasma total protein 237
blood pressure response 119
—, in crow 119
—, in hen 119
—, in pigeon 119
brain echolocation mechanisms 1
—, general characteristics 13
—, regulation scheme 15
brain stem 31
—, electrical activity 31
— —, effect of sex hormones 31

capsaicin 369
—, effect on isolated auricle 372
— —, on isolated ileum 369
cardiorespiratory responses 117
—, graded positive-pressure breath holding 117
cartilage mucoprotein$_1$, CMP$_1$ 355
cell types of Helix Pomatia L. 26, 27, 28
cherry stalk extract 183, 189
—, pharmacological effect of 183, 189
circulatory and respiratory parameters 130
cirrhosis 365
—, experimental 365
cold exposure of rabbits 63
—, effect on glycoproteins 63
— —, on lipoproteins 63
— —, on serum proteins 63
Columba Livia Domestica L. 47
conditioned evoked potentials 101
—, effect of hypnotic suggestion 101
— —, specific probability of appearance 104
conditioned fear 42
—, hypoglycaemia induced by 43
converting enzyme activity 365
—, in cirrhosis of rats 365
corticosteroid production 143
—, effect of ACTH 144
— —, hemiadrenalectomy 144
— —, immobilization 144
—, in vitro 143
corticosterone secretion 308
—, effect of venous congestion 309
CRF activity 47
—, content of medianeminence 50
— —, influence of insuline treatment 50
— — —, prednisolon treatment 51

dehydration 50, 51
—, effect on ACTH content 51
— —, on CRF content 50

depressor responses 259
—, bilateral interaction of 259
diencephalon 31
—, electrical activity 31
— —, effect of sex hormones 31
dynamics of sweep pulses 7

echolocational detection 1
—, discrimination 8
electrical activity 31
—, basomedial hypothalamus 33
—, brain stem 31
—, diencephalon 31
—, septo-preoptic region 33
EPSP 24
—, alterations of membrane potential 24
evoked response 37
—, inhibitory period 37
—, recovery period 37
explorative activity 287
—, lateral hypothalamic lesions 287

far-lateral hypothalamus 295
—, role in behaviour 295
— —, in food intake 296
— —, in water intake 296
feedback effect 141
—, role of corticosteroids 141
food intake 286
—, lateral hypothalamic lesions 287

glomerular filtration rate 211
—, influence of hypertonic saline 221
glutaminase activity 82, 84
glycogen 75
—, direct estimation of 78
glycograms 68

haemodilution 231
—, after continuous bleeding 238
— —, slow graded haemorrhage 233
—, after haemorrhage in pigeons 232
— —, in rats 232
haemorrhagic shock 87
—, effect on oxygen tension 87
—, clusion of small intestine 93
heat exposure of rabbits 63
—, effect on glycoproteins 63
— —, on lipoproteins 63
— —, on serum proteins 63
Helix Pomatia L. 19
—, identification of neurons 19
— —, action potential curve 21
— —, effect of mediator substances 25
— —, effect of polarizing current 22
—, nervus system 19
hepatic cirrhosis 365
horseshoe bat 3
$H_2$ — washout technique 322
hydration 50, 51
—, effect on ACTH content 51
—, effect on CRF content 50
hydrocortisone secretion 309
—, effect of venous congestion 309
hyperbaric oxygenation 60
—, survival time of rabbits in 60
— —, of rats in 60
hypertonic sodium chloride 220
—, effect on kidney 220
hypnotic suggestion 101
—, effect on conditioned evoked potentials 101
hypoglycaemia 41
—, induced by ACTH 41
— —, by psychic stress 42
— —, by systemic 42
hypothalamic lesions 286, 295
—, effect on explorative activity 286
— —, on food intake 287, 296
— —, on maternal activity 287
— —, on motility 287
— —, on nest building activity 297
— —, on pregnancy 296
— —, on rectal temperature 296
— —, on water intake 296
— —, on weight of endocrine glands 299

identification of neurons 21
—, in Helix Pomatia L. 21, 22
ileum 370
—, effect of capsaicin on 370
*Induktionsveränderungen der Glutamat-Oxalazetat-Transaminase* 133
insect catching 6
—, of rhinolophidae 6
—, of vespertilionidae 6
insulin treatment 50, 51
—, effect on ACTH content 51
— —, on CRF content 50

kidney 81
—, arterio-venous anastomoses 201
—, glutaminase activity 81, 84
— —, in renal failure 81
—, production of pressure substance 211

lateral hypothalamic structures 286
—, role in explorativ activity 287
— —, in food intake 287
— —, in maternal behaviour 286
— —, in motility 287
— —, in survival of offsprings 289
lipograms 71
liver glycogen assay 75
local venous congestion 305, 314, 321
—, effect on adrenal cortical blood flow 321
— —, on adrenal medullary blood flow 321
— —, on adrenal oxygen consumption 316, 317
— —, on arterio-venous oxygen saturation 316
— —, on corticosterone secretion 308
— —, on hydrocortisone secretion 309
lymph 346
—, hormones in 346

mammary glands 289
—, following lateral hypothalamic lesions 289
median eminence 337
—, effect of dexamethasone implants 337
— —, on thyroid$^{131}$J uptake 337
— — —, during exposure to cold 339
— —, on compensatory thyroid hypertrophic 339
melanocyte stimulating hormone 351
—, activity in hypophysis of carps 351
motility 286
—, effect of food restriction on 289
—, effect of hypothalamic lesions 287
Myotis Oxygnathus 2

nest building activity 297
—, influence of hypothalamic lesions on 298
oestradiol-17-beta 33

—, influence on evoked potentials 33
orientation in space of bats 2
—, during insect catching 4
—, during peaceful searching flight 3
ovariectomy 34
—, influence on evoked potentials 34
oxygen poisoning 59
—, thyroid function in 59
oxygen tension of thoracic duct lymph 87

peripheral vascular resistance 173
—, method of continuous recording 173
— —, theoretical principles 173
piegon 47
—, median eminence of 47
— —, effect on ACTH secretion 47
pituitary ACTH release 41, 141
—, effect of formaline injection 41, 332
—, following spinal cord hemisection 332
pituitary-thyroid function 335
—, influence of dexamethasone 335
prednisolon treatment 50, 51
—, effect on ACTH content 51
—, effect on CRF content 50
pregnancy 296
—, influence of far-lateral hypothalamic lesions 296
progesterone 36
—, influence on electrical activity 36
— —, of brain stem 36
— —, of diencephalon 36
prolactin 153
—, extraction from blood 158
—, specific action of 155
prolactin level 153
—, estimation in blood 153
protein-polysaccharide 355
—, light, PP-L 355
—, heavy, PP—H 355
protein polysaccharides 355
—, analysis of 357
—, effect on collagenous fibres 359
—, role in connective tissues 355

Prunus avium 183, 189
—, pharmacological effect of 183, 189
—, effect on cardiovascular system 189
— —, on smooth muscle 183

rectal temperature 296
—, changes after brain lesions 296
*règulation du débit sanguin cèrébral* 245
—, *l'effet de l'asphyxie* 247
— —, *à la pression artèrielle* 253
— —, *à la stimulation hypothalamique* 248, 251
renal blood flow 214
—, influence of hypertonic saline 221
renal venous blood 211
—, pressor activity of 211
renin 366
resistance recorder 174
—, amplifier 176
—, supply unit 175
—, tranducer 174
Rhinolophus Ferrum — Equinum 3

spinal cord hemisection 332
—, effect of formaline injection on ACTH release 332
sportsmen 118
—, blood pressure 123
—, gas exchange 123
—, heart rate 118
stopping 271
—, EEG changes 277
—, elicited by conditioned stimuli 271
—, in the course of extinction 277
stress-induced ACTH release 338
—, effect of dexamethasone on 338
"summary" electrical reactions 9
Sulman's procedure 158, 161
systemic stress 41
—, hypoglycaemia induced by 42

thalamic nuclei 271
—, role in arrest reaction 272
thyroid function 59, 337
—, effect of steroid implants in the brain on 337
—, oxygen poisoning 59
thyrotrophic hormone assay 167

ultrasonic signals of bats 2
—, orientation in space 5

vagal motor reflexes 257
—, depressor responses 258
vaginal evoked potentials 36
vago-pressor reflex 266
—, bilateral interaction of 266

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 1—17 (1969)*

# *Physiologia*

## BRAIN ECHOLOCATION MECHANISMS AND BIONICS

By

E. Sh. AIRAPETIANZ, A. I. KONSTANTINOV and D. P. MATJUSHKIN

DEPARTMENT OF HIGHER NERVOUS ACTIVITY,
LENINGRAD UNIVERSITY, USSR

(Received January 2, 1968)

The perception and analysis of various informations about the surrounding media are essential aspects of higher nervous activity of animals and man. The type of analysis of spatial signals is determined by the phylogenesis of the nervous system, its ontogenesis and ecological specialization of analysatory systems. The range of spatial information and means of its evaluation reflect basic factors of the evolution of analysatory systems and behaviour taken as a whole. The part of higher nervous activity known as spatial analysis has been the subject of special and systematic studies carried out in our laboratory headed by one of the authors (AIRAPETIANZ 1967). Investigation of the problem is closely related to the most urgent tasks of space study. Our researches have been based on the work of I. M. SECHENOV, I. P. PAVLOV and A. A. UKHTOMSKY.

During the last years attention of biologists and engineers has been focussed on some animals using a specific mode of orientation in space-echolocation.

Echolocation analysis is based on the emission of ultrasonic pulses with the sequential pick up and treatment of their echoes returned from the objects; a number of animals are using this particular way of "fumbling" space for detection and avoiding interceptions as well as for searching and catching food. The real "aces" of echolocation are small insectivorous bats of the suborder Microchiroptera whose mechanisms of space orientation have been the subject of our investigations for a few years. Our studies on echolocation were carried out in the following directions.

Firstly, a bioacoustic study of the location signals the bats are emitting during flight when they detect obstacles, catch insects and distinguish between different geometrical figures used as conditioned stimuli.

Secondly, electrophysiological investigations of the receiving (auditory) system of bat's echolocators, detection of nervous centres participating in the

pick up and analysis of ultrasonic signals (by the method of evoked potentials), investigation of the neuronal organization of the auditory system and properties of its separate neurons.

Thirdly, a morphophysiological study of auditory centres and their role in the process of echolocation.

### *1. Parameters of ultrasonic signals of bats*

According to the acoustic characteristics of location signals, all the bats on the territory of the Soviet Union may be divided into two isolated groups. The first group comprises bats of the family Vespertilionidae. The typical spec-

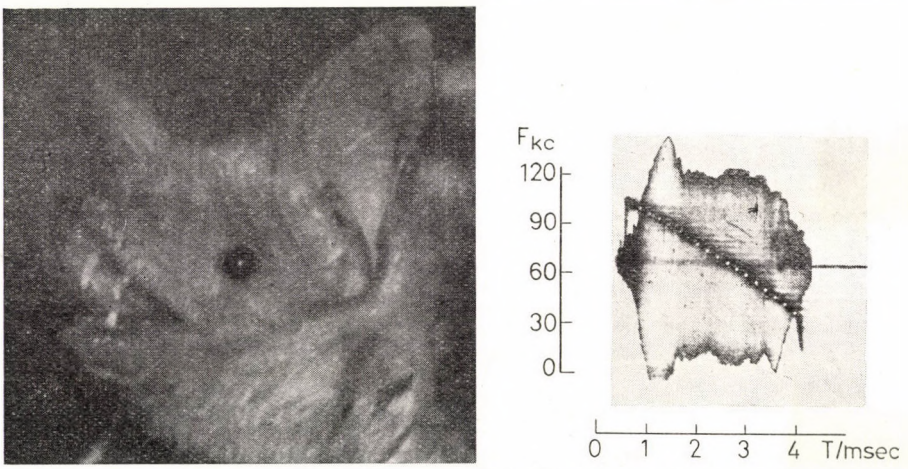


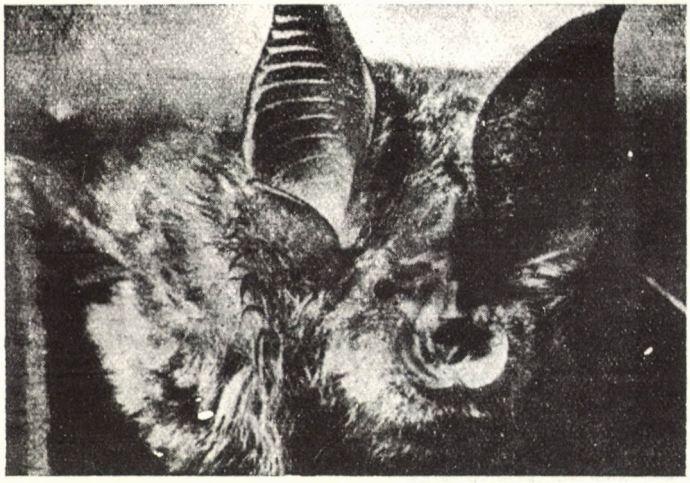

*Fig. 1.* Myotis oxygnathus and the oscillogram of its locational signal. The line intersecting the pulses indicates changes in the frequency sweep

imen is *Myotis oxygnathus* (Fig. 1). Like other species of Vespertilionidae *Myotis oxygnathus* emits through the mouth short pulses of 5 msec duration. Depending on the situation the pulse-repetition rate may vary from 8—10 pulses per sec during a peaceful searching flight to 100—150 pulses per second upon overcoming of obstacles or insect catching. The characteristic feature of pulses emitted by the bats of this group is a linear change of the frequency sweep within each pulse. Such pulses were called frequency modulated (Griffin 1958; Novick 1958). In our conditions in *Myotis oxygnathus* the frequency at the beginning of the pulse was 90—120 kc diminishing almost linearly to 20—30 kc at the end. Thus the frequency change covers about two octaves. Oscillograms of the typical pulses of *Myotis oxygnathus* are represented in Fig. 1.

The pulses of bats belonging to the second group comprising Horseshoe bats, Rhinolophidae, have different characteristics. According to Möhres (1953), Rhinolophidae emit location signals not through the mouth but through the nostrils, lying at a distance of half a wavelength of an emitted ultrasound in

the depth of a leathery process (Fig. 2). The latter serves for focussing an ultrasonic beam and changing the diagram of its direction.

It was generally acknowledged that Rhinolophidae send standard mono-frequent signals which have durations from 60 to 100 msec, i.e. 20—50fold of that in Vespertilionidae. The pulse repetition rate is constant, attaining 4—6 pulses per second. This mono-regime function of the echolocation system was



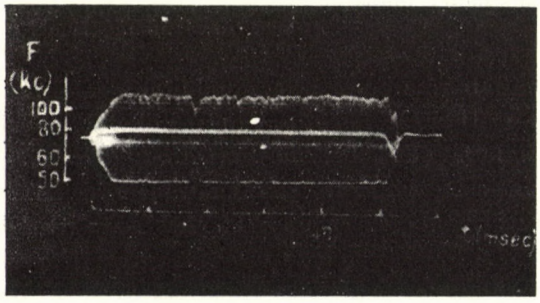



*Fig. 2.* The big Horseshoe bat (Rhinolophus ferrum-equinum) and the oscillogram of a single locational signal

regarded as a peculiarity of the Horseshoe bats but our experiments have shown that the echolocators of Rhinolophidae can work in two regimes.

For orientation in space during a peaceful searching flight a Horseshoe bat utters single sounds the average duration of which is 50 msec, and the maximal, 95 msec (Fig. 2). The pulse-to-pulse intervals are 45—50 msec on the average. Seeing an unknown object the animal begins to investigate it. Instead of single signals, it emits "packs" of shorter impulses interrupted by small

pauses (Fig. 3). The nearer the Horseshoe bat to the object the greater the number of pulses in the pack. Each pack may contain 2, 3, 4 or even 20 pulses. With the increase of their number in the pack, the duration of these pulses and pauses between them is reduced. Intervals between the packs remain almost the same as in the case of single pulses, or even decrease. As a result of such "splitting" of signals, the effectiveness of the Horseshoe bat's echolocator rises thus increasing the amount of information to be obtained. This is a particular echolocation regime earlier unknown for Rhinolophidae.

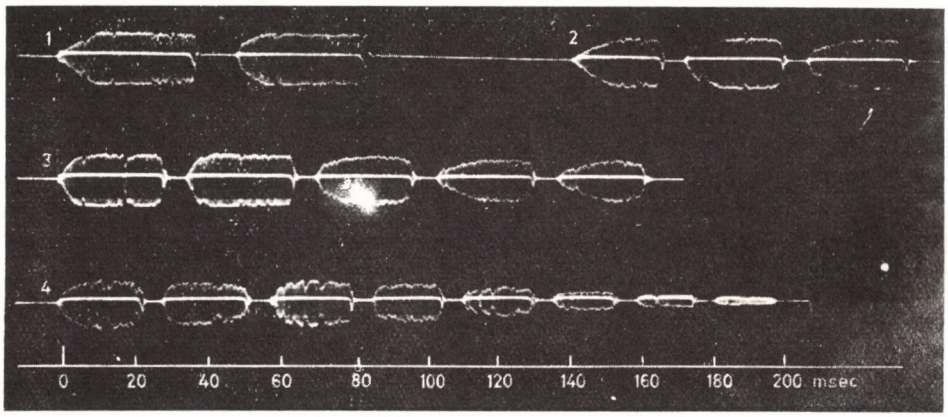



*Fig. 3.* Oscillograms of pulses of Rhinolophus ferrum-equinum emitted in "packs". 1, 2, 3, 4 — "packs" of 2, 3, 5 and 8 pulses. Pulses in the packs are divided by short pauses and packs of 2 and 3 pulses, by intervals

The pulse frequency sweep in Rhinolophidae has no such modulation as is typical for the pulses of Vespertilionidae. The frequency of pulses in the packs as well as in single signals remains constant attaining 80—100 kc in the different species. But sometimes at the beginning and always at the end of each pulse the frequency changes by 15—20 kc. The pulse amplitude in this region in some Rhinolophidae sharply increases forming a peak.

Hence, the forms of echolocation in bats of different families are different. However, all these bats live under similar conditions, detect and overcome various interceptions, catch the flying insects and, hence, must solve the same locational problems. They do solve them, but not always in the same way.

### *2. Hearing and echolocation during insect catching*

All bats of our fauna are insectivorous. Opinions differ as to the means the bats are applying for detecting and catching insects. Some of the authors (Kuzyakin 1950; Treat 1955) believe that only hearing helps bats to obtain food, while others (Griffin et al. 1960) have proved experimentally under labo-

ratory conditions the ability of bats to catch insects using only their echolocation apparatus. But what about natural conditions? Do different bats behave differently during pursuit? We have made an attempt at answering these questions when working in the field during the expedition in Nagorny Karabakh in 1966.

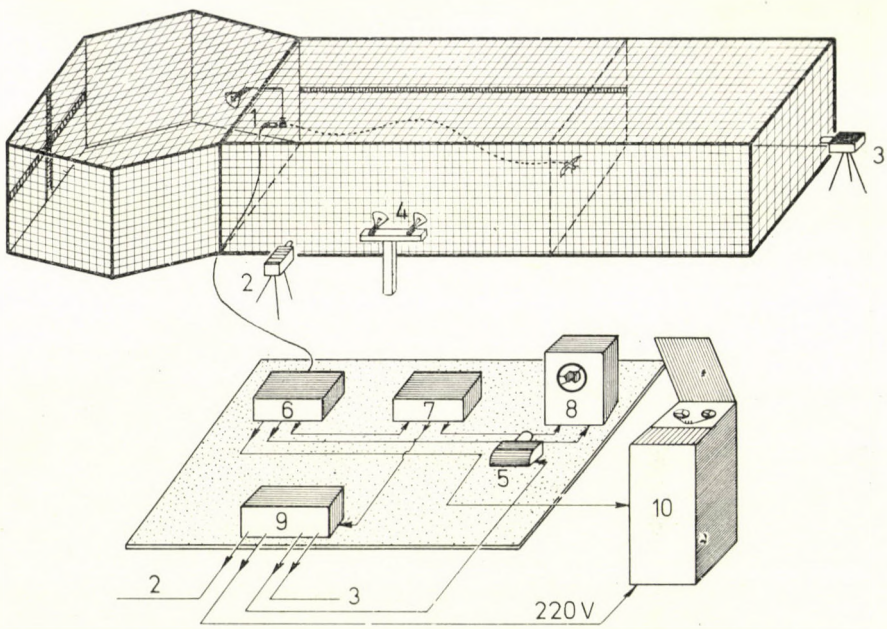



*Fig. 4.* The experimental flight room and the scheme of registering locational signals of bats. Abbreviations: 1 = condenser microphone with cathode follower; 2 and 3 = lateral and frontal cameras for registering the bat's flight; 4 = elimination lamps; 5 = camera for photographing oscilloscope screen; 6 = amplifier; 7 = deviometer performing an instant analysis of the frequency sweep of the signal; 8 = dual beam cathode oscilloscope; 9 = synchronized system; 10 = ultrasonic tape recorder

The experiments were carried out in a large flight chamber constructed in the open with a ceiling and walls covered with a thin nylon net (Fig. 4). The observations were made of catching manoeuvres of bats and their ways of discriminating free-flying and fixed insects. In the latter instance a living insect (mostly various night butterflies and dragonflies) was fastened to a thin wire the length of which limited the insect's flight and in the opposite end of the flight chamber a bat was set free.

The process of insect catching was recorded by two cameras, while the ultrasonic sounds emitted by the bats were picked up by a condenser microphone, amplified and fed into 1. an ultrasonic tape recorder, 2. a deviometer performing an instant analysis of the frequency sweep of the signal, and 3. a dual beam cathode oscilloscope from the screen of which the signal and its frequency characteristics were recorded on 35 mm film. All these apparatuses worked by a synchronized system (Fig. 4).

Various experiments with synchronous or sequential presentation of active (buzzing) and immobile insects, as well as insects buzzing but concealed from the bat have shown that bats detect a prey by hearing the sounds arising from the wing vibration of insects. This is the only common feature of insect

catching in Vespertilionidae and Rhinolophidae (KONSTANTINOV and SOKOLOV 1967).

After the aural detection of an insect, Vespertilionidae sharply increase the activity of their echolocation apparatus. At the searching stage *Myotis oxygnathus* emits 7—10 pulses per sec with durations of 3—4 msec each. The frequency of these pulses changes from 96 kc on the average at the beginning to 26 kc at the end. Approaching the buzzing insect to an average of 2 m 30 cm (1 m 80 cm to 3 m), depending on the species of insect, the bat suddenly increases the emittance rate of signals, reducing the intervals between them to an average of 40 msec (30 to 70 msec). Other parameters remain unaltered.

This second phase of echolocation activity was observed only during catching of buzzing insects. It was absent when the bat approached a passive insect and echolocation directly entered the third phase. The onset of the third phase is characterized on one hand by the appearance of a pulse several times higher in amplitude than the preceding as well as all the successive pulses and, on the other, by a decrease in the duration of the next pulse by 0.8 to 1 msec, i.e. by about one third. The change of the second into the third phase occurred at an average distance of 1 m 10 cm (80 cm to 1 m 40 cm) from the insect. A further approach led to a simultaneous decrease of intervals between pulses and their durations, as well as to the reduction of amplitudes of signals, whereas the range of frequency sweep remained almost unchanged.

The appearance of the second echolocation phase coincided in distance with the onset of the audial detection of buzzing insects in other experiments, while the third phase was the beginning of the echolocational detection (KONSTANTINOV 1968). The example of a phasic change in the parameters of location signals of *Myotis oxygnathus* during catching of buzzing insects is presented in Fig. 5.

Hence during pursuit of insects Vespertilionidae use a combined location: a target is detected by the method of passive location, i.e. by acoustic perception, whereas pointing and intercepting the prey are accomplished by means of echolocation.

Rhinolophidae act otherwise. They do not use echolocation but are catching insects with the aid of passive location, which means that they orient to sounds uttered by the insects themselves (butterflies). If the butterfly stops dead, i.e. folds its wings even at a distance of 15 to 20 cm from the Horseshoe bat, the latter loses interest and instantly flies away. Under natural conditions Rhinolo phidae do not respond to immobile insects.

A clue to the understanding of such great differences in the use of echolocation for insect catching by the bats Vespertilionidae and Rhinolophidae is probably due to the different food specialization of these bats. The majority of insect species serving for food to Vespertilionidae are not capable of discriminating ultrasounds (ROEDER 1965), and cannot therefore detect the bat by its

echo-signals, while night butterflies, which mostly serve as food for Rhinolophidae, are able to discriminate signals of bats even at a distance of 30 m and thus avoid encounter (ROEDER and TREAT 1964). Under such conditions the less pulses the bat emits, the longer it remains unnoticed and, hence, the more chances it will have to approach and catch its victim.

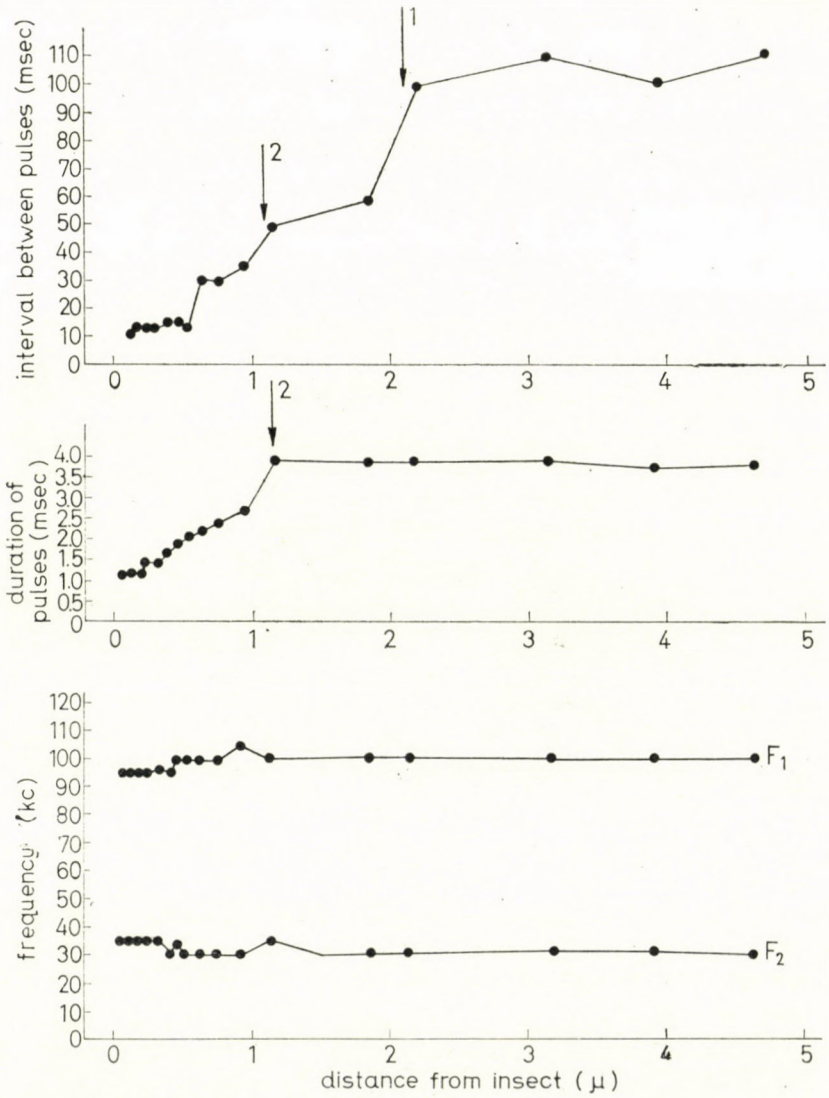



*Fig. 5.* Dynamics of changes in frequency of sequence, duration and frequency sweep of pulses of Myotis oxygnathus during insect catching. The first arrow in the upper graph indicates the moment of aural detection of an insect. The second arrow indicates the onset of the third catching stage — echolocational detection of an insect and preparation for the attack

### 3. *Echolocational discrimination*

Can bats by means of echolocation, i.e. by the difference in the returned signals, discriminate the quality of objects and judge about their size and shape? Apart from its scientific interest, the question has an importance for engineering and especially for radiolocational discrimination of objects. Now we can answer this question in the affirmative.

Simultaneously with American investigators (GRIFFIN et al. 1965) but using another technique we have shown that Vespertilionidae discriminate equivalent volumetrical figures (cubes, cylinders and pyramids) (AIRAPETIANZ and

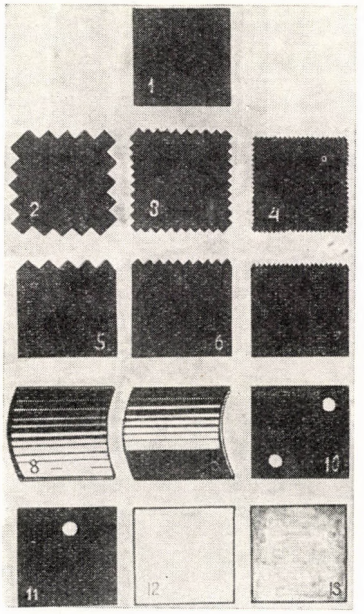



*Fig. 6.* Differentiation figures used for discrimination tests. All figures are of the same size. Square 1 (14 × 14) is a positive figure, the other squares are negative. Square 12 is made of plexiglass, square 13 of brass and all the remaining squares are made of aluminium

KONSTANTINOV 1965) as well as flat equivalent figures of different shape (squares, circles, triangles) or several squares of about equal size (KONSTANTINOV 1966; KONSTANTINOV and AKHMAROVA 1968). These experiments revealed an unusual differentiating ability of the echolocation system of bats. *Myotis oxygnathus*, for example, at a distance of 2.5 m and at the 80% level distinguished between two aluminium squares with the sides differing in 6 mm only, vision and olfaction having been eliminated. Even more striking results were obtained in further experiments which first revealed the possibility of echolocational discrimination in Rhinolophidae.

In Fig. 6 are shown different figures of identical area which were presented for discrimination to Vespertilionidae and Rhinolophidae. As a positive

(given with food) figure we used square 1. All the other targets were negative and were sequentially presented together with the positive figure. We have obtained statistically reliable evidence of the unusual "analytical" capabilities of the bats. Both species distinguished between squares with different edges, discriminated flat squares from concave or convex ones, and aluminium from equivalent plywood or plexiglass squares almost with equal success at the 70 to 100% level. *Myotis oxygnathus* showed higher and more stable indices, whereas the discrimination level of *Rhinolophus ferrum-equinum* was somewhat lower (70 to 90%) but still enough to speak about discrimination.

The bats only failed to discriminate equivalent squares made of different metals (e.g. aluminium square from iron or brass ones).

During these experiments, just as in the previous ones, vision and olfaction had been eliminated.

The mechanisms underlying echolocational discrimination of bats are still unknown, and form the subject of our investigations.

### *4. Electrophysiological investigations of the acoustic system of bats*

*a) "Summary" electrical reactions.* Electrophysiological experiments with Vespertilionidae and Rhinolophidae allowed to study the electrical reactions of the auditory nerve, dorsal colliculi and cortical auditory area to short ultrasonic signals (from 0.25 to 10 msec). Fig. 7 shows the action potential of the auditory nerve (A), evoked potential of the dorsal colliculus (B), and the response of the cortical auditory area to the shortest single ultrasonic stimulus (C). This is a general pattern of response to the shortest ultrasonic signals in the tested regions of the bats' auditory system (Airapetianz 1967; Vasilyev and Matjushkin 1967; Matjushkin 1969; Kotelenko and Matjushkin 1969; see also Grinnell 1963; Suga 1965).

In the case of longer signals we observed more complex reactions consisting of a strong response to the switching on of a stimulus, an on-effect, a weak response to a current stimulus (series of low oscillations), and of a more or less clear response to the switching off of a stimulus, off-effect. "D" in Fig. 7 designates the response to switch-on and switch-off of a stimulus revealed in the dorsal colliculus. Hence the auditory system of bats discriminates between the onset and the end of a signal as points carrying the greatest amount of information (Grinnell 1963; Vasilyev 1967).

Using evoked potentials as indices of responses of the acoustic system, we investigated frequency-threshold curves in the auditory nerve, dorsal colliculus and cortical auditory area (Fig. 7E). All these curves had the minima in the ultrasonic region and were found to be similar in each animal. The frequency-threshold curves of the auditory centres of Vespertilianidae and Rhinolophidae proved to be similar in shape (Fig. 8A). At the same time we found differences

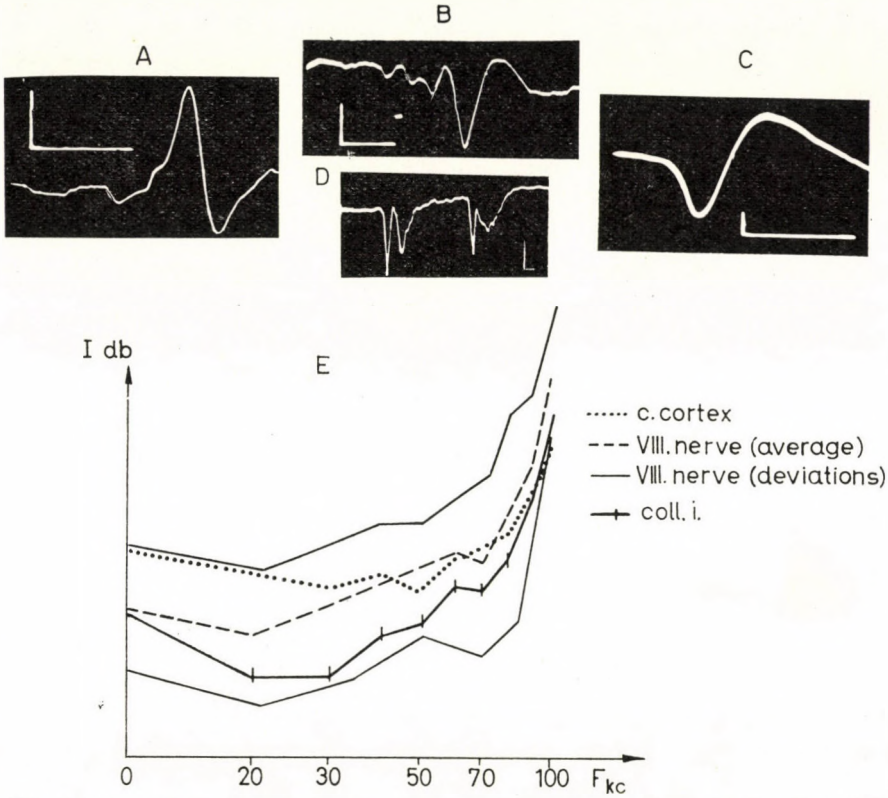



*Fig. 7.* **Electrical reactions of the auditory system of bats to single ultrasonic signals and frequency-threshold curves obtained from these reactions**
**A = action potentials of the auditory nerve (calibration: 50 $\mu$V, 1 msec); B = induced potential recorded from the surface of the dorsal colliculi (on-effect). (Calibration: 25 $\mu$V, 2 msec); C = induced potential in the auditory cortex (Calibration: 100 $\mu$V, 20 msec); D = on- and off-effects in the dorsal colliculi (Calibration: 100 $\mu$V, 2 msec); E = frequency-threshold curves of the auditory nerve (average and extreme variations), dorsal colliculi and cortex. The abscissa gives the frequency of ultrasound in kc/s, the ordinate, thresholds in db (above the level of 0.0002 dyne/cm$^2$)**

in the properties of the auditory system of these families. The amplitudes of electrical reactions indicating the number of responding elements in Vespertilionidae are comparable in a wide range of frequencies which is in accordance with the frequency-modulated character of signals, while in Rhinolophidae they have a clear maximum in the region of the main frequency of their signals (Fig. 8B, C). It should be pointed out that a response to the switch-off of a stimulus in Rhinolophidae is most easily obtainable when the height of ultrasounds corresponds to their cries, a fact showing that these animals are using off-effect in echolocation.

We also investigated the sensitivity of the auditory system to signals coming from different directions. A complex pattern of relationship was found in both families between the thresholds of reactions and the angle of outcoming

signals ranging within 180° across and down. The latter is considerably determined by the relief of the pinnae (GRINNELL and GRINNELL 1965). On their removal the pattern of the relationship becomes simpler. We also demonstrated an inhibiting interaction of signals of the right and left ears at the level of subcortical auditory centres amplifying (by contrast) the differences between signals (MATJUSHKIN and VASILYEV 1968; MATJUSHKIN and STOSMAN 1968).

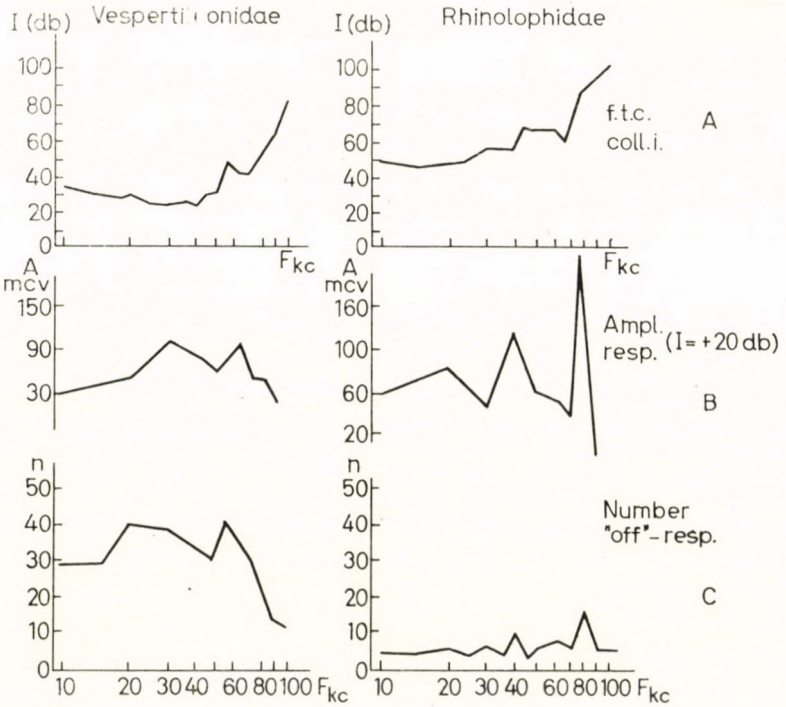



*Fig. 8.* Some qualitative characteristics of the bat's auditory system. A = Comparison of frequency-threshold curves (f.t.c.) by on-effects in Vespertilionidae and Rhinolophidae. B = Comparison of amplitudes of on-effects in the same animals. C = Comparison of the total number of off-effects of dorsal colliculi obtained from stimulation of Vespertilionidae and Rhinolophidae by ultrasounds of different frequency

Next, we studied the relation of the responses to the intensity of ultrasonic stimuli of different centres in the bat's auditory system. In a wide intensity range the reaction was the more marked the stronger the stimulus. However, beyond a certain limit this dependence is distorted showing inhibition. The latter effect could sometimes be detected in the acoustic nerve; it is clearly visible in the dorsal colliculus and is most notable in the cortical auditory centre.

Of special interest is the ability of different parts of the auditory system to respond by an evoked potential to a repeated stimulus following the first one after a short interval. It was found that the acoustic nerve and subcortical auditory centres of bats have a high lability. Restoration time of their excita-

bility does not exceed 10 msec (corresponding to the rhythm of 100 per sec). However, the cortical auditory area has a low lability with the restoration time of excitability of about 200 msec (corresponding to the rhythm of 5 per sec).

It is suggested that the relatively primitive and low labile auditory cortex of the bat somehow participates in the regulation of the echolocation process, but it cannot actively enter each working cycle of the echolocation (cry-pick up and echo-cry). This is demonstrated by the long latency period of the cortical evoked potential (10 msec).

*b) Experimental evidence obtained from recording activity of single neurons.* In a large experimental series we recorded reactions and studied various properties of more than 400 neurons of the dorsal colliculi and cortical auditory centre of Vespertilionidae and Rhinolophidae. We could observe a great diversity of properties, functional adjustment of neurons and their well expressed "individualization". In the same bat, the different neurons of each of the above mentioned centres differed in many parameters: in their frequency-threshold curves, thresholds of inhibition, frequency and intensity ranges of response, latent periods, restoration cycles (labilities), angular sensitivity, etc.

Hence, in the neuronal mass of higher auditory centres of bats one may single out particular "neuronal scales" for the judgement about the height and intensity of ultrasonic signals, intervals between signals (for instance, between a cry and its echo) and directions of outcoming signals. Fig. 9 shows frequency-threshold curves of some neurons presenting a scale for measuring the height of ultrasounds (A) and their intensity (B, C), and also electrograms showing reactions of neurons with a narrow intensity range of response (D).

We have discovered an interesting angular specialization of neurons (MATJUSHKIN and STOSMAN 1968). Fig. 10 represents threshold dependencies in neurons of the dorsal colliculus on the direction of a signal in the hemisphere. The difference in the angular adjustment of different neurons is clearly visible, i.e. the specialization of neurons in spatial analysis.

These findings may allow to describe some intimate mechanisms of treatment or analysis of signals in the auditory system of bats.

In conclusion let us discuss the self-protecting mechanisms of the bats' echolocation system from acoustical interferences. Our bioacoustical and electrophysiological experiments have shown that the echolocator of bats is provided with a perfect interference protection mechanism. It was found that one of the ways for the bat's echolocator to avoid unnecessary noise is the intensification of a useful signal (a cry and an echo, respectively) with a simultaneous decrease of general sensitivity of the reception device proportional to the amount of noise (MATJUSHKIN and VASILYEV 1968). The adjustment of the bat's auditory system to the pick up of useful signals that shows features of a dominant device has a great importance for an interference protective mechanism (MATJUSHKIN and VASILYEV 1968).

### 5. *General characteristics of nervous echolocation mechanisms*

The ultrasonic echolocation system, or, according to our terminology, the echolocation analyzer acts in a complex with other analyzers as a particularly specialized component of the analysers constellation. Echolocational "tentacles" are especially active in the initial phase of investigation of spatial elements and when it is necessary to obtain refined information about space.

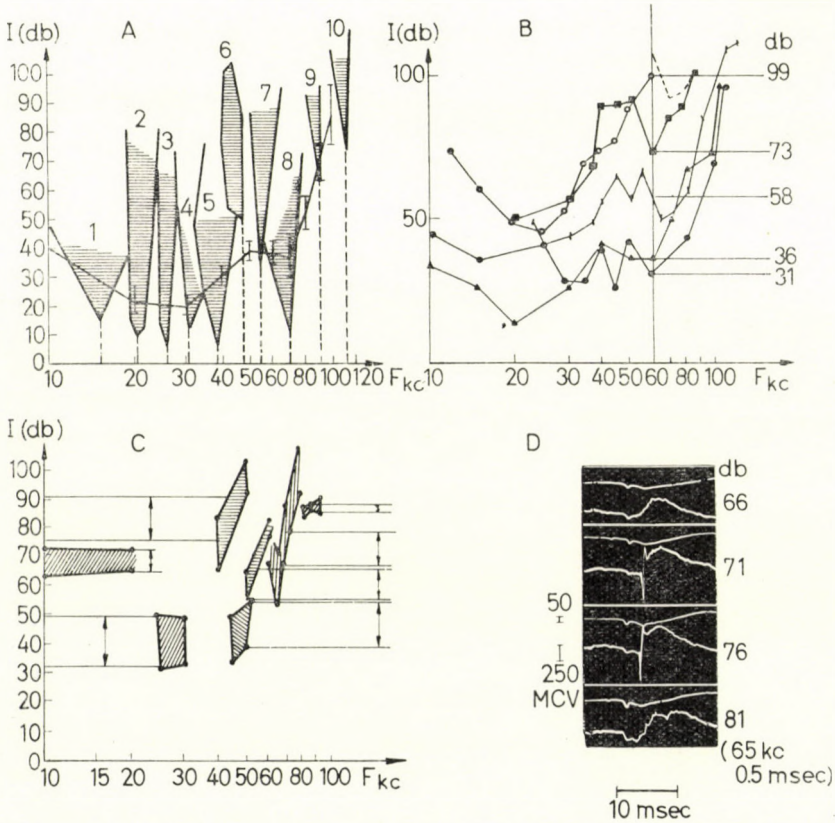



*Fig. 9.* Neuronal "scales" for evaluating the frequency and intensity of ultrasounds. A = family of frequency-threshold curves of ten-colliculi neurons "adjusted" to different frequencies. B = family of frequency-threshold curves of neurons actually responding to all frequencies within the range tested, but differing in intensity threshold. C = frequency-threshold curves of colliculus and cortical neurons with a limited (from top and bottom) intensity range of response to ultrasounds. D = electrogram of reactions of colliculus neuron with a limited in intensity range of response (parameters of the stimuli are specially marked in the figure)

The echolocation apparatus increases its activity when vision is excluded. However, under stable conditions its participation in spatial analysis decreases and is replaced by a motor analyzer (Konstantinov, 1964, 1965a; Airapetianz and Konstantinov 1965a, 1967).

The main central echolocational apparatus is situated in the subcortical layer, in the dorsal colliculi of the mesencephalon (KONSTANTINOV 1966b; AIRAPETIANZ and KONSTANTINOV 1967b). Although the participation of the cortical area in the process is beyond any doubt, there is no reliable evidence allowing to characterize the role of the cortex in echolocation. But it was shown that elimination of the cortex and its auditory projection in bats does not produce any considerable effect on their echolocation abilities (KONSTANTINOV 1965b).

As a scheme we propose a hypothesis about the construction and functioning of the echolocation analyzer. Let us assume that the activity of an animal

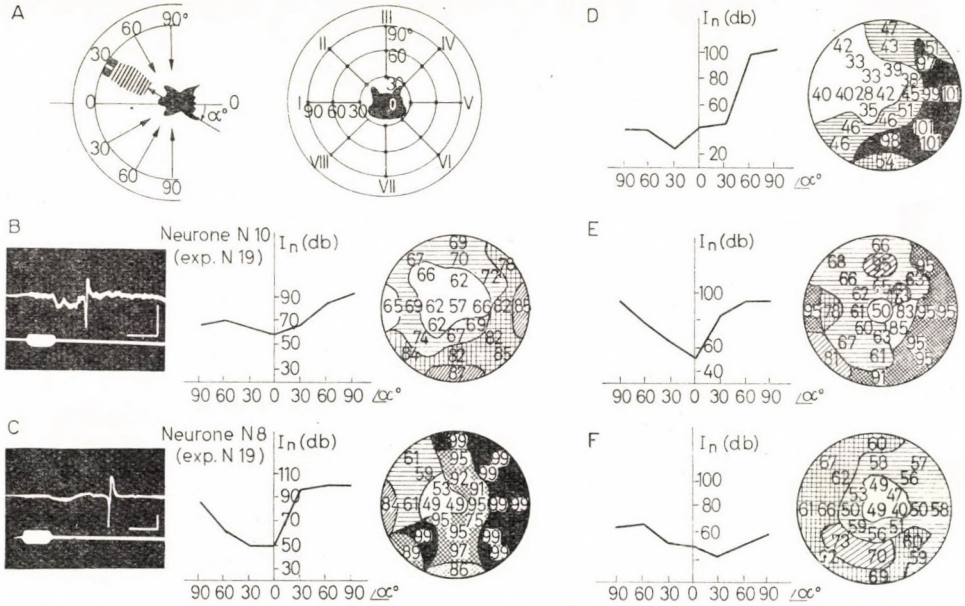



*Fig. 10.* Characteristics of neurons with different "angular" adjustments. A = scheme of the experimental method (directions of incoming signals relative to the bat's head). B, C, D, E, F = characteristics of single neurons: electrograms of their responses (to B and C), graphs showing threshold dependency for optimal frequencies from the angle in the horizontal plane, hemispheric ("circle") diagrams of threshold distribution from the optical frequency of these neurons. Threshold values in db in the diagrams are light in the high threshold regions. Calibration of the electrograms is 1 mV, 5 msec

is stimulated by hunger. Then stimuli from the digestive centre arrive simultaneously to the motor centres and the dorsal colliculus where commands are organized to generate ultrasounds and prepare the acoustic apparatus for the reception of emitted and returned pulses. The animal emits an ultrasonic pulse and picks it up while the contraction of entotic muscles prevents the animal from being deafened by its own cry (HENSON 1965). There follows an ultrasonic strike against the object tested and an echo arises. The next phase of the echolocation process occurs when the echo enters the acoustic apparatus. After

information about the target coded in nerve pulses has been obtained from the echo pulse through intermediate instances, it heads for the neuronal organization of the dorsal colliculi where it undergoes processing (decoding and analysis). Such is the structure of an elementary echolocational act.

In practice, however, echolocation analysis is brought about by a current of pulses following each other at intervals of a few milliseconds. Each incoming pulse ought to be treated immediately to allow much useful information to be

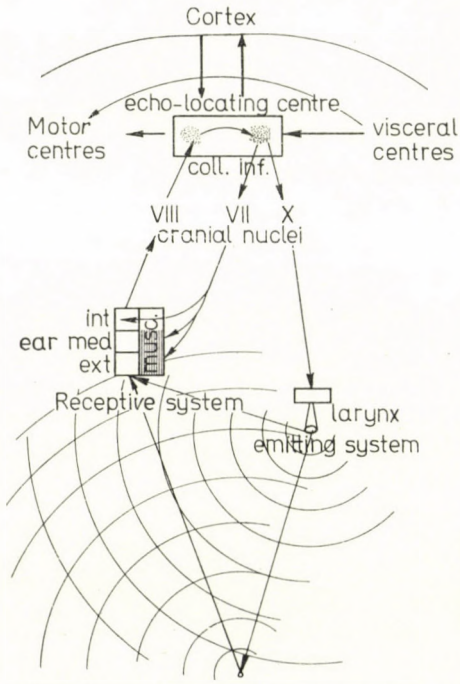



*Fig. 11.* Regulation scheme of the echolocational system

derived from the next pulse. It appears that, following the first pulse, the echolocation regulating apparatus begins to work — alterations concern the rhythm, intensity of pulses, etc.

*

It is customary to connect two ideas, two constructions; the radar and the bat. In fact, there is much in common between living echolocators and technical radars. Both emit energy quanta and detect by the echo targets and their distances. Both systems (each within its range) are using high frequencies and short pulses. Each has protection mechanisms. The question is whether the echolocation apparatus of winged animals has an advantage over our up-to-date electronic giants. The comparison of these two systems will not be in favour of nature. Bats obtain necessary information at a distance of a few metres,

whereas radiolocators pierce space at hundreds, thousands and even million kilometres, as has been proved by space investigations.

However, some properties of the bat's echolocation are an envy to engineers. What is that wise mechanism that the merciless evolution has supplied the brain of Chiroptera with?

Besides the over miniature construction of brain echolocation apparatus, bats possess echolocators of high resolution ability enabling them to detect and avoid intercepts 50 times lesser than the wavelength of emitted sound (KONSTANTINOV et al. 1967). The high resistance of echolocation gives these creatures a possibility to carry out a qualitative analysis of targets in condition of powerful high-frequent acoustic interference. And, finally, they show a most perfect system of analysis, differentiation and discrimination of targets by the shape, size and character of the reflecting surface. No modern radiolocation or ultrasonic system created by human beings is provided with such an ability.

However, in this contest of the creative powers of nature and human brain the victory will of course fall to man. Bionics was born to use the achievements of nature for technical progress. No doubt, more universal and less bulky radar systems will be constructed for the welfare of people, and they will be more perfect than those of any animal surprising us now.

We are proud of our science, biology, which takes part in the solution of such significant problems of human progress.

## Acknowledgement

The following workers of the Department of Higher Nervous Activity provided valuable assistance in this investigation: N. B. BURIKOVA, A. G. VASILYEV, S. S. GOLOVINA, L. M. KOTELENKO, B. V. SOKOLOV and I. M. STOSMAN.

## LITERATURE

AIRAPETIANZ, E. SH.: Experience of a comparative study of the substitution and replacement principle in the interanalysatory integration. In: Problems of the comparative physiology of analysors, Leningrd University 1960, 1: 9—40.

AIRAPETIANZ, E. SH. Brain mechanisms and space analysis. In: Research in brain signalisation apparatus. Nauka, Leningrd 1967. 11—22.

AIRAPETIANZ, E. SH.: Bionic aspects of research into mechanisms responsible for spatial information: Echo-location in bats. Sechenov physiol. USSR, **1967,** 53/4, 368—376.

AIRAPETIANZ, E. SH., KONSTANTINOV, A. I.: On the role of echolocation in spatial analysis of bats. Bionics, Nauka, Moscow **1965,** pp. 334—341.

AIRAPETIANZ, E. SH., KONSTANTINOV, A. I.: On the interaction of analysors in bats' echolocation. Research in brain signalization apparatus. Nauka, Leningrad **1967,** pp. 22—32.

AIRAPETIANZ, E. SH., KONSTANTINOV, A. I.: A study of nervous mechanisms of echolocation of bats. Questions of Bionics, Nauka, Moscow **1967,** pp. 445—453.

GRIFFIN, D. R.: Listening in the Dark. Yale University Press, New Haven **1958.**

GRIFFIN, D. R., WEBSTER, F. A., MICHAEL, CH. R.: The echolocation of flying insects by bats. Animal Behaviour **1960,** 8: 141—155.

GRIFFIN, D. R., FRIEND, J. H., WEBSTER, F. A.: Target discrimination by the echolocation of bats. J. exp. Zool. **1965,** 158: 155—168.

GRINNELL, A. D.: The neurophysiology of audition in bats. J. Physiol. (Lond.) **1963,** 167; v. 1, 38—127.

GRINNELL, A. D., GRINNELL, V. S.: J. Physiol. (Lond.) **1965,** 181: 830.

HENSON, O. W. JR.: The activity and function of the middle-ear muscles in echolocating bats. J. Physiol. (Lond.) **1965,** 180: 871.

KONSTANTINOV, A. J.: On the role of conditioned signalization in the spatial analysis in bats. J. high. nerv. Act. Acad. Sci. USSR, **1964,** 14: 701—706.

KONSTANTINOV, A. J.: The effect of exclusion of visual perception on the directed behaviour of bats. Vestn. Univ., Leningrad **1964,** 15: 72—75.

KONSTANTINOV, A. J.: The effect of partial and total exclusion of brain cortex on the echolocation of bats. Dokl. Akad. Nauk SSSR. Biol. **1965,** 161: 989—991.

KONSTANTINOV, A. J.: The effect of exclusion of dorsal colliculus on location-locomotor activity of bats. IV. Sci. Conf. Evolutional Physiology Leningrad **1965,** pp. 153—154.

KONSTANTINOV, A. J.: Discrimination of geometrical figures by bats using ultrasonic location. XXI. Cong. Problems of Higher Nervous Activity, Nauka, Moscow—Leningrad **1966,** p. 155.

KONSTANTINOV, A. I.: The bases of ultrasonic space orientation in bats. In: "Problems of the Comparative Physiology of Analysors", Leningrad University **1966,** 2: 93—111.

KONSTANTINOV, A. I.: The relationship of auditory perception and echolocation during detection and catching of insects by bats (Vespertilionidae). J. evol. Physiol. Biochem. (In press).

KONSTANTINOV, A. I., AKHMAROVA, N. I.: Discrimination (analysis) of targets by echolocation in Myotis oxygnathus. J. biol. Sci., Moscow Univ. **1968,** 4: 22—28.

KONSTANTINOV, A. I., SOKOLOV, B. V.: Active and passive location of bats during insect catching. III. Conf. Ecological Physiology, Biochemistry and Morphology, Novosibirsk **1967,** pp. 68—69.

KONSTANTINOV, A. I., SOKOLOV, B. V.: Characteristics of ultrasonic orientation signals of Horseshoe bats (Rhinolophidae). J. evol. Physiol. Biochem. **1969,** 1: 90—97.

KONSTANTINOV, A. I., SOKOLOV, B. V., STOSMAN, J. M. A.: Comparative study of the sensitivity of bat's echolocation. Dokl. Acad. Nauk SSSR, **1967,** 175/6, 1418—1421.

KOTELENKO, L. M., MATYUSHKIN,D.P.: Properties of neurons of bat's auditory cortex (responses to ultrasounds of different height and intensity. J. high. nerv. Act. Acad. Sci. USSR. **1969** (In press).

KUZYAKIN, A. P.: Bats. Sov. Nauka, **1950.**

MATJUSHKIN, D. P.: Some characteristics of the entrance of bat's auditory system (inhibition of cochlear neurons by strong adequate stimuli). J. Biol. Sci., Moscow Univ. **1969** (In press).

MATJUSHKIN, D. P., VASILYEV, A. G.: Bioelectrical responses to ultrasounds and their masking by white noise in the bat's auditory system. Vestn. Univ. Leningrad **1968,** 15: 94—101.

MATJUSHKIN, D. P., STOSMAN, I. M.: Sensitivity of dorsal Colliculus neurons of bats to ultrasonic signals coming from different directions. Dokl. Acad. Nauk SSSR, **1968,** 178: 973.

MATJUSHKIN, D. P., STOSMAN, I. M.: Neuronal mechanisms of the bat's brain detecting directions of incoming acoustic signals (in the spatial angle 180°). Sechenov physiol. J. **1969** (In press).

MÖHRES, F. P.: Über die Ultraschallorientierung der Hufeisennasen (Chiroptera-Rhinolophidae). Z. vergl. Physiol. **1953,** 34: 547—588.

NOVICK, A.: Orientation in paleotropical bats. I. Microchiroptera. J. Mammal. **1958,** 44: 2044—56.

ROEDER, K. D.: Biological Prototypes: Synthetics and systems. Plenum Press, New York **1962.**

ROEDER, K. D., TREAT, A. E.: The detection of bat cries by moths. In: ROSENBLITH W. (ed.): Sensory Communication, Technology Press, New York **1961.**

TREAT, A. E.: The response to sound of certain Lepidoptera. Ann. ent. Soc. Amer. **1955,** 48: 272—284.

SUGA, N.: Single unit activity in cochlear nucleus and inferior colliculus of echolocatory bats. J. Physiol. (Lond.) **1964,** 172: 449—472.

VASILYEV, A. G.: Electrical activity of dorsal colliculus in bats stimulated by ultrasounds. Dokl. Acad. Nauk, SSSR, **1967,** 175/3, 741—744.

VASILYEV, A. G.: A comparative description of the auditory system of bats Vespertilionidae and Rhinolophidae (Electrophysiological data). Dokl. Acad. Nauk SSSR, **1967,** 175/6: 1414.

VASILYEV, A. G., MATJUSHKIN, D. P.: To the characterization of the bat auditory system by reactions to ultrasonic stimuli. Sechenov Physiol. J. **1967**, 53/12: 1407—13.

E. Sh. AIRAPETIANZ,
A. I. KONSTANTINOV,
D. P. MATJUSHKIN,
Department of Higher Nervous Activity, Leningrad University
B—164, Universit. Nab., 7,
Leningrad, USSR

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1), pp. 19—30 (1969)*

# PHYSIOLOGICAL AND PHARMACOLOGICAL IDENTIFICATION OF NEURONS IN THE CENTRAL NERVOUS SYSTEM OF HELIX POMATIA L.

By

D. A. SAKHAROV and J. SALÁNKI

INSTITUTE OF DEVELOPMENTAL BIOLOGY, SOVIET ACADEMY OF SCIENCES, MOSCOW, AND BIOLOGICAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, TIHANY

(Received August 24, 1968)

Single units and neuron populations were identified by visual, electrophysiological and pharmacological methods in the pleural, parietal and visceral ganglia of *Helix pomatia* L.

Membrane and action potentials were recorded with intracellular electrodes; the shape of the action potential curve, the effect of polarizing currents, and the changes in PSP during alterations of the membrane potential have been studied.

It has been established that certain giant cells and cell populations exhibit well-defined characteristics while activated, allowing a clear distinction from adjacent neurons. In the case of other neurons identification is possible only on the basis of synaptic connections and tracts.

The response to ACh had no close relationship with the type of activity shown by the cells and with the reactions given to 5-HT or dopamine. At the same time, however, only oscillator type neurons could be stimulated by 5-HT, and the activity of the same cells was depressed by dopamine.

Since the pioneer work of ARVANITAKI and CHALAZONITIS (1955a, b) and TAUC (1955) carried out with intracellular electrodes, giant cells of snails have become an important object of neurophysiological investigation. Extensive studies have shown that the cell population of the ganglia in gastropods is heterogeneous, the cells being different in the type of electrical activity, the ionic mechanisms underlying the generation of action potential, in their pharmacological properties and a number of other parameters. This circumstance has led a number of investigators to construct cell maps which would allow the selection of neurons with the properties necessary for a given study.

In the present paper several characteristics of the single units and cell populations located on the dorsal surface of the subpharyngeal ganglion of *Helix pomatia* will be described and the possibilities for their identification by visual, electrophysiological and pharmacological methods will be discussed.

## Methods

The ring of ganglia around the pharynx of *Helix pomatia* L. was removed and fixed on a slide with rubber bands, with the dorsal surface of the ganglia upwards. During this procedure, the entire ganglionic ring and all the connections between its parts remained intact.

The preparation was placed into Meng's solution (MENG 1960), then the subpharyngeal complex was freed from the thick connective tissue covering it. A drawing was made of the preparation, particular care being taken for the lobules of the ganglia and the localization of the most prominent cells.

After accurate depiction the part of the preparation contaning the cells to be studied was freed from the thin connective tissue layer covering the cells, and the preparation was transferred into a 20 ml glass vessel through the front wall of which the preparation's position could exactly be established by the aid of a stereo-microscope, and the introduction of the microelectrode into the cell could be checked.

Membrane and action potentials were recorded with glass microelectrodes filled with 2.5 M KCl. Their resistance varied between 8 and 25 Mohm. An Alvar type direct current amplifier was used for amplification, with a cathode follower input. The deflections were observed on an Alvar oscilloscope and recorded in a signal storage. After the experiment, the necessary curves were recorded photographically. When the records were played back from the storage, amplification was not linear, hence the afterpotentials on the records are somewhat larger than on the oscilloscope.

By the aid of a supplementary bridge circuit it was possible to depolarize or hyperpolarize the cells through the microelectrode used for recording.

When studying the chemical susceptibility of the cells, the substances to be tested were dissolved in Meng's solution and added to the medium bathing the preparation. The amount of the substance varied between 0.04 and 0.00004 mg according to the susceptibility of the preparation. The following agents were tested: acetylcholine chloride (ACh; Fluka), 5-hydroxytryptamine creatinine sulphate (5-HT; Sigma), and dopamine (3-hydroxytryptamine HCl, DA; Sigma).

## Results

The subpharyngeal ganglionic complex of *Helix pomatia* consists of 7 partly accreted ganglia. Five are sited dorsally, i.e. the right and left pleural (RPlG and LPlG), the right and left parietal (RPaG and LPaG) and the visceral ganglion (VG) (Fig. 1, right bottom). In the present study the cells of these dorsally located ganglia have been investigated.

The terminology used by us was based on that introduced by FRAZIER et al. (1967) when mapping the neurons of Aplysia. Thus, each neuron was designated with the abbreviation of the ganglion in which it had been found, and a serial number was given to make a distinction between cells within the same ganglion. Groups of similar neurons were marked by capitals A, B, C, etc.

The location of the neurons and cell groups subjected to study is shown in Fig. 1. The anatomical picture was variable, particularly as regards the location of cells. Further information in this respect will be given below. Irrespective of the differences in localization, each cell indicated in the scheme could be found in the preparations.

### *I. Identification of the cells on the basis of physiological criteria*

A variety of information can be gained from the neuron with a single intracellular electrode. According to experience, the following characteristics are useful in identification: (a) the shape of the action potential curve; (b) the response of the cell to polarizing currents; (c) the changes in the EPSP during alterations of the membrane potential; (d) the response to mediator substances.

*(a) The shape of the action potential curve*

On the basis of the deflection shape the action potentials can be classified in two groups. In the first group there is a flattening of the curve on the initial segment of the descending leg; whereas in the second group this flattening is absent.

On the basis of the delayed descending part of the action potential it was possible to identify in the posterior mid-part of the right parietal ganglion the cell $RPa_1$, the only neuron in this zone exhibiting this characteristic. In another part of the same ganglion similar action potentials were recorded from the cells of group D.

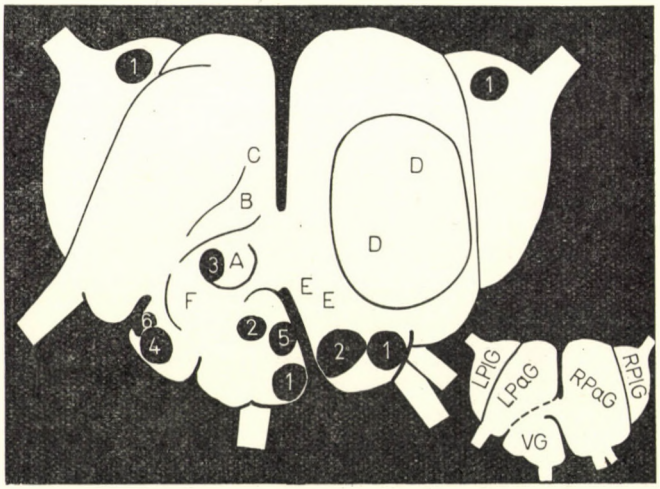



*Fig. 1.* Schematic representation of the preparation: identified cells and cell groups are designated by letters and figures as noted in the text. Right bottom: localization of the five dorsal ganglia of the subpharyngeal complex. LPlG — left pleural ganglion; RPlG — right pleural ganglion; LPaG — left parietal ganglion; RPaG — right parietal ganglion; VG — visceral ganglion

A characteristic feature of the action potentials of these units is the progressively increasing delay of the descending segment in successive spikes which followed one another with a high frequency (Fig. 2). The action potentials of these cells had usually a considerable overshoot, resulting in a potential of 80 to 100 mV.

The cells $RPl_1$, $LPl_1$, $V_4$, and sometimes also $V_1$, exhibited an after-hyperpolarization of considerable duration (0.3 sec). This hyperpolarization showed a tendency for summation, resulting in a progressive increase in the duration of hyperpolarization in successive action potentials. Similar properties were observed in the cells of group F which, however, were difficult to analyse

separately. Such action potentials had no large overshoot and their amplitude did not exceed 65 mV.

The neurons which on the basis of their pharmacological properties had been designated DINHI-cells by GERSCHENFELD (1964), could easily be identified by the aid of the action potential curve. Their amplitude varied considerably on changing the synaptic input. The number of these cells is moderate, on the basis of its uniform location it was possible to select one such neuron which was then marked as an identified cell ($V_6$), although it could not be exactly distinguished simply by visual methods from other giant cells surrounding it.

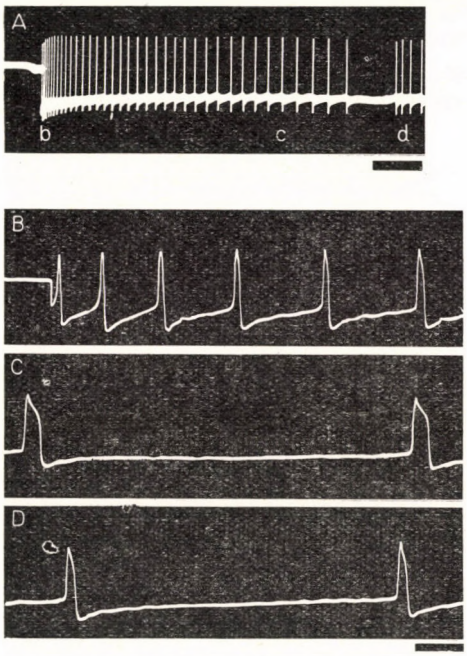



*Fig. 2.* Modification of the descending segment of the action potential curve during an impulse train in a cell belonging to group D. — *A*, train of impulses which had been elicited by the introduction into the cell of the electrode. Segments marked with b, c and d are represented in traces *B*, *C* and *D*, using a quicker moving beam; *B*, the shape of the spike in the initial phase of the impulse train; *C*, the shape of the spike at the end of the impulse train; *D*, the shape of the spike after a silent interval, i.e. in the initial phase of a subsequent impulse volley. Time marking: 5 sec (*A*), 100 msec (*B*, *C*, *D*). The amplitude of the spikes is 80 mV

### *(b) The effect of polarizing current*

Although the type of activity is characteristic of every cell (TAUC 1966), it is difficult to distinguish between neurons merely on the basis of spontaneous activity. Therefore, a study was made in which the response given to currents applied through the intracellular electrode was used for the differentiation of various cell types (TAUC 1957, 1966; ALVING 1968).

The classification is based on the ability of certain cells to produce over considerable periods of time action potentials by means of pacemaker activity. If a hyperpolarizing current is applied it is possible to ascertain whether the action potentials are generated under the effect of the EPSP or by auto-oscillation in the cell (TAUC 1957). This test is not very suitable if the cell receives frequently excitatory synaptic potentials which cause a lasting depolarization of the membrane. A more useful technique is based on the application of a depolarizing current which can be employed in both active and inactive (silent) cells.

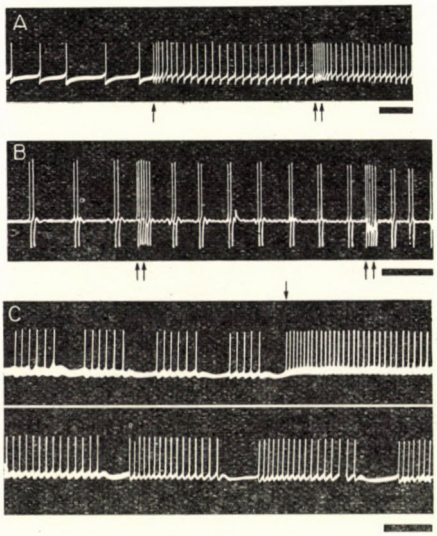



*Fig. 3.* Various types of oscillator neurons. *A*, monomodal oscillator cell of group D; *B*, bimodal oscillator which does not produce bursts ($RPa_2$ cell); *C*, bimodal oscillator which produces bursts; the second trace is a continuation of the first one ($RPa_1$ cell). Arrow marks the application of the depolarizing current, double arrow indicates an increase in the intensity of the depolarizing current. In tracing *B* the cell was already depolarized at the beginning of recording. Without depolarization it gave infrequent and irregular spikes. Time marks: 5 sec (*A*) and 3 sec (*B*, *C*). Amplitude of the action potential: 80 mV (*A*), 65 mV (*B*, in the initial segment of the tracing), 60 mV (*C*)

The cells can be classified in two main groups:

1. Non-oscillating cells which may be ranged into the following two categories: (a) inactivating with accommodation, (b) inactivating with depolarization.

2. Oscillating cells which may be either (a) monomodal, or (b) bimodal.

The latter can be divided into two subgroups, i.e. those which yield and those which do not yield long series of impulses.

Oscillating neurons have been found to retain, after an initial increase in frequency, their activity after a depolarizing current has been applied on the membrane; the frequency of impulses depends on the intensity of the depolarizing current (Fig. 3). The activity of non-oscillating neurons, on the other hand, ceases after an initial series of impulses (ALVING 1968).

There is reason to assume that non-oscillating neurons do not always belong to the category of driven cells the activity of which is determined by the synaptic input as it was supposed by ALVING (1968). These cells are not uniform, there being only a few among them which, owing to accommodation, becomes inactivated after an initial volley of impulses, thus, without an appreciable change in the membrane potential. These characteristics are shown by the cells of group E and the cell termed $V_1$. The remaining cells without pacemaker activity are inactivated by progressive depolarization, and after the polarizing current has been switched off the resting potential fails to return to its original value for a considerable length of time, indicating a failure of the repolarizing mechanisms. In some of the animals, the group D cells exhibited such characteristics.

The oscillating neurons show monomodal or bimodal oscillation; in the latter case the trains consisting of several impulses are periodically interrupted. Of the cells studied in these experiments, $LPl_1$, $RPl_1$, $V_2$, $V_4$, $V_6$, A, B, and F showed monomodal oscillation.

The cells showing bimodal oscillation are easy to be arranged in two categories. In some of these cells, as in cell $RPa_1$, the impulses within a train attain a considerable number, often several times ten. If a depolarizing current is applied, the duration of impulse trains increases and the frequency increases as well (Fig. 3*C*). In other cells exhibiting bimodal oscillation the intervals separate shorter trains consisting of a considerably smaller number of impulses, usually 2 to 4. Within a group of spike, the number of impulses and their frequency are practically not altered by depolarizing currents, the frequency of impulse volleys, however, increases. Cells $RPa_2$ and $V_5$ belong to this group (Fig. 3*B*).

### *(c) Changes in EPSP during alterations of the membrane potential*

The characteristic dependence of the EPSP on the membrane resting potential has been described by KANDEL and TAUC (1966) in the giant cells of the metacerebrum of *Helix pomatia*. If the response is typical, the amplitude of EPSP increases with the increase of the membrane potential. In contrast, in the metacerebral cells the amplitude of EPSP is increased when the cell's polarization is diminished provided that the membrane potential attains a value near the resting potential. Such anomalous EPSP show a shorter duration if the cell is hyperpolarized, and the duration is longer if the membrane is depolarized.

Thus, on the basis of the behaviour of EPSP in response to polarizing currents the cells can be divided in two groups. Of the cells studied in the present experiments an invariably "typical" response was given by the DINHI cells. This property serves as a basis for the differentiation of $V_6$ cells from the surrounding neurons which show an anomalous response (group F and cell $V_4$).

### *(d) The effect of mediator substances*

On the basis of the response given to ACh and the properties of the synaptic input, 5 types of neuron have so far been distinguished (TAUC 1967). For obvious reasons it is impossible to classify all the neurons in one of the above 5 groups, at least those cells which do not respond to ACh cannot belong to any of these groups.

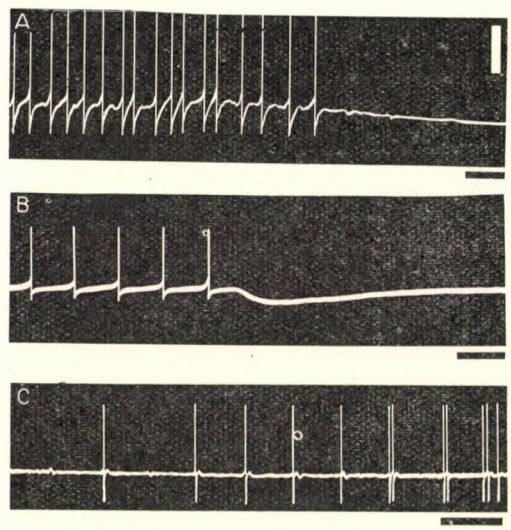



*Fig. 4.* Characteristic responses given by several types of cells to neurotransmitter substances. *A*, effect of ACh on a cell of group B; 0.02 mg of ACh was added to the medium bathing the preparation; *B*, effect of dopamine on a cell of group D; 0.008 mg of DA was added to the medium; *C*, effect on cell $RPa_2$ of 0.016 mg of 5-HT added to the medium. The cell was inactive until 5-HT had been added; the tracing shows a subsequent segment of activity. Time marking: 0.2 sec (*A*), 5 sec (*B*), 1 sec (*C*). Amplitude of the action potential: 80 mV (*B*), 65 mV (*C*). Calibration in tracing *A*: 20 mV

ACh has been shown to cause marked depolarization on the cell $RPa_1$. The giant cells $RPa_2$ and $V_5$, on the other hand, are usually less susceptible to ACh, although the indirect effect is clearly shown in the intense EPSP-s. Cell $V_3$ can be hyperpolarized by ACh. Cell $RPa_1$ which shows both IPSP and EPSP and is characterized by long-lasting inhibition can be classified in the group of CILDA cells. Also, the cells of group D which possess a similar synaptic input can be classified in this group; however, their response to ACh is an inhibitory one which is opposite to the reaction given by cell $RPa_1$ (Fig. 4*A*). That cell $RPa_1$ and the cells of group D belong to the CILDA pharmacological category is supported by the finding that these cells are inhibited by DA but stimulated by 5-HT (Fig. 4*B*).

A study of the response to ACh is of considerable aid also in the identification of cells belonging to groups A and B, which are often different in morpho-

logical appearance and spontaneous electrical activity. The existence of excitatory synaptic input and the marked hyperpolarization in response to ACh allow these cells to be classified among H-type cells. Groups C, E and F, on the other hand, are stimulated by ACh.

## *II. Further characteristics of the identified cells*

### *Cells $RPl_1$ and $LPl_1$*

Topographically, they are arranged symmetrically and their physiological properties are similar. The constancy of their size is remarkable, and they can clearly be distinguished among the much smaller adjacent neurons. They are usually located on the dorsal surface of the pleural ganglion, nevertheless, cell $RPl_1$ is sometimes found at the edge of RPaG instead of RPlG. This variability in localization has been mentioned in earlier histological studies (KUNZE 1918).

Both cells are usually inactive and show action potentials infrequently, in response to EPSP. In the resting state, thus, they behave as driven cells, however, the smallest depolarization is enough for their pacemaker properties to become prominent. In the action potential curve the components "a" and "s" are easily recognized, the latter, however, may be absent.

The effect of ACh has been studied in cell $RPl_1$, the one which exhibited depolarization in response to this agent. The desensitization phenomenon described for the neurons of molluscs (see TAUC 1966, 1967) could be observed after the repeated administration of ACh at 1 to 1.5 min intervals. The increase in susceptibility to ACh after a single treatment with a large dose of ACh (ZEIMAL and VULFIUS 1968) could also be registered. There was no IPSP in the spontaneous synaptic input.

### *Cell $V_1$*

This cell is of a considerable size, moderately pigmented, and well recognizable on the right posterior edge of the visceral ganglion. The response to depolarizing current has two different forms; this probably reflects two different levels of physiological activity. The first type is characterized by the appearance of a few action potentials in response to the depolarizing current, the afterpotentials summing up in a hyperpolarization which in turn inhibits the generation of the spikes.

In other preparations, and this was observed more frequently, the membrane potential successively diminished during such a burst of impulses. In a certain phase of this diminution of the membrane potential the cell exhibited a rapid spontaneous oscillation with variable amplitude. This reaction is shown only by cell $V_1$ and none of the other neurons produced a similar response. After the polarizing current has been switched off, the cell remains in a state of

marked depolarization and the membrane potential attains its original value with a considerable delay.

The response of the cell to hyperpolarization is also particular, the membrane potential being unstable, sometimes attaining a new level in response to the hyperpolarizing current, near to the value of the resting potential.

ACh in this cell produces a similar effect as does depolarizing current.

*Cell $V_2$*

This is not a giant cell, nevertheless, with some experience one will find it easily, left to cell $V_5$. It shows no pigmentation, somewhat merges into the superficial layer of cells, and is surrounded by tiny pigment-stained cells. It is characterized by frequent spontaneous activity. The spikes do not always contain "s" component, in other words, the potential generated in the axon hillock does not always propagate to the soma of the cell.

*Cell $V_3$*

This is a giant cell of variable size, often pigmented. It is sited at the left posterior edge of the frontal convexity of the visceral ganglion. $V_3$ is usually inactive. Spontaneous PSP-s at the resting level of the membrane potential tend to depolarize the cell and appear as EPSP-s. If, however, activity is generated artificially by reducing the membrane potential, the PSP-s become hyperpolarizing and inhibit activity. ACh elicits an H-effect. Such "latent" H-cells have been found also in the ganglion of Aplysia (FRAZIER et al. 1967).

*Cell $V_4$*

An outstanding feature of this cell is its extraordinary size. It is usually found at the site marked in the scheme (Fig. 1), although variations often occur. At this point, and particularly in front of cell $V_4$, there is a large group of neurons with similar properties. This group is designated group F, and cell $V_4$ is a representative of this group. Within the group, identification of the cells requires that the neurons to which they give efferents and from which they receive afferents be established. This needs further investigations.

*Cell $V_5$*

The size of this cell is sometimes gigantic, but often there is no giant cell at all in this region. On the other side of the borderline between VG and RPaG, however, there are sometimes cells with the same physiological properties as those of $V_5$; among them is cell $RPa_2$. Apparently, the cells lying in this region and displaying many common features, often vary in localization along the borderline. A bimodal oscillation in the form of short volleys in response to depolarization, a low susceptibility to ACh, and the frequent appearance of EPSP

trains are the most important characteristics of these cells. Activity is easily affected by polarizing currents and the effect of the latter on the cell is persistent.

*Cell $V_6$*

This cell is difficult to identify visually. It is usually located in the visceral ganglion, in the posterior portion of the sulcus facing the left parietal ganglion, on a small protrusion of the latter. It is easily distinguished from adjacent cells on the basis of physiological properties; other cells in this region belong to group F. Cell $V_6$ is characterized first of all by the great number and variety of synaptic input in which the trains of lasting IPSP-s with a duration of 200 to 300 msec and very large EPSP-s are particularly prominent. Occasionally, the excitatory input is strong enough to cause permanent depolarization which is responsible for the frequent and remarkably regular pacemaker activity. Such periods alternately follow periods of total inactivity due to IPSP-s, or periods in which the cell behaves as a driven cell responding only to EPSP-s with an action potential. It has been mentioned that this cell is characterized by the variability of the action potential's amplitude. This might mean that they are not generated by the soma but in some adjacent region of the axon hillock, while the pacemaker zone alternately approaches and moves off the soma, depending on the synaptic input.

*Cell $RPa_1$*

This is one of the giant cells of RPaG which is in the posterior medial zone. The physiological properties mentioned above indicate that this cell is identical with one of the identified neurons of *Aplysia*, designated by ARVANITAKI and CHALAZONITIS (1955a, b) as Br cell, by STRUMWASSER (1965) as parabolic burster, and cell R15 in the terminology of FRAZIER et al. (1967). A cell with similar properties has been found also in the right parietal ganglion of *Helix aspersa* (KERKUT and MEECH 1966); as estimated from its localization, it is homologous with cell $RPa_1$.

*Cell $RPa_2$*

This is a giant cell, lying between $RPa_1$ and the ganglion's medial border and displaying characteristics similar to those of cell $V_5$.

## Discussion

The present investigation hass hownt hat certain anatomically well defined cells possess individual physiological properties which allow the cells to be distinguished if not from all other neurons but at least from the cells lying nearby (cells $V_1$, $RPa_1$). Anatomically distinct cells often have similar physiological

and pharmacological properties in quite different parts of the ganglion (cells $RPl_1$, $LPl_1$, $V_5$). Further individual characteristics of these cells can be determined only after a close analysis of their connections with other neurons.

As to their connections, cells with identical physiological properties and classified therefore in the same group are also heterogeneous. Thus, the adjacent cells of group F show marked differences in the intensity of synaptic input and in their spontaneous activities, indicating that the connections are individual and do not correspond to the anatomical arrangement.

There are, however, cell groups in which the neurons apparently have no individual characteristics. Naturally, this is only an assumption; specific and detailed investigations are necessary to settle the problem. Nevertheless, cells of group D appear to be uniform, owing to the great similarities in both activity and synaptic input.

It seems that the pharmacological properties of the neurons, particularly their susceptibility to certain neurotransmitter substances, is not independent of the physiological characteristics of the cells. Such a correlation has been described also by others. Thus, for example, GERSCHENFELD and STEFANI (1966) found that all 5-HT susceptible cells in the ganglion of the mollusc *Cryptomphallus aspersa* show a number of common physiological properties, e.g. the membrane of all these cells possesses the ability of anomalous rectification, it is characterized by lasting inhibition, and can be inhibited by dopamine. It can be added according to the present findings that only those cells are sensitive to 5-HT which have pacemaker activity, and 5-HT specifically augments spontaneous oscillation. According to our experience, all cells which are stimulated by 5-HT respond with inhibition to dopamine. Nevertheless, in our preparations cells of this kind did not always exhibit long lasting inhibition.

A few cells and one cell group belong to the type which in many respects is similar to the meta-cerebral giant cells described by KANDEL and TAUC (1966). These cells are similar in the following general properties. The synaptic input consists only of EPSP-s, the duration of which is considerable at near threshold membrane potential values, but diminishes when polarity is increased, the action potential has no remarkable overshoot and usually does not exceed 65 mV; the hyperpolarization that follows the action potential generally shows a tendency for summation; the cells give a protracted response to polarizing currents and they are capable of producing monomodal oscillation the frequency of which depends on the intensity of the polarizing current; finally, in their respond to ACh they can be classified as D cells.

The neuron map presented here contains only a fraction of the neurons in the pleural, parietal and visceral ganglia of *Helix pomatia*, those which could be identified by the methods applied. This has been only an initiative work, and the terminology used for the designation of the various cells and cell groups allows that the map be further completed.

## LITERATURE

ALVING, B.: Spontaneous activity in isolated somata of Aplysia pacemaker neurons. J. gen. Physiol. **1968,** 51: 29—45.

ARVANITAKI, A., CHALAZONITIS, N.: Les potentiels biolectriques endocytaires du neurone géant d'Aplysia en activité autorythmique. C. R. Acad. Sci. (Paris) **1955a,** 240: 349—351.

ARVANITAKI, A., CHALAZONITIS, N.: Variations lentes et périodiques du potentiel de membrane associées à des groupes de pointes (neurone géant d'Aplysia). C. R. Acad. Sci. (Paris) **1955b**, 240: 462—464.

FRAZIER, W. T., KANDEL, E. R., KUPFERMANN, I., WAZIRI, R., COGGESHALL, R. E.: Morphological and functional properties of identified neurons in the abdominal ganglion of Aplysia californica. J. Neurophysiol. **1967,** 30: 1288—1351.

GLAIZNER, B.: Pharmacological mapping of cells in the suboesophageal ganglia of Helix aspersa. In: SALÁNKI, J. (Ed.) Neurobiology of Invertebrates. Plenum Press, New York and Akadémiai Kiadó, Budapest **1968,** pp. 267—284.

GERSCHENFELD, H. M.: A non-cholinergic synaptic inhibition in the central nervous system of a mollusc. Nature (Lond.) **1964,** 203: 415—416.

GERSCHENFELD, H. M., STEFANI, E.: An electrophysiological study of 5-hydroxytryptamine receptors of neurones in the molluscan nervous system. J. Physiol. (Lond.) **1966,** 185: 684—700.

HUGHES, G. M.: The left and right giant neurons (LGC and RGC) of Aplysia. In: SALÁNKI, J. (Ed.) Neurobiology of Invertebrates. Plenum Press, New York and Akadémiai Kiadó, Budapest **1968,** pp. 423—441.

KANDEL, E. R., FRAZIER, W. T., WAZIRI, R., COGGESHALL, R. E.: Direct and common connections among identified neurons in Aplysia. J. Neurophysiol. **1967,** 30: 1352—1376.

KANDEL, E. R., TAUC, L.: Anomalous rectification in the metacerebral giant cells and its consequence for synaptic transmission. J. Physiol. (Lond.) **1966,** 183: 287—305.

KERKUT, G. A., MEECH, R. W.: The internal chloride concentration of H and D cells in the snail brain. Comp. Biochem. Physiol. **1966,** 19: 819—832.

KERKUT, G. A., WALKER, R. J.: The specific chemical sensitivity of Helix nerve cells. Comp. Biochem. Physiol. **1962,** 7: 277—288.

KUNZE, H.: Über das ständige Auftreten bestimmter Zellelemente im Zentralnervensystem von Helix pomatia L. Zool. Anz. **1918,** 49: 123—137.

MENG, K.: Untersuchungen zur Steuerung der Herztätigkeit bei Helix pomatia L. Zool. Jahrb. **1960,** 68: 539—566.

STRUMWASSER, F.: The demonstration and manipulation of a circadian rhythm in a single neuron. In: ASCHOFF, J. (Ed.) Circadian Clocks, North-Holland Publ. Co., Amsterdam **1965.**

TAUC, L.: Étude de l'activité élémentaire des cellules du ganglion abdominal de l'Aplysie. J. Physiol. (Paris) **1955,** 47: 769.

TAUC, L.: Les divers modes d'activité du soma neuronique ganglionnaire de l'Aplysie et de l'Escargot. In: Microphysiologie des éléments excitables (67ème Coll. Int. C. N. R. S., Paris 1955) Paris: Centre Nat. Rech. Sci. **1957,** 91—119.

TAUC, L.: Physiology of nervous system: In: WILBUR, K. M., YONGE, C. M. (Eds) Physiology of Mollusca, Academic Press, New York **1966,** 11: 387—454.

TAUC, L.: Transmission in invertebrate and vertebrate ganglia. Physiol. Rev. **1967,** 47: 521—593.

ZEIMAL, E. V., VULFIUS, E. A.: The action of cholinomimetics and cholinolytics on the Gastropoda neurones. In: SALÁNKI, J. (Ed.) Neurobiology of Invertebrates, Plenum Press, New York and Akadémiai Kiadó, Budapest **1968,** pp. 225—265.

D. A. SAKHAROV
Institute of Developmental Biology, Soviet Academy of Sciences
Moscow, USSR

János SALÁNKI
MTA Biológiai Kutatóintézete, Tihany, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 31—39 (1969)*

# EFFECT OF SEX HORMONES ON THE ELECTRICAL ACTIVITY OF BRAIN STEM AND DIENCEPHALON IN CASTRATED FEMALE RATS

By

E. ENDRŐCZI

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received October 16, 1968)

The influence of oestradiol-17-$\beta$ and progesterone has been studied on the evoked potentials due to vaginal stimulation and electrical stimulation of brain stem reticular core in the mesencephalon and diencephalon of castrated female rats. Both vaginal and mesencephally evoked responses of the preoptic region were suppressed in castrated females. Oestradiol treatment resulted in the restoration of mesencephalic evoked potentials in the rostral and basal hypothalamic areas but did not affect vaginal evoked responses. Progesterone treatment led to a marked augmentation of the vaginal evoked potentials in the diencephalon and did not modify evoked responses of the preoptic region due to stimulation of the brain stem reticular formation. The results suggest that oestrogen facilitates brain stem and hypothalamic connections, and progesterone sensitizes sensory input in the brain stem and diencephalon, through oligosynaptic pathways.

Electrical recording methods provide valuable information of the endocrine influences on cerebral function. In the oestrous animal, but not during anoestrous, EEG afterreactions to vaginal probing have been recorded in the cat by PORTER et al. (1957), in the rabbit by SAWYER and KAWAKAMI (1959) and in the rat by BARRACLOUGH (1960). BARRACLOUGH and CROSS (1963) reported that progesterone was rather specifically inhibiting the unit activity to vaginal stimulation in the lateral hypothalamus. CROSS and SILVER (1965) found that endogenous progesterone in pseudopregnancy also reduced unit firing to vaginal stimulation in the lateral hypothalamus and that ovariectomy reversed this condition. Both oestrogen and progesterone treatment resulted in a lowering of the thresholds for EEG and behavioural arousal; the relative amount of paradoxical sleep periods was also increased within a few hours after treatment with the two steroids (SAWYER et al. 1966; COLVIN et al. 1968).

The presence of chemosensitive structures for sex steroids in the hypothalamus was indicated by numerous endocrinological investigations and the preoptic region seems to be strongly implicated in these neuroendocrine events (FLERKÓ 1962; EVERETT 1964; ENDRŐCZI 1967). Studies on hormonal control of sex behaviour revealed the leading role of the preoptic area both in triggering ovulation and in the maintenance of oestrous behaviour.

Recent observations on the integrative role of brain stem and diencephalon in the control of humorally conditioned and motivated behavioural

reactions have indicated the involvement in these processes of both ascending facilitatory and descending inhibitory connections. Hormonal sensitivity of these structures involved in the organization of motivated behavioural reactions is limited by genetic factors; this circumstance accounts for the wide variations in species differences (ENDRŐCZI 1967, 1968).

## Methods

The observations were performed on 64 female rats of 160 to 200 g ovariectomized one to three months before. All surgical procedures were carried out in ether anaesthesia and then the animals were immobilized by dl-tubocurarine. Electrical activity was recorded with 50 $\mu$ thick bipolar, stainless steel electrodes and monitored on a dual-beam cathode ray oscilloscope. Similar bipolar electrodes were used for electrical stimulation of the brain stem reticular formation. Vaginal stimulation was made with a bipolar, silver ball electrode isolated with polyvinyl except for the tips. All stimulations were performed with a Disa Multistim apparatus through isolation units.

Vaginal stimulation was made by the use of impulse train lasting for 200 msec; two consecutive trains were separated by a period of 8 to 10 sec at least.

Physiological saline solution and progesterone dissolved to 50 $\mu$g in 5 $\mu$l propylene glycol were injected intravenously through the cannula inserted into the jugular vein. Oestradiol-17-$\beta$ in oil or propylene glycol was given subcutaneously.

Evoked potentials were evaluated according to latency, shape and amplitude. For obtaining average responses at least fifty records were performed at different intervals before and after steroid treatment. The changes in the size of electrical potentials were expressed on the basis of the territory covered by the curve over the isoelectric line in both negative and positive direction.

After the experiment the animals were killed, the brain was removed and fixed in 10% formol solution, and electrode placement was controlled in 50 $\mu$ thick frozen sections.

## Results

### (i) *Changes in the evoked potential following ovariectomy*

Recordings in the preoptic region and the basomedial hypothalamus (n. ventromedialis hypothalami, tuberoinfundibular region) revealed that electrical potentials evoked by stimulation of mesencephalic reticular formation and vagina are markedly suppressed in the septo-preoptic area but not in the basomedial nuclei of castrated females. Bipolar recordings in different depths of both regions during stimulation of the mesencephalic reticular formation are shown in Fig. 1. Lower thresholds for these long-latency responses were found in the dorsomedial part of the recicular formation and the central grey matter in comparison to the thresholds for ventromedial and lateral reticular formation. The long-latency responses (5.5—6.5 msec) could be distinguished from the responses with short-latency (2.0—2.5 msec) evoked by stimulation of the region of specific projection pathways on the basis of both the latency and the shape of electrical potentials.

The evoked potentials of basomedial hypothalamic nuclei due to stimulation of the mesencephalic reticular formation did not change remarkably after ovariectomy. In the cases when the recording electrodes had been moved in

antero-posterior direction, the evoked responses of the suprachiasmatic area appeared also to be suppressed but to a lesser extent than those of the preoptic region (Figs 1 and 2).

Similarly, in the castrated females the vaginal evoked potentials diminished in the recordings obtained from preoptic and septal areas, as compared to the normal responses. There was no change in the vaginal evoked potentials

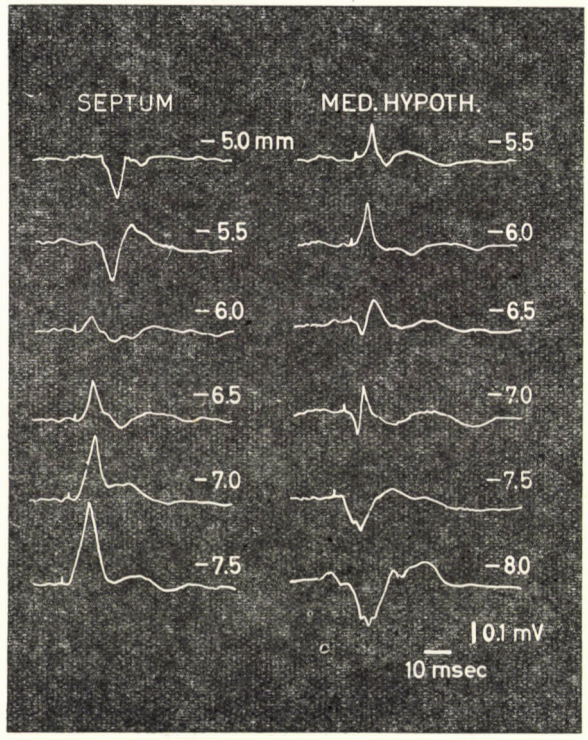



*Fig. 1.* Electrical potentials in the septo-preoptic region and the basomedial hypothalamus evoked by stimulation of mesencephalic reticular formation. Recordings in different depths with 50 $\mu$ thick bipolar electrodes. Normal female rat

recorded from the periventricular area and the dorsal subthalamic region. Latency of these responses varied between 15 and 17 msec, as of those recorded from the mesencephalic reticular formation.

### (ii) *Influence of oestradiol-17-$\beta$ on the electrical potentials evoked by stimulation of mesencephalic reticular formation and vagina*

Castrated rats were treated subcutaneously with 10 and/or 50 $\mu$g/100 g b.w. oestradiol-17-$\beta$ in oil for three days. In addition to this group other castrated females were injected with 50 $\mu$g/100 g b.w. oestradiol-17-$\beta$ in propylene glycol intraperitoneally and electrophysiological observations were carried

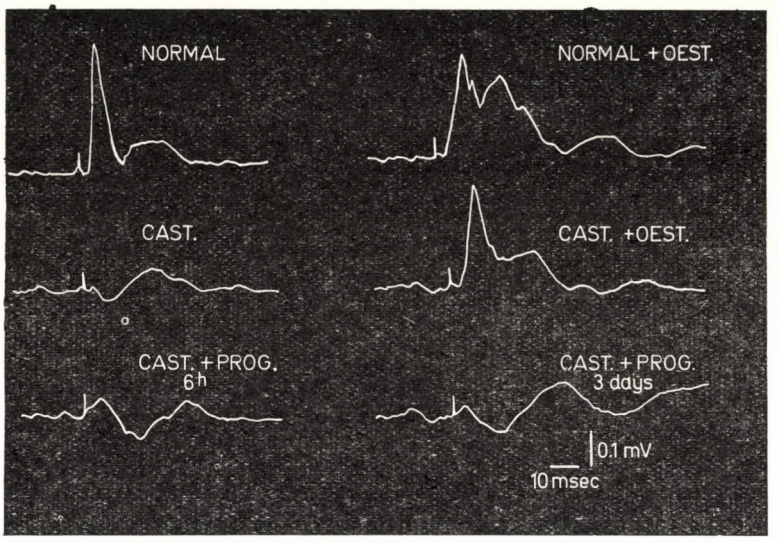



*Fig. 2.* Electrical potentials of septo-preoptic region after ovariectomy and following administration of sex steroids. 50 $\mu$g oestradiol-17-$\beta$ in oil was given subcutaneously 24 hours prior to the electrophysiological study. 50 $\mu$g per 100 g b.w. of progesterone in 5 $\mu$l propylene glycol was injected intravenously 6 hours and 3 days before observation. Bipolar stimulation of dorsal-medial part of the mesencephalic reticular formation was performed with maximal intensity

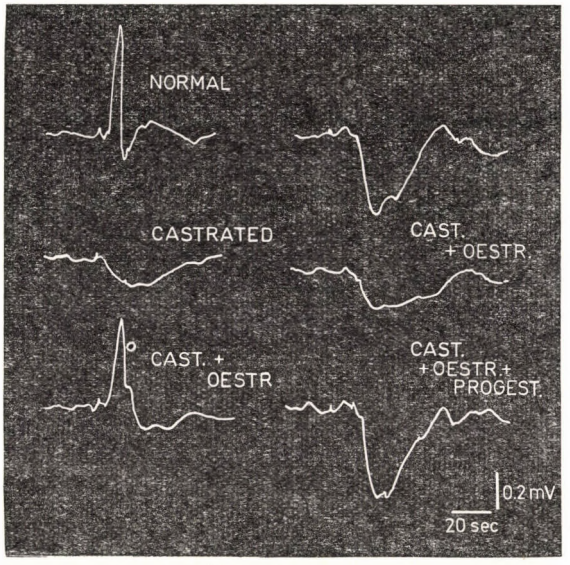



*Fig. 3.* Evoked responses in the preoptic region due to the stimulation of mesencephalic reticular formation (left) and the vagina (right column). Records on the top show normal responses, in the middle row, potentials are indicated after ovariectomy and following administration of 50 $\mu$g per 100 g b.w. of oestradiol-17-$\beta$ in oil 24 hours prior to the electrophysiological observation. Records on the bottom show the effect of oestradiol on the potential evoked by stimulation of the mesencephalic reticular formation, and the influence of 50 $\mu$g per 100 g b.w. of progesterone in propylene glycol injected 3 hours before the study. In the last case the animal was treated with 50 $\mu$g oestradiol 24 hours before the electrophysiological observation

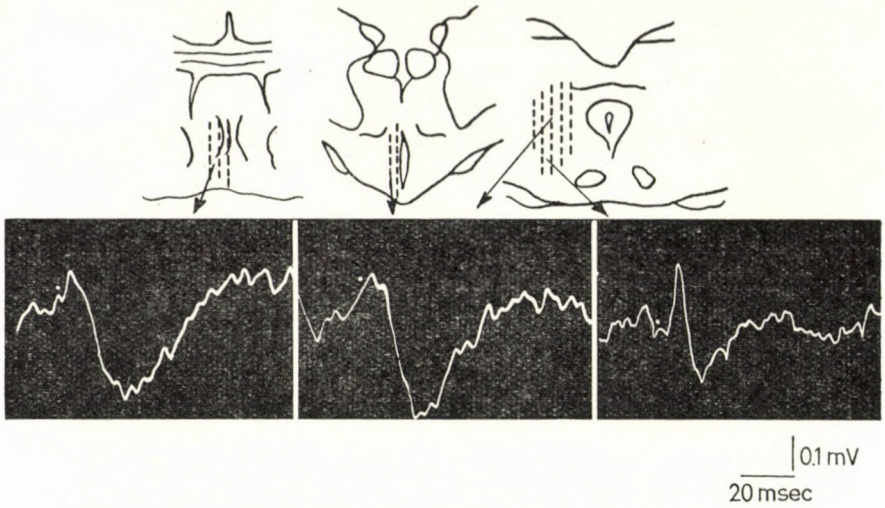



*Fig. 4.* Typical electrical potentials evoked by vaginal stimulation in normal female rats and their recording sites in the preoptic region, the basomedial and periventricular hypothalamus and the mesencephalic reticular formation

out 3, 24 and 36 hours later. Oestradiol treatment resulted in restoration of the preoptic potentials evoked by the stimulation of mesencephalic reticular formation on the 2nd day following injection. In contrast, there was no change in the preoptic response evoked by vaginal stimulation. The facilitatory effect of oestradiol on preoptic potentials due to stimulation of mesencephalic reticular formation was accompanied by a lowering of the stimulation threshold (Figs 3 and 4).

**Table I**

*Stimulation thresholds for maximal evoked responses*

(Impulse duration: 0.05 msec)

| | No. | Stimulation intensity in voltage Mesencephalic reticular formation | | | |
|---|---|---|---|---|---|
| | | Dorsomedial | | Ventrolateral | |
| Normal | 6 | 3.2 | 0.6 | 4.2 | 0.8 |
| Ovariectomized | 6 | 4.8 | 0.8 | 5.6 | 0.6 |
| (1) Normal + oestradiol-17 | 6 | 2.3 | 0.6 | 3.2 | 0.7 |
| (1) Ovariectomized + oestradiol-17 | 6 | 2.4 | 0.5 | 3.3 | 0.8 |
| (2) Normal + oestradiol-17 | 4 | 2.1 | 0.4 | 3.2 | 0.5 |
| (2) Ovariectomized + oestradiol-17 | 5 | 2.2 | 0.6 | 3.2 | 0.5 |

(1) 50 $\mu$g oestradiol-17 in 0.1 ml propylene glycol administered intraperitoneally 24 hours prior to the electrophysiological observations

(2) and 36 hours after injection

Oestradiol treatment did not affect conductivity and latency in the neuronal connections from brain stem to the preoptic and septal region. Furthermore, it failed to modify the electrical potentials of basomedial hypothalamic nuclei evoked by stimulation of the reticular formation (Table 1).

### (iii) *Influence of progesterone on the electrical activity of brain stem and diencephalon in normal and castrated females*

Intravenous injection of 50 $\mu$g/100 g b.w. of progesterone led to an augmentation of vaginal evoked potentials recorded in the brain stem reticular formation and the hypothalamus in both normal and ovariectomized rats. The facili-

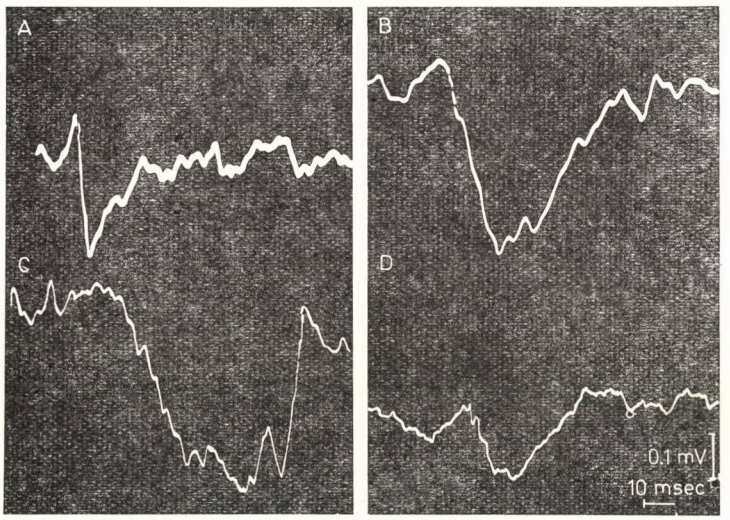



*Fig. 5.* Changes in the vaginal evoked potentials of the basomedial hypothalamus after the intravenous injection of 50 $\mu$g per 100 g body weight of progesterone. A: control, B: 20 min, C: 40 min and D; 120 min after progesterone injection. Bipolar recordings

tatory effect developed 5—10 min and lasted for 45—60 min. The recovery period of vaginal evoked potentials varied from 1500 till 2000 msec in both the untreated castrated and the normal females and this period was markedly reduced during the facilitatory period of progesterone action. While in the untreated animals two consecutive stimuli given within the recovery period resulted in the suppression of the second response, the second response was facilitated by progesterone treatment and after-discharges were also observed.

Progesterone treatment did not influence the evoked potentials due to stimulation of the brain stem reticular formation and recorded in the basomedial and anterior hypothalamic regions.

In the progesterone action on vaginal evoked potentials there was no significant difference between castrated and normal females, with the only

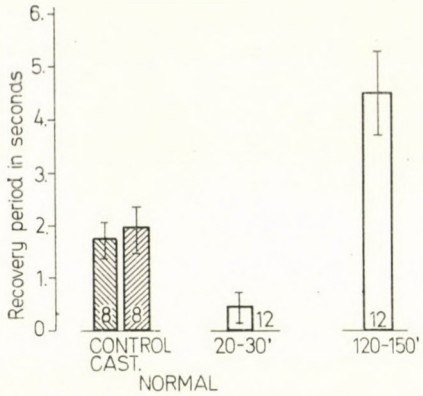



*Fig. 6.* Recovery period of evoked responses to vaginal stimulation. Recordings were performed in the basomedial hypothalamus before, and after 50 $\mu$g per 100 g b.w. of progesterone injection in two different intervals

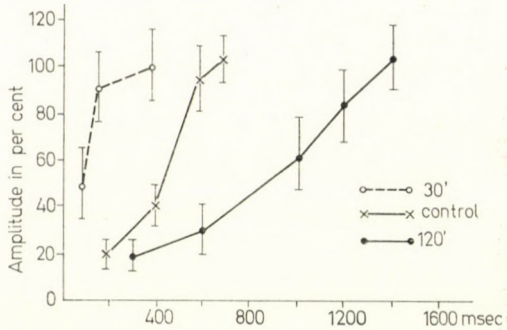



*Fig. 7.* Inhibitory period elicited by the conditioning stimulation of the medial forebrain bundle on the evoked potentials of the mesencephalic reticular formation, due to vaginal stimulation (as test-stimulus), before and after progesterone administration. 50 $\mu$g progesterone in 5 $\mu$l propylene glycol was injected intravenously and the inhibitory period was tested 30 and 120 min after administration. Conditioning stimulation was at maximal intensity. The figure shows values for 6 ovariectomized and 6 normal female rats

exception that the facilitatory period after the injection of progesterone was longer and the appearance of secondary after-discharges was more frequent in the castrated rats.

A decrease of vaginal evoked potentials and a considerable prolongation of the recovery period were observed in the second hour following the progesterone injection. A marked decrease in the amplitude of vaginal evoked responses could be found in both the anterior and the basomedial hypothalamic recordings and to a lesser extent in the brain stem reticular formation.

Testing the inhibitory influence of conditioning stimulation in the medial forebrain bundle region on vaginal evoked responses which were recorded in

the basomedial hypothalamus and the mesencephalic reticular formation by the separation of conditioning and testing stimuli at different intervals, it was found that the inhibitory period showed a biphasic change during progesterone treatment. In the first 30—45 min the inhibitory period was considerably reduced and became prolonged in the second and third hours in comparison to the pretreatment level (Figs 5, 6 and 7).

## Discussion

Earlier data indicated that vaginal stimulation led to after-reactions in the cat (PORTER et al. 1957; LISSÁK 1962), the rabbit (SAWYER 1966) and the rat (BARRACLOUGH 1960). The primary mechanism involved in the organization of after-reaction and sexual arousal has been studied by several investigators. Unit responses to vaginal stimulation of different hypothalamic areas yielded valuable informations, but most observations were carried out in anaesthetized preparations (CROSS and SILVER 1965; RAMIREZ et al. 1967; BARRACLOUGH and CROSS 1963).

A decrease of the threshold for EEG arousal as the result of oestrogen treatment has been confirmed by us after SAWYER and KAWAKAMI's (1959) pertaining study in the female rabbit (ENDRŐCZI 1967). The facilitatory action of progesterone on the EEG and behavioural arousal occurred in the period of progesterone action, and was followed by a marked suppression of the excitatory level of the brain stem ascending activatory system. According to our earlier observations the oestrogens primarily decreased the threshold of EEG arousal produced by a stimulation of the mesencephalic reticular formation and the descending inhibitory influence of forebrain structures on the brain stem was facilitated by progesterone (ENDRŐCZI 1967, 1968).

The present observations indicated a different rather than uniform action of sex steroids on the excitatory state of the central nervous system. Electrical potentials in the preoptic region which had been evoked by the stimulation of the brain stem reticular formation were successfully restored by oestrogen and they remained uninfluenced following progesterone administration. On the other hand, progesterone treatment facilitated vaginal evoked potentials but failed to influence hypothalamic responses to brain stem stimulation. On the basis of our electrophysiological findings we may emphasize that the oestrogens affect primarily the multisynaptic connections between brain stem reticular core and anterior hypothalamic region and that progesterone exerts its influence mostly on oligosynaptic pathways conveying sensory modalities from peripheral sources to the grey matter of the brain stem and the diencephalon.

The biphasic nature of progesterone action was also noted in the present investigations. The marked biphasic alteration in the inhibitory influence of the

medial forebrain bundle region on vaginal evoked potentials in the course of progesterone action has corroborated our earlier assumption that this steroid facilitates descending inhibitory influences of the forebrain structures (ENDRŐCZI 1967), and also the observation that the progesterone implant in the preoptic region enhanced sleeping behaviour (HEUSER et al. 1965). The secondary phase of progesterone action seems to be closely involved in the development of postcoital suppression in sexual arousal, although the understanding of this action of the steroid requires further investigations.

## LITERATURE

BARRACLOUGH, C. V.: Hypothalamic activation associated with stimulation of the vaginal cervix in prooestrous rats. Anat. Rec. **1960,** 136: 159.

BARRACLOUGH, C. A., CROSS, B. A.: Unit activity in the hypothalamus of the cyclic female rat: effect of genital stimuli and progesterone. J. Endocr. **1963,** 26: 339—359.

COLVIN, G. B., WHITMOYER, D. J. ROBERT, D. LISK, D. WALTER, C. H. SAWYER: Changes in sleep-wakefulness in female rats during circadian and estrous cycles. Brain Res. **1968,** 7: 173—181.

CROSS, B. A., SILVER, I. A.: Effect of luteal hormone on the behaviour of hypothalamic neurones in pseudopregnant rats. J. Endocr. **1965,** 31: 251—263.

ENDRŐCZI, E.: Neural and hormonal regulation of the patterns of sexual behaviour. In: K. Lissák (Ed.): Symposium on Reproduction, Akadémiai Kiadó, Budapest **1967,** pp. 39—63.

ENDRŐCZI, E.: Effects of hormones on the brain stem, the diencephalic and the limbic structures. In: Symposium on Hormones and Behavior. Mexico City **1968.** Excerpta med. Found. 1969.

EVERETT, J. W.: Central control of reproductive functions of the adenohypophysis. Physiol. Rev. **1964,** 44: 373—431.

FLERKÓ, B.: Hypothalamic control of hypophyseal gonadotrophic function. In: J. Szentágothai, B. Flerkó, B. Mess, B. Halász (Eds): Hypothalamic Control of the Anterior Pituitary. Akadémiai Kiadó, Budapest **1962,** pp. 192—264.

HEUSER, G., LING, G. M., BUCHWALD, N. A.: Sedation of seizures as dose-dependent effects of steroids. Arch. Neurol. (Chic.) **1965,** 13: 195—203.

LISSÁK, K.: Olfactory induced sexual behaviour in female cats. Brain-Gonad Symposium, Proc. int. Congr. Physiology, Leiden **1962,** Excerpta med. Found., Congr. Ser. Pp. 652—656.

PORTER, R. W., CAVANAUGH, E. B., CRITCHLOW, V. B., SAWYER, C. H.: Localized changes in electrical activity of the hypothalamus in estrous cats following vaginal stimulation. Amer. J. Physiol. **1957,** 189: 145—151.

RAMIREZ, D. V., KOMISARUK, B. R., WHITMOYER, D. I., SAWYER, C. H.: Effects of hormones and vaginal stimulation on the EEG and hypothalamic units in rats. Amer. J. Physiol. **1967,** 212: 1378—1384.

SAWYER, C. H., KAWAKAMI, M.: Characteristics of behavioral and electroencephalographic after-reactions to copulation and vaginal stimulation in the female rabbit. Endocrinology **1959,** 65: 622—630.

SAWYER, C. H., KAWAKAMI, M., KANEMATSU, S.: Neuroendocrine aspects of reproduction. Endocrines and the Central Nervous System. Res. publ. Ass. nerv. ment. Dis. **1966,** 43: 59—85.

Elemér ENDRŐCZI
Orvostudományi Egyetem Élettani Intézete
Pécs, Rákóczi ut 80,
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 41—46 (1969)*

# PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH) INDUCED HYPOGLYCAEMIA

By

L. KORÁNYI, E. ENDRŐCZI and Veronica TAMÁSY

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received October 14, 1968)

ACTH injected intraperitoneally was found to induce hypoglycaemia in both normal and adrenalectomized rats. A significant decrease of the blood sugar level was observed in adrenalectomized animals stressed with formalin injection and/or subjected to conditioned fear procedure. The hypoglycaemic effect could be prevented with dexamethasone implanted into the median eminence of the hypothalamus. Practically no decrease of the blood sugar level was found in response to ACTH, formalin injection, or conditioned fear 3 weeks after adrenalectomy. Hydrocortisone pretreatment for 3 days in animals markedly influenced the mechanism of hypoglycaemia induced by ACTH 60 days after surgery.

A number of data accumulated over the past years show that pituitary trophic hormones in addition to their specific action on the target organs exert a wide variety of extra-target effects. Concerning pituitary adrenocorticotrophic hormone preparations, it is well known that highly purified ACTH, even the synthetic eicosapeptide-corticotrophin, exert several extra-adrenal actions. For instance, injection of the peptide results in mobilization of depot fat, ketosis, hypoglycaemia by increasing insulin secretion, and hypocalcaemia in certain species, first of all in the rabbit (ANSELMINO and HOFFMANN 1933a and b; ENGEL and KOSTYO 1964; FRIESEN 1964; ENGEL and LEBOVITZ 1965; LEBOVITZ 1967; LUNDQUIST and RERUP 1967; TRYGSTAD 1967).

Oxidative deamination of the alpha-amino group or shortening of the ACTH peptide chain to 11 to 16 amino acid residuum completely abolished the effect on the adrenal cortex, while other extra-adrenal effects remained practically unchanged. Acetylation of the N-terminal serine of the peptide molecule converted the hypoglycaemic to a hyperglycaemic effect (LEBOVITZ 1967). On the other hand, as observed in experiments with crude and highly purified ACTH preparations, the lipid mobilizing and hypocalcaemic effects were due to impurities in the extracts (TRYGSTAD 1967).

The present study has been devoted to the question whether the effect of ACTH on insulin secretion is a result of aspecific and/or pharmacological factors, or is of a physiological significance, and whether systemic as well as psychic stress can liberate such an amount and type of endogenous ACTH which may induce hypoglycaemia.

## Methods

Ninety-nine adult male albino rats divided into eleven groups were used in the experiments.

The blood sugar level in blood samples taken from the tail vein of the animals was determined by the HAGEDORN and JENSEN method (1923).

Highly purified ACTH in doses of 100 $\mu$g per kg body weight, and cortrophine (Organon, Oss, Netherlands) in doses of 10 I.U. per kg body weight dissolved in physiological saline solution were given intraperitoneally. Formalin (0.1 ml of a 5% solution) was administered intramuscularly.

Conditioned fear was established in a conditioning box by pairing a sound signal of 10 sec duration with an electric shock (3 mA) applied to the paw of the animal through the grid floor.

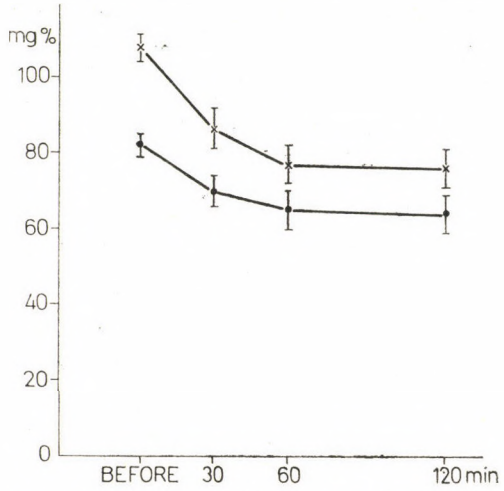



*Fig. 1.* Decrease of blood sugar level (mean ± standard error of the mean) following ACTH injection in normal (×) and adrenalectomized rats (●)

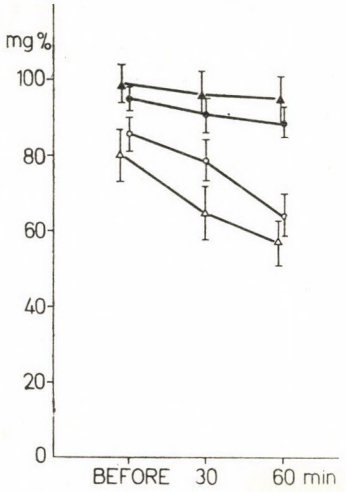



*Fig. 2.* Changes of blood sugar level (mean ± standard error of the mean) in adrenalectomized rats following formalin stress and conditioning procedure. Adrenalectomy + formalin (○); adrenalectomy + conditioned fear (△) one week after surgery. Adrenalectomy + formalin (●); adrenalectomy + conditioned fear (▲) 3 weeks after surgery

Ten trials in 60 sec intertrial intervals were given daily for 2 days. Blood sugar was estimated on the 3rd experimental day when sound signals without electric shock were presented.

Adrenalectomy was performed in ether anaesthesia and the animals were kept on 1% saline solution. Implantation of 5 to 10 $\mu$g of crystalline dexamethasone and cholesterol into the median eminence of the hypothalamus was made stereotaxically by a fine stainless steel capillary.

For histology the brains were fixed in formalin and the localization of steroid implants was checked in frozen sections.

## Results

ACTH injected intraperitoneally induced severe hypoglycaemia in both normal and adrenalectomized rats. The blood sugar level showed a decrease within 30 min and the effect lasted for more than 120 min (Fig. 1).

Formalin stress as well as conditioned fear also led to a rapid development of hypoglycaemia. When testing the effect of conditioned fear, blood samples were taken on the 3rd day of the conditioning procedure prior to and 30 as well as 60 min after presentation of the first trial, one week following adrenalectomy. No significant decrease of the blood sugar level in response to formalin injection or conditioned fear occurred 3 weeks after adrenalectomy (Fig. 2).

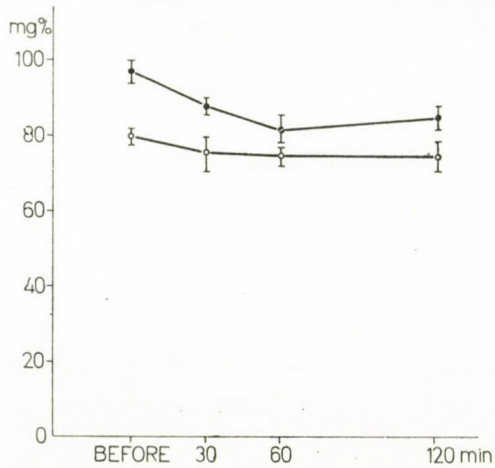



*Fig. 3.* Changes of blood sugar level (mean ± standard error of the mean) in rats following ACTH injection. 60 days after adrenalectomy (○); 63 days after adrenalectomy + hydrocortisone pretreatment (●)

Sixty days after adrenalectomy the blood sugar level was practically unchanged, but pretreatment with 5 mg of hydrocortisone daily for 3 days partially restored the sensitivity and a marked decrease of blood sugar level developed after ACTH had been injected. Sixty days after adrenalectomy the blood sugar decreased by 5 mg per 100 ml only, but on the 63rd day following the hydrocortisone pretreatment a 16 mg reduction was observed (Fig. 3).

In adrenalectomized animals with dexamethasone implant in the median eminence no significant decrease of the blood sugar level was induced by formalin or the conditioning procedure, as compared to the corresponding adrenalectomized control group, on the 3rd day following surgery (Figs 4 and 5).

## Discussion

Administration of both commercial and highly purified ACTH as well as systemic and/or psychic stress was found to induce hypoglycaemia in the adrenalectomized rat. The observation that this hypoglycaemia was prevented by dexamethasone, a powerful inhibitor of endogenous ACTH secretion, implanted into the median eminence of the hypothalamus, pointed to the fact

that for the phenomenon ACTH alone was responsible. Under physiological conditions, ACTH secretion does not induce hypoglycaemia owing to the previous activation of the sympathoadrenal system and adrenocortical steroid output. It follows that the insulin secretion induced by endogenous ACTH is a physiological mechanism even if the resulting blood sugar change is hyperglycaemia in the normal animal.

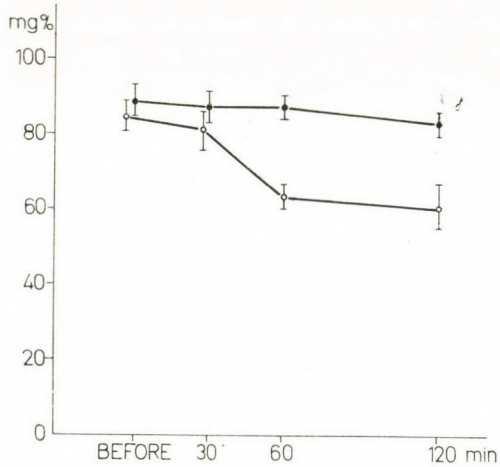



*Fig. 4.* Changes of blood sugar level (mean ± standard error of the mean) following formalin stress in adrenalectomized rats with dexamethasone implant in the median eminence of the hypothalamus (●), and adrenalectomized control animals (○)

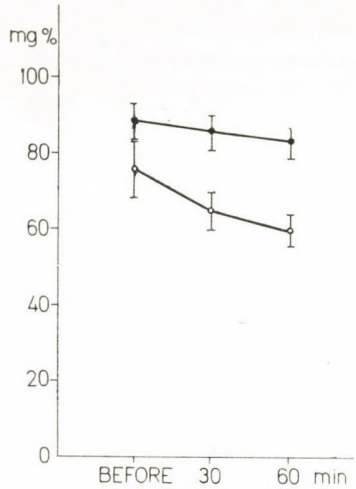



*Fig. 5.* Changes of blood sugar level (mean ± standard error of the mean) following conditioning procedure in adrenalectomized rats with dexamethasone implant in the median eminence of the hypothalamus (●), and adrenalectomized control animals (○)

Since the intimate mechanism of ACTH induced insulin secretion is unknown, we do not have to answer the question whether the action is bound to certain amino acids or some amino acid combination. Recent data have pointed to the important role of essential amino acids since while glucose infusion resulted in a slight elevation, the infusion of essential amino acids was associated with a striking increase in serum insulin content (Grasso et al. 1968).

Adrenal steroids play an important role in the maintenance of ACTH induced insulin secretion; this effect can be a permissive or a direct one. The influence of corticosteroids on the pancreas endocrine function has clearly been demonstrated by Houssay (1951, 1959) and Houssay et al. (1954a, b) who found that the action of corticosteroids and oestrogens manifested themselves with a diphasic mechanism. At first they increase the severity of experimental diabetes by increasing the blood sugar level, but later they have a preventive action on the incidence of diabetes in the rat. This action is probably a direct

one, since MALAISSE et al. (1967) found that the in vitro insulin output of pancreas slices under the effect of glucose was enhanced in cortisol treated animals.

In recent years many researchers have studied the effect of ACTH on neural mechanisms and behaviour. Wide varieties of extraadrenal actions and remarkable species differences have been reported in this respect (see for literature KORÁNYI et al. 1967; BOHUS and KORÁNYI 1968; DE WIED and PIRIE 1968). The hypoglycaemic phenomenon should always be taken into account in experiments on ACTH treated normal animals or when studying adrenalectomized animals. Further experiments are required to explore the modifying or mediating role of ACTH induced insulin secretion and consequential hypoglycaemia in the changes of higher nervous activity, behavioural and neural mechanisms observed in adrenalectomized or ACTH treated animals.

## LITERATURE


ANSELMINO, K. J., HOFFMANN, F.: Die pankreatrope Substanz aus dem Hypophysenvorderlappen. I. Über die Darstellung und die Eigenschaften der pankreatropen Substanz. Klin. Wschr. **1933a,** II. 1435—1436.

ANSELMINO, K. J., HOFFMANN, F.: Die pankreatrope Substanz. II. Über die Stoffwechselwirkungen der pankreatropen Substanz. Klin. Wschr. **1933b,** II. 1436—1438.

BOHUS, B., KORÁNYI, L.: Hormonal Conditioning of Adaptive Behavioural Processes. In: LISSÁK, K. (Ed.): Recent Development of Neurobiology in Hungary. Vol. II. Akadémiai Kiadó, Budapest **1969,** pp. 50—76.

ENGEL, F. L., KOSTYO, J. L.: Metabolic actions of pituitary hormones. In: PINCUS, G., THIMANN, K. V., ASTWOOD, E. B. (Eds): The Hormones, Physiology, Chemistry and Applications. Vol. V. Academic Press, New York **1964,** pp. 60—128.

ENGEL, F. L., LEBOVITZ, H. E.: Extra-target organ actions of anterior pituitary hormones. In: HARRIS, G. W., DONOVAN, B. T. (Eds): The Pituitary Gland. Vol. II. Butterworths, London **1966,** pp. 563—588.

FRIESEN, H.: Hypocalcaemic effect of pituitary polypeptides in rabbits. Endocrinology **1964,** 75: 692—697.

GRASSO, S., SAPORITO, N., MESSINA, A., REITANO, G.: Serum-insulin response to glucose and amino acids in the premature infant. Lancet **1968,** 2: 755—757.

HAGEDORN, H. C., JENSEN, B. M.: Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. Biochem. Z. **1923,** 135: 46—47.

HOUSSAY, B. A.: Action of sex hormones on experimental diabetes. Brit. med. J. **1951,** 4: 505—507.

HOUSSAY, B. A.: Comparative physiology of the endocrine pancreas. In: GORBMAN, A. (Ed.): Comparative Endocrinology. John Wiley & Sons, New York **1959,** pp. 639—667.

HOUSSAY, B. A., FOGLIA, V. G., RODRIGUEZ, R. R.: Production or prevention of some types of experimental diabetes by oestrogens or corticosteroids. Acta endocr. (Kbh.) **1954a,** 17: 146—149.

HOUSSAY, B. A., RODRIGUEZ, R. R., CARDEZA, A. F.: Prevention of experimental diabetes with adrenal steroids. Endocrinology **1954b,** 54: 550—555.

KORÁNYI, L., ENDRŐCZI, E., LISSÁK, K., SZEPES, É.: The effect of ACTH on behavioral processes motivated by fear in mice. Physiol. Behav. **1967,** 2: 439—445.

LEBOVITZ, H. E.: Some clinical implications of recent advances in the chemistry and physiology of protein hormones. In: BAJUSZ, E. (Ed.): An Introduction to Clinical Neuroendocrinology, S. Karger, Basel/New York **1967,** pp. 243—253.

LUNDQUIST, I., RERUP, C.: Blood glucose level in mice. On the nature of corticotrophin induced hypoglycaemia. Acta endocr. (Kbh.) **1967,** 56: 713—725.

MALAISSE, W. J., MALAISSE-LAGAE, F., MCCRAW, E. F., WRIGHT, P. H.: Insulin secretion in vitro by pancreatic tissue from normal, adrenalectomized and cortisol-treated rats. Proc. Soc. exp. Biol. (N. Y.) **1967,** 124: 924—28.

TRYGSTAD, O.: The lipid-mobilizing effect of some pituitary gland preparations. I. Evidence for a lipotrophic contamination with rabbit serum calcium-lowering effect in adrenocorticotrophin and human growth hormone preparations. Acta endocr. (Kbh.) **1967**, 56: 626—648.

DE WIED, D., PIRIE, G.: The inhibitory effect of ACTH 1—10 on extinction of a conditioned avoidance response: its independence of thyroid function. Physiol. Behav. **1968**, 3: 355—358.

Lajos KORÁNYI, Elemér ENDRŐCZI, Veronica TAMÁSY
Orvostudományi Egyetem Élettani Intézete
Pécs, Rákóczi ut 80,
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 47—57 (1969)*

# EFFECT OF THE MEDIAN EMINENCE OF THE PIGEON (COLUMBA LIVIA DOMESTICA L.) ON THE REGULATION OF ADENOHYPOPHYSIAL CORTICOTROPIN SECRETION

By

P. PÉCZELY

DEPARTMENT OF GENERAL ZOOLOGY AND COMPARATIVE ANATOMY,
LORÁND EÖTVÖS UNIVERSITY OF SCIENCES, BUDAPEST

(Received May 28, 1968)

The changes in the CRF activity of the median eminence and in the ACTH activity of the adenohypophysis of the pigeon have been studied in vitro in the case of adrenal hyperactivity and hypoactivity. At 30 minutes following the injection of 8 I.U. insulin the CRF content of the median eminence was reduced, at 1 hour the value was comparable to the control one, at 2 hours there was a marked decrease and at 3 hours a minimum value was found. The ACTH content of the adenohypophysis at 30 minutes was increased to about three times the control value, then in the next 3 hours it slowly decreased to below the control level. In response to ACTH treatment, water loading, administration of NaCl and prednisolone treatment the CRF content of the median eminence decreased, but it did not change in response to water deprivation. In response to ACTH treatment and water loading the ACTH content of the adenohypophysis decreased significantly, in response to NaCl administration and prednisolone treatment it increased, and it was unaffected by water deprivation. The results show that the pigeon's adrenal function is under the control of the hypothalamic-hypophysial system.

According to recent evidence in the literature, the mechanism of the central control of adrenal activity is essentially similar in birds and in mammals. The avian adenohypophysis contains considerable quantities of ACTH, which increase adrenal glycocorticoid production in vitro (DE ROOS and DE ROOS 1964; PÉCZELY and ZBORAY 1967). Apparently, ACTH is produced in the erythrosinophilic, more precisely in the purple-staining epsilon cells of the cephalic lobe. This is indicated by the fact that on metyrapone or reserpine treatment these cells undergo hypertrophy and degranulation (TIXIER-VIDAL 1963).

The importance of ACTH is shown by the atrophy of the interrenal matter of the avian adrenal (MILLER and RIDDLE 1942; NALBANDOV and CARD 1943; ASSENMACHER 1958; MILLER 1961) or the decrease of its corticoid production occurring after adenohypophysectomy (NAGRA et al. 1963; RESKO et al. 1964; FRANKEL et al. 1967).

As far as the role of the hypothalamus in the central regulation of adrenal activity is concerned, the scarce evidence available allows the only conclusion that the median eminence and the ventral hypothalamus are enhancing pituitary ACTH production (FRANKEL et al. 1967; RESKO et al. 1964; PÉCZELY

and ZBORAY 1967). In a previous study (PÉCZELY and ZBORAY 1967) we have obtained from the median eminence a crude extract which increased pituitary ACTH production; thus, the median eminence of the pigeon has CRF activity.

In the same study we have confirmed the hypothesis of RESKO et al. (1964) concerning the existence of extrahypophysial ACTH or a substance of ACTH activity bound to the median eminence. Furthermore, our results are in harmony with the statement made by CORBIN et al. (1965) that dexamethasone-21-phosphate inhibits adrenal steroidogenesis in both intact and adenohypophysectomized animals.

The existence of an extrahypophysial substance of ACTH activity represents a considerable difference from mammals as regards the central control of adrenal function. At the same time, it may offer an explanation for the great "autonomy" of the avian adrenal, which displayed no significant morphological change following hypophysectomy (NEWCOMER 1959; BAUM and MEYER 1956; ELTON et al. 1959). Also, the decrease of adrenal steroid production after hypophysectomy was slighter in birds than in mammals (FRANKEL et al. 1967; RESKO et al. 1964).

In the present experiments we have studied the changes in the CRF activity of the median eminence and of the ACTH activity of the adenohypophysis in the case of adrenal hypofunction and hyperfunction to observe the influence of stimulating and inhibiting factors of the hypothalamic-adrenocorticotropic system in the pigeon.

## Methods

A total of 226 sexually mature pigeons (*Columba livia domestica* L.), 131 males and 95 females, was used in the experiments. Average weight of the birds was 320 g. The ACTH assays were made in female rats of the Rehbrücke strain, weighing 180 to 220 g.

In the course of the investigations the pigeons were subjected to the following treatments.

Experimental group 1: Insulin treatment (46 birds). Five groups of pigeons were treated with insulin, the birds were killed following the intravenous injection of a single 8 I. U. dose of insulin at 30 minutes, 1 hour, 2 hours and 6 hours, or at 8 hours following the intravenous injection of a single 30 I. U. dose of insulin.

Experimental group 2: Prednisolone treatment (9 birds). The experimental birds were given orally 5 mg prednisolone in two doses (one at 10 a.m. and the other at 6 p.m.) daily for 20 days.

Experimental group 3: ACTH treatment (9 birds). 4 I. U. of ACTH was administered intramuscularly, 2 I. V. at 10 a.m., and 2 I. V. at 6 p.m., daily for 4 days.

Experimental group 4: NaCl treatment (8 birds). The pigeons were fed for 20 days corn soaked in 4% NaCl solution.

Experimental group 5: Hydration (10 birds). The birds received over 10 days daily 40 ml of tap water through an intragastric tube in two portions, one at 10 a.m., the other at 6 p.m.

Experimental group 6: Water deprivation (10 birds). The pigeons were deprived of drinking water for 8 days.

Both the pigeons and the rats were killed by decapitation between 11 a.m. and 2 p.m.

The median eminence homogenate from the experimental birds was added to the in vitro incubated adenohypophysis of intact pigeons and the incubate was assayed quantitatively for ACTH content by means of rat adrenal slices incubated in Krebs—Ringer bicarbonate. The ACTH content of the adenohypophysis of the treated pigeons was determined in the same way. The method has been described in detail (PÉCZELY and ZBORAY 1967).

The values have been calculated from the average corticosterone production by 25 mg rat adrenal tissue in 1 hour for μg/100 mg/hour. The adenohypophysis of necropsied pigeons weighed 3.6 mg, the excised median eminence tissue 3.0 mg, on the average.

Corticosterone production by the rat adrenal was determined on the basis of the works of GLICK et al. 1964; ZENKER and BERNSTEIN 1958; ESPINER 1965, by the fluorometric method described by us (PÉCZELY and ZBORAY 1967).

The control values were as follows. Reliable execution of incubation in vitro and of the fluorometric corticosterone determination was checked by the determination of the basal corticosterone secretion of the rat adrenal and by systemic control. The CRF content of the median eminence was determined in intact animals, and this served as the control value in the experiments. Pituitary ACTH production was estimated without adding median eminence extract, and to this value were related those for the CRF found in the control and the experimental animals. The effect of the experimental intervention was controlled in every case by histologic examination of the adrenals of the treated pigeons (Péterfi's embedding method, haematoxylin-eosin staining).

## Results

The intravenous injection of 8 I.U. of insulin rapidly stimulated the hypothalamic-corticotropin system. CRF activity of the median eminence decreased from the control 13.1 μg level to 8.4 in 30 minutes; this decrease of about 75% pointed to an intensive mobilization of the releasing factor from the median eminence. The intensive CRF transport was indicated by the 333% increase from 9.90 μg to 19.10 μg in adenohypophyseal ACTH content.

At 60 minutes, CRF activity of the median eminence was practically at the control level (12.85 μg). As compared to the 30-minute value, adenohypophyseal ACTH content was reduced, but still higher than the control value of 12.10 μg, pointing to a new equilibrium between increased synthesis and mobilization.

At 120 minutes following the intravenous injection of 8 I.U. insulin, the CRF content of the median eminence was again markedly reduced (8.75 μg); this was accompanied by a negligible increase in adenohypophyseal ACTH content (12.20 μg).

At 360 minutes the CRF content of the median eminence was further decreased (7.50 μg), and there was a significant reduction in the ACTH content of the adenohypophysis (9.10 μg). (Fig. 1).

At 8 hours following the intravenous injection of 30 I.U. of insulin an exhaustion of the hypothalamic-adrenocorticotropin system was observed. The CRF content of the median eminence was as low as 6.90 μg and the ACTH content of the adenohypophysis was also very low (7.50 μg) (Figs 2 and 3).

In response to prednisolone treatment, the CRF content of the median eminence decreased to a small extent, but significantly (11.60 μg); while the adenohypophyseal ACTH content increased very significantly to 13.48 μg.

In response to ACTH treatment, CRF content of the median eminence decreased (8.81 μg) and the ACTH content of the adenohypophysis suffered a significant decrease (6.95 μg).

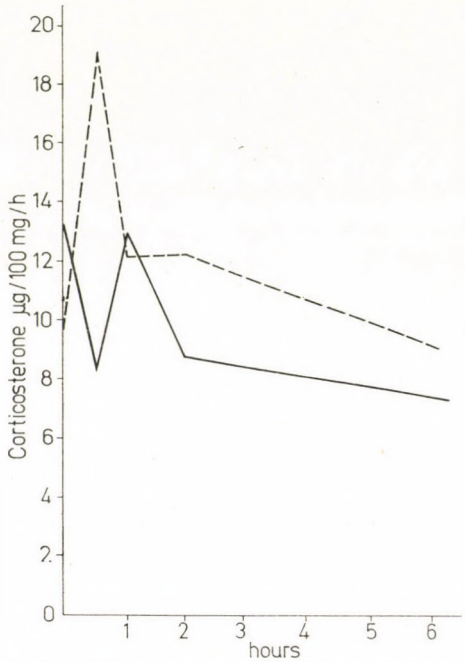



*Fig. 1.* Effect of a single 8 I.U. intravenous dose of insulin on CRF activity of median eminence and on ACTH content of adenohypophysis. ———— CRF in median eminence; – – – – ACTH in adenohypophysis

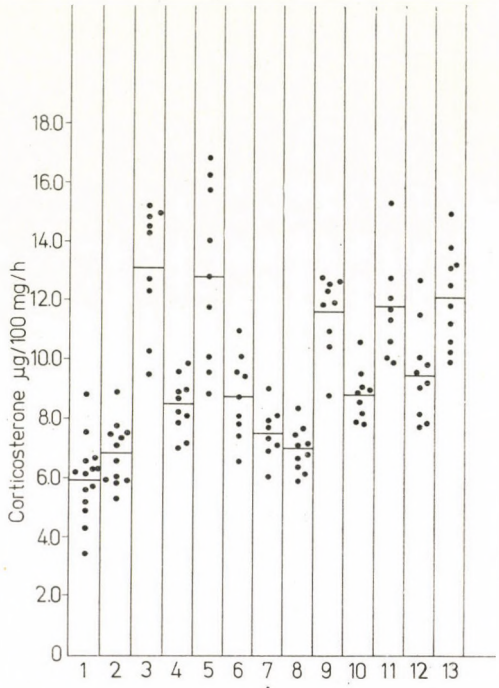



*Fig. 2.* CRF content of median eminence. 1. Basal corticosterone secretion of rat adrenal. 2. ACTH production of adenohypophysis. 3. Control CRF. 4. Insulin treatment, 30 minutes. 5. Insulin treatment, 1 hour. 6. Insulin treatment, 2 hours. 7. Insulin treatment, 6 hours. 8. Insulin treatment (30 I.U.), 8 hours. 9. Prednisolone treatment. 10. ACTH treatment. 11. NaCl administration. 12. Hydration. 13. Dehydration

NaCl loading reduced the CRF content of the median eminence to a smaller extent, yet significantly (11.75 $\mu$g), and the ACTH content of the adenohypophysis was significantly increased (11.75 $\mu$g).

Water loading caused a highly significant decrease in CRF content (9.60 $\mu$g), and a marked decrease in ACTH content (8.07 $\mu$g).

On water deprivation there was a slight, not significant decrease in CRF (12.15 $\mu$g), while the ACTH content was unchanged (10.00 $\mu$g) (Figs 2 and 3, Tables I and II).

As to adrenal morphology, 2 to 6 hours after 8 I.U. of insulin the interrenal matter displayed a slight, 8 hours after the injection of 30 I.U. of insulin, a marked hypertrophy. Prednisolone caused an atrophy of the deeper-lying interrenal cells, while ACTH a marked hypertrophy of the entire interrenal matter. In response to NaCl loading the peripheral zone atrophied, while the deeper

zone showed no major change. Hydration caused atrophy of the deeper zone and water deprivation a considerable hypertrophy of the interrenal matter.

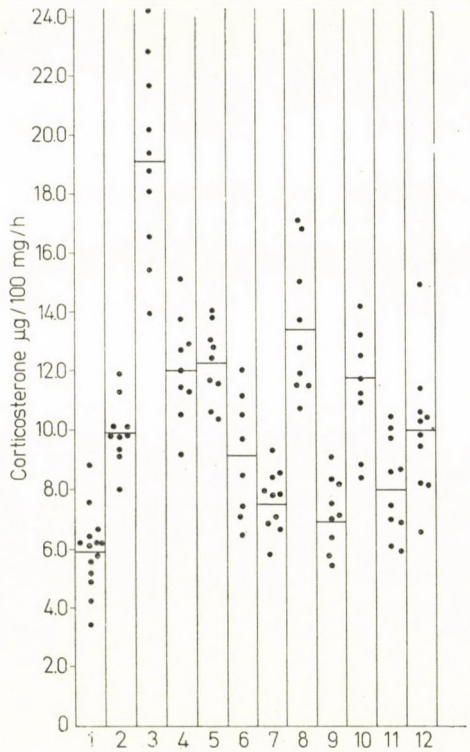



*Fig. 3.* ACTH content of adenohypophysis. 1. Basal corticosterone secretion of rat adrenal. 2. ACTH content of control adenohypophysis. 3. Insulin treatment, 30 minutes. 4. Insulin treatment, 1 hour. 5. Insulin treatment, 2 hours. 6. Insulin treatment, 6 hours. 7. Insulin treatment (30 I.U.), 8 hours. 8. Prednisolone treatment. 9. ACTH treatment. 10. NaCl administration. 11. Hydration. 12. Dehydration

## Discussion

The interventions applied in the present experiment have been shown to induce histological or functional changes in the adrenals. They were assumed to exert their effect through the hypothalamic-hypophysial system and so it seemed interesting to determine the CRF content of the median eminence and the ACTH content of the adenohypophysis in pigeons with adrenal hyperfunction and hypofunction.

Insulin treatment causes hypertrophy of the interrenal matter of the adrenal (Miller and Riddle 1942; Kumishima 1960; Miller 1961) in 2 to 4

**Table I**

*CRF activity of median eminence under different experimental conditions*
The difference is significant statistically when $t = 2.11-2.14$, $p = 0.05$

| | |
|---|---|
| Control / Insulin, 30 minutes | $t = -6.40$ |
| Control / Insulin, 1 hour | $t = -0.25$ |
| Control / Insulin, 2 hours | $t = -5.28$ |
| Control / Insulin, 6 hours | $t = -7.00$ |
| Control / Insulin, 8 hours | $t = -8.85$ |
| Control / Prednisolone | $t = -2.88$ |
| Control / ACTH | $t = -5.70$ |
| Control / NaCl | $t = -2.24$ |
| Control / Hydration | $t = -4.15$ |
| Control / Dehydration | $t = -1.14$ |

hours (KUMISHIMA 1960). It is known that stress activates the CRF-adrenocorticotropic system within a matter of minutes (VERNIKOS-DANELLIS 1965). As in the present study hyperactivity of the hypothalamic-adrenocorticotropic system was induced by endogenous adrenaline secretion evoked by insulin, it was reasonable to kill the first group of birds after a longer period of time (30 minutes).

In the birds killed at 30 minutes the CRF content of the median eminence was decreased, indicating a rapid mobilization of the accumulated releasing factor. This hypothesis was supported by the fact that at the same time the ACTH content of the adenohypophysis increased by about 333%, a change pointing to a greatly enhanced synthesis and release, with a dominance of synthesis.

One hour after the insulin injection, CRF activity of the median eminence was back at the control level, but the significantly increased pituitary ACTH content suggested the presence of an equilibrium of the increased CRF synthesis and release, or hypersecretion of ACTH.

At 2 hours CRF activity was reduced, but the ACTH content was still high above the control level. In this stage CRF secretion was increased, together with a decrease in the synthesis of the releasing factor.

**Table II**

*ACTH content of adenohypophysis under different experimental conditions*
The difference is significant statistically when $t = 2.10-2.12$, $p = 0.05$

| | |
|---|---|
| Control / Insulin, 30 minutes | $t = 8.38$ |
| Control / Insulin, 1 hour | $t = 3.24$ |
| Control / Insulin, 2 hours | $t = 4.13$ |
| Control / Insulin, 6 hours | $t = 2.15$ |
| Control / Insulin, 8 hours | $t = 5.21$ |
| Control / Prednisolone | $t = 4.11$ |
| Control / ACTH | $t = -5.26$ |
| Control / NaCl | $t = 2.42$ |
| Control / Hydration | $t = -3.08$ |
| Control / Dehydration | $t = 0.06$ |

At 6 hours both CRF activity and ACTH activity were significantly reduced. The low ACTH content of the adenohypophysis was suggestive of a decreased CRF synthesis and release. The decrease of CRF content in this case indicated a drop in the rate of releasing factor production.

Thus, 8 I.U. of insulin was found to stimulate the hypothalamic-adrenocorticotropic system but CRF synthesis and mobilization did not show a parallel change. At 6 hours following insulin administration the hypothalamic-adrenocorticotropic system exhibited signs of exhaustion, while histological adrenal hypertrophy takes 2 to 4 hours to appear (KUMISHIMA 1960). Therefore, the histologic changes in the adrenals are not suitable for the following up of such rapid changes, or for the demonstration of ACTH release.

After the administration of 30 I.U. of insulin, CRF activity of the median eminence practically ceased and pituitary ACTH content decreased, suggesting a total exhaustion of the hypothalamic-adrenocorticotropic system.

ACTH or prednisolone treatment, as well as water loading and NaCl administration presumably inhibited the hypothalamic-adrenocorticotropic system. The CRF activity of the median eminence decreased in every case; this

decrease was significant following ACTH treatment and water loading, and slighter after prednisolone or NaCl treatment. At the same time a different change occurred in the pituitary ACTH content; in response to ACTH treatment or water loading it decreased markedly, while it increased significantly on prednisolone or NaCl administration. With the exception of ACTH treatment every intervention lasting 10 to 20 days caused adrenal atrophy. It being improbable that the atrophied adrenals should have produce increased amounts of steroid and that the atrophy should not cease in response to an elevation of the endogenous blood ACTH level, ACTH release must have diminished in every case. Thus, the histological structure of the adrenals seems to have reflected the momentary relations of steroid production. The results suggest that ACTH treatment or water loading inhibit ACTH synthesis and release, while the administration of prednisolone or NaCl does not affect ACTH synthesis but inhibits its release into the blood. This would mean that in the median eminence there are "releasing" and "synthesis" factors, reacting independently of each other.

Our observations support the statement made by HALÁSZ and PUPP (1965) that in the hypophyseotropic area releasing and tropic factors would be formed, and are in harmony also with the observation made by FOLLET and FARNER (1966), FARNER and FOLLET (1966) who in studies concerned with the gonadotropic hormone production in birds suggested that independent hypothalamic factors would influence the release and synthesis of tropic hormones. Independent release and synthesis of ACTH has been suggested also by ESTEP et al. (1967) who combined in rats dexamethasone or puromycin treatment with morphine administration or laparotomy and found that dexamethasone + morphine treatment inhibited corticosterone secretion, but $^{14}C$ incorporation into the adenohypophysis was similar to that in the controls, suggesting that ACTH synthesis was unaffected.

The inhibitory action of ACTH on CRF may be brought into correlation with some internal feed-back effect, with the direct inhibitory effect of ACTH. The possibility of such a "short" feed-back was suggested by SZENTÁGOTHAI et al. (1962) and confirmed by MOTTA et al. (1965) in the rat by the inhibition of corticosterone secretion by ACTH microcrystals implanted into the median eminence.

The reduction of CRF activity by NaCl loading is difficult to explain. In this case the histological findings should be analysed. In birds, too, NaCl administration activates the Gomori-positive hypothalamic neurosecretory system and the neurohypophysis, but induces no demonstrable histological changes in the median eminence (FARNER and OKSCHE 1962). Thus, the decrease of the CRF content of the median eminence cannot be correlated with the changes in the Gomori-positive secretion content of the neurohaemal zone. At the same time, NaCl loading has been shown to affect the neuroendocrine system by

the karyometric studies made in the rat by LICHARDUS et al. (1965), which revealed that 10 days after drinking 2% NaCl solution a significant decrease in nuclear volume could be demonstrated in the posterior hypothalamic nucleus in the tuberal region. Thus, at least in mammals, NaCl not only acts on the hypothalamic Gomori-positive system, but also inhibits a cell group in the tuberal region. It may be assumed that NaCl administration has a similar effect in birds, and that this was somehow related with the decrease in CRF activity. The effect of NaCl loading on the histologic pattern of the hypothalamus and on the changes in CRF activity indicates that the Gomori-negative areas near the median eminence (tubero-infundibular group) may also play a role in the mechanism of CRF production and release. This statement is in harmony with the experimental results obtained by FRANKEL et al. (1967) according to which a lesion to the mediolateral area of the tuberal nucleus reduces the corticosterone level of adrenal venous blood. On the other hand, according to WILSON (1967) the FSH releasing factor would be produced in the medio-basal area of the infundibular nucleus.

The results of our investigations in vitro, analysed in the light of previous histological evidence, make it possible to arrive at the following conclusions. In response to 30 I.U. of insulin the CRF activity of the median eminence almost ceases, and in the anterior tip of the median eminence all the Gomori-positive secretion is evacuated (PÉCZELY 1967). ACTH and prednisolone treatment, as well as water loading inhibit the CRF activity of the median eminence, and the same treatments cause a marked accumulation of the Gomori-positive substance of the external zone. Acumulation of secretion and conglomeration of granules are characteristic of the outer, neurohaemal part of the external zone, while a reduced density of the secretion is characteristic of the reticular zone, indicating a cessation of secretion supply. These morphological changes unequivocally point to a reduction or cessation of secretion transport (PÉCZELY 1966a, b; 1967). The comparison of the effects of insulin, ACTH, prednisolone and hydration indicates close correlation between Gomori-positive secretion and CRF activity of the external zone of the median eminence.

We should deal separately with the adrenal hypertrophy following water deprivation, which is not accompanied by any change in hypothalamic-adrenocorticotropic activity. KAWASHIMA et al. (1964) found a depletion of Gomori-positive secretion in the anterior part of the median eminence of Zonotrichia. They correlated this finding with an activation of the adenohypophysis (as indicated by an increase of acid phosphatase and cateptic proteinase activity) and with adrenal hypertrophy. As no change takes place in the Gomori-positive content of the pigeon's median eminence either, it may be assumed that in this case we have to deal with an adrenal stimulating effect of either the neurohypophysis or the extrahypophysial ACTH.

## LITERATURE

ASSENMACHER, I.: Recherches sur le contrôle hypothalamique de la fonction gonadotrope préhypophysaire chez le canard. Arch. Anat. micr. Morph. exp. **1958,** 47: 447—572.

BAUM, G. J., MEYER, R. K.: Influence of diethylstilbestrol on lipids in intact and hypophysectomized cockerels. Endocrinology **1956,** 58: 338—347.

CORBIN, A., MANGILI, G., MOTTA, M., MARTINI, L.: Effect of hypothalamic and mesencephalic steroid implantations on ACTH feed back mechanism. Endocrinology **1965,** 76: 811—818.

ELTON, E. L., ZARROW, I. G., ZARROW, M. X.: Depletion of adrenal ascorbic acid and cholesterol. A comparative study. Endocrinology **1959,** 65: 152—160.

ESPINER, E. A.: The relation between free cortisol in urine and urinary free 11-hydroxycorticosteroids as measured by fluorescence. J. Endocr. **1965,** 33: 233—239.

ESTEP, H., MULLINAX, F. P., BROWN, R., BLAYLOCK, K., BUTS, E.: Increased ACTH release without increased synthesis. Endocrinology **1967,** 80: 719—724.

FARNER, D. S., FOLLET, B. K.: Light and other environmental factors affecting avian reproduction. VII. Biennal Symposium on Animal Reproduction 1965. J. Animal Sci. **1966,** 25: 90—118.

FARNER, D. S., OKSCHE, A.: Neurosecretion in Birds. Gen. comp. Endocr. **1962,** 2: 113—147.

FRANKEL, A. I., GRABER, J. W., NALBANDOV, A. V.: The effect of hypothalamic lesions on adrenal function in intact and adenohypophysectomized cockerels. Gen. comp. Endocr. **1967,** 8: 387—396.

FRANKEL, A. I., GOOK, B., GRABER, J. W., NALBANDOV, A. V.: The determination of corticosterone in plasma by fluorometric techniques. Endocrinology **1967,** 80: 181—194.

FOLLET, B. K., FARNER, D. S.: Pituitary gonadotropins in the Japanese quail *(Coturnix coturnix japonica)* during photoperiodically induced gonadal growth. Gen. comp. Endocr. **1966,** 7: 125—131.

GLICK, D., REDLICH, D., LEVINE, S.: Fluorometric determination of corticosterone and cortisol in 0.02 to 0.05 ml of plasma or submiligram samples of adrenal tissue. Endocrinology **1964,** 74: 653—655.

HALÁSZ, B., PUPP, L.: Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophyseotrophic area. Endocrinology **1965,** 77: 353—362.

KUMISHIMA, I.: Histological observations on the adrenal gland of the chick after injection of insulin and adrenalin. Arch. Histol. Jap. **1960,** 21: 129—145.

LICHARDUS, B., MITRO, A., CORT, J. H.: Size of cell nuclei in hypothalamus of the rat as a function of salt loading. Amer. J. Physiol. **1965,** 208: 1075—1077.

MILLER, R. A., RIDDLE, O.: The cytology of the adrenal cortex of normal pigeons and in experimentally induced atrophy and hypertrophy. Amer. J. Anat. **1942,** 71: 311—341.

MILLER, R. A.: Hypertrophic adrenals and their response to stress after lesion in the median eminence of totally hypophysectomized pigeon. Acta endocr. (Kbh.) **1961,** 37: 565—576.

MOTTA, M., MANGINI, G., MARTINI, L.: A "short" feed back loop in the control of ACTH secretion. Endocrinology **1965,** 77: 392—395.

NALBANDOV, A. V., CARD, L. E.: Effects of hypophysectomy on growing chicks. J. exp. Zool. **1943,** 94: 384—413.

NAGRA, C. L., BIRNIE, J. G., BAUM, G. J., MEYER, R. K.: The role of the pituitary in regulating steroid secretion by the avian adrenal. Gen. comp. Endocr. **1963,** 3: 274—280.

NEWCOMER, W. S.: The effects of hypophysectomy on some functional aspects of the domestic pigeon. Endocrinology **1959,** 65: 133—141.

PÉCZELY, P.: Effect of thirst and water load on the hypothalamic regulation of the adrenal in the pigeon. (*Columba livia domestica* L.). Acta morph. Acad. Sci. hung. **1966,** 14: 227—244.

PÉCZELY, P.: Effect of ACTH on the hypothalamic neurosecretion of the pigeon (*Columba livia domestica* L.) Acta biol. Acad. Sci. hung. **1966,** 16: 291—310.

PÉCZELY, P., ZBORAY, G.: CRF and ACTH activity in the median eminence of the pigeon. Acta physiol. Acad. Sci. hung. **1967,** 32: 229—239.

PÉCZELY, P.: Az eminentia mediana szerepe a madarak mellékveséjének központi szabályozásában. Állattani Közl. **1967,** 54: 121—128.

RESKO, J. A., NORTON, H. W., NALBANDOV, A. V.: Endocrine control of the adrenal in chickens. Endocrinology **1964,** 75: 192—200.

DE ROOS, R., DE ROOS, C. L.: Effects of mammalian corticotropin and chicken adenohypophyseal extracts on steroid genesis by chicken adrenal tissue in vitro. Gen. comp. Endocr. **1964,** 4: 602—607.

SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B., HALÁSZ, B.: Hypothalamic control of the anterior pituitary. Publishing House of the Hungarian Academy of Sciences, Budapest **1962.**

TIXIER-VIDAL, A.: Histophysiologie de l'adenohypophyse des oiseaux. In: J. BENOIT and C. DA LAGE (Eds): Cytologie de l'adenohypophyse. Coll. int. Centre nat. Rech. Sci. (Paris) **1963,** 128: 255—273.

VERNIKOS-DANELLIS, J.: Effect of stress, adrenalectomy, hypophysectomy and hydrocortisone on the corticotrophin releasing activity of the rat median eminence. Endocrinology **1965,** 76: 122—126.

WILSON, F. E.: The tubero-infundibular neuron system: a component of the photoperiodic control mechanism of the white-crowned sparrow, Zonotrichia leucophrys gambelli. Z. Zellforsch. **1967,** 82: 1—24.

ZENKER, N., BERNSTEIN, D. E.: The estimation of small amounts of corticosterone in rat plasma. J. biol. Chem. **1958,** 231: 695—703.

Péter PÉCZELY
Eötvös Loránd Tudomány Egyetem Általános Állattani és
Összehasonlító Anatómiai Intézete
Budapest VIII., Puskin u. 3.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 59—61 (1969)*

# OXYGEN POISONING AND THYROID FUNCTION

By

T. SZILÁGYI, S. TÓTH, L. MILTÉNYI and G. JÓNA†

INSTITUTE OF PATHOPHYSIOLOGY AND RADIOLOGICAL CLINIC, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received September 14, 1968)

Under hyperbaric oxygenation (4 atm pure oxygen) hyperthyroid rabbits and rats died sooner than the euthyroid controls. In survival, athyroid animals did not differ significantly from euthyroid ones. The causes of the potentiating effect of hyperthyroidism on oxygen poisoning are discussed.

CAMPBELL (1937) as well as GROSSMAN and PENROD (1949a) demonstrated earlier that survival of hyperthyroid rats after hyperbaric oxygenation is significantly shorter than by euthyroid animals and considerably shorter than by thyroidectomized ones. GROSSMAN and PENROD (1949b) also reported that rats cooled to 20°C tolerate hyperbaric oxygenation better than normothermic animals. According to POPOVIC, GERSCHMAN and GILBERT (1964), in the cooled ground squirrel *(Citellus tridecemlineatus)* oxygen poisoning manifests itself later than in control animals. Besides, during hibernation the ground squirrel is resistant to oxygen poisoning in an increased degree.

Thus, there exists a certain correlation between oxygen poisoning and the intensity of metabolism. In the present experiments we examined how rats and rabbits tolerate the influence of hyperbaric oxygenation under hyperthyroid, euthyroid and athyroid conditions.

## Methods

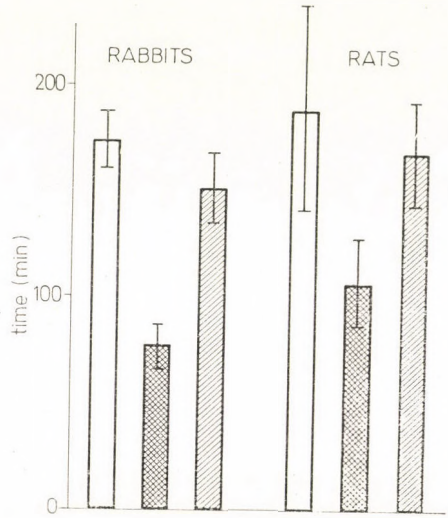
In the experiments female rabbits of identical stock, weighing 2500 ± 200 g were used. We induced the hyperthyroid condition by feeding 200 mg/kg bodyweight dry thyroid (Richter, Budapest) daily for 14—18 days. The main symptoms of hyperthyroidism were hypermotility, tremor, an average loss of 400 g weight, and a rise of colonic temperature by 0.5°C. In the other group of rabbits the thyroid gland was completely removed in cyclobarbital® anaesthesia. 14—18 days after the operation we noticed an average 300 g gain in bodyweight as well as a decrease of colonic temperature by 0.4°C. The rabbits were starved for a day before the experiment.

In the second series of experiments Wistar rats of both sexes were used; dried thyroid gland in a 2 per cent quantity was mixed to the standard food for 14—18 days. In the other group of rats the thyroid gland was extirpated under cyclobarbital® anaesthesia.

The rabbits were placed individually and the rats by threes into a 25 litre hyperbaric chamber previously flushed with pure oxygen. The experiments were carried out in circulating pure oxygen the pressure of which was increased by 0.4 atm/3 min to 4 atm where it was maintained throughout. Carbon dioxide was fixed by NaOH and vapour by silica gel. External temperature was 23 ± 2°C, the inner temperature did not change considerably during the experiment.

## Results and discussion

We observed in both series of experiments the appearance of symptoms of oxygen poisoning and the setting in of death, respectively. The symptoms ensued in the following order: tremor, generalized convulsions, dyspnoea, discharge of excrement and urine, death. As can be seen in Fig. 1, the hyperthy-




Average survival time of rabbits and rats under hyperbaric oxygenation (4 atm pure oxygen), each group comprising 11 animals

= euthyroid animals
= hyperthyroid animals
= athyroid animals

roid animals died sooner than the euthyroid control animals. Survival of thyroidectomized animals did not, however, differ considerably in the case of either species of animals in comparison to the controls. Under the conditions of the experiment, thyroidectomy failed to lengthen survival. It is worthy of note that the symptoms, the convulsions in the first place, appeared much sooner and more distinctly in the hyperthyroid animals than in the controls.

Oxygen poisoning is well known to damage the central nervous system (Bert 1878) and the first symptoms of this damage manifest themselves in the EEG: changes in amplitude and frequency, and convulsion potentials (Szám et al. 1966).

It has been shown (Tóth and Csaba 1966) that in animals with hyperthyroidism on the 14th—18th days of feeding with dry thyroid the brain stem 5-HT level increases considerably, while after thyroidectomy it hardly changes although the rate of metabolism decreases in a large measure. According to

KOENIG (1964), 5-HT is stored in the central nervous system in subcellular particles of lysosome character. Taking these facts into consideration, hyperthyroidism seems to enhance oxygen poisoning by the following mechanism.

In oxygen poisoning the damage to the lysosome membrane and the release of lysosomal enzymes play an important role (HALL and SANDERS 1966). On the effect of hyperbaric oxygenation, 5-HT is released from the lysosomes of hyperthyroid animals *sooner* and in *larger* quantities than in euthyroid state. According to CARLSSON (1964), 5-HT is involved in the control of motoric functions and is causing tremor, convulsions and extension. A similar synergistic effect was observed between hyperbaric oxygenation and endotoxin (SZILÁGYI et al. 1968).

In contrast to some reports (CAMPBELL 1937; GROSSMAN and PENROD 1949a) our experiments failed to reveal a resistance to oxygen poisoning in athyroid animals. This might have been due to the fact that we were recording the time of death setting in in the hyperbaric chamber, whereas in the mentioned experiments the survival of animals exposed to transitory hyperbaric oxygenation was studied.

## LITERATURE

BERT, P.: La pression barometrique-Baillière, Paris **1878.**

CAMPBELL, J. A.: Oxygen poisoning and the thyroid gland. J. Physiol. (Lond.) **1937,** 90: 91—92.

CARLSSON, A.: Functional significance of drug-induced changes in brain monoamino levels. Progr. Brain Res. **1964,** 8: 9—27.

GROSSMAN, M. S., PENROD, K. E.: The thyroid and high oxygen poisoning in rats. Amer. J. Physiol. **1949a,** 156: 182—184.

GROSSMAN, M. S., PENROD, K. E.: Relationship of hypothermia to high oxygen poisoning. Amer. J. Physiol. **1949b,** 156: 177—181.

HALL, I. H., SANDERS, A. P.: Effects of hyperbaric oxygenation on metabolism. III. Succinic dehydrogenase, acid phosphatase, cathepsin and soluble nitrogen. Proc. Soc. exp. Biol. (N. Y.) **1966,** 121: 1203—1206.

KOENIG, H.: Acidic glycolipoprotein granules (lysosomes) as probable binding sites of biogenic amines. Progr. Brain Res. **1964,** 8: 137—141.

POPOVIC, V., GERSCHMAN, R., GILBERT, D. L.: Effects of high oxygen pressure on ground squirrels in hypothermia and hibernation. Amer. J. Physiol. **1964,** 206: 47—50.

SZÁM, I., NIKOLITS, I., GOTTSEGEN, G.: Changes in cerebral electrical activity in experimental hyperoxia. Acta med. Acad. Sci. hung. **1966,** 22: 199—205.

SZILÁGYI, T., TÓTH, S., MILTÉNYI, L., JÓNA, G.: Effect of hyperbaric oxygenation on the local Shwartzman phenomenon. Acta microbiol. Acad. Sci. hung. **1968,** 15: 5—9.

TÓTH, S., CSABA, B.: The effect of thyroid gland on the 5-HT level of brain stem and blood in rabbits. Experientia (Basel) **1966,** 22: 755.

Tibor SZILÁGYI, Sándor TÓTH, László MILTÉNYI, Gábor JÓNA
Orvostudományi Egyetem Kórélettani Intézete és
Radiológiai Klinikája.
Debrecen 12, Hungary

*Acta Physiologica Academia Scientiarum Hungaricae, Tomus 35 (1) pp. 63—74 (1969)*

# EFFECT OF HEAT AND COLD EXPOSURE ON SERUM PROTEINS, GLYCOPROTEINS AND LIPOPROTEINS IN THE RABBIT

By

A. Lózsa Zita. Kereszti and G. Berencsi

INSTITUTE OF PUBLIC HEALTH, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received August 12, 1968)

Two groups of rabbits adapted to an environmental temperature of 18°C were placed in a cold room of 4°C and in a warm room of 30°C, respectively. After two weeks the groups were interchanged and the experiment was continued for two weeks more. The effect of cold and warm environment was studied on the blood serum macromolecules by means of paper electrophoresis. It has been found that the albumin level decreased whereas the alpha and beta globulin fractions increased in response to both cold and heat. The most marked shift in the serum protein pattern was observed on the 3rd day of exposure, after that the pattern gradually returned to normal. Similar changes were observed in the serum glycoprotein fractions. The alterations were not specific and were therefore regarded as a manifestation of the general adaptation syndrome. In this respect, the marked increase in the alpha-2 globulin level seemed to be of considerable importance since it was a reliable measure of the degree of stress.

In contrast, the changes in the lipoproteins proved to be highly specific. In cold, the alpha lipoprotein level showed a great increase, whereas in warm environment the beta lipoprotein showed considerably; none of these changes showed any tendency to become normal during the 2-week period of observation. In warm environment the abrupt increase in amount of beta lipoprotein was associated with splitting of the beta glycoprotein band and, thus, with the appearance of beta-2 glycoprotein, a fraction never observed in animals kept in indifferent or cold temperature. The alterations observed in the gamma globulin fraction were also specific. Its concentration decreased in warm and increased in cold environment.

The pathophysiological aspects of stress due to environmental cold and heat are discussed.

The effect of changes in environmental temperature on the living organisms has been studied from many aspects, but many questions have remained unclarified. Thus, the changes in serum protein fractions in response to environmental heat and cold were studied only by a few investigators; their results obtained with the method of salt precipitation cannot be evaluated in view of the present knowledge.

Using the paper electrophoretic method we have observed that various laboratory animals exhibit considerable seasonal variations in their serum protein components. If this fact is disregarded it may lead to substantial errors in research and to discrepancies in the literature. The practical significance of these observations, however, lies in the fact that man in his everyday life may be exposed to such alterations in environmental temperature which in laboratory animals already cause substantial shifts in the serum protein pattern. To what extent are extreme environmental cold and heat or gross changes in the temper-

ature harmful to man can be deduced mostly from empirical studies and statistical analyses, the pathophysiological events underlying these changes are, however, still obscure. The present experiments carried out on rabbits have furnished several new data to the problem.

## Methods

The experiments were performed on 20 grey rabbits of the same age and weighing 3 to 3.5 kg. The animals were selected from a breeding stock of young rabbits and care was taken that the electrophoretic fractions of their blood sera showed no significant difference; by this means it was possible to minimize individual variations in the course of experiments. Prior to the study, the animals had been kept at 18°C average temperature. The diet consisted of oat, fodder beet, lucerne; food and water were allowed ad libitum. The rabbits were divided in two groups. The first group was placed for 14 days in a warm room, whereas the other group was exposed to cold during this time; the two groups were then changed and the experiments conducted for a further 14-day period. Average temperature in the warm room was 30°C, and in the cold room, 4°C. Relative vapour content in the warm room was kept above 30%. The parameters of air in the rooms were continuously recorded by means of a thermo-baro-hygrograph.

The amount of ingested food was measured daily. Weight of the animals and rectal temperature were also determined every day. Blood was withdrawn from an ear vein prior to the study, then for 4 days after the exposure to cold or heat daily, later every second day, hence, 19 times in the course of the experiment. In order to avoid great loss of blood, the quantity obtained at every puncture was invariably 1 ml, thus, the total amount of blood lost during the study of 28 days was 19 ml. Red and white blood cell counts, serum total protein (Bürgi et al. 1967) and total hexose bound to protein (Holt 1954) were estimated and the serum was subjected to electrophoretic fractionation according to a method described previously (Lózsa 1961a). Staining and evaluation of the protein fractions (Lózsa 1961b) and of the carbohydrate part of glycoproteins (Lózsa et al. 1968) were also carried out by the previous methods. Lipoproteins were determined according to the prestaining method of McDonald and Ribeiro (1959) adapted to the rabbit.

The experiments were performed in February, 1967, and were repeated in February, 1968. Informative studies were undertaken also in summer 1967 and 1968.

## Results

*Food intake.* When kept in indifferent temperature (18°C) the rabbits ingested approximately 300 g of a mixed diet daily. Animals in the first group, when exposed to warm temperature, ingested about 48% of this amount during the first days of exposure, and their intake attained 75% of the initial value on the 14th day of the experiment. The rabbits were then transferred into the cold room, where their food intake increased rapidly and stabilized at a level of 152% of control values. Rabbits in the second group, when placed in the cooled room, ingested 135% of the amount eaten at indifferent temperature; when transferred to the warm place, their food intake decreased to 45% of the initial value, and then increased to 78% until the 14th day of experiment. If the changes in food intake are not compared to the control values but the two extremes found in cold and heat are compared, it can be established that rabbits kept in a cold place ingest about twice to three times as much food as rabbits maintained in warm environment.

*Body weight.* The alterations in food intake had a marked effect on body weight. As shown in the middle part of Fig. 1, animals in the first group lost

about 200 g in the warm room, but then gained about 600 g when transferred into a cold place. Rabbits of the second group also gained an average of 500 g when placed in cold environment. This gain, however, was lost in a few days after the animals had been transferred into the warm place. Further weight loss was small, indicating a certain degree of adaptation as the basal metabolic rate had stabilized at a lower level.

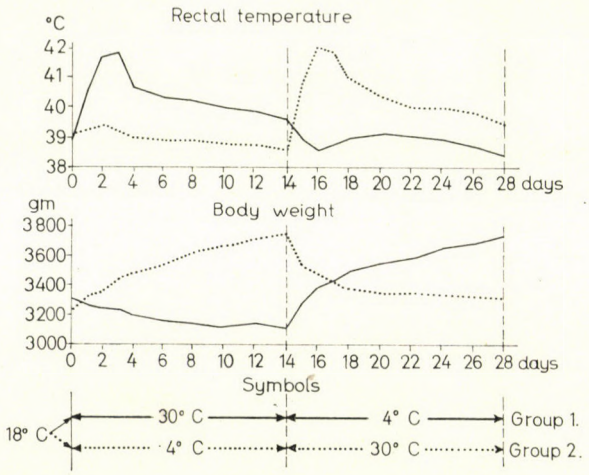



*Fig. 1.* Rectal temperature and body weight in two groups of rabbits exposed to different environmental temperatures, as indicated in the legend for symbols

*Body temperature.* The initial rectal temperature attained 39°C which in cold, as shown in the upper part of Fig. 1, exhibited little variation and diminished by 0.5°C only during the course of the experiment. In warm environment, on the other hand, body temperature increased rapidly, attaining 42°C on the 2nd or 3rd day, but subsequently diminished as a sign of adaptation, never attaining the initial values measured at indifferent temperature. The greatest increase was 3.5°C.

*Red blood cell count* was 5.5 to 5.8 million per $\mu$l at the beginning of the experiment, and 5.0 to 5.2 million at the end of it. Thus, collection of blood samples for analytical purposes did not give rise to anaemia.

*White blood cell count.* The initial value of 8000 to 10 000 per $\mu$l increased to 9000 to 13 000 by the end of the experiment. The increase was too small to be ascribed to some intercurrent infection which might have interfered with the results of electrophoretic investigations.

*Serum proteins.* The changes in the level of serum proteins are shown in Fig. 2. At first sight it was clear that the albumin decreased and the globulins increased in both the warm and the cold environment. The shift was maximal on the 3rd day, and then a successive normalization of the serum protein pattern could be revealed. An exact analysis of the curves, however, disclosed well-

defined differences in quantitative changes, too. The reduction in albumin and the increase in alpha-2 and beta globulins were more marked in the warm than in the cold environment. In contrast, alpha-1 and gamma globulins exhibited a more pronounced increase in response to cold than to heat. The greatest changes measured on the 3rd and 17th day of the experiments, as compared to the

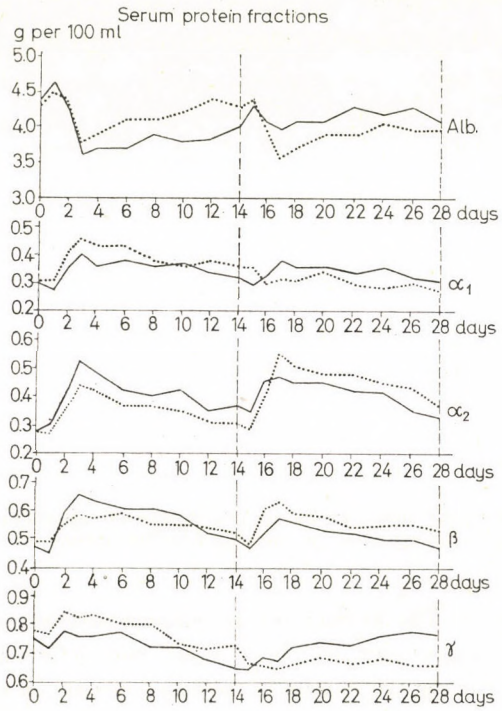



*Fig. 2.* Absolute values for serum protein fractions in rabbits exposed to warm and cold environmental temperature. Symbols, as in Fig. 1

initial values, were significant statistically. In the case of the alpha-2 globulin fraction the difference was highly significant, whereas the gamma globulin exhibited a statistically significant difference only between the initial value and those obtained on the 14th and 28th days of the experiment. At any rate, the gamma globulin level showed a tendency to increase in cold, and decrease in warm environment.

*Total protein.* The changes in total protein concentration being essentially the same as those shown by the albumin, no special curve was drawn. The similarity was not surprising since in the rabbit about 70% of the serum total protein is albumin. Nevertheless, the increase in globulin levels did not compensate for the marked diminution of albumin. As in the case of albumin, total protein levels exhibited a greater reduction in warm than in cold environment.

*Glycoproteins.* The relative amounts of glycoproteins are shown in Fig. 3. The obtained curves are very similar to those for the protein levels. Considering that alpha glycoproteins constitute the main component of the carbohydrate pattern, their concentration can be determined with a much greater accuracy than the amount of alpha proteins which are but a small fraction of the total protein. Therefore, it is important that the changes observed in the glycopro-

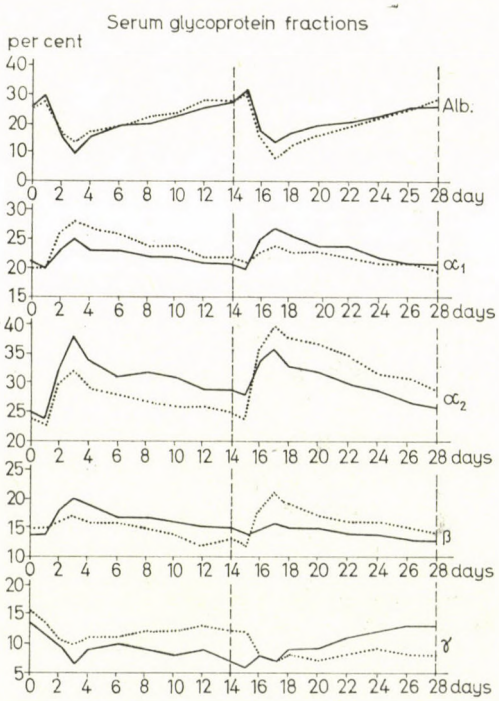



*Fig. 3.* Relative amount of serum glycoproteins in rabbits exposed to warm and cold environmental temperature. Symbols, as in Fig. 1

tein level are in the same direction as those in protein concentration. These data obtained by means of the PAS staining method underline the significance of the differences, and in view of these results it may be emphasized that in warm environment the increased levels of the alpha-2 and beta globulins and in cold environment the elevated alpha-1 and gamma globulin concentrations are not only changes in the same direction but there exist certain qualitative differences between these alterations. That serum proteins and glycoproteins exhibited similar changes is clearly shown by the curves which invariably displayed a break on the first day after the animals had been transferred to another environmental temperature, i.e. on the 1st and 15th days of the experiment.

The beta glycoprotein underwent specific changes which could not be demonstrated in the Figures representing only the quantitative alterations but

which deserve special attention. In cold and indifferent environments there was always but one beta band in the electropherogram. If, however, the rabbits were transferred from indifferent temperature to a warm place, or from cold to warm, the beta glycoprotein band splitted in two distinct bands on the second

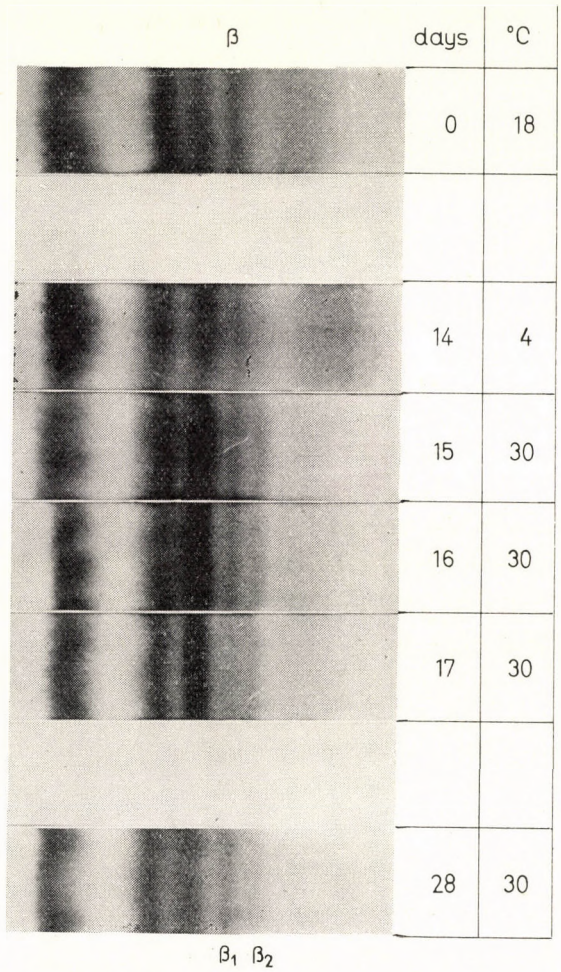



*Fig. 4.* Glycograms obtained from the serum of a rabbit from the second group. After adaptation to cold for 14 days the animal was transferred to a warm place. In addition to quantitative changes in all the fractions there appeared a new band as the beta-2 glycoprotein

and first day, respectively. This is shown by the glycograms in Fig. 4, prepared from the serum of a rabbit in the second group. The beta-2 band was present until the animal had been kept in a warm environment. When transferred to a cold place, the amount of beta-2 glycoprotein was successively reduced and by the 6th day the two separate bands had united again. Thus, the presence of this

fraction is specific to high environmental temperature. In the case of the quantitative determinations, it was evaluated together with beta-1 glycoprotein. The beta-2 band could be revealed by PAS staining but not by acid fuchsin, thus the analysis of protein fractions failed to disclose a beta-2 globulin even in warm environment. To check the validity of the results the experiments were repeated twice in summer when the "indifferent" temperature in the stables attained 28°C. It has been found that the beta-2 glycoprotein was present in the sera of all animals, and no further change was observed after the animals had been transferred into an even warmer place.

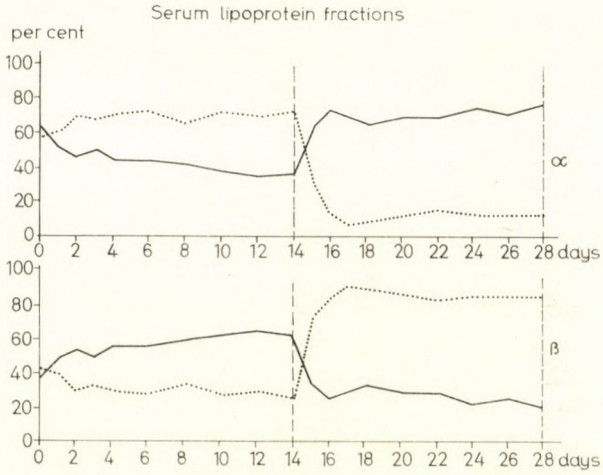



*Fig. 5.* Relative amount of serum lipoproteins in rabbits exposed to warm and cold environmental temperatures. Symbols, as in Fig. 1

*Total hexose.* The values for total hexose showed essentially the same alterations as those exhibited by the total protein and albumin level; the observed changes were therefore not plotted graphically. This finding indicated that in the glycoproteins the binding between the protein and carbohydrate constituent is very strong and the two components are always subjected to the same changes in the cases of dysproteinaemia. The only exception to this rule in our experiment seems to be the appearance of the above mentioned beta-2 glycoprotein fraction in response to warm environmental temperature.

*Lipoproteins.* The changes in the relative concentration of lipoproteins are shown in Fig. 5. There being only two fractions the total amount of which is considered 100%, any decrease in one of the fractions is necessarily associated with an increase in the other; thus, the two curves are mirror images of each other. The ratio of alpha to beta lipoprotein, which depends on a number of factors and particularly on age, attained 60 : 40 in the young animals used in the present study in the initial phase of the experiments. The ratio was depressed to 36 : 64 in the warm environment and increased to 73 : 27 in response to exposure to

cold. Both changes were significant statistically. These shifts in the ratios persisted throughout the exposure and no tendency for normalization could be detected as a possible sign of acclimatization.

On the 14th day of the experiment, exposing the rabbits to a temperature difference as great as 26°C, extreme changes in opposite direction occurred in both groups. In the group transferred from the cold into the warm room the ratio of alpha to beta lipoprotein was reduced from 73 : 27 to 8 : 92 as early as on the 3rd day, whereas in the other group the ratio increased from 36 : 64 to 75 : 25. In both groups, these changes developed promptly. There was no break on the curves on the first day after the change in environmental temperature as in the case of proteins and glycoproteins, indicating that the lipoid constituent is loosely bound to the protein molecule and its regulation is independent of the other two components. The shift in the relative amount of lipoproteins observed in the second period of the experiments persisted throughout, without any tendency of the lipoprotein pattern to normalize.

These severe changes are demonstrated in the lipograms represented in Fig. 6, prepared from the serum of a rabbit in the second group.

It is clearly seen that from the normal pattern obtained at indifferent environmental temperature, the beta lipoprotein fraction almost disappeared in response to environmental cold, and on the 14th day a marked alpha band predominated. After the animal had been transferred to 30°C, the alpha lipoprotein displayed a considerable decrease and at the same time a marked beta lipoprotein band appeared. This abrupt increase in the amount of beta lipoprotein coincided with the above discussed splitting of the beta glycoprotein band indicating a remarkable change in the composition or structure of the molecule.

In the lipograms only the alpha and beta lipoproteins were estimated quantitatively, and the chylomicron zone which is composed predominantly of triglycerides was not evaluated. Nevertheless, it is remarkable that this band running after the lipoproteins disappeared almost totally in response to cold, and, as shown in Fig. 6, a considerable increase was observed when the animals were transferred into a warm place. Subsequently, however, this fraction diminished, indicating some kind of adaptation, which was never observed with the lipoproteins.

## Discussion

The curves representing the changes of serum proteins and glycoproteins show a characteristic break on the first day of exposure to another environmental temperature. This is then followed by a drop in the amount of albumin and an increase in globulins, and finally the pattern becomes normal. It is easy to recognize on these curves the different phases of the alarm reaction and the

general adaptation syndrome, i.e. shock, counter shock and resistance, as described by SELYE (1950). At first it appears that the changes in serum proteins and glycoproteins are in the same direction both after exposure to cold

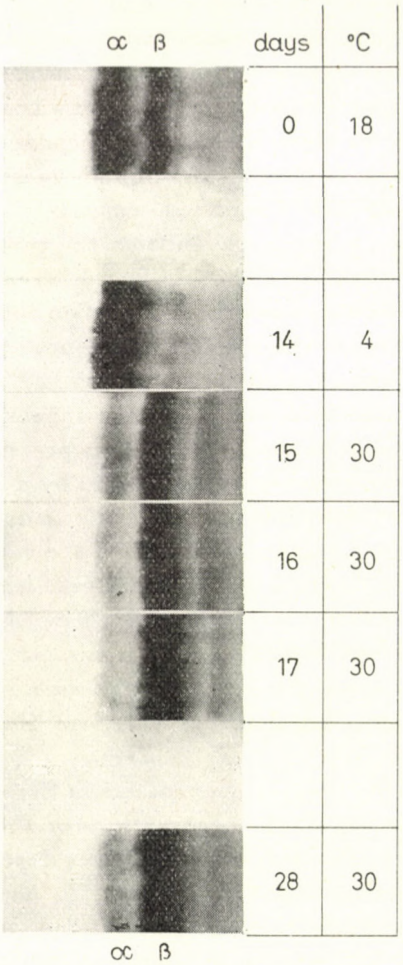



*Fig. 6.* Lipograms prepared from the serum of a rabbit of the second group. After acclimatization to cold for 14 days the animal was transferred to a warm place. The ratio of alpha to beta lipoproteins showed a rapid and marked inversion

and warm, i.e. they are not specific and can be regarded as a manifestation of the general adaptation syndrome. The most characteristic change is the statistically highly significant increase in the level of alpha-2 globulin and alpha-2 glycoprotein, which reflects also the degree of stress, the changes being more marked in response to exposure to 30°C than to 4°C.

The electrophoretic studies, however, disclosed some alteration in the serum which seemed to be specific and could not be regarded as a manifestation

of general adaptation. The most pronounced, rapid and sustained alterations in response to the change in environmental temperature were observed with the lipoproteins. Apparently, specific changes are superimposed also on the non-specific alterations shown by the proteins and glycoproteins. In cold environment, both the protein and the carbohydrate component of alpha-1 globulin increased more markedly than at warm temperature. On the other hand, the beta globulin and glycoprotein level rose more in warm than in cold. It is reasonable to assume that for these alterations the changes in the alpha and beta lipoproteins were responsible. Still, the lipoid constituent which is loosely bound to the protein molecule responded sensitively to the alteration in environmental temperature, whereas the shift in the proteins and glycoproteins appeared with a delay of about 24 hours. The different response of two components of the same molecule allows the assumption that the rapid and specific change in the lipoprotein pattern was due to lipoproteins with abnormal composition or structure. This is supported by the finding that in response to heat an unknown beta-2 glycoprotein was found, the appearance of which coincided with the increase in the concentration of beta lipoproteins. This beta-2 fraction arose from the so far homogeneous beta globulin by a decrease of its mobility indicating a drop in the charge of the molecule and, hence, a change in its structure. In all the lipoproteins so far studied a certain amount of carbohydrate could be detected (PUTNAM 1960), therefore it was not surprising that in this phase of the experiments there appeared a new carbohydrate band in the glycoprotein pattern. It was, however, remarkable that the protein component of the new lipoprotein was not detectable by the usual staining techniques used in electrophoretic studies. This again indicated an abnormal structure of the molecule.

The regulation of the level of lipoproteins in the serum has not been sufficiently clarified. According to the observations of MITCHELL and LONGWELL (1964), the consequences of a cold stressor impact depend greatly on the fact, whether the animals were well-fed or starved. In the present experiments, the amount of food ingested by the rabbits and the body weight of the animals exhibited great differences according to the environmental temperature. In the periods of considerable weight gain the ratio alpha to beta lipoproteins was high, whereas in the catabolic phases characterized by weight loss the ratio was small. On the other hand, it is known that the basal metabolic rate is augmented in cold and diminished in warm environment; this may be also important in the regulation of lipoproteins. SFIKAKIS et al. (1965) have shown that in man the ratio of alpha to beta lipoproteins is increased in hyperthyroidism and diminished in hypothyroid conditions, although the changes in body weight were opposite to those found in the present experiments made on rabbits. Thus, it seems likely that, in cold, it is not the gain in weight but the considerable increase of lipoid metabolism which is the most important factor.

The rabbits, although increasing the amount of depot adipose tissue, utilize more fat than normally because it is the most suitable means by which they are able to increase heat production. This is clearly demonstrated also by the changes revealed in the lipograms, i.e. by the disappearance of the chylomicron zone in cold. The high alpha to beta lipoprotein ratio clearly indicated these events. In warm environment, the opposite was taking place in that fat utilization decreased, triglycerides were present in the blood in increased amounts, and the ratio alpha to beta lipoproteins diminished. The enzyme systems governing the metabolism of fat are affected not only by thyroxin but also by the catecholamines (RADOMSKI 1966), thus, it is a complex process which is still obscure in many aspects.

The changes in lipoproteins had entirely different characteristics than the alterations in proteins and carbohydrates, as shown by the fact that the level of the lipoproteins, although being considerably altered by the exposure to cold and warm, failed to show any tendency towards normalization. A new equilibrium of alpha and beta lipoproteins had been attained in both warm and cold environment, and this was maintained throughout the time of exposure. This particular condition may play a role in the pathomechanism of atherosclerosis. The observation of BARR et al. (1955) that beta lipoproteins are substantially elevated in arteriosclerosis has repeatedly been confirmed. This condition is characterized by the deposition in the wall of blood vessels of mucopolysaccharides, calcium salts, and also of lipoproteins of a high molecular weight, i.e. beta lipoproteins. Thus, high environmental temperature, by causing a sustained elevation of serum beta lipoprotein level, may be a predisposing factor in the genesis of atherosclerotic vascular changes and is perhaps responsible for the high incidence of vascular diseases among subjects who are working under high temperatures.

The changes observed in the gamma globulin level failed to correspond with the usual course and events of the general adaptation syndrome. Alterations of this fraction were also specific as the amount of gamma globulins decreased in warm, and increased in cold environment. Similar changes were recorded recently in man (BLATT and KERKAY 1967). Considering that the basal metabolic rate is depressed in warm environment and elevated in a cold one, furthermore, that the gamma globulin level is low in hypothyroidism and high in hyperthyroidism (LAMBERG and GRÄSBECK 1955), the possibility cannot be excluded that, similarly as in the case of lipoproteins, the thyroid is in some way involved in the regulation of the gamma globulin levels. DONHOFFER (1957) also emphasized the importance of the thyroid in the acclimatization to changes in environmental temperature. The epidemiological significance of depressed gamma globulin level and diminished antibody production in response to warm environmental temperature will be discussed in a subsequent paper.

## LITERATURE

BARR, D. P., RUSS, E. M., EDER, H. A., KENDELL, F. E., ABEL, L. L.: Protein-lipid relationships in experimental canine atherosclerosis. Circulat. Res. **1955,** 3: 199—202.

BLATT, W. F., KERKAY, J.: The effect of repeated heat and cold exposure on serum protein composition in man. Canad. J. Physiol. Pharmacol. **1967,** 45: 571—575.

BÜRGI, W., RICHTERICH, R., BRINER, M.: UV-photometric determination of total cerebrospinal fluid proteins with a modified biuret reagent. Clin. chim. Acta **1967,** 15: 181—184.

DONHOFFER, SZ.: Kórélettan. Medicina, Budapest **1957.**

HOLT, C.: Eine Methode zur Bestimmung des neutralen Hexoseanteiles der Proteine des Blutserums. Klin. Wschr. **1954,** 32: 661—662.

LAMBERG, B. A., GRÄSBECK, R.: The serum protein pattern in disorders of thyroid function. Acta endocr. (Kbh.) **1955,** 19: 91—100.

LÓZSA, A.: Papirelektroforézis készülék és metodika nagyszámu szérumminta fehérjefrakcióinak szeparálására és festésére. Kisérl. Orvostud. **1961a,** 13: 86—97.

LÓZSA, A.: A savanyu fuchsin tulajdonságai és kötődése a szérumfehérjékhez papirelektroforézisnél. Kisérl. Orvostud. **1961b,** 13: 98—111.

LÓZSA, A., KERESZTI, Z., BERENCSI, G.: Methodologische Beiträge zur elektrophoretischen Bestimmung der Glykoproteide des Blutes. Ärztl. Lab. **1968,** 14: 292—297.

MCDONALD, H. J., RIBEIRO, L. P.: Ethylene and propylene glycol in the pre-staining of lipoproteins for electrophoresis. Clin. chim. Acta **1959,** 4: 458—459.

MITCHELL, CH. E., LONGWELL, B. B.: Related effects of acute exposure to cold and fasting on free fatty acid release by adipose tissue. Proc. Soc. exp. Biol. (N. Y.) **1964,** 117: 590—593.

PUTNAM, F. W.: The plasma proteins. Academic Press, New York **1960.**

RADOMSKI, M. W.: Effect of cold exposure on serum lipids and lipoproteins in the rat. Canad. J. Physiol. Pharmacol. **1966,** 44: 711—719.

SELYE, H.: Stress. Acta Inc., Montreal **1950.**

SFIKAKIS, P., FRIGELI, TH., DALAKOS, TH., KOUTRAS, D. A.: Serum proteins, glycoproteins and lipoproteins in thyroid disease and their relation to thyroid autoantibodies. Z. Immunitätsforsch. **1965,** 129: 462—468.

Albert LÓZSA, Zita KERESZTI, György BERENCSI
Orvostudományi Egyetem Közegészségtani Intézete,
Szeged, Dóm tér 10.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 75—80 (1969)*

# COMPARISON OF LIVER GLYCOGEN ASSAY METHODS

By

LI BOK NAM, I. SZELÉNYI and T. ZELLES*

INSTITUTE OF PATHOPHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received September 12, 1968)

The glycogen content of alkaline and acid tissue extracts was determined by various methods. The two techniques of extraction yielded identical amounts of glycogen. The higher values for glycogen obtained previously with the iodine reagent have been ascribed to the use of commercial glycogen standards for the determination of tissue concentrations. Commercial preparations have been shown to give lower values with the iodine reagent than glycogen obtained from tissue. It is suggested that the preparation of glycogen standards from the same tissue is necessary when using the iodine reagent which otherwise is a suitable method for the estimation of tissue glycogen content.

There are numerous methods available for the estimation of glycogen (GOOD et al. 1933; BOETTINGER 1946; WAGTENDONK et al. 1946; SEIFTER et al. 1950; VAN DER KLEIJ 1951; KEMP and KITS VAN HEIJNINGEN 1954; VAN DER VIES 1954, GYERMEK and FEKETE 1955; MONTGOMERY 1957; KRISMAN 1962), and this often makes it difficult to compare the results.

The various methods differ in two points. First, in the mode by which glycogen is separated from other tissue constituents, i.e. by precipitation with alcohol or some other substance, or by some other method. Second, in that glycogen is estimated directly by some chemical method or after it has been converted into sugar. The most suitable procedures seem to be those of VAN DER VIES (1954) and GYERMEK and FEKETE (1955); both techniques are simple and consist in a direct estimation of glycogen.

The purpose of the present study was to compare under identical experimental conditions some typical methods of glycogen determination and to obtain information about the factor(s) responsible for the differences in the results yielded by these techniques.

## Methods

The experiments were undertaken on inbred albino rats weighing 100 to 120 g. The animals were fed a normal standardized semisynthetic diet (LATI, Budapest). After decapitation, the liver was removed instantaneously and two strips were cut out from its substance, according to the technique of NIJS et al. (1949). The two samples were weighed and subjected rapidly to analysis in duplicata.

*(1) Hydrolysis with 30% KOH.* One specimen was weighed and then kept for 20 min in

* With the technical assistance of Mrs. F. Gál.

boiling 30% KOH, 3 ml per g of tissue, then immediately precipitated in a twofold volume of concentrated alcohol. The tubes were allowed to stand for 24 hours, then centrifuged for 7 min at 3000 r.p.m. The precipitate was dissolved in a small amount of water and again washed with alcohol (2 : 1 v/v). For glycogen determination 4 different methods were applied; they will be described in detail below and in Table I.

*(2) Extraction with 10% trichloracetic acid.* The other tissue sample was homogenized with 10% TCA to give a fine suspension, then centrifuged for 7 min at 3000 r.p.m. per min. The precipitate was discarded as preliminary tests had shown that it contained only traces of glycogen. The supernatant was divided in two parts. In the first portion, glycogen was estimated directly, whereas the other one was treated with alcohol as described above.

The glycogen extracted by one of the above methods was estimated directly by the method of WAGTENDONK et al. (1946), that of VAN DER VIES (1954), or by the iodine reagent, or by the nephelometric technique of GYERMEK and FEKETE (1955).

When employing the indirect method, glycogen was converted into hexose by hydrolysis with N $H_2SO_4$ for 2 hours. The amount of monosaccharides thus obtained was established by the o-toluidine method of HULTMAN (1957) and the anthrone method of MORRIS (1948), as given in Table I.

**Table I**

*Schematic representation of rat liver glycogen extraction and assay methods*
(Figures in brackets refer to the values in Table II)

| Extraction method | Hydrolysis with 30% KOH | | | | Homogenization with 10% TCA | | | |
|---|---|---|---|---|---|---|---|---|
| Precipitation | Alcohol | | | | Alcohol | | | |
| Washing | Alcohol | | | | Alcohol | | | |
| Conversion into monosaccharides | Hydrolysis (2 hours) | | | | Hydrolysis (2 hours) | | | |
| Product of conversion | Glucose | Glucose | Glycogen | | Glucose | Glucose | Glycogen | |
| Reagent | o-Toluidine | Anthrone | Iodine | Alcohol | o-Toluidine | Anthrone | Iodine | Alcohol |
| Estimation | Photometry (1) | Photometry (2) | Photometry (3) | Nephelometry (4) | Photometry (5) | Photometry (6) | Photometry (7) | Nephelometry (8) |

*Preparation of glycogen.* The liver of several rats was removed rapidly and halved. The two portions were extracted by the above methods and then, after washing in alcohol, both samples were dried to constant weight in an exsiccator.

Protein impurities were usually negligible, in the case of TCA precipitation invariably below 1%. Nevertheless, the protein content was always determined by a micro-Kjeldahl technique and, if necessary, a correction was made for the impurities. The glycogen samples thus prepared were compared with Merck's commercial preparation.

## Results

Glycogen extracted from the liver of 15 rats was estimated. As shown in Table II, of the methods of direct assay, estimation with the iodine reagent yielded values almost twice as high as those obtained by the nephelometric technique based on a similar principle.

**Table II**

*Concentrations of glycogen in the liver tissue of 15 rats as revealed by two different methods of extraction and four different methods of estimation (Mean ± S.D.).*
(Figures in brackets refer to the schematic representation of the techniques in Table 1. All values are given in g per 100 g wet weight)

| o-Toluidine | | Nephelometry | | Anthrone | | Iodine reagent | |
|---|---|---|---|---|---|---|---|
| Extraction | | Extraction | | Extraction | | Extraction | |
| with KOH | with TCA | with KOH | with TCA | with KOH | with TCA | with KOH | with TCA |
| (1) | (5) | (4) | (8) | (2) | (6) | (3) | (7) |
| 4.61 | 5.18 | 6.82 | 7.17 | 6.92 | 7.33 | 13.00 | 12.44 |
| ±0.40 | ±1.21 | ±1.82 | ±1.53 | ±1.33 | ±1.43 | ±2.90 | ±3.27 |

Determination with the anthrone reagent which measures the amount of hydrolyzed glycogen yielded essentially the same results as those obtained by nephelometry. The lowest values were found with the o-toluidine method which

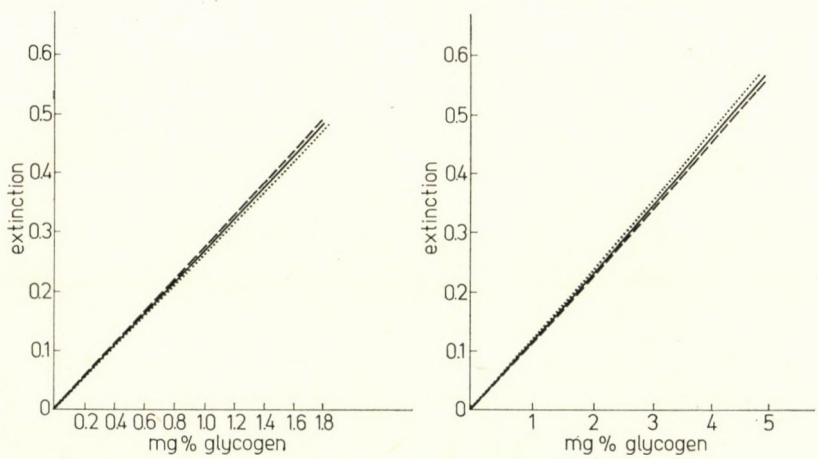



*Fig. 1.* Determination of hydrolysed glycogen on the basis of monosaccharide content. Left results obtained with anthrone, right, with o-toluidine reagent. Solid line represents the extinction of the commercial glycogen preparation; broken line and dotted line show extinctions of glycogen obtained by acid and alkaline extraction, respectively

is also based on the determination of the hexose content. When glycogen was estimated in two different extracts but by the same method, the obtained values were in good agreement; thus, the two modes of extraction yielded the same amount of glycogen. Statistical analysis failed to reveal any significant difference between the results obtained by the two types of extraction.

Figures 1 and 2 show the values obtained with Merck's commercial glycogen preparation and the standards extracted by us; both preparations were assayed with anthrone and o-toluidine after having been converted into mono-

saccharides, and directly by means of the iodine reagent and the nephelometric technique. Figure 1 demonstrates that the extinctions of the different glycogen preparations, when computed for equivalent weight, were essentially the same, irrespective of the employed method of extraction. Figure 2 shows that glycogen extracted with alkali had a somewhat lower extinction value, as compared to the preparation extracted with acid and to the commercial preparation; the difference was, however, not significant statistically.

With the iodine reagent the two extracts had identical, whereas the commercial preparation lower extinction values (Fig. 2).

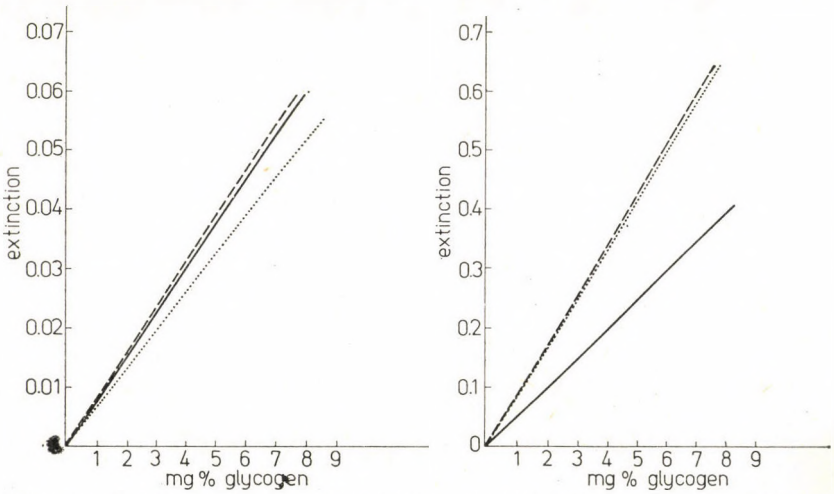



*Fig. 2.* Direct estimation of glycogen by nephelometry and iodine reagent. Symbols, as in Fig. 1

## Discussion

As some authors (Bloom et al. 1951; Carrol et al. 1956) found more glycogen in alkaline than in acid extracts, it seemed interesting to compare these by means of different methods of assay.

The two extraction procedures yielded essentially identical values; the slight differences obtained might be attributed to the circumstance that the molecular weight of glycogen is reduced more rapidly when the material is treated with alkali at a high temperature than if TCA is used for extraction. The reduction is probably due to hydrolysis and according to Bailey and Whelan (1961) the colour intensity of the iodine starch complex, a polysaccharide with a structure similar to that of glycogen, increases when in the molecule the number of monosaccharide units increases from 18 up to 72. Thus, the amount of glucose yielded by alkaline extraction depends on the duration of the procedure. It appears therefore that acid extraction is a more suitable method for the isolation of glycogen from tissue samples. Bryce et al. (1958),

STETTEN et al. (1958, 1961) and WHISTLER and BEMILLER (1962) have come to a similar conclusion.

Determination of the glycogen thus extracted can be undertaken most suitably by the iodine reagent. This method will, however, yield reliable values only if commercial reference standards are replaced by standard preparations extracted from the same tissue in which the glycogen level is estimated, i.e. in the present experiments from rat liver. With the other methods of glycogen assay (o-toluidine, anthrone reagent, nephelometry) we obtained a much smaller difference between the commercial preparation and the standards elaborated by us. Considering that the values represented in Table II have been obtained with the commercial reference standard, and this preparation yields values approximately 40% lower than the glycogen extracted from rat liver when using the iodine reagent, the readings obtained with the iodine method are in fact close to those revealed by the anthrone reagent or the nephelometric method.

The present results have led to the conclusion that acid extraction followed by the iodine method offers many advantages, particularly owing to its reliability and the simple and rapid technique. The procedure, however, needs to be improved, especially for use in studies devoted to changes in tissue glycogen content.

## LITERATURE

BAILEY, J. M., WHELAN, W. J.: Physical properties of starch. I. Relationship between iodine stain and chain length. J. biol. Chem. **1961,** 236: 969—973.

BLOOM, W. L., LEWIS, G. T., SCHUMPERT, M. Z., SHEN, T. M.: Glycogen fractions of liver and muscle. J. biol. Chem. **1951,** 188: 631—636.

BOETTINGER, E. CS.: Diphenylamine as colorimetric agent in determination of glycogen. J. Cell. Comp. Physiol. **1946,** 27: 1—8.

BRYCE, W. A. J., GREENWOOD, C. T., JONES, I. G.: Physicochemical studies on starches. XVI. The molecular weight and apparent molecular-weight distribution of rabbit-liver. J. Amer. chem. Soc. **1958,** 3845—3851.

CARROL, N. V., LONGLEY, R. W., ROE, J. H.: Determination of glycogen in liver and muscle by use of anthrone reagent. J. biol. Chem. **1956,** 220: 583—593.

HULTMAN, E.: A new method for determining aldosaccharides. Scand. J. clin. Invest. **1957,** 9: 315—316.

GOOD, C. A., KRAMER, H., SOMOGYI, M.: Determination of glycogen. J. Biol. Chem. **1933,** 100: 485—491.

GYERMEK, L., FEKETE, GY.: Studies with a simple method of liver glycogen estimation. Acta physiol. Acad. Sci. hung. **1955,** 8: 259—267.

KEMP, A., KITS VAN HEIJNINGEN, A. J. M.: A colorimetric micro-method for the determination of glycogen in tissue. Biochem. J. **1954,** 56: 646—648.

KRISMAN, R. C.: A method for the colorimetric estimation of glycogen with iodine. Ann. Biochem. **1962,** 4: 17—23.

MONTGOMERY, R.: Determination of glycogen. Arch. Biochem. **1957,** 67: 378—386.

MORRIS, D. L.: Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science **1948,** 107: 254—255.

NIJS, A., AUBERT, X., DUVE, C.: The distribution of glycogen in the liver of rabbits. Biochem. J **1949,** 45: 245—246.

SEIFTER, S., DAYTON, S., NOVIC, B., MUNTWYLER, E.: Estimation of glycogen with anthrone reagent. Arch. Biochem. **1950,** 25: 191—200.

STETTEN, M. R., KATZEN, H. M., STETTEN, W.: A comparison of the glycogens isolated by acid and alkaline procedures. J. biol. Chem. **1958,** 232: 475—478.

STETTEN, M. R., KATZEN, H. M.: Degradation of glycogen by alkali. J. Chem. Soc. **1961,** 83: 2912—2918.
VAN DER KLEIJ, B. J.: A rapid determination of glycogen in tissues. Biochim. Biophys. Acta (Amst.) **1951,** 7: 481—482.
VAN DER VIES, I.: Two methods for the determination of glycogen in liver. Biochem. J. **1954,** 57: 410—416.
WAGTENDONK, W. J., VAN SIMONSEN, D. H., HACKETT, P. L.: A rapid microdetermination of glycogen in tissue slices. J. biol. Chem. **1946,** 163: 301—306.
WHISTLER, R. L., BEMILLER, J. N.: Extraction of glycogen with dimethyl-sulfoxide. Arch. Biochem. **1962,** 98: 120—123.

Li BOK NAM, István SZELÉNYI, Tivadar ZELLES
Orvostudományi Egyetem Kórélettani Intézete és
Dietetikai Tudományos Munkacsoportja
Budapest, IX. Hőgyes Endre u. 9.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 81—86 (1969)*

# GLUTAMINASE ACTIVITY OF THE KIDNEY IN EXPERIMENTAL RENAL FAILURE OF RATS

By

L. MOLNÁR, Erika BENEDEK and I. TARABA

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received June 8, 1968)

Two hours ligation of the renal artery in rats induces a postischaemic disturbance similar to human acute renal failure. On the first postoperative day azotaemia and acidosis developed. Diminution of alkaline phosphatase activity indicated a decreased activity of the tubular cells, but glutaminase activity was unchanged.

It is assumed that glutaminase activity has no role in the development of postischaemic acidosis. It is supposed that glutaminase is not sensitive to anoxia or perhaps acidosis enhances glutaminase resynthesis and this was compensating the ischaemic disturbance.

Temporary occlusion of the renal artery in animals induces a postischaemic condition similar to human acute renal failure (SELKURT 1945; BÁLINT et al. 1960; BÁLINT et al. 1961); after occlusion of the renal artery for two hours oliguria, anuria, azotaemia develop and the animals die with symptoms of uraemia. The mechanism of this renal failure has been discussed earlier (TARABA, et al. 1966; BENEDEK et al. 1966).

The kidney is well known to play an important role in the maintenance of the acid–base balance, and that acids are disposed of without a loss of alkali reserve. This is done either by the excretion of weak acids, or by the substitution of ammonium ion for sodium ion in the excretion of their salts. Ammonia is produced mostly in the cells of the renal proximal tubules (WEISS and LONGLEY 1960). The source of urinary ammonia are the amino acids of blood, and the major fraction, about 60% urinary ammonia of the whole originates from glutamine (VAN SLYKE et al. 1943). Ammonia formation from glutamine is catalysed in 95% by the enzyme glutaminase I (PITTS et al. 1963). Ammonia is readily penetrating the cell membrane and in the tubular lumen it reacts with hydrogen ions to yield ammonium ion. The quantity of ammonium and the activity of glutaminase are considerably increased in acidosis (DAVIES and YUDKIN 1952; RECTOR et al. 1955; HODLER 1964).

The present experiments were designed to examine the correlation of acidosis and glutaminase I activity in postischaemic renal failure.

## Methods

Adult white rats of both sexes with body weight from 160 to 250 g were used. In superficial ether anaesthesia the left renal artery was ligated for two hours. (For details, see BENEDEK et al. 1966.)

The animals were divided into two groups.

1. In this group of 25 animals the right kidney was removed simultaneously with the arterial ligation. One, two or three days later blood was taken with heart puncture, the animals were killed by decapitation and the left kidney was removed and homogenized immediately.

2. Animals in the second group with intact right kidney were killed on the second or third day after arterial ligation and the left kidney was homogenized immediately.

Heart puncture was done by an air-tight glass syringe. NPN was determined according to CLEGHORN and JENDRASSIK (1934). The blood pH was determined by a RADELKIS OP 203 pH meter. The removed kidney was weighed, potterized in a dish of ice and suspended 1 in 10 of a mixture of 85.5 g saccharose, 200 ml of 0.05 M Tris buffer and 0.372 g of trisodium acetate in 1000 ml distilled water and centrifuged at room temperature.

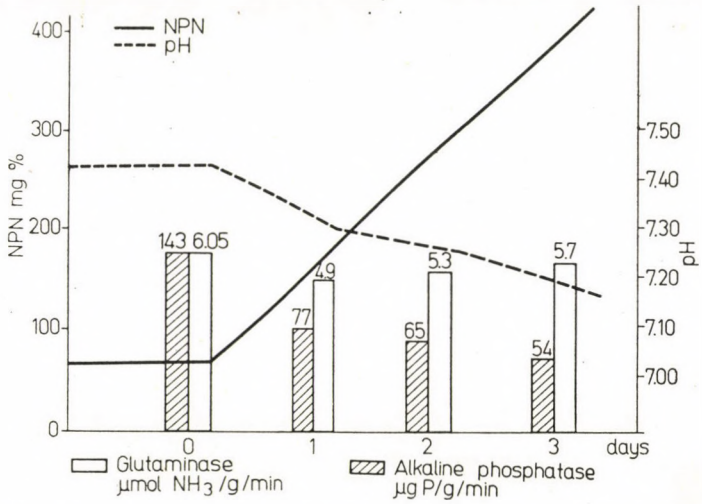



*Fig. 1.* Alkaline phosphatase and glutaminase activity, NPN, and pH in unilaterally nephrectomized rats in the postischaemic period

Alkaline phosphatase activity was estimated by a modification of BODANSKY's method (BÁLINT 1962). Glycerophosphatase was used as a substrate. The samples were incubated for 60 minutes at 37°C, the resulting phosphorus formed molybdenum blue with ammonium molybdate and the result was read at 710 m$\mu$ in a spectrophotometer. Activity was expressed in $\mu$g phosphorus broken down by 1 g kidney tissue in 1 minute.

The tissue homogenate-buffer-substrate solution used for glutaminase I estimation consisted of 0.01 ml kidney tissue homogenate, 0.5 ml phosphate buffer pH 7.3, 0.05 ml 0.1 M glutamine substrate, and 0.5 ml of distilled water (HODLER 1964).

Samples were incubated for 30 minutes at 37°C, and ammonia was determined by KISFALUDY's micromethod (1960). Glutaminase activity was expressed in $\mu$M ammonia/g kidney tissue/minute.

Statistical evaluation was done by Student's *t* test (FISCHER 1946).

## Results

In the first group on the first and second days 10 animals each were sacrificed; by the third day only 5 rats survived. Fig. 1 represents the results.

The first signs of renal failure appeared after a day of ischaemia, and on the next day renal failure was complete. NPN increased to 180 mg per 100 ml blood on the first day, to 294 mg per 100 ml on the second and to 409 mg per

100 ml on the third postoperative day ($p < 0.01$). In the meantime, severe acidosis developed; blood pH was 7.31 on the first, 7.18 on the third postoperative day. The difference was significant statistically ($p < 0.01$).

Alkaline phosphatase activity decreased from 143 $\mu$g P/g/min to 77 $\mu$g P/g/min in one day. On the second and third days there was a further decrease but the differences between the second and first days, as well as between the second and third days were not significant statistically.

Glutaminase activity did not vary during the observation period except for a slight diminution on the first day, not reaching statistical significance. On the second and third days activity was near to normal (6.05 : 5.3 : 5.7 $\mu$Mol $NH_3$/g/min, respectively).

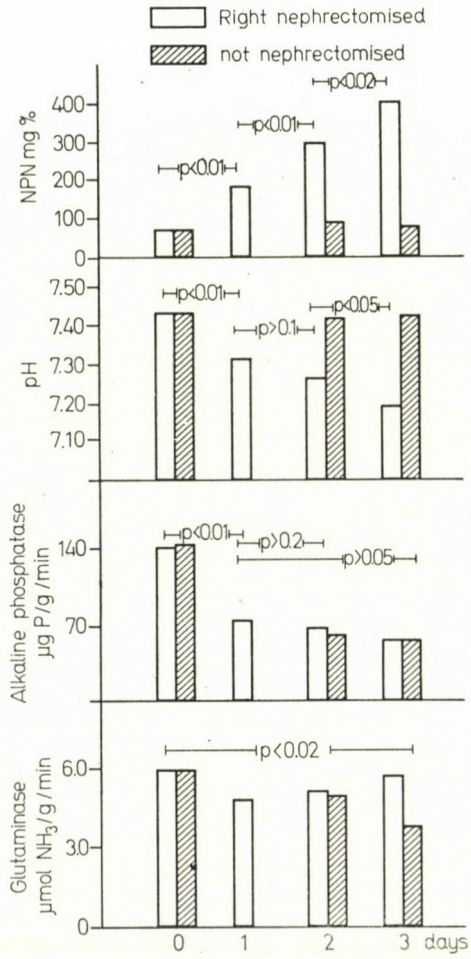



*Fig. 2.* Alkaline phosphatase and glutaminase activity, NPN, and pH, in rats with and without nephrectomy, in the postischaemic period

**Table I**

| | Control | Right nephrectomized | | | Not nephrectomized | |
|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 2nd day | 3rd day |
| n | 19 | 10 | 10 | 5 | 10 | 6 |
| NPN, mg per 100 ml | 65±4 | 180±12 | 294±20 | 409±40 | 79±5 | 69±4 |
| pH | 7.43±0.02 | 7.31±0.03 | 7.26±0.02 | 7.18±0.02 | 7.42±0.01 | 7.42±0.02 |
| Alkaline phosphatase, μg/P/g/min | 143±9 | 77±8 | 65±4 | 54±10 | 58±4 | 54±2 |
| Glutaminase μmol $NH_3$/g/min | 6.05±0.42 | 4.94±0.80 | 5.35±0.67 | 5.70±1.10 | 5.10±0.26 | 3.80±0.49 |

In the second goup 10 rats were sacrificed on the second day and 6 rats on the third day. The had nor uraemia, the blood NPN level was normal and there was no acidosis.

In the left kidney alkaline phosphatase activity decreased as in the previous group, but glutaminase activity diminished as well. The difference between the control and the first day level was not significant, while there was a significant difference between the control and the third day level ($p < 0.02$).

Results are shown in Fig. 2.

## Discussion

Ligation of the renal artery for 2 hours in unilaterally nephrectomized animals induces a condition similar to human acute renal failure, with an increase of the NPN value, severe acidosis and death on the second or third postischaemic day.

In postischaemic renal failure, acidosis is due to a decreased glomerular filtration and/or to impaired tubular activity.

In the present experiments both glomerular and tubular factors probably played a role in the development of acidosis. It has been shown that glomerular filtration is diminished in postischaemic renal failure (BÁLINT et al. 1964), and, since alkaline phosphatase activity is characteristic of the tubular function (GOEBEL and PUCHTLER 1954—55; ZIMMERMANN 1957; BENEDEK et al. 1966), the observed low phosphatase activity was suggestive of tubular failure.

It has been assumed that glutaminase might be damaged by ischaemia and thus the renal control of acidosis is lost. However, in the animals of the first group acidosis developed although glutaminase activity did not change.

This means that the glutaminase is not affected by a lack of oxygen, an observation made also by WICKHAM and SHARMA (1965). It seems also likely that acidosis may develop even if glutaminase activity is normal.

To explain the present observations it could also be assumed that glutaminase activity is decreased by ischaemia but this is compensated by the acidosis which is known to enhance the enzyme's activity, as may clearly be observed on the third day (POLLÁK et al. 1965). This assumption has been supported by the decrease of glutaminase activity in the animals of the second group which were not acidotic.

The present results thus allow the conclusion that glutaminase is not sensitive to anoxia, as it did not decrease on the first and second postoperative days when phosphatase activity had already decreased. In the second group the decreased glutaminase activity on the third day was most probably a result of the decreased enzyme resynthesis. In the animals of the first group uraemia developed, but owing to the acidosis no decrease of glutaminase activity could be observed.

## LITERATURE

BÁLINT, P., FEKETE, Á., SZALAY, E., TARABA, I.: Einfluss der Narkose auf den Verlauf der akuten Niereninsuffizienz. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **1960,** 239: 497—506.

BÁLINT, P., FEKETE, Á., MISIK, S., TARABA, I.: Nierenfunktion im Verlauf des akuten Nierenversagens. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **1961,** 242: 261—271.

BÁLINT, P.: Klinikai laboratóriumi diagnosztika. Medicina, Budapest **1962,** pp. 491—492.

BÁLINT, P., FEKETE, Á., TARABA, I.: Circulation and renal function in postischaemic renal failure. Acta med. Acad. Sci. hung. **1964,** 20: 433—443.

BENEDEK, E., FEKETE, Á., MOLNÁR, L., TARABA, I.: Alkaline phosphatase activity of the renal tubules in experimental postischaemic renal failure of rats. Acta physiol. Acad. Sci. hung. **1966,** 30: 175—182.

CLEGHORN, R. A., JENDRASSIK, L.: Photometrische Stickstoffbestimmung. Biochem. Z. **1934,** 274: 189—196.

DAVIES, B. M. A., YUDKIN, J.: Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. Biochem. J. **1952,** 52: 407—412.

FISCHER, R. A.: Statistical Methods for Research Workers. 10th ed. Oliver and Boyd, London—Edinburgh **1946.**

FULGRAFF, G., PITTS, R. F.: Kinetics of ammonia production in the acidotic dog. Amer. J. Physiol. **1965,** 209: 1206—1212.

GOEBEL, A., PUCHTLER, H.: Über das Verhalten der histochemisch nachweisbaren alkalischen Phosphatase und 5 — Nucleotidase im Sauerstoffmangel und im Niereninfarkt. Virchows Arch. path. Anat. **1954—55,** 326: 119—134.

HODLER, J.: Die Aktivität der Glutaminase I, der Transaminasen sowie der Glutaminsäure- und Milchsäuredehydrogenase in der erkrankten Niere des Menschen. Schweiz. med. Wschr. **1964,** 94: 11—22.

HODLER, J.: Untersuchungsmöglichkeiten bei renalen Acidosen und ihr Beitrag zur klinischen Differentialdiagnose der Schrumpfnieren. Schweiz. med. Wschr. **1964,** 94: 79—86.

KISFALUDY, S.: Bestimmung des Blutammoniaks mit der Ninhydrinreaktion. Acta med. Acad. Sci. hung. **1960,** 16: 175—187.

LEVEY, S., PERSKY, L., SZARNECKI, N.: Transaminase and glutaminase activity in kidney injury. Arch. Surg. **1963,** 86: 209—213.

ODERT, H.: Die Abhängigkeit der Ammoniaproduktion von der GFR bei Ureterabklemmung und bei Durchblutungsdrosselung in der Hundenniere. Pflügers Arch. ges. Physiol. **1966,** 292: 129—139.

PITTS, R. F., HAAS, J., KLEIN, J.: Relation of renal amino and amide nitrogen extraction to ammonia production. Amer. J. Physiol. **1963,** 204: 187—191.
PITTS, R. F.: Renal production and excretion of ammonia. Amer. J. Med. **1964,** 36: 720—742.
PITTS, R. F.: The renal metabolism of ammonia. Physiologist **1966,** 9: 97—109.
PITTS, R. F., STONE, W. J.: Renal metabolism and excretion of ammonia. Proc. 3rd Int. Congr. Nephrology, Washington 1: 123—135, **1966.** Karger, Basel—New York **1967.**
POLLAK, V. E., MATTENHEIMER, H., DE BRUIN, H., WEINMAN, K.: Experimental metabolic acidosis: The enzymatic basis of ammonia production by the dog kidney. J. clin. Invest. **1965,** 44: 169—181.
PREUSS, H. G., DAVIS, B. B., MAHER, J. F., BISE, B. W., SCHREINER, G. E.: Ammonia metabolism in renal failure. Ann. intern. Med. **1966,** 65: 54—61.
RECTOR, F. C., SELDIN, P. W., COPENHAVER, J. H.: The mechanism of ammonia excretion during ammonium chloride acidosis. J. clin. Invest. **1955,** 34: 20—26.
SELKURT, E. E.: The changes in renal clearance following complete ischaemia of the kidney. Amer. J. Physiol. **1945,** 144: 395—403.
TARABA, I., BENEDEK, E., MOLNÁR, L., STIASZNY, L.: Postischaemic renal failure in unanaesthetized dogs. Acta med. Acad. Sci. hung. **1966,** 23: 53—60.
ULLRICH, K. J., HILGER, H. H., KLÜMPER, J. D.: Sekretion von Ammoniumionen in den Sammelrohren der Säugetierniere. Pflügers Arch. ges. Physiol. **1958,** 267: 144—150.
VAN SLYKE, D. D., PHILIPS, R. A., HAMILTON, P. B., ARCHIBALD, R. M., FUTCHER, P. H., HILLER, A.: Glutamine as source material of urinary ammonia. J. biol. Chem. **1943,** 150: 481.
WEISS, M. B., LONGLEY, J. B.: Renal glutaminase. I. Distribution and ammonia excretion in the rat. Amer. J. Physiol. **1960,** 198: 223—226.
WICKHAM, J. E. A., SHARMA, G. P.: Endogenous ammonia formation in experimental renal ischaemia. Lancet **1965,** 1: 195—98.
ZIMMERMANN, H.: Experimentelle histologische, histochemische und funktionelle Untersuchungen zur Frage der Nierenschädigung nach temporärer Ischämie. Beitr. path. Anat. **1957,** 117: 65—89.

László MOLNÁR, Erika BENEDEK, István TARABA
Orvostudományi Egyetem Élettani Intézete
Budapest VIII., Puskin u. 9.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 87—92 (1969)*

# EFFECT OF HAEMORRHAGIC SHOCK ON OXYGEN TENSION OF THORACIC DUCT LYMPH*

By

S. NAGY, T. BARANKAY and Klára TÁRNOKY

INSTITUTE OF EXPERIMENTAL SURGERY, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received July 26, 1968)

Tissue hypoxia has a key role in the mechanism of shock. Direct quantitative measurement of tissue oxygen tension by electrodes is not possible since the electrode cannot be calibrated accurately for use in vivo and considerable damage to the tissues occurs with its insertion. We have therefore analyzed the oxygen tension of lymph as an aliquot of interstitial fluid assuming that it is in equilibrium with the interstitial oxygen tension. The thoracic duct was cannulated in dogs by a stainless steel tube and lymph was led directly into the thermostated measuring cuvette of a membrane-covered polarographic platinum electrode connected to a Beckman Physiological Gas Analyzer. Lymph oxygen tension was measured continuously. Normal oxygen tension of lymph was approximately the same or slightly below mixed venous or hepatic venous oxygen tension. In haemorrhagic shock (blood pressure 30—40 mm Hg) lymph oxygen tension fell rapidly to very low levels (1—5 mm Hg, in some cases to zero) and was now much below the level of venous oxygen tension. The effect of reinfusion of blood, administration of low molecular weight dextran and of vasoactive drugs was also studied. It is concluded that measurement of lymph oxygen tension provides good information on the functional behaviour of the microcirculation and the level of tissue oxygen tension in shock.

The decrease of cardiac output and tissue blood flow in shock results in widespread tissue hypoxia. The latter has a key role in the mechanism of shock. Our knowledge on the level of tissue oxygen tension in shock is, however, scanty due to methodological difficulties. Polarographic oxygen electrodes of the Clark-type cannot sufficiently be miniaturized to be introduced into the tissues without causing considerable damage which alters local oxygen tension. Bare-tipped electrodes, owing to polarization effects, are not stable for longer periods. Calibration for in vivo use of tissue electrodes cannot be done accurately. Results of electrode measurements of tissue oxygen tension are usually given as percentage changes which can be most misleading. (A change of $pO_2$ from 1 mm Hg to 2 mm Hg is very different from a change from 25 to 50 mm Hg; CATER 1964.) In the present study the oxygen tension of lymph was analyzed. Lymph is tissue fluid that has entered the lymphatic capillaries (MAYERSON 1962) and its oxygen tension according to the few known data (BERGOFSKY et al. 1962; BERGOFSKY et al. 1964; GROTH et al. 1965; WITTE et al. 1967) probably repre-

* Presented in a condensed form at the Meeting of the Hungarian Physiological Society, Debrecen, July 5, 1968.

sents the mean oxygen tension of the tissues from which it is derived. No data are available on the oxygen tension of lymph in shock. Lymph flow in shock was studied extensively by WESSELY (1958).

## Methods

The experiments were performed on 32 dogs of 14.9 kg mean body weight (range 9.7—18.7 kg). The animals were lightly anaesthetized with sodium pentobarbital. In 11 experiments the thoracic duct was cannulated in the neck close to its entrance into the subclavian vein. In the other experiments it was cannulated in the caudal part of the right chest. During thoracotomy intermittent positive pressure ventilation was applied with a respirator. After cannulation the thoracotomy wound was closed, the cannula led through the chest wall, the air removed from the thorax and the animals resumed spontaneous breathing. In preliminary experiments it was shown that polyethylene or other plastic tubings are not suitable for lymph collection for $pO_2$ measurement as their permeability to atmospheric oxygen makes impossible the measurement of a few millimeters of Hg of oxygen tension occurring in shock. A stainless steel tube (length 13 cm, diameter 1 mm) with a short polyethylene tubing at its end was used for cannulation in such a manner that the polyethylene part was pushed totally into the duct together with a small length of the steel tube on which the cannula was secured by a ligature. The stainless steel tube was directly connected to the flow chamber (volume 30 $\mu$l) of a Radiometer E 5046 type polarographic electrode (diameter of platinum cathode 20 $\mu$, with a polypropylene membrane of 20 $\mu$ thickness). The electrode was kept at 37°C and connected to a Beckmann Model 160 Physiological Gas Analyser. The oxygen consumption of the electrode was tested in static samples and found to be negligible even for the slowest lymph flows observed in the experiments.

The lymph which passed through the electrode chamber was collected in test tubes graduated in 0.1 ml divisions. Collection periods timed by a stop-watch were 15 or 30 minutes each. The lymph was not returned to the animals.

After a control observation period the animals were bled from the cannulated femoral artery into a reservoir set to maintain mean arterial pressure between 30 and 40 mm Hg. This pressure was maintained for 2 to 3 hours depending on the amount of spontaneous reinfusion. After the hypotensive period all blood remaining in the reservoir was reinfused intravenously. The animals received 5 mg/kg heparin intravenously at the beginning of the bleeding.

Blood samples were taken anaerobically every thirty minutes from the pulmonary artery, hepatic vein and femoral artery and their oxygen tension was analyzed polarographically in a Godart Haemoxytensiometer. The venous samples were drawn through KIFA catheters placed into the pulmonary artery and hepatic vein under X-ray control at the beginning of the experiment.

Statistical analyses were made by the method of WILCOXON et al. (1963). For the method of calculation and interpretation of percentiles see HERRERA (1958).

## Results

In Table I are shown the values for oxygen tension in thoracic duct lymph (cannulated in the neck) and in mixed venous and hepatic venous blood. It can be seen that mean lymph $pO_2$ was slightly higher than venous $pO_2$ but the difference was not significant statistically. Percentiles give an estimate of the scatter of the results.

When the thoracic duct was cannulated in the caudal part of the right chest, normal lymph $pO_2$ values were statistically significantly less than venous values (Table II). At this region the duct carries lymph almost exclusively from the splanchnic area (YOFFEY and COURTICE 1956).

Bleeding the animals into a reservoir set to maintain mean arterial pressure between 30 and 40 mm Hg resulted within a few minutes in a very rapid

**Table I**

*Oxygen tension of thoracic duct lymph, pulmonary arterial and hepatic venous blood*
(thoracic duct cannulated in the neck)

| | Pulmonary artery blood, mm Hg (n = 11) | Hepatic venous blood, mm Hg (n = 11) | Lymph, mm Hg (n = 11) |
|---|---|---|---|
| Mean | 41.9 | 40.2 | 45.2 |
| 20th and 80th percentiles | (35.9 47.2) | (36.1 43.8) | (35.2 60.0) |

P > 10%

P > 10%

**Table II**

*Oxygen tension of thoracic duct lymph, pulmonary arterial and hepatic venous blood*
(thoracic duct cannulated in the thorax)

| | Pulmonary artery blood, mm Hg (n = 14) | Hepatic venous blood, mm Hg (n = 14) | Lymph, mm Hg (n = 22) |
|---|---|---|---|
| Mean | 38.1 | 37.9 | 31.1 |
| 20th and 80th percentiles | (31.9 43.5) | (31.7 42.3) | (22.2 41.4) |

P < 2%

P < 5%

**Table III**

*Oxygen tension of thoracic duct lymph, pulmonary arterial and hepatic venous blood after 25 to 35 minutes of haemorrhagic hypotension*

| | Pulmonary artery blood, mm Hg (n = 14) | Hepatic venous blood, mm Hg (n = 14) | Lymph, mm Hg (n = 14) |
|---|---|---|---|
| Mean | 20.2 | 20.1 | 4.8 |
| 20th and 80th percentiles | (16.5 25.0) | (13.1 28.1) | (1.6 7.8) |

P < 1%

P < 1%

decline of lymph oxygen tension. After 25 to 35 minutes lymph $pO_2$ averaged 4.8 mm Hg which was much below the venous $pO_2$ at that time (Table III).

The decline of lymph $pO_2$ to extremely low levels characterized the course of haemorrhagic shock. In Table IV the average of the lowest values attained in shock is shown. This was 2.4 mm Hg. In 6 cases out of 24 the lowest value attained was zero.

**Table IV**

*Lowest values for lymph oxygen tension during haemorrhagic shock and the time needed to reach them*

| | Lymph, mm Hg (n = 24) | Time elapsed from beginning of bleeding, minutes (n = 24) |
|---|---|---|
| Mean | 2.4 | 36.4 |
| 20th and 80th percentiles | (0.9 6.2) | (18.4 51.2) |

The effect of reinfusion of the shed blood was also studied. It was interesting to note that spontaneous reinfusion which is regarded as a sign of irreversibility was usually accompanied by a rise in lymph $pO_2$. The rise was, however, small and transitory. Reinfusion of the total amount of blood in the reservoir intravenously to the animals was followed in most cases by a prompt rise in lymph oxygen tension (Table V). In many cases characteristic oscillations occurred with a frequency of about 1—2 per minute and amplitudes of 10—20 mm Hg.

**Table V**

*Effect of reinfusion of blood on lymph oxygen tension in haemorrhagic shock*

| | Lymph oxygen tension 15 minutes after reinfusion, mm Hg (n = 12) | Rise in lymph oxygen tension on reinfusion of blood, mm Hg (n = 12) |
|---|---|---|
| Mean | 36.1 | 26.2 |
| 20th and 80th percentiles | (22.4 48.4) | (19.2 36.2) |

The course of a typical experiment is demonstrated in Fig. 1.

The infusion of a low molecular weight dextran (Rheomacrodex, Pharmacia) was also examined. In every instance it was followed by a rise in lymph oxygen tension. It was particularly interesting in a few cases that small doses (5 ml/kg from a 10% solution) also produced this effect without simultaneously raising arterial blood pressure.

In some experiments administration of phenoxybenzamine (3 mg/kg) or chlorpromazine (2 mg/kg) was studied. The drugs were given after reinfusion. No elevation of lymph $pO_2$ was found, in some cases a decline occurred along with a decrease of blood pressure.

Lymph flow which averaged 0.48 ml/min (range, 0.20—0.93) in the control state showed a temporary increase immediately after the bleeding had been started. After a few minutes, however, the flow declined and remained below

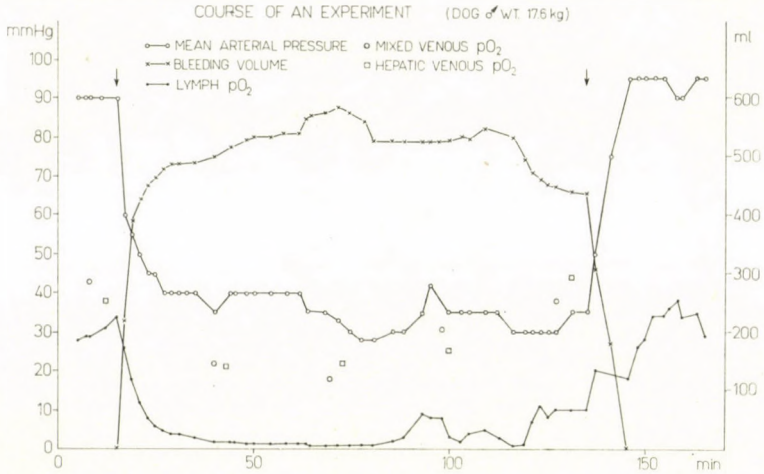



*Fig. 1*

the control level throughout the hypotensive period reaching the average lowest value of 0.19 ml/min (range, 0.11—0.40) between one and one and a half hours after bleeding had been started. Reinfusion of blood was followed by a rise in lymph flow to an average value of 0.45 ml/min (range 0.23—0.68) approaching thus the control values.

## Discussion

In these experiments lymph oxygen tension rapidly responded in a predictable manner to stimuli which are known to alter tissue oxygen tension in shock. It is known that there are $pO_2$ gradients which normally are of the order of tens of mm's of Hg from capillary venous blood to peripheral parts of tissue cylinders supplied by a capillary (Forster 1965). The differences found in the composition of plasma and lymph are due mainly to the metabolic activity of the parenchymatous cells (Földi 1967). The fact that lymph $pO_2$ rapidly becomes much lower than the corresponding venous $pO_2$ in shock suggests that its value indicates more closely mean tissue oxygen tension than the venous values do. The extremely low levels and, indeed, zero levels of $pO_2$ of lymph suggest that oxygen tension in the cells approaches or becomes zero very early in shock at least in the splachnic area where the lymph we have

studied mainly originates from. Measurement of lymph $pO_2$ provides good information on the performance of the microcirculation with respect to its main function, the supply of oxygen to the tissue cells. It has the advantage of avoiding trauma to the tissues which is very extensive with tissue electrodes (JAMIESON 1964), and also of furnishing information from a larger area of tissue. The fact that measurements can be quantitative permits the correlation of data from different experiments.

## Acknowledgements

We are indebted to AB Pharmacia, Uppsala, Sweden, for the supply of Rheomacrodex, and to Miss J. GALOTTI and Miss R. Kószó for technical assistance.

## LITERATURE

BERGOFSKY, E. H., JACKSON, J. H., FISHMAN, A. P.: The use of lymph for the measurement of gas tensions in interstitial fluid and tissues. J. clin. Invest. **1962,** 41: 1971—1980.

BERGOFSKY, E. H., WANG, M. C., YAMAKI, T., JACOBSON, J. H.: Tissue oxygen and carbon dioxide tensions during hyperbaric oxygenation. J. Amer. med. Ass. **1964,** 189: 841—844.

CATER, D. B.: The measurement of $pO_2$ in tissues. In: DICKENS, F., NEIL, E. (Eds), Oxygen in the Animal Organism. Pergamon Press, Oxford **1964,** 239—246.

FORSTER, R. E.: Oxygenation of the tissue cell. Ann. N. Y. Acad. Sci. **1965,** 117: 730—735.

FÖLDI, M.: General Discussion. In: COLLETTE, J. M., JANTAT, G., SCHOFFENIELS, M. (Eds), New Trends in Basic Lymphology. Birkhäuser Verlag, Basel, Stuttgart **1967,** pp. 228—235.

GROTH, C. G., LÖFSTRÖM, B., WERNER, B.: Oxygen tension of thoracic duct lymph in man. Acta chir. scand. **1965,** 129: 586—590.

HERRERA, L.: The precision of percentiles in establishing normal limits in medicine. J. lab. clin. Med. **1958,** 52: 34—40.

JAMIESON, D.: General Discussion. In: DICKENS, F., NEIL, E. (Eds), Oxygen in the Animal Organism. Pergamon Press, Oxford **1964,** pp. 247—263.

MAYERSON, H. S.: Formation of lymph. In: ABRAMSON, D. I. (Ed.), Blood Vessels and Lymphatics. Academic Press, New York **1962,** pp. 714—718.

WESSELY, J. Lymph circulation of dogs in experimental thermal haemorrhagic and tourniquet shock. Acta physiol. Acad. Sci. hung. **1958,** 327—351.

WILCOXON, F., KATTI, S. K., WILCOX, R. A.: Critical values and probability levels for the Wilcoxon rank sum test and the Wilcoxon signed rank test. American Cyanamid Co. and Florida State University, Pearl River and Tallahassee, **1963,** p. 64.

WITTE, C. L., CLAUSS, R. H., DUMONT, A. E.: Respiratory gas tensions of thoracic duct lymph. Ann. Surg. **1967,** 166: 254—262.

YOFFEY, J. M., CORTUCE, F. C.: Lymphatics, Lymph and Lymphoid Tissue, 2nd edition. Arnold, London **1956,** p. 510.

Sándor NAGY, Tamás BARANKAY, Klára TÁRNOKY,
Szegedi Orvostudományi Egyetem Sebészeti Műtéttani Intézet,
Szeged, Postafiók 464.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) pp. 93—97 (1969)*

# EFFECT OF EXCLUSION OF SMALL INTESTINE IN HAEMORRHAGIC SHOCK

By

M. GERGELY and S. NAGY

FIRST DEPARTMENT OF SURGERY AND INSTITUTE OF EXPERIMENTAL SURGERY, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received September 12, 1968)

The effect of a surgical exclusion of the small intestine was studied in dogs with haemorrhagic shock. The intervention failed to improve survival rates. Contrary to literary data on the mechanism of haemorrhagic necrosis of intestinal mucosa in shock, such a change was found in the excluded small intestinal segment in 50% of the cases.

The cause of the irreversibility of shock is still unknown. In recent years an increasing number of data has been published supporting or denying the role of the gastrointestinal tract in the outcome of shock. According to FINE and his group (1961) the irreversibility in different kinds of shock is due to the endotoxins of the intestinal bacterial flora. Absorption of these highly toxic substances is facilitated by the hypoxic damage of the intestinal mucosa due to the failing circulation (LILLEHEI 1957). The increase of permeability of the intestinal mucosa under such conditions has been verified experimentally (BOUNOUS et al. 1965). The importance of the gastrointestinal tract in shock was most clearly demonstrated in LILLEHEI's experiments (1957), in which dogs in otherwise lethal haemorrhagic shock could be protected in 90% of the cases by the perfusion of the superior mesenteric artery with blood from a donor dog. Death from endotoxin shock was prevented in the experiments of EVANS et al. (1966a) by extensive resection of the small intestine or by exclusion of the small intestine (EVANS et al. 1967). Contrary data were also published by the same group (EVANS et al. 1966b).

The aim of the present experiments was to examine the effect of small bowel exclusion in haemorrhagic shock, and to determine to what extent the results obtained in endotoxin shock apply to haemorrhagic shock.

## Methods

Twenty-one dogs of both sexes weighing 4.7—15.0 kg were used. In the control series 10 dogs were fasted for 18 hours after which both femoral arteries and a femoral vein were exposed and cannulated with polyethylene tubing under local anaesthesia. Arterial blood pressure was measured with a Statham P23 Db transducer. The animals were bled into a reservoir to a blood pressure level between 30 and 40 mm Hg. This usually took about 10 minutes. The hypotension was maintained for 2 hours. During this period spontaneous reinfusion usually started. All the blood in the reservoir was reinfused intravenously after two hours. The cannulas were then removed, the wounds sutured. Survival was examined at 8, 24 and 48 hours after the experiment. One dog (No. 11, see Table I) was submitted to a sham operation.

The animals in the other series were fasted for 18 hours and thereafter the small intestine was excluded aseptically according to EVANS et al. (1967) under intravenous sodium pentobarbital anaesthesia (30 mg/kg). The blood and nerve supply of the intestines were preserved. The jejunum was transected about 4—5 cm distal to the duodenojejunal flexure and the ileum at about the same distance from the ileocolic junction. The oral end of the jejunum and the distal end of the ileum were anastomosed by a two-layer end-to-end suture. The cut ends of the excluded small intestine were used as jejunostomy and ileostomy openings on the two sides of the skin incision. The animals were given 500 ml of isotonic NaCl infusion during and after the operation. Next day they received water orally and from the following day on liquid and soft food. The plasma volume of 7 operated animals was determined (NAGY et al. 1965) before the operation and 6—8 days later immediately before they were subjected to the shock procedure described above.

## Results

Results are shown in the Tables. Exclusion of the small intestine failed to exert any beneficial influence on the outcome of haemorrhagic shock. The operated animals usually succumbed earlier to the shock than did the controls; 5 dogs died while they were bled or during the next hour.

It was thought that the failure of small bowel exclusion to protect the animals against shock might have been due to an increased loss of intestinal fluid and diarrhoea. Plasma volume measurements, however, have not supported this assumption as in contrast to our expectation plasma volumes measured 6 to 8 days after the operation were higher than before the operation.

At autopsy a characteristic haemorrhagic mucosal necrosis was found in every case in the re-established intestinal tract. The lesion extended from the beginning of the duodenum to the rectum. The most severe picture was always seen in the duodenum. In half of the cases the excluded small intestine was also necrosed; here the most pronounced changes occurred in the jejunum, i.e. in the oral part of the excluded segment (Figs 1, 2; Tables I, II).

## Discussion

The general condition of the operated animals deteriorated rapidly. A week after the operation there was a mean loss of body weight of 1.28 kg (range 0.55—2.25 kg). EVANS et al. (1966a, b; 1967) did not mention such a phenomenon even though they administered infusions only for two days after the operation after which the animals were kept on a standard diet. They also failed to observe diarrhoea, a symptom which occurred in all of our operated animals. They administered the E. coli endotoxin 0.55 B5 in a dose of 2 mg/kg intravenously. The 10% survival in their control series was improved to 52% in the bowel excluded group which meant a 48-hour survival of 16 animals out of 31.

The results of EVANS et al. become quite inexplicable when we consider another of their papers (EVANS et al. 1966b) in which they are discussing the relation between gastrointestinal secretion and haemorrhagic mucosal necrosis

*Fig. 1.* Typical haemorrhagic mucosal necrosis in the reconstituted gastrointestinal tract

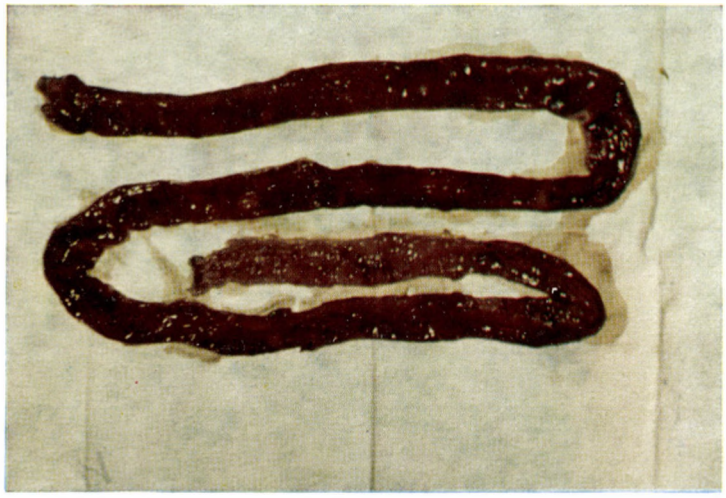


*Fig. 2.* Excluded small intestine. Haemorrhagic mucosal necrosis, the intensity of which diminishes in the distal direction

**Table I**

*Haemorrhagic shock in control dogs*

| Number, sex | Body weight, kg | Duration of survival | Maximal bleeding volume, ml | Spontaneous reinfusion in per cent of maximal bleeding volume |
|---|---|---|---|---|
| 1. m | 7.00 | 8 hours | 340 | 30 |
| 2. m | 7.40 | less than 8 hours | 320 | 28 |
| 3. f | 13.40 | less than 8 hours | 770 | 31 |
| 4. m | 11.50 | less than 48 hours | 445 | 20 |
| 5. m | 15.00 | less than 48 hours | 725 | 36 |
| 6. f | 8.00 | less than 24 hours | 415 | 26 |
| 7. f | 8.40 | less than 48 hours | 410 | 34 |
| 8. m | 11.20 | survived | 515 | 22 |
| 9. m | 9.50 | less than 24 hours | 355 | 24 |
| 10. f | 9.00 | less than 8 hours | 550 | 32 |
| 11.* f | 7.95 7.60 (499) (560) | survived | 265 | 0 |

* Subjected to a sham operation (laparotomy, exteriorization of intestine) one week before shock. The sham operation lasted as long as the exclusion operation. Pertinent plasma volumes in brackets

of the intestine in canine shock. In a series of 10 dogs exclusion of the small intestine prevented only the haemorrhagic mucosal necrosis but was without effect on survival. The dose of their endotoxin of unspecified origin was 1.5 mg/kg and killed all animals within 48 hours.

The only significant positive result of our experiments was the surprising fact that in 5 cases haemorrhagic enteritis and severe mucosal necrosis developed in the excluded intestinal segment (Fig. 2). This was in contrast not only to the results of EVANS et al. (1966a) but also to the currently accepted theory of the genesis of this lesion. According to the investigations of GURD's group (BOUNOUS et al. 1965; 1966) the enzyme systems of the intestinal mucosa are damaged in shock, the production of mucin is decreased, the permeability increased and the mucosa is digested by the trypsin contained in the intestinal lumen. This theory is supported by experiments demonstrating the protective effect of a locally applied trypsin inhibitor (Trasylol). An even stronger evidence was presented by those experiments in which ligation of the main pancreatic duct prevented the mucosal necrosis and prolonged survival in haemorrhagic

**Table II**

*Haemorrhagic shock in small*

| Number, sex | Body weight (kg) | | Interval between operation and shock, days | Survival |
|---|---|---|---|---|
| | before operation | after operation | | |
| 1. f | 7.00 | 6.45 | 7 | less than 8 hours |
| 2. f | 5.15 | 4.60 | 7 | died during experiment |
| 3. f | 6.75 | 5.10 | 8 | less than 24 hours |
| 4. m | 4.70 | 4.15 | 7 | less than 48 hours |
| 5. m | 12.20 | 11.00 | 6 | died during experiment |
| 6. f | 13.00 | 11.60 | 6 | less than 8 hours |
| 7. m | 10.50 | 8.25 | 7 | died during experiment |
| 8. f | 9.25 | 7.95 | 8 | less than 8 hours |
| 9. f | 9.50 | 7.40 | 7 | less than 8 hours |
| 10. f | 14.20 | 12.25 | 8 | less than 24 hours |

shock elicited 5—6 days later. This correlation of the morphological changes with survival has not been corroborated by other investigators. For example the haemorrhagic mucosal necrosis could be prevented by total gastrectomy or gastric resection and vagotomy (Evans et al. 1966b), hyperbaric oxygen (Evans et al. 1967), tris buffer (Selmonosky et al. 1963), pretreatment with Trasylol (Bounous et al. 1965), but without a simultaneous general protective effect.

In the interpretation of mucosal necrosis in our experiments several facts should be considered. The blood and nerve supply of the excluded segment remained intact, the intestinal contents were not removed actively, the pH was not neutralized, antibiotics were not applied locally, the enzymes which were present in the lumen before the operation were not inactivated. Thus intestinal exclusion alone did not eliminate all the factors which were previously made responsible for the mucosal changes by other investigators.

It appears that the contradictory observations described in the literature can at least partly be explained by the different experimental conditions employed. This stresses the difficulty of the subject. Our results add to the contradictory observations and call for further experimental work.

## Acknowledgement

The authors are indebted to Miss R. Kószó for technical assistance.

*intestine excluded dogs*

| Degree of intestinal lesion in the excluded segment | Maximal bleeding volume, ml | Spontaneous reinfusion per cent of maximal bleeding volume | Plasma volume, ml | | Haematocrit, per cent | |
|---|---|---|---|---|---|---|
| | | | before operation | after operation | before operation | after operation |
| min. | 260 | 32 | — | — | — | — |
| — | 140 | — | — | — | — | — |
| min. | 130 | 65 | — | — | — | — |
| min. | 190 | — | 294 | 338 | 48 | 40.5 |
| max. | 425 | 36 | 613 | 600 | 44.5 | 42 |
| max. | 450 | 42 | 586 | 633 | 54 | 45 |
| — | 185 | — | 556 | 448 | 42.5 | 37.5 |
| max. | 340 | 20 | 598 | 718 | 35.5 | 43.5 |
| moderate | 325 | 25 | 763 | 534 | 39.5 | 44.5 |
| max. | 785 | — | 863 | 891 | 44.5 | 41.5 |

## LITERATURE


BOUNOUS, G., GROWN, R. A., MULDER, D. S., HAMPSON, L. G., GURD, F. N.: Abolition of tryptic enteritis in the shocked dog. Arch. Surg. **1965,** 91: 371—375.

BOUNOUS, G., MCARDLE, A. H., HODGES, D. M., HAMPSON, L. G., GURD, F. N.: Biosynthesis of intestinal mucin in shock: Relationship to tryptic haemorrhagic enteritis and permeability to curare. Ann. Surg. **1966,** 164: 13—22.

EVANS, W. E., SHORE, R. T., DAVIS, J. C.: Graded enteroectomy in Escherichia coli shock. Arch. Surg. **1966a,** 93: 196—199.

EVANS, W. E., SHORE, R. T., CAVEY, L. C., DAVIS, J. C.: Effect of gastrointestinal secretions on the formation of haemorrhagic intestinal necrosis in Escherichia coli endotoxin shock. Amer. J. Surg. **1966b,** 111: 799—804.

EVANS, W. E., SHORE, R. T., CAVEY, L. C., DAVIS, J. C.: Effect of intestinal exclusion in Escherichia coli endotoxin shock. Arch. Surg. **1967,** 95: 511—516.

FINE, J.: Endotoxins in traumatic shock. Fed. Proc. **1961,** 20: 166—170.

FINE, J.: The intestinal circulation in shock. Gastroenterology **1967,** 52: 454—458.

LILLEHEI, R. C.: The intestinal factor in irreversible haemorrhagic shock. Surgery **1957,** 42: 1043—1054.

NAGY, S., TÁRNOKY, K., PETRI, G.: Adjustment of the circulation to haemorrhage in dogs during sympathetic nervous blockade. Acta physiol. Acad. Sci. hung. **1965,** 28: 379—387.

SELMONOSKY, C. A., GOETZ, R. H., STATE, D.: The role of acidosis in the irreversibility of experimental haemorrhagic shock. J. Surg. Res. **1963,** 3: 491—496.



Mihály GERGELY, Sándor NAGY,
Orvostudományi Egyetem I. sz. Sebészeti Klinikája és
Sebészeti Műtéttani Intézete,
Szeged, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (1) p. 99 (1969)*

# *Recensio*

G. E. SCHREINER (Ed.)

## Proceedings of the Third International Congress of Nephrology, Washington, D.C. 1966

Vol. 1: Physiology. J. S. Handler (Ed.) XVI + 288 p., 117 fig., 24 tabl. US $14.00
Vol. 2: Morphology, Immunology, Urology. R. H. Heptinstall (Ed.) XVI + 363 p., 209 fig., 45 tab. US $14.00
Vol. 3: Clinical Nephrology. L. E. Becker (Ed.) XVI + 382 p., 163 fig., 75 tab., US $14.00
Complete Proceedings: US $36.50. S. Karger AG, Basel 1967

The present book contains a selection of the papers given at the Third International Congress of Nephrology held in 1966 in Washington, D.C. Volume I deals with physiological, Volume II with morphological, immunological and urological problems, and the subject of Volume III is clinical nephrology.

The editors succeeded in selecting from the immense material presented at the Congress those leading lectures which constitute the most important chapters of today's nephrology. The result is a monograph useful for everybody who wants to enrich his knowledge in experimental or clinical nephrology.

The value of the book is in part due to the circumstance that each chapter has been written by leading experts of the subject and therefore the reader not only learns the most recent results attained in the various fields of nephrology but he may read between the lines the problems to be solved in the near future and the perspectives of this branch of theoretical and practical medicine. In this respect, Volume III is a particularly exciting reading, since the long-standing aim of medical sciences, i.e. the substitution of the function of vital organs, is perhaps nearest to its solution in the replacement of kidney function. The sections dealing with vivodialysis and transplantation are fascinating for every physician.

The perfect set-up of the volumes praises the high-standard workmanship of Messrs. S. Karger.

P. BÁLINT

INDEX

PHYSIOLOGIA

*Airapetianz E. Sh., Konstantinov A. I., Matjushkin D. P.:* Brain Echolocation Mechanisms and Bionics ..... 1

*Sacharov D. A., Salánki J.:* Physiological and Pharmacological Identification of Neurons in the Central Nervous System of Helix Pomatia L. ..... 19

*Endrőczi E.:* Effect of Sex Hormones on the Electrical Activity of Brain Stem and Diencephalon in Castrated Female Rats ..... 31

*Korányi L., Endrőczi E., Tamásy Veronica:* Pituitary Adrenocorticotrophic Hormone (ACTH) Induced Hypoglycaemia ..... 41

*Péczely P.:* Effect of the Median Eminence of the Pigeon (Columba Livia Domestica L.) on the Regulation of Adenohypophysial Corticotropin Secretion ..... 47

*Szilágyi T., Tóth L., Miltényi L., Jóna G.†:* Oxygen Poisoning and Thyroid Function ..... 59

*Lózsa A., Kereszti Zita, Berencsi G.:* Effect of Heat and Cold Exposure on Serum Proteins, Glycoproteins and Lipoproteins in the Rabbit ..... 63

*Li Bok Nam, Szelényi I., Zelles T.:* Comparison of Liver Glycogen Assay Methods ..... 75

*Molnár L., Benedek Erika, Taraba I.:* Glutaminase Activity of the Kidney in Experimental Renal Failure of Rats ..... 81

*Nagy S., Barankay T., Tárnoky Klára:* Effect of Haemorrhagic Shock on Oxygen Tension of Thoracic Duct Lymph ..... 87

*Gergely M., Nagy S.:* Effect of Exclusion of Small Intestine in Haemorrhagic Shock ..... 97

RECENSIO

*Schreiner G. E.* (Ed.): Proceedings of the Third International Congress of Nephrology, Washington, D.C. 1966. (P. Bálint) ..... 99

# INSTRUCTION TO AUTHORS

ACTA PHYSIOLOGICA is devoted to publication of original findings in the field of physiology, pathophysiology and pharmacology. Papers may be submitted in English, French, German and Russian.

Papers should be sent to the Editor-in-Chief:
Professor K. Lissák, M. D.
Institute of Physiology
University Medical School
Rákóczi út 80.
Pécs, Hungary

MANUSCRIPT REQUIREMENTS. Manuscripts of full papers should not exceed 15 typewritten pages. The maximum length allowed for short communication is 5 pages including Tables and Literature, and for book review not more than 2 pages.

Manuscripts should be submitted double spaced with wide margins (at least 4 cm) on pages of uniform size.

Manuscripts should consist of (i) title of the paper; (ii) authors' names; (iii) laboratory of origin with city and state; (iv) abstract (in a duplicate copy) which does not exceed 1 page; (v) introduction; (vi) method(s); (vii) results; (viii) discussion; (ix) acknowledgements; (x) literature; (xi) authors' full names and complete addresses for mailing. The name and address of the author to whom correspondence are to be sent should be given; (xii) text for illustration should be given on separate sheets double spaced with wide margins.

*Literature* references should be listed in alphabetical order according to the surname of the first author. References must be complete including initials of the authors cited, title of article, journal name abbreviated as listed in the World List of Scientific Periodicals, year, volume, the first and last page numbers.

*Book references* should be in the following order: author's name, initials, title, publisher, city of publication, year, e.g.: LISSÁK, K., ENDRŐCZI, E.: The Neuroendocrine Control of Adaptation, Pergamon Press, Oxford **1965.**

Reference to authors contributing to multiauthor books should include: author, initials, title of article, name of Editor, title of book, publisher, city of publication, year and page numbers referred to, e.g.:

LEVINE, S.: Influence of gonadal hormones in infancy on adult behavior. In: LISSÁK, K. (Ed.): Symposium on Reproduction. Congress of the Hungarian Society for Endocrinology and Metabolism, Pécs 1967, Akadémiai Kiadó, Budapest **1967,** pp. 229—241.

A paper which has been accepted for publication but which has not appeared may be cited in the literature followed by the words, "In press". Responsibility for the accuracy of the references rest with the author.

Citation such as "unpublished observation", "personal communication", "in preparation", "to be published" should not be entered in the list of references, but may be noted in the text in parentheses.

*Reference in the text:* the name of author(s), (if there are not more than two contributors) and year should be given, e.g.: BIGNAMI (1960), SMITH and SMITH (1968), and if more than two, then only the first author's name should be given followed by "et al.", e.g.: GUZMAN-FLORES et al. (1960).

*Illustrations:* sharply contrasting photographs which must be kept to a minimum of figures on glossy paper are required and should be identified on the reverse with figure number (Arabic numbers) and author(s) name; when necessary the top should be clearly marked. Illustrations should be larger than the final size of reproduction but not more than 18 × 24 cm.

*Tables:* each table should have a brief heading, numbered with Roman numerals. Statistical measures of variation SD, SE etc. should be identified.

Standard nomenclature, abbreviations and symbols should be used; infamiliar or new terms, abbreviations and trade names should be defined when first used. The Editorial Board reserves the right to make literary corrections.

*Proofs:* galley proofs will be sent to the first-named author of the paper unless otherwise indicated. Corrections to the proofs must be restricted to printer's errors only. Other than these, any substantial alterations may be charged to the author. Proofs must be sent back by return mail.

*Reprints:* a total of 100 reprint will be provided to the first-named author. Additional copies can be ordered at reasonable price by ordering the reprints with the proof.

*Printed in Hungary*

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1969. I. 17. — Terjedelem: 9 (A/5) ív, 46 ábra (2 színes), 1 melléklet

69.66945 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY
## and Cognate Medical Sciences

Chairman: D. WHITTERIDGE, F. R. S., B. Sc., D. M. (Oxon).
Press Editor: HUGH BEGBIE, M. A., Ph. D., M. B., Ch. B.

**Publication Dates: January, April, July, October**
**Annual Subscription: £6**
**Single Number: £1. 10s**

The *Quarterly Journal of Experimental Physiology* has always specialised in the publication of original articles with high-grade half-tone illustrations both in colour and black and white. All aspects of human and animal physiology are covered and the journal is essential for anyone wishing to keep abreast of current research in this field.

**Published by**

**E & S LIVINGSTONE LTD**
**15—17**

**TEVIOT PLACE**
**EDINBURGH**

ACTA PHYSIOLOGICA

РЕЗЮМЕ

ТОМ. 35. ВЫП. 1.

## МОЗГОВЫЕ МЕХАНИЗМЫ ЭХОЛОКАЦИИ И БИОНИКА

Э. Ш. АЙРАПЕТЬЯНЦ, А. И. КОНСТАНТИНОВ,
Д. П. МАТЮШКИН

Эксперименты проводились на летучих мышах 2-х семейств **Vespertilionidae** и **Rhinolophidae.** Описываются различия в параметрах локационных сигналов и в способах преследования насекомых у тех и других летучих мышей. Методом условных рефлексов установлено, что летучие мыши обоих семейств только с помощью эхолокации способны распознавать мышени, различающиеся по размерам, по форме и качеству материалов.

В электрофизиологических экспериментах исследованы электрические реакции слухового нерва заднего холма и корковой слуховой области, возникающие в ответ на ультразвуковые стимулы. В нейронной массе высших слуховых центров могут быть выделены своеобразные «нейронные шкалы» для оценки частоты и силы, интервалов и направления прихода ультразвуковых сигналов.

Приводится схема возможного функционирования эхолокационной системы у летучих мышей.

## ФИЗИОЛОГИЧЕСКАЯ И ФАРМАКОЛОГИЧЕСКАЯ ИДЕНТИФИКАЦИЯ НЕЙРОНОВ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ HELIX POMATIA L.

Д. А. САХАРОВ, Я. ШАЛАНКИ

В плевральных, париетальных и висцеральных ганглиях Helix pomatia авторы идентифицировали отдельные нейроны и клеточные группы визуально, а также на основе их электрофизиологических и фармакологических свойств.

Мембранные и акционные потенциалы были регистрированы внутриклеточными электродами. Изучены следующие свойства: Форма акционного потенциала, действие поляризационного тока, изменения ПСП при изменении мембранного потенциала.

Было установлено, что отдельные гигантские клетки и группы клеток обладают хорошо характеризуемым типом активности, что облегчает их различение от окружающих клеток. У других клеток точная идентификация возможна лишь при знании синаптических связей и путей.

Ответ на ACh в общем не показывает тесной связи с типом активности клеток, ни с реакцией на 5HT и на допамин. В то же время применением 5HT удалось стимулировать лишь клетки осцилляторного типа, а допамин тормозила активность этих же клеток.

## ДЕЙСТВИЕ ПОЛОВЫХ СТЕРОИДОВ НА ЭЛЕКТРИЧЕСКУЮ АКТИВНОСТЬ СТВОЛА МОЗГА И ПРОМЕЖУТОЧНОГО МОЗГА

Э. ЭНДРЁЦИ

Автор изучал действие 17-$\beta$-эстрадиола и прогестерона на реакции, вызываемые со стороны влагалища или сетчатого образования ствола мозга у нормальных и кастрированных крыс. После кастрации реакции regio preopticus были как при раздражении влагалища, так и при раздражении ствола мозга пониженными. Дача эстрадиола нормализовала реакцию на раздражение ствола мозга но не влияла на ответы, вызываемые раз-

дражением влагалища. Прогестерон облегчал реакцию на раздражение влагалища на уровне гипоталамуса и среднего мозга. Действие прогестерона двуфазное: вначале он вызывает фацилитацию реакции, а затем — выраженное торможение ее. В развитии торможения предположительно играет роль нисходящая тормозная система переднего мозга. Это предположение подкрепляется тем фактом, что тормозящее действие кондиционирующего раздражения медиального пучка переднего мозга выраженно усиливается во второй фазе прогестеронового эффекта.

## ГИПОГЛИКЕМИЯ, ОБУСЛОВЛЕННАЯ АДРЕНОКОРТИКОТРОПНЫМ ГОРМОНОМ ГИПОФИЗА (АКТГ)

Л. КОРАНЬИ, Э. ЭНДРЁЦИ, В. ТАМАШИ

У нормальных и адреналектомированных животных внутривенное введение АКТГ приводит к развитию гипогликемии. У адреналектомированных животных значительное понижение уровня сахара в крови наблюдается при формалиновом стрессе или после кондиционированной реакции страха. Имплантацией дексаметазония в среднюю возвышенность гипоталамуса можно предотвратить эту гипогликемию.

Через 3 недели после удаления надпочечников, ни после кондиционированной реакции страха, ни после инъекции формалина или АКТГ не развивается гипогликемии. В противоположность этому, у этих животных предварительная дача гидрокортизона в существенной степени влияет на возникновение гипогликемии, вызванной введением АКТГ.

## ДЕЙСТВИЕ СРЕДНЕГО ВОЗВЫШЕНИЯ НА РЕГУЛИРОВАНИЕ СЕКРЕЦИИ КОРТИКОТРОПИНА АДЕНОГИПОФИЗОМ У ДОМАШНЕГО ГОЛУБЯ

П. ПЕЦЕЛИ

Изменения активности CRF среднего возвышения и активности АКТГ аденогипофиза домашнего голубя при гипер- и гипофункции надпочечников были изучены прижизненным методом. Под влиянием 8 М. Е. инсулина, через полчаса после введения содержание CRF в среднем возвышении сильно уменьшается, по истечении часа оно соответствует контрольным величинам, через два часа вновь сильно понижается, и по истечении 3 часов показывает уже совершенно минимальные величины. Через полчаса после введения инсулина содержание АКТГ в аденогипофизе повышается приблизительно до трехкратной контрольной величины, затем, в первый и второй часы наблюдается медленное понижение, а по истечении 3 часов оно падает ниже контрольной величины. Под влиянием дачи АКТГ, нагрузки с водой, при введении поваренной соли и преднизолона содержание CRF в среднем возвышении уменьшается, под влиянием жажды, однако, оно не изменяется. Содержание АКТГ в аденогипофизе под влиянием введения АКТГ и нагрузки с водой значительно уменьшается, после введения хлорида натрия и преднизолона оно повышается, а при жажде — не изменяется. Эти результаты показывают, что у этого вида птиц функция надпочечников находится под контролем гипоталамо-гипофизарной системы.

## ОТРАВЛЕНИЕ КИСЛОРОДОМ И ФУНКЦИЯ ИТОВИДНОЙ ЖЕЛЕЗЫ

Т. СИЛАДЬИ, Ш. ТОТ, Л. МИЛТЕНЬИ,
Г. ЙОНА

При вдыхании гипербарического кислорода (4 атм. чистого кислорода) кролики и крысы с гипертиреозом погибают раньше, чем евтиреоидные контрольные животные. Время выживания животных с атиреозом не отличается в существенной мере от времени выживания евтиреоидных кроликов или крыс. Обсуждается потенцирующее действие гипертиреоза на отравление кислородом.

## ИЗМЕНЕНИЯ ФРАКЦИЙ СЫВОРОТОЧНЫХ БЕЛКОВ, ГЛИКОПРОТЕИНОВ И ЛИПОПРОТЕИНОВ У КРОЛИКОВ ПОД ВЛИЯНИЕМ ТЕПЛОЙ И ХОЛОДНОЙ СРЕДЫ

А. ЛОЖА, З. КЕРЕСТИ, Д. БЕРЕНЧИ

Одну группу кроликов, адаптированных к температуре среды в 18 °C, авторы поместили в пространство с температурой в 4 °C, а другую группу — в пространство с температурой в 30 °C. По истечении двух недель животных обменяли друг с другом, и в течение дальнейших двух недель изучали действие холодной и теплой среды на макромолекулы сыворотки. Согласно результатам, полученным при помощи метода бумажного электрофореза, количество альбумина уменьшается, а концентрация альфа- и бетаглобулинов повышается, как в холодной, так и в теплой среде. Максимальные отклонения отмечаются на третий день экспозиции, после этого спектр сывороточных белков нормализуется. Это же наблюдается и в связи с гликопротеинами. Изменения представляют собой неспецифические реакции и их следует рассматривать как частичные явления общего синдрома адаптации. В этой области наибольшее значение имеет сильное увеличение концентрации альфа-2-глобулина, верно отражающее также интенсивность нагрузки.

В противоположность этому, изменения липопротеинов совершенно специфичны. Под влиянием холода наблюдается сильное повышение количества альфалипопротеина, а в теплой среде — сильное повышение беталипопротеина, причем, этот сдвиг на протяжении двух недель не показывает никакого признака нормализации. В теплой среде внезапное повышение уровня беталипопротеинов сопровождается раздвоением полосы бетагликопротеинов, в результате чего появляется новая фракция, — бета-2-гликопротеин, — никогда не наблюдаемая в нейтральной или холодной среде. Специфическое изменение показывает также гаммаглобулин: под влиянием тепла его количество уменьшается, а в холодной среде оно увеличивается.

На основе этих данных указывается на патофизиологические отношения нагрузки, вызванной теплой или холодной средой.

## СРАВНЕНИЕ МЕТОДОВ ОПРЕДЕЛЕНИЯ ГЛИКОГЕНА ПЕЧЕНИ

ЛИ БОК НАМ, И. СЕЛЕНЬИ, Т. ЗЕЛЛЕШ

ТЕХНИЧЕСКИЙ СОТРУДНИК Ф. ГАЛ

Содержание гликогена в щелочной и кислотной вытяжках ткани печени было определено различными методами. Было установлено, что при обоих методах можно получить аналогичные количества гликогена. Высокие величины, полученные раньше с помощью йода, авторы объясняют тем, что количественное определение тканевого гликогена было сопоставлено с торговым стандартным гликогеном. При применении реагента-йода, последний дает более низкие величины, чем гликоген из ткани. На основе этих данных авторы придерживаются того мнения, что при методе, использующем в качестве реагента йод для определения содержания гликогена в тканях, приходится применять стандартный гликоген, экстрагируемый из аналогичной ткани.

## ИЗМЕНЕНИЕ АКТИВНОСТИ ГЛЮТАМИНАЗЫ ПРИ ПОСТИШЕМИЧЕСКОМ ПОРАЖЕНИИ ПОЧЕК У КРЫС

Л. МОЛЬНАР, Э. БЕНЕДЕК, И. ТАРАБА

У крыс лигированием почечной артерии на протяжении 2 часов была создана картина, подобная острому поражению почек у человека. В дни после лигирования повышается остаточный азот и уже на первый день после травмирования развивается тяжелый ацидоз. На ишемическое поражение почечных канальцев указывает значительное уменьшение активности щелочной фосфатазы. Активность глютаминазы-I не изменяется.

На основе экспериментальных данных кажется, что в развитии постишемического тяжелого ацидоза поражение энзиматической системы, образующей аммоний (глютаминаза), не играет роль. Резистентности глютаминазы к недостатке $O_2$, по-видимому, способствует то обстоятельство, что развивающийся ацидоз стимулирует образование энзима, и это может компенсировать также ишемическое поражение.

## ВЛИЯНИЕ ГЕМОРРАГИЧЕСКОГО ШОКА НА КИСЛОРОДНОЕ ДАВЛЕНИЕ В ЛИМФЕ ГРУДНОГО ПРОТОКА

Ш. НАДЬ, Т. БАРАНКАИ, К. ТАРНОКИ

Тканевая гипоксия играет решающую роль в механизме шока. Прямое, количественное измерение кислородного давления тканей с помощью электродов невозможно, так как для прижизненного применения нельзя точно калибрировать электроды и их введение сопровождается значительным повреждением тканей. Авторы в своих экспериментах изучали давление лимфы. Они рассматривали лимфу как аликвотный делитель интерстициальной жидкости и полагали, что кислородное давление обеих жидкостей находится в равновесии. В грудной проток собак они ввели канюлю из нержавеющей стали и вводили лимфу непосредственно в термостабилизированную измерительную посуду полярографического платинового электрода, снабженного мембраной. Электрод был соединен с физиологическим газовым анализатором Бекмана. Кислородное давление лимфы было измерено непрерывно. В норме кислородное давление лимфы приблизительно было аналогично с кислородным давлением смешанной венозной крови или крови из печеночной вены, или оно было несколько ниже. При геморрагическом шоке (кровяное давление 30—40 мм рт. ст.) кислородное давление лимфы быстро понижалось и достигало очень низких величин (1—5 мм рт. ст., в отдельных случаях 0), и было существенно ниже кислородного давления венозной крови. Изучалось также действие реинфузии крови, вливания декстрана с низким молекулярным весом, и действие вазоактивных средств. Было установлено, что измерение кислородного давления лимфы предоставляет хорошие информации о функциональном состоянии микроциркуляции и кислородного давления тканей при шоке.

## ДЕЙСТВИЕ ВЫКЛЮЧЕНИЯ ТОНКОЙ КИШКИ НА ГЕМОРРАГИЧЕСКИЙ ШОК

М. ГЕРГЕЙ, Ш. НАДЬ

Значение кишечного тракта при шоке кажется доказанным фактом. Приводится краткая сводка литературных данных. Изучалось действие включения тонкой кишки, — примененного другими исследователями при шоке от эндотоксина, — при геморрагическом шоке собак. Выключение тонкой кишки не оказало благоприятного влияния на время выживания животных. В противоположность литературным данным о механизме геморрагического некроза слизистой, наблюдаемого при шоковом состоянии собаки, авторам удалось выявить это изменение в половине случаев также на изолированном участке тонкой кишки.

Die *Acta Physiologica* veröffentlichen Abhandlungen aus dem Bereiche der experimentellen medizinischen Wissenschaften in deutscher, englischer, französischer oder russischer Sprache.

Die *Acta Physiologica* erscheinen in Heften wechselnden Umfanges. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an folgende Adresse zu senden:

*Acta Physiologica, Pécs, Rákóczi út 80.*

An die gleiche Anschrift ist auch jede für die Redaktion und den Verlag bestimmte Korrespondenz zu senden.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

---

Les *Acta Physiologica* paraissent en français, anglais, allemand et russe et publient des mémoires du domaine des sciences médico-expérimentales.

Les *Acta Physiologica* sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à la rédaction à l'adresse suivante:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Toute correspondance doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forint par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. — Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

---

«*Acta Physiologica*» публикуют трактаты из области экспериментальной медицинской науки на русском, немецком, английском и французском языках.

«*Acta Physiologica*» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации рукописи следует направлять по адресу:

*Acta Physiologica, Pécs, Rákóczi út 80.*

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «*Acta Physiologica*» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «*Kultúra*» (Budapest I., Fő ucta 32. Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

*42,— Ft*

## Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA

Ndermarja Shtetnore e Botimeve
*Tirana*

AUSTRALIA

A. Keesing
Box 4886, GPO
*Sydney*

AUSTRIA

Globus Buchvertrieb
Salzgries 16
*Wien I*

BELGIUM

Office International de Librairie
30, Avenue Marnix
*Bruxelles 5*
Du Monde Entier
5, Place St. Jean
*Bruxelles*

BULGARIA

Raznoiznos
1, Tzar Assen
*Sofia*

CANADA

Pannonia Books
2, Spadina Road
*Toronto 4, Ont.*

CHINA

Waiwen Shudian
*Peking*
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Smečkách 30
*Praha 2*
Poštovná Novinová Služba
Dovoz tisku
Vinohradská 46
*Praha 2*
Mad'arská Kultura
Václavské nám. 2
*Praha I*
Poštová Novinová Služba
Dovoz tlače
Leningradská 14
*Bratislava*

DENMARK

Ejnar Munksgaard
Nörregade 6
*Copenhagen*

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
*Helsinki*

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
*Paris 5*

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
*Leipzig 701*
Zeitungsvertriebsamt
Fruchtstrasse 3—4
*1004 Berlin*

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
*7 Stuttgart S.*

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
*Wellingborough, Northants.*
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
*Oxford*

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
*Amsterdam C*
Martinus Nijhof
Lange Voorhout 9
*The Hague*

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP
GPO Post Box 1374
*Bombay I*

ITALY

Santo Vanasia
Via M. Macchi 71
*Milano*
Libreria Commissionaria Sansoni
Via La Marmora 45
*Firenze*

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
*Tokyo*
Maruzen and Co. Ltd.
P. O. Box 605
*Tokyo-Central*
Far Eastern Booksellers
Kanda P. O. Box 72
*Tokyo*

KOREA

Chulpanmul
*Phenjan*

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
*Oslo*

POLAND

RUCH
ul. Wronia 23
*Warszawa*

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
*Bucureşti*

SOVIET UNION

Mezhdunarodnaya Kniga
*Moscow G—200*

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
*Stockholm*

USA

Stechert Hafner Inc.
31, East 10th Street
*New York, N. Y. 10003*
Walter J. Johnson
111, Fifth Avenue
*New York, N. Y. 10003*

VIETNAM

Xunhasaba
19, Tran Quoc Toan
*Hanoi*

YUGOSLAVIA

Forum
Vojvode Mišića borj 1
*Novi Sad*
Jugoslovenska Knjiga
Terazije 27
*Beograd*

*16. VI. 1969* Index: 26.023

# ACTA PHYSIOLOGICA

## ACADEMIAE SCIENTIARUM HUNGARICAE

ADIUVANTIBUS

P. BÁLINT, SZ. DONHOFFER, E. ERNST, B. ISSEKUTZ SEN., L. KESZTYŰS, J. KNOLL, F. OBÁL

REDIGIT

K. LISSÁK

TOMUS XXXV FASCICULUS 2

AKADÉMIAI KIADÓ, BUDAPEST

1969

# ACTA PHYSIOLOGICA

## A MAGYAR TUDOMÁNYOS AKADÉMIA KÍSÉRLETES ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21.

Főszerkesztő:
LISSÁK KÁLMÁN
akadémikus

Technikai szerkesztő:
KORÁNYI LAJOS

Az *Acta Physiologica* német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudományok köréből.

Az *Acta Physiologica* változó terjedelmű füzetekben jelenik meg: több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az *Acta Physiologica* előfizetési ára kötetenként belföldre 120 forint, külföldre 165 forint. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla 43-790-057-181 sz.) vagy annak külföldi képviseleteinél és bizományosainál.

---

Die *Acta Physiologica* veröffentlichen Abhandlungen aus dem Gebiete der experimentellen medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die *Acta Physiologica* erscheinen in Heften wechselnden Umfanges. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an folgende Adresse zu senden:

*Acta Physiologica, Pécs, Rákóczi út 80.*

An die gleiche Anschrift ist auch jede für die Redaktion und den Verlag bestimmte Korrespondenz zu senden.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 101—108 (1969)*

# *Physiologia*

## THE EFFECT OF HYPNOTIC SUGGESTION ON CONDITIONED EVOKED POTENTIALS

By

I. MÉSZÁROS

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received May 18, 1968)

Evoked cortical conditioned responses were established in healthy individuals, using brief clicks as the conditioned stimulus and stroboscopic flash applied with a delay of 200 msec as reinforcement. When elicited in the hypnotic state, the conditioned response proved to be firmer than in the wakeful state. Elimination by verbal suggestion of the photic stimulus used previously as the reinforcing stimulus did not abolish the positive conditional response, whereas disturbing of the interval between the unconditioned and conditioned stimulus by suggestion caused the response to disappear. It has been concluded that, in the establishment of conditioned evoked responses, the time factor is labile and can easily be influenced.

It has been shown (MÉSZÁROS and ÁDÁM 1968) that in humans the application of a brief click which is followed at a fixed delay (200 msec) by a stroboscopic flash results, after a sufficient number of reinforcements, in a conditioned evoked potential which appears 200 msec after the presentation of the auditory stimulus. Similar conditioned responses were successfully established in laboratory animals to the electrical stimulation of afferent nerves, under both acute and chronic conditions (ÁDÁM and MÉSZÁROS 1962; ÁDÁM and KUKORELLI 1964).

The experiments carried out in human subjects have made it possible to direct the attention of the test individuals to one or other of the applied stimuli. Considering the difficulties in controlling the concentration of attention to the stimulus it seemed purposeful to undertake the experiments in the hypnotic state when the attention of the test subject can be directed by verbal suggestion to the required stimulus with perhaps the greatest efficiency.

### Methods

Several preliminary seances were carried out with each subject participating in the experiments to secure the necessary depth of sleep. The observations were started either with the hypnotic induction of sleep or with experiments carried out in the wakeful state

(1) In the first case, the electrodes and the earphone were placed on the head of the subject in hypnosis. Then the evoked potentials elicited by the auditory stimulus presented alone were recorded in bipolar parieto-occipital leads, averaging 40 responses; this was followed by estimation of the spontaneous cortical activity and then by the control of the responses given to the photic (unconditioned) stimulus when applied alone. If the amplitude of the re-

sponse showed a tendency to diminish, the clicks were presented in groups of 40 at intervals of 2 sec and reinforced by the stroboscopic flash applied with a fixed delay of 200 msec. Conditioning was invariably followed by 40 conditional trials. If the conditioned response was firmly established, only the conditional stimulus was presented in groups of 40 trials.

(2) The procedure was the same in the group where the observations were commenced in the wakeful state. After checking the responses given to the isolated auditory and then photic stimuli, the potential elicited by the click was again controlled in the alert subject. Then sleep was induced by hypnosis, and the previously established conditional response was elicited in several series of trials. Further reinforcement was undertaken if necessary.

A total of 27 trials was made in 6 healthy individuals. In 5 subjects, similar conditioned cortical evoked potentials had been established in preliminary experiments.

The obtained deflections were averaged in groups of 40 with a NTA 256 type computer. Technical details of recording, averaging and mathematical elaboration of the results have been described elsewhere (MÉSZÁROS and ÁDÁM 1968).

## Results

The first important finding in these experiments was that hypnosis *per se* did not significantly affect the shape of the "conditioned" deflection recorded by scalp electrodes above the cortex, although the base line of the averaged curve recorded in the hypnotic state contained considerably less waves, the scatter of the points was smaller, consequently, the evoked potentials were easier to analyse, than without hypnosis.

The conditioned response could be recognized without difficulty, and the components of the unconditioned deflection arose more markedly from the background activity (Fig. 1).

In one of the test subjects the conditioned evoked response was established during hypnosis; it was remarkable that the response appeared earlier and became more rapidly established than in the other subjects.

The 1st, 2nd and 3rd conditioned deflection appearing in response to the conditional stimulus [waves III, V and VII according to the terminology proposed by CIGÁNEK (1961)] were compared in the wakeful and hypnotic state on basis of the frequency of appearance of these waves ($\hat{p}$). The frequency of the "spontaneous" deflections recorded prior to the conditioned response was also established, and by comparing the values for $\hat{p}$ the specific probability ($p_s$) for each wave could be calculated. This latter value is a reliable measure of that particular component of the frequency of appearance of the deflections which was due to the specific stimulus. From the values for $\hat{p}$ angular transformation was done within each group and between the hypnotic and wakeful group; statistical significance of the obtained data was checked by the U-test (P).

The probability that at least one of the conditioned deflections appeared in response to the conditioned stimulus was $p_s = 0.78$ as the average of all the experiments carried out in the wakeful state. In a previous series of experiments this value had been 0.77. At the same time, however, the frequency of the appearance of individual deflections had been somewhat lower than the

values obtained in the present experiments. This was in part ascribed to the circumstance that, in the majority of the test subjects, the present observations constituted a continuation of previous experiments which must have facili-

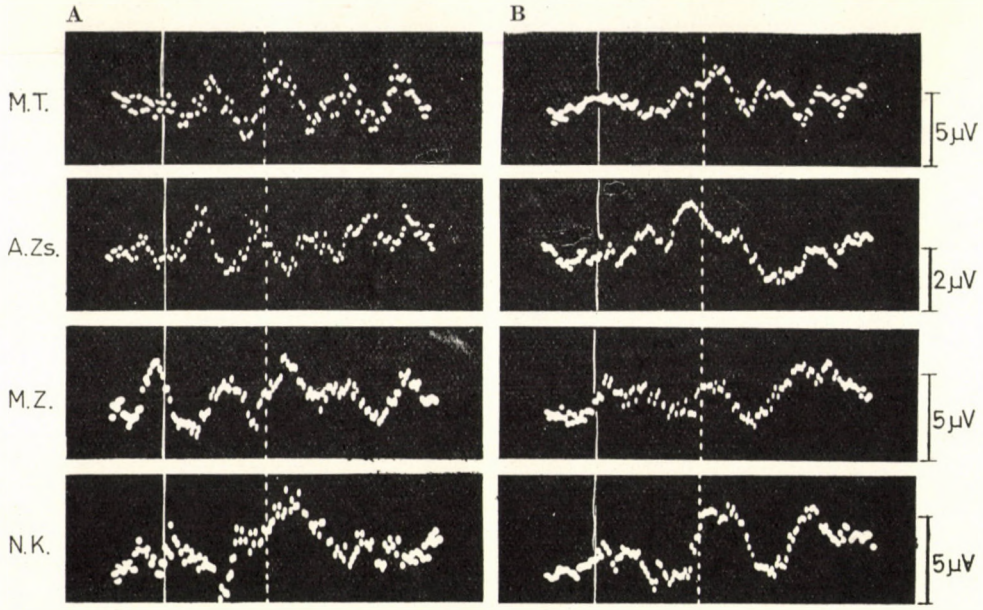



*Fig. 1.* Effect of hypnosis on the conditioned evoked response. The records were obtained in 4 test subjects who were wakeful at the start of the experiment (A) and then were hypnotized and subjected to the isolated auditory (conditioned) stimulus (B). The vertical solid line indicates the time of the auditory stimulus, and the vertical broken line represents the time when the photic stimulus was applied in the course of reinforcements

tated the establishment of the conditioned response; on the other hand, conditioning in the hypnotic state had undoubtedly contributed to the firm establishment of the response. The specific probability of the responses given to isolated auditory stimulation in the hypnotic state, when the subject was instructed: "Take care of the light", was $p_s = 0.99$; the difference as compared to parallel responses obtained in the same series of trials but in the wakeful state, was significant statistically ($P < 0.05$).

Next, the effect of hypnosis was studied on the frequency of appearance of the waves (1st, 2nd and 3rd) constituting the conditioned response, and of the deflection preceding the conditioned evoked potential (x). The frequency rate of waves appearing on the segment of the tracing which showed spontaneous deflections was $\hat{p} = 0.01$ in the hypnotic state and 0.27 in wakefulness. These and further values were calculated only from such experiments in which the trials were done both in the wakeful and hypnotic state in the same test subject, a total of 25 series of 40 being undertaken in the awake subjects and 29 similar series during hypnosis (Fig. 2).

The specific probability of the wave preceding the conditioned response (x) to appear in the record was $p_s = 0.78$ in the wakeful state and 0.84 in the hypnotized subjects; the difference was not significant statistically. The specific probability of the three subsequent waves, however, was considerably increased by hypnosis. The 1st wave appeared with a specific probability of 0.62 in the alert subject, and this value was increased to 0.93 by the hypnotic suggestion; this meant a 50% increase in the frequency of appearance of the response as compared to the wakeful state.

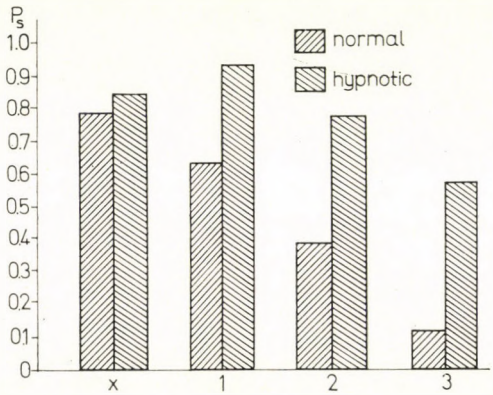



*Fig. 2.* Specific probability of the appearance of the individual components of the conditioned response in wakeful and hypnotized subjects

The value for $p_s$ referring to the 2nd wave of the conditioned evoked response attained 0.38 in the awake subject, and this was increased to 0.77 during hypnosis; the rise was 103%. The greatest difference was recorded with the 3rd wave where the specific probability was 0.11 in the wakeful state and 0.57 after hypnotic suggestion; the increase in this case attained 418%.

The differences in the frequency values ($\hat{p}$) were not significant statistically between the individual waves of the deflection recorded in wakeful and hypnotized subjects. The results allowed the assumption that the differences would have become significant with a sufficiently high number of trials. This was supported by the fact that the difference was significant mathematically if the appearance of at least one of the waves had been considered a positive response.

If the frequency of appearance of the various constituents of the response is analysed within the different series and compared to the appearance of "spontaneous" cortical electrical activity, statistical analysis reveals significant differences in all values except one. In the alert state, the 3rd wave of the conditioned response did not appear significantly more frequently than

the spontaneous deflections in these tracings. In both the wakeful and the hypnotic state, the 1st wave appeared with the highest frequency, whereas the 2nd, and particularly the 3rd waves were recorded less frequently (Table I).

**Table I**

*Values for frequency of appearance ($\hat{p}$) and specific probability ($p_s$) of the individual deflections of the conditioned evoked response in a wake and in hypnotized subjects*

| Deflections | $\hat{p}$ | | $p_s$ | |
|---|---|---|---|---|
| | Awake | Hypnotized | Awake | Hypnotized |
| Spontaneous activity | 0.27 | 0.01 | | |
| x | 0.84 | 0.84 | 0.78 | 0.84 |
| 1st | 0.72 | 0.93 | 0.62 | 0.93 |
| 2nd | 0.55 | 0.78 | 0.38 | 0.77 |
| 3rd | 0.35 | 0.57 | 0.11 | 0.57 |

This difference was apparent also in the significance of the appearance of the waves recorded in the wakeful state when compared to the spontaneous activity, for the value of P in the case of the 1st wave was less than 0.01, for the 2nd wave it was $<0.05$, whereas in the case of the 3rd one statistical significance was not attained. Such a difference between the three waves was absent in the hypnotic group, since the value for P was invariably $\leqslant 0.001$ with all the waves. Remarkably, the frequency of appearance of the wave preceding the conditioned evoked response (x) was higher than the value obtained for any of the other waves and was equal to the specific probability of the overall response when the appearance of any of the waves was considered to mean a positive response ($p_s = 0.78$).

In addition to studying the effect of hypnotic suggestion on the focussing of attention, the influence of other verbal suggestions has also been investigated. In the above discussed experiments the instruction given to the subjects was: "Take care of the light"; in subsequent studies a trial was made on the effect of opposite instructions such as: "There will be no light", on the conditioned evoked potentials. The test subjects said after the experiments that, in contrast to the previous trials where they had always "remembered" the flash, the experience of light had been absent; still, the conditioned response invariably occurred in these experiments, although occasionally with a smaller or higher amplitude (Fig. 3). It should be noted that in these trials the conditioned response was evoked by presenting the conditioned stimulus without stroboscopic flash. The instruction was given 10 times and in one case only was the conditioned response absent in the tracing. After these experiments,

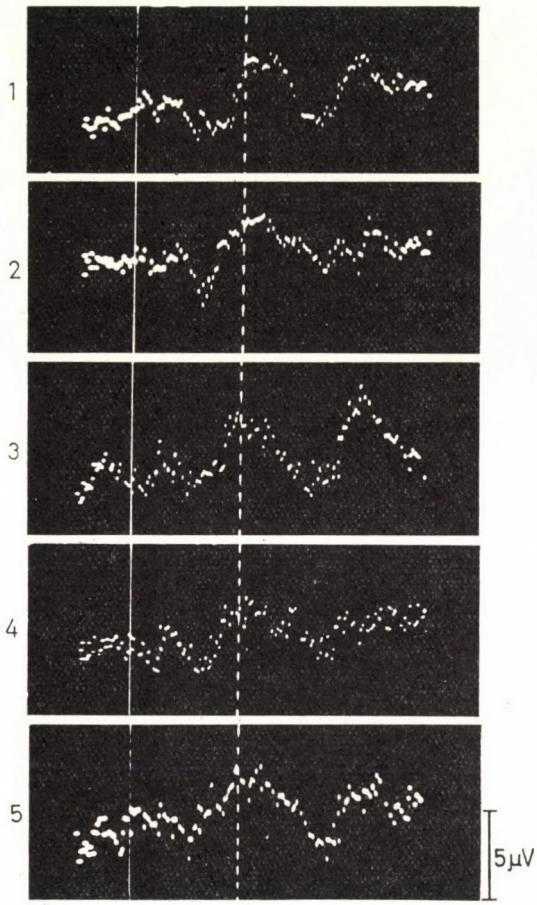



*Fig. 3.* Effect of various hypnotic suggestions on conditioned evoked potentials. In traces 1, 3 and 5 the instruction was "Take care of the light"; in trace 2, "There will be no light"; in trace 4, "You have lost your sense of time, you see the flash either too early or too late". Symbols, as in Fig. 1

the suggestion was given to the subjects: "You have lost your sense of time, you see the flash either too early or too late." After the experiment, the subjects reported that this suggestion had been associated with unpleasant sensations. They had experienced the flash quite clearly, but the lights had appeared irregularly and with variable intensity after the clicks. The amplitude of the conditioned response diminished after such suggestions. Subsequently the suggestion was given: "You have regained your sense of time" which, according to the subjects, had a reassuring effect, and the previous tension vanished. The conditioned response reappeared in these trials. In several experiments the disturbing sensation of tension had to be relieved by suggestion; this, however, did not affect the abolition of the conditioned response.

## Discussion

According to the present findings and to data obtained by others (BECK and DUNSTMAN 1966), hypnosis does not affect the conditioned evoked responses elicited by photic stimuli. At the same time, however, the stability of the conditional response is significantly augmented by hypnosis.

The frequency of appearance of the individual deflections of the conditioned response considerably increased in the hypnotic state. This finding indicates that the significance of all the three phases of the conditioned evoked response obtained with a fixed delay after the presentation of the auditory conditioned stimulus was increased by the concentration of attention during hypnosis. The analysis of specific probabilities of the deflections seems to have confirmed the results obtained in the previous study devoted to conditioned evoked responses in man (MÉSZÁROS and ÁDÁM 1968) according to which the appearance of the 1st deflection (wave III in CIGÁNEK's terminology) is the most important event in the conditioned cortical evoked response.

The fact that the elimination by verbal suggestion of the sensation of light during the trial did not abolish the evoked response but appeared with a somewhat diminished, occasionally also augmented, amplitude indicates that the conditioned evoked potential had been firmly established. This is supported by the finding that on discontinuing the suggestion of the reinforcing stimulus the response was not abolished. It is likely that the changes in the amplitude were due to the circumstance that in these experiments the intensive flash was no more expected by the subjects. The suggestion resulted in a weakening of the attention which presumably led to the diminution of the activity level of the reticular formation. Numerous data are indicating that such changes in the reticular activity may result in both diminution and increase of the amplitude of evoked cortical responses.

SPONG et al. (1965) found that a concentration of attention augments the amplitude of evoked responses. This observation seems to have been supported by TIZARD (1966) who observed an increase in the psychogalvanic reflex when the subject's attention was increasingly foccussed on the experiment. NAQUET and PRUVOT (1956), on the other hand, obtained a diminution of the amplitude of cortical evoked potentials in response to increased reticular activity. It is justified to assume that the changes in postsynaptic activity responsible for the present conditioned evoked potentials are identical with those eliciting the unconditioned cortical response, thus, there is ground for believing that changes in the activity of the reticular formation produce similar alterations of the evoked response, independently whether this is conditioned or unconditioned.

The conditional response has been shown to disappear on verbal suggestion of disturbances in the sense of time. The test subject had the unpleasant

feeling that he did not perceive the usual delay of the photic stimulus, i.e. the rhythm between the click and the stroboscopic flash to which he had been accustomed. The fact that the averaged curve did not show a conditioned evoked response does not necessarily mean that there was absolutely no positive response, since the deflections may have appeared at variable intervals after the auditory stimulus and therefore were not visible in the averaged curve, similarly as spontaneous activity was also screened in the records. This is supported by the finding that the scatter of the base line was greater than usual in these tracings. Obviously, the increase in reticular formation activity which must have followed the hypnotic suggestion, might also have played a significant part in the observed diminution of the response's amplitude. It seems certain, nevertheless, that in the conditioned response established by us in these experiments the time factor (fixed delay), which was interfered with in the above discussed trials, played the most important part, and this factor can be influenced by hypnotic suggestion.

## LITERATURE

ÁDÁM, G., MÉSZÁROS, I.: The role of midbrain reticular formation in visceral afferentation. Excerpta med. Int. Congr. Ser. No. 48, **1962.**

ÁDÁM, G., KUKORELLI, T.: Conditioning of evoked potentials: an experimental model of learning. Acta physiol. Acad. Sci. hung. Suppl. **1964,** 24, 17.

BECK, E. C., DUNSTMAN, R. E.: Hypnotic suggestions and visually evoked potentials. Electroenceph. clin. Neurophysiol. **1966,** 20: 396—400.

CIGÁNEK, L.: Die Elektroencephalographische Lichtreizantwort der menschlichen Hirnrinde. Verlag der Slowakischen Akademie der Wissenschaften, Bratislava **1961.**

MÉSZÁROS, I., ÁDÁM, G.: Conditioned cortical evoked potentials in man. Acta physiol. Acad. Sci. hung. **1968,** 34: 137—146.

NAQUET, R., PRUVOT, PH.: Essai d'interprétation de quelques variations des réponses EEG induites par la stimulation lumineuse intermittente chez l'homme. C. R. Soc. Biol. (Paris) **1956,** 150: 1232—1233.

SPONG, P., HAIDER, M., LINDSLEY, D. B.: Selective attentiveness and cortical responses to visual and auditory stimuli. Science **1965,** 1948: 393—397.

TIZARD, B.: Repetitive auditory stimuli and the development of sleep. Electroenceph. clin. Neurophysiol. **1966,** 20: 112—121.

István MÉSZÁROS
Orvostudományi Egyetem Élettani Intézete
Budapest VIII., Puskin u. 9, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 109—116 (1969)*

# MORTALITY OF VARIOUS AVIAN AND MAMMALIAN SPECIES FOLLOWING BLOOD LOSS

By

A. G. B. Kovách, Erzsébet Szász and N. Pilmayer

EXPERIMENTAL RESEARCH DEPARTMENT, UNIVERSITY MEDICAL SCHOOL,

AND DEPARTMENT OF ANIMAL PHYSIOLOGY, LORÁND EÖTVÖS UNIVERSITY, BUDAPEST

(Received August 22, 1968)

The response to blood loss has been studied in the pigeon, duck, hen, crow, pheasant, rat, cat and dog under general anaesthesia. In every species the blood loss amounted to 1% body weight per hour.

The species tested were found to differ in tolerance of blood loss. The pigeon and the duck are resistant, the hen and the crow occupy an intermediate position, and the pheasant together with the mammals belongs in the sensitive group. Dibenamine has no influence on the resistance to blood loss in the pigeon. Dibenamine (20 mg/kg) pretreatment does not block the pressor response to adrenaline and noradrenaline in the pigeon. After carotid clamping, as a result of the so-called "cerebral ischaemic reflex", a significant rise in blood pressure occurs after bleeding in the pigeon and the crow. No pressor response to carotid clamping could be observed in the hen.

It has been shown (Kovách and Szász 1969) that pigeons were more resistant to haemorrhage than mammals; some pigeons survived a slow blood loss of 8 ml per 100 g, one equal to their total blood volume. The calculated residual blood volume was significantly less than that compatible with the survival of mammals.

These investigations have now been extended to other birds and the resistance to blood loss has been compared under similar conditions in birds and mammals. Particular interest has been paid to the behaviour of flying, domestic and diving birds.

## Methods

The birds involved in the experiments were pigeons, ducks, hens, crows and pheasants; the mammals were rats, cats and dogs. The tests for tolerance of blood loss were done under general anaesthesia in each species.

*Pigeons*, a total of 30, of either sex, from a mixed stock with an average body weight of 350 g, were anaesthetized with 1 g/kg of urethane intramuscularly, treated with 0.2 ml/100 g of heparin and bled from a cannula inserted into a carotid artery. The blood loss, amounting to 1% body weight per hour, totalled 2 to 10% of the body weight.

*Crows*, a total of 19, of either sex and different ages, weighing 300 to 420 g, caught in fields and kept for unknown periods of time in cages before the experiment, were anaesthetized with 40 mg/kg of chloralose[1] injected into a wing vein, treated intravenously with 0.5 ml of heparin,[2] were bled from a carotid artery. The first bleeding amounted to 2% of the body weight. This was followed by blood losses amounting to 1% body weight at 1 hour intervals.

[1] α-D(+)-Glucochloralose reinst. E. Merck, Darmstadt.
[2] Heparin: Gedeon Richter A.G., Budapest.

*Hens*, a total of 43, farm-grown, 1500 to 2600 g in weight, were anaesthetized with 40 mg/kg of chloralose injected into a wing vein and treated with 1 ml of heparin intravenously, were bled through a polyethylene cannula inserted into a carotid artery. The blood loss amounted to 1% of the body weight per hour. In another group of 16 hens the interval between bleedings was merely 15 minutes.

*Pheasants*, a total of 27, of both sexes weighing 800 to 1200 g, raised in the Zoological Garden, were anaesthetized with 40 mg/kg of chloralose intravenously, treated with 0.5 ml/kg of heparin, were bled from the carotid, letting every hour 1% body weight of blood.

*Ducks*, a total of 31, of both sexes, weighing 2500 to 3300 g, obtained from a farm, were anaesthetized with 30 to 40 mg/kg of chloralose intravenously and bled from the cannulated wing artery, withdrawing 1% of body weight of blood per hour until death had ensued.

*Rats*, a total of 39 of both sexes weighing 150 to 220 g of our own stock were anaesthetized with 100 to 150 mg/100 g of urethane and without heparinization bled from the tail vein at a rate of 1% body weight per hour.

*Cats*, a total of 43, of both sexes, 2.8 to 3.0 kg in weight under 100 mg chloralose + 200 mg/kg urethane anaesthesia and 0.5 ml/kg heparinization were bled from the common carotid at a rate of 1% body weight per hour.

*Dogs*, a total of 56, of either sex, weighing 11 to 14 kg, were anaesthetized with 100 mg/kg of chloralose, heparinized (0.2 ml/kg) and bled from the femoral artery at a rate of 1% body weight per hour.

In pigeons, hens and crows in the normotensive and in the posthaemorrhagic hypotensive state the carotid was clamped for 30 seconds to evoke the so-called "cerebral ischaemic reflex" and then the changes in arterial pressure were studied. In the crows their only carotid artery, in pigeons and hens after ligation of one carotid the other was clamped.

In a group of 11 pigeons 20 mg/kg of Dibenamine was injected intravenously at a slow rate and 2 or 3 hours later the birds were tested for the pressor response to adrenaline; comparing the result with their pre-Dibenamine response in this group the effect of Dibenamine on survival following blood loss was also studied.

Statistical evaluation was done by Student's *t*-test (HETÉNYI, 1954).

## Results

The effect of bleeding in the different species are shown in Table I and Fig. 1.

Bled at the slow rate of 1% body weight per hour, dogs began to die after 3% blood loss. Only 31 out of 56 survived a 4% blood loss, 5% blood loss killed 75%, 6% blood loss 91% of the animals. A loss of 7% body weight of blood killed the survivors within a few minutes.

As illustrated in Table I and Fig. 1, cats and rats are more sensitive to blood loss than dogs. Even the 2% loss began to kill the former and all cats died after 6%, and all rats after 5% blood loss.

Like in the previous studies, pigeons significantly differed in behaviour. Of the 30 pigeons tested 1 died 4 hours after having lost 4% body weight of blood. After 6% blood loss the mortality rate was 17%. At 7 hours 5 more birds (altogether 33%) died. An 8% loss killed 60% of the 30 birds but 12 were still alive at that time. Of these, the loss of 9% body weight of blood killed 10 pigeons, and 2 survived even a 10% loss. As seen in Table I, the sensitivity to blood loss did not change on treatment with 20 mg/kg of Dibenamine. The blood pressure elevation induced by adrenaline and noradrenaline (2.5 to 5 $\mu$g/kg) was not influenced by 20 mg/kg of Dibenamine. Blood loss tolerance of the ducks resembled that of pigeons. The loss of 4% body weight of blood

killed 2; a 5% loss, fatal for mammals, was survived by 84% of the ducks. A 6% loss killed 4, one of 7%, 7 birds, 52% of the total number of ducks. Blood loss amounting to 8% of the body weight killed 74%, and shortly after the 9% loss all ducks died. Thus, the difference from mammals was marked.

The crows and hens showed similar results (Table I). In these two species the sensitivity to bleeding varied greatly. Some birds behaved in this respect like mammals, others like the ducks and pigeons. The rapidly bled group of hens, with only 15-minute intervals between the 1% losses, died in the same way as the mammals.

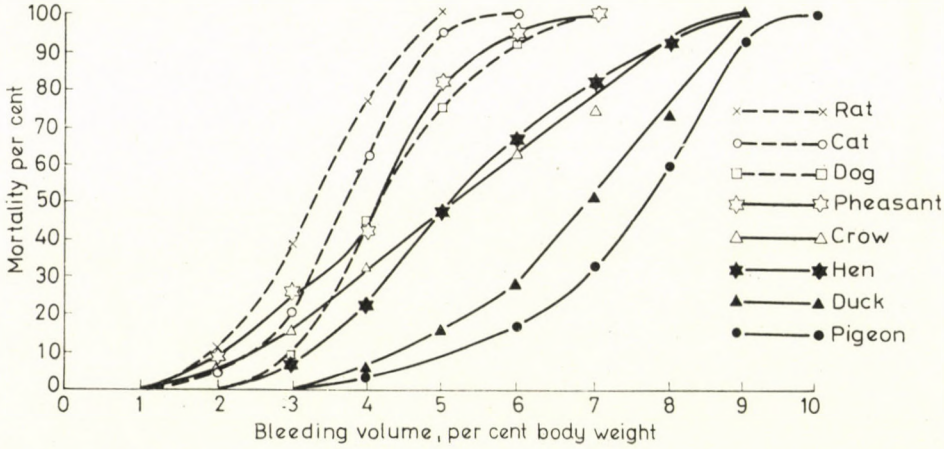



*Fig. 1.* Mortality after identical blood losses in various avian and mammalian species (abscissa). Every hour 1% body weight of blood was withdrawn from every animal (ordinate). The percentage of animals lost during the hour following bleeding has been recorded and plotted

Pheasants even when bled slowly did not tolerate blood loss better than the mammals, differing in this respect from the other bird species tested.

In Table II is presented the blood pressure response to carotid clamping before and after bleeding, in pigeons, crows and hens. In the pigeons, arterial pressure increased from the pre-bleeding value of 96 $\pm$ 7 mm Hg to 113 mm Hg. On bleeding, arterial blood pressure decreased gradually but its increase in response to carotid clamping became more marked; after 2% loss the rise amounted to 26 mm Hg, after 4% loss to 33, after 6% to 39, and after 7% loss to 63 mm Hg. On continued bleeding the pressor response to carotid clamping did not increase further. Owing to the decrease in the number of surviving birds, the increase was not significant statistically but was demonstrable in every case. The tabulated data show that the maximum arterial blood pressure evoked by carotid clamping was the same before and after bleeding.

**Table I**

*Mortality rate of various species following blood loss of different extent.*

| Bleeding volume, in per cent of body weight | Absolute and percentual mortality | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pigeon | | Crow | Pheasant | Hen 15′ | Hen 1 hour | Duck | Rat | Cat | Dog |
| | Control | Diben-amine | | | | | | | | |
| 1 | — | — | — | — | — | — | — | — | — | — |
| 2 | — | 1 (9.1) | 1 (5.3) | 2 (8.7) | 1 (6.3) | — | — | 4 (10.3) | 2 (4.6) | — |
| 3 | — | — | 2 (16) | 4 (26) | 3 (25) | 2 (7.4) | — | 11 (39) | 7 (21) | 5 (8.9) |
| 4 | 1 (3.3) | — | 3 (32) | 4 (43) | 5 (56) | 6 (22) | 2 (6.4) | 15 (77) | 18 (63) | 20 (45) |
| 5 | — | — | 3 (48) | 9 (82) | 4 (81) | 7 (48) | 3 (16) | 9 (100) | 14 (95) | 17 (75) |
| 6 | 4 (17) | 1 (18) | 3 (63) | 3 (95) | 2 (94) | 5 (67) | 4 (29) | 0 | 2 (100) | 9 (91) |
| 7 | 5 (33) | 2 (36) | 2 (74) | 1 (100) | 1 (100) | 4 (82) | 7 (52) | 0 | 0 | 5 (100) |
| 8 | 8 (60) | 1 (45) | 4 (95) | 0 | 0 | 3 (93) | 7 (74) | 0 | 0 | 0 |
| 9 | 10 (93) | 1 (55) | 1 (100) | 0 | 0 | 2 (100) | 8 (100) | 0 | 0 | 0 |
| 10 | 2 (100) | 5 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 30 | 11 | 19 | 23 | 16 | 27 | 31 | 39 | 43 | 56 |

Per cents in brackets.

**Table II**

*Blood pressure response to carotid clamping following different grades of blood loss in the pigeon, crow and hen*

| Blood loss, in per cent of body weight | Pigeon | | | | Crow | | | Hen | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of cases | Arterial blood pressure, mm Hg (± standard deviation) | Increase of blood pressure after carotid clamping, mm Hg | Statistical evaluation, P % | Number of cases | Arterial blood pressure, mm Hg | Increase of blood pressure after carotid clamping, mm Hg | Number of cases | Arterial blood pressure, mm Hg | Increase of blood pressure after carotid clamping, mm Hg |
| 0 | 11 | 96 ± 7 | 17 ± 5 | 0.1— 1 | 16 | 107 ± 11 | 13 ± 8 | 17 | 120 ± 12 | 2 |
| 1 | — | — | — | — | — | — | — | 17 | 99 ± 23 | 0 |
| 2 | 10 | 76 ± 9 | 26 ± 7 | 0.1— 1 | 11 | 75 ± 8 | 29 ± 11 | 14 | 84 ± 19 | 0 |
| 3 | — | — | — | — | 11 | 75 ± 7 | 26 ± 8 | 14 | 69 ± 11 | —2 |
| 4 | 9 | 65 ± 4 | 33 ± 11 | 1 — 2 | 8 | 55 ± 3 | 21 ± 7 | 12 | 63 ± 10 | —1 |
| 5 | — | — | — | — | 4 | 54 ± 7 | 11 ± 3 | 8 | 64 ± 8 | 0 |
| 6 | 8 | 54 ± 6 | 39 ± 13 | 1 — 2 | — | — | — | 2 | 81 | 0 |
| 7 | 8 | 52 ± 8 | 63 ± 18 | 1 — 2 | — | — | — | 1 | 78 | 0 |
| 8 | 7 | 55 ± 7 | 37 ± 17 | 5 —10 | — | — | — | — | — | — |
| 9 | 4 | 52 ± 3 | 44 ± 14 | 5 | — | — | — | — | — | — |

In the crows arterial pressure responded to carotid clamping similarly as in the pigeons, except that after 4 to 5% blood loss the pressor response was less marked than in pigeons (Table II).

In hens carotid clamping evoked no pressor response either in the control period or after the bleeding (Table II).

## Discussion

It has been shown that the species tested varied greatly in tolerance of blood loss. With the exception of the duck, all birds tolerated blood loss much less than pigeons. In the case of hens and crows the intermediate position may be due to their life conditions. The domesticated hen may be classified as a non-flying bird. The great individual differences among crows may have been due to the fact that these were captive birds and differed greatly in age. Experience has shown that crows did not tolerate life in a cage. As the interval between catching and experimentation varied, some of the crows might have been underfed, weakened and less resistant to blood loss.

Pheasants, closely related to hens, behaved similarly to mammals, presumably because they had been raised in the zoo, had lived under aphysiological conditions for generations and had lost their original resistance. The duck, next in resistance to the pigeon, is a diving bird and this circumstance may explain its increased resistance. It is not clear what accounts for the differences in resistance between the various birds. Experience has shown so far that the flying or diving birds have increased resistance to blood loss. It is, however, possible that this difference in homeostatic capacity is due to some other factor, viz.: a) a difference in initial blood volume; b) a redistribution of flow; c) increased replacement of blood volume by haemodilution; and d) resistance to hypoxia.

In a previous paper (Kovách and Szász, 1969) the problem of the birds' blood volume has been discussed in detail. According to Bond (1958) in certain birds circulating blood volume is higher than in mammals; in the pigeon it is $9.2 \pm 0.3$ ml/100 g. Anderson (1966) described the blood volume of pigeons to be 7.0 ml/100 g and presents data also for other species. Potsubay and Duduk (1966) found 7.07 to 9.54 ml/100 g for chicks weighing 800 to 1200 g. Bond (1958) found lower values in hens and pheasants. It is unclear whether the adaptation to flying or diving is associated with a high blood volume. In any case, a difference in blood volume between birds and mammals does not yet suffice to explain the increased resistance to blood loss.

Significant differences have been found in the effect of carotid clamping. Pigeons and crows displayed a pressor response while the hens failed to do so, in agreement with the observation of Van der Linden (1934) who found no

pressor response to carotid clamping in the hen. In birds the pressor receptor zone, corresponding to the carotid sinus of mammals, is in the chest, near to the origin of the common carotid (HEYMANS and NEIL, 1958; ADAMS, 1959) thus before the clamped site. VAN DER LINDEN (1934) and ARA (1934) clamped the carotid behind the pressor receptor zone and obtained a reflex hypertension that was less pronounced than in mammals. The evoked pressor effect is presumably not the carotid sinus reflex, but the acute bulbar ischaemia described in 1934 by VAN DER LINDEN, or the cerebral ischaemic reflex described by SAGAWA et al. (1961) as well as GINNIS and RINGER (1965). This reflex may be elicited in the flying pigeon and crow, but not in the non-flying hen; the difference may be due to differences in cerebral blood supply (TERNI, 1931; MURATORI, 1937).

The maximum rise in blood pressure induced by carotid clamping in the pigeon was independent of the actual blood pressure. A lower level of arterial blood pressure was associated with an increased cerebral ischaemic pressor response. Our observations indicated that after bleeding the cerebral blood supply is sufficient even at the 30 mm Hg arterial blood pressure level, because this alone does not evoke the cerebral ischaemic reflex, while simultaneous carotid clamping elicits it at once. According to our findings the pigeon does not make full use of the possibilities of vasoconstriction in posthaemorrhagic hypotension. It is unclear what role this phenomenon plays in the regulation of circulation, especially in cerebral blood flow, in birds during flying.

In the pigeon Dibenamine failed to inhibit the vasoconstriction induced by adrenaline or noradrenaline, i.e. the alpha receptor stimulating effect of these drugs. According to THOMPSON and COON (1948), Dibenamine does not block the pressor effect of adrenaline in the chick either, in contrast to the other adrenolytics such as dihydroergotamine or tolazoline which exert an inhibitory effect. In contrast to this, BARGER and DALE in 1907 found a response to ergot alcaloids similar to that to Dibenamine. It is not known whether this lack of a pressor blocking effect of Dibenamine is characteristic of the other avian species and there are no data concerning the physiological significance or mechanism of the difference demonstrable between fowls and mammals.

According to STEKIEL et al. (1967), phenoxybenzamine does not influence plasma volume in the normal dog, but after bleeding it increases the mobilization of fluid into the blood stream. In our pigeons Dibenamine pretreatment did not influence the outcome of shock at a uniform rate of bleeding. This may be explained by assuming that the homeostatic fluid mobilization capacity is more active in the pigeon than in mammals, so that these birds escape that very situation in which Dibenamine could exert its action. On the other hand, similarly to what has been observed in the absence of the adrenaline pressor blocking effect, Dibenamine might have no adrenolytic effect in the pigeon.

## LITERATURE

ADAMS, W. E.: The comparative morphology of the carotid body and carotid sinus. Thomas, Springfield **1959.**

ANDERSEN, H. T.: Physiological adaptations in diving vertebrates. Physiol. Rev. **1966,** 46: 212—243.

ARA, G.: Il reflesso di Pagano-Hering nel pollo. Arch. Fisiol. **1934,** 33: 332—336.

BARGER, G., DALE, H. H.: Ergotoxine and some other constituents of ergot. Biochem. J. **1907,** 2: 240—299.

BOND, F. Ch.: Comparative study of blood volume in representative aquatic and nonaquatic birds. Amer. J. Physiol. **1958,** 194: 519—521.

DALE, H. H.: On some physiological actions of ergot. J. Physiol. (Lond.) **1906,** 34: 163—206.

GINNIS, C. H. jr., RINGER, R. K.: Arterial occlusion and baroreceptors in the chicken. Fed. Proc. **1965,** 24: 524.

HETÉNYI, G.: Kísérleti eredmények statisztikai értékelése. In: KOVÁCH, A. G. B. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1954,** Vol. 2. Pp. 665—700.

HEYMANS, C., NEIL, E.: Reflexogenic areas of the cardiovascular system. Churchill, London **1958.**

KOVÁCH, A. G. B., SZÁSZ, Erzsébet: Survival of pigeon after graded haemorrhage. Acta physiol. Acad. Sci. hung. **1969.** In press.

MURATORI, G.: Contributi morfologici allo studio dei recettori aortico-arteriosi dei reflessi cardiopressoregulatori. Arch. ital. Anat. Embriol. **1937,** 38: 387—405.

POTSUBAY, J., DUDUK, V.: Determination of blood volume with T-1824 and degree of exsanguination in growing chickens. Acta vet. Acad. Sci. hung. **1966,** 16: 373—382.

SAGAWA, K., ROSS, J., GUYTON, A. C.: Quantitation of cerebral ischemic pressor response in dogs. Amer. J. Physiol. **1961,** 200: 1164—1168.

STEKIEL, W. J., LOGIC, J. R., ERDÉLYI, A., ROZEK, L. F.: Effect of phenoxybenzamine on plasma volumes during hemorrhagic shock. Amer. J. Physiol. **1967,** 213: 1089—1094.

TERNI, T.: Il simpatico cervicale degli Amniotti (Ricerche di morfologia comparata). Z. Anat. Entwickl. Gesch. **1931,** 96: 289—426.

THOMPSON, R. M., COON, J. M.: Effect of adrenolytic agents on the response to pressor substances in the domestic fowl. Fed. Proc. **1948,** 7: 259—260.

VAN DER LINDEN, P.: Recherches sur les réflexes sinocarotidiens chez les oiseaux. Arch. int. Physiol. **1934,** 11: 59—82.

A. G. B. KOVÁCH, Erzsébet SZÁSZ, N. PILMAYER
Experimental Research Dept., University Medical School,
Budapest VIII., Üllői út 78/a, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 117—132 (1969)*

# CARDIORESPIRATORY RESPONSES TO GRADED POSITIVE-PRESSURE BREATH HOLDING, I.

By

V. GRUBICH, J. MALOMSOKI and M. NEMESSURI

NATIONAL INSTITUTE FOR MEDICINE OF PHYSICAL EDUCATION AND SPORTS, BUDAPEST

(Received October 6, 1968)

Graded increase of intrapulmonic pressure elicited by positive-pressure breath holding (PPBH) has been found to cause general as well as organ-specific cardiorespiratory changes in sportsmen. Depending on the pressure of forced expiration, heart rate increased slightly immediately before and more markedly during PPBH. After cessation of the PPBH a bradycardia irrespective of the degree of exertion ensued, then heart rate normalized as if by counterregulation. This response was the most subtly differentiated of all in this study. In spite of that, the response occurred in only 3 or 4 steps, though static work load, *i.e.* PPBH had been increased in 6 steps. This manifested itself also in the duration of the cardiac cycle. The shortening of the cardiac cycle during PPBH and the rebound-like prolongation of it observed after cessation of PPBH maneuvre, seem to have been due mostly to changes in the duration of the electric diastole, although the contribution of electric systolic time was statistically significant, too.

A similar dynamism was observed in the changes of blood pressure and gas exchange; the 6-step work load again caused a response which occurred in fewer steps. The smallest changes were observed in diastolic pressure. The reaction of the systolic an diastolic pressures was made up of 3 distinct phases. As the pressure of forced expiration was increased, the counterregulation following the PPBH ensued with successively increasing delay and intensity. The increase in respiratory gas exchange in response to the growing load was of nearly equal extent in the initial 4 phases of exertion, but it rose considerably and much steeper during the subsequent phases. By increasing intrapulmonic pressure a larger fraction of excess oxygen uptake fell to the period immediately preceding the maneuvre, whereas excess carbon dioxide release, which during great exertions increased steeper than the curve of oxygen uptake, could not be accounted for merely by the energy cost of PPBH. The respiratory response consisted similarly of only 3 phases. Thus, static neuromotor gradation was more differentiated than the responses observed in the cardiorespiratory system.

Positive-pressure breath holding (PPBH) is a type of static work, and as such, it is known to give rise to an overall increase in cardiovascular activity. There is a relationship in the degree of the increase between motor and vegetative functions. The question arose whether and to what extent the cardiorespiratory parameters such as heart rate, duration and various components of the electric cardiac cycle, blood pressure and respiratory gas exchange rate, follow the pattern of a strictly established PPBH maneuvre.

It has been assumed that 1. in addition to the general control of cardiorespiratory functions, there exists a specific regulation the observed responses of which differ more or less according to the organ involved; 2. regulation of the cardiac cycle responds more sensitively and corresponds better to motor

activation than the regulation of blood pressure and gas exchange; and finally 3. sensomotor activation will be graded more sensitively than cardiorespiratory response.

## Methods

Fifteen sportsmen aged 17 to 20 years performed PPBH maneuvre in a stepwise fashion, increasing the pressure with 20 milliatmosphere (mat) above atmospheric pressure in each successive stage. After a rest of 3 min, the subjects maintained an expiratory pressure corresponding to 20, 40, 60, 80, 100 and 120 mat, resp., for periods of 20 sec each; one experimental period was 3 min in duration.

In another series of experiments 6 sportsmen of the same age performed the maneuvre by elevating the intrapulmonary pressure first to 80 and then to 100 mat; the recovery period between the two performances was 7 min which allowed complete restoration of the previous metabolic rate. After the last forcible expiration the subjects held their breath for a period of 20 sec.

Heart rate and the duration of the various phases of the cardiac cycle were determined by making use of the second standard lead ECG. Blood pressure was measured at 20-sec intervals. A rubber mask was fixed on the face and gas exchange rate was determined by a Zeiss interferometer. The amount of oxygen and carbon dioxide in the expired air was calculated from the percentage of the respective gases (Method of WOLLSCHITT et al. 1935, modified by MALOMSOKI, 1963). The volume of expired air was indicated by a Dräger Volumeter. Pressure in the lungs was kept above the atmospheric level by a spirotonometer (GRUBICH 1955, 1958). A special mouthpiece, which could be changed and easily sterilized was constructed (GRUBICH and VÁRADI, unpublished). This consisted of a nickel-plated metal mouthpiece mounted on a tube; it could be turned to and from the mouth without interfering with the function of the valves through which the subjects were breathing.

Values for gas exchange rate were read at every 30 sec, whereas the values immediately preceding the maneuvre refer to a period of 40 sec, since the 20 sec of PPBH would have rendered it difficult to make determinations during the remaining 10 sec. Thus, the interval between two maneuvres was exactly 2 min and 40 sec, and this together with the PPBH maneuvre of 20 sec made up periods of exactly 3 min.

The obtained results were analysed statistically, using Student's *t*-test.

## Results

### *1. Heart rate*

The resting value of heart rate was 86/min on the average, presumably owing to the load caused by the breathing mask (Table I). Just before beginning the maneuvre heart rate increased to 93/min ("start" condition). When intrapulmonary pressure was increased by 20 mat, an average heart rate of 89/min was obtained, which was higher than that at rest but somewhat lower than the value observed immediately before the experiment. As the pressure of expiration was gradually increased, the heart rate during the maneuvre increased further, successively attaining an average of 135/min. The first measured values in the phase of recovery were remarkably uniform in the different subjects, the difference between the highest and lowest value being only 4 beats. In the first 30 sec of recovery after 40 and 80 mat the heart rate diminished to 83/min (control 86/min). In the latter case a further decrease

**Table I**

*Heart rate during and after PPBH*
(Mean ± S.D.)

| Intrapulmonary pressure (mat) | During | After | | | | |
|---|---|---|---|---|---|---|
| | | 30 sec | 60 sec | 90 sec | 120 sec | 160 sec |
| — | — | — | 86 ± 13 | — | 86 ± 9 | 93 ± 10 |
| 20 | 89 ± 13 | 86 ± 11 | 84 ± 11 | 86 ± 8 | 87 ± 12 | 92 ± 13 |
| 40 | 98 ± 15 | 83 ± 11 | 83 ± 12 | 85 ± 13 | 87 ± 12 | 95 ± 10 |
| 60 | 106 ± 14 | 85 ± 6 | 84 ± 10 | 86 ± 14 | 84 ± 12 | 91 ± 13 |
| 80 | 111 ± 19 | 83 ± 11 | 81 ± 10 | 84 ± 10 | 88 ± 10 | 93 ± 14 |
| 100 | 115 ± 13 | 87 ± 12 | 83 ± 18 | 82 ± 8 | 88 ± 14 | 111 ± 15 |
| 120 | 135 ± 5 | 87 ± 7 | 86 ± 7 | 95 ± 8 | 97 ± 10 | 98 ± 8 |
| Increase during maneuvres | Rest—20 mat | 20—40 mat | 40—60 mat | 60—80 mat | 80—100 mat | 100—120 mat |
| | 3 | 9 | 8 | 5 | 4 | 20 |
| | 0.001 > P | 0.001 > P | 0.001 > P | 0.20 > P > 0.10 | 0.50 > P > 0.20 | 0.001 > P |

to 81/min was recorded by the end of the first minute of recovery. Even when intrapulmonary pressure was maintained at 100 mat, heart rate was 82/min one and half min after cessation of the forced expiration. After this initial depression heart rate again increased prior to the next maneuvre exceeding considerably the resting level. The differences between the values recorded during PPBH were mostly significant statistically.

The duration of the electric systole and diastole of the successive periods of 20 sec prior to, during and after the PPBH maneuvre was measured in 8 subjects. The obtained data are shown in Table II.

There was no substantial difference in the duration of the cardiac electric cycle during the 20-sec periods preceding the forced expiration at different intrapulmonary pressures: prior to the 20 mat maneuvre mean cycle duration was 619 msec, it varied between 651 and 638 before forced expiration against up to 100 mat and decreased to 542 msec only before the last trial of 120 mat; this diminution was obviously due to preceding static muscular work. Though work load was graded in 6 steps, the changes in the duration of the cardiac cycle displayed only a 3-step response. The values observed during PPBH were 665 and 640 during 20 and 40 mat, 569 and 542 during 60 and 80 mat, and 448 and 443 during 100 and 120 mat, respectively. In the recovery period following the maneuvre the duration of the heart cycle showed a graded increase from 685 to 787 msec between 20 and 80 mat. This tendency was observed also after 100 and 120 mat PPBH, the cardiac cycle during exertion became, however, so short that the subsequent increase was relatively smaller than in the case of smaller pressure loads. Thus, the cardiac cycle as compared to previous values was apparently prolongated at each of the successive pressure steps.

Duration of the electric diastole in the periods preceding forced expiration varied between 291 and 302 msec in 4 of the 6 stages of the experiment, but it was 260 msec before the maneuvre when intrapulmonary pressure was increased to 20 mat, and 219 msec with the largest pressure load (120 mat). During the PPBH maneuvre electric diastole became longer in response to the lowest pressure value, it did not change when an overpressure of 40 mat was applied, and became successively shorter and shorter when the pressure load was further increased. In comparison to the duration at rest the respective changes in the diastole times were: +44, −2, −50, −78, −106 and −37 msec. Before 120 mat the diastole was rather short (219 msec), this is the reason why the subsequent shortening was small. Following the maneuvre prolongation of the electric diastole was insignificant at the lowest pressure value, but at the higher levels of positive pressures it became more and more marked.

Mean duration of the electric systole preceding the maneuvre showed a progressive diminution with increasing intrapulmonary pressures. During forced expiration a slight prolongation of 26 msec was recorded when over-

**Table II**

*Duration of cardiac cycle, electric diastole and systole (msec) in response to graded PPBH maneuvre*
(b, before; d, during; a, after)

| Intrapulmonary pressure (mat) | | RR | | TQ | | QT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | $Mean_b - Mean_d$ | P | $Mean_b - Mean_a$ | P |
| | b | 619 | 97.0 | 260 | 40.7 | 357 | 56.0 | 26 | 0.001 > P | | |
| 20 | d | 665 | 227.6 | 304 | 101.8 | 383 | 128.3 | | | −5 | |
| | a | 685 | 79.0 | 314 | 37.3 | 378 | 43.6 | | | | P > 0.05 |
| | b | 651 | 79.1 | 299 | 36.3 | 357 | 43.4 | −18 | 0.05 > P > 0.02 | | |
| 40 | d | 640 | 261.8 | 297 | 121.5 | 339 | 138.7 | | | 27 | |
| | a | 725 | 189.3 | 364 | 95.0 | 366 | 95.6 | | | | 0.001 > P |
| | b | 640 | 100.2 | 291 | 45.5 | 350 | 54.8 | −20 | 0.001 > P | | |
| 60 | d | 569 | 88.2 | 241 | 37.3 | 330 | 51.2 | | | 18 | |
| | a | 735 | 70.3 | 379 | 36.2 | 348 | 33.3 | | | | 0.001 > P |
| | b | 638 | 168.8 | 302 | 79.9 | 354 | 93.7 | −38 | 0.001 > P | | |
| 80 | d | 542 | 114.4 | 224 | 47.2 | 316 | 66.7 | | | 34 | |
| | a | 787 | 381.0 | 429 | 208.0 | 350 | 169.7 | | | | 0.001 > P |
| | b | 638 | 252.0 | 301 | 118.8 | 339 | 133.9 | −27 | 0.001 > P | | |
| 100 | d | 448 | 121.1 | 195 | 52.7 | 312 | 84.4 | | | 49 | |
| | a | 687 | 86.3 | 331 | 41.6 | 361 | 45.4 | | | | 0.001 > P |
| | b | 542 | 50.2 | 219 | 20.3 | 328 | 30.4 | −34 | 0.001 > P | | |
| 120 | d | 443 | 66.9 | 189 | 27.5 | 294 | 44.4 | | | 23 | |
| | a | 714 | 97.7 | 392 | 53.6 | 317 | 43.4 | | | | 0.001 > P |

**Table III**

*Systolic blood pressure in response to PPBH maneuvre*

| Intrapulmonary pressure (mat) | During maneuvre | | After maneuvre | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 20 sec | | 40 sec | | 60 sec | | 80 sec | | 100 sec | | 120 sec | | 140 sec | | 160 sec | |
| | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| — | — | — | 118 | 8 | — | — | — | — | 118 | 10 | — | — | — | — | 114 | 11 | — | — |
| 20 | 124 | 10 | 117 | 12 | 115 | 9 | 113 | 11 | 111 | 9 | 113 | 10 | 114 | 8 | 110 | 8 | 113 | 8 |
| 40 | 126 | 7 | 117 | 9 | 113 | 8 | 110 | 4 | 114 | 8 | 112 | 9 | 113 | 9 | 112 | 6 | 112 | 6 |
| 60 | 135 | 12 | 120 | 12 | 116 | 8 | 114 | 9 | 114 | 9 | 114 | 8 | 112 | 9 | 114 | 16 | 114 | 7 |
| 80 | 148 | 10 | 129 | 14 | 121 | 12 | 114 | 4 | 113 | 5 | 113 | 7 | 113 | 5 | 113 | 8 | 116 | 5 |
| 100 | 150 | 10 | 133 | 13 | 124 | 9 | 117 | 10 | 116 | 10 | 117 | 11 | 116 | 11 | 117 | 9 | 115 | 14 |
| 120 | 151 | 14 | 134 | 12 | 124 | 4 | 116 | 8 | 11 | 9 | — | — | — | — | — | — | — | — |

| Increase during maneuvre | Rest—20 mat | 20—40 mat | 40—60 mat | 60—80 mat | 80—100 mat | 100—120 mat |
|---|---|---|---|---|---|---|
| | 10 | 2 | 9 | 13 | 2 | 1 |
| | $0.001 > P$ | $0.50 > P > 0.20$ | $0.01 > P > 0.001$ | $0.001 > P$ | $0.10 = P$ | $P > 0.50$ |

pressure was 20 mat, and a statistically significant decrease was noted in each subsequent step compared to the preceding value. The values obtained after the maneuvre became slightly shorter after the first load (20 mat), while a progressive prolongation was recorded after successively increasing pressure loads.

### 2. *Blood pressure*

Mean systolic pressure prior to forced expiration diminished or remained unchanged on subsequent trials (Table III). The values obtained at the end of each maneuvre, on the other hand, showed a progressive increase. Counter-regulation could clearly be recognized after each pressure level. This appeared within 20 sec when the pressure load was 20 or 40 mat, only after 40 sec when the load was 60 mat, and it took 1 min when the pressure load was 80 mat or more.

Average diastolic pressure (Table IV) did not fall prior to, and was invariably increased during the PPBH maneuvre. A subsequent reduction indicating counterregulation was observed almost exclusively after experiments in which intrapulmonary pressure was raised only slightly. During the maneuvre the pulse pressure diminished when exertion was small but it increased if positive pressure was higher. A more marked diminution of the pulse pressure was the result of the counterregulatory phase of systolic pressure.

### 3. *Gas exchange*

In order to compare the respective periods of 30 and 40 sec, the rate of gas exchange was expressed in ml per sec. When the maintenance of 120 mat was required the majority of experimental subjects was notable to close the mouth sufficiently owing to the discomfort caused by the breathing mask; the leakage of gas in these cases made it impossible to obtain reliable results. Therefore, these data were neglected in the statistical evaluation. Consequently the values given in Table V represent the average of 3 measurements only.

Oxygen uptake (Table V) rose already prior to the maneuvre, the resting values becoming subsequently higher in the successive steps of overpressure. After the maneuvre, the rate of oxygen uptake was practically the same in the case of the 4 initial steps; a considerable increase was observed only at 100 and 120 mat overpressures. The original values of rest were, however, never reached during the phase of recovery, instead, the differences became more and more pronounced (see the values measured 2 minutes after the maneuvre).

The dynamism of carbon dioxide production (Table VI) showed a pattern similar to that of oxygen uptake. The progressive increase of the values in the phase of hyperventilation preceding the maneuvre could be observed also

**Table IV**

*Diastolic blood pressure in response to PPBH maneuvre*

| Intrapulmonary pressure (mat) | During maneuvre | | After maneuvre | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 20 sec | | 40 sec | | 60 sec | | 80 sec | | 100 sec | | 120 sec | | 140 sec | | 160 sec | |
| | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| — | — | — | 81 | 9 | — | — | — | — | 81 | 8 | — | — | — | — | 79 | 6 | — | — |
| 20 | 92 | 6 | 84 | 6 | 76 | 8 | 84 | 5 | 79 | 8 | 80 | 7 | 81 | 5 | 82 | 6 | 84 | 3 |
| 40 | 93 | 7 | 82 | 7 | 79 | 3 | 79 | 12 | 82 | 8 | 81 | 6 | 82 | 8 | 80 | 7 | 81 | 9 |
| 60 | 93 | 8 | 86 | 3 | 84 | 6 | 85 | 3 | 82 | 7 | 81 | 6 | 82 | 7 | 84 | 6 | 80 | 13 |
| 80 | 97 | 8 | 91 | 8 | 84 | 9 | 82 | 6 | 84 | 5 | 82 | 9 | 81 | 8 | 82 | 5 | 84 | 5 |
| 100 | 102 | 3 | 87 | 8 | 86 | 2 | 82 | 10 | 84 | 8 | 84 | 6 | 82 | 6 | 82 | 6 | 83 | 5 |
| 120 | 101 | 9 | 87 | 7 | 85 | 10 | 86 | 9 | 83 | 6 | — | — | — | — | — | — | — | — |

| Increase during maneuvre | Rest—20 mat | 20—40 mat | 40—60 mat | 60—80 mat | 80—100 mat | 100—200 mat |
|---|---|---|---|---|---|---|
| | 13 | 1 | 0 | 4 | 5 | —1 |
| | $0.001 > P$ | $P > 0.50$ | — | $0.10 > P > 0.05$ | $0.001 > P$ | $P > 0.50$ |

**Table V**

*Oxygen uptake (ml per sec) in response to PPBH maneuvre*

| Intrapulmonary pressure (mat) | After PPBH | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30 sec | | 60 sec | | 90 sec | | 120 sec | | 160 sec | |
| | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| — | — | — | 7.45 | 2.42 | — | — | 7.43 | 2.35 | 8.83 | 2.49 |
| 20 | 10.69 | 3.63 | 9.93 | 3.69 | 9.23 | 2.90 | 7.89 | 2.57 | 8.88 | 2.45 |
| 40 | 10.87 | 3.20 | 9.73 | 2.49 | 8.18 | 2.51 | 8.02 | 1.93 | 9.64 | 3.33 |
| 60 | 10.52 | 4.11 | 10.23 | 3.66 | 8.72 | 2.26 | 8.02 | 2.66 | 9.52 | 2.74 |
| 80 | 9.76 | 3.04 | 8.73 | 3.15 | 8.73 | 2.53 | 8.36 | 2.07 | 10.61 | 3.71 |
| 100 | 11.10 | 2.66 | 10.91 | 3.83 | 9.70 | 2.84 | 8.79 | 2.44 | 12.59 | 4.93 |
| 120 | 11.25 | 3.51 | 11.15 | 2.27 | 9.62 | 1.42 | 9.77 | 2.30 | 9.10 | 1.31 |

**Table VI**

*Carbon dioxide output (ml per sec) in response to PPBH maneuvre*

| Intrapulmonary pressure (mat) | After PPBH | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30 sec | | 60 sec | | 90 sec | | 120 sec | | 160 sec | |
| | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| — | — | — | 5.19 | 3.67 | — | — | 5.15 | 1.90 | 6.14 | 1.40 |
| 20 | 7.01 | 1.31 | 6.33 | 1.99 | 6.05 | 1.74 | 5.48 | 1.72 | 6.08 | 1.42 |
| 40 | 7.07 | 1.93 | 6.63 | 1.59 | 5.49 | 1.52 | 5.49 | 1.29 | 6.34 | 2.09 |
| 60 | 7.04 | 2.53 | 6.82 | 2.06 | 6.05 | 1.51 | 5.50 | 2.03 | 6.23 | 1.92 |
| 80 | 6.46 | 1.77 | 6.54 | 1.41 | 6.06 | 1.57 | 6.06 | 1.57 | 6.96 | 1.31 |
| 100 | 7.52 | 3.02 | 7.79 | 3.14 | 6.96 | 2.27 | 6.04 | 1.77 | 8.20 | 1.61 |
| 120 | 8.46 | 3.71 | 9.86 | 2.02 | 8.22 | 2.23 | 7.96 | 1.42 | 8.19 | 1.13 |

with carbon dioxide production. The lowest values recorded 2 min after PPBH, were successively more and more different from the values of rest. Particularly when overpressure attained 120 mat, the increase in carbon dioxide output was greater than that observed in oxygen consumption. This manifested itself in a marked increase of the respiratory quotient, too.

Table VII shows the efficiency of ventilation, i.e. the percentile utilization of oxygen from the inspired air, which was directly measured in the course of the experiments. Extraction was somewhat increased after the maneuvre and used to reach a maximum in about half to 1 min after cessation of the maneuvre. At higher pressure levels, maximum efficiency was observed successively earlier, and with 120 mat it already preceded the performance of the maneuvre.

**Table VII**

*Percentile oxygen uptake from inspired air after PPBH maneuvre*

| Intrapulmonary pressure (mat) | 30 sec | | 60 sec | | 90 sec | | 120 sec | | 160 sec | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| — | — | — | 4.29 | 0.68 | — | — | 4.51 | 0.86 | 4.50 | 0.95 |
| 20 | 5.21 | 0.94 | 5.32 | 0.61 | 4.86 | 0.72 | 4.48 | 0.66 | 4.43 | 0.74 |
| 40 | 5.26 | 0.62 | 5.27 | 0.69 | 4.77 | 0.66 | 4.56 | 1.07 | 4.49 | 0.79 |
| 60 | 4.99 | 1.01 | 5.02 | 1.02 | 4.67 | 0.82 | 4.50 | 0.78 | 4.41 | 0.46 |
| 80 | 5.15 | 0.86 | 5.09 | 0.62 | 4.79 | 0.72 | 4.51 | 0.75 | 4.67 | 0.77 |
| 100 | 5.20 | 0.69 | 4.88 | 0.71 | 4.69 | 0.55 | 4.70 | 0.76 | 5.13 | 1.04 |
| 120 | 4.63 | 0.79 | 4.49 | 1.31 | 4.29 | 1.01 | 4.03 | 1.00 | 3.81 | 1.02 |

The change in the metabolic rate during the PPBH maneuvre ought to be calculated by distracting the values during rest from the total exchange measured after the efforts. However, prior to the exertion there was always a marked hyperventilation which improved the energetical conditions for the subsequent maneuvre. This is the reason why hyperventilatory excess was added to the value measured during the recovery phase of the maneuvre. Excess gas exchange, thus, refers to the 3-min periods. The differences caused by incomplete recovery were disregarded; these latter, however, did not substantially affect the tendency of changes. The excess values for oxygen uptake (Table VIII) were almost identical up to an intrapulmonary pressure of 80 mat, although a slight rise had been expected. The changes were not significant statistically. Above 80 mat, however, excess oxygen uptake increased significantly (147 ml), and a further increase was observed after a pressure value of 120 mat (118 ml). As regards excess carbon dioxide output, above 80 mat a sudden increase was found. The increase was 120 ml in response to the pressure level of 100 mat, and 229 ml after 120 mat.

**Table VIII**

*Excess gas exchange (ml) at different intrapulmonary pressures during PPBH maneuvre*

| | 20 | 40 | 60 | 80 | 100 | 120 | 80 | 100 | Apnea |
|---|---|---|---|---|---|---|---|---|---|
| | milliatmosphere (mat) | | | | | | | | |
| Oxygen | 297 | 284 | 317 | 299 | 446 | 564 | 333 | 605 | 13 |
| Carbon dioxide | 179 | 174 | 191 | 184 | 304 | 533 | 200 | 392 | — |

A separate series of experiments was devoted to find a reason for the sudden increase between 80 and 100 mat (see right part of Table VIII). To imitate the conditions encountered in every-day life, the intervals between

the successive maneuvres were kept short, not allowing time for complete regeneration. The difference was, however, substantial even when time allowed complete regeneration. The difference was at the border of statistical significance for the values of excess oxygen uptake, and concerning carbon dioxide output it was significant statistically ($0.05 > P > 0.02$). An apnea lasting for the same period as PPBH (20 sec) caused only insignificant changes in metabolic rate.

## Discussion

The changes in heart rate observed during the experiments clearly indicated that the apparent ergotropic (pressor) dominance during the PPBH maneuvre was not associated with an appreciable diminution of trophotropic (depressor) activity. The two activities showed a physiological instability during isometric work load, marked ergotropic activity was occasionally interrupted by the trophotropic effect. This is particularly easy to recognize when the values for duration of the cardiac cycle are compared. A considerable variation was observed already at rest, and this physiological oscillation was apparent also during and after the exercise. Thus it seems that both activities are present at the same time, but during forced expiration the pressor effect becomes predominating.

Following an intrapulmonic pressure of 20 mat considerable depressor activity was found in comparison to the values at rest. Thus, the pressor response of the preparative phase exceeded the actual load imposed by the 20 mat maneuvre.

Duration of the cardiac cycle after the maneuvres increased almost linearly between 20 and 80 mat. This fact indicated an increasing depressor effect. The effect was relatively less marked after the pressure levels of 100 and 120 mat. However, if compared to the previous change we find a similar predominance of depressor activity after intrapulmonary pressures of 80, 100 and 120 mat.

Regulation seems to be different when PPBH is performed at low, medium and high intrapulmonary pressures, resp. At low pressures, the tendency of regulation was depressor in character, which was further increased following the maneuvre. At higher pressure values depressor activity was enhanced only after cessation of the overpressure maneuvre. PPBH performed at higher levels of pressure elicited a marked response followed by intensive depressor activity, an obvious overshoot of the counterregulatory mechanisms. Counterregulation manifested itself in a waveline deviation of values. The counterregulatory phase observed after static work was very similar to that observed by Nemessuri and Stadler (1963) during light dynamic work, which manifested itself in appropriate changes in the heart rate and was regarded as a reliable indicator of the sportsman's endurance.

GEBERT and GROBER (1925) assumed that changes in the duration of the cycle were due to alterations in diastolic time, and that the duration of the electric systole was not altered. Others found a prolongation of the electric systole during forced expiration (TSCHERNOGOROFF and EGOROFF 1929, BORST 1935, SCHMOLKA and LAMBERT 1935, GRŐFER 1951, GROSS 1952, BÜRGER 1957). These authors performed the maneuvre restricted to a certain pressure range, usually 40 mm Hg. ALBERT and KLAUS (1938) studied the effect of 20 and then of 60 mm Hg intrapulmonary pressure and observed a rise in heart rate of 12 and 30/min, respectively. The present results also indicate that changes in the duration of the cardiac cycle are due first of all to alterations in diastolic time. Nevertheless, the electrical systole, too, seems to have significantly contributed to the observed changes in the cycle. At low intrapulmonary pressures the QT period was statistically significantly prolonged. An increase in the static load resulted in a significant shortening of the electric systole during the maneuvre. Systolic time corresponded to the over-all tendency of regulation and depended on the intensity of forced expiration; a similar change in the opposite direction was observed after cessation of the maneuvre, i.e. systolic time was longer.

According to CSINÁDY and PURJESZ (1940) and PLENCZNER (1943) blood pressure shows considerable variations during forced expiration. The present results have shown that systolic pressure in the last seconds of PPBH increases progressively as the load is increased. After cessation of the maneuvre, increased blood pressure returns to the control level. The delay of return is successively increasing as intrapulmonary pressure, i.e. the force of impeded expiration, is stepwise increased. Thus, the phase of counterregulation ensues later and later with the increase of exertion. Mean diastolic pressure, too, increases parallel with the increase of the work load. A counterregulatory phase could be observed practically only after lower intrapulmonary pressures. The static work load presented in 6 distinct steps, thus, gave rise to a response which consisted of 3 phases only (see the values measured during the maneuvre). These phases, however, did not coincide with respect to the different cardio-respiratory parameters. As regards systolic pressure, the lowest degree of regulation was observed at intrapulmonic pressure levels of 20 and 40 mat, it was marked when intrapulmonary pressure was maintained at 60 mat, and the last phase occurred at 80 and 120 mat, resp., the respective degrees of diastolic response were, however, 20 to 60 mat, 80 mat, and 100 to 120 mat. In other words, there are intrinsic differences in the regulation of cardiovascular response.

The values for gas exchange rate measured before the rise in intrapulmonary pressure showed a progressive increase as work load was increased. As regards the time distribution of excess gas exchange, in the case of oxygen an increasing fraction of total excess uptake coincided with the preparatory

phase in the subsequent maneuvres; this increase in the fraction of excess uptake occurred in 3 phases, viz. at 20 to 40 mat, at 60 to 100 mat; and at 120 mat (Table IX). Carbon dioxide output did not show a similar pattern. The observed phenomenon might be attributed to the psychic effect of the start.

**Table IX**

*Fraction of excess gas exchange in the phase preceding PPBH maneuvre, per cents*

| | 20 | 40 | 60 | 80 | 100 | 120 |
|---|---|---|---|---|---|---|
| | milliatmosphere (mat) | | | | | |
| Oxygen | 19 | 20 | 28 | 28 | 28 | 37 |
| Carbon dioxide | 23 | 21 | 25 | 23 | 23 | 23 |

The response of respiratory gas exchange given to the first 4 stages of the experiment was practically identical, thus, the respiratory system failed to follow the pattern of the 4-step increase of motor activity. A significant change was only observed during the last two stages of static loading. Thus excess oxygen consumption and carbon dioxide output displayed a 3-stage response. The phases of respiratory regulation did not coincide with those observed in the cardiovascular parameters. In respiration, the first phase was observed between pressure values of 20 to 80 mat, the second at 100, and the third at 120 mat.

The relatively higher output of carbon dioxide during PPBH performed at higher pressures was probably not only the consequence of metabolic changes due to the exertion. Owing to an accumulation of metabolic end-products in the course of increased anaerobic metabolism, carbon dioxide is released also from the bicarbonate pool of the blood. The nature and significance of this carbon dioxide release will be studied in further experiments.

The changes in the cardiorespiratory parameters have been expressed also as per cents of the control level and are represented diagrammatically in Fig. 1. The smallest change was observed in diastolic blood pressure, the minimum and maximum changes in the various stages of the experiment having been 14 and 25%, respectively (range, 11%). The static work load applied in 6 stages gave rise to a regulatory response which manifested itself in 3 phases. The changes in systolic pressure varied between 5 and 28%, with a range of 23%; the number of phases was also 3. However, there was a shift of phasing as compared to the alterations of diastolic pressure. The most differentiated response was observed in the heart rate concerning which perhaps the greatest number of phases occurred, the smallest change having been 3% and the greatest 56% (range, 53%). The response of respiratory gas exchange also showed a 3-phase pattern; excess oxygen uptake varied

between 24 and 47%, with a range of 23%. The absolute changes were greater than of carbon dioxide which showed a minimum increase of 20% and a maximum of 66%, with a range of 46%. This change was the greatest relatively.

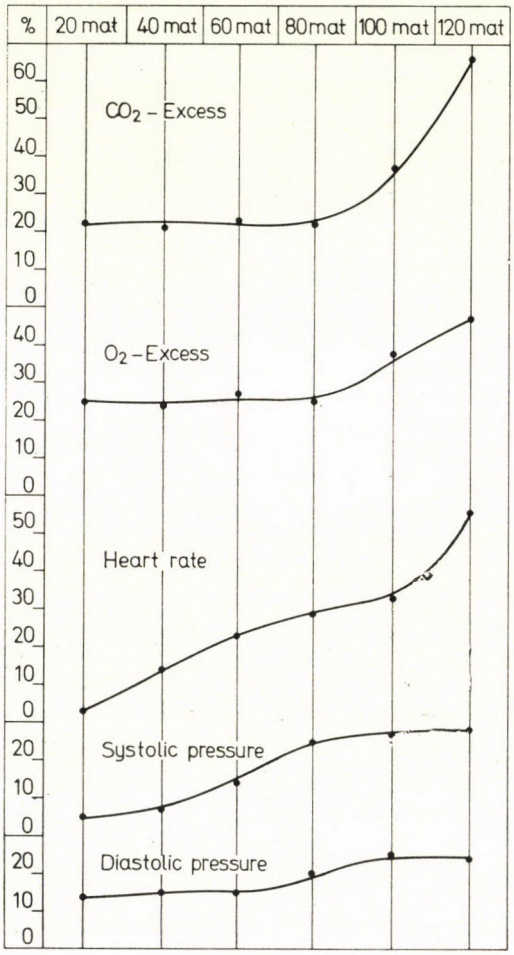



*Fig. 1.* Changes in circulatory and respiratory parameters in response to PPBH, in per cent of the initial values. (Intrapulmonary pressure: mat)

At higher intrapulmonary pressure levels the curves of excess carbon dioxide production and of heart rate showed a close similarity. The marked increase in carbon dioxide output and heart rate was most likely to result from an increase in metabolic rate due to the growing exertion.

On the basis of these results phase sensitivity of pressor receptors seemed to be limited, as the 6-stage graded exertion elicited a cardiorespiratory response

pattern which consisted of fewer phases. Obviously, the reaction as a whole is controlled by an intricate neurohumoral mechanism. The integration of vegetative and motor systems and the functional connections between them are still unclear (LISSÁK and ENDRŐCZI 1965). The increase and decrease in the activity of the motor system give rise to similar activity changes in the cardiorespiratory functions. The latter, however, do not always occur according to the pattern of the former, as was shown by the present results. Only the main features of the vegetative response seem to follow the changes in motor activity. Thus, motor functions, cardiovascular system, and respiration appear to be governed by more or less independent mechanisms.

This is particularly evident when the whole process is decomposed into 4 periods, viz. activation, compensation, damping, and overshoot-rebound (NEMESSURI 1968). In motor analysis the first 3 periods are generally studied.

PPBH is a static work load in which the period of activation coincides with the steep rise of intrapulmonic pressure. In the compensatory phase motor activity maintained for 20 sec at a level characterized by the respective pressure. Spirotonometric data showed a slight oscillation of muscular activity, obviously due to the balance of the correcting feedback mechanisms. In the phase of damping, motor innervation ceased abruptly.

The analysis of cardiorespiratory responses of the same periods indicated that regulation took the form of antagonistically organized effects, in the midst of a continuous competition between activating and inhibitory systems.

In the period of activation one would expect a pronounced increase in pressor activity due to the static work. Certain level of depressor activity was, however, present also during the period of activation. The present results have shown that the onset of PPBH was more likely to trigger depressor than pressor activity. In this respect, the changes in the duration of the cardiac cycle were particularly demonstrative. The changes in the electric systole and diastole equally supported this conception.

During the phase compensation a continuous competition between pressor and depressor activity could be demonstrated. The method of continous registration made it possible to study in great detail the dynamic change of myocardial contractibility.

In the period of damping depressor activity became predominant which manifested itself in bursts. This predominance of depressor activity was periodically interrupted by pressor activity.

In the fourth period the predominance of depressor activity caused many of the parameters to fall below the initial values, displaying the so-called counterregulation and overshoot. The observed changes in the duration of the cardiac cycle were in conformity with this theoretical pattern of regulation. The overshoot was followed by a rebound-like increase in pressor activity boosting the parameters above resting values.

The present results clearly indicate that the responses of circulation and respiration given to PPBH are governed by general as well as by organ-specific regulatory mechanisms. It seems that the static work of the neuromotor system has a more differentiated gradation than the neurovegetative one.

## LITERATURE

ALBERT, A. H., KLAUS, E. J.: Die morphologischen und funktionellen Herzveränderungen im dosierten Valsalvaschen Versuch bei jugendlichen Wettkampfsportlern. Untersuchungen im Stufen-Valsalva. Dtsch. Arch. klin. Med. **1938,** 183: 315—332.

BORST, A.: Wert und Bedeutung von Kreislaufbelastungsproben bei Fliegeruntersuchungen (Pressdruckprobe und "Aufrichtereaktion"). Verh. Dtsch. Ges. inn. Med. **1935,** 47: 58—61.

BÜRGER, M., MICHEL, D.: Funktionelle Engpässe des Kreislaufs. Lehmann, München **1957.**

CSINÁDY, E., PURJESZ, B.: Erfahrungen über die Brauchbarkeit des Bürgerschen Untersuchungsverfahrens. Arbeitsphysiologie **1940,** 11: 138—147

GEBER, N. and GROBER, K. **1925;** GROSS, D. **1952;** GRÖFER, H. **1951** CIT BÜRGER, M. and MICHEL, D. **1957.**

GRUBICH, V.: A tüdő vitális nyomáskapacitása. Testneveléstud. **1955,** 1: Suppl. 39—49.

GRUBICH, V.: Spirotonométer, készülék az intrapulmonalis nyomás mérésére, illetve a spirotonometriás terhelés végrehajtására. Eü. Közl. **1958,** 8: 272—273.

LISSÁK, K., ENDRŐCZI, E.: The neuroendocrine control of adaptation. Academic Press, Budapest **1965.**

MALOMSOKI, J.: A fizikai terheléssel egybekötött gázanyagcsere és vizsgáló módszerei. Testnev.-Sportegészs. Szle **1963,** 4: 149—156.

NEMESSURI, M. Az erőnlét. **1968** (In press)

NEMESSURI, M., STADLER, E.: Adaptation to muscle activity of circulation control. Acta physiol. Acad. Sci. hung. **1963,** 22: 17.

PLENCZNER, S.: A vérkeringési szervek és a sport. Országos Ifjúsági Egészségügyi Bizottság, Budapest **1943.**

SCHLOMKA, G., LAMMERT, C.: Das Belastungselektrokardiogramm. Das Verhalten der Herzstromkurve bei der Pressdruckbelastung nach Bürger (Valsalva EKG). Arbeitsphysiologie **1935,** 8: 742—777.

TSCHERNOGOROFF, J. A., EGOROFF, P. J.: Zum Problem der Herzfunktionsprüfung mittels des modifizierten Valsalva Versuchs. Z. ges. exp. Med. **1929,** 67: 206—218.

WOLLSCHITT, H., BOETHE, W., RUSKA, H.: Der Zeisssche Laboratoriumsinterferometer als Stoffwechselmessgerät. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **1935,** 177: 635—654.

Vilmos GRUBICH, Jenő MALOMSOKI, Mihály NEMESSURI
Országos Testnevelés- és Sportegészségügyi Intézet,
Budapest XII., Alkotás u. 48, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 133—139 (1969)*

# INDUKTIONSVERÄNDERUNGEN DER GLUTAMAT-OXALAZETAT-TRANSMINASE IN DER HENNE UNTER WIRKUNG VON 11-DEHYDROKORTIKOSTERON

Von

J. PAVEL

INSTITUT FÜR BIOCHEMIE, LANDWIRTSCHAFTLICHE HOCHSCHULE, BRNO, ČSSR

(Eingegangen am 17. Juli 1968)

Es wurden die adaptiven Aktivitätsveränderungen der Glutamat-Oxalazetat-Transaminase (GOT) nach Verabreichung von 11-Dehydrokortikosteron in einer täglichen Dosis von 1 mg/100 g Körpergewicht, ein, drei und sechs Tage hindurch in 15 verschiedenen Organen und Geweben bei weißen Plymouth-Hennen untersucht. Signifikante Unterschiede zeigten sich erst nach drei Tagen. Die GOT-Aktivität war in der Leber und im Eigelb (Dotter) stark signifikant, im Herz- und Lungengewebe sowie im Knochenmark signifikant erhöht.

Unsere bisherigen Studien über die kortikosteroide Kontrolle der Tätigkeit einiger Enzyme bei Geflügel (PAVEL und SVOZIL 1968a, b) ergaben viele gemeinsame Züge, gleichzeitig aber auch einige spezifische Abweichungen in der Kortikoidwirkung bei Labortieren und Vögeln.

Im vorliegenden Beitrag beschäftigen wir uns mit der Wirkung des 11-Dehydrokortikosterons auf die Aktivität der Glutamat-Oxalazetat-Transaminase (GOT) bei der weißen Plymouth-Henne.

Die Grundbedingung für die kortikoide Wirksamkeit der 21 C-Steroide ist die Anwesenheit der $\Delta$4-3-Ketogruppen und die $\alpha$-Ketogruppierung auf C-17 (s. z. B. MAŠEK [zit. HOŘEJŠÍ] 1963).

BLEHA und KÜCHEL (1967) charakterisieren die glukokortikoide Wirkung der Nebennierensteroide durch ihre eiweißkatabolische und glukoneogenetische Aktivität, deren Mechanismus nach den angeführten Autoren auf der erhöhten Aktivität einer Reihe von Enzymen so z. B. von SGOT, SGPT, Glukose-G-Phosphatase, Tryptophanperoxydase und 1,6-Diphosphofruktokinase beruht, die direkt oder indirekt auf den Metabolismus der Aminosäuren einwirken, während KANDRÁČ (1964) die Wirkung damit erklärt, daß die Glukokortikoide die Struktur der Enzyme verändern, wie z. B. bei der Konversion der kristallinen Glutamatdehydrogenase zu L-Alanindehydrogenase.

In diesem Zusammenhang sind die Ergebnisse von UMBREIT und TONHÁZY (1951, zit. KNOX u. Mitarb. 1956) interessant; diese Autoren fanden nach Adrenalektomie eine unveränderte Transaminaseaktivität in Leber, Nieren und Muskeln. Nach zehntätiger Kortisonbehandlung mit 1 mg täglich zeigte sich jedoch in der Rattenleber eine um 290% erhöhte D-Aminosäure-

oxydase-Aktivität. Diese Wirksamkeit des Kortisons dürfte als spezifisch bezeichnet werden. KERPPOLA (1953) beobachtete bei Kaninchen nach Kortisonbehandlung eine Senkung der Phosphorylaseaktivität, während nach CHOW (zit. KNOX u. a., 1956) die Glutaminaseaktivität in der Rattenleber selbst nach 15tägiger Verabreichung von Kortison unbeeinflußt bleibt. Anderseits konnten wieder HADNAGY u. a. (1960) durch Hydrokortison eine Erhöhung der Alanintransaminaseaktivität im Rattenleberhomogenat hervorrufen. Auch LIN und KNOX (1957) beobachteten nach Hydrokortisonbehandlung einen annähernd zehnfachen Anstieg der Tyrosin-$\alpha$-ketoglutarattransaminaseaktivität in der Rattenleber, wobei das Maximum bereits in fünf Stunden verzeichnet wurde.

TEEBOR, BECKER und SEIDMAN (1967) fanden nach Kortikosteroidverabreichung in der Rattenleber einen signifikanten Anstieg der Tryptophanpyrrolase- und Tyrosintransaminaseaktivität. Diese Induktion der Tryptophanpyrrolase war jedoch nach 70%iger Hepatektomie, als es zur Regeneration kam, wesentlich geschwächt, d. h. daß der Regulationsmechanismus bereits während der Vorbereitung zur Zellteilung verändert war.

## Methode

Die Experimente wurden an 21 weißen Plymouth-Legehennen durchgeführt. Die Hennen wurden in sieben Gruppen eingeteilt und folgendermaßen behandelt.

Gruppe A — unbehandelte Kontrolle
Gruppe B — eintägige Verabreichung des Trägers
Gruppe C — eintägige Verabreichung des Hormons
Gruppe D — dreitägige Verabreichung des Trägers
Gruppe E — dreitägige Verabreichung des Hormons
Gruppe F — sechstägige Verabreichung des Trägers
Gruppe G — sechstägige Verabreichung des Hormons

Die Behandlung erfolgte mit 11-Dehydrokortikosteron (FLUKA) in einer täglichen Dosis von 1 mg je 100 g Lebendgewicht, in 3,2 ml Sonnenblumenöl (Träger) aufgelöst und in zwei Dosen in zwölfstündigen Intervallen intramuskulär injiziert. Bei den Gruppen B, D und F wurde 3,2 ml Sonnenblumenöl intramuskulär verabreicht.

Die GOT-Aktivität wurde nach REITMAN und FRANKEL (1957) teils im Blutserum und im Plasma, teils in den Homogenaten von Schenkelmuskel, Brustmuskel, Bauchmuskel, Leber, Herz, Lungen, Magen, Eierstock, Nieren, Milz, Hirn, Eigelb (Dotter) und Knochenmark bestimmt. Die festgestellte Aktivität wurde in m.-Einheiten U/37°C/l h/1 g frischen Gewebes bzw. 1 ml Serum (Plasma) ausgedrückt.

Die Ergebnisse wurden außer der graphischen Darstellung des zeitlichen Verlaufes der GOT-Veränderungen mit dem Studentschen *t*-Test bewertet.

## Ergebnisse und Diskussion

Die Ergebnisse in den Kontrollgruppen sind in Abb. 1 veranschaulicht. Die höchsten GOT-Werte wurden in Herz, Nieren, Leber, Gehirn sowie einigen Muskeln und die niedrigsten im Plasma und Serum festgestellt.

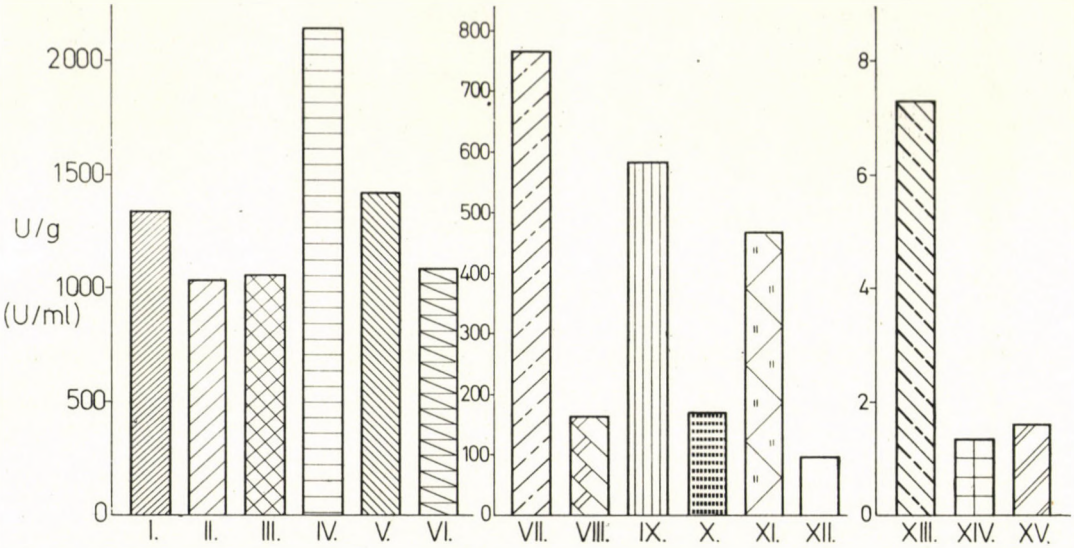



*Abb. 1.* GOT-Aktivität in den einzelnen Geweben bei Hennen der Kontrollgruppen (U/g) (I — Schenkelmuskel; II — Bauchmuskel; III — Leber; IV — Herz; V — Nieren; VI — Gehirn; VII — Brustmuskel; VIII — Lungen; IX — Magen; X — Eierstock; XI — Milz; XII — Knochenmark; XIII — Eigelb [Dotter]; XIV — Plasma; XV — Serum; U/ml)

Abb. 2 zeigt die GOT-Aktivität in Brustmuskel, Leber und Lungen unter Hormonbehandlung und in den nur mit Sonnenblumenöl behandelten Tieren. Bei den hormonbehandelten Vögeln konnte eine signifikante Erhöhung der GOT-Aktivität verzeichnet werden, doch war auch die Wirkung des Sonnenblumenöls signifikant und in einigen Fällen kam es sogar zu größeren Abweichungen als in den hormonbehandelten Gruppen. Diese letztere Erscheinung war ausschließlich bei eintägiger Behandlung festzustellen, später war die Aktivitätserhöhung nach Hormonbehandlung immer höher als nach Verabreichung von Sonnenblumenöl. Es kann angenommen werden, daß es bei eintägiger Verabreichung des im Träger gelösten Hormons zu Richtungsdivergenzen kam und zwar so, daß der Träger eine erhöhte Aktivität hervorrief, während das Hormon allein deren Herabsetzung bewirkte. Die Gesamtabweichung hing dann verständlicherweise von der Größe der beiden gegenseitig wirkenden Einflüße ab.

Abb. 3 zeigt diese zeitlichen Veränderungen der GOT-Aktivität im Eierstock sowie im Eigelb (Dotter). Hier stieg die Wirksamkeit des 11-Dehydrokortikosterons mit der Zeit in dem Maße, daß sich nach 6 Tagen die ursprüngliche Aktivität auf fast das Zehnfache erhöhte, wobei die Wirksamkeit des Trägers belanglos war. Als besonders interessant dürfte der Verlauf der GOT-Aktivität im Blutplasma bezeichnet werden. Nach eintägiger Hormonverabreichung war eine doppelte und nach dreitägiger Verabreichung eine drei-

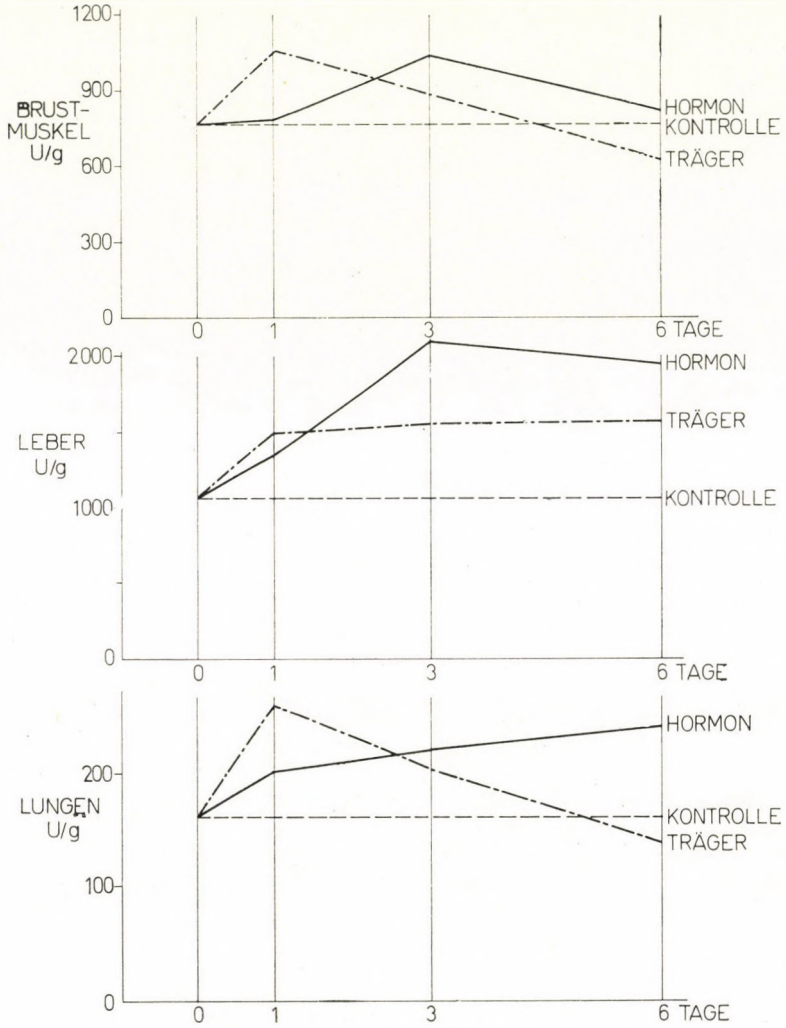



*Abb. 2* und *Abb. 3.* GOT-Aktivität bei ein-, drei- und sechstätiger Behandlung mit 11-Dehydrokortikosteron und mit Sonnenblumenöl

fache Erhöhung des Enzymspiegels zu verzeichnen, dieser Wert stieg jedoch nach sechstägiger Behandlung nicht mehr an. Da der Enzymspiegel im Blut bekanntlich den des ganzen Organismus widerspiegelt, muß eine drei bis sechs Tage dauernde 11-Dehydrokortikosteronbehandlung als ausschlaggebend für die GOT-Aktivität im Organismus erachtet werden.

Die exakte Bewertung der soeben beschriebenen Veränderungen der GOT-Aktivität nach Applikation des 11-Dehydrokortikosterons in allen testierten Geweben — folglich auch in jenen, denen bisher keine Aufmerksamkeit geschenkt

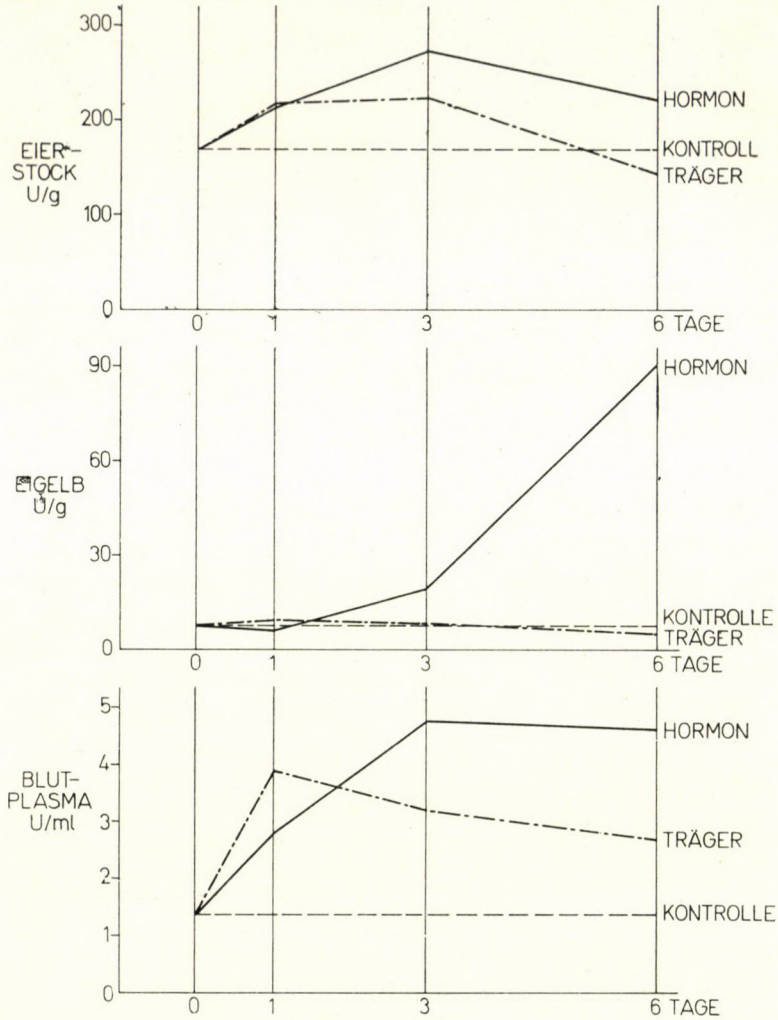



*Abb. 3*

wurde —, die in Tabelle I ersichtliche statistische Auswertung der Unterschiede zwischen der Kontroll- und Hormonalgruppen, sowie zwischen Kontroll- und Träger-Gruppen ergaben, daß eine eintägige 11-Dehydrokortikosteronbehandlung in keinem von den untersuchten Geweben eine erweisbare Beeinflussung der GOT-Aktivität zeitigte. Nach dreitägiger Hormonverabreichung war die GOT-Aktivität in Leber und Blutplasma sowie im Knochenmark und Eigelb signifikant erhöht.

Nach sechstägiger Hormonbehandlung war die GOT-Aktivität in Leber und Eigelb stark signifikant, im Herz und in der Lunge nicht signifikant

**Tabelle 1**

*Signifikanz der Aktivitätsunterschieoe zwischen den einzelnen Gruppen.*
++ ... P = 0,01; ▨ ... erhöht; + ... P = 0,05

| Gruppe | Schenkelmuskel | Brustmuskel | Bauchmuskel | Leber | Herz | Lungen | Muskuläser Magen | Eierstock | Niere | Milz | Hirn | Eigelb (Dotter) | Knochenmark | Plasma | Blutserum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A – B | – | – | + | + | – | + | – | – | – | – | – | – | – | + | + |
| A – C | – | – | + | – | – | – | – | – | – | – | – | – | – | + | – |
| A – D | – | – | – | + | – | – | – | – | – | – | – | – | + | + | ++ |
| A – E | – | – | + | ++ | – | – | – | + | – | – | – | + | + | ++ | + |
| A – F | – | – | – | + | – | – | – | – | – | – | – | – | – | + | + |
| A – G | – | – | – | – | + | + | – | – | – | – | – | ++ | – | ++ | ++ |
| B – C | – | – | – | – | + | – | – | – | – | – | – | – | – | – | + |
| D – E | – | – | – | ++ | + | – | + | – | – | – | + | + | + | + | + |
| F – G | – | – | – | + | + | + | – | + | + | – | – | ++ | – | + | – |

und im Eierstock und in den Nieren wahrscheinlich erweisbar erhöht. In diesen letzten Organen rief nämlich die Trägersubstanz eine Depression des Enzymspiegels hervor, während das im Träger gelöste Hormon zu einer Erhöhung der Aktivität führte, die zwar die erweisbaren Unterschiede von den Kontrollgruppen nicht erreichte, jedoch signifikant höher als in der Träger-Gruppe war.

Die Wirkung des 11-Dehydrokortikosterons manifestierte sich erst nach einer bestimmten Zeit. Bei eintägiger Verabreichung wurde die GOT-Aktivität kaum beeinflußt, im Widerspruch zu der Erhöhung binnen fünf Stunden der Tyrosin-$\alpha$-ketoglutarattransaminase-Aktivität (Lin und Knox 1957).

Aufgrund der beobachteten Beeinflussung der GOT-Aktivität durch 11-Dehydrokortikosteron dürfte in Übereinstimmung mit Bleha und Küchel (1967) festgestellt werden, daß die glukokortikoide Wirkung auf einer erhöhten Enzymaktivität beruht; dies wurde mit wenigen Ausnahmen in allen untersuchten Geweben und Organen der Versuchsvögel bestätigt.

## LITERATUR


Bleha, O., Küchel, O.: Klinická endokrinologie v praxi. Statni zdravotnické nakladatel, Prag **1967,** S. 493.

Hadnagy, C., Kelemen, L., Gyergyay, F.: Alanintransaminase-Aktivitätserhöhung und Verfettung des Lebergewebes nach Cortisonbehandlung. Wirkung von Vitamin $B_{12}$. Int. Z. Vitaminforsch. **1964,** 34: 385—391.

Horejsi, J., Mašek, K., Pudlák, P.: Základy klinické biochemie ve vnitrnim lekarstvi. Statni zdravotnické nakladatel., Prag **1963,** S. 573.

Kandráč, M. S.: Klinik der Kortikoide. In: I. Staatliche Konferenz über die Steroidtherapie, Prag **1964.**

Kerppola, W.: Inhibition of phosphorylase with cortisone and its activation with adrenaline in rabbit. Acta med. scand., **1953,** Suppl. 287: 29—30.

Knox, W. E., Auerbach, V. H., Lin, E. C. C.: Enzymatic and metabolic adaptations in animals. Physiol. Rev. **1956,** 36: 164—254.

LIN, E. C. C., KNOX, W. E.: Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. Biochim. biophys. Acta (Amst.) **1957,** 26: 85—88.

PAVEL, J., SVOZIL, B.: Serum enzyme response to the application of adrenal cortical hormones in egg-laying hens. Sborník VŠZ v Brne, Reihe A, **1968,** 4.

PAVEL, J., SVOZIL, B.: Přispěvek k poznani kortikosteroidni kontroly aktivity některých enzymů v krevnim séru u kuřic plemene plymutky bilé, Živočis, výr. (Prag) **1968,** in Druck.

REITMAN, S., FRANKEL, S.: A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminase. Amer. J. clin. Path. **1957,** 28: 56.

TEEBOR, G. W., BECKER, F. F., SEIDMAN, I.: Hydrocortisone induction of tyrosine transaminase in regenerating rat liver. Nature (Lond.) **1967,** 5113: 396—397.

Jiři PAVEL,
Landwirtschaftliche Hochschule.
Brno, Zemedilská 1,
ČSSR

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 141—148 (1969)*

# EVALUATION OF THE ROLE OF THE FEEDBACK EFFECT OF CORTICOSTEROIDS IN THE CONTROL OF PITUITARY ACTH RELEASE

By

B. Bohus

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received October 16, 1968)

In order to elucidate the role of the negative feedback effect of corticosteroids in the control of stress-induced ACTH release, the influence of corticosterone pretreatment on pituitary-adrenal function has been investigated in rats exposed to immobilization or hemiadrenalectomy.

While leaving the acute stress-induced ACTH release unaffected, administration of corticosterone 1 hour prior to immobilization or before hemiadrenalectomy resulted in a marked suppression of ACTH secretion during the subnormal response period which follows the maximal activation of the pituitary-adrenal axis. The second increase of ACTH release after the subnormal phase was also diminished in rats pretreated with corticosterone.

Repeated exposure to stressful stimuli (environmental changes) did not elicit ACTH release in the subnormal phase 12 hours after hemiadrenalectomy in rats treated with corticosterone while adrenal sensitivity to exogenous ACTH remained unchanged.

It has been concluded that the negative feedback effect of corticosterone plays a role in the control of the biphasic pituitary-adrenal response to continuously acting stressors like immobilization or hemiadrenalectomy. The possible mode and site of action of corticosteroids has been discussed.

It has been supposed that pituitary adrenocorticotrophic function is influenced by the feedback effect of corticosteroids, but their exact role in the control of stress-induced ACTH release is not well understood. The hypothesis that ACTH release is regulated by the circulating corticosteroid level was advanced by Sayers and Sayers (1947), who suggested that exposure of rats to stress brings about an increased utilization of corticosteroids by the peripheral tissues and the resulting decrease of the blood corticoid level acts as a stimulus of ACTH release from the anterior pituitary. This hypothesis has not been confirmed.

However, the importance of the blood corticosteroid level in regulating the stress-induced ACTH release was re-emphasized by Yates et al. (1961). They suggested that the control of stress-induced ACTH release involves a negative feedback effect of blood corticosteroids and that the immediate effect of stress is "to increase the set-point of the controller". Further experiments on rats (Hodges and Jones 1963; Smelik 1963a; Stark and Fachet 1963), on cats and dogs (Bohus et al. 1965) and on human beings (Estep et al. 1963) failed to support the "reset" hypothesis. These observations showed that the ACTH release due to stress is independent of the blood corticosteroid

level within the physiological range. This finding is of general validity, since it was shown that exposure to different stimuli provokes ACTH release in the rat in spite of the acute elevation of the blood corticosterone level (BOHUS 1968).

On the other hand, the corticosteroids may still play some role in the regulation of ACTH release, since in the rat adrenalectomy induces an overactivity of the anterior pituitary in response to stress (HODGES and VERNIKOS 1959) and this overactivity is inhibited by a small dose of corticosterone (HODGES and JONES 1964). Furthermore, SMELIK (1963b) reported that the inhibitory influence of a high dose of corticosterone on stress-induced ACTH release appears with a certain delay. VERNIKOS-DANELLIS and TRIGG (1967) also suggested that the inhibition of ACTH release by the maximum endogenous blood corticosterone level is slow to develop.

Since there are reports on a decline in ACTH release in response to continuously acting stresses some time after the onset of exposure (KNIGGE et al. 1959; HENKIN and KNIGGE 1963), it seemed interesting to study whether the corticoids are involved in this inhibitory period of ACTH secretion. Accordingly, the effect of corticosterone administration on ACTH release has been investigated in rats exposed to continuously acting stressors.

## Methods

Male albino rats of an inbred strain were used. The rats were fed purina chow and allowed to drink water ad libitum.

Corticosterone (Organon, Oss), dissolved in ethanol and diluted with saline, was injected subcutaneously in a dose of 5 mg/100 g b.w. Saline containing the same amount of ethanol was injected subcutaneously to the control rats.

Stressful stimuli applied 60 min after corticosterone or saline injection were as follows.

(i) Hemiadrenalectomy; removal of the left adrenal in ether anaesthesia from the lumbar approach;

(ii) Immobilization; the rats were kept in plastic tubes completely hindering locomotion.

Pituitary-adrenal activity was assessed 0.5, 2, 4, 8, 12, 18 and 24 hours after the onset of immobilization or hemiadrenalectomy. The rats were decapitated and adrenal corticosteroid production in vitro was determined by the method of VAN DER VIES et al. (1960). Total corticosteroid production in vitro expressed in $\mu$g/100 mg/adrenal/hour served as the index of pituitary ACTH release (VAN GOCH et al. 1963).

Adrenal response to ACTH was investigated 12 hours after surgery in two groups of hemiadrenalectomized rats treated with either corticosterone or saline. A group of non-stressed rats served as normal controls. The rats were anaesthetized with intraperitoneal pentobarbital (4.5 mg/100 g b.w.) and 30 min later saline or purified ACTH (Organon, Oss) was injected into one of the tail veins. The rats were decapitated 15 min thereafter and the adrenal corticosteroid production in vitro was determined.

The effect of repeated stress on pituitary ACTH release was assessed 12 hours after surgery in two groups of hemiadrenalectomized rats treated with corticosterone or saline. Non-operated rats served as control. The repeated stress consisted of a change in environment; the rats were moved from their home-cages to another room. 15 min later they were decapitated and pituitary ACTH release was determined by estimating adrenal corticosteroid production in vitro.

## Results

Corticosterone administration (5 mg/100 g b.w.) resulted in a depression of ACTH secretion as judged from the decrease in corticosteroid production in vitro of rats decapitated stress-free (Fig. 1). The decrease was apparent 1 hour after corticosterone administration; maximal suppression occurred between the 8th and 12th hours. At 24 hours corticosterone was not effective any more.

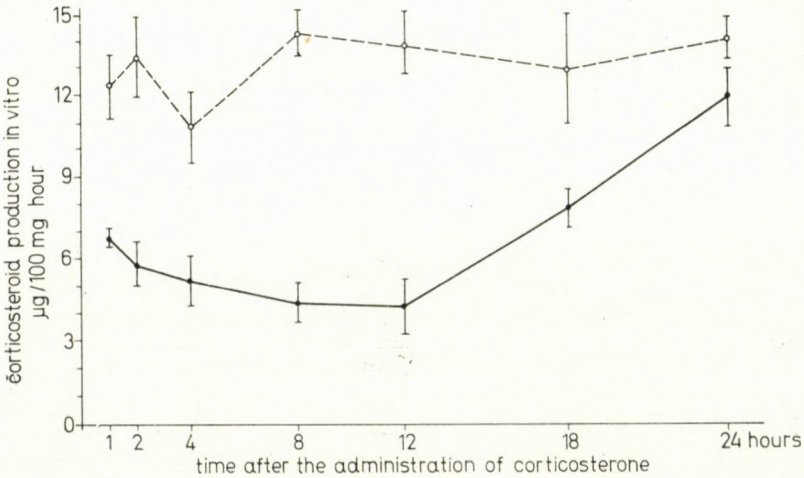



*Fig. 1.* Corticosteroid production in vitro of adrenals of stress-free decapitated rats following the administration of 5 mg/100 g b.w. of corticosterone (●——●) or physiological saline (○– – –○). Bars indicate standard error of the mean (6 observations per point)

Hemiadrenalectomy brought about a significant increase in ACTH release 0.5 hour after surgery in rats treated with saline (Fig. 2). Following the maximal ACTH release a continuous decline of adrenal corticosteroid production in vitro was observed at 2, 4, 8 and 12 hours. Maximal decrease of ACTH secretion appeared at 12 hours, when corticosteroid production in vitro was below the level observed in rats decapitated stress-free ($6.7 \pm 1.2$ vs. $11.8 \pm 1.4$ $\mu$g/100 mg adrenal/hour). A second rise of pituitary ACTH release was observed 18 and 24 hours after hemiadrenalectomy in the rats treated with saline. However, in this period the peak of stress-induced ACTH release observed at 0.5 hour was not attained.

ACTH release provoked by hemiadrenalectomy in rats treated with corticosterone 1 hour prior to surgery was 0.5, 2, 4 and 8 hours after operation in the same range as in rats treated with saline. However, depression of ACTH secretion in the 12th hour was more marked ($p < 0.01$) in corticosterone-treated than in saline-treated rats. Adrenal corticosteroid production in vitro

showed an increase in the 18th hour but the values remained significantly ($p < 0.01$) below those for the corresponding saline-treated controls. There was no difference in adrenal corticosteroid production between the two groups hemiadrenalectomized 24 hours before.

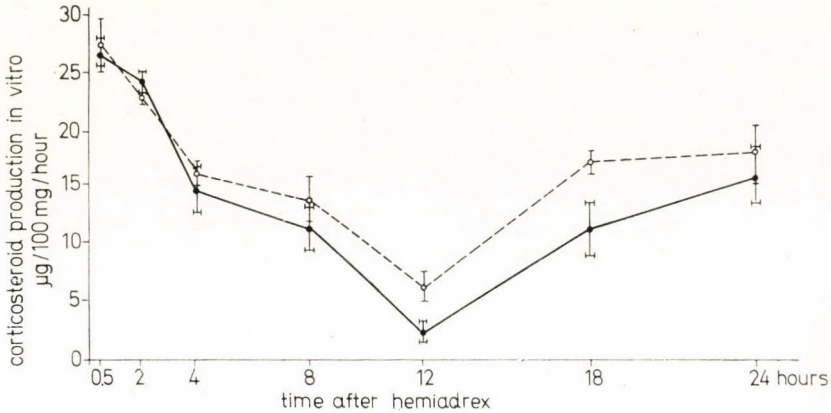



*Fig. 2.* Effect of hemiadrenalectomy on adrenal corticosteroid production in vitro of rats treated with corticosterone (●——●) or saline (○– – –○) 1 hour prior to surgery. Bars indicate standard error of the mean (6 observations per point)

Immobilization resulted in an increase of ACTH release in rats treated with saline 1 hour before (Fig. 3). A decline in corticosteroid production was observed 2 and 4 hours after immobilization. The maximal depression of stress-

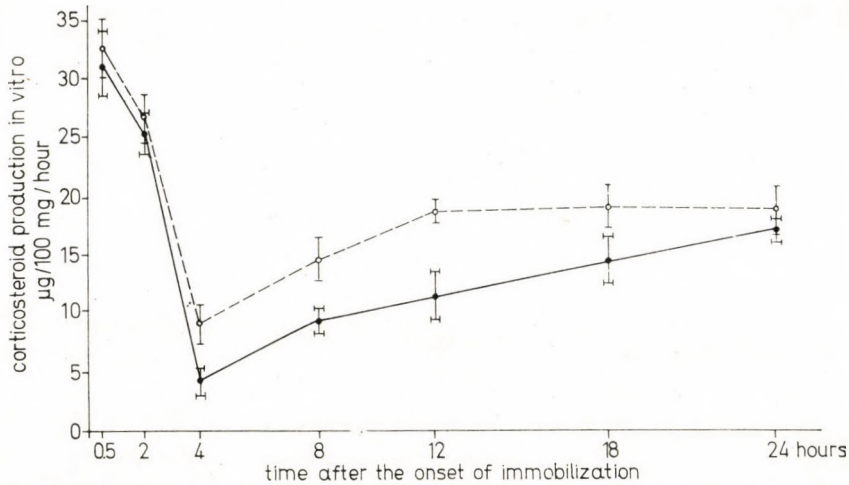



*Fig. 3.* Effect of continuous immobilization on adrenal corticosteroid production in vitro of male rats treated with corticosterone (●——●) or saline (○– – –○) 1 hour prior to immobilization. Bars indicate standard error of the mean (6 observations per point)

induced ACTH release appeared in the 4th hour. Then a second rise of ACTH secretion occurred in the 8th and 12th hours and corticosteroid production remained at that level for the next 12 hours.

Maximal pituitary ACTH release as indicated by adrenal corticosteroid production was not affected by the previous administration of corticosterone. A more marked decline ($p < 0.01$) was, however, observed 4 hours after the onset of immobilization in rats treated with corticosterone. The second rise in

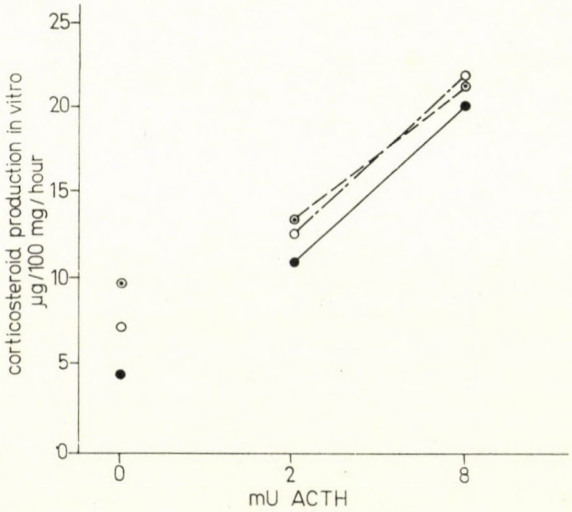



*Fig. 4.* Effect of ACTH on adrenal corticosteroid production of hemiadrenalectomized rats treated with corticosterone (●——●) or saline (○—·—○). Comparison between rats hemiadrenalectomized 12 hours before and non-operated (⊙— —⊙) rats. 5 observations per point

**Table I**

*Effect of repeated stress of environmental change on the pituitary-adrenal activity of hemiadrenalectomized rats 12 hours after surgery*

| Treatment* | Surgery | Adrenal corticosteroid production** | |
|---|---|---|---|
| | | Before stress | After stress |
| Corticosterone | Hemiadrex | 2.1 ± 0.9*** (5)**** | 3.6 ± 2.0 (6) |
| Saline | Hemiadrex | 6.2 ± 1.2 (6) | 10.4 ± 1.6 (6) |
| None | None | 11.6 ± 2.0 (9) | 23.4 ± 1.6 (8) |

* Corticosterone (5 mg/100 g b.w.) or saline, given 1 hour prior to hemiadrenalectomy
** µg/100 mg adrenal/hour
*** Mean ± standard error of mean
**** Number of observations

ACTH release was also apparent in corticosterone-treated rats, but adrenal corticosteroid production remained significantly below the corresponding control values at 0.01 (8th and 12th hour) and 0.05 (18th hour) levels.

The repeated stress of exposure to a new environment provoked significantly ($p < 0.01$) less ACTH release in saline-treated rats 12 hours after hemiadrenalectomy than in the non-operated rats (Table I). The response to repeated stress was absent in corticosterone-treated rats 12 hours after hemiadrenalectomy.

Adrenal response to ACTH administered intravenously in pentobarbital anaesthesia 12 hours after hemiadrenalectomy showed the same pattern in corticosterone- and in saline-treated rats (Fig. 4). The ACTH sensitivity of the adrenals of hemiadrenalectomized rats was similar to that of the non-operated controls.

## Discussion

The pituitary-adrenal axis showed a biphasic response to continuously acting stressor such as hemiadrenalectomy or continuous immobilization. Maximal ACTH release occurred immediately after the onset of stimuli, then a subnormal phase of ACTH secretion developed; the latter phase seemed to depend on the stimulus applied. The decline was followed by a second rise of ACTH release but the rate of pituitary-adrenal activation never attained the maximal ACTH output during the early period of exposure to stress. A similar response pattern was observed by Knigge et al. (1959) in rats exposed to immobilization and by Reck and Fortier (1960) following hemiadrenalectomy.

In addition, the present experiments suggest that the negative feedback effect of corticosteroids may play a role in the control of pituitary ACTH release in response to continuously acting stressors. The phase of subnormal ACTH secretion 12 hours after hemiadrenalectomy or 4 hours after the onset of immobilization was more pronounced in rats treated with corticosterone. Furthermore, the second rise in ACTH release after the subnormal phase was moderately diminished by corticosterone pretreatment.

Since corticosterone administration leads to an increase in plasma corticosterone concentration, one may suggest that this increased plasma steroid level is acting through a negative feedback mechanism to diminish ACTH secretion in the subnormal phase and during the second rise of ACTH release. If this is the case, then the suppression of ACTH secretion during the subnormal phase might be due to the increased output of adrenocortical hormones in consequence of maximal ACTH release in the early period of stimulus exposure. The second rise of ACTH release after the subnormal phase, to the level beyond the maximal ACTH output, may be the result of both the continuous stimulatory influence of the stressor breaking through the complete steroid

inhibition, and the suppressive effect of adrenal steroid secretion. As a corollary to this supposition, a constant high plasma corticosterone level would determine the feedback effect of steroids.

This assumption, however, is not consistent with the observations of SMELIK (1963b) and HODGES and JONES (1964), who showed a lack of correlation between the blood corticosterone level and the degree of pituitary inhibition. Suppression of ACTH release appears long after the increase in the blood corticosterone level induced by injected steroid has subsided. A similar time-pattern was observed in the present experiments, where the maximal suppression of ACTH secretion in non-stressed, corticosterone-treated rats occurred 8 to 12 hours after the injection of the steroid. Thus, it may be suggested that the negative feedback action of corticosteroids on ACTH release is independent of the actual plasma corticosteroid level.

SMELIK (1963b) suggested that the inhibition of pituitary ACTH release depends on the accumulation of corticosteroids by certain structures controlling pituitary-adrenal activity. This assumption agrees well with those findings which showed that corticosteroid implantation into the basal-medial hypothalamus and into certain fore- and midbrain structures increase the local concentration of steroids but inhibit the stress-induced ACTH release (ENDRŐCZI et al. 1961; DAVIDSON and FELDMAN 1963; CORBIN et al. 1965; BOHUS and LISSÁK 1967). Electrophysiological experiments revealed that evoked potentials induced by sciatic nerve stimulation in the anterior hypothalamus of the rat are seen enhanced by a single dose of hydrocortisone but depressed only after a lag of 2 hours. Furthermore, forebrain inhibition of mesencephalic reticular potentials evoked by sciatic nerve stimulation is enhanced by corticosteroids (ENDRŐCZI et al. 1968). The present experiment, showing the unchanged ACTH sensitivity of the adrenals during the subnormal phase of ACTH secretion, may also suggest the involvement of a level higher than the adrenal cortex in the feedback effect of corticosteroids.

Recent experiments, however, suggest that a possible accumulation of corticosterone in some structures controlling pituitary-adrenal activity is not the sole factor to determine the slow feedback effect of corticosteroids during the action of continuously operating stressor stimuli. The phase of subnormal ACTH secretion may take different times to appear after the onset of stress exposure, as a subnormal ACTH secretion was observed 4 hours after immobilization and 12 hours following adrenalectomy. These facts emphasize that the neural processes evoked by noxious stimuli are also decisive factors in the feedback action of corticosterone. It remains to be shown whether the corticosteroid "sensitivity" of the stimulatory neural mechanisms is different or, else, the corticosteroid feedback effect modifies the actual neural pattern by enhancing the inhibitory mechanisms participating in the control of pituitary-adrenal activity.

## LITERATURE

BOHUS, B., ENDRŐCZI, E., LISSÁK, K.: Studies on the control of the pituitary-adrenal system: stress and humoral feed-back control. Acta physiol. Acad. Sci. hung. **1965,** 27: 279—284.

BOHUS, B., LISSÁK, K.: The sites of feedback action of corticosteroids at extrahypothalamic level. Gen. comp. Endocr. **1967,** 9: 434—435.

BOHUS, B.: Failure of corticosterone to suppress pituitary ACTH release in rats exposed to various noxious stimuli. Acta physiol. Acad. Sci. hung. **1968,** 34: 241—244.

CORBIN, A., MANGILI, G., MOTTA, M., MARTINI, L.: Effect of hypothalamic and mesencephalic steroid implantations on ACTH feedback mechanisms. Endocrinology **1965,** 76: 811—818.

DAVIDSON, J. M., FELDMAN, S.: Cerebral involvement in the inhibition of ACTH secretion by hydrocortisone. Endocrinology **1963,** 72: 936—946.

ENDRŐCZI, E., LISSÁK, K., TEKERES, M.: Hormonal "feedback" regulation of pituitary-adrenocortical activity. Acta physiol. Acad. Sci. hung. **1961,** 18: 291—299.

ENDRŐCZI, E., LISSÁK, K., KORÁNYI, L., NYAKAS, Cs.: Influence of corticosteroids on the hypothalamic control of sciatic-evoked potentials in the brain stem reticular formation and the hypothalamus. Acta physiol. Acad. Sci. hung. **1968,** 33: 375—382.

ESTEP, H. L., ISLAND, D. P., NEY, R. L., LIDDLE, G. E.: Pituitary-adrenal dynamics during surgical stress. J. clin. Endocr. **1963,** 23: 419—425.

VAN GOCH, J. J., DE WIED, D., SCHÖNBAUM, E.: Adrenocorticotropic activity in the rat assessed by in vivo and in vitro indices. Amer. J. Physiol. **1963,** 205: 1083—1088.

HENKIN, R. I., KNIGGE, K. M.: The effect of sound on the hypothalamo-pituitary-adrenal axis. Amer. J. Physiol. **1963,** 204: 710—714.

HODGES, J. R., VERNIKOS, J.: Circulating corticotrophin in normal and adrenalectomized rats after stress. Acta endocr. (Kbh.) **1959,** 30: 188—196.

HODGES, J. R., JONES, M. T.: The effect of injected corticosterone on the release of adrenocorticotrophic hormone in rats exposed to acute stress. J. Physiol. (Lond.) **1963,** 167: 30—37.

HODGES, J. R., JONES, M. T.: Changes in pituitary corticotrophic function in adrenalectomized rats. J. Physiol. (Lond.) **1964,** 173: 190—200.

KNIGGE, K. M., PENROD, C. H., SCHINDLER, W. J.: In vitro and in vivo adrenal corticosteroid secretion following stress. Amer. J. Physiol. **1959,** 196: 579—582.

RECK, D. C., FORTIER, C.: Adrenal ascorbic acid and corticosteroidogenesis following unilateral adrenalectomy in the rat. Proc. Soc. exp. Biol. (N. Y.) **1960,** 104: 610—613.

SAYERS, G., SAYERS, M. A.: Regulation of pituitary ACTH activity during the response to stress. Endocrinology **1947,** 40: 265—273.

SMELIK, P. G.: Failure to inhibit corticotrophin secretion by experimentally induced increase in corticoid level. Acta endocr. (Kbh.) **1963a,** 44: 36—46.

SMELIK, P. G.: Relation between blood level of corticoids and their inhibitory effect on the hypophyseal stress response. Proc. Soc. exp. Biol. (N. Y.) **1963b,** 113: 616—619.

STARK, E., FACHET, J.: Effect of blood corticosteroid levels on ACTH release caused by stress. Acta med. Acad. Sci. hung. **1963,** 9: 366—370.

VERNIKOS-DANELLIS, J., TRIGG, L. N.: Feedback mechanisms regulating pituitary ACTH secretion in rats bearing transplantable pituitary tumors. Endocrinology **1967,** 80: 345—350.

VAN DER VIES, J., BAKKER, R. F. M., DE WIED, D.: Correlated studies on plasma-free corticosterone and on adrenal steroid formation in vitro. Acta endocr. (Kbh.) **1960,** 34: 513—523.

YATES, F. E., LEEMAN, S. E., GLENISTER, D. W., DALLMAN, M. F.: Interaction between plasma corticosterone concentration and adrenocorticotropin-releasing stimuli in the rat: evidence for the reset of an endocrine feedback control. Endocrinology **1961,** 69: 67—80.

Béla BOHUS
Orvostudományi Egyetem Élettani Intézete,
Pécs, Rákóczi út 80, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 149—151 (1969)*

# EFFECT OF ANGIOTENSIN SUPERFUSION ON THE RATE OF ALDOSTERONE PRODUCTION BY INCUBATED RAT ADRENALS

By

A. Spät, J. Sólyom, J. Sturcz, Ildikó Mészáros and E. Ludwig

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received November 30, 1968)

The effect of angiotensin II on adrenal steroid production in vitro has been studied in the rat. To compensate for the destruction of angiotensin due to adrenal angiotensinase activity angiotensin was added continuously to the incubation medium during the whole period of incubation. Superfusion of angiotensin failed to affect adrenal aldosterone production.

The role played by angiotensin II in the regulation of aldosterone secretion in the rat is being currently questioned. The demonstration of a direct action of angiotensin on the adrenals would constitute an important proof in favour of the aldosteronotrophic effect of angiotensin II. As shown by Gláz and Sugár (1962), Kaplan and Bartter (1962) and in previous experiments from this laboratory (Spät et al. 1965) angiotensin II does not augment the rate of aldosterone production of incubated rat adrenals. Müller (1965) observed no increase up to 80 $\mu$g/ml angiotensin concentrations in the incubation medium, whereas 320 $\mu$g/ml had an enhancing effect.

Since Bumpus et al. (1964) found a high angiotensinase activity in the rat adrenal, it seems reasonable to assume that the angiotensin added to the incubation medium is destroyed at such a high rate that enhancement of aldosterone production occurs only initially which cannot be detected by the methods used in such studies. Inhibition of angiotensinase by EDTA, DFP or other agents may interfere also with the synthesis of steroid hormones and thus it is not a suitable procedure. In the present experiments, angiotensin II was continuously added to the incubation medium bathing the adrenal slices.

## Methods

A total of 60 male albino rats with an average weight of 140 g was used in the experiments. Six adrenals were pooled in 10 flasks and incubated in Krebs—Ringer bicarbonate-glucose medium; these served as controls. To another 10 such pools angiotensin II (Hypertensin, Ciba) was added to the medium at a concentration of 1 $\mu$g/ml, so that each flask contained 6 adrenals and 10 $\mu$g of synthetic angiotensin. Continuous supply of angiotensin to the latter pools was secured by adding 0.5 $\mu$g/min of angiotensin II to the medium by a micro-infusion pump throughout the 120 min of incubation. The total amount of angiotensin II in

each pool attained 60 $\mu$g; this was added after dissolving in 1 ml of Krebs—Ringer bicarbonate-glucose solution. Incubation was undertaken at 38 °C, in a mixture of 95% $O_2$ and 5% $CO_2$.

Steroid content of the incubation medium was assayed after extraction with chloroform, Bush-type paper chromatography and the tetrazolium blue reaction (for details see SPÄT et al. 1968).

## Results

The rate of aldosterone production by the control adrenals was 1.02 $\pm$ 0.12 (S.E.) $\mu$g/100 mg/h, which was associated with a corticosterone production of 5.08 $\pm$ 0.36 $\mu$g/100 mg/h. Aldosterone synthesis was unaffected by

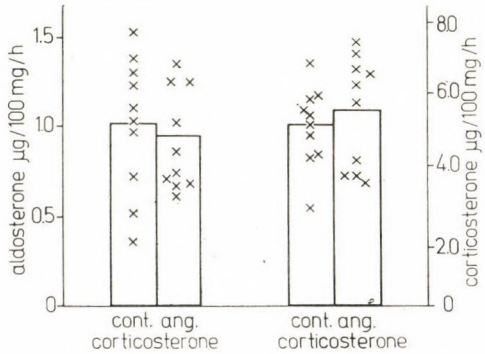



*Fig. 1.* Effect of angiotensin II superfusion on the rate of aldosterone and corticosterone synthesis by the incubated rat adrenal

the superfusion of angiotensin II, as the rate of aldosterone production in the experimental series attained 0.95 $\pm$ 0.08 $\mu$g/100 mg/h; the secretion of corticosterone was somewhat augmented (5.50 $\pm$ 0.56 $\mu$g/100 mg/h), but the difference was not significant statistically ($P > 0.30$; Fig. 1).

## Discussion

The high angiotensinase activity in the rat adrenal was described by BUMPUS et al. (1964) who found a 20 min half-life for angiotensin II incubated in adrenal gland homogenate. GLÁZ and SUGÁR (1962), KAPLAN and BARTTER (1962) and ourselves (SPÄT et al. 1965) failed to demonstrate any enhancing effect of angiotensin on the rate of aldosterone production by incubated rat adrenal slices. In these experiments, the failure of angiotensin to augment aldosterone synthesis might have been due to a rapid destruction of the hormone by tissue angiotensinase. Taking into considering the 20 min half-life of angiotensin it must have been present in this experiment in the medium at sufficiently high concentrations. The finding that aldosterone was not

increased by angiotensin even when this had been superfused continuously indicates that, in the rat, the failure of angiotensin II to affect adrenal aldosterone secretion is not or not only due to the presence of angiotensinase in the adrenals.

## LITERATURE

BUMPUS, F. M., SMEBY, R. R., PAGE, I. H., KHAIRALLAH, P. A.: Distribution and metabolic fate of angiotensin II and various derivatives. Canad. med. Ass. J. **1964,** 90: 190—193.

GLÁZ, E., SUGÁR, K.: The effect of synthetic angiotensin II on synthesis of aldosterone by the adrenals. J. Endocr. **1962,** 24: 299—302.

KAPLAN, N. M., BARTTER, F. C.: The effect of ACTH, renin, angiotensin II and various precursors on biosynthesis of aldosterone by adrenal slices. J. clin. Invest. **1962,** 41: 715—724.

MÜLLER, J.: Aldosterone stimulation in vitro I. Acta endocr. (Kbh.) **1965,** 48: 283—296.

SPÄT, A., STURCZ, J., SÓLYOM, J.: Effect of hypervolaemia on aldosterone production in the rat. Acta physiol. Acad. Sci. hung. **1968,** 33: 431—434.

SPÄT, A., STURCZ, J., SZIGETI, R.: New observations on the function of the angiotensin-aldosterone system. Acta physiol. Acad. Sci. hung. **1965,** 27: 199—203.

András SPÄT, János SÓLYOM, József STURCZ, Ildikó MÉSZÁROS,
Endre LUDWIG
Orvostudományi Egyetem Élettani Intézete,
Budapest VIII., Puskin u. 9, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 153—166 (1969)*

# ESTIMATION OF THE BLOOD PROLACTIN LEVEL

## A CRITICAL ANALYSIS OF LITERARY DATA AND OF THE SULMAN—SIMKIN METHOD OF EXTRACTION

By

M. KURCZ, I. NAGY, Cs. KISS and L. HALMY

NATIONAL INSTITUTE OF PUBLIC HEALTH, HEIM PÁL CHILDREN'S HOSPITAL, AND POSTGRADUATE MEDICAL SCHOOL, BUDAPEST

(Received August 23, 1968)

Recent data on the methodological problems of estimating prolactin in blood by the pigeon crop sac technique are reviewed. The results yielded by the different methods are conflicting, partly owing to methodological errors.

From model experiments *in vivo* and *in vitro* it has been concluded that the extraction procedure described originally by SULMAN (1956) and later recommended by SIMKIN and GOODART (1960) is unsuitable for the determination of prolactin in blood; data obtained by this method may be regarded as artifacts.

The criteria of the specificity of the prolactin effect with respect to the estimation of the hormone in native blood, its disappearance rate and the causes why several research groups were unable to detect prolactin in blood are discussed.

The obtained results suggest that the blood prolactin level is lower than the sensitivity threshold of the pigeon crop sac micro-test, therefore the value obtained after the injection of native plasma is not a reliable indicator of the blood prolactin content.

A further advance in the pathophysiology of adenohypophyseal trophic hormones cannot be expected before some reliable method has been elaborated for their determination in blood. Such a sensitive method would be of particular value in clinical medicine, since the procedures now available, being based upon the observation of the target organs, furnish indirect data concerning the concentration of the hormones in body fluids.

According to previous experiments (KURCZ et al. 1964, 1965, 1966, 1967) prolactin cannot be measured in blood since the sensitivity threshold of the most widely used assay method, the pigeon crop sac micro-test, is higher than the concentration of prolactin in blood. Recently, however, numerous investigators have reported on the estimation of prolactin in human blood (KRAWCZUK et al. 1962; STEHLIKOVA et al. 1965; BERSWORDT-WALLRABE and JANTZEN 1964; BERSWORDT-WALLRABE et al. 1965; SÁVELY et al. 1965a, b; GÁTI et al. 1967; FLASKAMP et al. 1968; TALAŠ et al. 1968). All these teams used the pigeon crop sac for the determination of prolactin, injecting blood without pretreatment or after having subjected it to the procedure suggested by SIMKIN (1958). In 1966, MEITES and NICOLL reported that they were unable to detect prolactin in blood with SIMKIN's method (1960, 1963) but they omitted to discuss the cause of their negative results.

In view of the above literary data it seemed interesting to reinvestigate the problem of blood prolactin content. The results obtained in this study and model experiments have confirmed our belief that data yielded by the pigeon crop sac method should be considered artifacts.

### *Survey of literary data*

The papers reporting on blood prolactin estimation contain numerous contradictions and errors. Prolactin could not be detected in the blood of children and males by SIMKIN and GOODART (1960), SIMKIN and ARCE (1963), and GÁTI et al. (1967), nor in the blood of non-lacting women by SÁVELY et al. (1965). On the other hand, FLASKAMP (1968) found 24 I.U. per 100 ml of plasma in healthy children, more than the 10 I.U. per 100 ml of blood obtained by GÁTI et al. (1967) during the menstrual cycle in 24-year-old women. In women during lactation 0.05 I.U. was found (SÁVELY et al. 1965a); unfortunately, the authors omitted to give the volume in which they found this value. In another paper of the same group a value of 0.5 I.U. is mentioned (SÁVELY et al. 1965b). SIMKIN and GOODART (1960) were unable to detect prolactin in blood during the first half of the menstrual cycle, whereas SIMKIN and ARCE (1963) could demonstrate prolactin activity during the first 5 days and the last 10 days of the cycle. In the second half of the 28-day cycle, GÁTI et al. (1967) found an activity of about 10.0 I.U. per 100 ml, SIMKIN et al. (1960, 1963) 2—4 I.U. per 100 ml, and TALAŠ et al. (1968) approximately 0.3 I.U. per 100 ml of blood. The greatest differences were found by STEHLIKOVÁ et al. (1965) who obtained in the secretory phase of the cycle 0.0012 to 0.06 I.U., in the lactation period 84 to 780 I.U., and in patients with galactorrhoea 0.36 to 6.000 I.U. per 100 ml of blood.

SÁVELY et al. (1965), STEHLIKOVÁ et al. (1965), GÁTI et al. (1967), TALAŠ et al. (1968) succeeded in demonstrating prolactin in human blood only with the aid of the preparatory procedure described by SULMAN (1956) and recommended by SIMKIN and GOODART (1960); whereas others (BERSWORDT-WALLRABE and JANTZEN 1964; FLASKAMP et al. 1968) obtained positive results both with and without the preparation of the material. According to FLASKAMP et al. (1968) the procedure of preparation causes considerable losses, whereas SIMKIN and GOODART (1960) recovered completely the prolactin added to the blood of children.

A similar discrepancy exists in the calibration curves used by the different authors. SIMKIN (1958) in his paper describing the crop sac method emphasized the importance of the volume injected into the skin of the pigeon. For instance, 0.05 I.U. of prolactin in a volume of 0.75 ml caused almost the same gain in weight of the crop sac as did 5 I.U. dissolved in 0.1 ml. In our opinion, this holds even more for the method based on planimetry (GROSVENOR and

TURNER 1958). Despite these well-known facts, several authors (HERLYN et al. 1965) plotted the calibration curve after injecting 4 times 1 ml and later (FLASKAMP et al. 1968) 4 times 0.1 ml; in spite of this difference, the two calibration curves agree well.

Calculation and presentation of the calibration curve are in most of the papers insufficient or erroneous. Thus, it happened that amounts five times less than the lower threshold of the method's sensitivity were "estimated" (GÁTI et al. 1967). SÁVELY et al. (1965a) reported on the weight of 3 pigeon crop sacs of which one gave an opposite result. Although the observations were interpreted with a certain reservation, the supplementary histological examination was not convincing either. The results obtained by TALAŠ et al. (1968) have to be considered unreliable since the material containing the hormone was injected *subcutaneously* instead of *intradermally*. Finally, the majority of the values obtained by STEHLIKOVÁ et al. (1965) lay beyond the calibration curve and the concentrations were determined by extrapolation.

Thus, there are considerable differences in the use of the pigeon crop test and in its evaluation between the various investigators, and the extraction procedure of SULMAN and SIMKIN which has never been subjected to careful control, seems to be the source of substantial errors.

### *Criteria of the specific effect of prolactin*

BAHN and BATES (1956) presented a systematic analysis of those earlier and rather conflicting results which had been obtained with the pigeon crop sac technique when used for the estimation of prolactin in serum and urine. They suggested that the specific histological criterion was a diffuse cytoplasmic basophilia of the epithelial cells of the crop sac. This has been accepted by PASTEELS (1963) as well as NICOLL and BERN (1964) and rejected by PREISZ et al. (1967), while others have not even mentioned it although they subjected the epithelium of the crop sac to histological examination (TALAŠ et al. 1968; SÁVELY et al. 1965). BAHN and BATES (1956) stated in addition that blood and urine extracts whether injected locally or intramuscularly do not give rise to basophilia of the crop sac epithelium.

Similar conclusions were drawn from a gross study of the dried and spanned crop sac (KURCZ 1966). The mosaic structure characteristic of the prolactin effect was never observed after the injection of blood or other extracts containing no prolactin. The local injection of these materials caused an inflammatory reaction which increased the weight of the crop sac and made preparation more difficult, but the macroscopic alterations were different from those elicited by prolactin.

In the present study prolactin was determined in large series after the intradermal injection of native blood serum; as described (KURCZ 1966)

**Table I**

*Determination of prolactin in native blood serum*

| Serum | Volume injected | Number of determinations* | Results of pigeon crop sac assay | | |
|---|---|---|---|---|---|
| | | | positive | negative | doubtful |
| *Human* | | | | | |
| Woman | 0.4 ml | 4 | — | 4 | — |
| Woman | 0.8 ml | 8 | — | 8 | — |
| Woman | 1.6 ml | 10 | — | 10 | — |
| Man | 0.4 ml | 2 | — | 2 | — |
| Man | 0.8 ml | 4 | — | 4 | — |
| Man | 1.6 ml | 10 | — | 10 | — |
| *Sheep* | | | | | |
| ♀ | 0.4 ml | 3 | — | 3 | — |
| ♀ | 0.8 ml | 6 | — | 6 | — |
| ♂ | 0.4 ml | 2 | — | 2 | — |
| ♂ | 0.8 ml | 6 | — | 6 | — |
| *Rat* | | | | | |
| ♀ | 0.4 ml | 20 | — | 20 | — |
| ♀ | 0.8 ml | 8 | — | 8 | — |
| during lactation | | | | | |
| ♀ | 1.6 ml | 10 | — | 10 | — |
| with pituitary tumour | | | | | |
| ♀ | 0.8 ml | 8 | — | 8 | — |
| ♀ | 1.2 ml | 8 | — | 8 | — |
| Ovine prolactin preparation | 0.02 to 0.06 I.U. | 40 | 36 | 2 | 2 |
| Rat pituitary extract | 6.8 mg of wet weight | 50 | 50 | — | — |

* For the determination carrier-pigeons aged 6 to 8 weeks and weighing 250 to 350 g were used. The serum samples were divided in four parts and equal amounts were injected daily for 4 days intracutaneously, in the region lying above the susceptible part of the crop sac. Necropsy was undertaken 24 hours after the last injection (Grosvenor and Turner 1958, Kurcz 1966).

a positive response was recorded when the mosaic structure characteristic of prolactin was seen on the crop sac. Results are summarized in Table I; it is seen that no positive response occurred after the injection of native serum.

In a subsequent series, ovine prolactin was dissolved in human serum, and activity was established after incubation for 12, 24 and 48 hours at 37 °C. During the subsequent days the serum samples were stored at 0 °C. Results are shown in Table II. As it is seen, all the prolactin added to the serum could be demonstrated after 48 hours. The dried crop sac showed the characteristic mosaic-like structure, and with a few exceptions the area showing a positive response could be measured reliably. The practically complete recovery of the

**Table II**

*Determination of prolactin after incubation in vitro in the presence of blood serum*

| Origin of serum | Amount of dissolved prolactin (I.U. per 0.4 ml) | Duration of incubation (hours) | Number of determinations | Positive response | Recovered amount of prolactin* (I.U.) |
|---|---|---|---|---|---|
| Human (3) | 0.5 | 12—24—48 | 12 | 12 | 0.5 |
| Rabbit (1) | 0.25 | 12—24—48 | 12 | 12 | 0.25 |
| Ovine (3) | 0.12 | 12—24—48 | 20 | 20 | 0.12 |
| Rat (5) | 0.06 | 12—24—48 | 20 | 16 | ~0.06 |
| Rat during lactation (5) | 0.25 | 24 | 5 | 5 | 0.25 |
| Rat with pituitary tumour (4)+ | 0.25 | 24—48 | 16 | 16 | 0.25 |

* Within a confidence limit of ±20%.

\+ Blood obtained from rat in which a pituitary tumour had been induced by diethylstilboestrol treatment (KURCZ et al. 1967).

amounts of prolactin added to the sera indicates that the statement of SULMAN (1956) emphasized by SIMKIN and GOODART (1960) that "this procedure (viz. the precipitation with acetone) inactivates the hormone decomposing enzymes of the blood" does not hold true for prolactin. The presence of inactivating enzymes would render it impossible to establish the concentration of prolactin in native blood.

### *Failure of Sulman and Simkin's preparatory procedure in the extraction of prolactin from blood*

As prolactin could not be detected in native blood, the possibility arose that the amount injected (0.4 to 1.6 ml) did not suffice for inducing the specific reaction and that the inflammatory reaction caused by the inert proteins may interfere with an evaluation of the response produced by prolactin. To test this possibility, blood samples were prepared in three different laboratories according to SULMAN, SIMKIN and GÁTI et al., with the only difference that the volume of blood was 20 ml, thus twice the amount given in the original papers; also, the "dissolved" final precipitation was not divided in smaller quantities but was injected intradermally into the same pigeon.

As seen in Tables III and IV the final precipitate allegedly containing prolactin failed to give a positive reaction in 34 cases and in 4 blood samples the result was dubious.

**Table III**

*The method of Sulman and Simkin for the extraction of prolaction from blood*
20.0 ml of full blood + 40.0 ml of acetone of analytical purity + 0.2 ml of glacial acetic acid)

Percolation on filter paper

↓ Filtrate

↓ Precipitate No. 1 (discarded)

+ 120 ml of acetone and 20 ml of ether
Precipitation for 12 hours at 0 °C
Precipitate No. 2 is decanted
Washing with ether
Evaporation
Solution of precipitate in 1.6 ml of 0.05 N NaOH
Neutralization with 1 N HCl
Distilled water added to a volume of 2 ml

### *Addition of prolactin to blood in vitro*

Prolactin of known activity was added to blood samples and recovery from the final precipitate was studied. Coagulation of blood was inhibited by heparin. The materials examined were:

| | | |
|---|---|---|
| physiological saline | + | purified ovine prolactin |
| ovine blood | + | purified ovine prolactin |
| ovine serum | + | purified ovine prolactin |
| human blood | + | purified ovine prolactin |
| human blood | + | human pituitary extract |
| rat blood | + | rat pituitary extract |

**Table IV**

*Determination of "prolactin" in human blood plasma by Sulman and Simkin's method*

| Test subjects | Number of cases | Results | | |
|---|---|---|---|---|
| | | negative | doubtful | positive |
| *I. Determination in 20 ml of blood* | | | | |
| Healthy women on the 18th to 21st day of the cycle | 11 | 10 | 1 | — |
| Women in the lactation period | | | | |
| 1st and 2nd day } after delivery | 5 | 5 | — | — |
| 3rd day } after delivery | 3 | 3 | — | — |
| 5th and 6th day } after delivery | 10 | 8 | 2 | — |
| Women with galactorrhoea | 2 | 2 | — | — |
| *II. Determination in 60 ml of blood* | | | | |
| Healthy women on the 18th to 21st day of the cycle | 3 | 2 | 1 | — |

Results are summarized in Table V. The majority of added prolactin was recovered only when a protein-free physiological saline solution was subjected to the procedure of "purification" and "concentration". Recovery even in this case was not more than 50%, firstly because much prolactin is absorbed by the filter paper, secondly because prolactin is destroyed by drying with ether and solution in alkali. Recovery was considerably lower when the prolactin was dissolved in serum, and it was completely lost when prolactin was added to blood. As shown in Table V the largest fraction remained in "precipitate No. 1" in which it could be demonstrated after having dissolved an aliquot amount in 0.05 N NaOH, centrifuged, adjusted to pH 7 and injected into the pigeon.

The retention of prolactin in various interphases of the preparation procedure was checked also by acrylamide gel electrophoresis. A maximum amount was dissolved, 20 mg, i.e. 400 I.U. per 1 ml of ovine serum, then a 5 $\mu$l sample was subjected to electrophoresis. If the material had completely dissolved, bands corresponding to 100 $\mu$g were obtained, as shown in Fig. 1. However, the whole amount of the final precipitate contained only insignificant amounts of protein at the site where prolactin had to appear.

### *Decrease of prolactin activity on treatment with ether and alkali*

The final precipitate takes a long time to dissolve in 0.05 N NaOH, the alkali used by the majority of authors. It is, however, known that in alkaline medium prolactin is destroyed and its activity is lost. The rate of

**Table V**

*Attempts for the recovery of known amounts of prolactin added in vitro to native blood samples, after preparation with the usual technique*

| Number of cases | Activity added to blood (I.U.) | Activity calculated from the activity of serum (I.U.) | Activity of precipitate No. 1 (I.U.)* | Activity in final precipitate (I.U.)** |
|---|---|---|---|---|
| 1. Human blood + human pituitary extract + | | | | |
| 6 | 1 | 0.6 ± 0.2 | 0.5 | 0 |
| 6 | 23 | 20.0 ± 3.0 | 12.0 | 0 |
| 2. Human blood + purified ovine prolactin + + | | | | |
| 4 | 200 | 200 | 100 | 0 |
| 2 | 400 | 400 | 200 | 1 to 2 |
| 3. Ovine blood + purified ovine prolactin | | | | |
| 3 | 200 | 200 | 120 | 0 |
| 3 | 400 | 400 | 200 | 0.5 |
| 4. Ovine serum + purified ovine prolactin | | | | |
| 3 | 200 | 200 | 100 | 0.5 |
| 3 | 400 | 400 | 200 | 1.5 |
| 5. Rat blood + purified pituitary extract§ | | | | |
| 2 | 5 | 5 | 1 to 2 | 0 |
| 6. Physiological saline + purified ovine prolactin | | | | |
| 2 | 200 | — | 50□ | 100 |
| 2 | 400 | — | 50□ | 200 |

* Within a confidence limit of ±20%
** Within a confidence limit of ±40%
\+ Prepared according to the method of Apostolakis (1965)
\+ + Panlitar, Armour Ltd., 15. I.U. per mg, and Prolactin, Ferring Ltd., 22 I.U. per mg
§ Extract prepared from a neoplastic hypophysis
□ Activity of the filter paper

destruction depends on the pH of the medium, the temperature, and the duration of exposition (Reisfeld et al. 1964; Lewis and Cheever 1965, 1967). The inactivating effect of alkali has not been analysed quantitatively chiefly because the error due to this procedure is insignificant as compared to those caused by the preceding phases; on the other hand, the time necessary for complete inactivation has not been mentioned by any of the quoted authors. Nevertheless, this factor also represents a source of error, the more so as the final precipitate could never be dissolved completely in NaOH.

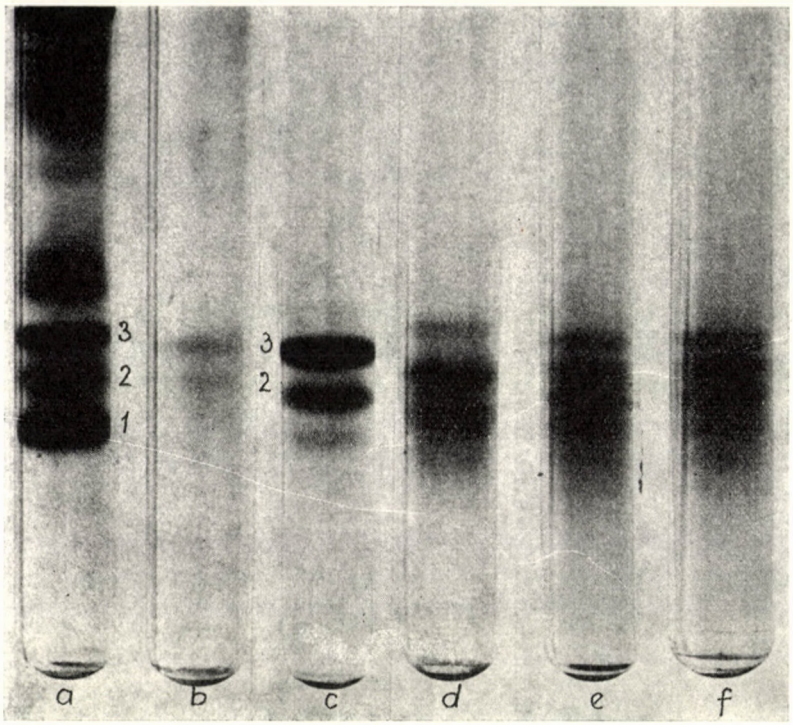



*Fig. 1.* Checking of the various steps of SULMAN's extraction procedure by means of acrylamide gel electrophoresis

(a) Preparation containing 1 ml of ovine serum and 20 mg of ovine prolactin. 100 mg of "precipitate No. 1" was dissolved in 0.05 N NaOH, then neutralized. Amounts of 5 $\mu$l were applied on the gel. Above the albumin band (1) the bands characteristic of sheep prolactin (2, 3) are seen. Thus, prolactin does not pass through the filter paper.

(b) "Final precipitate" of the same material. Prolactin (2, 3) is present only in traces.

(c) 100 $\mu$g of ovine prolactin preparation.

(d) 100 $\mu$g of ovine prolactin was incubated with 0.05 N NaOH for 15 hours at 4 °C. Slight destruction of the preparation.

(e, f) 2 mg of preparation added to 10 ml of ether. The decanted material was dried at room temperature. Marked destruction

Another source of error is in the drying in ether. The common ether for anaesthesia, of analytical purity, always contains some peroxide which by oxidation may diminish the biological activity of prolactin. To check this possibility, biological activity was established in an aliquot amount of prolactin by acrylamide gel electrophoresis. To the other aliquot (2 mg) 10 ml ether of analytical purity was added, and after decantation the material was evaporated to dryness. The specific activity of the preparation thus treated was diminished, and considerable changes were observed in the electrophoretic pattern (Fig. 1).

### *Addition of prolactin to blood in vivo*

To confirm the validity of our results obtained *in vitro*, in rats, the blood prolactin level was elevated by the subcutaneous, intraperitoneal and intravenous injection of prolactin containing preparations of known activity. Results are shown in Table VI. The administered activity disappeared from the blood with a half-time of 5 min and 30 sec, as reported previously (KURCZ and OROSZ 1965) and confirmed by GROSVENOR (1967). For several minutes after its injection, prolactin can easily be estimated in 0.4 ml samples of native serum, whereas prolactin is absent from the final precipitate if the material is subjected to the usual purification procedure. Part of the administered prolactin is extracted in urine, without diminution of the biological activity. This can be demonstrated in native urine without difficulty, whereas about 50% of the activity is lost if the urine is subjected to the preparation procedure.

**Table VI**

*Disappearance rate of prolactin from blood after the injection of exogenous prolactin*

| | Amount of prolactin injected (I.U.) | Mode of administration | Time of blood sampling after the injection | Number of determinations | Amount of prolactin in (I.U. per ml) | |
|---|---|---|---|---|---|---|
| | | | | | serum | urine |
| *A* | 20 | subcutaneously | 24 hours | 2 | 0 | 0 |
| | 40 | subcutaneously | 24 hours | 6 | 0 | 0 |
| | 40 | subcutaneously | 5 hours | 6 | 0 | 0 |
| | 40 | intraperitoneally | 45 min | 4 | ~0.09 | — |
| | 375 | intravenously | — | 4 | 47.5 | — |
| | 375 | intravenously | 15 min | 4 | 3.8 | 10.0 |
| | 375 | intravenously | 30 min | 4 | 1.0 | — |
| | 375 | intravenously | 60 min | 4 | 0.25 | — |
| | 375 | intravenously | 120 min | 4 | <0.02 | — |
| *B* | 1000 | intravenously | 10 min | 3 | 0 | >100 |

*A*: Determination from native serum, injecting 4 times 0.1 ml of the original or diluted serum
*B*: Determination after preparation, in 5 ml of full blood. The whole amount of precipitate No. 2 had been injected

In the present experiments *in vivo*, in contrast with the previous ones, prolactin was added to blood under physiological circumstances, so that it could mix with the blood constituents similarly to the endogenous hormone. In spite of this, after the usual preparation procedure prolactin could not be detected in the blood.

Of course, the possibility could not be excluded that the injection of a heterologous protein caused a reaction which might have interfered with the results. Therefore, experiments in which the disappearance of homologous prolactin is being studied are in progress. Unfortunately, the administration of human prolactin is not yet possible.

## Discussion

The results unequivocally showed that the data obtained with the extraction procedure of SULMAN and SIMKIN must have been artifacts.

There are at least 3 arguments speaking against the reliability of the results obtained after the injection of native blood. These are

(a) the rapid disappearance rate of prolactin;

(b) the erroneous evaluation of the pigeon crop sac reaction without consideration of the criteria set by BAHN and BATES;

(c) the great number of negative results obtained in animal experiments.

It is known from the experiments of GROSVENOR (1967) and from our own (KURCZ and OROSZ 1965) that the half-life of prolactin in blood is 5 to 10 min. Assuming that 20 I.U. of prolactin are contained in 100 ml of plasma, there must be 500 I.U. in the whole amount of circulating blood. As the amount inactivated in the body within 10 min is about 500 I.U., the human adenohypophysis would have to produce 3,000 I.U. of prolactin every hour. Considering that the biochemically homogeneous preparation of highest purity (REISFELD et al. 1964) contains 30 I.U. per mg, the above production rate means that the adenohypophysis would have to synthetize 100 mg of prolactin every hour; this is of course impossible, as the adenohypophysis cannot produce such an amount of protein.

The positive results indicating the presence of prolactin in native blood were obtained with the pigeon crop sac micro-method. It is of prime importance when working with this technique that the material containing the prolactin be injected into the skin lying above the susceptible area of the crop sac. BAHN and BATES (1956) showed unequivocally that after the intramuscular injection of serum, urine or nonspecific substances the crop sac does not show any specific reaction. The intradermal injection of the same materials in the area of the crop sac (so-called local injection) gives rise to a certain reaction which, however, is not like the response elicited by prolactin and cannot therefore be regarded as specific. It is characteristic of the prolactin effect that the epithelial cells of the crop gland not only increase in number, but also show an intense cytoplasmic basophilia. The specific reaction is easily recognized by the naked eye on the dried crop sac and is well distinguished from the inflammatory immunological reaction (KURCZ 1966). The authors detecting prolactin

in human blood neglected to check these criteria or found them negative (PREISZ et al. 1967).

Finally, mention should be made of the numerous unsuccessful attempts to detect prolactin in the blood of intact animals. Thus, BATES et al. (1966) were unable to detect prolactin in the blood of rats, and WOLTHUIS (1963), using the very sensitive method elaborated by him, could detect an appreciable activity only in the blood of rats bearing a pituitary graft. TALWALKER et al. (1964) could not find prolactin in the blood of either intact, pregnant, lactating or oestradiol-treated rats or in rats with a pituitary graft. On the other hand, they could detect prolactin in the blood of rats with a transplanted autonomic mammotropic tumour (TALWALKER et al. 1964; MIZUNO et al. 1964). In previous experiments we were also unable to detect prolactin in the blood of normal rats or rats possessing a pituitary neoplasm (KURCZ 1966; KURCZ et al. 1967). MEITES and NICOLL (1966) have emphasized that further confirmatory results are necessary for proving that the experiments indicating the presence of prolactin activity in blood in fact detected the hormone; their own results were consequently negative in this respect.

The problem of prolactin estimation in blood will be solved by the radioimmunoassay; the first results achieved by this method have already been published (APOSTOLAKIS 1965; LARON and APOSTOLAKIS 1966; ARAI and LEE 1967; KWA and VERHOFSTAD 1967a, b, c; KWA et al. 1967; BRYANT et al. 1968).

*

She authors would like to thank the Enddctinplogy Section, National Institute of Health, U.S.A., for the prolactin preparation.

## LITERATURE

ARAI, Y., LEE, T. H.: A double-antibody radioimmunoassay procedure for ovine pituitary prolactin. Endocrinology **1967,** 81: 1041—1046.

APOSTOLAKIS, M.: The extraction of prolactin from human pituitary glands. Acta endocr. (Kbh.) **1965,** 49: 1—16.

BAHN, R. C., BATES, R. W.: Histologic criteria for detection of prolactin: lack of prolactin in blood and urine of human subjects. J. clin. Endocr. **1956,** 16: 1337—1346.

BERSWORDT-WALLRABE, Ilse, JANTZEN, K.: Prolactin-Gehalt im Serum vor und nach der Ovulation, gemessen am Taubenkropftest. Arch. Gynäk. **1964,** 202: 30.

BERSWORDT-WALLRABE, I., HERLYN, U., JANTZEN, K.: A modification of the pigeon crop sac assay for determination of lactogenic hormone (prolactin) in human serum. Acta endocr. (Kbh.) **1965,** Suppl. 100, 169.

BATES, R. W., MILKOVIC, S., GARRISON, M. M.: Concentration of prolactin, growth hormone and ACTH in blood and tumor of rats with transplantable mammotropic pituitary tumors. Endocrinology **1962,** 71: 943—948.

BRYANT, G. C., TECH, B., GREENWOOD, F. C., LINZELL, J. L.: Radioimmunoassay of ovine, caprine and bovine prolactin in plasma: physiological and experimental changes in levels. Third Int. Congr. Endocrinology, Mexico **1968.**

FLASKAMP, D., BERSWORDT-WALLRABE, I., JANTZEN, K., HERLYN, U., GELLER, H. F.: Untersuchungen zur Frage der laktotropen Aktivität des somatotropen Hormons. Arch. Gynäk. **1968,** 205: 267—273.

GÁTI, I., DOSZPOD, J., PREISZ, J.: LTH production and steroid excretion during the menstrual cycle. Acta physiol. Acad. Sci. hung. **1967,** 32: 115—122.

GROSVENOR, C. E.: Disappearance rate of exogenous prolactin from serum of female rats. Endocrinology **1967,** 80: 195—200.

GROSVENOR, C. E., TURNER, C. W.: Assay of lactogenic hormone. Endocrinology **1958,** 63: 530—534.

HERLYN, U., GELLER, H. F., BERSWORDT-WALLRABE, I., BERSWORDT-WALLRABE, R.: Pituitary lactogenic hormone release during onset of pseudopregnancy in intact rats. Acta endocr. (Kbh.) **1965,** 48: 220—224.

KRAWCZUK, A., JÓZWIK, M., SZAMATOWITZ, M.: Sbornik XV. jubileuszowogo sjazda ginekolow polskich. Gdansk **1962,** pp. 18—20.

KYPY, M.: Гиноаламическая регуляция секреции пролоктина. Москва 1964. (Thesis).

KURCZ, M.: Specificity of the micro-test of the pigeon's crop-sac for prolactin determination. Ann. Univ. Sci. (Budapest) **1966,** 8: 150—154.

KURCZ, M.: Studies on the neuro-humoral regulation of lactation. In: Lissák, K. (Ed.): Symposium on reproduction. Akadémiai Kiadó, Budapest **1967.** Pp. 175—211.

KURCZ, M., KABAK, J. M.: A hypophysis prolaktin tartalmának változása az eminentia medialis és a hypothalamus középső területének roncsolása után. Kísérl. Orvostud. **1965,** 16: 82—87.

KURCZ, M., OROSZ, A.: Die "Anti-Prolaktin" Aktivität des Organismus. 2nd Endocrinological Days, Debrecen **1965.**

KURCZ, M., TIBOLDI, T., OROSZ, A., KOVÁCS, K., JULESZ, M.: Der Prolaktingehalt der durch Östrogenverabreichung in den intrasellaren und in die vordere Augenkammer transplantierten Rattenhypophysen hervorgerufenen Tumoren. Endokrinologie **1966,** 50: 316—325.

KURCZ, M., KOVÁCS, K., TIBOLDI, T., OROSZ, A.: Effect of androgenisation on adenohypophyseal prolactin content in rats. Acta endocr. (Kbh.) **1967,** 54: 663—667.

KWA, H. G., VERHOFSTAD, F.: Prolactin levels in the plasma of female ($C_{57}BL \times CBA$) $F_1$ mice. J. Endocr. **1967,** 38: 81—82.

KWA, H. G., VERHOFSTAD, F.: Prolactin levels in the plasma of female rats. J. Endocr. **1967,** 39: 455—456.

KWA, H. G., VERHOFSTAD, F.: Radioimmunoassay of rat prolactin. Biochim. biophys. Acta (Amst.) **1967,** 133: 186—188.

KWA, H. G., BENT, E. M., VERHOFSTAD, F.: Radioimmunoassay of mouse prolactin based on a protein isolated from the "granular" fraction of prolactin-producing pituitary tumour grafts. Acta endocr. (Kbh.) **1967,** Suppl. 119, 125.

LARON, Z., APOSTOLAKIS, M.: Immunological properties of a human pituitary prolactin preparation. J. Endocr. **1966,** 35: 117—118.

LEWIS, U. J., CHEEVER, E. V.: Evidence for two types of conversion reactions for prolactin and growth hormone. J. biol. Chem. **1965,** 240: 247—252.

LEWIS, U. J., CHEEVER, E. V.: Effect of toluene on bovine growth hormone and prolactin in pituitary extracts. Endocrinology **1967,** 81: 1338—1348.

MEITES, J., NICOLL, C. S.: Adenohypophysis: Prolactin. Ann. Rev. Physiol. **1966,** 28: 57—88.

MIZUNO, H., TALWALKER, P. K., MEITES, J.: Influence of hormones on tumor growth and plasma prolactin levels in rats bearing a pituitary "mammotropic" tumor. Cancer Res. **1964,** 24: 1433—1436.

NICOLL, C. S., BERN, H. A.: "Prolactin" and the pituitary glands of fishes. Gen. comp. Endocr. **1964,** 4: 457—471.

PASTEELS, J. L.: Recherches morphologiques et expérimentales sur la sécrétion de prolactine. Arch. Biol. (Liège) **1963,** 74: 439—553.

PREISZ, J., GÁTI, I., SAÁRY, Z., DOSZPOD, J.: A specifikus LTH-hatás histológiai kritériumáról. Congress of the Hungarian Society for Endocrinology and Metabolism. Pécs **1967.**

REISFELD, R. A., WILLIAMS, D. E., CIRILLO, V. J., TONG, G. L., BRINK, N. G.: Characterization of sheep prolactin. J. biol. Chem. **1964,** 239: 1777—1782.

SÁVELY, C., MÖDLINGER-ODORFER, M., SZÉCSÉNYI-NAGY, L.: Gleichzeitiges Vorkommen eines Chiari-Frommel-Syndroms und einer Hypothyreose. Endokrinologie **1965a,** 48: 129—141.

SÁVELY, C., MÖDLINGER-ODORFER, M., SZÉCSÉNYI-NAGY, L.: Chiari-Frommel syndroma és hypothyreosis együttes előfordulása. A Péterfy Sándor utcai Kórház-Rendelő Évkönyve, Budapest, **1965b.**

SIMKIN, B.: Pigeon crop weight responses to single local intradermal injections of prolactin. Exp. Med. Surg. **1958,** 16: 183—189.

SIMKIN, B., GOODART, D.: Preliminary observations on prolactin activity in human blood. J. clin. Endocr. **1960,** 20: 1096—1106.

SIMKIN, B., ARCE, R.: Prolactin activity in blood during the normal human menstrual cycle. Proc. Soc. exp. Biol. (N. Y.) **1963,** 113: 485—488.

SULMAN, F. G.: Chromatophorotropic activity of human blood: Review of 1200 cases. J. clin. Endocr. **1956,** 16: 755—774.

STEHLIKOVÁ, J., TALAŠ, M., JEZDINSKÝ, J.: Titrace luteotrophniho hormonu v krvi žen. Čs. Gynek. **1965,** 30: 164—167.

TALAŠ, M., STEHLIKOVÁ, J., ZEDINSKÝ, J.: Pigeon crop weight responses to the application of extracts from the blood of women with Stein-Leventhal syndrome, galactorrhoea and of those in the secretory phase of the menstrual cycle (Including critical remarks of the method by Simkin and Goodart). Endokrinologie **1968,** 52: 387—394.

TALWALKER, P. K., RATNER, A., MEITES, J.: In vivo and in vitro prolactin secretion by transplanted rat "mammotropic" pituitary tumors. Cancer Res. **1964,** 24: 1723—1726.

WOLTHUIS, O. L.: The effects of sex steroids on the prolactin content of hypophysis and serum in rats. Acta endocr. (Kbh.) **1963,** 43: 137—146.

Mihály KURCZ,
Országos Közegészségügyi Intézet,
Budapest IX., Gyáli út 2—6, Hungary

Iván NAGY,
Heim Pál Gyermekkórház, Központi Laboratórium,
Budapest VIII., Üllői, út 86, Hungary

Csaba KISS
László HALMY } Orvostovábbképző Intézet,
Budapest XIII., Szabolcs u. 33, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 167—172 (1969)*

# A SIMPLE METHOD FOR THYROTROPIC HORMONE ASSAY

By

Alice L. REVICZKY and L. SZÁNTÓ

RESEARCH SECTION AND SECOND SECTION OF MEDICINE,
NATIONAL INSTITUTE OF RHEUMATISM AND MEDICAL HYDROLOGY, BUDAPEST

(Received October 15, 1968)

The histoautoradiographic assay modified by employing the method of MESS and HÁMORI (1961) has been instrumental estimation for routine clinical tests. Up to 0.3 mIU of TSH, the $^{131}$I uptake by the thyroid of 8-day chick embryos was proportionate, but beyond that value iodine release resulted and $^{131}$I uptake was inversely proportionate to the dose of TSH. The simplified method meets the requirements of bioassays.

Serum assays for thyrotropic hormone (TSH) are gaining significance both in thyroid diagnostics and in the control of the response to treatment. It is important therefore to possess a reproducible analytical method easy to carry out and suitable in routine work.

Since TSH has not been produced in a chemically pure form, it can be assayed by biological methods only, on the basis of changes of the thyroid tissue or in the single steps of iodine and phosphorus metabolism. The method is the more sensitive, the smaller the experimental animal and the more successfully endogenous TSH may be suppressed. These two criteria have been observed by TSUI and OGURA (1960) in their study of $^{131}$I uptake and release by thyroid slices in response to TSH administration. Excellent results have been reported also by LEMARCHAND-BÉRAUD et al. (1966), as well as ODELL et al. (1965) who combined the immunological process with the principle of the isotope dilution method and developed an in vitro technique for serum TSH assay.

Our method was based on the histoautoradiographic assay of MESS and HÁMORI (1961). As test animal, the 8-day chick embryo was used, which may be considered intact as far as endogenous TSH production is concerned. Changes in the $^{131}$I uptake by their thyroid glands were regarded as characteristic of TSH effects. Efforts were made to simplify the method by instrumental estimation.

## Methods

Pre-hatched Leghorn eggs, weighing 55 to 60 g containing embryos at the 33—34 or 35—36 stage of life, were used. For plotting the standard curve 20, for assaying two sera usually 15 eggs were needed.

Thyrotrophin was diluted with physiological NaCl solution to a final value of 0.3 mIU in 0.1 ml. The eggs were washed with alcohol, then by means of a sterile syringe and needle

0.1 ml of a less than 2 weeks old 12 $\mu$C $K^{131}I$ solution of 12 $\mu$C was injected into the eggs. Except for the 5 controls, in addition to the isotope solution. 0.15, 0.30, 0.45 mIU of standard thyrotrophin in 0.05, 0.10 and 0.15 ml volume was injectied into groups of 5 eggs, respectively. The puncture was sealed by paraffin wax and the eggs were incubated at 37.5 °C for 24 hours.

Serum TSH was determined according to MESS and HÁMORI (1961) in 4 ml samples. After separation with ammonium sulphate and dialysis the membrane content was saturated with acetone, 24 hours later the supernatant was poured into ice-cold acetone and the flaky precipitate slowly formed in the refrigerator was centrifuged, then taken up in 0.4 ml physiological NaCl solution and after determination of the total volume in each of 2 eggs 0.05 ml, in each of 3 eggs 0.10 ml, and in each of 2 eggs 0.20 ml were injected.

The embryonic thyroids removed 24 hours later were placed into Supremax incinerator tubes. After determining their background, the thyroids covered by 0.5 ml of 2 n $Na_2CO_3$ solution were dried at 105 °C, then the samples were incinerated at a temperature not exceeding 600 °C. The cooled ashes were dissolved in 1 ml distilled water and activity in the samples were tested for 5 minutes with an NK-108 type Energia selective counter calibrating the scintillator so that background activity amounted to 50 to 60 cpm.

## Results

Values for the treated animals were related to those for the daily controls, thus,

$$\text{T/C quotient} = \frac{\text{cpm TSH-treated}}{\text{control}}$$

or

$$\text{T/C quotient} = \frac{\text{cpm blood serum}}{\text{cpm control}}$$

With the TSH mIU values on the abscissa and the T/C quotients on the ordinate (Fig. 1), a curve was plotted, which increased up to 0.3 mIU, but decreased above that. The increase in function in response to TSH was reversed at that value and the embryonic thyroids started releasing instead of accumulating iodine.

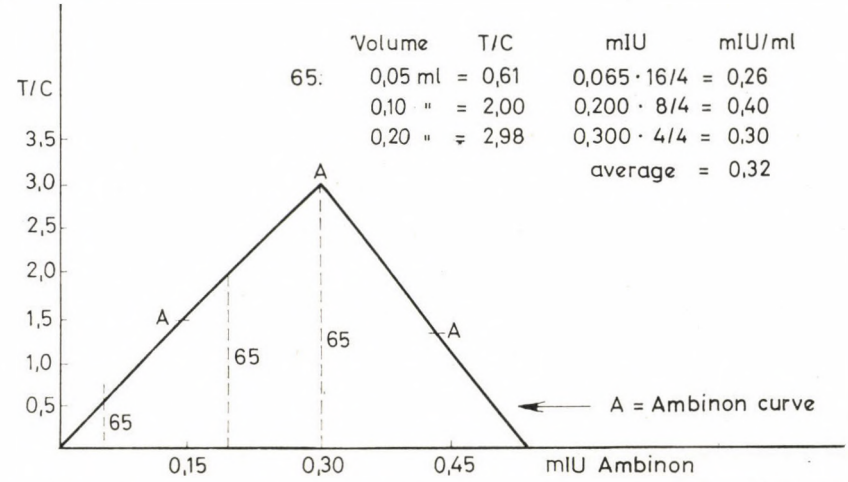



*Fig. 1.* TSH standard curve, with the corresponding serum extract values. Of 4 ml serum 0.8 ml extract was made. The T/C values of the aliquots are on the ascending branch of the curve

If in response to increasing amounts of serum extracts the T/C values increased, they were represented on the ascending, if they decreased, on the descending, part of the curve, related to 1 ml of serum (Fig. 1).

Embryonic glands in general, even in response to TSH gave low cpm values, though activity in the eggs was high. The question therefore arose whether we did not measure radioactive contamination instead of estimating the biological characteristic of the TSH effect. The distribution of radioactivity in the eggs is shown in Table I.

**Table I**

*$^{131}I$ distribution in eggs, at 1 hour and 24 hours. The embryonic glands contain 0.047% and 0.055% of the radioactivity, respectively*

| Time | Components | Weight of components, g | cpm. per cent measured |
|---|---|---|---|
| 1 hour | Shell | 8 | 18.6 |
| | Eggwhite | 28 | 62.1 |
| | Membrane | 25 | 17.6 |
| | Embryo | 1 | 0.047 |
| 24 hours | Shell | 7 | 0.029 |
| | Eggwhite | 30 | 87.8 |
| | Membrane | 25 | 12.1 |
| | Embryo | 1.5 | 0.055 |

In response to TSH the cpm values usually increased in the glands and in the same series the results of parallel measurements agreed well. At the lower stage of development 5 minutes of measurement, at the higher stage 1 minute sufficed to characterize radioactivity. In 10-day embryos the thyroid response increased steeply (Fig. 2).

In the next step the T/C values obtained at different points of time were compared. The ranges changed according to the stage of development (Fig. 3).

The same three normal subjects were tested repeatedly for serum TSH. In these tests we determined our own range of values to serve as controls; they were between 0.14 and 0.26 mIU in 1 ml serum (Table II).

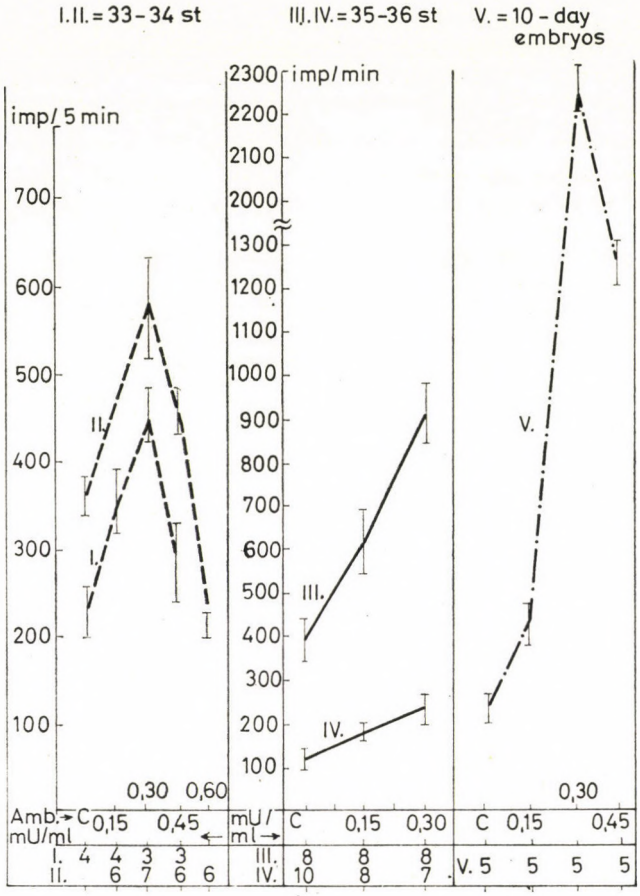



*Fig. 2.* Results of parallel measurements in groups I to V according to the stage of development. The two lowermost rows show the number of eggs used. Groups I and II are at a lower stage of development and cpm should be measured for 5 minutes, while groups III and IV require 1 minute measurement. Group V: 10-day embryos. S.D. values are shown

**Table II**

*TSH values for normal subjects*

| Name | Mean TSH, mIU/ml | Number of experiments | Range, mIU |
|---|---|---|---|
| P.M. | 0.20 | 8 | 0.14—0.26 |
| R.A. | 0.18 | 7 | 0.14—0.21 |
| K.J. | 0.19 | 6 | 0.14—0.24 |

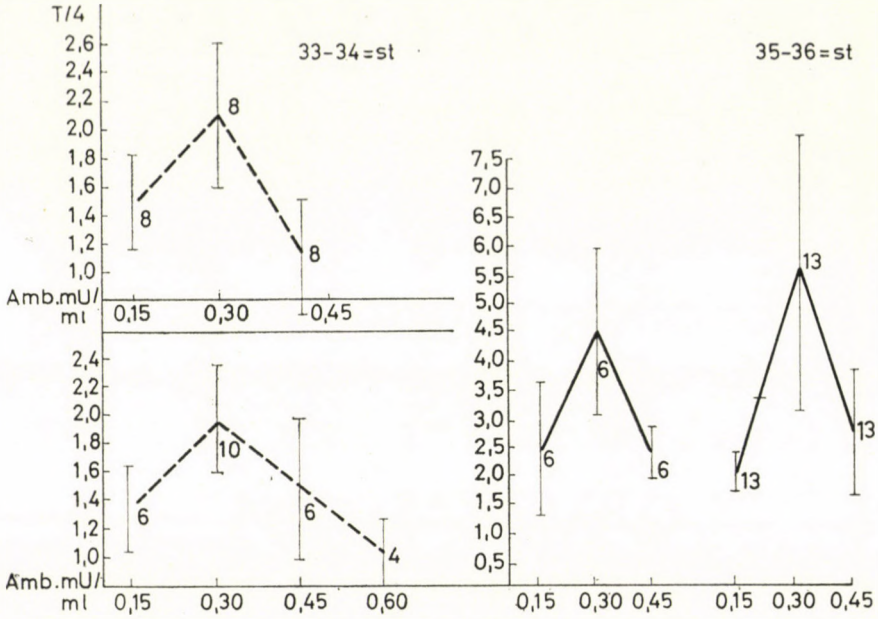



*Fig. 3.* T/C values in different series. At the lower stage of development 2.4 to 2.6, at higher stages 7.5 are the maximum values. S.D. and number of experiments are shown

## Discussion

The simplified method has been found to meet the requirements of biological assay. Beside the advantages there were recurring problems such as a suitable quality of chick embryos, because otherwise the mortality rate was high, especially in the summer. Similarly, virus infection and other factors may also kill the embryos. If 20 $\mu$C or more of $^{131}$I are administered, radiation

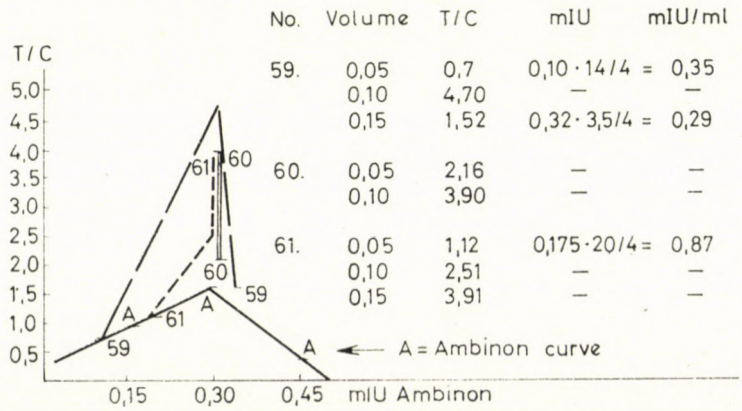



*Fig. 4.* Abnormal behaviour of serum extracts. Maximum surpasses the TSH peak in the absence of iodine release

injury may result. Another important factor is the reliability of the measuring instruments, with special reference to the reproducibility of the low activities.

The effect of drug pretreatment on the results or on the quantity of the TSH carrier protein fraction has hardly been investigated by us, yet an observation made several times is shown in Fig. 4. Sera from certain individuals, especially from patients treated for hyperthyroidism, enhance $^{131}I$ uptake by the embryonic thyroid. In such cases the I-release phase is absent and instead the glands may incorporate $^{131}I$ far in excess of the observed peak value.

## LITERATURE

LEMARCHAND-BÉRAUD, Th., SCAZZIGA, B. R., VANNOTTI, A.: Étude de la thyréostimulin plasmatique dans les affections thyroidiennes. Schweiz. med. Wschr. **1966,** 22: 718—722.

MESS, B., HÁMORI, J.: Bioassay of thyrotrophic hormone in blood by autoradiography in embryonic chick thyroid. Acta physiol. Acad. Sci. hung. **1961,** 20: 299—303.

ODELL, W. D., WILBER, J. F., PAUL, W. E.: Radioimmunoassay of human thyrotropin in serum. Metabolism **1965,** 14: 465—467.

TSUJI, S., OGURA, H.: Experimentelle und klinische Studien über die Messung der Plasmakonzentration des thyreotropen Vorderlappenhormons. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol. **1960,** 238: 391—418.

Alice L. REVICZKY
László SZÁNTÓ
Országos Reuma és Fürdőügyi Intézet
Budapest II., Frankel Leó u. 17—19, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 173—182 (1969)*

# A METHOD FOR THE CONTINUOUS RECORDING OF PERIPHERAL VASCULAR RESISTANCE

By

T. JANCSÓ and G. GALGÓCZY

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received May 11, 1968)

A method using mercury manometers has been developed for the direct and continuous recording of the vascular resistance of peripheral vascular areas. The theoretical and practical aspects of the method are described together with its detailed mathematical analysis and circuit diagrams. The reliability of the method has been tested in model and animal experiments, the results of which indicated the apparatus to be suitable for use in studies concerned with the qualitative and quantitative analysis of the laws of haemodynamical regulation manifesting themselves in changes of vascular resistance.

It is known that the various organs participate in the circulatory reactions by changing their vascular resistance, but this parameter could be recorded directly only under conditions greatly different from physiological ones, for example by the volume constant pump (TEXTER et al. 1964) or by a constant pressure perfusion system (KOEPCHEN et al. 1967).

The difficulty is due to the fact that the biological meaning of the expression "resistance" is well known, but its numerical value is given by the mathematical quotient of the perfusion pressure and the flow, and it is only recently that by means of analogous computers a continuous recording of the quotient of two figures independent of each other and changing in time has become possible. It is therefore easy to see why we have found no method in the literature by means of which the changes in vascular resistance could be recorded under near-physiological conditions.

For this reason we have developed a new method by which the quotient of the blood pressure gradient measured by a mercury manometer and the flow in a given vascular area measured by a differential manometer can be recorded continuously.

## Methods

### *Theoretical principles of the method*

It is text-book evidence (BÁLINT 1965) that the vascular resistance in a vascular area may be expressed by the formula

$$R = \frac{P_1 - P_2}{Q} \tag{1}$$

where $R$ is the resistance, $P_1 - P_2$ the arteriovenous pressure difference and $Q$ the blood flow per unit of time.

The fundamental requirement for the continuous recording of resistance is obviously a device supplying voltages proportionate to the changes in resistance, corresponding to the formula

$$U_r = K \cdot R \tag{2}$$

The setup working on grounds of these principles is shown diagrammatically in Fig. 1. The first equation for the output voltage is

$$U_r = a \cdot U_2 \tag{3}$$

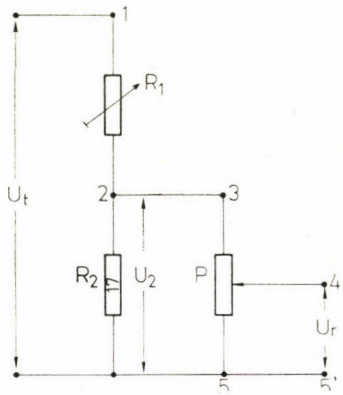



*Fig. 1.* Theoretical circuit diagram of continuous resistance recording. $U_t$ = supply voltage; $R_1$ = changeable resistance; $U_2$ = voltage falling at resistance; $U_r$ = voltage led off from the slide of potentiometer $P$

where $0 \leq a \leq 1$, because the voltage led off from the slide of the potentiometer $P$ fed by potential $U_2$ may change between 0 and $U_2$.(*a* means the so-called transfer coefficient representing the position of the slide; BENYÓ and GYÜRKI 1966.)

In the point of division of the resistance chain $R_1$ and $R_2$,

$$U_2 = U_t \cdot \frac{R_2}{R_1 + R_2} \tag{4}$$

If $R_1 = 100\ R_2$, then with 1% error

$$U_2 = U_t \cdot \frac{R_2}{R_1} \tag{5}$$

If we substitute equation (5) into (3) and make the multiplication, we get

$$U_r = U_t \cdot \frac{aR_2}{R_1} \tag{6}$$

If the transfer coefficient $a$ is proportionate to the blood pressure gradient and the changes of resistance $R_1$ to flow, the output voltage ($U_r$) will be proportionate to the resistance of the vascular area examined, because $U_t$ and $R_2$ are constant.

### *Construction and operation of the resistance recorder*

A) *Transducer*

It follows from the definition of resistance that for its measurement it is necessary to record simultaneously the flow in the vascular area examined and its corresponding pressure gradient. For this purpose we have constructed the transducer shown in Fig. 2.

Essentially, the device is a tube containing an adjustable stricture ($R$), attached to a mercury differential manometer ($D$) and a blood pressure meter ($P$). The copper (or platinum) electrodes soldered in the glass tubes are so connected that manometer $D$ should substitute $R_1$ and the blood pressure meter the potentiometer $P$ in the circuit diagram shown in Fig. 1. (Identical numbers indicate corresponding points.) In the resting position the mercury levels are just touching the electrodes 1 and 5.

If a liquid is flown in the horizontal tube ($Q$), a pressure difference proportionate to the intensity of flow will appear at the stricture. The mercury will break away from electrode 1, and between them a column of liquid proportionate in length to the rate of flow will take place. As the electrical resistance of physiological solutions is several orders magnitude higher than that of mercury, we have realized the requirement that the changes of $R_1$ be proportionate to the rate of flow.

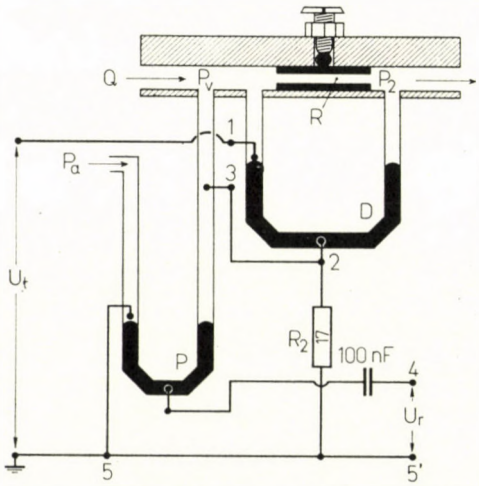



*Fig. 2.* Diagram of trausducer. $P$ = manometer measuring blood pressure gradient; $D$ = differential manometer for measuring flow; $Q$ = flow and its direction; $P_a$ = arterial blood pressure; $R$ = adjustable stricture. Other signs as in Fig. 1

In biological experiments we divide a vein coming from the vascular area tested, and attach its proximal and distal stumps to the tube so that the direction of flow should correspond to the arrows. The free end of manometer $P$ is attached to a major artery by means of a polyethylene tube; then $P$ will show the arteriovenous pressure difference. On the basis of the circuit, $P$ may be considered a liquid potentiometer having mercury as its slide. As the position of the mercury is adjusted by the arteriovenous pressure difference, it is evident that the transfer coefficient $a$ is proportionate to the pressure gradient. Thus, voltage $U_r$ appearing at points 4 and 5′ follows the changes in resistance.

During experimentation the gauge of the rubber inlay stricture $R$ is adjusted by means of a screw so that with a normal flow $D$ should show a pressure difference of 4 to 6 mm Hg. Before use, the system is filled up with physiological solution. If blood enters the manometer arms, due to the sedimentation of erythrocytes the resistance of the electrolyte will change. This may be avoided if the upper one-third of the manometer tubes together with the stricture are bent backward 180° behind the plane of the manometers. In this way the blood owing to its specific gravity being higher than that of the physiological solution will rise only in proportion to the differences in pressure and will not pass through the bend.

B) *Supply unit*

The transducers of the resistance recording device contain blood–mercury–copper (or platinum) galvanic systems. Therefore the supply current is 7 KHz sinus AC. This makes it possible to detach by condenser circuiting the direct current levels and to use a stable AC amplifier for the slight amplification of the output potential. The circuit diagram of the supply unit is shown in Fig. 3.

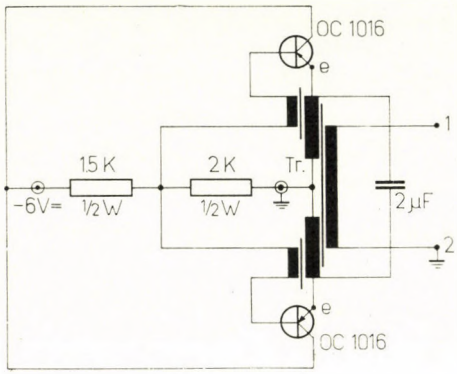



*Fig. 3.* Diagram of supply unit. 1 and 2 = output points; Tr = 5-coil transformer

This is a push-pull oscillator with high-performance transistors. To points 1 and 2 is connected the transducer shown in Fig. 2. The high performance and the low (5 Ohm) output resistance make it possible simultaneously to operate from this one oscillator several flow, blood pressure or resistance recorders.

C) *Amplifier*

Owing to the high output resistance of the transducer in the resistance recorder, as well as in view of the amplification and rectification required for recording, we use the amplifier and rectifier unit illustrated in Fig. 4.

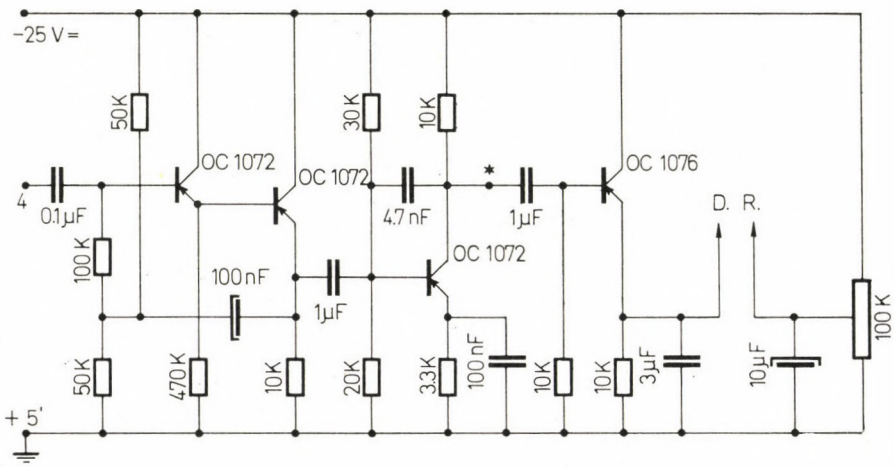



*Fig. 4.* Diagram of amplifier and rectifier unit. 4 and 5 = input points; D.R. = output to DC direct writer

To the input points 4 and 5 of the amplifier are attached the identically numbered output points of the transducer. Input resistance is about 10 Mohm, voltage is amplified about tenfold. The 100 K potentiometer on the output makes it possible to set the baseline at the onset of the experiment.

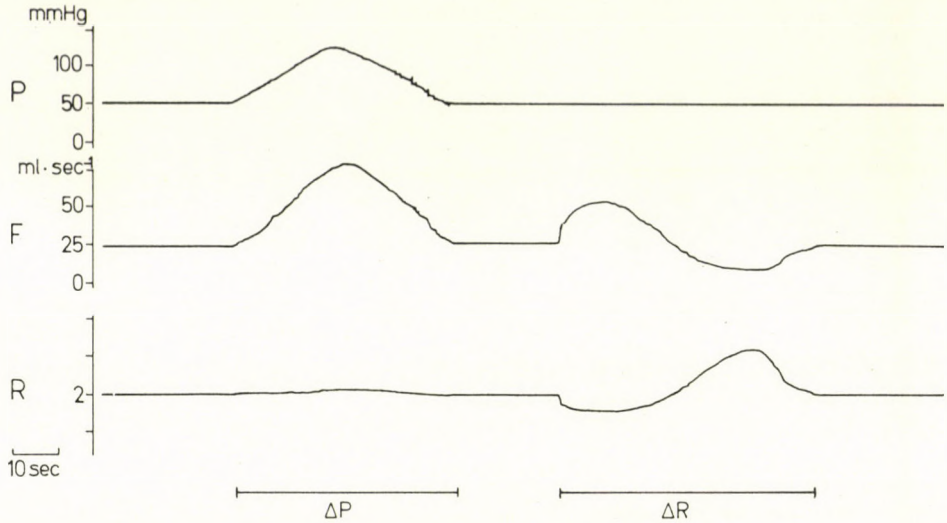



*Fig. 5.* Continuous resistance recording in model experiment. $P$ = pressure curve; $F$ = flow curve; $R$ = resistance curve; $\Delta P$ = domain of pressure change; $\Delta R$ = domain of resistance change

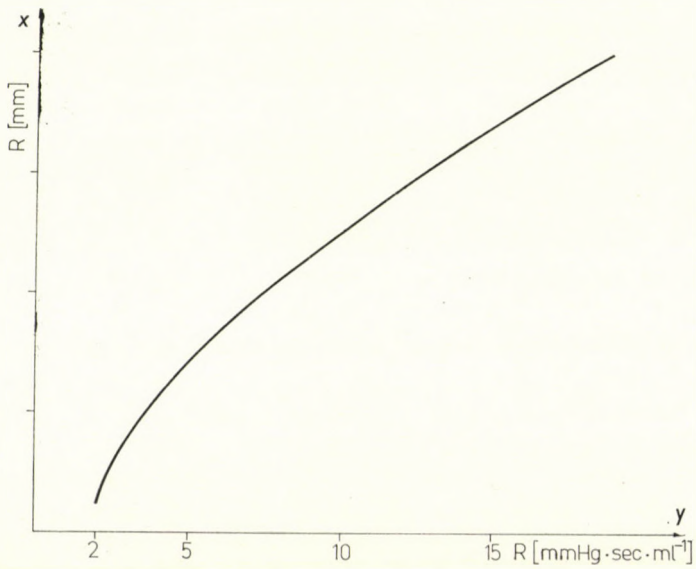



*Fig. 6.* Measuring characteristic of resistance recorder. Ordinate: Excursion of direct writer in mm; Abscissa: calculated resistance

### *Model experiments*

To control the operation of the resistance recorder under well-defined physical conditions, through changeable stricture from a dish a physiological solution was circulated under different pressures. The resistance corresponded in the biological experiments to the resistance of the vascular area tested. Then the resistance recorder was switched on. Flow was recorded by means of a differential manometer (ISSEKUTZ 1954), and measured in a calibrated cylinder. The perfusion pressure was transformed into electrical signal by a Statham element and together with the informations corresponding to resistance and flow it was recorded with the DC channels of a Galileo polygraph. The result of such an experiment is seen in Fig. 5.

In the first half of the figure ($\Delta P$) the perfusion pressure was changed; this was followed by a proportionate increase of flow, while resistance remained unchanged. In the second step we increased or decreased resistance by adjusting the stricture ($\Delta R$), as a result of which at constant pressure flow changed in the opposite directions.

The results of these model experiments were evaluated also by determining the absolute pressure and flow values for the different points of the resistance curve and calculating from them the numerical value of $R$ in mm Hg $\cdot$ sec $\cdot$ ml$^{-1}$. On the $x$ axis of the coordinate system are the calculated $R$ values, on the $y$ axis the oscillations read in mm, from which the characteristic of the resistance recording by the system could be calculated (Fig. 6).

The curve was approaching linearity, but on prolonged examination the characteristic proved to be nonlinear. The causes of this will be dealt with later.

## Results

### *Animal experiments*

To prove the reliability of the method, text-book results were reproduced in animal experiments. In dogs anaesthetized with chloralose—urethane (60 mg/kg—600 mg/kg) we tested the effect of known vasoactive agents on the circulation of the hind limb. The blood of the right external iliac vein was passed through the resistance recorder and led from there into the right jugular vein. To one branch of the $T$ cannula ligated in the right common carotid artery a Statham element was attached to record blood pressure, to the other was attached the manometer of the resistance recorder (Fig. 2, $P_a$). Flow was estimated by a differential manometer having the same stricture as that used for resistance measuring. As anticoagulant 10 mg/kg of heparin was used.

Three representative segments of the curves are shown in Fig. 7.

In part $A$ it is visible that 2 $\mu$g/kg of intravenous adrenaline increased blood pressure in the known way. During the first 15 sec there was no change in flow, then a marked increase ensued, lasting about 1 minute. Resistance increased during the first 15 sec, then decreased when flow increased, and in the third phase it reached the base line from a somewhat higher level.

In the experiment marked $B$ the same animal was treated with 0.15 mg/kg of propranolol 1 minute before injecting adrenaline. The induced bradycardia is well visible on the blood pressure curve. In this case, too, 2 $\mu$g/kg of adrenaline elevated blood pressure, but did not increase the rate of flow. The resistance curve showed only a one-phase, lasting increase.

Series $C$ presents the effect of ATP. Resistance decreased parallel with the blood pressure while flow remained practically unchanged.

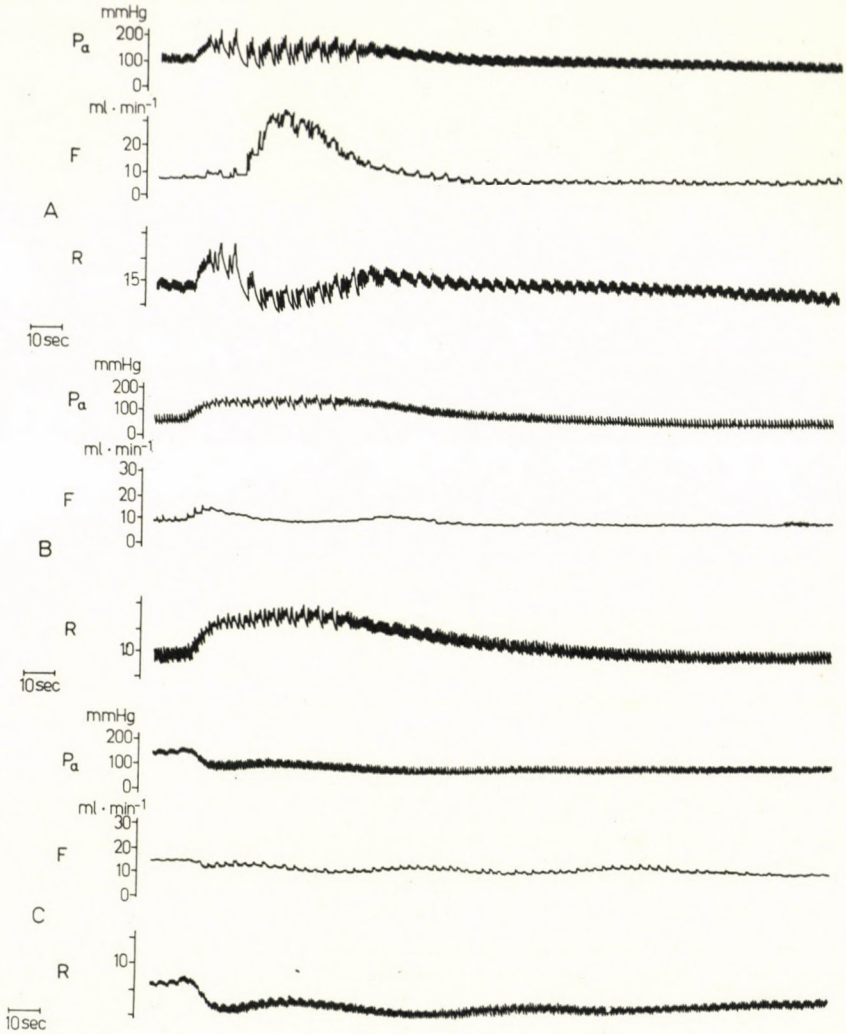



*Fig. 7.* Changes in circulatory parameters of the hind limb in response to vasoactive drugs. *A* = response to 2 μg/kg adrenaline; *B* = response to 2 μg/kg adrenaline following pretreatment with 0.15 mg/kg propranolol; *C* = response to 0.15 mg/kg ATP; $P_a$ = arterial pressure; *F* = flow in hind limb; *R* = resistance curve of hind limb

## Discussion

As vascular resistance represents the basic process of haemodynamic regulation, it is essential to follow its changes in circulatory investigations. A fine analysis of regulation in time requires continuous recording. We therefore adhered to certain fundamental principles of the analogous computers [(1/x) function generator, co-acting potentiometer, operational amplifier; BENYÓ

and GYÜRKI 1966] and constructed a device performing continuous quotient recording by means of the simplest mechanoelectric transducers.

In connection with the transducers it is to be noted that we have selected mercury manometers, because with their help the voltage inversely proportional to flow can be produced in the simplest way (Fig. 1, $U_2$), and it is the transfer coefficient of the mercury liquid potentiometer alone that follows directly the changes in the blood pressure gradient. For these reasons the resistance recorder is extremely simple, and this compensates well for the known disadvantages of mercury manometers (relatively low volume elasticity coefficient, large free oscillation; LICHTNECKERT 1954). By adjustment to proper sizes, these were reduced to a minimum.

As to the principle of operation, continuous quotient recording was realized by continuous multiplication by the reciprocal of flow, because the co-acting potentiometer can be used for multiplication only without a servo unit.

From a comparison of equations (4) and (5) it follows that we should always take into account some distortion when producing a voltage proportional to the reciprocal of flow ($U_2$). This error can be reduced below a predetermined value by properly selecting the ratio of $R_1$ to $R_2$. In our system the impedance of 1 mm physiologic solution is 100 ohm at 7 kHz, therefore if $R_2$ is 10 ohm, distortion is 10%, when in the flow meter the mercury is 1 mm from electrode 1 (Fig. 2). As the stricture of the differential manometer is so adjusted that with normal flow the mercury is 5 mm from the mentioned electrode, the error due to distortion is 2%.

Resistance $R_2$ cannot be decreased any further because of the limited performance of the supply unit. The special construction of the amplifier was justified by several points. During measurement the co-acting potentiometer of high output resistance must not be overloaded, because this would lead to a distortion of the linear characteristic (BENYÓ and GYÜRKI 1966), therefore the well-known Darlington circuit is used for attachment. In addition, the amplifier compensates for the inevitable losses due to rectification, and allows to use a writer apparatus of medium sensitivity for the direct recording of resistance.

In model experiments (Fig. 5) the apparatus was found to be suitable for the continuous recording of vascular resistance. From the resistance curve qualitative conclusions may be drawn without any additional procedure. If we determine one point of the curve from the simultaneously measured true pressure and flow values in the form of a concrete $R$ value, in the knowledge of the system's characteristic the results can be evaluated quantitatively.

In Fig. 6 the resistance measuring characteristic is shown. It has been said above that in the first approach it was linear. The causes of the non-linearity found on finer analysis are as follows.

a) The variable distortion of the resistance chain $R_1 - R_2$ cannot be eliminated.

b) The nonlinear characteristic of the transistors in the amplifier may also play a role.

c) The divergence from linearity is due decisively to the fact that at the amplifier output rectification and zeroing are done by a Wheatstone bridge-like circuit, the outgoing characteristic of which is hyperbolic in shape. This distortion is slight, because during measurement the output voltages fall to a relatively linear part of the characteristic of the Wheatstone bridge (balanced Wheatstone bridge).

In animal experiments we have invariably succeeded in reproducing some well-known results. We have studied the total circulation of the hind limb which is governed decisively by the laws for muscle circulation. We have shown that in response to intravenous injection of ATP vascular resistance decreases, but due to a simultaneous decrease in perfusion pressure there is no increase in flow. Similarly, adrenaline was shown to induce the well-known increase in blood pressure and muscle flow (GOTTSTEIN et al. 1955). In resistance we could observe (we wish to emphasize that it is by our method for the first time that such an observation could be made) the damped oscillations well known in regulation technique and in biocybernetics (OBÁL 1966). The increase of flow by the beta receptors has been confirmed by recording the known phenomenon that propranolol inhibited the increase of flow caused by adrenaline and induced a one-phase increase in vascular resistance (SCHMIDT-VANDERHEYDEN and KOEPCHEN 1967).

Our apparatus offers two possibilities for biological measurement, viz. connecting the flow recorder between the proximal and distal stumps of a cut artery, or between those of a vein. In the former case we measure the resistance of the vascular area behind the artery, in the latter that before the vein. As the arteries are joined by nerves involved in regulatory processes, it is better to cut a vein. Other outgoing veins should, of course, be ligated. To avoid venous congestion, the resistance of the measuring stricture must be set so that in the normal flow domain the flow meter differential manometer should show a pressure difference of 4 to 6 mm Hg. In order to ensure the same load in every experiment, the stricture should be adjustable at will. The transducer should be placed at the level of the heart.

## LITERATURE

BÁLINT, P.: Az élettan tankönyve. Medicina, Budapest **1965**, p. 1019.

BENYÓ, Z., GYÜRKI, J.: Villamos analóg számológépek. Mérnöki Továbbképző Intézet, Budapest **1966**, p. 355.

GOTTSTEIN, U., HILLE, H., OBERDORF, A.: Die Wirkung von Adrenalin und Noradrenalin auf die Durchblutung der Skelettmuskulatur und der Haut. Pflügers Arch. ges. Physiol. **1955**, 261: 78—89.

ISSEKUTZ, B. jr.: Véráramlás mérése. In: Kovách, A.: A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1954**, Vol. 2, pp. 110—145.
ISSEKUTZ, B. sen.: Gyógyszertan és gyógyítás. Medicina, Budapest **1959**, Vol. 1, p. 903.
KNOLL, J.: Gyógyszertan. Medicina, Budapest **1965**, p. 842.
KOEPCHEN, H. P., SELLER, H., POSTER, J.: Hochempfindliche Widerstandregistrierung bei kleinen Flüssen. Pflügers Arch. ges. Physiol. **1967**, 294: 72—78.
LICHTNECKERT, I.: Manométerek, galvanométerek, optikai regisztrálások. In: Kovách, A.: A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1954**, Vol. 2, pp. 1—78.
OBÁL, F.: The fundamentals of the central nervous control of vegetative homeostasis. Acta physiol. Acad. Sci. hung. **1966**, 30: 15—29.
SCHMIDT-VANDERHEYDEN, W., KOEPCHEN, H. P.: Zum Mechanismus der Adrenalindilatation der Skelettmuskelgefässe. Pflügers Arch. ges. Physiol. **1967**, 298: 1—11.
TEXER, E. C. jr., CHOU, C. C., MERILL, S. L., LAURENTA, H. C., FROLICH, E. D.: Direct effects of vasoactive agents on segmental resistance of the mesenteric and portal circulation. J. Lab. clin. Med. **1964**, 64: 624—633.

Tamás JANCSÓ,
OMI, Experimental Toxicological Department,
Budapest IX., Nagyvárad tér 2, Hungary
Gábor GALGÓCZY,
OMI, Respiratory Function Laboratory,
Budapest IX., Nagyvárad tér 2, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35 (2), pp. 183—188 (1969)*

# *Pharmacologia*

## PHARMACOLOGY OF CHERRY (PRUNUS AVIUM) STALK EXTRACT, I

### EFFECT ON SMOOTH MUSCLE

By

E. HETÉNYI and T. VÁLYI-NAGY

INSTITUTE OF PHARMACOLOGY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received April 24, 1968)

The effect of cherry-stalk (Pruni avium stipes) extract on smooth muscle preparations has been investigated. The extract termed CN, consisting of flavonoid components was found reversibly to abolish at concentrations of 50 to 100 $\mu$g/ml the activity of the rat and guinea pig uterus and to counteract oxytocin-induced contractions. Similar results were obtained on the rat ileum, while in the guinea pig intestine 500 $\mu$g/ml caused contraction. At 100 $\mu$g/ml concentration CN abolished the oxytocin and acetylcholine contractions practically completely and caused 50% inhibition of $BaCl_2$ contractions. Using an automatic superfusion titration apparatus a procedure for the biological evaluation of CN on the rat uterus has been elaborated.

A great number of our drugs are prepared from natural substances and their chemical analysis serves as a basis for the development of new synthetic compounds. Cherry-stalk tea has been known for long as a diuretic agent and mainly as a substitute for imported tea (AUGUSZTIN et al. 1948; HALMI and NOVÁK 1963).

The first pharmacological and toxicological investigations were performed in the University Pharmacological Institutes of Pécs and Budapest. It was unequivocally established in both laboratories that cherry-stalk extract (CN in the following) exerted a positive inotropic effect on heart preparations (SZEGI and SZEKERES, personal communications). SZEKERES later confirmed the coronary-dilator effect of CN. MAGDA et al. reported on their first results in 1960. Our own investigations were started in 1961.

Simultaneously with the pharmacological investigations, chemical research was also started (Research Institute of the Organic Chemical Industry, Budapest, and Institute of Organic Chemistry, Lajos Kossuth University, Debrecen); these investigations have shown that CN consists of various flavonoid components (BOGNÁR et al., in preparation).

After some preliminary studies experiments have been started in this institute as to the detailed pharmacological analysis of CN. The present paper deals with the effect of CN on smooth muscles.

## Methods

Preparation of CN substance* involved the following main steps. 1. Extraction with acetone of air-dried ground cherry-stalks. 2. Removal with light petrolatum of the dark-green wax-like ballast substances from the distilled extract. The product obtained is a greenish-yellow powder.

The stock solution used in the experiments was prepared from CN substance as follows. 250 mg of the substance were suspended in 25 ml of physiological saline under continuous stirring at 50 to 60 °C. This was followed by filtration and filling up the filtrate to 25 ml. The filter paper with the insoluble part of the extract was dried to constant weight, and weighed again. This procedure allowed us to calculate the real concentration of the solution. The solution was crystal-clear, light yellow in colour, and had a pH of 7.2.

As smooth muscle preparations virginal rat and guinea pig uterus and rat and guinea pig small intestine were used. The uterus was suspended in oxygenated Gaddum solution (BURN et al. 1962), the intestine in Tyrode solution. After preincubation in Gaddum solution at 4 °C for 24 hours the uterus showed no spontaneous motility when placed into a low-

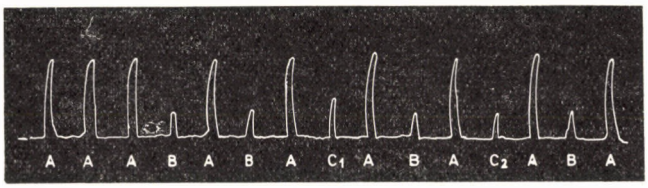



*Fig. 1.* Titration of CN on rat uterus. Automatic titration apparatus, superfusion method. A = standard pituitary extract solution; B = standard pituitary extract + standard CN; C = standard pituitary extract + CN at unknown concentration. For details, see text

temperature (25 °C) bath and thus proved suitable for the titration of spasmolytic effects. Contractions were evoked by 0.003 I.U./ml of posterior pituitary extract or $BaCl_2$ or acetylcholine, according to the usual procedure (PATAKY 1954; ISSEKUTZ 1957). For titration an automatic superfusion device was used (KÖVÉR and BALLA 1960); it delivered to the uterus preparation at definite intervals a standard pituitary extract solution of known concentration (A). As a result, stable and equal contraction occurred. Thereafter, a mixture of standard pituitary extract and a standard CN solution (B) was added to the muscle from another reservoir. The experimental arrangement allowed an alternating treatment of the uterus with the A and B solutions. After appropriate sensitivity had set in, solutions ($C_1$, $C_2$, etc.) containing various concentrations of CN + pituitary extract were employed alternately with the (A) and (B) solutions, and the degree of inhibition was determined (Fig. 1). The procedure allowed the quantitative evaluation of new solutions containing unknown amounts of the CN preparation. A biological standardization procedure was developed at the Biological Drug Testing Department of the Hungarian Institute of Public Health. Similarly to the biological assay of digitalis preparations, the method is based on the determination of the dose causing heart failure in guinea pigs (KERTAI, personal communication).

## Results

CN brough about an unequivocal and clear-cut inhibition of the spontaneous motility of the rat uterus. In 15 experiments (and in a reproducible way in a total of 42 experiments) a concentration of 50 to 100 $\mu$g/ml abolished

* The procedure for preparation of the extract is under patent protection No. 150.217 in Hungary, No. 219.195 in Austria, No. 1,117.822 in West Germany, and No. 908.612 in Great Britain.

the spontaneous motility. The inhibition was reversible since, after washing, contractions continued at the original level. The effect could be reproduced several times during a given experiment; no ineffectiveness was ever observed (Fig. 2).

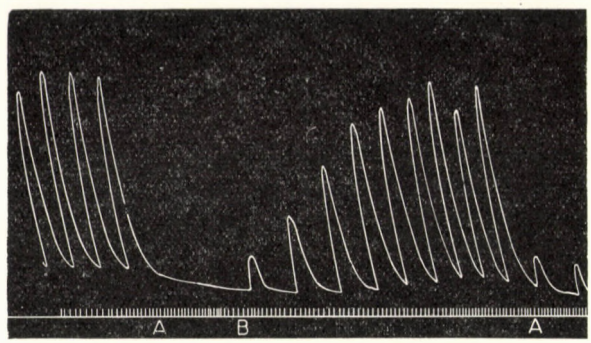



*Fig. 2.* Inhibitory effect of CN on the spontaneous motility of rat uterus. A = 50 $\mu$g/ml CN; B = washing; Time: 6 sec

Contractions were evoked by 0.002 to 0.003 I.U./ml of pituitary extract in uterus preparations exhibiting no spontaneous movement after 24 hour incubation in Gaddum's solution at 25 °C. After reaching constant contraction heights the uterus was incubated for two minutes in Gaddum solution containing 50 to 100 $\mu$g/ml of CN. Thereafter, pituitary extract was added in the same concentration as previously. No contractions were observed (Fig. 3).

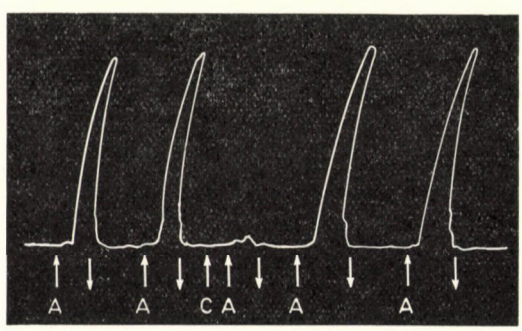



*Fig. 3.* Abolition of pituitary extract contraction of rat uterus by CN. A = 0.002 I.U. of pituitary extract; C = 50 $\mu$g/ml of CN

In all of the 25 experiments (with repetitions, a total of 114) CN inhibited or completely abolished the contractions, depending on the dose used. The results of one experiment concerning the relationship between the dose employed and the effect obtained are shown in Fig. 4.

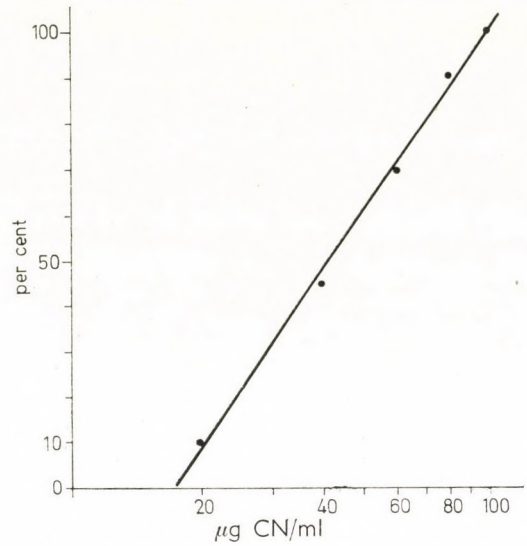



*Fig. 4.* Relationship between dose and effect of CN. Ordinate: inhibition of pituitary extract spasm, decrease in mm of the contraction under the effect of CN in per cents of the initial value. Abscissa: Concentration of CN in organ bath

The inhibitory effect of CN on various preparations exhibited slight variations independently of the dose applied. This might have been due to differences in sensitivity of the various uterus preparations. According to our observations the same amount of CN caused a higher degree of inhibition in pre-oestrous preparations.

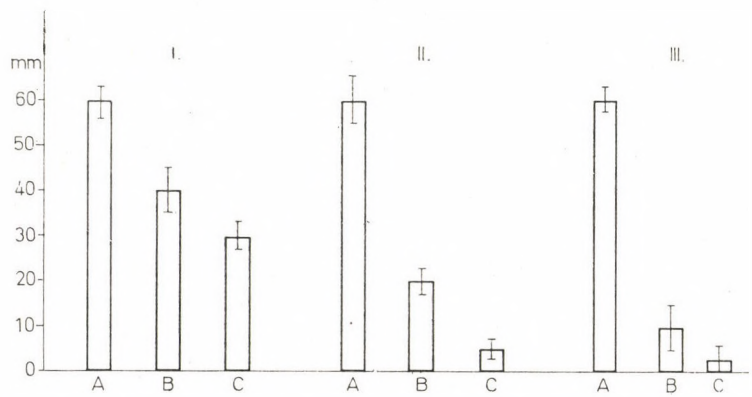



*Fig. 5.* Inhibition by CN of spasms of various origin. I/A = 40 μg/ml $BaCl_2$; I/B = 40 μg/ml $BaCl_2$ + 50 μg/ml CN; I/C = 40 μg/ml $BaCl_2$ + 100 μg/ml CN; II/A = 0.04 μg/ml acetylcholine; II/B = 0.04 μg/ml acetylcholine + 50 μg/ml CN; II/C = 0.04 μg/ml acetylcholine + 100 μg/ml CN; III/A = 0.01 I.U./ml pituitary extract; III/B = 0.01 I.U./ml pituitary extract + 50 μg/ml CN; III/C = 0.01 I.U./ml pituitary extract + 100 μg/ml CN. Ordinate: Height of contraction in mm. Rat uterus, mean of 10 experiments each

In the further experiments the effect of CN was investigated on contractions induced by $BaCl_2$ and acetylcholine. The effect of doses of pituitary extract and acetylcholine causing equal contractions (0.01 I.U./ml, and 0.04 $\mu$g/ml, respectively) was completely abolished by an identical amount, i.e. by 100 $\mu$g/ml of CN. The effect of 40 $\mu$g/ml of $BaCl_2$ causing similar contractions was inhibited to 50% only (Fig. 5). In some experiments the comparison of CN with papaverine revealed CN to possess about 60% of the papaverine activity.

In five experiments CN at higher concentrations (500 $\mu$g/ml) evoked a contraction of the guinea pig ileum, in contrast with the results obtained on uterus preparations. However, in the rat ileum even higher CN concentrations (1 mg/ml) failed to cause any contraction. Conditions identical with those found with the rat uterus were seen on the guinea pig uterus; CN counteracted the pituitary extract contractions in every one of ten experiments. This question will yet be touched upon in the Discussion.

Since the effect of CN is a reversible one, the smooth muscle preparation can also be used for titration. These experiments were performed by using an automatic titration apparatus, as described in the methodological part of this paper. It was stated that the method is suitable for comparison of CN solutions with unknown biological activity (Fig. 1).

## Discussion

Flavonoid compounds are known to possess a marked spasmolytic activity (Böhm 1960). Since CN mainly consists of flavonoid components, its effect on smooth muscles could be expected. Our investigations fully confirmed this assumption, and the effects observed support the flavonoid character of CN also from the biological side. These facts mean a further corroboration of our experiments revealing the same concentration of CN to cause contractions of the guinea pig ileum while leaving the rat ileum unaffected. This question is of importance because the action of flavonoids is known to be different in carnivores and herbivores (Jeney and Uri 1954).

The distinct coronary dilator effect of CN (Hetényi and Vályi-Nagy 1969) has been confirmed also by other authors. We assume that one of the factors figuring in the effect of CN enhancing cardiac function is the coronary dilator effect, i.e. the smooth muscle effect, of the preparation.

On the basis of the inhibition of spontaneous uterine motility and of the inhibition of spasmogenic effects CN seems to exert a considerable effect on those molecular processes which are figuring in the background of these events. Our previous studies with flavonoid compounds have shown that a 7-oxiflavone derivative profoundly influenced the potassium permeability

of red blood corpuscles (HETÉNYI 1966), as well as the electrophysiological events occurring during muscular contraction (HETÉNYI 1968). Our investigations with CN in this direction are promising but need further proofs.

The above statements might serve to prove that the study of the molecular level of the effects of natural substances may not only lead to new drugs, but also to the elucidation of finer physiological and pharmacological mechanisms.

## Acknowledgement

We are indebted to Mr. L. KRÁLIK, scientific research worker at the Research Institute of the Organic Chemical Industry, and inventor of CN, for supplies of the preparation.

## LITERATURE

AUGUSZTIN, B., JÁVORKA, S., GIOVANNINI, R., ROMM, P.: Magyar Gyógynövények. Földművelési Minisztérium, Budapest **1948.**

BÖHM, K.: Die Flavonoide. VII. Flavonoid Wirkungen auf die glatte Muskulatur. Arzneimitt.-Forsch. **1960,** 10: 188—192.

BURN, J. H., FINNEY, D. J., GOODWIN, L. G.: Biological Standardization. Oxford University Press, London **1952,** p. 440.

HALMAI, J., NOVÁK, I.: Farmakognozia. Medicina, Budapest **1963,** p. 718.

HETÉNYI, E.: Effect of flavonoid compounds on potassium transport. Acta physiol. Acad. Sci. hung. **1966,** 30: 387.

HETÉNYI, E.: Influence of flavonoid compounds on muscular contractions of different origin. Acta physiol. Acad. Sci. hung. **1968,** 34: 213—219.

HETÉNYI, E., VÁLYI-NAGY, T.: Pharmacology of cherry (Prunus avium) stalk extract. II. Cardiovascular effects. Acta physiol. Acad. Sci. hung. **1969,** 35: 189—197.

ISSEKUTZ, B. sen.: Az általános gyógyszertan és gyógyszerkutatás vizsgáló módszerei. In: KOVÁCH, A. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1957,** Vol. III, pp. 696—940.

JENEY, E., URI, J.: A flavonfestékek farmakológiai és biológiai hatásai. MTA. Orv. Oszt. Közl. **1954,** 5: 193—219.

KÖVÉR, A., BALLA, L.: Egyetemes biológiai titráló. Kísérl. Orvostud. **1960,** 12: 318—327.

MAGDA, K., RAUSCH, J., SZEGI, J., NAGY, J., KRÁLIK, L.: A Pruni avium stipes száraz acetonos kivonatának hatástani vizsgálata. A Magyar Élettani Társaság XXVI. Vándorgyűlése. Akadémiai Nyomda, Budapest **1960,** p. 177.

PATAKY, I.: Izolált szervek vizsgálatának módszerei. In: KOVÁCH, A. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1954,** Vol. 1 pp. 633—689.

Ede HETÉNYI, Tibor VÁLYI-NAGY
Orvostudományi Egyetem Gyógyszertani Intézete,
Debrecen 12, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(2), pp. 189—197 (1969)*

# PHARMACOLOGY OF CHERRY (PRUNUS AVIUM) STALK EXTRACT, II

## CARDIOVASCULAR EFFECTS

By

E. HETÉNYI and T. VÁLYI-NAGY

INSTITUTE OF PHARMACOLOGY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received April 24, 1968)

The pharmacological effects of the extract of cherry (Prunus avium) stalk were investigated. The extract at a concentration of 100 $\mu$g exhibited a positive inotropic effect on frog hearts damaged by quinine and posterior pituitary extract. The degree of this positive inotropic action depended to a great extent on the qualitative and quantitative conditions of the damage. On Trendelenburg's frog hind limb preparation the drug abolished the adrenaline-induced vasoconstriction without showing any own effect on the vascular bed. On Langendorff's rat heart preparation the extract decreased coronary resistance by 30% on the average. On Starling's heart-lung preparation it restored to normal the functioning of the barbiturate-weakened heart, in half of the experiments even above the initial value. Blood pressure was not influenced by small doses (1 to 2 mg/kg) and slightly decreased by high doses (some 10% decrease after 5 to 10 mg/kg). In the open-chest dog the extract increased coronary flow and heart muscle contractions while on the cat's papillary muscle preparation the extract exhibited but a slight positive inotropic effect. The hypoxic ECG changes induced in the rat by posterior pituitary extract or vasopressin were normalized by 400 $\mu$g/kg of the cherry-stalk extract for 5 to 10 minutes, and the T-wave elevation at the beginning of anoxia was also abolished. The results obtained and the possible mechanisms of action are discussed and the amelioration of heart function is ascribed to a coronary dilating effect, on the one hand, and to the modifying influence on the metabolism of heart muscle and blood vessel walls, on the other.

The results of our experiments with extract of dewaxed cherry-stalk (CN in the following) have been described in a previous paper (HETÉNYI and VÁLYI-NAGY 1969). CN which mainly consists of flavonoid components (BOGNÁR et al., in preparation) exhibited a considerable spasmolytic effect and was observed to exert cardiovascular actions, in full agreement with the results of other pharmacological investigations (SZEKERES, KOVÁCS and SZENTIVÁNYI, personal communications). The present paper reports on some circulatory effects of CN.

## Methods

A stock solution was made from CN substance prepared as described previously (HETÉNYI and VÁLYI-NAGY 1969); for the investigations appropriate dilutions were used. These were crystal-clear, light yellow in colour, with a pH of 7.2.

(i) Acute toxicity was determined in rats and mice. CN in a dose of 300 mg/kg intravenously or intraperitoneally showed no toxicity during the 24 hours observation period.

(ii) Frog heart preparation according to Straub was prepared in the usual way (PATAKY 1954). In one series of experiments the perfusing fluid was oxygenized, while in another series no oxygen was used. Heart functioning was damaged by Ringer's solution containing 2 mg

per 100 ml of quinine hydrochloride or 0.2 to 0.4 I.U. posterior pituitary extract (Richter, Budapest). The experiments were performed from May to September and from January to March.

Frog hind limb preparation perfused according to Trendelenburg was used to study the effect of CN on blood vessels (PATAKY 1954).

(iii) Cat papillary muscle preparation was obtained under urethane anaesthesia from the right ventricle of animals weighing 2 to 3 kg. The preparation was suspended in oxygenized Cattel-Gold solution, stimulated with square wave pulses (4 V, 500 sec, 80/min), and the isometric contractions were recorded photokymographically (PATAKY 1954).

(iv) Experiments on mammalian heart were performed in the usual way (PATAKY 1954) on rats of both sexes weighing 200 to 250 g. Changes in coronary flow were expressed on the basis of resistance changes. These were calculated according to the formula

$$\text{Resistance} = \frac{t_2}{t_1} \times \frac{T - t_1}{T \cdot t_2}$$

where $T$ = the pressure of Locke's solution in the perfusion system (200 mm Hg in our experiments); $t$ = the reduced perfusion pressure measured by the manometer after compression of the pre-cardiac section (100 mm Hg); $t_1$ = value before the administration of the drug; $t_2$ = value after the administration of the drug.

(v) Heart-lung preparation according to Starling was prepared on the cat. The changes in heart work were expressed by the efficiency index (SZEGI and RAUSCH 1958).

(vi) Coronary flow in situ was measured in open-chest dogs under chloralose anaesthesia; the coronary sinus was cannulated and flow was estimated by means of a rotameter (SZENTIVÁNYI and JUHÁSZ-NAGY 1961).

(vii) Heart contractility in situ was recorded by means of a cardiomyograph (SZEKERES et al. 1958).

(viii) The coronary effect of CN was studied by electrocardiography in male rats weighing 250 g anaesthetized with 100 mg/kg of thialbarbital. Coronary spasm was evoked by posterior pituitary extract or vasopressin. Evaluation was made according to NIESCHULZ et al. (1955).

## Results

On freshly excised oxygenized intact isolated frog hearts which were neither hypodynamic nor damaged by drugs, CN in amounts of 1 to 500 $\mu$g was practically without effect. Higher doses, i.e. 1 to 2 mg, exerted a negative effect. Suspension of oxygenation, i.e. a moderate degree of damage, enhanced the positive inotropic effects. These results agreed with the observation made in other tests that CN similarly to the other flavonoids has no influence on the normal functioning of intact structures. No definite seasonal variations could be observed, as CN is usually ineffective on intact hearts, although during the winter period from December to February a negative inotropic effect seemed to be elicited by doses 300 to 500 $\mu$g lower than those mentioned above. In hearts damaged in the way to be described below no positive effect could be elicited in the winter period in contrast with the 20 experiments made in the summer.

In frog hearts damaged by quinine or posterior pituitary extract (20 experiments each), considerably better effects were observed. On the quinine-treated heart CN exerted a clear-cut positive inotropic effect and it restored to normal the quinine-induced decrease in amplitude for a long period of time. In this case the amplitudes began to increase immediately after the application of CN, in contrast with the control experiments; and after reaching

or slightly exceeding the pre-quinine level the amplitude remained permanently at the same level.

Similar results were obtained after damaging the heart by pituitary extract; in these cases, however, the positive inotropic effect was more marked and could be observed in nearly every case (Figs 1 2).

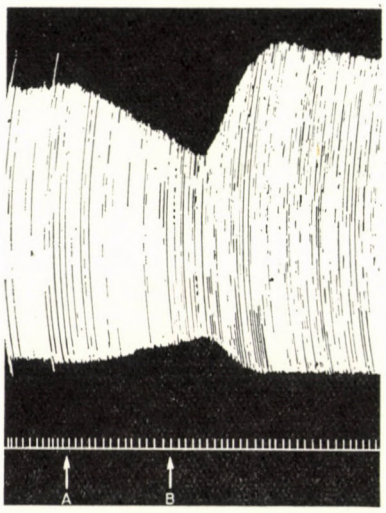



*Fig. 1.* Effect of CN on the quinine-damaged isolated frog heart. A: 40 $\mu$g quinine-HCl. B: 50 $\mu$g CN. Time: 6 sec

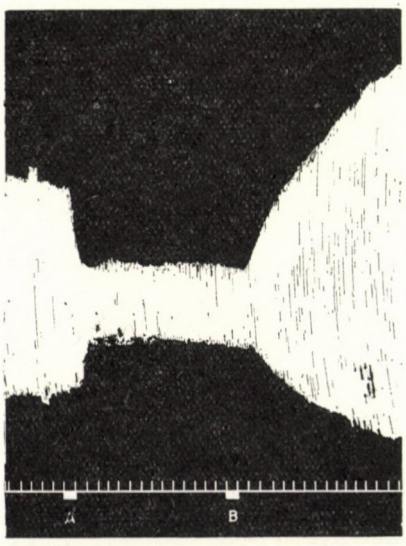



*Fig. 2.* Effect of CN on the isolated frog heart damaged by posterior pituitary extract. A: 0.5 I.U. pituitary extract. B: 50 $\mu$g CN. Time: 6 sec

Thus, CN was ineffective on the intact heart. On the heart damaged by various means a dose of 50 to 100 μg exerted a positive inotropic effect in the majority of cases. Doses below 20 to 30 μg were ineffective, while doses above 250 μg failed further to augment the positive inotropic effect. Amounts of 0.5 to 1 mg exerted a negative inotropic effect.

In ten experiments high doses (5 to 10 mg) of CN caused cardiac arrest in diastole; after vigorous washing, however, the hearts resumed beating.

The relationship between positive inotropic effect and the various kinds of damage will be touched upon in the Discussion.

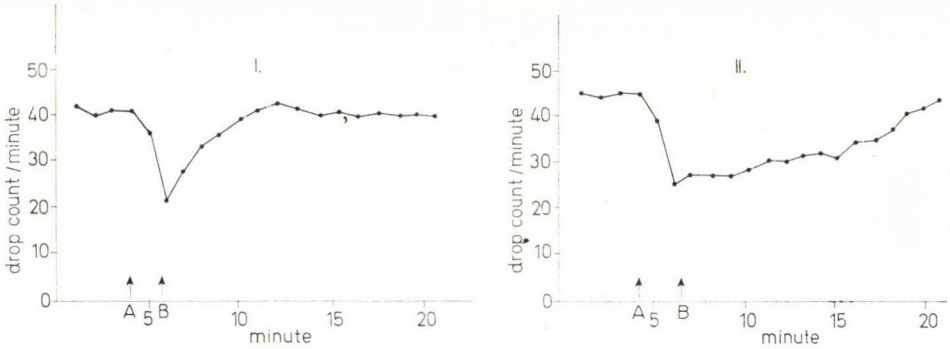



*Fig. 3.* Effect of CN on vasoconstriction by adrenaline. Frog hind limb preparation. I/A: 10 μg adrenaline; B: 400 μg of CN. II/A: 10 μg of adrenaline; B: 0.1 ml of physiological saline

Twelve experiments were performed on frog hind limbs perfused according to Trendelenburg. Twenty to 500 μg of CN did not influence the diameter of blood vessels, but the drug counteracted the adrenaline-induced vasoconstriction in 5 out of 12 experiments (Fig. 3).

In the cat papillary muscle preparation 50 to 100 μg/ml of CN in the organ bath caused a 30% amplitude increase in five out of eight cases.* High doses (1 to 2 mg/ml) decreased the amplitude by 50% negative effect, while 50 μg/ml of CN considerably increased the positive effect of adrenaline on the papillary muscle and caused the augmented contractions to last three to four times longer.

On rat hearts isolated according to Langendorff a total of 30 experiments was performed. In every case a dose of 200 to 400 μg of CN markedly decreased coronary resistance in a reversible way. The average decrease was 32%. Amelioration of heart function, i.e. a positive inotropic effect, was observed in ten cases and mainly in experiments where perfusion was very slow. This means that, in order to evoke an inotropic effect, CN has to be in contact with the heart muscle for a longer period of time. Results are summarized in Table I.

* Recent experiments on guinea pig papillary muscle yielded similar results (Dr. M. Reiter, Institute of Pharmacology, Munich, personal communication).

**Table I**

*Effect of CN on coronary resistance*

| Number of experiments | CN, μg | Mean resistance | σ | Decrease of resistance, per cent |
|---|---|---|---|---|
| 20 | 200 | 0.62 | ±0.09 | 38 |
| 10 | 400 | 0.74 | ±0.09 | 26 |

Table I reveals the optimum dose of CN to lie around 200 μg. On increasing the dose up to 500 μg, we obtained a lesser degree of resistance diminution. In one case we applied 1 mg of CN several times repeatedly; this resulted in a deterioration of cardiac function without coronary dilation. In these experiments the $ED_{50}$ of CN on Langendorff's rat heart was 150 μg. Compared with the results obtained on frog heart and cat papillary muscle, these observations indicate that not only the direct heart muscle effect but also the coronary dilator effect has a significant part in the manifestation of the CN action.

On the cat heart-lung preparation 50 to 100 μg of CN given into the venous reservoir considerably and permanently increased the efficacy of the barbiturate-weakened or spontaneously failing heart in each of the 16 experiments. In some cases a dramatic effect was observed on hardly functioning heart; it set in rapidly and the preparation remained functioning well until the end of the 20 to 30 minutes observation period. In half of the cases the efficiency index even exceeded the initial value.

In the course of the same experiments, the heart failure induced by repeated administration of phenobarbital was always counteracted by CN. Results are summarized in Table II.

**Table II**

*Effect of CN on the efficiency index of phenobarbital-weakened heart-lung preparation*

| Number of experiments | Efficiency index | | | | | |
|---|---|---|---|---|---|---|
| | At the beginning of the experiment | | After 10 mg/kg of phenobarbital | | After 70 μg/kg of CN | |
| | mean | σ | mean | σ | mean | σ |
| 16 | 0.80 | 0.16 | 0.21 | 0.13 | 0.69 | 0.20 |

The effect of CN on blood pressure, heart muscle contractility and coronary flow was studied *in situ*. Our observations confirmed the results of other authors (KOVÁCS and SZEKERES, personal communications) in that 1 to 2 mg/kg of CN given intravenously to cats anaesthetized with chloralose had no effect

on the blood pressure, while a dose of 5 to 10 mg/kg caused a mean decrease of 10% (7 experiments). Functioning of the heart weakened by thialbarbital gradually and permanently improved under the effect of 1 to 2 mg/kg of CN, with a concomitant diminution of left atrial pressure (5 experiments).

Coronary flow in the open-chest dog was augmented by 0.5 to 1.0 mg/kg of CN injected into the femoral vein (10 experiments), in agreement with the above findings and with the observations of other authors (SZENTIVÁNYI, personal communication) made on isolated mammalian hearts, as well as with the investigations of KOVÁCS et al. (personal communication) who showed that 400 μg/kg of CN when injected directly into the circumflex ramus of the left coronary artery, exerted a vasodilator action. Coronary flow (ml/min) was doubled and the coronary resistance decreased by 50%.

Fifty experiments were performed in rats to study the influence of CN on the pathological ECG pattern brought about by 2 to 3 I.U./kg of posterior pituitary extract, or 0.1 ml of vasopressin, given by the intravenous route. These drugs evoked a characteristic short-lasting elevation of the T-wave; higher doses (5 to 10 I.U./kg) caused marked and prolonged ST-depression and bradycardia. In ten experiments carried out in an atmosphere of 6 to 8 volume per cent oxygen the ECG pattern was the same as that seen after the administration of pituitary extract or vasopressin. In these cases CN in a dose of 400 μg/kg was administered by the intravenous route 1 minute prior to the injection of 2 to 3 I.U./kg of pituitary extract (protective experiment) or after the pathological ECG pattern had developed. In ten similar experiments as a pretreatment papaverine in a dose of 4 μg/kg was given by the intravenous route.

In the protective experiments the posterior pituitary induced elevation of the T-wave was counteracted in 15 out of 20 cases. In the remaining five experiments the effect was transient and uncertain; in these cases CN was considered ineffective. The protective effect of papaverine was 60% only. A somewhat similar observation was made in 10 experiments in which we administered 500 μg of CN by the oral route every other day for three weeks. In four of the animals treated in this way the T-wave elevation evoked by pituitary extract failed to appear (40% vs. 90% in the controls). Far reaching conclusions cannot be drawn from these few experiments but they call for further investigation of the preventive administration of CN.

To animals without any pretreatment, 5 I.U./kg of posterior pituitary extract was administered and then 1 to 2 minutes following the depression of ST 400 μg/kg CN was given intravenously. In 13 out of 20 experiments there was a normalization of the pathologic pattern for 3 to 6 minutes. In the remaining seven cases CN was ineffective. The influence of CN on the heart muscle damaged by hypoxia is summarized in Table III. Similar results have been obtained on alert rabbits (SZEKERES, personal communication).

**Table III**

*ECG changes brought about by posterior pituitary extract after papaverine and CN pretreatment*

| | Number of experiments | Elevation of T-wave | Per cent | |
|---|---|---|---|---|
| Zero minute:<br>2 I.U./kg of posterior pituitary extract | 10 | 9 | 90 | Control |
| Zero minute:<br>4 mg/kg of papaverine<br>1 minute:<br>2 I.U./kg of posterior pituitary extract | 10 | 6 | 60 | |
| Zero minute:<br>400 μg/kg of Cardinovin<br>1 minute:<br>2 I.U./kg of posterior pituitary extract | 20 | 5 | 25 | |

All the substances were administered by the intravenous route.

## Discussion

The extract CN used in the present study seemed to represent the most active one among those displaying a biological activity. In the case of CN we are probably faced by the beneficial synergetic effect of several components and many of the actions can be explained by the flavonoid character of the preparation, this group of compounds being known to have cardiovascular effects (Böhm 1960).

On the frog heart the cardiovascular effect of CN manifests itself primarily on the intact moderately hypoxic heart muscle in the form of a positive inotropic effect. Development of this effect depends on some unknown biochemical prerequisites. The moderate damage can be assumed to mean a decreased oxidative capacity, without substrate deficiency. Under the effect of CN, the energetically important substrates become utilizable under conditions of reduced oxidation. All this is well in accordance with the important part of flavonoids in oxido-reductive processes (Böhm 1959; Jeney and Uri 1954). — Also, positive inotropic effect was elicited by CN in the winter on either intact or drug-inhibited frog hearts under conditions otherwise identical with those prevailing in summer; the winter frog may be in such a biochemical

insufficiency state (substrate deficit) which prevents the development of CN action.

In warm-blooded animals CN considerably increased heart work in the Starling preparation and dilated the coronaries of the Langendorff heart. The latter finding as well as the results with coronary perfusion obtained by us and corroborated by other authors (KOVÁCS et al. 1960; SZEKERES 1964) prove that one of the most important aspects of the CN action is an augmentation of coronary flow. In addition, the data obtained on Starling's preparation indicate a direct heart muscle effect. Our assumption concerning the vasodilator action was corroborated also by the spasmolytic effect exerted by CN on smooth muscles (HETÉNYI and VÁLYI-NAGY 1969). Accordingly, one of the causes of the heart function increasing effect is the coronary dilation brought about by the drug; in the dog 20 mg/kg of CN increased cardiac output by 15 to 25% without affecting the heart rate and without causing any pathological change in the ECG (FÖLDVÁRI, personal communication).

The ECG changes brought about by posterior pituitary extract, vasopressin or hypoxia did not mean a coronary spasm but the bioelectric summation of the consecutive hypoxic changes in cardiac metabolism. The normalization of the ECG pattern under the effect of CN was indicative of the drug's influence on the metabolic disturbances of the heart muscle, as corroborated by the positive inotropic effects of CN on the frog heart as well as by its increasing effect on the efficacy of the cat heart-lung preparation. In all probability, a direct action on the heart muscle was figuring in both cases. To elucidate the mechanism of action, investigations performed at the molecular level are needed. Our studies in this direction revealed the influence of some flavonoids on potassium permeability, depolarization during excitation, etc. (HETÉNYI 1966, 1968); all these, however, need some further experimental corroboration. Finally, CN administered by the oral route in a daily dose of 100 mg for three weeks significantly decreased the serum cholesterol and triglyceride levels in rabbits fed 1 g of cholesterol daily (FÖLDVÁRI, personal communication).

All these observations are indicative of an effect of CN on cell metabolism, which might prove to be of importance in cardiovascular diseases.

## LITERATURE

BÖHM, K.: Die Flavonoide. III. Flavonoide und zelluläre Oxydation. Arzneimitt.-Forsch. **1959,** 9: 778—785.

BÖHM, K.: Die Flavonoide. VIII. Flavonoid-Effekte an Herz und Kreislauf. Arzneimitt.-Forsch. **1960,** 10: 547—554.

HETÉNYI, E.: Effect of flavonoid compounds on potassium transport. Acta physiol. Acad. Sci. hung. **1966,** 30: 387.

HETÉNYI, E.: Különböző eredetű izomkontrakciók befolyásolása flavonoid vegyületekkel. Kísérl. Orvostud. **1968,** 20: 534—539.

HETÉNYI, E., VÁLYI-NAGY, T.: Pharmacology of cherry (Prunus avium) stalk extract. I. Effect on smooth muscle. Acta physiol. Acad. Sci. hung. **1969**, 35: 183—188.
JENEY, E., URI, J.: A flavonfestékek farmakológiai és biológiai hatásai. MTA. Orv. Oszt. Közl. **1954**, 5: 193—219.
NIESCHULZ, O., POPENDIKER, K., HOFFMANN, I.: Weitere Untersuchungen über N-methyl-piperidyl-3-methyl-phenothiazin. Arzneimitt.-Forsch. **1955**, 5: 680—695.
PATAKY, I.: Izolált szervek vizsgálatának módszerei. In: Kovách, A. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1954**, Vol. 1, pp. 633—689.
SZEGI, J., RAUSCH, J.: Wirkung von Strophanthin auf normale und gekühlte Herz-Lungen Präparate von Katzen. Acta physiol. Acad. Sci. hung. **1958**, 13: 79—87.
SZEKERES, L., FALLER, I., LICHNER, Gy.: Empfindlichkeit der einzelnen Reizbildungszentren des Herzens gegenüber Kohlensäure und Hypoxia. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **1958**, 233: 343—347.

Ede HETÉNYI, Tibor VÁLYI-NAGY
Orvostudományi Egyetem Gyógyszertani Intézete,
Debrecen 12, Hungary

# *Recensiones*

HUDLICKÁ, O. (ED.)

## Circulation in skeletal muscle

Proceedings of an International Symposium held at Smolenice, Czechoslovakia, 1966. Pergamon Press, Oxford 1968. IX + 356 pages, with 171 figures. Price £6.00

The organizers of this Symposium realized an important task since before this book there was no contemporary review of the subject. The 55 participants of the Symposium, all of them well-known workers involved in these problems, presented their papers in five main sessions and took part in the discussions.

The first session under the chairmanship of S. M. HILTON covered central nervous regulation of skeletal muscle circulation. The second session with E. M. RENKIN as chairman discussed the problems of capillary circulation and transcapillary exchange. The third session under the chairmanship of H. BARCROFT reviewed interrelations between blood flow and metabolism, and exercise hyperaemia. The fourth session with C. HYMAN as chairman dealt with the interrelations of blood flow, metabolism, and reactive hyperaemia. The fifth session with B. FOLKOW as the chairman covered the problem of autoregulation in skeletal muscle. Finally, there was a general discussion concentrated on the problems of vascular tone and postconcentration hyperaemia and some of the most controversial questions of muscle circulation.

The book is a valuable source of up-to-date information for everyone interested in the problems of circulation in skeletal muscle.

K. LISSÁK

BRESLER, J. B. (ED.)

## Environments of man

Addison-Wesley Publishing Company, Reading, Massachusetts 1968. XI 289 pages. Price 35 s.

The book, concerned with the problems of human ecology, is an auxiliary text for the student of biology and as such elucidates the connections between biology, geology, anthropology and social sciences. Man's environment being composed of physical, biological and social factors, the book contains 29 modern papers from 17 different journals of general science, biology, medicine, dentistry, anthropology, and social problems. The student will be able to realize how environment affects man, how man affects his environment, and how man affects other people in his environment.

The text is supplemented with a glossary of terms used in the selected papers, a summary of statistical principles, and a list of supplementary readings.

K. LISSÁK

WETTERER, E. and KENNER, TH.

## Grundlagen der Dynamik des Arterienpulses

Springer-Verlag, Berlin, Heidelberg, New York, 1968.
379 pages, with 126 figures. Price DM 32.— US $8.00

The book is dedicated to the memory of Otto FRANK, Phillipp BROEMSER, Otto Friedrich RANKE and William Ferguson HAMILTON, the classical figures of circulation research, in pulse-dynamic. In consequence of the recent progress in methods and registration techniques, such apparently clarified fields like the arterial pulse gave rise to new interest in research and led to new results.

The authors are reviewing the literature up to 1967 and present the detailed results of their own research in 45 chapters. Thus the book offers an up-to-date and complete picture of the dynamics of the arterial pulse and the problems connected with it.

The work offers good information for physiologists and workers in circulatory system, but is very useful in clinical medicine too.

K. LISSÁK

STRAUB, F. B. and FRIEDRICH, P. (EDS)

## Symposium on Modern Methods in the Investigation of Protein Structure

Publishing House of the Hungarian Academy of Sciences, Budapest 1967. 93 pages

The book contains six comprehensive lectures on the most up-to-date problems in the various fields of protein chemistry.

H. HANSON and S. FITTKAU deal with the enzymatic sequence analysis of peptides, W. MEJBAUM with the use of Chinese tannin in studies of the polypeptide-like products of protein breakdown. B. K. VAINSHTEIN analyses the problems of protein morphology and conformation, with special reference to the use in such investigations of X-ray analytical, electron diffraction and electron microscopic methods.

P. ELŐDI discusses the correlations between optical rotational dispersion and the conformation of polypeptide chains, deviating in many points from previously accepted theories. G. SZABOLCSI gives an account of investigations concerned with the structure, separation and activity of chemically modified enzymes, with special reference to the mechanism of enzyme reactions. Finally, A. E. BRAUNSTEIN gives an excellent survey of the latest methods and results concerning the identification of the site of action of enzymes, especially of some well-known enzymes available also in crystalline form.

There are comprehensive references to each paper.

I. ALKONYI

INDEX

PHYSIOLOGIA

*Mészáros I.:* The Effect of Hypnotic Suggestion on Conditioned Evoked Potentials ... 101

*Kovách A. G. B., Szász Erzsébet, Pilmayer N.:* Mortality of Various Avian and Mammalian Species Following Blood Loss ... 109

*Grubich V., Malomsoki J., Nemessuri M.:* Cardiorespiratory Responses to Graded Positive-Pressure Breath Holding, I ... 117

*Pavel J.:* Induktionsveränderungen der Glutamat-Oxalazetat-Transaminase in der Henne unter Wirkung von 11-Dehydrokortikosteron ... 133

*Bohus B.:* Evaluation of the Role of Feedback Effect of Corticosteroids in the Control of Pituitary ACTH Release ... 141

*Spät A., Sólyom J., Sturcz J., Mészáros Ildikó, Ludwig E.:* Effect of Angiotensin Superfusion on the Rate of Aldosterone Production by Incubated Rat Adrenals ... 149

*Kurcz M., Nagy I., Kiss Cs., Halmy L.:* Estimation of the Blood Prolactin Level: A critical Analysis of Literary Data and of the Sulman—Simkin Method of Extraction 153

*Reviczky L. Alice, Szántó L.:* A Simple Method for Thyrotropic Hormone Assay ... 167

*Jancsó T., Galgóczy G.:* A Method for the Continuous Recording of Peripheral Vascular Resistance ... 173

PHARMACOLOGIA

*Hetényi E., Vályi-Nagy T.:* Pharmacology of Cherry (Prunus avium) Stalk Extract, I. Effect on Smooth Muscle ... 183

*Hetényi E., Vályi-Nagy T.:* Pharmacology of Cherry (Prunus avium) Stalk Extract, II. Cardiovascular Effects ... 189

RECENSIONES

*Hudlická O.* (Ed.): Circulation in Skeletal Muscle. Pergamon Press, Oxford, 1968. (K. Lissák) ... 199

*Bresler J. B.* (Ed.): Environments of Man. Addison-Wesley Publishing Company, Reading, Massachusetts, 1968. (K. Lissák) ... 199

*Wetterer E., Kenner Th.:* Grundlagen der Dynamik des Arterienpulses. Springer V. Berlin, Heidelberg, New York, 1968. (K. Lissák) ... 199

*Straub F. B.* and *Friedrich P.:* Symposium on Modern Methods in the Investigation of Protein Structure. Budapest, October 6—8, 1965. (I. Alkonyi) ... 200

# INSTRUCTION TO AUTHORS

ACTA PHYSIOLOGICA is devoted to publication of original findings in the field of physiology, pathophysiology and pharmacology. Papers may be submitted in English, French, German and Russian.

Papers should be sent to the Editor-in-Chief:
Professor K. Lissák, M. D.
Institute of Physiology
University Medical School
Rákóczi út 80.
Pécs, Hungary

MANUSCRIPT REQUIREMENTS. Manuscripts of full papers should not exceed 15 typewritten pages. The maximum length allowed for short communication is 5 pages including Tables and Literature, and for book review not more than 2 pages.

Manuscripts should be submitted double spaced with wide margins (at least 4 cm) on pages of uniform size.

Manuscripts should consist of (i) title of the paper; (ii) authors' names; (iii) laboratory of origin with city and state; (iv) abstract (in a duplicate copy) which does not exceed 1 page; (v) introduction; (vi) method(s); (vii) results; (viii) discussion; (ix) acknowledgements; (x) literature; (xi) authors' full names and complete addresses for mailing. The name and address of the author to whom correspondence are to be sent should be given; (xii) text for illustration should be given on separate sheets double spaced with wide margins.

*Literature* references should be listed in alphabetical order according to the surname of the first author. References must be complete including initials of the authors cited, title of article, journal name abbreviated as listed in the World List of Scientific Periodicals, year, volume, the first and last page numbers.

*Book references* should be in the following order: author's name, initials, title, publisher, city of publication, year, e.g.: LISSÁK, K., ENDRŐCZI, E.: The Neuroendocrine Control of Adaptation, Pergamon Press, Oxford **1965.**

Reference to authors contributing to multiauthor books should include: author, initials, title of article, name of Editor, title of book, publisher, city of publication, year and page numbers referred to, e.g.:

LEVINE, S.: Influence of gonadal hormones in infancy on adult behavior. In: LISSÁK, K. (Ed.): Symposium on Reproduction. Congress of the Hungarian Society for Endocrinology and Metabolism, Pécs 1967, Akadémiai Kiadó, Budapest **1967,** pp. 229—241.

A paper which has been accepted for publication but which has not appeared may be cited in the literature followed by the words, "In press". Responsibility for the accuracy of the references rest with the author.

Citation such as "unpublished observation", "personal communication", "in preparation", "to be published" should not be entered in the list of references, but may be noted in the text in parentheses.

*Reference in the text:* the name of author(s), (if there are not more than two contributors) and year should be given, e.g.: BIGNAMI (1960), SMITH and SMITH (1968), and if more than two, then only the first author's name should be given followed by "et al.", e.g.: GUZMAN-FLORES et al. (1960).

*Illustrations:* sharply contrasting photographs which must be kept to a minimum of figures on glossy paper are required and should be identified on the reverse with figure number (Arabic numbers) and author(s) name; when necessary the top should be clearly marked. Illustrations should be larger than the final size of reproduction but not more than 18 × 24 cm.

*Tables:* each table should have a brief heading, numbered with Roman numerals. Statistical measures of variation SD, SE etc. should be identified.

Standard nomenclature, abbreviations and symbols should be used; infamiliar or new terms, abbreviations and trade names should be defined when first used. The Editorial Board reserves the right to make literary corrections.

*Proofs:* galley proofs will be sent to the first-named author of the paper unless otherwise indicated. Corrections to the proofs must be restricted to printer's errors only. Other than these, any substantial alterations may be charged to the author. Proofs must be sent back by return mail.

*Reprints:* a total of 100 reprint will be provided to the first-named author. Additional copies can be ordered at reasonable price by ordering the reprints with the proof.

*Printed in Hungary*

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1969. II. 25. – Terjedelem: 9 (A/5) ív, 34 ábra

69.67209 Akadémiai Nyomda, Budapest – Felelős vezető: Bernát György

# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY
## and Cognate Medical Sciences

Chairman: D. WHITTERIDGE, F. R. S., B. Sc., D. M. (Oxon).
Press Editor: HUGH BEGBIE, M. A., Ph. D., M. B., Ch. B.

**Publication Dates: January, April, July, October**
**Annual Subscription: £6**
**Single Number: £1. 10s**

The *Quarterly Journal of Experimental Physiology* has always specialised in the publication of original articles with high-grade half-tone illustrations both in colour and black and white. All aspects of human and animal physiology are covered and the journal is essential for anyone wishing to keep abreast of current research in this field.

**Published by**

**E & S LIVINGSTONE LTD**
**15—17**

**TEVIOT PLACE**
**EDINBURGH**

ACTA PHYSIOLOGICA

ТОМ 35 – ВЫП. 2

# РЕЗЮМЕ

## ДЕЙСТВИЕ ГИПНОЗА НА ВЫЗВАННЫЕ УСЛОВНЫЕ ПОТЕНЦИАЛЫ

И. МЕСАРОШ

У здоровых людей в бодрствующем состоянии и под гипнозом изучены изменения вызванных условных потенциалов, выработанных при сочетании условного звукового раздражения и последующего с запаздыванием в 200 мсек подкрепляющего проблеска света. Под гипнозом условные потенциалы более прочны, чем в бодрствующем состоянии. Экспериментальные результаты указывают на то, что выключение светового раздражения, посредством вербального вну шения,примененного предварительно в качестве подкреиления, в процессе вызывания рефлекса не выключает положительного условного рефлекса, в то время как нарушение времени запаздывания путем внушения приводит к прекращению ответной реакции. На основе этих наблюдений кажется вероятным, что при выработке условного вызванного потенциала фактор времени является более лабильным, поддающимся воздействию фактором.

## СМЕРТНОСТЬ РАЗЛИЧНЫХ ВИДОВ ПТИЦ И МЛЕКОПИТАЮЩИХ ПОСЛЕ ОБЕСКРОВЛИВАНИЯ

А. Г. Б. КОВАЧ, Е. САС и Н. ПИЛМАЙЕР

Авторы проводили сравнение чувствительности голубя, утки, куры, вороны, фазана, крысы, кошки и собаки к обескровливанию при наркозе. Обескровливание проводилось у всех исследовавшихся видов одинаковым методом: количество взятой крови составляло 1% веса тела животных в час.

В отношении выносливости к кровопусканию отдельные исследовавшиеся виды животных в значительной степени отличаются друг от друга. Наиболее выносливыми оказались голубь и утка, куры и вороны занимают промежуточное место, а фазаны относятся, вместе с млекопитающими, к группе чувствительных видов. Дача дибенамина не оказывает влияния на резистентность голубя. Предварительная дача дибенамина у голубя не блокирует прессорного эффекта, вызванного адреналином и норадреналином. При сжатии сонной артерии в результате так наз. «церебрального ишемического рефлекса» у голубя и вороны возникает также после кровопускания значительное повышение кровяного давления. У куры после сжатия сонной артерии не наблюдается прессорного ответа.

## ОРГАНОСПЕЦИФИЧЕСКОЕ УПРАВЛЕНИЕ НЕСКОЛЬКИМИ КАРДИОРЕСПИРАТОРНЫМИ РЕАКЦИЯМИ НА УВЕЛИЧЕНИЕ СЖАТИЯ ВОЗДУХА В ЛЕГКИХ

В. ГРУБИХ, Й. МАЛОМШОКИ и М. НЕМЕШШУРИ

Увеличение сжатия воздуха вызывает у спортсменов общие и органоспецифические кардиореспираторные изменения.

Частота пульсовых ударов сердца повышается в соответствии со степенью сжатия непосредственно перед сжатием воздуха в меньшей мере, а во время сжатия более значительно. После сжатия наступает брадикардия, независимо от степени нагрузки, а затем

пульс нормализуется посредством противорегуляции. Реакция частоты сердца больше всего дифференцирована, однако, она реагирует на статическую работу, повышающуюся шестью ступенями, лишь в 3—4 ступенях. Это сказывалось также в продолжительности сердечных циклов. Укорочение цикла во время сжатия и его удлинение в виде обратного размаха после сжатия обуславливаются в первую очередь изменением электрического времени диастолы, однако в возникновении этих изменений статистически достоверно участвуют также изменения электрического времени систолы.

Подобная динамика наблюдается также в поведении кровяного давления и газообмена; шестиступенчатая нагрузка и здесь реализуется в меньших ступенях. Меньше всего изменяется диастолическое давление. Реакция, проявляющаяся в изменении систолического и диастолического кровяного давления, реализуется тремя ступенями. В динамике кровяного давления, обусловленной сжатием воздуха, после более сильных сжатий отмечается запоздание противорегуляции, и это становится все более интенсивным. Добавочные величины газообмена, вызванные нагрузкой, при первых четырех ступенях нагрузки одинаковы, затем они скачкообразно нарастают. При более значительных давлениях сжатия бóльшая часть добавочного потребления кислорода приходится на стартовое состояние; в то же время добавочная отдача двуокиси углерода, — показавшая по сравнению с потреблением кислорода в связи с повышенной нагрузкой более крутый подъем, — предположительно, происходит частично не из обмена веществ работы сжатия. Реакция газообмена также лишь трехступенчатая. Значит, статическая нейродвигательная градация более дифференцирована, чем исследовавшиеся кардиореспираторные ответы.

## ИНДУКЦИЯ ТРАНСАМИНАЗЫ ГЛУТАМАТА-ОКСАЛАЦЕТАТА В ОРАНИЗМЕ КУР, ВЫЗВАННАЯ АППЛИКАЦИЕЙ 11 ДЕГИДРОКОРТИКОСТЕРОНА

И. ПАВЕЛ

Были исследованы адаптивные изменения активности трансаминазы глутамата оксалацетата (GOT) в 15 различных органах и тканях у кур породы белый плимутрок после аппликации 11 дегидрокортикостерона, а именно, в интервалах одного, трех и шести дней, при чем дневная дача составляла 1 мг действенного вещества на 100 г живого вещества. Получились следующие результаты:

1) При сравнении групп птиц с разным сроком аппликации тесткортикостероида было доказано, что главные изменения индукции возникли только в интервалах от трех до шести дней проведенной аппликации.

2) При вариационно-статистической оценке результатов показалось, что очень сильному влиянию (P = 0,01) была подвергнута активность GOT в ткани печени, далее в плазме крови и в желтке яйца, между тем как только доказательное влияние (P = 0,95) подтверждалось в ткани сердца, легких и в костном мозге.

3) Индукционные воздействия аппликации дач 11-дегидрокортикостерона в организме кур в общем проявились по мере повышения активности GOT.

## РОЛЬ МЕХАНИЗМА ОБРАТНОЙ СВЯЗИ КОРТИКОСТЕРОИДОВ В РЕГУЛИРОВАНИИ СЕКРЕЦИИ АКТГ В ГИПОФИЗЕ

Б. БОХУШ

Роль механизма отрицательной обратной связи кортикоидов в регулировании секреции АКТГ, вызванной стрессом, была изучена после предварительного введения кортикостерона у крыс с односторонней адреналектомией и у иммобилизованных крыс.

Предварительная дача кортикостерона не влияет на острое повышение секреции АКТГ, в то время как у иммобилизованных крыс и у животных с односторонней адреналэктомией в фазе субнормального ответа на максимальную активацию гипофизарно-надпочечной системы кортикостерон вызывает существенное торможение секреции АКТГ. Наблюдаемое цосле субнормальной фазы второе повышение выделения АКТГ также понижается в связи с предварительным введением кортикостерона.

Результаты опытов указывают на то, что механизм отрицательной обратной связи, обусловленной кортикостероном, влияющий на непрерывные стрессорные воздействия, как напр., иммобилизация животных или односторонее удаление их надпочечников, проявляется в регулировании двуфазного ответа гипофизарно-надпочечной системы.

## ДЕЙСТВИЕ СУПЕРФУЗИИ АНГИОТЕНСИНА НА СЕКРЕЦИЮ АЛЬДОСТЕРОНА НАДПОЧЕЧНИКАМИ КРЫС

А. ШПЕТ, Я. ШОЙОМ, Й. ШТУРС, И. МЕСАРОШ и Э. ЛЮДВИГ

Изучалось действие ангиотенсина на стероидопродукцию надпочечников у крыс. В целях уравновешивания расщепления вследствие ангиотенсиназной активности надпочечников, в течение инкубации непрерывно вливали ангиотенсин в инкубационный раствор. Суперфузия ангиотенсина не повысила секреции альдостерона.

## ДАННЫЕ К СОДЕРЖАНИЮ ПРОЛАКТИНА В КРОВИ (ЛИТЕРАТУРНЫЕ ДАННЫЕ И КРИТИЧЕСКИЙ АНАЛИЗ МЕТОДА ЭКСТРАГИРОВАНИЯ ПО СУЛЬМАН—СИМКИНУ)

М. КУРЦ, И. НАДЬ, Ч. КИШШ и Л. ХАЛМИ

Подробно анализуются новые литературные данные, в которых с помощью теста на зобе голубя определяется пролактин в крови. Эти литературные данные противоречивы и содержат ряд ошибок.

На основе опытов in vivo и in vitro на модели, описанная Сульманном (1956 г.) и предложенная Симкин и Гударт (1960 г.) процедура экстрагирования непригодна для обратного получения пролактина крови. Полученные этим методом данные следует считать артефактами.

В связи с определением пролактина в нативной крови обсуждаются критерии специфичности действия пролактина, коэффициент исчезновения пролактина, а также отрицательные результаты определений, в которых не удалось выявить пролактина в крови.

На основе результатов своих собственных экспериментов авторы приходят к заключению, что содержание пролактина в крови является более низким, чем порог чувствительности микротеста на зобе голубя, поэтому и концентрации, полученные после введения нативной плазмы, нельзя рассматривать как реальные величины.

## ПРОСТОЙ МЕТОД ДЛЯ ОПРЕДЕЛЕНИЯ ТИРЕОТРОПНОГО ГОРМОНА

А. РЕВИЦКИ и Л. САНТО

Авторы переработали гисто-авторадиографический метод *Месса* и *Хамори* (1961- для инструментального измерения, благодаря чему получается возможность для исполь) зования этого метода при повседневных клинических исследованиях. До количества 0,3 мМЕ Амбинона поглощение $J^{131}$ щитовидной железой восьмидневных цыплят оказалось соразмерным, однако, при повышении дозы Амбинона наблюдался перелом и инкорпорация $J^{131}$ щитовидной железой показывала обратную пропорциональность с дозами Амбинона. По мнению авторов упрощенный метод соответствует требованиям биологических методов определения.

## МЕТОД ДЛЯ НЕПРЕРЫВНОЙ РЕГИСТРАЦИИ СОПРОТИВЛЕНИЯ КРОВОТОКУ

Т. ЯНЧО и Г. ГАЛГОЦИ

С помощью ртутных манометров было сконструировано измерительное приспособление, предоставляющее возможность для непрерывной прямой регистрации сопротивления кровотоку периферических сосудистых областей (органов). Излагаются принци-

пиальные и технические основы метода с помощью подробного математического анализа и схем соединения. Чтобы доказать правильность своего метода, авторы проводили опыты на модели и на животных, из результатов которых они сделают вывод, что данное приспособление пригодно для количественного и качественного анализа закономерностей регулирования гемодинамики, проявляющихся в изменениях сопротивления кровотоку.

## ФАРМАКОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЫТЯЖКИ ИЗ СТЕБЛЯ ЧЕРЕШНИ (PRUNI AVIUM STIPES) I ДЕЙСТВИЕ НА ГЛАДКИЕ МЫШЦЫ

З. ХЕТЕНЬИ и Т. ВАЙИ-НАДЬ

Действие вытяжки стебля черешни (Pruni avium stipes) полученной патентован, ным методом и состоящей преимущественно из флавоноидных компонентов (Кардиновин)-было исследовано на препаратах гладких мышц. В концентрации 50—100 $\mu$г вытяжки на мл питательной жидкости Кардиновин обратимо прекращал самопроизвольную активность матки крысы и морской свинки. Препарат предотвращал сокращение, вызываемое Гландуитрином. На тонкой кишке крысы были получены подобные результаты, тогда как на кишке морской свинки после применения больших доз (500 $\mu$г/мл) возникли сокращения. Доза в 100 $\mu$г/мл практически полностью купировала судороги, вызываемые одинаковой дозой Гландуитрина, а сокращение от $BaCl_2$ предотвратила лишь в 50% случаев. На матке крысы с помощью автоматического суперфузионного прибора для титрования была осуществлена биологическая титрация Кардиновина. Обсуждаются полученные результаты, доказывающие также с биологической стороны флавоноидный характер Кардиновина. В заключение авторы обсуждают необходимость исследования природных веществ с помощью современных методов, и указывается на несколько фармакологических экспериментов такого рода, проводившихся авторами.

## ФАРМАКОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЫТЯЖКИ ИЗ СТЕБЛЯ ЧЕРЕШНИ (PRUNI AVIUM STIPES) II КАРДИОВАСКУЛЯРНЫЕ ДЕЙСТВИЯ

З. ХЕТЕНЬИ и Т. ВАЙИ-НАДЬ

Авторы исследовали фармакологическое действие вытяжки стебля черешни полученной запатентованным методом и состоящей преимущественно из флавоноидных компонентов (Кардиновин). На сердце лягушки, пораженном хинином и Гландуитрином (Pruni avium stipes) 100 $\mu$г вытяжки оказывают положительное инотропное действие. Возможность вызывания положительных инотропных эффектов в значительной мере зависела от качественных и количественных условий поражения. На препарате сосуда задней конечности лягушки (Тренделенбург) Кардиновин прекращает сосудистый спазм, вызванный адреналином, но само по себе он безэффективен. На сердечно-коронарном препарате крысы (Лангендорфф) вытяжка в значительной мере понижает сопротивление венечного сосуда кровотоку (в среднем на 30%). В экспериментах на сердечно-легочном препарате (Старлинг) она нормализует работу сердца, ослабленного барбитуратом, и в половине случаев повышает ее даже выше исходной величины. На кровяное давление, измеренное кровавым путем, в дозе 1—2 мг Кардиновин не оказывает действия, лишь большие дозы (5—10 мг/кг) вызывают понижение кровяного давления примерно на 10%. На собаке со скрытой грудной клеткой Кардиновин повышает кровоток в коронарии и учащает систолы (при регистрации на кардиомиографе), однако, на сосочковую мышцу сердца кошки он оказывает лишь слабое положительно инотропное действие. У крысы Кардиновин в дозе 400 г на кг веса на 5—10 минут нормализует патологический — гипоксический — ЭКГ, вызванный дачей Гландуитрина (или вазопрессина) и предотвращает повышение зубца Т, наблюдаемое в начале гипоксии. В заключение обсуждаются полученные результаты и предположительные механизмы действия Кардиновина. Уменьшение сердечной деятельности, предположительно, является общим результатом эффектов, отчасти расширяющих венечные сосуды, отчасти улучшающих обмен веществ миокарда и стенки сосудов. Упоминается также, что исследуемое вещество — Кардиновин — вызывает и значительное понижение уровня холестерина в крови.

The *Acta Physiologica* publish papers on experimental medical science in English, French German or Russian.

The *Acta Physiologica* appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the *Acta Physiologica* is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. — Account No. 43-790-057-181) or with representatives abroad.

---

Les *Acta Physiologica* paraissent en français, anglais, allemand et russe et publient des mémoires du domaine des sciences médico-expérimentales.

Les *Acta Physiologica* sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à la rédaction à l'adresse suivante:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Toute correspondance doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forint par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. — Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

---

«*Acta Physiologica*» публикуют трактаты из области экспериментальной медицинской науки на русском, немецком, английском и французском языках.

«*Acta Physiologica*» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации рукописи следует направлять по адресу:

*Acta Physiologica, Pécs, Rákóczi út 80.*

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «*Acta Physiologica*» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «*Kultúra*» (Budapest I., Fő utca 32. — Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

## Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA

Ndermarja Shtetnore e Botimev
*Tirana*

AUSTRALIA

A. Keesing
Box 4886, GPO
*Sydney*

AUSTRIA

Globus Buchvertrieb
Salzgries 16
*Wien I*

BELGIUM

Office International de Librairie
30, Avenue Marnix
*Bruxelles 5*

Du Monde Entier
5, Place St. Jean
*Bruxelles*

BULGARIA

Raznoiznos
1, Tzar Assen
*Sofia*

CANADA

Pannonia Books
2, Spadina Road
*Toronto 4, Ont.*

CHINA

Waiwen Shudian
*Peking*
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Směčkách 30
*Praha 2*

Poštová Novinová Služba
Dovoz tisku
Vinohradská 46
*Praha 2*

Mad'arská Kultura
Václavské nám. 2
*Praha I*

Poštová Novinová Služba
Dovoz tlače
Leningradská 14
*Bratislava*

DENMARK

Ejnar Munksgaard
Nörregade 6
*Copenhagen*

FINLAND

Akateeminen Kirjkauppa
Keskuskatu 2
*Helsinki*

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
*Paris 5*

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
*Leipzig 701*

Zeitungsvertriebsamt
Fruchtstrasse 3—4
*1004 Berlin*

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
*7 Stuttgart S.*

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
*Wellingborough. Northants.*

Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
*Oxford*

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
*Amsterdam C.*

Martinus Nijhof
Lange Voorhout 9
*The Hague*

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP
GPO Post Box 1374
*Bombay I*

ITALY

Santo Vanasia
Via M. Macchi 71
*Milano*

Libreria Commissionaria Sansoni
Via La Marmora 45
*Firenze*

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
*Tokyo*

Maruzen and Co. Ltd.
P. O. Box 605
*Tokyo-Central*

Far Eastern Booksellers
Kanda P. O. Box 72
*Tokyo*

KOREA

Chulpanmul
*Phenjan*

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
*Oslo*

POLAND

RUCH
ul. Wronia 23
*Warszawa*

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
*Bucureşti*

SOVIET UNION

Mezhdunarodnaya Kniga
*Moscow G—200*

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
*Stockholm*

USA

Stechert Hafner Inc.
31, East 10th Street
*New York, N. Y. 10003*

Walter J. Johnson
111, Fifth Avenue
*New York, N. Y. 10003*

VIETNAM

Xunhasaba
19, Tran Quoc Toan
*Hanoi*

YUGOSLAVIA

Forum
Vojvode Mišića broj 1
*Novi Sad*

Jugoslovenska Knjiga
Terazije 27
*Beograd*

*30. VI. 1969* Index: 26.023

# ACTA PHYSIOLOGICA

## ACADEMIAE SCIENTIARUM HUNGARICAE

ADIUVANTIBUS

P. BÁLINT, SZ. DONHOFFER, E. ERNST, B. ISSEKUTZ SEN., L. KESZTYŰS, J. KNOLL, F. OBÁL

REDIGIT

K. LISSÁK

TOMUS XXXV FASCICULI 3—4

AKADÉMIAI KIADÓ, BUDAPEST

1969

# ACTA PHYSIOLOGICA

## A MAGYAR TUDOMÁNYOS AKADÉMIA KÍSÉRLETES ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21.

Főszerkesztő:
LISSÁK KÁLMÁN
akadémikus

Technikai szerkesztő:
KORÁNYI LAJOS

Az *Acta Physiologica* német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudományok köréből.

Az *Acta Physiologica* változó terjedelmű füzetekben jelenik meg: több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az *Acta Physiologica* előfizetési ára kötetenként belföldre 120 forint, külföldre 165 forint. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla 43-790-057-181 sz.) vagy annak külföldi képviseleteinél és bizományosainál.

---

Die *Acta Physiologica* veröffentlichen Abhandlungen aus dem Gebiete der experimentellen medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die *Acta Physiologica* erscheinen in Heften wechselnden Umfanges. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an folgende Adresse zu senden:

*Acta Physiologica, Pécs, Rákóczi út 80.*

An die gleiche Anschrift ist auch jede für die Redaktion und den Verlag bestimmte Korrespondenz zu senden.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

*Physiologia*

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 201—210 (1969)*

# ARTERIO-VENOUS ANASTOMOSES IN THE KIDNEY

## I. RENAL BLOOD FLOW FOLLOWING THE INFUSION OF SERUM ALBUMIN*

By

P. GÖMÖRI, L. HÁRSING,** K. KÁLLAY, J. BARTHA** and L. DEBRECZENI

SECOND DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 28, 1968)

In previous studies performed with the corrosion method, the presence of arterio-venous anastomoses in the kidney has been demonstrated in a variety of conditions associated with shock. In the present observations attempts were made to furnish functional evidence of the existence of such anastomoses. As the infusion of serum albumin has been shown to reduce PAH extraction and this might be the result of the opening of arterio-venous shunts, in the present study total renal blood flow was measured by an electromagnetic flow-meter, and nutritive blood flow by SAPIRSTEIN's $^{86}$Rb extraction method as modified by HÁRSING and PELLEY (1965). The difference between the values yielded by the two methods has been termed "inert" blood flow. In the normal dog, this value amounted to 5% of the total blood flow, whereas following the administration of serum albumin it increased to 28% while medullary blood flow did not change. The result is interpreted as functional evidence of the opening of arterio-venous anastomoses in the kidney.

The question of arterio-venous anastomoses in the kidney has repeatedly been dealt with in earlier works from this laboratory, with the aim to clarify the role of renal blood flow in the changes occurring in the kidney during circulatory failure. In experiments performed with the corrosion technique of MUNKÁCSI (1957) it has been demonstrated that the kidney becomes ischaemic in shock due to ischaemia, trauma, haemorrhage and dehydration (GÖMÖRI et al. 1962). It was surprising that, in the corrosion preparations, filling was observed also in the renal vein. Since owing to its high viscosity, the substance used in these studies could not pass the capillaries, thus in the normal kidney the veins are not filled, the appearance of the injected material on the venous side had to be interpreted to point to the presence of arterio-venous communications. Their existence has repeatedly been confirmed morphologically (GÖMÖRI et al. 1964c, 1965), and the presence in various parts of the kidney of corticomedullary and intracortical communications in the form of short channels in the cortex corticis has been shown. These experiments were

* This paper was presented at the Congress of the Hungarian Physiological Society, Debrecen/Hungary, July 1968.

** Institute of Physiology, University Medical School, Budapest.

the first to demonstrate that certain glomeruli open directly into veins (paravenous glomeruli) and practically act as shunts (MUNKÁCSI et al. 1963). The results were essentially in agreement with earlier data of TRUETA (1948), DANIEL (1952), CLARA (1956) and SPANNER (1957) and have recently been confirmed by SIEGELMAN and GOLDMAN (1968) in man.

There was, however, no functional evidence of the existence of by-passes. The present studies have been based on the earlier findings by CARGILL (1948); reduction of the renal arterio-venous difference of PAH after the infusion of human serum albumin in patients with nephrosis; a reliable explanation for this finding was that the infusion of albumin had opened arterio-venous shunts in the kidney, causing part of the blood to be diverted from the parenchyma and to flow through by-passes directly into the veins. These experiments were repeated in dogs; the effect of human serum albumin was tested on the extraction rate of PAH and of oxygen in the kidney (GÖMÖRI et al. 1964b) and it was found that human serum albumin causes a significant depression of $E_{PAH}$ and $E_{O_2}$. Similar results have been obtained with dihydralazine. It is known that PAH is not completely extracted in either the human or the canine kidney; the average PAH extraction rate was 73% in the dog (GÖMÖRI et al. 1964a). The diminution of $E_{PAH}$ was thought to be due to the fact that part of the blood was flowing through regions which did not extract PAH. ELPERS and SELKURT (1963) observed a similar diminution in $E_{PAH}$ in response to the infusion of bovine or canine serum albumin to dogs. As $Tm_{PAH}$ did not change in their experiments it was concluded that the observed diminution of $E_{PAH}$ could not be the result of some deterioration of tubular transport.

In view of these results it seemed interesting to investigate the problem by up-to-date techniques and to seek further functional evidence of the existence of arterio-venous communications in the kidney. In the present experiments attempts have been made not only to establish whether human serum albumin diminished $E_{PAH}$ in the dog, but also to reveal whether or not the difference between total renal blood flow as estimated by measuring arterial inflow and nutritive blood flow representing the amount of blood flowing through the parenchyma was affected by the infusion of serum albumin. Thus, if total blood flow and nutritive blood flow are determined simultaneously, the difference between the two values is equal to the amount of blood diverted from the functioning renal parenchyma and can therefore be termed "inert" blood flow; its increase might be the result of several factors, the opening of arterio-venous shunts, among others.

The experiments were undertaken by techniques allowing reliable measurement of both total and nutritive blood flow. The amount of blood entering the kidney can be measured under near physiological conditions by an electromagnetic flow-meter. Blood flow through the parenchymal capillaries

was determined by SAPIRSTEIN's $^{86}$Rb method (1956, 1968) modified by HÁRSING and PELLEY (1965). Obviously, an increase in the difference between total and parenchymal blood flow might be the result of the opening of vascular by-passes. The purpose of the present experiments was to determine the difference between total and nutritive blood flow ("inert" blood flow) under resting conditions and during the infusion of human serum albumin; it has been assumed that this experimental arrangement would furnish functional evidence of the existence of arterio-venous communications in the kidney.

## Methods

Experiments were performed on 25 mongrel dogs of either sex, weighing 11.0 to 17.0 kg (mean: 14.5 kg) and anaesthetized with chloralose (0.10 g/kg). Blood pressure was measured by an Elema-Schönander EMT 35 electromanometer through a polyethylene catheter introduced via the femoral artery into the aorta up to the level of the diaphragm; mean arterial pressure was determined by electric integration. Blood flow of the left kidney ($RBF_{Dir}$) was measured by an electromagnetic flow-meter (Nykotron) placed on the renal artery after the kidney had been exposed through a lumbar incision. In the aim of obtaining the extraction ratio of PAH ($E_{PAH}$) venous blood samples were obtained through an indwelling catheter introduced via the right external jugular vein and passed under fluoroscopic control into the left renal vein; the testicular or ovarian veins were ligated. The position of the catheter was repeatedly checked by palpation, and also at the end of the experiment. Concentration of PAH in renal venous and simultaneously obtained arterial blood samples was estimated by the method of BRATTON and MARSHALL (1939). Haematocrit was determined in arterial blood samples by a Hawksley microhaematocrit centrifuge (12,000 g, for 5 min). Nutritive blood flow in the left kidney ($RBF_{Rb}$) was estimated by the method of SAPIRSTEIN (1956, 1958) modified by HÁRSING and PELLEY (1965). The method allows the measurement of total "nutritive" or "capillary" blood flow, cortical blood flow ($CBF_{Rb}$) and medullary blood flow ($MBF_{Rb}$). $^{86}$Rb was injected directly into the right atrium through a catheter introduced via the external jugular vein. The dilution of $^{86}$Rb was established in arterial blood samples collected by a fractionating collector from the cannulated common carotid at 1-sec intervals. Urine was collected through indwelling catheters introduced into the ureters through a low abdominal midline incision. To secure adequate urine flow and maximal medullary blood flow, 10% mannitol in physiological saline and the necessary amount of PAH were infused into the femoral vein by a constant-rate pump (10 ml/min); as an increase in medullary blood flow may depress the $E_{PAH}$. Blood pressure, $RBF_{DIr}$ and ECG were recorded continuously by a Mingograf 81 (Elema), usually at 2.5 mm/sec paper speed.

After completion of the above procedures the infusion of mannitol was started, and the experiments were begun 25 to 30 min later. In two 5 min control periods (periods 1 and 2), urine was collected, blood samples were withdrawn for the estimation of $E_{PAH}$, and arterial blood pressure and left renal blood flow ($RBF_{Dir}$) were recorded continuously; average values for these two periods are shown in the Tables as "Preinfusion period". Subsequently, in 13 dogs, a solution containing 25% human serum albumin in physiological saline in addition to PAH and 10% mannitol was infused. The total amount of albumin infused was 3 g/kg of body weight; as the rate of infusion was kept constant, the time necessary for the infusion of this amount of albumin varied between 20 and 25 min. Then the infusion was continued with the original solution containing only mannitol and PAH. During the last 5 min of the albumin infusion, urine was collected and blood samples were withdrawn for the estimation of $E_{PAH}$ (period 3), then followed a fourth 5-min period and immediately after this 50 to 100 $\mu$C of $^{86}$Rb in a volume of 1 ml was injected into the right atrium, and collection of blood samples was started from the carotid artery (20 sec). Exactly 60 sec after the injection of $^{86}$Rb the animal was killed by the intravenous injection of saturated KCl solution. Thus, the entire experimental period lasted for about 40 min.

Subsequently, the kidneys were removed, decapsulated, and slices weighing 700 to 1000 mg were cut out from the cortex, and the outer and inner medulla. The tissue samples and the kidney residue were dissolved in concentrated $HNO_3$. Radioactivity of the kidney and of the tissue samples was determined by a scintillation counter. Nutritive blood flow

**Table I**

*Control experiments. Infusion of 10% mannitol*

| No. | Preinfusion period | | | | During | | | |
|---|---|---|---|---|---|---|---|---|
| | Blood pressure (mm Hg) | $RBF_{Dir}$ (ml/min/ 100 g) | Resistance ($R_{Dir}$/kg) | $E_{PAH}$ | Blood pressure (mm Hg) | $RBF_{Dir}$ (ml/min/ 100 g) | Resistance ($R_{Dir}$/kg) | $RBF_{Rb}$ ml/min/ 100 g |
| 5 | 174 | 523 | 1.99 | 0.58 | 162 | 348 | 2.79 | 287 |
| 7 | 179 | 421 | 2.54 | 0.65 | 160 | 291 | 3.30 | 304 |
| 10 | 127 | 481 | 1.58 | 0.58 | 110 | 461 | 1.43 | 383 |
| 12 | 117 | 244 | 2.87 | 0.72 | 105 | 169 | 3.72 | 166 |
| 22 | 117 | 490 | 1.43 | 0.65 | 107 | 391 | 1.65 | 215 |
| 24 | 147 | 345 | 2.55 | 0.58 | 140 | 262 | 3.20 | 226 |
| 25 | 173 | 349 | 2.98 | 0.78 | 160 | 443 | 2.17 | 348 |
| 29 | 233 | 418 | 3.34 | 0.54 | 186 | 535 | 2.08 | 555 |
| 31 | 162 | 391 | 2.47 | 0.62 | 145 | 324 | 2.69 | 435 |
| 33 | 130 | 283 | 2.77 | 0.54 | 142 | 391 | 2.18 | 562 |
| 34 | 142 | 533 | 1.60 | 0.64 | 140 | 483 | 1.74 | 320 |
| 39 | 160 | 270 | 3.56 | 0.69 | 155 | 290 | 3.20 | 305 |
| Mean | 155 | 395 | 2.47 | 0.63 | 143** | 365 | 2.51 | 342 |
| ±S.D. | 33 | 99.4 | 0.71 | 0.07 | 24.8 | 105 | 0.74 | 124.6 |

*Symbols.* Values not marked by * are not significant statistically as compared to values
cally different from preinfusion values at

---

was calculated on basis of the equation:

$$RBF_{Rb} = \frac{60 \times Q}{{}_0\int^t C_a \times dt}$$

where $Q$ is the radioactivity in the kidney, cortical and medullary tissue samples (cpm/100 g); $C_a$ the radioactivity of arterial blood samples (cpm/ml); and $t$ the mean circulation time; the latter was obtained on semilogarithmic paper by extrapolating the descending part of the arterial dilution curve to the abscissa. The abscissa was drawn at the value corresponding to 1% of the maximal concentration of $^{86}Rb$. $C_a \times dt$ is equal to the area under the dilution curve which was calculated by adding the concentration values in every second. Values for blood flow were computed for 100 g of kidney, cortex or medulla. Blood flow through cortex ($CBF_{Rb}$) and medulla ($MBF_{Rb}$), i.e. the intrarenal distribution of blood flow in 100 g of kidney was calculated by assuming that the cortical tissue is equal to 64%, and the medullary tissue to 32%, of the total weight of the kidney (HÁRSING et al. 1966). The remaining 4% were shared by the adipose and other non-specific tissues the blood flow of which was not determined.

The same measurements were undertaken in 12 control dogs with the difference that albumin had not been infused. The duration of the experiments was the same as in the other series.

Renal vascular resistance was calculated on basis of the equation:

$$R_{kg} = \frac{\text{mean arterial pressure (mm Hg)}}{\text{blood flow (ml/sec/100 g)}} \times 10$$

*in physiological saline, at a rate of 10 ml/min*

dilution of $^{86}Rb$

| Resistance ($R_{Rb}$/kg) | $\frac{RBF_{Rb}}{RBF_{Dir}}$ | Cortical flow ($CBF_{Rb}$) ml/min/100 g | | Medullary flow ($MBF_{Rb}$) ml/min/100 g | | $\frac{CBF/100\ g}{RBF_{Dir}/100\ g}$ | $E_{PAH}$ |
|---|---|---|---|---|---|---|---|
| | | cortex | kidney | medulla | kidney | | |
| 3.38 | 0.83 | 341 | 218 | 216 | 69 | 0.63 | 0.65 |
| 3.16 | 1.04 | 306 | 195 | 337 | 108 | 0.67 | 0.63 |
| 1.72 | 0.83 | 456 | 292 | 284 | 91 | 0.63 | 0.55 |
| 3.80 | 0.98 | 191 | 122 | 138 | 44 | 0.73 | 0.75 |
| 3.00 | 0.55 | 238 | 152 | 197 | 63 | 0.39 | 0.62 |
| 3.70 | 0.86 | 247 | 158 | 212 | 68 | 0.61 | 0.60 |
| 2.76 | 0.79 | 388 | 248 | 344 | 110 | 0.58 | 0.71 |
| 2.00 | 1.04 | 590 | 377 | 556 | 178 | 0.71 | 0.55 |
| 2.00 | 1.34 | 495 | 317 | 370 | 118 | 0.98 | 0.58 |
| 1.51 | 1.44 | 630 | 401 | 502 | 161 | 1.03 | 0.53 |
| 2.62 | 0.67 | 380 | 242 | 244 | 78 | 0.50 | 0.55 |
| 3.04 | 1.05 | 335 | 214 | 284 | 91 | 0.74 | 0.59 |
| 2.72 | 0.95 | 383 | 245 | 307 | 98 | 0.68 | 0.61 |
| 0.77 | 0.26 | 137.5 | 87.8 | 124 | 39.8 | 0.18 | 0.07 |

obtained in the preinfusion period. Values marked by *, **, and *** are statisti- the 0.05, 0.01 and 0.001 levels, respectively.

and was expressed in R units (1 R = 1328 dyne · sec · $cm^{-5}$). When calculating resistance, renal venous pressure was considered zero. Only those experiments were considered where $E_{PAH}$ was higher than 0.50 and mean blood pressure higher than 100 mm Hg in the preinfusion periods.

The values for the preinfusion period were compared statistically to the experimental periods, the differences were evaluated by STUDENT's *t*-test. Parallel readings obtained in the dogs subjected to albumin infusion and in the control dogs were also evaluated statistically by the *t*-test.

## Results

Results have been summarized in Tables I and II. In the control group (Table I), blood pressure gradually decreased until the end of the experiments from an average of 155 to 143 mm Hg; this slight but statistically significant reduction was obviously due to the anaesthesia. $RBF_{Dir}$ did not change in the course of the experiments, the average measured in the preinfusion periods attaining 395 ml/min/100 g and that obtained in the last period, 365 ml/min/100 g. Renal vascular resistance showed no significant change, and $E_{PAH}$ also remained essentially unchanged.

In the experimental group receiving the serum albumin (Table II) blood pressure was somewhat more reduced (from 151 to 135 mm Hg) than in the control dogs. Renal blood flow showed considerable changes in comparison

**Table II**

*Effect of human serum albumin on renal blood flow in the dog. In the preinfusion period, the perimental periods, 25% albumin was added to the infusion administered*

| No. | Preinfusion period | | | | During | | | |
|---|---|---|---|---|---|---|---|---|
| | Blood pressure (mm Hg) | $RBF_{Dir}$ (ml/min/100 g) | Resistance ($R_{Dir}$/kg) | $E_{PAH}$ | Blood pressure (mm Hg) | $RBF_{Dir}$ (ml/min/100 g) | Resistance ($R_{Dir}$/kg) | $RBF_{Rb}$ (ml/min/100 g) |
| 2 | 183 | 630 | 1.74 | 0.69 | 185 | 896 | 1.24 | 434 |
| 3 | 174 | 286 | 3.65 | 0.70 | 141 | 354 | 2.39 | 320 |
| 4 | 167 | 742 | 1.35 | 0.55 | 162 | 885 | 1.10 | 535 |
| 6 | 130 | 215 | 3.63 | 0.66 | 137 | 262 | 3.13 | 264 |
| 9 | 145 | 399 | 2.18 | 0.62 | 110 | 621 | 1.07 | 480 |
| 14 | 106 | 703 | 0.90 | 0.75 | 106 | 956 | 0.67 | 640 |
| 15 | 141 | 547 | 1.54 | 0.61 | 107 | 586 | 1.10 | 334 |
| 20 | 184 | 552 | 2.00 | 0.66 | 182 | 640 | 1.70 | 322 |
| 23 | 157 | 500 | 1.89 | 0.64 | 112 | 437 | 1.55 | 278 |
| 35 | 147 | 529 | 1.67 | 0.71 | 130 | 836 | 0.94 | 627 |
| 40 | 162 | 368 | 2.64 | 0.63 | 145 | 564 | 1.54 | 423 |
| 41 | 147 | 397 | 2.23 | 0.56 | 145 | 572 | 1.52 | 590 |
| 43 | 122 | 418 | 1.75 | 0.73 | 95 | 736 | 0.77 | 470 |
| Mean | 151 | 483 | 2.09 | 0.65 | 135** | 642***+++ | 1.44***+++ | 440 |
| ±S.D. | 23.3 | 156 | 0.81 | 0.06 | 29 | 214.5 | 0.68 | 131 |

*Symbols.* Values marked by *, **, or *** are different statistically from the preinfusion value are different statistically from parallel readings obtained in the control experiments to reach statis

with the preinfusion values and the parallel readings of the control experiments. The average of 483 ml/min/100 g obtained in the preinfusion periods increased to 642 ml/min/100 g. Renal vascular resistance was 2.09 R/kg in the preinfusion periods and this diminished to 1.44 R/kg by the end of the experiment. $E_{PAH}$ was significantly depressed, from an average of 0.65 to 0.52.

As in the preinfusion periods not all parameters could be determined, of considerable value is the comparison between parallel readings of the control group and the group receiving albumin; the $^{86}Rb$ method did not allow to measure nutritive blood flow in the preinfusion periods. $RBF_{Dir}$ attained 365 ml/min/100 g at the end of the control experiments, whereas in the experimental group an average of 642 ml/min/100 g was obtained after the infusion of albumin. Nutritive blood flow was 342 ml/min/100 g at the end of the control experiments, and 440 ml/min/100 g in the parallel series, after the infusion of albumin. Thus, in the control group, the difference between total blood flow and nutritive blood flow was small (23 ml), whereas in the dogs receiving albu-

*animals received 10% mannitol in physiological saline, at a rate of 10 ml/ at a rate of 10 ml/ min; during the exmin, until 3 g/kg of albumin had been introduced*

dilution of $^{86}Rb$

| Resistance ($R_{Rb}$/kg) | $\frac{RBF_{Rb}}{RBF_{Dir}}$ | Cortical flow ($CBF_{Rb}$) ml/min/100 g | | Medullary flow ($MBF_{Rb}$) ml/min/100 g | | $\frac{CBF/100\ g}{RBF_{Dir}/100\ g}$ | $E_{PAH}$ |
|---|---|---|---|---|---|---|---|
| | | cortex | kidney | medulla | kidney | | |
| 2.55 | 0.48 | 497 | 318 | 362 | 116 | 0.36 | 0.40 |
| 2.64 | 0.91 | 385 | 246 | 232 | 74 | 0.70 | 0.55 |
| 1.82 | 0.61 | 585 | 373 | 506 | 162 | 0.42 | 0.59 |
| 3.12 | 1.01 | 294 | 188 | 237 | 76 | 0.71 | 0.59 |
| 1.38 | 0.77 | 544 | 347 | 416 | 133 | 0.56 | 0.56 |
| 0.99 | 0.67 | 785 | 501 | 435 | 139 | 0.53 | 0.60 |
| 1.93 | 0.57 | 411 | 263 | 222 | 71 | 0.45 | 0.37 |
| 3.40 | 0.50 | 364 | 237 | 267 | 85 | 0.37 | 0.50 |
| 2.43 | 0.64 | 338 | 216 | 194 | 62 | 0.50 | 0.36 |
| 1.25 | 0.75 | 810 | 516 | 347 | 111 | 0.62 | 0.61 |
| 2.06 | 0.75 | 485 | 309 | 356 | 114 | 0.55 | 0.41 |
| 1.47 | 1.03 | 755 | 481 | 341 | 109 | 0.84 | 0.60 |
| 1.21 | 0.64 | 572 | 365 | 328 | 105 | 0.50 | 0.60 |
| 2.02+ | 0.72+ | 525+ | 327+ | 326 | 104 | 0.53+ | 0.52** |
| 0.76 | 0.18 | 172.5 | 109.8 | 93 | 29.8 | 0.14 | 0.10 |

at the 0.05, 0.01 and 0.001 levels, respectively. Values marked by +, ++, or +++ (Table I) at the 0.05, 0.01 and 0.001 levels, respectively. The other values failed tical significance

min, direct blood flow exhibited a more pronounced increase in response to albumin than did nutritive blood flow, the former increasing to 642 ml/min/100 g, and the latter to 440 ml/min/100 g; thus the difference between the two values was 202 ml. In the control group, 71.6% of the nutritive blood flow perfused the cortex, and 28.4% of it the medulla, whereas in the experimental group the corresponding values were 74.3% and 25.7%. Following the infusion of albumin, cortical nutritive blood flow ($CBF_{Rb}$) was increased while medullary flow ($MBF_{Rb}$) remained unchanged, as compared to the control series. The ratio of cortical and total blood flow ($CBF_{Rb}/RBF_{Dir}$) was in satisfactory agreement with $E_{PAH}$ both in the control group and after the infusion of albumin. Haematocrit in the control group decreased from 33 to 31%, whereas in the albumin group it was reduced from 34 to 28%; these changes were obviously due to hydraemia caused by the experimental procedure. Urine flow was not consistently altered in the control group, however in the experimental group it was diminished from an average of 8.7 ml/min/100 g to 4.7 ml/min/100 g.

## Discussion

The present experiments show that "inert" blood flow in the kidney is increased by the infusion of albumin from 5.0% to 28.0%. Considering that the values for nutritive blood flow ($RBF_{Rb}$) found in the control and experimental groups were not statistically different, the excess in flow ($RBF_{Dir} - RBF_{Rb}$) found in the animals receiving albumin was most likely due to the opening of vascular by-passes. Thus, the present experiments seem to have furnished functional evidence of the existence of shunts in the kidney during the intravenous infusion of serum albumin.

Similar results were obtained by HÁRSING et al. (1964) under different experimental conditions. In this study, "inert" blood flow was 9.1% in the control experiments and this was increased to 34.5% by the administration of acetylcholine into the renal artery. Renal blood flow was measured by cannulation of the renal vein, and parenchymal blood flow was determined by estimating $C_{PAH}$. Acetylcholine did not reduce parenchymal blood flow, thus, the increase in "inert" blood flow in other words the by-pass was due to the excess in total blood flow. In the same experiments, the effect of papaverine was also tested. Total direct blood flow was increased but $C_{PAH}$ was diminished and the possibility could not be ruled out that the changes were due to the deteriorating effect of papaverine on tubular transport. In a subsequent study, HÁRSING et al. (1967) succeeded in demonstrating that under stop-flow conditions the ratio of cortical blood flow to total renal blood flow was diminished from 0.78 to 0.48, and this was associated with a decrease of $E_{PAH}$ from 0.75 to 0.52; thus, the changes elicited by ureteric occlusion were similar to those observed in the present experiments in response to albumin. The above experiments of HÁRSING et al. (1967) too, were interpreted by assuming that part of the blood flowing into the kidney was diverted through vascular shunts from the functioning parenchyma.

Similar conclusions were drawn by JIRKA et al. (1961) who found a discrepancy between the values of blood flow obtained by direct measurement and by cineradiography in haemorrhagic hypotension. On the basis of experiments employing erythrocytes labelled with $^{51}Cr$ and with $^{131}I$ serum albumin, SHALDON et al. (1963) also concluded to the opening of shunts in the kidney. In contrast to these observations, MUNCK et al. (1962) found a close correlation between the oxygen saturation of blood derived from the renal cortex and renal venous blood in haemorrhagic hypotension. Obviously, this finding is irreconcilable with the opening of arterio-venous shunts in the kidney.

The present results, together with the earlier findings of HÁRSING et al. (1967) point to the possibility of some of the blood flow by-passing the functioning parenchyma. It is believed that functional evidence of the opening of vascular shunts can be obtained only in model experiments (albumin

infusion, administration of acetylcholine or papaverine, stop-flow), and it is questionable whether shunts can be demonstrated functionally in shock due to trauma, tourniquet, haemorrhage or dehydration. The method employed in the present experiments seems to be suitable for the approach of this problem, and further studies are in progress by us to reveal arterio-venous connections under different experimental conditions.

It should be noted that $E_{PAH}$ was somewhat lower in the preinfusion periods, in both the control and the experimental series, than the values obtained by other authors under similar circumstances. The reason for this is unknown; it is to be emphasized, however, that, similarly to earlier studies of HÁRSING and BARTHA (1966), the ratio of cortical to total blood flow ($CBF_{Rt}/RBF_{Dir}$) was in satisfactory agreement with the values for $E_{PAH}$.

## LITERATURE


BRATTON, A. E., MARSHALL, E. K.: A new coupling component for sulfanylamide determination. J. biol. Chem. **1939,** 128: 537—550.

CARGILL, W. H.: Effect of the intravenous administration of human serum albumin on renal function. Proc. Soc. exp. Biol. (N. Y.) **1948,** 68: 189—192.

CLARA, M.: Die arteriovenösen Anastomosen. 2. ed. Springer Verlag, Wien **1956.**

DANIEL, P. M., PEABODY, C. N., PRICHARD, M. L.: Cortical ischaemia of the kidney with maintained blood flow through the medulla. Quart. J. exp. Physiol. **1952,** 37: 11—18.

ELPERS, M. J., SELKURT, E. E.: Effect of albumin infusions on renal function in the dog. Amer. J. Physiol. **1963,** 205: 153—161.

GÖMÖRI, P., MUNKÁCSI, I., NAGY, Z., TAKÁCS, L., KÁLLAY, K.: Ischaemia and arterio-venous anastomoses of the kidney in shock, haemorrhage, dehydration and arterial hypoxia in dogs. Acta med. Acad. Sci. hung. **1962,** 18: 119—125.

GÖMÖRI, P., NAGY, Z., JAKAB, I.: The problem of the arterio-venous anastomoses in the kidney. I. Renal extraction of PAH and $O_2$ in dehydration, haemorrhagic hypotension and arterial hypoxia. Acta med. Acad. Sci. hung. **1964a,** 20: 153—158.

GÖMÖRI, P., KÁLLAY, K., NAGY, Z., SZABÓ, Z.: The problem of the arterio-venous anastomoses of the kidney. II. Effect of human serum albumin and dihydralazine on the opening of renal shunts. Acta med. Acad. Sci. hung. **1964b,** 20: 159—168.

GÖMÖRI, P., ZOLNAI, B., NAGY, Z., JAKAB, I., MÉSZÁROS, A.: The problem of renal ischaemia and of the arterio-venous anastomoses of the kidney. III. New corrosion studies in dehydration, haemorrhagic, traumatic, and ischaemic shock, arterial hypoxia and after serum albumin treatment. Acta med. Acad. Sci. hung. **1964c,** 20: 169—183.

GÖMÖRI, P., NAGY, Z., ZOLNAI, B., JAKAB, I., MÉSZÁROS, A.: The problem of the arterio-venous anastomoses of the kidney. IV. Extraction of PAH and $O_2$ and corrosion studies in the denervated kidney in traumatic shock and following human serum albumin administration. Acta med. Acad. Sci. hung. **1965,** 21: 197—203.

HÁRSING, L., KÖVÉR, GY., MÁLYUSZ, M., TÓTH, G., HARZA, T.: Die Wirkung von vasodliatorischen Substanzen (Papaverin, Acetylcholin) auf die Nierenfunktion. Pflügers Arch. ges. Physiol. **1964,** 281: 346—355.

HÁRSING, L., PELLEY, K.: Die Bestimmung der Nierendurchblutung auf Grund der Ablagerung und Verteilung von $^{86}Rb$. Pflügers Arch. ges. Physiol. **1965,** 285: 302—312.

HÁRSING, L., BARTHA, J.: Renal blood flow and p-aminohippurate extraction in osmotic diuresis. Acta physiol. Acad. Sci. hung. **1966,** 30: 225—232.

HÁRSING, L., BARTHA, J., HARZA, T., PELLEY, K.: Sodium and potassium concentration and blood flow in the renal cortex and medulla during different diuretic responses. Acta physiol. Acad. Sci. hung. **1966,** 30: 215—224.

HÁRSING, L., SZÁNTÓ, H., BARTHA, J.: Renal circulation during stop flow in the dog. Amer. J. Physiol. **1967,** 213: 935—938.

JIRKA, J., GANZ, V., FENCL, V., CORT, J. H., TRÁVNICEK, R.: Measurement of renal blood flow in the intact kidney by local thermodilution during haemorrhagic hypotension. Lancet **1961,** 2: 692—693.

MUNCK, O., LASSEN, N. A., DEETJEN, P., KRAMER, K.: Evidence against renal hypoxia in acute haemorrhagic shock. Pflügers Arch. ges. Physiol. **1962,** 274: 356—363.

MUNKÁCSI, I.: Über die Verwendung der Kunststoffe Polyvinylchlorid (PVC) und Piacryl in der Korrosionstechnik. Z. wiss. Mikr. **1957,** 63: 352—356.

MUNKÁCSI, I., NAGY, Z., TAKÁCS, L., KÁLLAY, K., GÖMÖRI, P.: Vascular shunts in the renal cortex of the dog. Rev. Canad. Biol. **1963,** 22: 353—363.

SAPIRSTEIN, L. A.: Fractionation of the cardiac output of rats with isotopic potassium. Circulat. Res. **1956,** 4: 689—692.

SAPIRSTEIN, L. A.: Regional blood flow by fractional distribution of indicators. Amer. J. Physiol. **1958,** 193: 161—168.

SHALDON, S., SILVA, H., LAWSON, T. R., WALKER, J. G.: Measurement of renal red cell and plasma transit times in acute renal failure. Proc. Soc. exp. Biol. (N. Y.) **1963,** 112: 359—361.

SIEGELMAN, S. S., GOLDMAN, A. G.: The Trueta phenomenon. Angiographic documentation in man. Radiology **1968,** 90: 1084—1089.

SPANNER, R.: Die Abkürzungskreislauf der menschlichen Niere. Beitrag zur Kenntnis der Leistungszweiteilung ihres Gefäss-systems. Klin. Wschr. **1937,** 16: 1421—1423.

SPANNER, R.: Über Gefässkurzschlusse in der Niere. Verh. anat. Ges. **1937—38,** 45: 81—90.

TRUETA, J., BARCLAY, A. E., DANIEL, P., FRANKLIN, K. J., PRICHARD, M.: Studies of the renal circulation. 2nd ed. Blackwell, Oxford **1948.**

Pál GÖMÖRI, Kálmán KÁLLAY, Lóránd DEBRECZENI
Orvostudományi Egyetem II. sz. Belklinikája
Budapest VIII., Szentkirályi u. 46.

László HÁRSING, Jenő BARTHA
Orvostudományi Egyetem Élettani Intézete
Budapest VIII., Puskin u. 9.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 211—218 (1969)*

# PRESSOR ACTIVITY OF RENAL VENOUS BLOOD IN ANAESTHETIZED DOGS

By

L. TAKÁCS, L. DEBRECZENI and I. LULITY

SECOND DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received September 17, 1968)

An increase in the pressor activity of untreated renal venous plasma following the reduction of renal blood flow (to about 20 per cent of the control value) has been demonstrated in anaesthetized dogs. The phenomenon is not due to hemodynamic changes (concentration) but is the result of an increase in the production of pressor substances.

In the diagnosis of renovascular hypertension it is essential to demonstrate morphologically the stenosis and to clarify the relationship between hypertension and stenosis. This is usually done by estimating the renin or angiotensin level in renal venous blood. The methods are more or less cumbersome and all of them are based on the bio-assay of angiotensin generated during incubation. However, the actual pressor content of renal venous blood has not been determined and the presence of other pressor or depressor substances has not been taken into consideration, either.

McPHAUL et al. (1964, 1965, 1966) have shown that the direct determination of the pressor activity of renal venous blood/plasma is a reliable method for the appraisal of a "significant" stenosis of the renal artery, and the pressor activity is in good correlation with the results of surgery.

In the present studies in acute dog experiments, under standard conditions the influence of a significant decrease in renal blood flow (RBF) on the pressor activity of renal venous blood has been investigated.

## Methods

*Kidney experiments.* Thirty-three mongrel dogs of either sex, weighing 9 to 15 kg, were used. Under chloralose anaesthesia (0.10 g/kg body weight) the trachea was cannulated. Blood pressure was measured in the carotid artery with a mercury manometer. To replace the blood loss heparinized blood diluted to about one-half with Locke's solution was infused into the femoral vein. The left kidney was exposed by lumbar incision. After ligating the testicular (ovarian) vein a cannula having a T extension was inserted into the renal vein, directing the blood to the jugular vein. The T extension allowed direct measurement of renal blood flow (RBF) (SELKURT 1946; BÁLINT 1965).

In the control experiments RBF was measured after cannulation, and after waiting for 20 to 30 minutes again. Blood samples were taken from the renal artery (A), from the renal vein ($V_1$), then 30 minutes later again from the renal vein ($V_3$).

After having obtained the A and $V_1$ samples a clamp was placed on the renal artery to study the pressor substance production, and flow was reduced to 1/4—1/5 in about 2 to

**Table I**

*Pressor substance production in control kidney*

DOG (n = 5)

| No. | Body weight Kg | Blood pressure mm Hg | | Direct renal blood flow ml/min | | |
|---|---|---|---|---|---|---|
| | | $V_1$ | $V_3$ | $V_1$ | $V_2$ | $V_3$ |
| 49 | 11.0 | 150 | 145 | 111 | — | 99 |
| 50 | 11.0 | 150 | 150 | 123 | — | 102 |
| 51 | 10.0 | 120 | 110 | 136 | — | 96 |
| 52 | 16.0 | 120 | 110 | 96 | — | 81 |
| 53 | 13.5 | 130 | 115 | 68 | — | 56 |
| $\bar{x}$ | 12.3 | 136 | 126 | 106.8 | — | 86.8 |
| S.D. | ±2.4 | ±15.3 | ±19.8 | ±26.2 | — | ±19.0 |

RAT (n = 20)

| No. | A mm Hg | $V_1$ mm Hg | $V_2$ mm Hg | $V_3$ mm Hg | Basal blood pressure, mm Hg | |
|---|---|---|---|---|---|---|
| | | | | | onset of titration | end of titration |
| 49 | 30.5 ± 9.2 | 33.7 ± 7.4 | — | 36.0 ± 5.9 | 75.7 ± 6.5 | 76.2 ± 3.0 |
| 50 | 26.5 ± 3.8 | 29.7 ± 3.1 | — | 42.0 ± 7.5 | 52.5 ± 10.0 | 60.2 ± 17.1 |
| 51 | 16.7 ± 7.7 | 18.0 ± 2.8 | — | 28.0 ± 13.3 | 62.0 ± 10.1 | 69.0 ± 17.3 |
| 52 | 24.5 ± 10.0 | 25.0 ± 13.2 | — | 23.5 ± 9.8 | 55.2 ± 16.2 | 62.2 ± 21.2 |
| 53 | 29.5 ± 10.2 | 41.0 ± 10.1 | — | 35.5 ± 7.9 | 63.7 ± 11.7 | 74.5 ± 17.6 |
| $\bar{x}$ | 25.5 | 29.5 | — | 33.0 | 61.9 | 68.4 |
| S.D. | ±9.6 | ±10.9 | — | ±10.6 | ±13.1 | ±16.0 |

*Symbols:*

A = femoral arterial plasma
$V_1$ = control renal (femoral) venous plasma
$V_2$ = renal or femoral venous plasma obtained immediately after reduction of arterial inflow
$V_3$ = renal or femoral venous plasma obtained 30 minutes after reduction of arterial inflow
The plasma samples were titrated in 0.5 ml volumes

3 minutes. A renal venous blood sample was taken immediately afterwards ($V_2$) and 30 minutes after the onset of flow reduction ($V_3$). Flow was controlled at 10-minute intervals.

*Hind limb experiments.* In six dogs anaesthetized with chloralose (0.08 g/kg body weight) and morphine (0.02 g/dog), at the hip joint every muscle, nerve and blood vessel was ligated and cut. The hind limb so isolated remained in contact with the trunk exclusively through the femur; supplied with blood from the cannulated femoral artery and vein. Blood samples from the femoral vein were taken at the same intervals and in the same sequence as in the renal experiments.

**Table II**

*Pressor substance production in kidney with reduced renal blood flow*

DOG (n = 9)

| No. | Body weight Kg | Blood pressure mm Hg | | Direct renal blood flow ml/min | | |
|---|---|---|---|---|---|---|
| | | $V_1$ | $V_3$ | $V_1$ | $V_2$ | $V_3$ |
| 27 | 12.5 | 130 | 120 | 118 | 10 | 40 |
| 28 | 12.0 | 140 | 110 | 150 | 28 | 22 |
| 29 | 11.0 | 140 | 100 | 72 | 8 | 9 |
| 31 | 13.0 | 150 | 140 | 104 | 22 | 20 |
| 32 | 14.0 | 180 | 150 | 114 | 20 | 9 |
| 34 | 12.0 | 170 | 145 | 156 | 21 | 12 |
| 35 | 12.0 | 160 | 160 | 88 | 15 | 10 |
| 36 | 12.0 | 150 | 140 | 90 | 20 | 16 |
| 37 | 12.0 | 160 | 140 | 123 | 27 | 31 |
| $\bar{x}$ | 12.2 | 153 | 134 | 113.0 | 19.0 | 18.8 |
| S.D. | ±0.9 | ±15.8 | ±19.6 | ±27.9 | ±6.8 | ±10.7 |

RAT (n = 36)

| No. | A mm Hg | $V_1$ mm Hg | $V_2$ mm Hg | $V_3$ mm Hg | Basal blood pressure, mm Hg | |
|---|---|---|---|---|---|---|
| | | | | | onset of titration | end of titration |
| 27 | 22.5 ± 6.6 | 32.0 ± 11.9 | 41.0 ± 12.3 | 45.0 ± 13.1 | 76.2 ± 4.8 | 81.7 ± 10.0 |
| 28 | 18.5 ± 4.4 | 22.5 ± 3.4 | 26.5 ± 3.4 | 35.0 ± 6.1 | 65.0 ± 0.0 | 67.5 ± 9.6 |
| 29 | 33.5 ± 3.0 | 42.5 ± 6.7 | 48.5 ± 7.2 | 56.5 ± 15.9 | 66.7 ± 6.5 | 75.7 ± 8.8 |
| 31 | 24.0 ± 3.6 | 30.0 ± 10.7 | 30.0 ± 10.4 | 44.5 ± 2.5 | 65.0 ± 12.9 | 82.5 ± 8.7 |
| 32 | 28.0 ± 4.9 | 31.5 ± 7.7 | 34.5 ± 10.6 | 58.5 ± 5.7 | 65.5 ± 14.3 | 68.7 ± 16.7 |
| 34 | 27.0 ± 8.9 | 26.5 ± 7.0 | 39.5 ± 4.7 | 48.5 ± 14.4 | 66.2 ± 11.8 | 85.0 ± 9.1 |
| 35 | 15.5 ± 7.7 | 16.5 ± 7.0 | 27.5 ± 11.7 | 58.5 ± 31.5 | 65.0 ± 12.2 | 80.0 ± 20.8 |
| 36 | 29.0 ± 12.5 | 43.5 ± 14.2 | 48.5 ± 19.9 | 64.0 ± 11.8 | 72.5 ± 5.0 | 80.0 ± 7.1 |
| 37 | 19.7 ± 5.2 | 19.7 ± 5.0 | 23.2 ± 3.2 | 35.0 ± 7.4 | 73.0 ± 4.0 | 76.0 ± 7.4 |
| $\bar{x}$ | 24.2 | 29.4 | 35.5 | 49.5 | 68.4 | 77.2 |
| S.D. | ±8.2 | ±11.7 | ±12.9 | ±16.3 | ±9.1 | ±12.2 |

*Preparation of samples.* The blood samples were cooled and immediately centrifuged at 4 °C at 5000×g for 10 minutes in a 17″ MSE Type MK2 refrigerator centrifuge. One ml samples of plasma were added into the pre-cooled dishes and these were placed in a deep-freezer. The plasma samples were thawed after 1 to 3 days at room temperature and tested for pressor activity.

*Bioassay.* In 127 male rats of our own stock, weighing 150 to 180 g, the kidneys were removed under ether anaesthesia. After fasting for 18 to 24 hours the animals were anaesthetized with 0.60 g/kg body weight urethane subcutaneously, then were treated subcutaneously with 15 mg/kg of 0.5 per cent chlorisondamine chloride (Ecolid, CIBA), or with 2.5 mg/kg

hexamethonium and 0.5 mg/kg of 0.05 per cent atropine. The volume of the ganglionic blocking agents was adjusted to 1.0 ml with 5 per cent polyvinylpyrrolidone solution. About 40 to 60 minutes following the administration of drugs, bilateral vagotomy was performed and the trachea was cannulated. Blood pressure was measured in the carotid artery with a mercury manometer and was recorded on smoked paper, using a Zimmermann kymograph. A thin polyethylene tube was inserted into the left femoral vein to ensure rapid and reliable injection of the substances to be titrated.

The plasma samples were injected in 0.5 ml volume and were washed in with 0.1 ml of Locke's solution. As required by combination of treatment the samples were usually titrated in 4 rats.

*Evaluation.* The elevation of blood pressure by the arterial (A) and venous ($V_1$, $V_2$, $V_3$) plasmas administered in varying order was considered the measure of pressor activity.

Experience has shown that in one rat only 4 plasma samples may be titrated, because basal blood pressure gradually increased with the administration of each sample.

Plasma pressor activity was characterized by the mean of the delta $p$ values and by the standard deviation; the results were evaluated by the Student's $t$ test.

## Results

### A) *Effect of the reduction of RBF on the pressor activity of renal venous plasma*

1. It has been studied how plasma pressor activity changed under the effect of spontaneous variations in RBF (under control conditions) (Table I). At 30 minutes, sample $V_3$ — with the RBF unchanged ($p > 0.10$) — had a higher pressor effect than the arterial control (A) ($p < 0.05$), but did not differ from sample $V_1$, as opposed to the data in Table II.

2. In preliminary experiments RBF was reduced in 11 dogs to an average of 18.5 per cent of the initial value and was kept at that level for 30 minutes. The plasma samples were tested in 34 rats. The pressor responses were: to

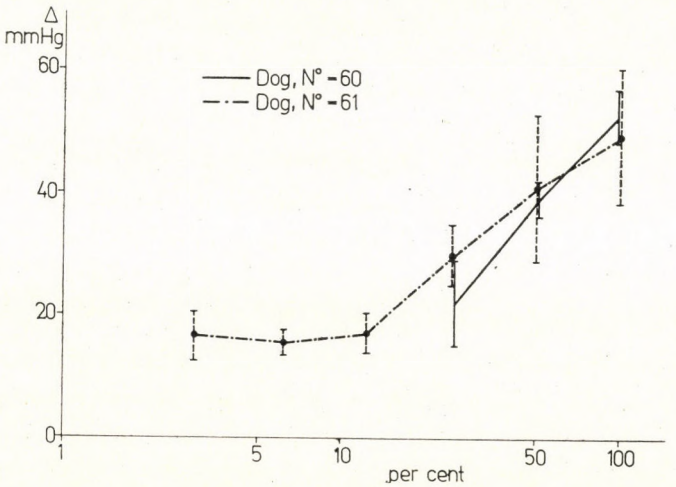



*Fig. 1.* Log dose-response curve. Abscissa: logarithm of percentage plasma concentration. Ordinate: △ blood pressure ± S.D. The samples were administered in 0.5 ml volume

arterial sample (A) 26.8±9.2 mm Hg; to the control renal venous plasma ($V_1$) 30.5±9.9 mm Hg; 30 minutes after the reduction of RBF, to sample $V_3$ 44.0±12.8 mm Hg. The differences were significant statistically between samples A—$V_3$ and $V_1$—$V_3$ ($p < 0.001$).

3. The series of experiments was repeated with the difference that a renal venous blood sample ($V_2$) was obtained immediately after the reduction of RBF. As shown in Table II, under control conditions there was a difference in pressor response between the arterial (A) and renal venous ($V_1$) samples ($p < 0.05$). The difference increased immediately on the reduction of RBF ($V_2$) and was highly significantly augmented at 30 minutes ($V_3$). The differences

**Table III**

*Pressor substance production in the isolated hind limb*

DOG (n = 6)

| No. | Body weight Kg | Blood pressure mm Hg | | Blood flow ml/min | | |
|---|---|---|---|---|---|---|
| | | $V_1$ | $V_3$ | $V_1$ | $V_2$ | $V_3$ |
| 42 | 11.0 | 130 | 115 | 67 | 10 | 12 |
| 43 | 11.0 | 115 | 80 | 36 | 9 | 4 |
| 45 | 11.0 | 140 | 130 | 87 | 11 | 8 |
| 46 | 12.5 | 135 | 115 | 66 | 12 | 10 |
| 47 | 18.0 | 120 | 110 | 54 | 12 | 18 |
| 48 | 11.5 | 115 | 95 | 45 | 6 | 8 |
| $\bar{x}$ | 12.5 | 126 | 108 | 59 | 9.9 | 10.1 |
| S.D. | ±2.8 | ±10.7 | ±17.6 | ±18.0 | ±2.3 | ±4.6 |

RAT (n = 23)

| No. | A mm Hg | $V_1$ mm Hg | $V_2$ mm Hg | $V_3$ mm Hg | Basal blood pressure, mm Hg | |
|---|---|---|---|---|---|---|
| | | | | | onset of titration | end of titration |
| 42 | 23.5 ± 6.6 | 23.5 ± 2.5 | 23.0 ± 4.8 | 26.7 ± 3.6 | 70.2 ± 3.8 | 70.5 ± 13.8 |
| 43 | 24.6 ± 2.9 | 26.7 ± 13.6 | 24.0 ± 3.5 | 26.7 ± 12.0 | 63.3 ± 20.2 | 73.3 ± 16.1 |
| 45 | 23.7 ± 6.6 | 26.2 ± 6.6 | 27.5 ± 11.7 | 28.7 ± 8.8 | 63.2 ± 4.7 | 65.0 ± 7.5 |
| 46 | 24.7 ± 7.3 | 26.5 ± 9.3 | 25.5 ± 8.4 | 34.0 ± 11.2 | 58.0 ± 15.9 | 85.7 ± 17.5 |
| 47 | 29.7 ± 5.6 | 26.2 ± 4.4 | 26.2 ± 3.9 | 28.0 ± 5.2 | 70.2 ± 6.6 | 74.0 ± 10.5 |
| 48 | 44.7 ± 6.2 | 38.0 ± 14.9 | 44.7 ± 4.1 | 34.7 ± 16.7 | 53.5 ± 15.1 | 71.0 ± 8.5 |
| $\bar{x}$ | 28.7 | 27.9 | 28.7 | 29.9 | 63.1 | 73.0 |
| S.D. | ±9.5 | -±9.6 | ±9.5 | ±9.8 | ±12.4 | ±12.9 |

between A$-V_2$, A$-V_3$ ($p < 0.001$), $V_1-V_2$ ($p < 0.05$), $V_1-V_3$ and $V_2-V_3$ ($p < 0.001$) were significant.

4. After 30 minutes of reduced RBF, renal venous plasma from 2 dogs was diluted with Locke's solution to 6.25, 12.5, 25.0 and 50 per cent dilutions. Using these and whole plasma, dose-response curves were plotted in several rats. In Fig. 1 the pressor responses to the different plasma dilutions are characterized by the mean of the values and their standard deviations measured in the rats (in the case of dog No. 60 titration was done in six rats, in that of dog No. 61, in eight rats). In the linear segment the common slope of the two curves was calculated by determining the mean and the standard deviation of the slopes measured in each rat between two doses. The logarithm of the samples containing 12.5 per cent or more plasma showed good linear correlation with the increase of pressor activity revealing at the same time the presence of vasoactive substances in proportion with the plasma content.

In the linear segments the slopes of the two curves were $44.6 \pm 21.8$.

*B)* To decide whether in response to the RBF reduction, pressor substance production was induced only in the kidney, in six dogs left hind limb flow was significantly reduced and the pressor responses to the plasma samples were compared (Table III). There was no significant difference between the pressor responses to the different plasma samples.

## Discussion

A reduction of RBF for 30 minutes (to about 20 per cent the initial value) was found to increase the pressor activity of renal venous plasma. As the control arterial plasma also had pressor activity (Lewis 1960; Lustinec 1965), the venous samples were always compared with the pressor responses to arterial plasma. Reduction of flow in the hind limb produced no similar effect.

In 2 dogs an attempt was made to determine the quantity of pressor substance from the log-dose-response diagram plotted from renal venous plasmas after 30 minutes of reduced RBF (Fig. 1). The common slope was in this case 44.6 in the linear segment of the curve. As seen in Table II, the control renal venous plasma ($V_1$) induced a $29.4 \pm 11.7$ mm Hg increase in the test animal's blood pressure while after 30 minutes of RBF reduction renal venous plasma ($V_3$) induced a $49.5 \pm 16.3$ mm Hg increase. On the basis of the slope (assuming that the pressor response to $V_3$ falls to the linear part of the curve) this means that 0.5 ml of the $V_3$ plasma was equipotent with 5.6 ml of the control renal venous plasma. Calculating in a similar way, the renal venous plasma obtained immediately after the reduction of RBF ($V_2$) in a volume of 0.5 ml produces the same effect as 2.7 ml of the control renal venous plasma ($V_1$). This value (2.7 : 0.5) means a 5.4-fold concentration of the active agent,

which corresponds to the ratio of flow reduction (5.9) and thus may be caused by haemodynamic changes (concentration). As opposed to this, the renal venous plasma $V_3$ contained 11.3 times as much pressor substance as the control ($V_1$), and 2.0 times as much as plasma $V_2$.

Several vasoactive agents have been demonstrated in the kidney. The pressors are the renin-angiotensin system (GOLDBLATT et al. 1934; GOLDBLATT 1964; PAGE and MCCUBBIN 1965; VANDER 1967), as well as the non-identified pressor substance of KHAIRALLAH and PAGE (1965); the depressors are the medullary extracts (MUIRHEAD et al. 1960; TOBIAN et al. 1964; LEE et al. 1965; STRONG et al. 1966). ROBLERO and CROXATTO (1968) demonstrated in the incubated dog and rat plasma two pressor substances, the activity of which decreased following nephrectomy. Both proved to be non-dialysable proteins, evoking a pressor response in the nephrectomized rat, but differing in other properties.

In our experiments the nature of the pressor substance has not been studied. MCPHAUL et al. (1966) holds unlikely that the directly measured increased pressor activity of the renal venous plasma from patients suffering from renovascular hypertension would be due to an increase of the renin or angiotensin level. On the other hand, EBIHARA and GROLLMAN (1968) concluded in dog experiments that the pressor agent cannot be distinguished from angiotensin.

Ultimately, in our model experiments the observations of MCPHAUL et al. (1966) in renovascular hypertension that were confirmed recently in dog experiments by EBIHARA and GROLLMAN (1968) could readily be reproduced. For diagnostic purposes the assay of untreated plasma pressor activity presents advantages, being easy to carry out and, not being restricted to one compound, it may be used for the determination of the resultant of several pressor and depressor substances.

## Acknowledgements

We are indebted to Mrs. V. VAJDA and Mr. A. KARAI for the skilful technical assistance.

## LITERATURE

BÁLINT, P.: Nierenclearance. Technik, Bewertung, Ergebnisse in Klinik und Experiment. G. Fischer, Jena **1965.**

EBIHARA, A., GROLLMAN, A.: Pressor activity of renal venous effluent following constriction of renal artery in dogs. Amer. J. Physiol. **1968,** 214: 1—5.

GOLDBLATT, H., LYNCH, J., HANZAL, R. F., SUMMERVILLE, W. W.: Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J. exp. Med. **1934,** 59: 347—379.

GOLDBLATT, H.: Hypertension due to renal ischemia Bull. N. Y. Acad. Med. **1964,** 40: 745—747.

KHAIRALLAH, PH. A., PAGE, I. H.: A new vasopressor agent in blood extracts. Nature (Lond.) **1965,** 206: 835—836.

LEE, J. B., COVINI, B. C., TAKAMA, B. H., SMITH, E. R.: Renomedullary vasodepressor substance: medullin. Circulat. Res. **1965,** 17: 57—77.

LEWIS, G. P.: Active polypeptides derived from plasma proteins. Physiol. Rev. **1960,** 40: 647—676.

LUSTINEC, K.: Vasoconstrictor properties of blood in perfusion experiments. Physiol. bohemoslov. **1965,** 14: 583—593.

MCPHAUL, J. J., Jr., MCINTOSH, D. A., WILLIAMS, L. F., GRITTI, E. J., MALETTE, W. G., GROLLMAN, A.: Pressor activity of renal venous blood in hypertension. Ann. intern. Med. **1964,** 61: 847—848.

MCPHAUL, J. J., Jr., MCINTOSH, D. A., WILLIAMS, L. F., GRITTI, E. J., MALETTE, W. G., GROLLMAN, A.: Remediable hypertension due to unilateral renal disease: correlation of split function tests and pressor assays of renal venous blood. Arch. intern. Med. **1965,** 115: 644—646.

MCPHAUL, J. J., Jr., MCINTOSH, D. A., WILLIAMS, L. F., GRITTI, E. J., GROLLMAN, A.: Correlation of the pressor activity of the renal venous effluent with excretory function and other tests in focal, parenchymal and vascular renal disease. Circulation **1966,** 33: 781—788.

MUIRHEAD, E. E., JONES, F., STIRMAN, J. A.: Antihypertensive property in renoprival hypertension of extract from renal medulla. J. Lab. clin. Med. **1960,** 56: 167—180.

PAGE, I. H., MCCUBBIN, J. W.: The physiology of arterial hypertension. In (Eds): W. F. Hamilton and Ph. Dow: Handbook of Physiology, American Physiological Society, Washington, D. C. **1965,** Sect. 2. Vol. 3. Chapter 61. Pp. 2163—2208.

ROBLERO, J., CROXATTO, H.: Vasoactive substances formed in blood serum by incubation. N. Y. St. J. Med. **1968,** 68: 235—241.

SELKURT, E. E.: Comparison of renal clearances with direct renal blood flow under control conditions and following renal ischemia. Amer. J. Physiol. **1946,** 145: 376—386.

STRONG, C. G., BOUCHER, R., NOWACZYNSKI, W., GENEST, J.: Renal vasodepressor lipid. Proc. Mayo Clin. **1966,** 41: 433—452.

TOBIAN, L., SCHONNING, S., SEEFELDT, C.: The influence of arterial pressure on the antihypertensive action of a normal kidney, a biological servomechanism. Ann. intern. Med. **1964,** 60: 378—383.

VANDER, A. J.: Control of renin release. Physiol. Rev. **1967,** 47: 359—382.

Lajos TAKÁCS, Loránd DEBRECZENI, István LULITY
Orvostudományi Egyetem II. sz. Belklinikája
Budapest VIII., Hungary Szentkirályi u. 46

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 219—229 (1969)*

# THE EFFECT OF HYPERTONIC SODIUM CHLORIDE INFUSED INTO THE RENAL ARTERY

By

I. FORGÁCS, R. CHÂTEL and MÁRIA VISY

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received April 27, 1968)

In dogs maintained previously on a low-sodium diet, the infusion of 8.5% sodium chloride into the left renal artery at a rate sufficient to elevate the filtered load of sodium by about 50% caused depression of RBF (venous outflow) and GFR to a fraction of preinfusion levels, and in some cases to zero. The same amount of saline failed to affect the renal vascular tone in another group of dogs whose kidney had been depleted of renin by giving large amounts of sodium chloride for several days prior to the experiment. When, however, the rate of saline infusion was doubled vasoconstriction was observed also in these dogs. In both groups, the excretion of sodium on the left side increased to values many times the control readings; this was due to a substantial decrease in tubular reabsorption. The vascular changes in the contralateral kidney, as revealed by $C_{PAH}$ and $C_{creat}$, were insignificant, and the rise in sodium excretion was not greater than what could have been accounted for by the elevation of plasma sodium level.

It has been concluded that the marked vasoconstriction occurring in the kidney in response to the infusion of strongly hypertonic sodium chloride into the renal artery was due to the intrarenal liberation of renin, possibly as a result of the altered sodium content of the tubular fluid.

Evidence has been accumulated in the past years indicating that the renin-angiotensin system plays a key role in the regulation of sodium excretion and the maintenance of isovolaemia. There are at least three possibilities by which this system may regulate sodium and fluid balance, viz. (1) angiotensin affects directly the renal tubular handling of sodium (LEYSSAC et al. 1961; LAULER et al. 1963; PETERS 1963; VANDER 1963; TOBIAN 1964; LANGFORD 1964; GROSS et al. 1964; LEYSSAC 1964; 1965; BARRACLOUGH 1965; BARRACLOUGH et al. 1967); (2) the effect of the renin-angiotensin system on the tubular transport of sodium is mediated by aldosterone (KUHN et al. 1961; FRASER et al. 1965; GROSS et al. 1965; COHEN et al. 1967, etc.); (3) the renin-angiotensin system exerts its control on sodium excretion by vascular effects, i.e. through changes in the filtered load (BOCK et al. 1958; THURAU and SCHNERMANN 1965; CANNON et al. 1966; CORTNEY et al. 1966; HORSTER et al. 1966; SCHNERMANN et al. 1966; EIGLER 1967). On the other hand, it has been shown by numerous investigators that sodium depletion increases, whereas sodium loading diminishes, the renin stores of the kidney (GORDON and HARTROFT 1961; BROWN et al. 1964; TOBIAN 1964; BUNAG et al. 1966; VANDER and LUCIANO 1967).

The present experiments were devoted to the effects on renal haemodynamics of high amounts of hyperosmotic saline solution infused directly into the renal artery of dogs. In this way it was possible to ensure marked increases in the sodium concentration of blood plasma reaching the kidney, without considerably elevating the over-all sodium concentration. The extra-renal effects of sodium chloride loading were thus minimized, and the observed changes could be ascribed to local reactions.

## Methods

The experiments were performed on 19 male mongrel dogs weighing 15 to 25 kg. Nine dogs (Group A) received a diet restricted in sodium (approximately 1 g of NaCl per day) for 5 days prior to the experiment; free access to water was allowed. Ten dogs (Group B) were given a normal diet and water ad libitum, and 8 to 10 g of NaCl intravenously, as a hyperosmotic solution throughout 5 days preceding the experiment.

The experimental procedure was as follows. Under pentobarbital anaesthesia (30 mg/kg), the left renal pedicle was approached by an abdominal midline incision. The left renal vein was cannulated and connected with the external jugular vein by means of a siliconized polyethylene tube. Renal venous outflow was measured by a stop-watch and graduated cylinder through a T-extension of the tube, the latter serving also for withdrawal of renal venous blood samples, 10 ml each. A 18-gauge needle was inserted into the left renal artery about 0.5 cm below its origin from the aorta. Urine was collected through indwelling catheters introduced into the ureters.

Clearance substances (creatinine, PAH) were infused, after an appropriate priming dose, into the left femoral vein at a constant rate, to maintain concentrations of 15 to 20 mg per 100 ml for creatinine, and 1 to 2 mg per 100 ml of PAH. One femoral artery was cannulated for the collection of arterial blood samples, 10 ml each, and a mercury manometer was attached to the other femoral artery.

In Group A, after control measurements had been made, hyperosmotic (8.5%) sodium chloride was infused into the renal artery for 5 to 10 min at a rate sufficient to elevate plasma sodium concentration in the kidney by 50% (average rate of infusion: 6.4 mEq/min of Na).

The dogs previously loaded with sodium (Group B) received the same dose of intra-arterial sodium chloride; in this group, the infusion was thereafter repeated at a higher rate, delivering 12.8 mEq/min of Na to the left kidney.

The following parameters were measured and calculated in the control, infusion, and postinfusion periods. In the left kidney, renal blood and plasma flow ($RBF_{dir}$, $RPF_{dir}$), renal vascular resistance ($R_{ren}$), urine flow (V), clearance and extraction ratio of creatinine ($C_{creat}$, $E_{creat}$) and PAH ($C_{PAH}$, $E_{PAH}$), the amount of sodium filtered and excreted per min (filtered load, $U_{Na}V$), and the fraction of filtered sodium rejected by the tubules ($TRF_{Na}$). The same parameters, except RBF, RPF, $R_{ren}$ and extration ratios of creatinine and PAH, were determined in the right kidney. In addition, plasma sodium concentration and hematocrit value were repeatedly checked in arterial blood samples. All values referring to the kidneys were computed for 100 g of kidney weight, except for renal vascular resistance which was computed for 1 kg of kidney weight. For sake of convenience calculations were made with the reciprocal of resistance, i.e. conductance, which was expressed in $\frac{1}{R}$ units.

Creatinine in blood plasma and urine was determined by the technique described by BÁLINT and VISY (1965), and PAH was estimated by the method of SMITH et al. (1945). Sodium concentration was measured by flame photometry.

Statistical analysis of the results was undertaken by STUDENT's *t*-test. The differences were regarded as significant statistically if $P < 0.05$; the values obtained in the left, experimental kidney in the infusion and postinfusion periods were compared to control (preinfusion) values, and the differences (d) were evaluated. In addition, comparison was made between parallel readings obtained in the left (experimental) and right (control) kidney.

## Results

The results are shown in Tables I through IV. Control readings represent the average of two urine collection periods, and three renal venous outflow measurements. Values for P refer to the differences found between the infusion and postinfusion periods, and the respective control values.

Values for PAH and creatinine clearance in the left, cannulated kidney and in the contralateral, intact kidney were in satisfactory agreement in the control periods (Table I), indicating that the experimental procedure had not markedly affected the function of the left kidney. Parameters of renal function in both kidneys were consistent with the data of BÁLINT and FORGÁCS (1967) obtained under similar experimental conditions in normal dogs.

*The effect of hypertonic sodium chloride infusion in sodium-deficient dogs* (Table II). Elevation of the filtered load of sodium by 50% by infusing hypertonic sodium chloride into the renal artery had dramatic effects in the

**Table I**

*Statistical analysis of the values obtained in the left and right kidney in the control period in Groups A and B*

| | | Group A Sodium depletion $Mean_{left}$-$Mean_{right}$ | Group B Sodium loading $Mean_{left}$-$Mean_{right}$ |
|---|---|---|---|
| Urine flow | P< | 0.99 | 0.10 |
| $C_{creat}$ | P< | 0.99 | 0.80 |
| $U_{Na}V$ | P< | 0.30 | 0.40 |
| Filtered Na | P< | 0.60 | 0.70 |
| $TRF_{Na}$ | P< | 0.10 | 0.80 |

kidney, although the volume of saline solution was insignificant in comparison to the total amount of plasma reaching the organ. Five minutes after the infusion had been started, $RBF_{dir}$ fell to 35% of control readings, and to 14% in the next 5 min. In 3 dogs, blood flow completely ceased. As arterial blood pressure did not change significantly, conductance $\left(\frac{1}{R_{ren}}\right)$ also decreased to a fraction of control values. The extraction ratio of PAH was reduced from an average of 0.82 to 0.49. Creatinine clearance diminished to 45% of control readings. Urine flow did not decrease significantly.

The filtered load of sodium was calculated as the product of the plasma concentration in the left renal vein (the DONNAN factor was disregarded) and of $C_{creat}$. Owing to the substantial reduction of GFR in response to saline infusion, the filtered load of sodium was somewhat reduced despite the infusion

**Table II**

*The effects of infusion of 8.5% sodium chloride*

| | BP mm Hg | $RBF_{dir}$ ml/min | $RPF_{dir}$ ml/min | $1/R_{ren}$ | $E_{creat}$ | $E_{PAH}$ | $P_{Na}$ mEq/L |
|---|---|---|---|---|---|---|---|
| *Control* | | | | | | | |
| Mean | 122 | 346 | 200 | 0.53 | 0.19 | 0.82 | 133 |
| S.D. | 28 | 73 | 45 | 0.13 | 0.07 | 0.02 | 6 |
| n: 9 | | | | | | | |
| *Infusion* (5 min) | | | | | | | |
| Mean change ($\bar{d}$) | +3 | −226 | −135 | −0.35 | +0.03 | −0.12 | +10 |
| P< | 0.70 | 0.001 | 0.001 | 0.001 | 0.10 | 0.10 | 0.02 |
| n: 9 | | | | | | | |
| *Infusion* (10 min) | | | | | | | |
| Mean change ($\bar{d}$) | +1 | −297 | −172 | −0.47 | +0.02 | −0.33 | +11 |
| P< | 0.80 | 0.001 | 0.001 | 0.001 | 0.90 | 0.05 | 0.05 |
| n: 9 | | | | | | | |
| *Postinfusion* (5 min) | | | | | | | |
| Mean change ($\bar{d}$) | −10 | −253 | −158 | −0.36 | −0.11 | −0.42 | +11 |
| P< | 0.10 | 0.01 | 0.01 | 0.01 | 0.90 | 0.05 | 0.01 |
| n: 5 | | | | | | | |
| *Postinfusion* (10 min) | | | | | | | |
| Mean change ($\bar{d}$) | −25 | −229 | −129 | −0.27 | −0.11 | −0.30 | + 8 |
| P< | 0.03 | 0.005 | 0.005 | 0.005 | 0.90 | 0.02 | 0.03 |
| n: 5 | | | | | | | |
| *Postinfusion* (15 min) | | | | | | | |
| Mean change ($\bar{d}$) | −15 | −177 | − 96 | −0.15 | −0.07 | −0.19 | + 6 |
| P< | 0.40 | 0.01 | 0.001 | 0.05 | 0.05 | 0.05 | 0.02 |
| n: 5 | | | | | | | |

of hypertonic saline. Obviously, this was the net effect of saline infusion, and it should be borne in mind that the amount of filtered sodium must have substantially increased until in the kidney vasoconstriction had ensued which, according to random measurements of RBF during the infusion of the 8.5% saline solution, never occurred promptly. In face of diminished filtration, the marked (11-fold) increase of sodium excretion pointed to depressed tubular reabsorption, as reflected by the sharp (16-fold) increase in $TRF_{Na}$.

*into the left renal artery in sodium-depleted dogs*

| V ml/min | | $C_{creat}$ ml/min | | $U_{Na}V$ μEq/min | | Filtered Na mEq/min | | $TRF_{Na}$ % | |
|---|---|---|---|---|---|---|---|---|---|
| Left | Right | Left | Right | Left | Right | Left | Right | Left | Right |
| 1.61 | 1.63 | 44 | 45 | 14 | 9 | 5.78 | 6.13 | 0.24 | 0.15 |
| 0.58 | 0.52 | 16 | 15 | 13 | 8 | 2.39 | 2.06 | 0.19 | 0.10 |
| −0.39 | −0.04 | −24 | + 4 | +146 | +16 | −0.97 | +1.07 | + 3.60 | +0.29 |
| <0.30 | 0.90 | 0.01 | 0.30 | 0.05 | 0.20 | 0.40 | 0.05 | 0.01 | 0.10 |
| −0.98 | +0.02 | −43 | −11 | +126 | +26 | −5.14 | −1.01 | +14.82 | +0.40 |
| <0.001 | 0.70 | ⩽ 0.001 | 0.005 | 0.005 | 0.01 | ⩽0.001 | 0.02 | 0.005 | 0.001 |

In contrast, in the right kidney, $C_{PAH}$ and $C_{cr\cdot at}$ remained essentially unchanged. Alterations in the filtered and excreted sodium reflected a sodium diuresis, obviously due to the elevation of plasma sodium concentration.

In the postinfusion periods, blood flow in the left kidney was gradually restored to preinfusion levels, however, RBF did not exceed 50% of the control values until 15 min after the cessation of infusion. In the 3 dogs where a complete shutdown of renal blood flow was observed, the kidney remained ischaem-

**Table III**

*The effects of infusion of 8.5% sodium chloride*

| | BP mm Hg | $RBF_{dir}$ ml/min | $RPF_{dir}$ ml/min | $1/R_{ren}$ | $E_{creat}$ | $E_{PAH}$ | $P_{Na}$ mEq/L |
|---|---|---|---|---|---|---|---|
| *Control* | | | | | | | |
| Mean | 127 | 401 | 224 | 0.54 | 0.19 | 0.76 | 148 |
| S.D. | 23 | 110 | 19 | 0.18 | 0.03 | 0.06 | 13 |
| n: 10 | | | | | | | |
| *Infusion I* | | | | | | | |
| Mean change ($\bar{d}$) | +2 | − 73 | − 42 | −0.14 | +0.02 | −0.04 | + 6 |
| P< | 0.40 | 0.10 | 0.05 | 0.05 | 0.20 | 0.20 | 0.10 |
| n: 10 | | | | | | | |
| *Infusion II* | | | | | | | |
| Mean change ($\bar{d}$) | +5 | −261 | −134 | −0.35 | +0.13 | −0.10 | +13 |
| P< | 0.30 | 0.005 | 0.02 | 0.02 | 0.30 | 0.20 | 0.01 |
| n: 9 | | | | | | | |
| *Postinfusion* | | | | | | | |
| Mean change ($\bar{d}$) | 0 | −190 | −102 | −0.26 | −0.03 | −0.10 | + 8 |
| P< | 0.98 | 0.01 | 0.02 | 0.02 | 0.01 | 0.05 | 0.01 |
| n: 10 | | | | | | | |

ic despite that the infusion of hypertonic saline had been discontinued. Creatinine clearance and urine flow showed a further diminution even as compared to the infusion periods. The filtered load of sodium attained an average of as low as 1% of preinfusion value, while $TRF_{Na}$ exhibited a 63-fold increase. In the right kidney, the amount of filtered sodium decreased by 15% in face of a 30% reduction of $C_{creat}$; the excretion of sodium in the urine at the same time increased to values 4 times those observed in the control period.

*The effect of hypertonic sodium chloride infusion in sodium-loaded dogs* (Table III). Elevation of the filtered load of sodium by about 50% by infusing the same amount of hypertonic sodium chloride into the renal artery in Group A caused a slight but statistically not significant reduction of $RBF_{dir}$. Creatinine clearance somewhat increased, while the extraction ratio of PAH remained essentially unaltered. Both $TRF_{Na}$ and sodium excretion increased considerably.

When the rate of infusion of hypertonic sodium chloride was doubled (12.8 mEq/min of Na) to cause an about 100% elevation of the filtered sodium, RBF fell to 35% of the control value, and the clearance of creatinine was

*into the left renal artery in sodium-loaded dogs*

| V ml/min | | $C_{creat}$ ml/min | | $U_{Na}V$ μEq/min | | Filtered Na mEq/min | | $TRF_{Na}$ % | |
|---|---|---|---|---|---|---|---|---|---|
| Left | Right | Left | Right | Left | Right | Left | Right | Left | Right |
| 1.29 | 1.24 | 57 | 54 | 73 | 85 | 8.54 | 7.95 | 0.95 | 0.88 |
| 0.41 | 0.71 | 24 | 19 | 55 | 68 | 2.50 | 3.34 | 0.76 | 1.08 |
| +3.02 | +0.31 | +15 | + 8 | + 652 | + 39 | +5.01 | +3.70 | +4.22 | −0.02 |
| 0.005 | 0.02 | 0.10 | 0.05 | 0.01 | 0.20 | 0.005 | 0.30 | 0.005 | 0.99 |
| +3.54 | 1.13 | −18 | +11 | +1090 | +167 | +1.42 | +2.64 | + 7.50 | +0.97 |
| 0.10 | 0.02 | 0.10 | 0.50 | 0.01 | 0.05 | 0.60 | 0.20 | 0.005 | 0.05 |
| +0.74 | +0.98 | −26 | + 5 | + 390 | +167 | −3.74 | +1.72 | +11.90 | +1.56 |
| 0.20 | 0.02 | 0.02 | 0.50 | 0.02 | 0.05 | 0.05 | 0.20 | 0.001 | 0.01 |

reduced to 68%. Renal handling of sodium exhibited similar changes as in Group A.

The postinfusion period was characterized by restoration of the values to control levels.

Statistical analysis of the differences between Groups A and B in the control, infusion and postinfusion periods revealed the following results (Table IV). (1) The preinfusion values for the haemodynamic parameters were not significantly different in the two groups; plasma sodium concentration, the amount of filtered and excreted sodium, and the tubular rejection fraction were statistically significantly higher in Group B. (2) When infusing identical amounts of hypertonic sodium chloride into the renal artery, RBF, conductance of the renal vessels, $C_{creat}$, and the amount of filtered and excreted sodium were significantly lower in Group A. As the rise in $TRF_{Na}$ was about the same in the two groups, the infusion values were not significantly different, indicating that the difference in sodium excretion rate was due to haemodynamic rather than tubular factors. (3) In the third heading of the Table, the responses obtained after the infusion of 6.4 mEq/min of Na in Group A, and to 12.8 mEq/

**Table IV**

*Statistical analysis of the values obtained in the control, first and second infusion periods in sodium-depleted (A) and sodium-loaded (B) dogs*

| | | Control $Mean_A$-$Mean_B$ | Infusion I $\bar{d}_A-\bar{d}_B$ | Infusion II $\bar{d}_A-\bar{d}_B$ |
|---|---|---|---|---|
| BP | P< | 0.20 | 0.40 | 0.70 |
| RBF | P< | 0.10 | 0.01 | 0.70 |
| RPF | P< | 0.20 | 0.02 | 0.70 |
| 1/R | P< | 0.90 | 0.02 | 0.99 |
| $E_{creat}$ | P< | 1.00 | 0.60 | 0.50 |
| $E_{PAH}$ | P< | 0.01 | 0.20 | 0.10 |
| $P_{Na}$ | P< | 0.001 | 0.30 | 0.60 |
| V | P< | 0.20 | 0.001 | 0.05 |
| $C_{creat}$ | P< | 0.20 | 0.001 | 0.97 |
| $U_{Na}V$ | P< | 0.001 | 0.001 | 0.01 |
| $F_{Na}$ | P< | 0.01 | 0.005 | 0.40 |
| $TRF_{Na}$ | P< | 0.001 | 0.60 | 0.10 |

min in Group B, were compared. Except for the excretion rate of sodium, there was no statistically significant difference between the two groups, thus, the infusion of 12.8 mEq/min of Na into the renal artery gave rise to changes in Group B that were comparable to the changes elicited by half of this amount of sodium in Group A.

## Discussion

The present results indicate that the infusion of hypertonic sodium chloride into the renal artery of dogs maintained previously on a low-sodium diet causes a marked vasoconstriction in the kidney. At the same time, the excretion of sodium increases, despite large decreases in GFR, hence, tubular reabsorption of sodium is significantly depressed.

There are two possibilities by which the infusion of saline could have brought about vasoconstriction in the kidney, viz. (1) the hypertonic solution *per se* affected the renal vascular tone; (2) the increase in sodium concentration was perceived by some intrarenal receptor, the stimulation of which resulted in the liberation of a vasoactive agent, the latter eliciting a local vasoconstrictor response in the kidney.

A direct vasoconstrictor effect of hypertonic sodium chloride seems highly improbable in view of the results obtained in Group B: in the animals pretreated with large amounts of sodium to deplete the kidney of renin, the infusion of identical amounts of the same saline solution caused a much

slighter increase in renal vascular tone, than in Group A. Thus, the vasoconstrictor effect of hypertonic saline depended on the amount of renin present in the kidney, and the dose of sodium chloride sufficient to block renal flow in the kidney rich in renin had no such effect in the renin-depleted kidney. However, increasing the dose of infused sodium to 12.8 mEq/min resulted in a vasoconstriction that was comparable to that obtained in Group A following the infusion of 6.4 mEq/min of sodium. Thus, renal vasoconstriction seems to be proportional to the amount of sodium infused, and inversely proportional to the amount of renin present in the kidney; whether or not vasoconstriction ensues in response to saline depends on the available renin stores.

The marked increase in sodium excretion in face of diminished urine flow and filtered sodium points to reduced tubular reabsorption and increased intratubular concentration of sodium. In a series of micropuncture studies, THURAU et al. (THURAU and SCHNERMANN 1965; THURAU 1966; CORTNEY et al. 1966; HORSTER et al. 1966) have demonstrated that, in rats previously kept on a low-sodium diet, elevation of the intratubular concentration of Na in the early distal convolution causes a reduction in GFR, which has been ascribed to the liberation of renin from the JG cells, an effect mediated by the macula densa receptors. VANDER (1963), KRÜCK (1966), and CANNON et al. (1966) observed diminution of GFR in response to exogenous angiotensin. In rats, PETERS (1963) demonstrated that the infusion of small amounts of angiotensin reduces $C_{PAH}$ but does not alter $C_{in}$. ENDES et al. (1963) were able to show that the acute administration of large quantities of sodium chloride results in hypergranulation of the JG cells in the rat.

In view of these findings it seems likely that in the present experiments the vasoconstriction that followed the infusion of 8.5% sodium chloride into the renal artery was due to the intrarenal liberation of renin in response to the substantial increase in the amount and concentration of sodium in the tubular fluid delivered to the macula densa.

The assumption that the concentration of sodium in the tubular fluid was significantly increased after the infusion of hypertonic saline is supported by the following considerations. The excretion of sodium was found substantially elevated which, owing to the reduction of filtered load, must have been the result of diminished tubular reabsorption; this is clearly indicated by the gross increase in $TRF_{Na}$. Elevation of plasma sodium has been shown by KAMM and LEVINSKY (1965) and GOLDBERG et al. (1967) to reduce the fractional reabsorption of sodium in the proximal tubule; it is therefore reasonable to assume that the amount of sodium delivered to the ascending limb of HENLE's loop was significantly increased in the present experiments. As the infusion of 8.5% saline increased the interstitial sodium concentration as well, which was further elevated by the subsequent diminution of RBF causing

the medullary washout mechanism to be practically abolished, the transtubular sodium gradient must have increased, particularly in the postinfusion periods when the plasma sodium concentration had already returned to normal levels.

The most plausible explanation, thus, for the marked vasoconstriction observed in the kidney in response to intra-arterial sodium chloride is that the initial rise in the filtered sodium causes release of renin which, by the liberation of angiotensin, reduces RBF and GFR, thereby compensating for the increased glomerular load of sodium. Although an acute elevation of plasma sodium concentration of such a degree does not occur under physiological conditions, the present results seem to support the concept that the renin-angiotensin system, by altering renal vascular tone, and, hence, glomerular filtration, plays a prominent part in the regulation of sodium excretion.

## Acknowledgements

The authors are indebted to Mrs. S. BÁCSALMÁSY and Mrs. Á. MÁRKUS for skilful technical assistance.

## LITERATURE

BÁLINT, P., FORGÁCS, I.: Parameters of renal function in the anaesthetized and unanaesthetized dog. Acta physiol. Acad. Sci. hung. **1967,** 31: 99—106.

BÁLINT, P., VISY, M.: "True creatinine" and "pseudo-creatinine" in blood plasma of the dog. Acta physiol. Acad. Sci. hung. **1965,** 28: 265—272.

BARRACLOUGH, M. A.: Dose-dependent opposite effects of angiotensin on renal sodium excretion. Lancet **1965,** 2: 987—988.

BARRACLOUGH, M. A., JONES, N. F., MARSDEN, C. D.: Effect of angiotensin on renal function in the rat. Amer. J. Physiol. **1967,** 212: 1153—1157.

BOCK, K., DENGLER, H., KRECKE, H. J., REICHEL, G.: Untersuchungen über die Wirkung von synthetischem Hypertensin II auf Elektrolythaushalt, Nierenfunktion and Kreislauf beim Menschen. Klin. Wschr. **1958,** 36: 808—814.

BROWN, J. J., DAVIES, D. L., LEVER, A. F., ROBERTSON, J. I. S.: Influence of sodium deprivation and loading on the plasma-renin in man. J. Physiol. (Lond.) **1964,** 173: 408—419.

BUNAG, R. D., PAGE, I. H., MCCUBBIN, J. W.: Influence of dietary sodium on stimuli causing renin release. Amer. J. Physiol. **1966,** 211: 1383—1386.

CANNON, P. J., AMES, R. P., LARAGH, H. J.: Indirect action of angiotensin infusion to inhibit renal tubular sodium reabsorption in dogs. Amer. J. Physiol. **1966,** 211: 1021—1030.

COHEN, E. L., CONN, J. W., ROVNER, D. R.: Postural augmentation of plasma renin activity and aldosterone excretion in normal people. J. clin. Invest. **1967,** 46: 418—428.

CORTNEY, M. A., NAGEL, W., THURAU, K.: A micropuncture study of the relationship between flow-rate through the loop of Henle and sodium concentration in the early distal tubule. Pflügers Arch. ges. Physiol. **1966,** 287: 286—295.

EIGLER, F. W.: Regulierung von Glomerulumfiltrat und arteriellen Blutdruck durch den Natriumgradienten an den Macula densa-Zellen: eine Hypothese über die Stimulierung des Renin-Angiotensin-Systems. Zugleich ein Beitrag zur Genese verschiedener Hochdruckformen. Klin. Wschr. **1967,** 45: 23—30.

ENDES, P., DÉVÉNYI, I., GOMBA, SZ., DAUDA, GY.: Veränderungen der granulierten Zellen des juxtaglomerulären Apparates bei akuter Salz- und Wasserbelastung. Z. ges. exp. Med. **1963,** 137: 396—400.

FRASER, R., JAMES, V. H. T., BROWN, J. J., ISAAC, P., LEVER, A. F., ROBERTSON, J. I. S.: Effect of angiotensin and of frusemide on plasma aldosterone, corticosterone, cortisol, and renin in man. Lancet **1965,** 2: 989—991.

GOLDBERG, M., GOLDSTEIN, S. S., GOLDSHALL, S., PUSCHETT, J. B.: Separate effects of plasma sodium concentration ($P_{Na}$) and glomerular filtration rate (GFR) on proximal tubular sodium reabsorption ($T_{Na}$) in the dog. J. clin. Invest. **1967,** 46: 1060.

GORDON, M., HARTROFT, P. M.: Renal juxtaglomerular cells in acute dietary sodium deficiency. Fed. Proc. **1961,** 20: 404.

GROSS, F., SCHAECHTELIN, G., BRUNNER, H., PETERS, G.: The role of renin-angiotensin system in blood pressure regulation and kidney function. Canad. med. Ass. J. **1964,** 90: 258—262.

GROSS, F., BRUNNER, H., ZIEGLER, M.: Renin-angiotensin system, aldosterone, and sodium balance. In: PINCUS, G. (Ed.): Recent progress in hormone research. Academic Press, New York, **1965,** 21: 119—177.

HORSTER, M., NAGEL, W., THURAU, K.: Frühdistale Na-Konzentration und GFR in der Rattenniere unter dem Einfluss von Furosemid und Chlorothiazid. Pflügers Arch. ges. Physiol. **1966,** 289: R 68.

KAMM, D. E., LEVINSKY, N. G.: Inhibition of renal tubular sodium reabsorption by hypernatremia. J. clin. Invest. **1965,** 44: 1144—1150.

KRÜCK, F.: Das Renin-Angiotensin System. In: KRÜCK, F. (Ed.): Aktuelle Probleme der Nephrologie. Springer Verlag, Berlin **1966.** Pp. 55—72.

KUHN, C., HARTROFT, P. M., PITCOCK, J. A.: Effects of renal extracts on juxtaglomerular cells, renin content and zona glomerulosa. Fed. Proc. **1961,** 20: 404.

LANGFORD, H.: Tubular action of angiotensin. Canad. med. Ass. J. **1964,** 90: 332—333.

LAULER, D. P., VAGNUCCI, A. I., BERGARI, A. E., HICKLER, R. B., THORN, G. W.: Angiotensin natriuresis: direct tubular action. Clin. Res. **1963,** 11: 409.

LEYSSAC, P. P., LASSEN, U. V., THAYSEN, H. J.: Inhibition of sodium transport in isolated renal tissue by angiotensin. Biochem. biophys. Acta (Amst.) **1961,** 48: 602—603.

LEYSSAC, P. P.: The in vivo effect of angiotensin on the proximal tubular reabsorption of salt in rat kidneys. Acta physiol. scand. **1964,** 436—448.

LEYSSAC, P. P.: The in vivo effect of angiotensin and noradrenaline on the proximal tubular reabsorption of salt in mammalian kidneys. Acta physiol. scand. **1965,** 64: 167—175.

PETERS, G.: Renal tubular effect of $val_5$-angiotensin II amide in rats. Proc. Soc. exp. Biol. (N. Y.) **1963,** 112: 771—775.

SCHNERMANN, J., NAGEL, W., THURAU, K.: Die frühdistale Natriumkonzentration in Rattennieren nach renalen Ischaemie und Hämorrhagischer Hypotension. (Ein Beitrag zur Pathogenese der postischaemischen und posthämorrhagischen Filtraterniedrigung.) Pflügers Arch. ges. Physiol. **1966,** 287: 296—310.

SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B., GRABER, M.: The renal clearance of substituted hippuric acid derivates and other aromatic acids in dog and man. J. clin. Invest. **1945,** 24: 388—404.

THURAU, K., SCHNERMANN, J.: Die Natriumkonzentration an den Macula densa-Zellen als regulierender Faktor für das Glomerulumfiltrat (Mikropunktionsversuche). Klin. Wschr. **1965,** 43: 410—413.

THURAU, K.: Die intrarenale Rolle der Renin-Angiotensin System für die Regulation des Glomerulumfiltrates und der Natriumausscheidung. In: KRÜCK, F. (Ed.): Aktuelle Probleme der Nephrologie. Springer-Verlag, Berlin **1966,** Pp. 75—88.

TOBIAN, L.: Sodium, renal arterial distension and the juxtaglomerular apparatus. Canad. med. Ass. J. **1964,** 90: 160—162.

TOBIAN, L., COFFEE, K., FERREIRA, D., MEULI, J.: The effect of renal perfusion pressure on the net transport of sodium out of distal tubular urine as studied with the stop-flow technique. J. clin. Invest. **1964,** 43: 118—128.

VANDER, A. J.: Inhibition of distal tubular sodium reabsorption by angiotensin II. Amer. J. Physiol. **1963,** 205: 133—138.

VANDER, A. J., LUCIANO, J. R.: Effects of mercurial diuresis and acute sodium depletion on renin release in dog. Amer. J. Physiol. **1967,** 212: 651—656.

Iván FORGÁCS, Rudolf CHÂTEL, Mária VISY,
Orvostudományi Egyetem Éllettani Intézete,
Budapest VIII., Puskin u. 9, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 231—243 (1969)*

# COMPARATIVE STUDY ON HAEMODILUTION AFTER HAEMORRHAGE IN THE PIGEON AND THE RAT

By

A. G. B. Kovách and Terézia Bálint

EXPERIMENTAL RESEARCH DEPARTMENT AND GERONTOLOGICAL RESEARCH UNIT, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received November 21, 1968)

In 33 rats and 27 pigeons bleeding standardized to body weight was carried out and haemodilution, haemoglobin content, haematocrit value and erythrocyte count have been studied together with the plasma total protein, albumin, globulin and potassium levels.

If haemorrhage was rapid (within 10 min) no difference was observed in the rate of haemodilution in rats and pigeons.

If bleeding was sustained (over 1 hour) characteristic differences between the two species could be revealed which became apparent after the first 15 min. While at the beginning the rate of haemodilution was about the same, after 15 to 20 min there was no further haemodilution in the rat, whereas in the pigeon blood was further diluted at an unchanged rate. Haemodilution was the result of the inflow of isotonic fluid with a low protein content.

The higher tolerance to bleeding in flying and diving birds observed in previous experiments seems to be due to the more marked haemodilution observed in these species which allow more efficient homeostatic regulation of vascular volume.

It has been demonstrated in a previous paper (Kovách and Szász 1968) that blood loss is tolerated much better by pigeons than by mammals. If haemorrhage is sufficiently graded, almost the whole calculated blood volume can be removed without the animal dying. The duck also exhibited a high resistance to bleeding, nevertheless, not all avian species proved to be equal in this respect (Kovách et al. 1968).

The question arises what are the factors responsible for the difference between the pigeon and mammals. In a previous work (Kovách and Szász 1968) it has been discussed in detail that there may be a difference between the pigeon and mammals in the rate of fluid replacement and the regulation of blood volume.

It is common knowledge that hypovolaemia due to haemorrhage is compensated on the basis of Starling's principle by transcapillary reabsorption of interstitial fluid to fill the intravascular compartment. Bleeding is associated with an increased sympathetic activity (Fedina et al. 1965) and elevated blood catecholamine levels (Glaviano et al. 1960), leading to constriction of the resistance and capacitance vessels and to an increase in the ratio of precapillary and postcapillary resistance, the latter causing the capillary pressure to diminish and to the net transfer of fluid from the extravascular into the

intravascular compartment (LUNDGREN et al. 1964; HADDY et al. 1965). In mammals, the rate of interstitial fluid reabsorption is 0.10 to 0.15 ml/kg/min in the case of small haemorrhages (PRICE et al. 1941; LAWSON and REHM 1945), and 0.25 ml/kg/min when the amount of blood lost is higher (ADOLPH et al. 1933). The above mechanisms are capable of restoring under experimental conditions about 10% of the total volume of blood measured in the control period (SHOEMAKER 1967). DUNN et al. (1958) claimed that bleeding is accompanied not only by ultrafiltration but also by the inflow of depot protein.

The present experiments have been undertaken to clarify the factors responsible for the difference in the resistance to haemorrhage observed in the pigeon and various mammalian species. To approach this problem blood haemoglobin, haematocrit, erythrocyte count, plasma protein, albumin, globulin, K and Na levels were repeatedly checked after haemorrhage in pigeons and rats.

## Methods

Experiments were performed on 33 inbred albino rats of either sex, weighing 274 g on the average (range: 120 to 350 g), and on 27 pigeons of different strains and of either sex, with an average weight of 410 g (range: 280 to 490 g). The animals were anaesthetized and bled through a polyethylene cannula inserted into the left common carotid artery. The pigeons were anaesthetized with 25% urethan given intramuscularly in a dose of 1.2 g/kg, and the rats with 0.5% sodium pentobarbital given intraperitoneally. The pigeons received 0.5 ml and the rats 0.3 ml of heparin to inhibit coagulation of blood.

Two types of haemorrhage were applied. The first type of bleeding was graded and was employed in 10 pigeons and 20 rats. According to body weight, appropriate amounts of blood were removed 4 times at intervals of 5 min; then after an interval of 30 min, further amounts of blood were removed every 5 min.

The other type of haemorrhage was continuous and rapid; 17 pigeons and 13 rats belonged to this group. Bleeding was continued for 10 min. The blood flowing out uninterruptedly from the carotid artery was collected in 5 ml fractions in the pigeons, and in 2 ml fractions in the rats.

Haemoglobin content of blood was determined indirectly. Blood was diluted in a slightly alkaline medium and its extinction was measured with an UVIFOT photometer at 546 m$\mu$ wave length.

Red blood cell counts were determined using Hellige pipettes, in a Bürker counting chamber.

Haematocrit value was estimated after centrifugation for 15 min at 5000 v.p.m.

Total protein and albumin levels were estimated with the biuret reagent, in the case of albumin after shaking with ether.

Plasma Na and K levels were determined by a Zeiss flame photometer.

Arterial blood pressure was monitored in both pigeons and rats by a mercury manometer attached to the common carotid artery by means of a polyethylene tubing.

Statistical analysis was undertaken by using STUDENT's *t*-test, as described by HETÉNYI (1954a).

## Results

Table I summarizes the values obtained in the control period. In both the pigeon and the rat, the initial erythrocyte count, haemoglobin content and bleeding volume were in good agreement with data in the literature (KOVÁCH 1954b, SCHERMER 1954). The values for haematocrit were somewhat higher

**Table I**

*Normal values*

| | Pigeon | Rat |
|---|---|---|
| Blood pressure (mm Hg) | 101.8 ± 12.6 (43) | 130.0 ± 19.6 (10) |
| Erythrocyte count (million per μl) | 3.43 ± 0.38 (39) | 7.98 ± 0.82 (39) |
| Haemoglobin (g per 100 ml) | 14.8 ± 0.51 (50) | 13.9 ± 0.97 (39) |
| Haematocrit (per cent) | 51.1 ± 4.1 (23) | 43.6 ± 2.7 (37) |
| Plasma Na (mEq per l) | 147 ± 10.3 (56) | 140 ± 18 (34) |
| Plasma K (mEq per l) | 2.39 ± 0.44 (56) | 3.75 ± 0.44 (33) |
| Plasma protein (g per 100 ml) | 3.12 ± 0.49 (43) | 6.0 ± 0.7 (9) |
| Plasma albumin (g per 100 ml) | 1.45 ± 0.92 (41) | 2.62 ± 0.44 (18) |
| *Bleeding volume* (ml per 100 g body weight) | | |
| Pigeon, rapid bleeding 7.24 ± 0.65 (17) | | |
| Pigeon, substained bleeding 9.45 ± 1.6 (10) | | |
| Rat, rapid bleeding 4.27 ± 0.66 (20) | | |
| Rat, substained bleeding 4.19 ± 1.6 (13) | | |

The number of measurements is given in brackets.

than the usual figures (Kovách 1954b) whereas total protein and albumin levels were somewhat lower (Schermer 1954).

a) *Slow graded haemorrhage.* Results are shown in Tables II through V.

As seen in Table II in the pigeon red blood cell count was significantly depressed during the first 5 min of bleeding from an initial value of 3.41±0.1 million per μl. As haemorrhage was continued this value was depressed to 1.20±0.28 million, i.e. to 35.2% of the control reading. Red blood cell count considerably diminished also in the rat. From an initial value of 8.26±0.21 million per μl it decreased to considerably lower values and after 10 min the difference attained statistical significance. Fifty-five min after the onset of bleeding the rats died with a red blood cell count of 6.94±0.20 million, which was 84% of the control value.

*Haemoglobin content* of blood (Table II) in the pigeon attained 14.51± 0.45 g per 100 ml in the control periods, and was statistically significantly depressed as early as 5 min after the onset of haemorrhage. Sixty-five min later, haemoglobin was reduced to 52.4% of the initial value. In the rat too, bleeding caused a statistically significant reduction of the blood haemoglobin level, but the rate of decrease was considerably different in the two observed species. Starting from the values obtained 10 min after the onset of haemorrhage there was a statistically demonstrable difference in the course of reduction of blood haemoglobin content. In the rat the initial haemoglobin

**Table II**

*Changes in red blood cell count and haemoglobin content during substained haemorrhage. $P_0$ refers to differences against control, $P_{p-r}$ to differences between pigeon and rat*

| | | | Start | 5 min | 10 min | 15 min | 45 min | 50 min | 55 min | 60 min | 65 min |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Red blood cell count | Pigeon | Mean | 3.41 | 3.18 | 2.91 | 2.48 | 2.14 | 1.89 | 1.97 | 1.53 | 1.20 |
| | | S.E.M. | 0.11 | 0.10 | 0.16 | 0.11 | 0.12 | 0.11 | 0.07 | 0.15 | 0.28 |
| | | n | 9 | 9 | 9 | 9 | 7 | 7 | 6 | 5 | 3 |
| | | $P_0<$ | | 0.05 | 0.05 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.01 |
| | Rat | Mean | 8.26 | 8.16 | 7.73 | 7.23 | 7.22 | 7.11 | 6.94 | | |
| | | S.E.M. | 0.21 | 0.18 | 0.22 | 0.21 | 0.21 | 0.12 | 0.20 | | |
| | | n | 17 | 17 | 17 | 14 | 12 | 8 | 7 | | |
| | | $P_0<$ | | 0.60 | 0.02 | 0.001 | 0.01 | 0.01 | 0.01 | | |
| | | $P_{p-r}<$ | | 0.60 | 0.001 | 0.60 | 0.90 | 0.40 | 0.90 | | |
| Haemoglobin | Pigeon | Mean | 14.51 | 13.66 | 12.67 | 11.47 | 9.44 | 8.86 | 8.86 | 8.35 | 7.60 |
| | | S.E.M. | 0.45 | 0.52 | 0.47 | 0.55 | 0.56 | 0.60 | 0.45 | 0.42 | 0.60 |
| | | n | 9 | 9 | 9 | 9 | 7 | 7 | 6 | 5 | 3 |
| | | $P_0<$ | | 0.02 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| | Rat | Mean | 14.0 | 13.52 | 12.85 | 12.14 | 12.15 | 12.09 | 11.29 | | |
| | | S.E.M. | 0.23 | 0.26 | 0.15 | 0.19 | 0.22 | 0.26 | 0.22 | | |
| | | n | 17 | 17 | 17 | 14 | 12 | 9 | 7 | | |
| | | $P_0<$ | | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | | |
| | | $P_{p-r}<$ | | 0.20 | 0.20 | 0.01 | 0.001 | 0.001 | 0.001 | | |

**Table III**

*Changes in haematocrit value and plasma protein content during sustained haemorrhage. $P_0$ refers to differences against control, $P_{p-r}$ to differences between pigeon and rat*

| | | | Start | 5 min | 10 min | 15 min | 45 min | 50 min | 55 min | 60 min | 65 min |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Haematocrit value | Pigeon | Mean | 49.60 | 47.10 | 43.60 | 39.80 | 32.50 | 30.25 | 28.86 | 26.50 | 24.00 |
| | | S.E.M. | 1.38 | 1.35 | 1.34 | 1.55 | 1.55 | 1.45 | 1.28 | 0.91 | 0.43 |
| | | n | 9 | 9 | 9 | 9 | 7 | 7 | 6 | 5 | 3 |
| | | $P_0<$ | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| | Rat | Mean | 44.11 | 41.83 | 39.22 | 37.07 | 38.46 | 36.50 | 36.25 | | |
| | | S.E.M. | 0.72 | 0.72 | 0.46 | 0.50 | 0.83 | 0.27 | 0.56 | | |
| | | n | 17 | 17 | 17 | 14 | 13 | 9 | 7 | | |
| | | $P_0<$ | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | | |
| | | $P_{p-r}<$ | | 0.70 | 0.20 | 0.10 | 0.001 | 0.001 | 0.001 | | |
| Plasma protein content | Pigeon | Mean | 2.69 | 2.76 | 2.31 | 1.87 | 1.54 | 1.45 | 1.34 | 1.28 | 0.97 |
| | | S.E.M. | 0.13 | 0.13 | 0.18 | 0.17 | 0.11 | 0.05 | 0.05 | 0.10 | 0.13 |
| | | n | 7 | 4 | 6 | 7 | 6 | 7 | 6 | 5 | 2 |
| | | $P_0<$ | | 0.50 | 0.02 | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 | 0.05 |
| | Rat | Mean | 6.23 | 5.96 | 5.63 | 5.38 | 5.40 | 5.01 | 5.47 | | |
| | | S.E.M. | 0.17 | 0.16 | 0.17 | 0.27 | 0.11 | 0.20 | 0.10 | | |
| | | n | 13 | 12 | 12 | 9 | 9 | 6 | 2 | | |
| | | $P_0<$ | | 0.30 | 0.01 | 0.02 | 0.01 | 0.01 | 0.40 | | |
| | | $P_{p-r}<$ | | 0.90 | 0.60 | 0.90 | 0.05 | 0.40 | 0.10 | | |

**Table IV**

*Changes in plasma albumin and globulin levels during sustained haemorrhage. $P_0$ refers to differences against control, $P_{p-r}$ to differences between pigeon and rat*

| | | | Start | 5 min | 10 min | 15 min | 45 min | 50 min | 55 min | 60 min |
|---|---|---|---|---|---|---|---|---|---|---|
| Albbumin | Pigeon | Mean | 1.46 | 1.51 | 1.46 | 1.02 | 0.78 | 0.67 | 0.58 | 0.52 |
| | | S.E.M. | 0.19 | 0.22 | 0.18 | 0.20 | 0.15 | 0.13 | 0.19 | 0.18 |
| | | n | 5 | 3 | 4 | 5 | 5 | 5 | 4 | 3 |
| | | $P_0<$ | | 0.80 | 0.30 | 0.20 | 0.02 | 0.02 | 0.05 | 0.05 |
| | Rat | Mean | 3.28 | 2.98 | 2.80 | 2.58 | 2.30 | 2.04 | 1.80 | |
| | | S.E.M. | 0.13 | 0.15 | 0.16 | 0.28 | 0.17 | 0.17 | 0.25 | |
| | | n | 13 | 12 | 12 | 9 | 9 | 6 | 2 | |
| | | $P_0<$ | | 0.001 | 0.01 | 0.05 | 0.001 | 0.01 | 0.01 | |
| | | $P_{p-r}<$ | | 0.05 | 0.20 | 0.60 | 0.20 | 0.20 | 0.05 | |
| Globulin | Pigeon | Mean | 1.24 | 1.19 | 0.95 | 0.81 | 0.80 | 0.78 | 0.81 | 0.79 |
| | | S.E.M. | 0.20 | 0.27 | 0.15 | 0.11 | 0.27 | 0.12 | 0.21 | 0.29 |
| | | n | 5 | 3 | 4 | 5 | 4 | 5 | 4 | 3 |
| | | $P_0<$ | | 0.60 | 0.20 | 0.05 | 0.05 | 0.20 | 0.20 | 0.05 |
| | Rat | Mean | 2.95 | 2.97 | 2.83 | 2.84 | 3.10 | 2.97 | 3.67 | |
| | | S.E.M. | 0.20 | 0.20 | 0.20 | 0.26 | 0.15 | 0.28 | 0.26 | |
| | | n | 13 | 12 | 12 | 9 | 9 | 6 | 2 | |
| | | $P_0<$ | | 0.70 | 0.40 | 0.90 | 0.20 | 0.60 | 0.20 | |
| | | $P_{p-r}<$ | | 0.40 | 0.80 | 0.50 | 0.05 | 0.20 | 0.05 | |

**Table V**

*Changes in plasma K levels during sustained haemorrhage. $P_0$ refers to differences against control $P_{p-r}$ to differences between pigeon and rat*

| | | Start | 5 min | 10 min | 15 min | 45 min | 50 min | 55 min | 60 min | 65 min |
|---|---|---|---|---|---|---|---|---|---|---|
| Pigeon | Mean | 1.99 | 1.98 | 2.16 | 2.52 | 2.36 | 2.58 | 2.94 | 3.78 | 4.62 |
| | S.E.M. | 0.12 | 0.13 | 0.15 | 0.27 | 0.15 | 0.11 | 0.10 | 0.19 | 0.22 |
| | n | 9 | 9 | 9 | 9 | 7 | 7 | 6 | 5 | 3 |
| | $P_0<$ | | 0.80 | 0.01 | 0.05 | 0.02 | 0.01 | 0.001 | 0.001 | 0.01 |
| Rat | Mean | 3.75 | 3.98 | 4.67 | 5.43 | 4.87 | 6.15 | 8.13 | | |
| | S.E.M. | 0.11 | 0.13 | 0.26 | 0.32 | 0.44 | 0.45 | 0.80 | | |
| | n | 15 | 15 | 15 | 12 | 9 | 6 | 4 | | |
| | $P_0<$ | | 0.01 | 0.001 | 0.001 | 0.02 | 0.01 | 0.01 | | |
| | $P_{p-r}<$ | | 0.02 | 0.01 | 0.01 | 0.10 | 0.10 | 0.001 | | |

content was 14.0±0.23 g and this was reduced to 11.29±0.22 g per 100 ml when the animals died, i.e. 55 min after the onset of haemorrhage, haemoglobin attained 80.6% of the initial value.

Table III shows the changes observed in the haematocrit value. In the pigeon, the control value of 49.6±1.38% decreased until the 65th min to 24.0±1.87%, i.e. to 48.4% of the original value. In the rat 44.1±0.72% was found in the control period and this was reduced to 36.25±0.56%. The fall in haematocrit value was significant statistically in both species, and after the 45th min on there was a statistically significant difference in the rate of decrease between the rat and pigeon, the latter species showing a more marked depression.

*Plasma total protein* values are also shown in Table III. In the pigeon, they decreased from 2.69±0.13 to 0.97 g per 100 ml, while in the rat a diminution from 6.23±0.17 to 5.47±0.10 g per 100 ml was observed, the percentile change in the former case was 74.0% but only 12.2% in the latter. In both species, the differences as compared to the initial values were significant statistically.

*Plasma albumin* and *globulin* levels are shown in Table IV. Albumin was reduced from 1.46±0.19 g per 100 ml to 0.52±0.18 in the pigeon, i.e. to 35.6% of the value measured prior to haemorrhage. In the rat, the control value of 3.28±0.13 g per 100 ml diminished to 1.80±0.25, i.e. to 54.9% of the control reading. Plasma globulin in the pigeon attained 1.24±0.20 g per 100 ml in the control period, and this was reduced to 0.79 g per 100 ml (63.7%). In the rat, globulin levels did not exhibit a diminution in response to haemorrhage, moreover, prior to death they showed a slight, statistically not significant, rise.

The plasma A/G ratio was significantly depressed in both species towards the end of bleeding. In the pigeon, there was a slight initial increase the control of 1.18 rising to 1.53 in the 10th min, however, subsequent bleeding caused the ratio to decrease to 0.65 at the end of haemorrhage (65 min); this decrease was due to the circumstance that the albumin level was more markedly depressed by the blood loss than was the globulin content. In the rat, the initial rise in the A/G ratio was absent and the diminution was continuous. The value recorded prior to bleeding attained 1.10, and this was reduced until the death of the animals to 0.43.

The plasma K level increased markedly in both species, as shown in Table V. The control value in the pigeon attained 1.99±0.12 mEq/l and this rose continuously until death when the value 4.62±0.22 mEq/1. The rise was significant statistically after the 10th min. A similar pattern was observed in the rat, the control reading of 3.75±0.11 mEq/l being elevated to 8.13± 0.80 by the end of the experiment. In this case, statistical significance was reached as early as 5 min after the onset of haemorrhage.

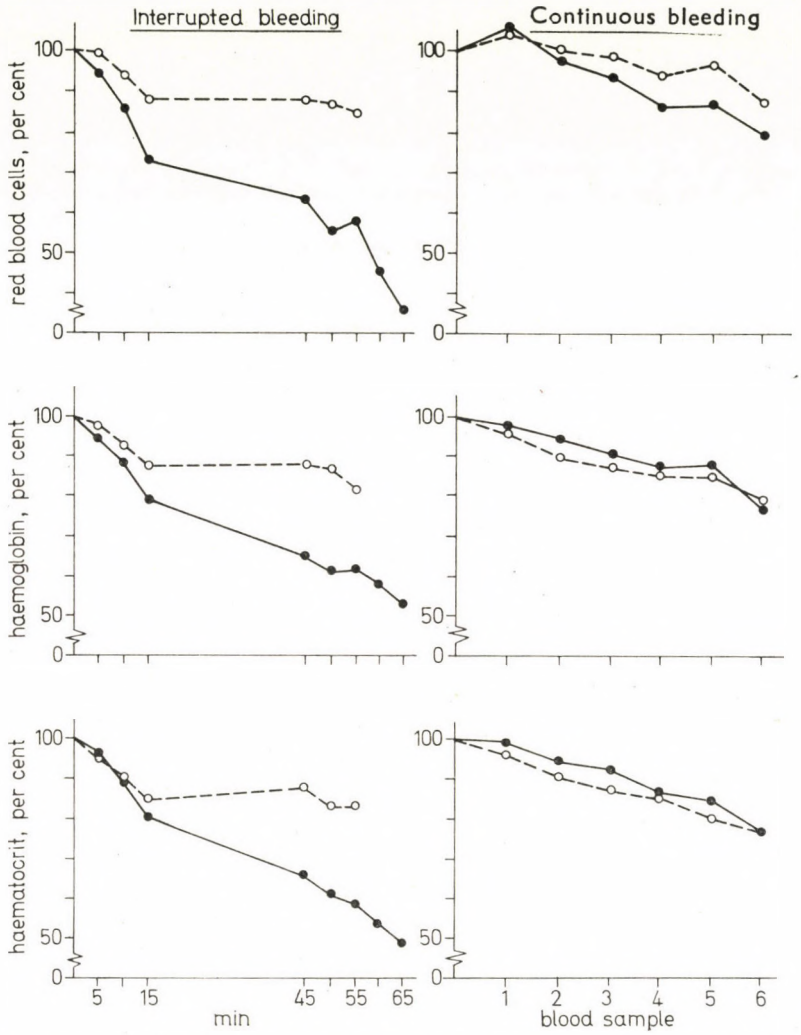



*Fig. 1.* Changes in red blood cell count, haemoglobin content and haematocrit value in response to haemorrhage in pigeon and rat. R i g h t, sustained blood loss within 1 hour; l e f t, rapid haemorrhage within 10 min. o——o pigeon, o- - - -o rat. Values are expressed in per cent of the control

All these changes are represented diagrammatically in Figs 1 and 2.

b) *Continuous bleeding*. In these experiments the animals were bled within 10 min; the removed blood was collected in 6 fractions. The obtained results are shown in Figs 1 and 2.

Red blood cell count in the pigeon was $3.45 \pm 0.15$ million per $\mu$l, which was statistically significantly reduced to $2.74 \pm 0.25$ million per $\mu$l. In the rat, however, the decrease from the control value of $8.01 \pm 0.20$ million per $\mu$l to $7.01 \pm 0.50$ million per $\mu$l was not significant statistically.

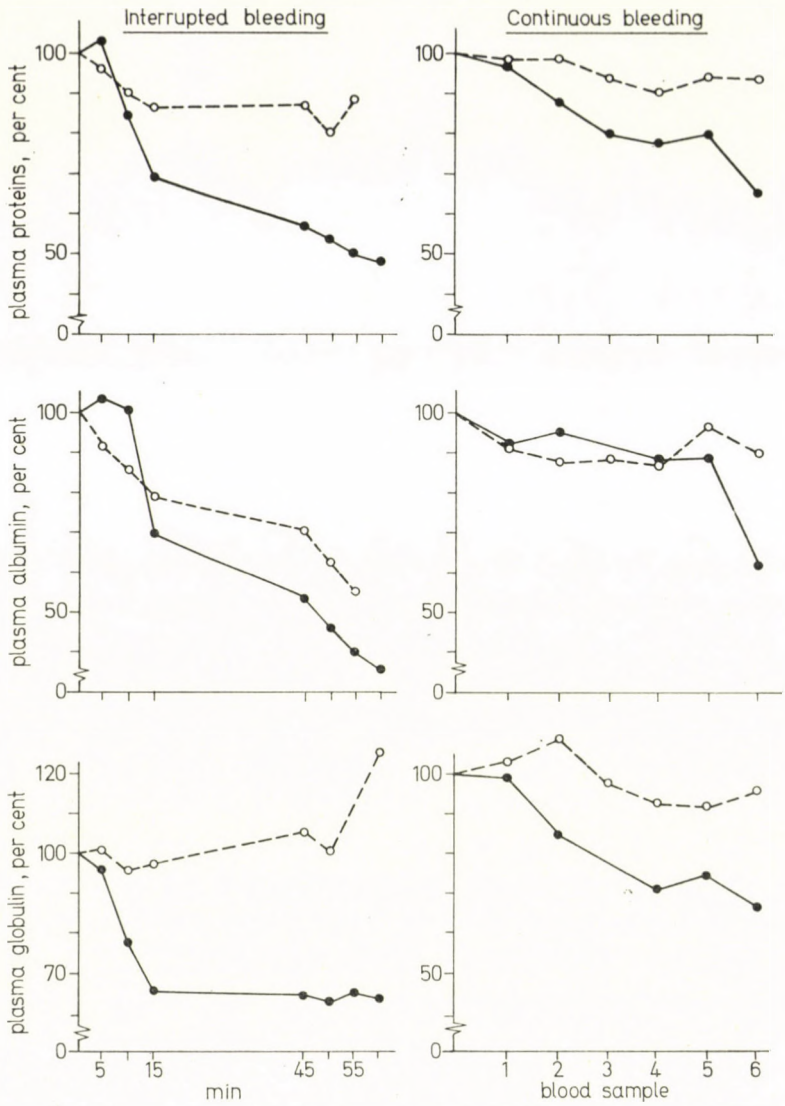



*Fig. 2.* Percentile changes in plasma total protein, albumin and globulin levels in response to haemorrhage. R i g h t, sustained blood loss within 1 hour; l e f t, rapid blood loss within 10 min. For symbols, see Fig. 1

Haemoglobin content in the pigeon underwent a gradual decrease from 14.07±0.34 g to 10.83±0.22 g per 100 ml of blood in the last fraction. A similar and statistically also significant diminution was obtained in the rat, the control value being 13.58±0.30 g per 100 ml and the value prior to death, 10.84± 0.62 g per 100 ml. The haematocrit value was considerably depressed by rapid haemorrhage. In the pigeon, the initial value of 52.23±1.0% was reduced

to 40.0±3.08%, whereas in the rat the control value of 43.0±0.75% diminished to 33.0±0.58%; the changes were significant statistically in both cases.

The plasma total protein level showed significant changes in the pigeon, where the control value of 3.77±0.11 g per 100 ml was reduced to 2.43±0.19 g per 100 ml; in the rat the protein level was essentially unaffected by rapid bleeding.

Plasma albumin and globulin levels exhibited statistically significant alterations only in the pigeon during the short period of bleeding. The small diminution observed in the rat was not significant statistically.

Plasma K levels underwent a statistically significant elevation in both species.

## Discussion

The present results indicate that the haemodilution occurring in response to bleeding is considerably more marked in the pigeon than in the rat. The difference between the two species becomes less apparent when haemorrhage is rapid (lasting 10 min in the present experiments) than in case of sustained blood loss (approximately 1 hour in the present experiments).

During the first 10 to 15 min in the initial phase of sustained blood loss haemodilution was closely similar in the pigeon and the rat. Compensatory haemodilution, however, almost completely ceased in the rat after 15 min, whereas in the pigeon blood was further diluted practically at an unchanged rate.

When blood volume is suddenly diminished by removing a substantial amount of blood, homeostatic mechanisms become operating to compensate for the blood loss and to maintain arterial blood pressure and cardiac output. An important factor of this mechanism is the diminution of the capacity of the vascular bed mainly as a result of increased venomotor tone; the significance of this compensatory adjustment lies in the rapidity of the response. This mechanism, however, is not suitable for the compensation of large blood losses. The finding that plasma volume is expanded during haemorrhage indicates that, in addition to the above homeostatic adjustment, there is another mechanism developing more slowly which helps in maintaining circulation despite a considerable reduction of blood volume. There seem to be two factors playing a part in the restoration and increase of blood volume after haemorrhage. The first is the net transfer of fluid from the extravascular compartment where considerable amounts of fluid are stored and can be mobilized in case of necessity. The other possibility is the reduction of fluid loss through the kidneys; according to GAUER and HENRY (1963) this antidiuretic response is governed by cardiovascular receptors. The transfer of fluid from the extravascular into the intravascular compartment through the capillary membrane is governed by cardiovascular receptors. The transfer of fluid from the extra-

vascular into the intravascular compartment through the capillary membrane has been thoroughly studied by FOLKOW and MELLANDER (1960) and ÖBERG (1964).

It has been known for long that blood loss in mammals is followed by a redistribution of extracellular fluid (GREGERSEN and RAWSON 1959). Decrease of the red blood cell count and dilution of plasma proteins has been used as a qualitative and quantitative measure of transcapillary fluid transfer. ADOLPH et al. (1933) found in the dog that, 20 min after the removal of 20 to 35 ml per kg of body weight of blood, about 35% of the blood loss was replaced by plasma. PRICE et al. (1941), LAWSON and REHM (1945) observed in an aesthetized dogs that the rate of fluid replacement was 0.15 ml/kg/min. KAUFMANN et al. (1956) measured a 30 to 40% restitution of blood volume within 10 to 20 min after the removal of 5 to 15 ml/kg of blood. Similar observations were made in man. EBERT et al. (1941) found a 20% restitution of plasma volume after blood losses ranging between 150 and 1200 ml. KAUFMANN and MÜLLER (1958) removed 600 to 700 ml of blood and measured a 50% restitution within 15 to 20 min. It has been repeatedly emphasized that the post-haemorrhagic increase in plasma volume slows down after 20 to 30 min. From this it has been concluded that the replacement of blood loss is a self-limiting process. Obviously there must be several factors involved in the transcapillary transfer of fluid from the interstitium. Thus, for instance, the dilution of plasma proteins changes the osmotic gradient, affecting thereby, the reabsorption of extravascular fluid. In addition to the pressure gradient, the permeability of the capillaries and the surface area of the capillary membrane actually available for fluid transfer are also factors of prime importance in determining the amount of fluid reabsorbed into the vascular bed. The importance of nervous mechanisms in the compensation of the blood loss was first suggested by CHIEN (1958). The anatomical and physiological basis for such a mechanism seems to be well established since it has been demonstrated that the tone of the precapillary sphincters and the ratio of precapillary and postcapillary tone are under nervous control (BÜCHERL and SCHWAB 1952, FOLKOW and MELLANDER 1960, ROSELL and UVNES 1962, RENKIN and ROSELL 1962, COBBOLD et al. 1963). The data referring to this question have been thoroughly reviewed by ÖBERG (1964).

The rate of haemodilution and the time of cessation of this process in the rat as observed in the present experiments are in satisfactory agreement with the above data obtained in other mammalian species.

It is clear from the present results that the replacement of plasma lost by haemorrhage shows considerable differences in the pigeon and the rat. The haemodilution ensuing immediately after the start of bleeding followed a similar pattern in the two species in the initial phase of the experiments, but the process ceased rapidly in the rat whereas in the pigeon blood was further

diluted at an unchanged rate and thus the final values of haemodilution attained substantially higher figures. This would mean that the capacity to compensate for blood loss is far less limited in the pigeon than in the rat. This might be due to a difference in the amount of fluid stores available for such purposes, but there are no data indicating a difference in this respect between mammalian and avian species. It is possible that the pigeon is capable of mobilizing a part of the intracellular fluid, too, which obviously could be responsible for a significantly more efficient compensatory mechanism. In this respect the finding seems to be of considerable interest that, in the pigeon, the dilution of plasma proteins was significantly more pronounced than in the rat.

Finally, the question arises whether the fluid moving into the intravascular compartment is derived from every part of the body or some organs participate more in this response. In mammals the skeletal muscles may liberate significant amounts of fluid in case of necessity (MELLANDER and LEWIS 1963). FOLKOW et al. (1966) demonstrated that the value for capillary filtration coefficient was considerably higher in the duck than in mammals whereas in the turkey a value close to that observed in mammals was found.

It is thus reasonable to assume that the pigeon's increased resistance to haemorrhage is due, at least in part, to a quantitatively more efficient adjustment of blood volume than that found in mammals. Another responsible factor may be the consistently higher cardiac output of pigeons demonstrated in a previous work from this laboratory (KOVÁCH and SZÁSZ 1968).

It might be asked whether the increased mobilization of extravascular fluid and the significantly more efficient cardiovascular homeostatic adjustment is related to the circumstance that the pigeon is a flying bird. Of the various avian species it is first of all the pigeon and the duck which possess a significant resistance to bleeding as compared to mammals, whereas the domestic fowl and the crow are more sensitive than the former and less than the latter (KOVÁCH et al. 1968). To decide the problem comparative studies are needed involving more animal species and by experiments undertaken after appropriate changes in the mode of living.

## Acknowledgements

The skilful technical assistance of Miss M. KELEMEN is gratefully acknowledged.

## LITERATURE

ADOLPH, E. F., GERBASI, M. J., LEPORE, M. J.: The rate of entrance of fluid into the blood in hemorrhage. Amer. J. Physiol. **1933**, 104: 502—517.

BÜCHERL, E., SCHWAB, M.: Der Sauerstoffverbrauch des ruhenden Skelettmuskels bei reflektorisch nervöser Vasokonstriktion. Pflügers Arch. ges. Physiol. **1952**, 254: 337—343.

CHIEN, S.: Quantitative evaluation of the circulatory adjustment of splenectomized dogs to hemorrhage. Amer. J. Physiol. **1958**, 193: 605—614.

COBBOLD, A., FOLKOW, B., KJELLMER, I., MELLANDER, S.: Nervous and local chemical control of precapillary sphincters in skeletal muscle as measured by changes in filtration coefficient. Acta physiol. scand. **1963,** 57: 180—192.

DUNN, J. R., DEAVERS, S., HUGGINS, R. A., SMITH, E. L.: Effect of hemorrhage on red cell and plasma volume of various organs of the dog. Amer. J. Physiol. **1958,** 195: 69—72.

EBERT, R. V., STEAD, E. A., GIBSON, J. G. Jr.: Response of normal subjects to acute blood loss. Arch. intern. Med. **1941,** 68: 578—590.

FEDINA, L., KOVÁCH, A. G. B., VANIK, M.: Sympathetic activity in haemorrhagic shock. Acta physiol. Acad. Sci. hung. **1965,** Suppl. 26: 35.

FOLKOW, B., MELLANDER, S.: Aspects of the nervous control of the precapillary sphincters with regard to the capillary exchange. Acta physiol. scand. **1960,** Suppl. 50., 175. 52—54.

FOLKOW, B., FUX, K., SONNENSCHEIN, R. R.: Responses of skeletal musculature and its vasculature during "diving" in the duck. Acta physiol. scand. **1966,** 67: 327—342.

GAUER, O. H., HENRY, J. P.: Circulatory basis of fluid volume control. Physiol. Rev. **1963,** 43: 423—481.

GLAVIANO, V. V., BASS, N., NYKIEL, F.: Adrenal medullary secretion of epinephrine and norepinephrine in dogs subjected to hemorrhage. Circulat. Res. **1960,** 8: 564—571.

GREGERSEN, M. I., RAWSON, R. A.: Blood volume. Physiol. Rev. **1959,** 39: 307—342.

HADDY, F. J., SCOTT, J. B., MOLNÁR, J. I.: Mechanism of volume replacement and vascular constriction following hemorrhage. Amer. J. Physiol. **1965,** 208: 169—181.

HETÉNYI, G.: Kísérleti eredmények statisztikai értékelése. In: KOVÁCH, A. (Ed.): A kísérleti orvostudomány vizsgáló módszerei II. Budapest, Akadémiai Kiadó, **1954**/a, pp. 667—702.

KAUFMANN, W., KLUSSMANN, F. W., KOCH, J., LÜTCKE, A.: Volumenersatz nach Hämorrhagien vor und nach Ausschaltung der Milz. Pflügers Arch. ges. Physiol. **1956,** 263: 253—271.

KAUFMANN, W., MÜLLER, A. A.: Expansion des Plasmavolumens nach rascher Verminderung des zirkulierenden Blutmenge. Z. Kreislauf-Forsch. **1958,** 47: 719—728.

KOVÁCH, A. G. B., SZÁSZ, E.: Survival of pigeon after graded hemorrhage. Acta physiol. Acad. Sci. hung. **1968,** 34: 301—309.

KOVÁCH, A. G. B., SZÁSZ, E., PILMAYER, N.: The mortality of various avian and mammalian species following blood loss. Acta physiol. Acad. Sci. hung. **1969,** 35: 109—116

LAWSON, H., REHM, W. S.: The effect of hemorrhage and replacement on the apparent volume of plasma and cells. Amer. J. Physiol. **1945,** 144: 199—205.

LUNDGREN, O., LUNDWALL, J., MELLANDER, S.: Range of sympathetic discharge and reflex vascular adjustments on skeletal muscle during hemorrhagic hypotension muscle. Acta physiol. scand. **1964,** 62: 380—390.

MELLANDER, S., LEWIS, D. H.: Effect of hemorrhagic shock on the reactivity of resistance and capacitance vessels and on capillary filtration transfer in cat skeletal muscle. Circulat. Res. **1963,** 13: 105—118.

ÖBERG, B.: Effects of cardiovascular reflexes on net capillary fluid transfer. Acta physiol. scand. **1964,** Suppl. 62: 229. 1—98.

PRICE, P. B., HANLON, C. R., LONGMIRE, W. P., METCALF, W.: Experimental shock. I. Effects of acute hemorrhage in healthy dogs. Bull. Johns Hopkins Hosp. **1941,** 69: 327—362.

RENKIN, E. M., ROSELL, S.: The influence of sympathetic adrenergic vasoconstrictor nerves on transport of diffusible solutes from blood to tissues in skeletal muscle. Acta physiol. scand. **1962,** 54: 223—240.

ROSELL, S., UVNÄS, B.: Vasomotor nerve activity and oxygen uptake in skeletal muscle of the anesthetized cat. Acta physiol. scand. **1962,** 54: 209—222.

SHERMER, S.: Die Blutmorphologie der Laboratoriumstiere. Johann Ambrosius Barth Verlag, Leipzig **1954,**

SHOEMAKER, W. C.: Shock, chemistry, physiology and therapy. Thomas, Springfield. **1967,** P. 19.

SÓS, J., S. LÖDI, I.: Kísérleti állatok biológiai és fiziológiai standard értékei. In: KOVÁCH, A. (Ed.): A kísérleti orvostudomány vizsgáló módszerei I. Budapest, Akadémiai Kiadó, **1954**/b, pp. 104—140.

Arisztid G. B. KOVÁCH, Terézia BÁLINT
Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma
Budapest VIII., Üllői út 78/a, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 245—256 (1969)*

# ÉTUDES DES PROBLÈMES DE LA RÉGULATION DU DÉBIT SANGUIN CÉRÉBRAL

## I.: SUR LE RÔLE DE L'HYPOTHALAMUS DANS LA RÉGULATION DU DÉBIT SANGUIN DU TRONC CÉRÉBRAL

Par

L. MOLNÁR et J. CZOPF

CLINIQUE DES MALADIES NERVEUSES ET MENTALES DE L'UNIVERSITÉ DE PÉCS

(Reçu le 4 janvier 1969)

Chez des lapins anesthésiés la stimulation électrique de l'hypothalamus n'influence le débit de la région ponto-bulbaire que dans le cas où la pression artérielle systémique et la respiration sont fortement modifiées par la même stimulation. Il n'y a pas de corrélations systématiques entre les changements du débit circulatoire et ceux de l'activité électrique et de la respiration.

L'effet dilatateur du $CO_2$ sur les vaisseaux du tronc cérébral peut être affaibli ou au contraire renforcé par la stimulation électrique de l'hypothalamus.

Les résultats des observations suggèrent que des influx hypothalamiques influencent les relations fonctionnelles néo-cortico-bulbaires en assurant l'équilibre de ce système réverbérant.

Les résultats de nos études concernant les modifications circulatoires du cerveau dans des conditions les plus différentes, physiologiques et pathologiques, nous ont suggéré une conception de la régulation du débit sanguin cérébral. Tout en admettant l'importance de la pression artérielle systémique et celle des processus métaboliques, nous avons cru avoir démontré l'intervention des influx nerveux, en particulier le rôle joué d'un système réverbérant néo-cortico-bulbaire dans cette régulation (MOLNÁR 1967). Ces observations ne nous ont cependant pas renseignés sur les détails de la fonction de ce système réverbérant. Nous avons dû, bien entendu, prendre en considération les influences exercées par le fonctionnement des autres structures du cerveau sur les relations fonctionnelles du système cortico-bulbaire. C'est ainsi que nous nous sommes attachés à rechercher le rôle que joue éventuellement l'hypothalamus dans la régulation du débit circulatoire du tronc cérébral.

### Méthodes

Notre expérimentation a porté sur 42 lapins anesthésiés au mélange de chloralose (70 mg/kg) et d'uréthane (0,5 g/kg). Dans la grande majorité de ces expériences, chez 38 animaux, le débit sanguin et l'activité électrique de la même région du cerveau, la pression artérielle systémique et la fréquence respiratoire ont été enregistrés simultanément.

*Pour mesurer le flux sanguin* nous avons utilisé la méthode développée par SEYLAZ (1965), dont la description détaillée se trouve dans son travail original ainsi que dans plusieurs de nos publications précédentes (cf. MOLNÁR, 1967).

*La dérivation de l'activité électrique* s'est effectuée par des fins filaments à extrémités dorées, ceux-ci étant introduits avec les thermistances utilisées pour l'enregistrement des variations du courant sanguin, à l'aide d'un appareil de type Horsley—Clarke, dans la structure choisie du cerveau.

*La pression artérielle systémique* a été mesurée, par l'intermédiaire d'un cathéter introduit dans l'artère fémorale, à l'aide d'un système à capsule transductrice.

Une thermistance placée dans la canule trachéale a indiqué *la fréquence et l'amplitude des mouvements respiratoires.*

Des électrodes d'argent, enrobées de verre, ont été employées *pour l'excitation,* mono- ou bipolaire, *de l'hypothalamus.* Le stimulateur utilisé nous a permis de changer les paramètres d'excitation (la fréquence, le voltage et la durée d'un stimulus), l'un indépendamment des autres.

*L'examen microscopique* des coupes histologiques nous a renseigné sur *la localisation anatomique des électrodes et des thermistances.*

Grâce à un dispositif convenable nous avons pu mélanger du gaz carbonique avec de l'air aux concentrations voulues. Nous avons fait inhaler du $CO_2$ de 7 p. 100 en air.

## Résultats

Nos premières expériences nous ont montré que des excitations électriques de l'hypothalamus produisant quelquefois des modifications importantes de la pression artérielle systémique n'influençaient guère le débit sanguin de la protubérance et du bulbe. Mais déjà lors de ces expériences nous avons pu constater que les paramètres de la stimulation avaient une importance décisive dans l'évolution du changement éventuel du flux sanguin. Dans le cas, où la stimulation était effective déterminant une modification du débit, ce dernier suivait fidèlement, dans la plupart des expériences, la variation de la pression artérielle produite par la même stimulation (Fig. 1).

Les observations présentées ci-dessus nous ont suggéré, d'une part, que le débit sanguin du bulbe et de la protubérance était encore plus stable que celui des autres régions du cerveau et, d'autre part, que la pression artérielle systémique jouait peut-être un rôle plus important dans la régulation de ce même débit que dans celle du flux sanguin des autres centres cérébraux.

Les mêmes expériences, dont nous avons parlé jusqu'ici nous ont démontré que l'arrêt respiratoire (par clampage de la canule trachéale), ainsi que l'inhalation du $CO_2$ entraînaient une augmentation du débit sanguin aussi dans le bulbe et la protubérance.

Des stimulations de l'hypothalamus, n'ayant aucun effet sur le débit de la région ponto-bulbaire et ne produisant qu'un changement passif de ce débit, ont néanmoins influencé l'allure de l'augmentation du flux sanguin entraînée par l'arrêt respiratoire. Les mêmes stimulations n'ont cependant pas modifié quantitativement chaque fois l'effet de l'asphyxie (Fig. 2).

Il restait à éclaircir le problème dans quelle mesure c'est le changement de la pression artérielle qui influençait le débit augmenté par l'arrêt respiratoire, ou bien il s'agissait d'une coïncidence de deux phénomènes (changement

de la pression artérielle et celui du débit sans être cependant en relation causative l'un avec l'autre), dont l'origine pouvait être tout de même commune.

Pour résoudre cette question d'une part, et pour pouvoir mieux juger le rapport entre le changement circulatoire et l'état fonctionnel des territoires étudiés d'autre part, nous avons effectué aussi des stimulations, utilisant des paramètres subcritiques ou correspondant aux valeurs de seuil, ainsi que des excitations périphériques, et nous avons toujours enregistré l'activité électrique de la région, dont le débit était examiné.

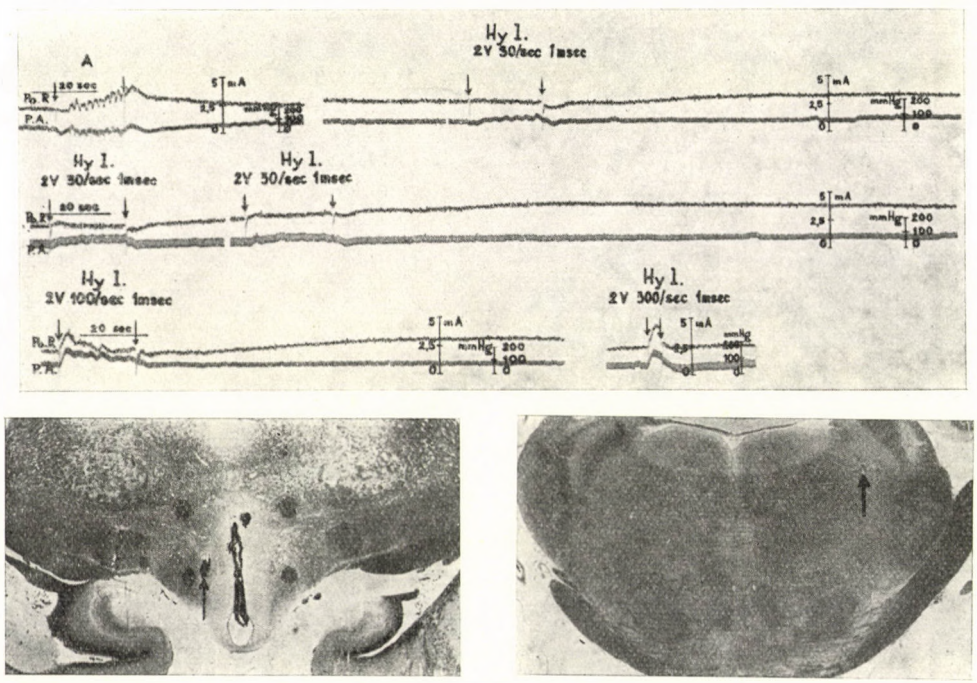



*Fig. 1.* Effet de l'asphyxie, produite par le clampage de la canule trachéale *(A)* et des excitations électriques de l'hypothalamus sur le débit de la protubérance (Po.R) et la pression artérielle fémorale (P.A.). *Hyl.* durée des stimulations. Noter la passivité du flux sanguin durant les deux dernières stimulations effectuées avec du courant de haute fréquence. Les microphotographies montrent la localisation anatomique de l'électrode stimulatrice dans l'hypothalamus, ainsi que celle de la thermistance dans le tronc cérébral

La comparaison de l'effet des stimulations électriques de l'hypothalamus à celui des excitations douloureuses périphériques (pincement de l'oreille) s'est montrée très instructive. Des stimulations hypothalamiques n'ayant pas d'influence ni sur la pression artérielle systémique ni sur le débit sanguin, diminuaient néanmoins l'effet vasodilatateur de l'arrêt respiratoire. Par contre, le pincement de l'oreille provoquant une chute importante de la pression artérielle, suivie d'une faible diminution du courant sanguin, n'avait pas notablement modifié l'effet de l'asphyxie (Fig. 3).

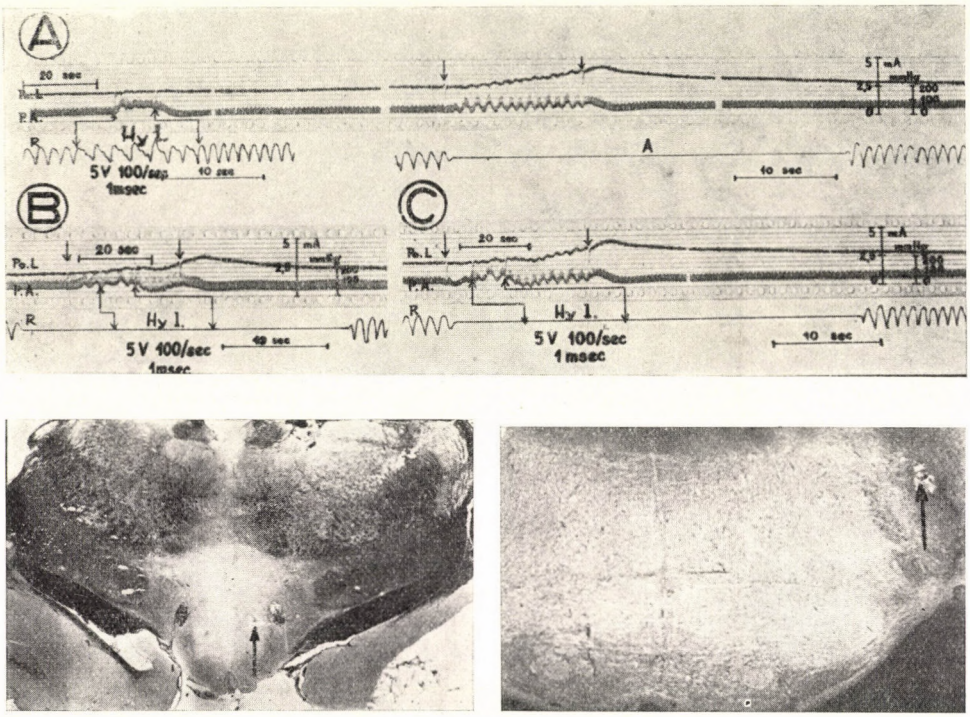
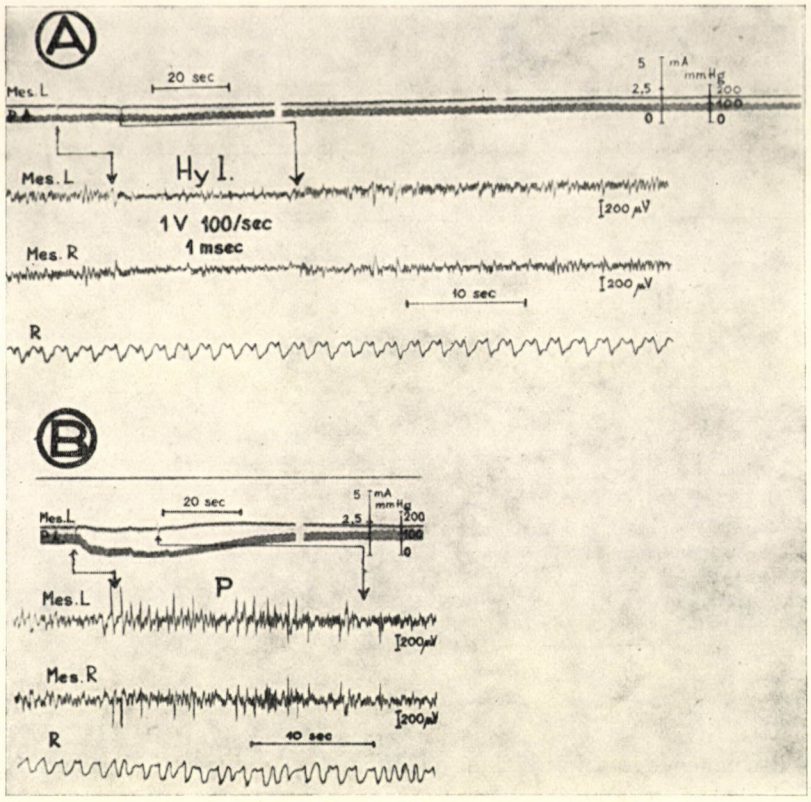


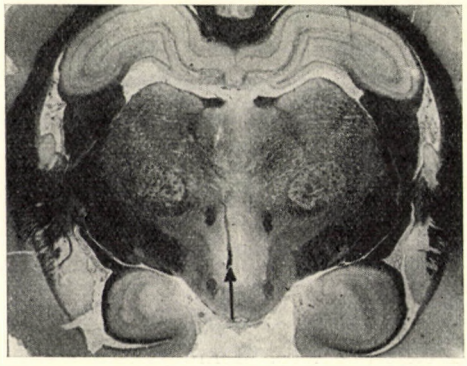

*Fig. 2. A* La stimulation hypothalamique n'influence pas le débit de la protubérance (Po.L.) quoiqu'elle produise une augmentation importante de la pression artérielle (P.A.). Pendant l'asphyxie (A) le débit s'accroît. — *B* la même stimulation affaiblit légèrement, — *C* ne modifie pas l'effet de l'asphyxie (R respiration)

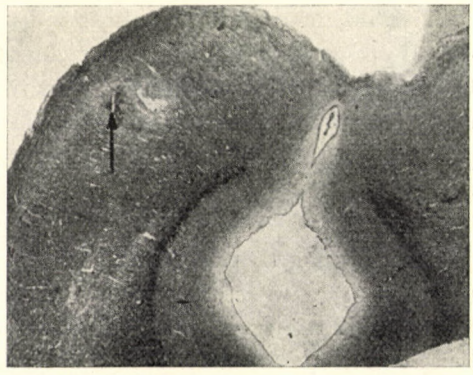
Lors de ces expériences nous avons souvent eu l'occasion de constater que la diminution de l'amplitude et l'accroissement de la fréquence de l'activité électrique, la désynchronisation, n'était pas obligatoirement accompagnée d'une augmentation du débit sanguin. Bien au contraire, la désynchronisation de l'activité électrique apparaissait, dans la majorité des cas, en même temps qu'une diminution plus ou moins prononcée du flux sanguin.

Des stimulations hypothalamiques produisant une désynchronisation de l'activité électrique ainsi qu'une diminution du débit se sont montrées efficaces même durant l'augmentation de flux sanguin provoquée par le clampage de la canule trachéale. C'est ainsi que les stimulations affaiblissaient l'effet que l'arrêt respiratoire exerçait sur le courant sanguin (Fig. 4).

Bien que le clampage de la canule trachéale ait toujours entraîné une augmentation du flux sanguin, cet effet vasodilatateur ne pouvait pas être considéré comme tout à fait physiologique. Dans ce cas il ne s'agit sûrement pas de l'effet d'une simple accumulation du $CO_2$, celle-ci n'étant qu'un des facteurs mis en jeu durant l'asphyxie. C'est pour cette raison que nous avons

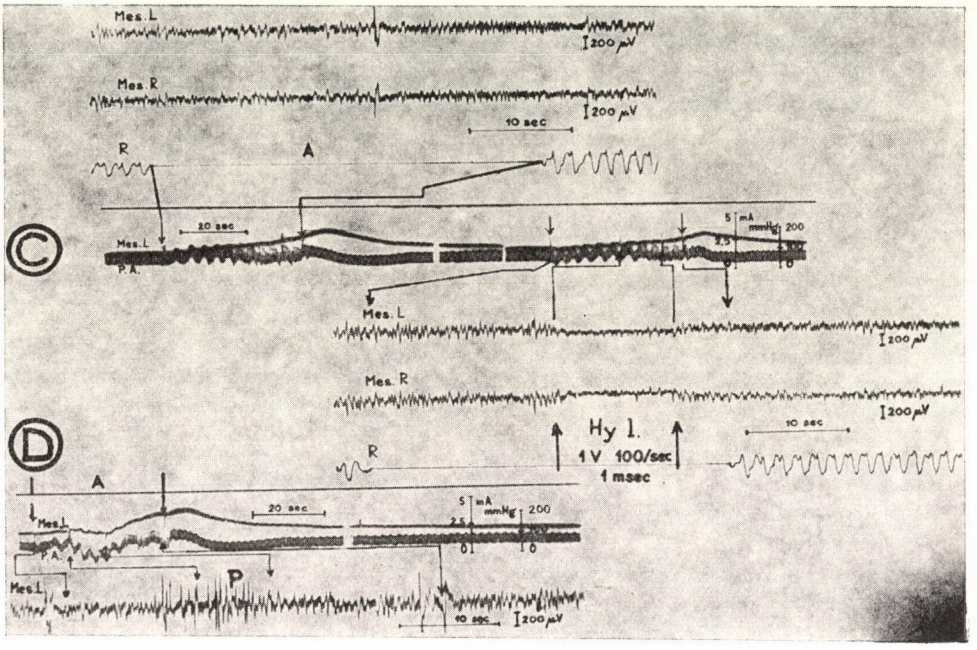




*3a*

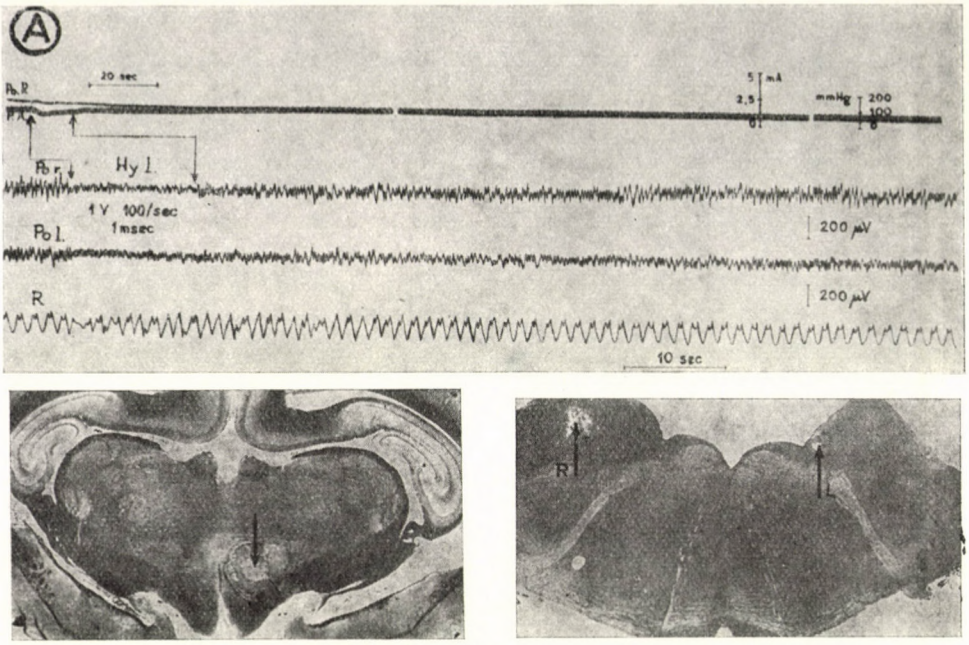
*Fig. 3. A* la stimulation hypothalamique ne modifie ni le débit du mésencéphale (Mes.L) ni la pression artérielle (P.A.), mais produit une désynchronisation de l'activité électrique du mésencéphale du côté gauche (Mes.L) et droit (Mes.R). La respiration (R) ne change pas. — *B* le pincement de l'oreille (P) provoque chez le même animal une chute importante de la pression artérielle, celle-ci étant suivie d'une faible diminution du débit sanguin. — *C* pendant l'asphyxie (A) le débit du mésencéphale augmente. La stimulation hypothalamique effectuée pendant l'arrêt respiratoire affaiblit l'augmentation du débit. — *D* le pincement de l'oreille (P), bien que produisant un abaissement transitoire du flux sanguin, n'influence pas l'effet vasodilatateur de l'asphyxie (A)



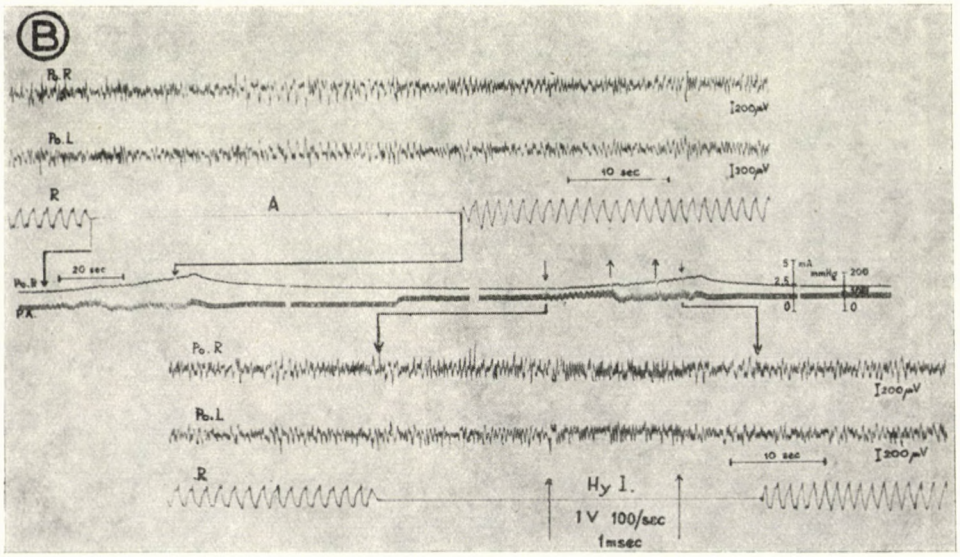

*3b*

étudié l'influence des stimulations électriques de l'hypothalamus sur l'effet de l'inhalation du gaz carbonique.

L'influence des stimulations hypothalamiques sur l'effet vasodilatateur du $CO_2$ dépendait pratiquement dans tous nos cas des paramètres d'excitation. Cela veut dire qu'en changeant les paramètres d'excitation ou en déplaçant l'électrode excitatrice d'une fraction de millimètre, l'action du gaz carbonique pouvait être affaiblie ou, au contraire, renforcée par l'excitation de l'hypothalamus (Fig. 5). L'influence de l'excitation n'a guère dépendu de la modification de la pression artérielle qu'elle a provoquée. De même, l'influence provoquée par la stimulation sur la respiration, quelle que soit sa nature (arrêt respiratoire, diminution ou augmentation de l'amplitude respiratoire, hyperventilation), n'était non plus en corrélation directe avec l'influence de l'excitation qu'elle a exercée sur l'effet du $CO_2$ (Fig. 6). Même dans des cas, où la stimulation hypothalamique a produit un arrêt de la respiration, l'effet de l'inhalation du gaz carbonique a pu être affaibli par la même excitation (Fig. 5).

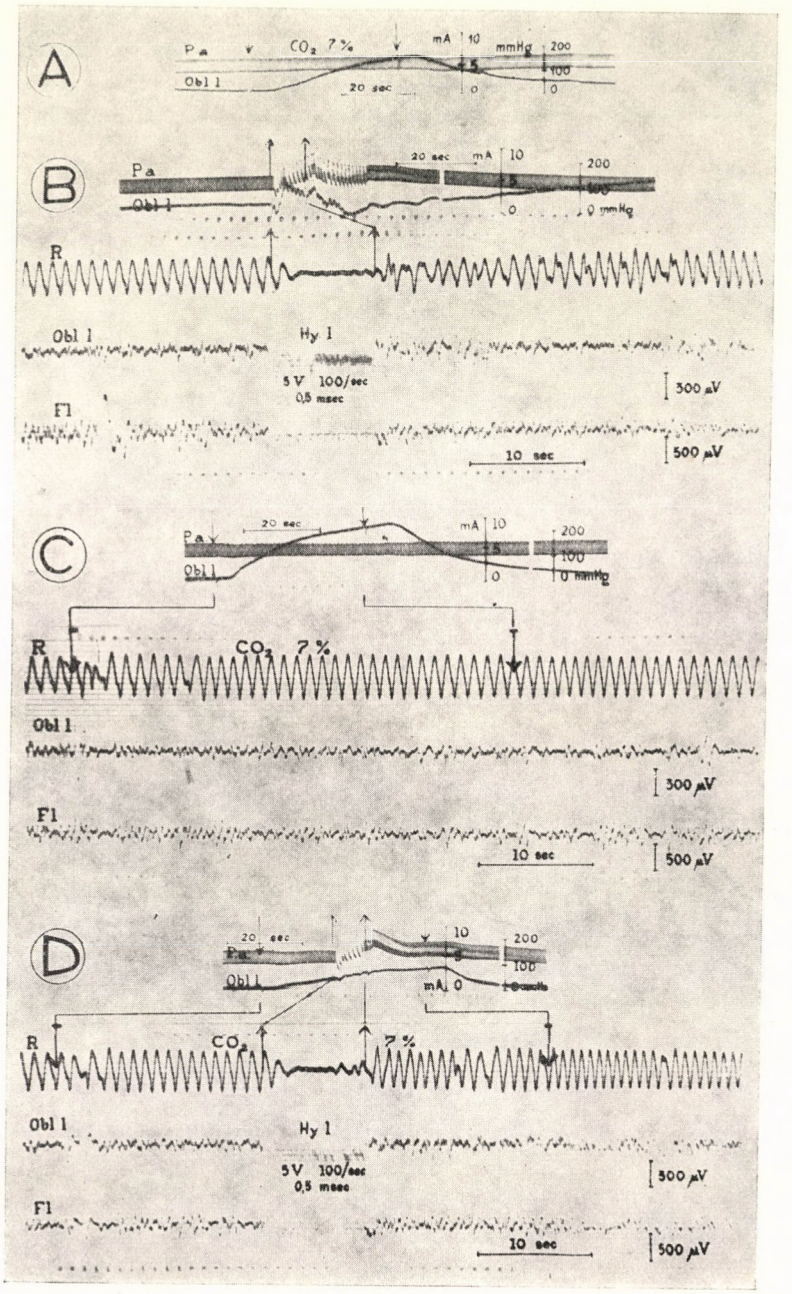



*4a*

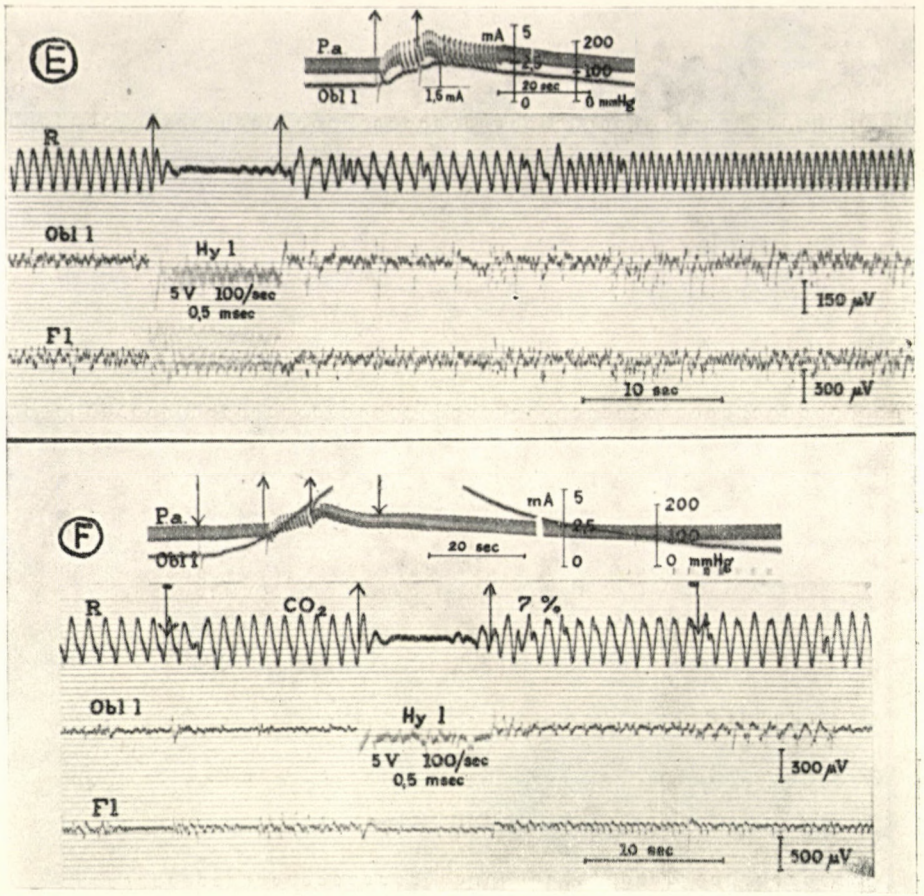


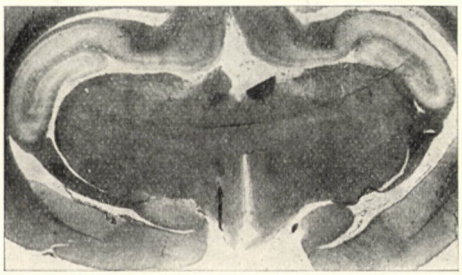

*4b*

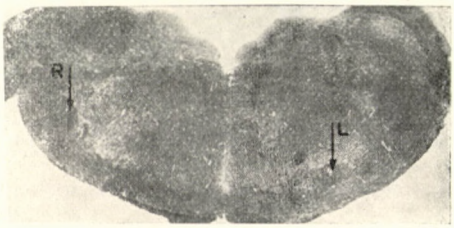
*Fig. 4. A* Sous l'effet d'une stimulation hypothalamique le débit de la protubérance (Po.R) baisse très faiblement, la pression artérielle (P.A.) diminue, l'activité électrique de la protubérance se désynchronise et du côté droit (Po.r.) et du coté gauche (Po.l.); la respiration (R) devient irrégulière. — *B* chez le même animal l'asphyxie (A) entraîne une augmentation du flux sanguin qui s'affaiblit par la stimulation de l'hypothalamus; dans ce cas la région stimulée a été coagulée à la fin de l'expérience


A
Pa
CO2 7%
mA 10
mmHg 200
100
Obl 1
20 sec
0
0
B
Pa
20 sec
mA 10
200
Obl 1
0
0 mmHg
R
Obl 1
Hy 1
5 V 100/sec
0,5 msec
300 µV
Fl
10 sec
500 µV
C
Pa
20 sec
mA 10
200
100
Obl 1
0
0 mmHg
R
CO2 7%
Obl 1
300 µV
Fl
10 sec
500 µV
D
20 sec
Pa
10
200
100
Obl 1
mA 0
R
CO2
7%
Obl 1
Hy 1
5 V 100/sec
0,5 msec
500 µV
Fl
10 sec
500 µV

*5a*






*5b*

*Fig. 5. A* Le débit du bulbe (Obl. l.) augmente, la pression artérielle (P.a.) ne change pas sous l'effet de l'inhalation du $CO_2$ — *B* l'excitation de l'hypothalamus provoque une augmentation considérable de la pression artérielle, accompagnée d'un accroissement de courte durée, suivi d'une diminution beaucoup plus importante, du débit sanguin; pendant la stimulation un arrêt respiratoire se manifeste, l'activité électrique du bulbe (Obl. l.) ne change pas, celle de la région frontale gauche (Fl.) se désynchronise. — *C* après la stimulation hypothalamique le $CO_2$ a un effet vasodilatateur plus important. — *D* cet effet est affaibli, d'une façon marquée, par la stimulation effectuée pendant l'inhalation du gaz carbonique. — *E* la même stimulation, mais pratiquée après le déplacement de l'électrode stimulatrice, produit, simultanément avec l'augmentation de la pression artérielle, un accroissement du débit et — *F* renforce, si elle est effectuée pendant l'inhalation du $CO_2$, l'effet vasodilatateur de celui-ci

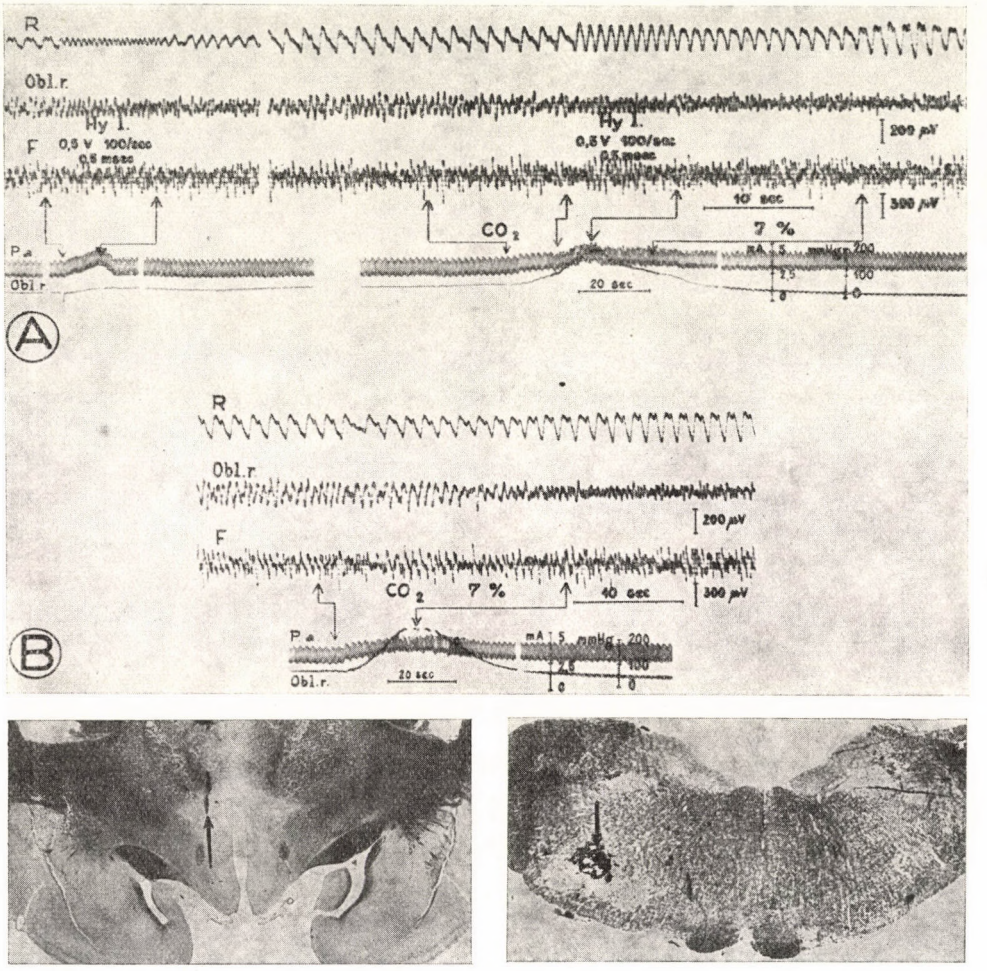



*Fig. 6. A* Sous l'effet d'une stimulation hypothalamique, provoquant une augmentation importante de la pression artérielle (P.a.) le débit du bulbe (Obl. l.) ne change que faiblement. La fréquence respiratoire (R) est accélérée, l'activité électrique du bulbe (Obl.r.) se désynchronise, celle de la région frontale (F) ne change pas. La même stimulation, effectuée pendant l'inhalation du $CO_2$, affaiblit l'effet vasodilatateur de ce dernier. — *B* effet de l'inhalation du gaz carbonique sur la respiration, l'activité électrique, la pression artérielle et le débit du bulbe

## Remarques et conclusions

Les résultats de nos observations montrent que la stimulation électrique de l'hypothalamus, chez des lapins anesthésiés, n'influence le flux sanguin de la protubérance et du bulbe que dans le cas, où la pression artérielle ou la respiration ou bien toutes les deux sont fortement modifiées par la même stimulation. Ce fait semble prouver que le débit sanguin du tronc cérébral se

trouve sous l'influence passive de la pression artérielle systémique et de la $pCO_2$ du sang et du tissu cérébral examiné. Mais, de l'autre côté, nous n'avons pas trouvé de corrélations systématiques entre les éventuelles modifications du courant sanguin et celles de l'activité électrique de la région étudiée. Le changement du débit, quel qu'il soit (augmentation ou diminution), peut être accompagné d'une synchronisation ou d'une désynchronisation de l'activité électrique. Bien que rarement, le sens du changement du débit, même dans le tronc cérébral, peut être l'inverse de celui de la modification de la pression artérielle systémique, cette dernière étant alors contrebalancée ou même hypercompensée par des vaisseaux du tronc cérébral.

Il ressort quand même clairement des résultats de nos observations que la pression artérielle joue un rôle important dans la régulation de la circulation sanguine du tronc cérébral, du moins chez des lapins anesthésiés. C'est ainsi que même l'effet du gaz carbonique, qui s'est révélé dans nos expériences comme un agent très puissant dans la détermination du débit sanguin du tronc cérébral, peut subir une modification sous l'action d'une stimulation hypothalamique produisant un changement de la pression artérielle systémique.

L'analyse détaillée de nos enregistrements obtenus lors de cette série d'expériences nous convainquent tout de même qu'il serait une simplification d'attribuer une importance décisive à la pression artérielle systémique dans la régulation du débit sanguin du tronc cérébral.

En effet, comme nos observations nous le montrent, une chute considérable de la pression artérielle, produite par l'excitation hypothalamique ou périphérique, n'influence pas toujours le débit de la région ponto-bulbaire et ne modifie pas, dans toutes les circonstances, l'effet vasodilatateur de l'asphyxie ou de l'inhalation du $CO_2$. Bien au contraire, ce dernier effet peut être renforcé par des stimulations de l'hypothalamus produisant une diminution de la pression artérielle systémique.

En possession des données présentées dans ce travail, on peut conclure que le débit circulatoire du tronc cérébral est fonction de plusieurs facteurs, dont tous ont une importance dans la régulation de la circulation sanguine de cette partie du cerveau.

Les influx d'origine hypothalamique affaiblissent souvent l'effet vasodilatateur du $CO_2$ dans le tronc cérébral. Ce fait nous suggère que c'est grâce à l'intervention de l'hypothalamus que le débit du tronc cérébral est stabilisé sur un niveau convenable, et de ce fait, les conditions d'un fonctionnement normal de cette partie du cerveau sont assurées malgré des changements éventuellement importants des processus métaboliques. De cette façon, l'hypothalamus peut influencer les relations fonctionnelles néo-cortico-bulbaires. De plus, nous croyons que le fonctionnement de l'hypothalamus peut garantir l'équilibre fonctionnel du système réverbérant néo-cortico-bulbaire.

## BIBLIOGRAPHIE

MOLNÁR, L.: Sur le contrôle nerveux de la circulation sanguine régionale des centres cérébraux. Akadémiai Kiadó, Budapest. **1967.**

SEYLAZ, J.: Développement d'une méthode de mesure instantanée, localisée et semi-quanti. tative du débit sanguin in vivo. — Applications diverses. Biophysik **1965**, 2: 330—339.

László Molnár
Orvostudományi Egyetem Ideg-elmeklinika Debrecen,
József Czopf
Orvostudományi Egyetem Ideg-és elmeklinika Pécs,

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 257—270 (1969)*

# BILATERAL INTERACTION OF VAGAL VASOMOTOR REFLEXES

By

GY. SUCH and F. OBÁL

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received September 12, 1968)

In cats anaesthetized with chloralose, the central stump of both sectioned vagus nerves was stimulated separately and simultaneously using identical stimulus parameters. The blood pressure response allowed the following conclusions.

(1) In the case of identical and homogeneous depressor and pressor reflexes the interaction may be manifested with both occlusion and facilitation;

(2) Unilateral stimulation activates only a fraction of bilateral reflex capacity;

(3) Bilateral reflex processes resulting in changes in the same direction act synergetically;

(4) Reflexes which are not homogeneous but give rise to changes in the same direction, when occurring simultaneously, may result in inhibition;

(5) The so-called bilateral antagonism is not identical with the antagonism of symmetrical reflex centres. In such cases the two vagi do not activate analogous mechanisms, and the bilateral interaction usually results in algebraic summation, and occasionally in facilitation.

It has been shown earlier (SUCH et al. 1958) that in the anaesthetized cat alternative stimulation with the same parameters of symmetrical nerves or receptor fields mostly results in vasomotor responses of variable intensity In the case of the vagus nerves the variability may manifest itself in responses of different direction. The eventual role of the interaction of nervous processes elicited by the separate stimulation of symmetrical afferent nerves has not been established satisfactorily. Therefore, in the present experiments attempts have been made to study the problem of the bilateral interaction of vasomotor reflexes elicited by stimulation of the two vagi. The question bears particular importance in view of the observations of GRASTYÁN et al. (1952) in that separate stimulation of the vagal stumps gives rise to antagonism in the bilateral centres.

Considering that nervous interactions are highest in intensity when simultaneous processes occur in the CNS. The effect of separate vagal stimulation on blood pressure has been compared to the response given to the simultaneous stimulation of the vagus nerves. The obtained results served as a basis for conclusions concerning the characteristics of central nervous mechanisms mirorring themselves in reflex responses.

## Methods

Experiments were undertaken in 25 cats. Anaesthesia was induced by α-chloralose; or in some cases by thialbarbital + α-chloralose, or pentobarbital given intraperitoneally, or intravenously under light ether anaesthesia. Observations were made also on unanaesthetized cats immobilized with gallamine triethiodide. Both vagi were exposed on the neck at a length of about 3 cm, and then divided at the level of the basis of the neck. The central stumps were stimulated with silver electrodes, with a distance of about 2.5 to 3.0 mm between the two poles. To minimize the spreading of the stimulus, liquid paraffin was used as an insulator under the electrodes. Square-wave pulses were produced by a two-channel DISA-Multistim apparatus. Blood pressure was recorded in the femoral artery by a mercury manometer. Respiratory movements were recorded after inserting a cannula into the trachea, using a Marey capsula and a Palmer kymograph. To inhibit coagulation of blood, 500 I.U. per kg of body weight of heparin were given intravenously.

## Results

### *Bilateral interaction of depressor responses*

In light or medium depth chloralose anaesthesia, stimulation of the central vagal stumps induces a depression of blood pressure which cannot be abolished with atropine and is due to the inhibition of central vasoconstrictor tone. Bradycardia which could eventually be causally related to the decrease in blood pressure has not been observed in these experiments. Figure 1 shows a typical example of the bilateral interaction of the depressor vasomotor responses. Separate stimulation of the both vagal stumps for periods of 10 sec elicited a drop in blood pressure which was similar in pattern but slightly different in intensity when comparing the responses obtained from the two sides; the depressor reaction was invariably associated with apnoea. The responses to simultaneous stimulation were greater than any of the separate reactions, but in intensity they were smaller than the sum of the latter. The lesser effectivity of simultaneous stimulation, as compared to the sum of separate responses, could not be attributed to haemodynamic factors since the lowest value observed after simultaneous stimulation was approximately 100 mm Hg, whereas the lowest value to which blood pressure can be reflectorily reduced is 40 mm Hg. In other experiments stimuli which caused a 5 to 10% reduction of blood pressure also gave rise to occlusion when applied simultaneously. Unfrequently and particularly with responses of low intensity, simultaneous stimulation resulted in an algebraic summation of the separate responses or, eventually, in slight facilitation (Fig. 2). The interrelationship in these experiments was rather variable; it was considerably affected by the parameters of the stimulus and, very likely, the reactivity of the centres. Table I summarizes the relationship between the stimulus parameters and the bilateral interrelations in 3 cats. Although on 50 c/s stimuli in two cats facilitation appeared this response was not a typical one, since occlusion was observed

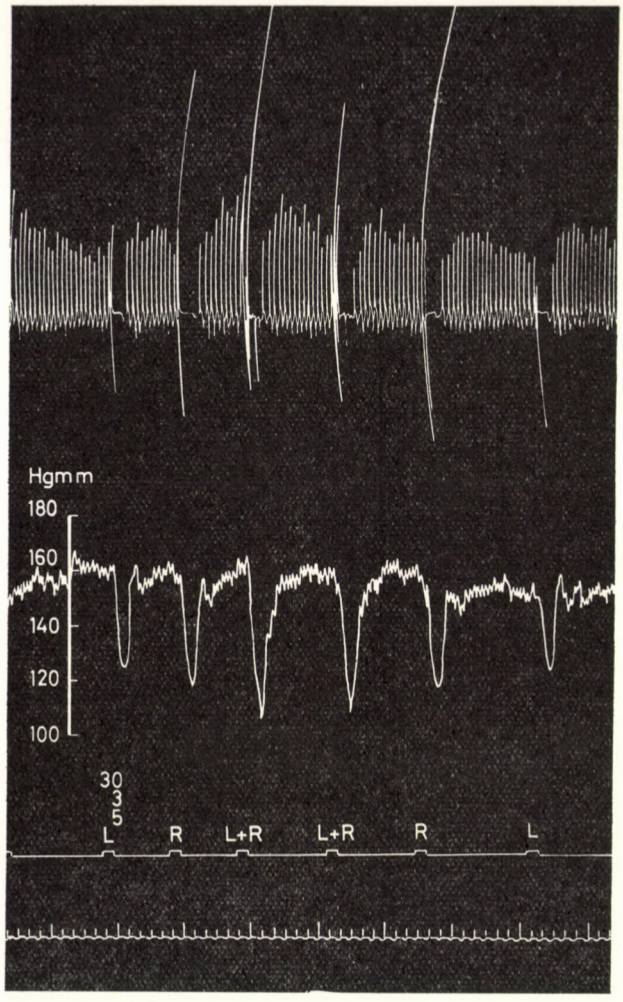



*Fig. 1.* Cat weighing 4.8 kg, anaesthetized with 50 mg/kg of intraperitoneal chloralose. The bilateral interaction of depressor responses manifested itself with occlusion. Tracings from top to below, represent respiration, blood pressure, duration of stimulus, 10-sec time marking. *R*, stimulation of right vagus; *L*, stimulation of left vagus; *R* + *L*, simultaneous stimulation of both vagi. Stimulus parameters: 30 c/s, 3 msec, 5 V

in much more instances, and facilitation of the depressor response did not occur except for the 3 cases represented in Table I.

Bilateral interrelations manifested themselves occasionally with a prolongation of normalization time.

Bilateral synergism is illustrated in Fig. 3. In this experiment, blood pressure was kept at a low value by continuous stimulation of the right vagus nerve, then a contralateral stimulus of 10 sec duration was applied at the lowest

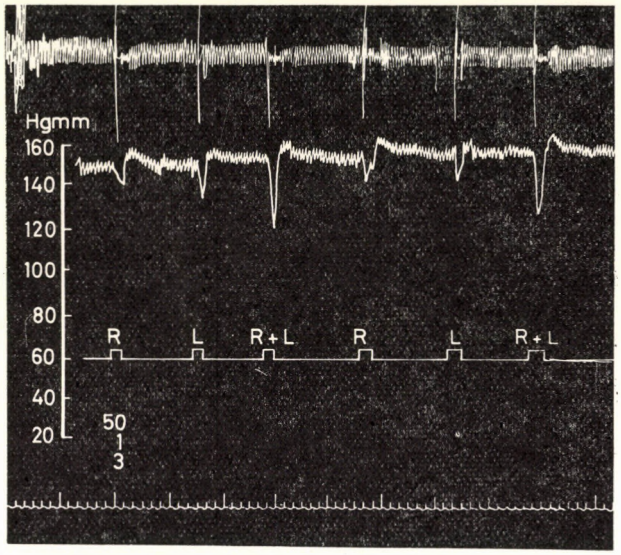



*Fig. 2.* Cat weighing 3.8 kg, anaesthetized with 45 mg/kg of intravenous chloralose. Moderate bilateral facilitation. Stimulus parameters: 50 c/s, 1 msec, 3 V

**Table I**

*Bilateral interaction of vagal depressor responses to stimuli at different parameters*

| Stimulus parameters | *R* | *L* | *B* | $B-(R+L)$ |
|---|---|---|---|---|
| Cat weighing 3.2 kg, anaesthetized with 45 mg/kg of intravenous chloralose | | | | |
| 5 c/s, 1 msec, 1 V | 8 | 9 | 12 | −5 |
| 10 c/s, 1 msec, 1 V | 8 | 12 | 14 | −6 |
| 50 c/s, 1 msec, 1 V | 8 | 10 | 20 | +2 |
| 100 c/s, 1 msec, 1 V | 8 | 10 | 14 | −4 |
| Cat weighing 3.8 kg, anaesthetized with 41 mg/kg of intravenous chloralose | | | | |
| 10 c/s, 1 msec, 2 V | 12 | 11 | 16 | −7 |
| id. | 11 | 10 | 18 | −3 |
| 20 c/s, 1 msec, 2 V | 9 | 8 | 20 | +3 |
| id. | 9 | 8 | 22 | +5 |
| Cat weighing 2.9 kg, anaesthetized with 40 mg/kg of intravenous chloralose | | | | |
| 10 c/s, 1 msec, 3 V | 7 | 7 | 10 | −4 |
| 25 c/s, 1 msec, 3 V | 8 | 9 | 12 | −5 |
| 50 c/s, 1 msec, 3 V | 10 | 14 | 30 | +6 |
| id. | 8 | 15 | 32 | +9 |

*Symbols. R*, stimulation of right vagus; *L*, stimulation of left vagus; *B*, simultaneous stimulation of both vagi. Negative sign indicates occlusion, positive sign, facilitation. Responses are given in mm Hg of blood pressure. Duration of stimulus: 10 sec.

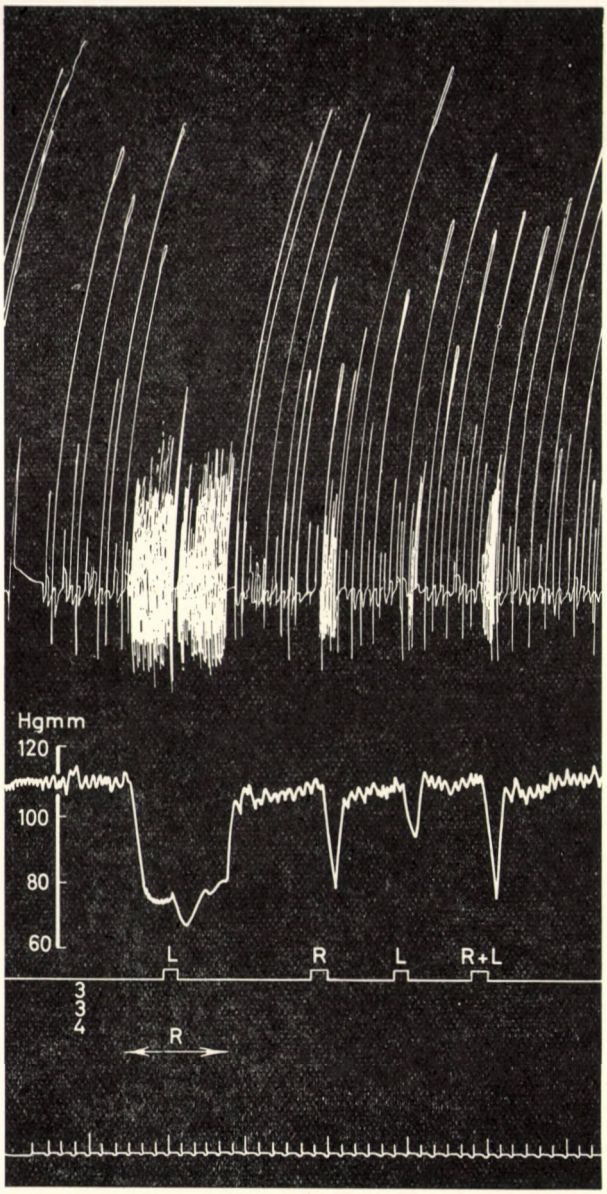



*Fig. 3.* Cat weighing 3 kg, anaesthetized with 50 mg/kg of intraperitoneal chloralose. For details, see text. Stimulus parameters: 3 c/s, 3 msec, 4 V

point of the blood pressure curve, resulting in further blood pressure reduction. Thus, with a stable stimulus parameter, the maximum reflectory response elicited from one side could be enhanced by contralateral stimulation, although, as seen in Figure 3, left vagal stimulation alone caused a reaction attaining about half of that elicited from the right side.

The reduction of the time interval between alternating stimuli had no appreciable effect on the intensity of the responses elicited from the two sides. The effect of alternating left and right vagal stimulation without any time interval between the stimuli induced, in the case of symmetrical responses, changes in blood pressure identical with those elicited by stimulation of any of the vagus nerves for the same length of time.

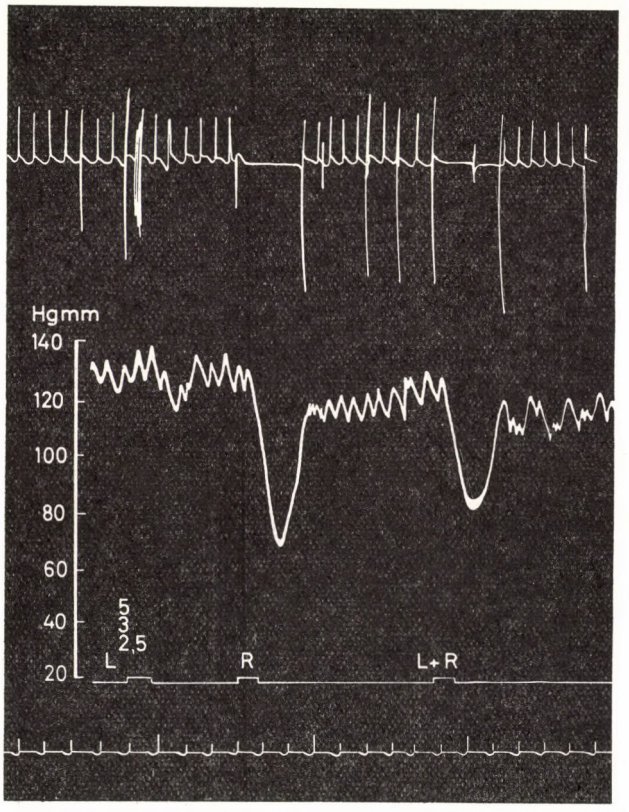



*Fig. 4.* Cat weighing 3 kg, anaesthetized with 45 mg/kg of intravenous chloralose. Inhibition-like bilateral interaction. Stimulus parameters: 5 c/s, 3 msec, 2.5 V

The bilateral interaction of strongly asymmetric and diverging depressor reactions may manifest itself in the form of inhibition (Fig. 4). In simultaneous combination, stimuli apparently ineffective on blood pressure may depress central nervous activity. In such cases there is reason to believe that unilateral vagal stimulation gives rise to inhomogeneous nervous processes, as indicated by the slight tachypnoea accompanying the blood pressure decrease elicited from the left side in Fig. 4.

*Interactions during so-called "bilateral antagonism"*

In superficial anaesthesia induced by 40 to 45 mg/kg of chloralose alternating stimulation of the two vagus nerves frequently elicited opposite responses. In other words, stimulation at identical parameters of one vagus caused

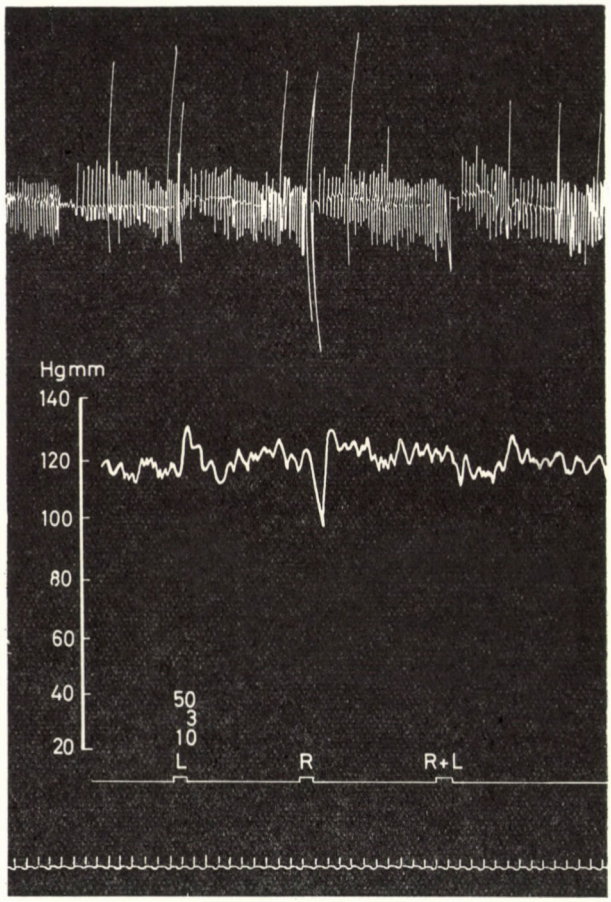



*Fig. 5.* Cat weighing 2.7 kg, anaesthetized with 40 mg/kg intravenous chloralose. Arithmetical summation of opposite vagal responses. Stimulus parameters: 50 c/s, 3 msec, 10 V

a depressor response, whereas from the other side a pressor reaction was evoked. This phenomenon was a stable property and the antagonism of the two sides could not be reversed by increasing the voltage of the stimulus. In these cases the vagus from which the depressor response was elicited played a predominating role, whereas the pressor reactions evoked from the contralateral side were less stable and the rise in blood pressure turned transitorily

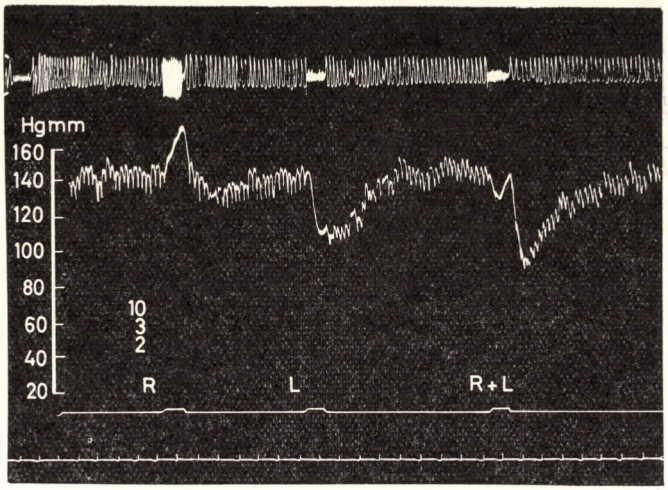



*Fig. 6.* Cat weighing 3.2 kg, anaesthetized with 45 mg/kg of intraperitoneal chloralose. Vasomotor responses in opposite directions. The intense after-discharge of the depressor reaction has modified the arithmetical summation of the two responses. Stimulus parameters: 10 c/s, 3 msec, 2 V

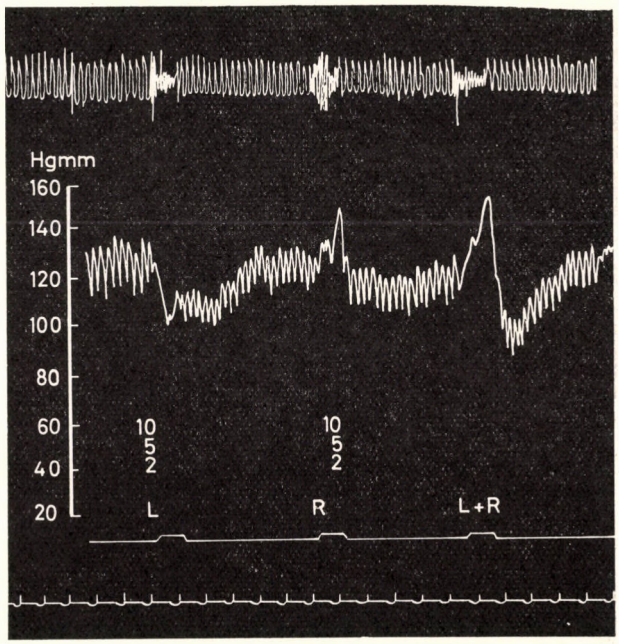



*Fig. 7.* Cat weighing 3.2 kg, anaesthetized with 45 mg/kg of intraperitoneal chloralose. Facilitation of pressor response in the case of opposite reactions. Stimulus parameters: right vagus (1), 10 c/s, 5 msec, 2 V; left vagus (2), 10 c/s, 3 msec, 2 V

into a decrease if bilateral stimulation was applied or the stimulus parameters were altered.

The interaction of reflex processes in the opposite direction usually resulted in algebraic summation (Fig. 5), although differences in the time course

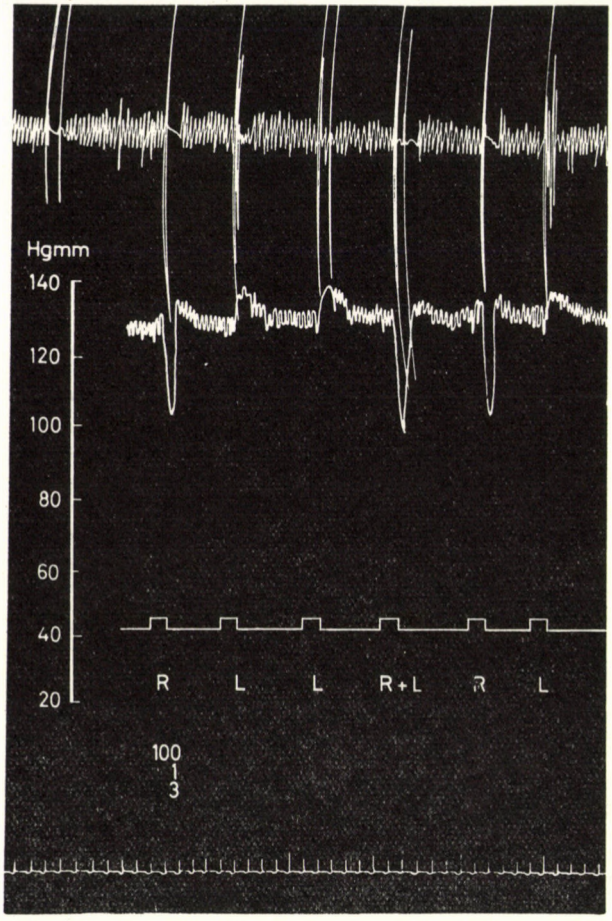



*Fig. 8.* Cat weighing 3.8 kg, anaesthetized with 40 mg/kg of intravenous chloralose. Bilateral interaction opposite of reflexes manifested itself with facilitation of the depressor response. Stimulus parameters: 100 c/s, 1 msec, 3 V

of the heteronomous reflex responses could considerably alter the result. The secondary decrease in blood pressure observed after the cessation of simultaneous bilateral stimulation (Fig. 6) may be accounted for by the intense afterdischarge of the depressor mechanisms. At the same time, however, in the case of responses to simultaneous stimulation, summation occurs not only with opposite responses but also with simultaneous vasodilator reactions.

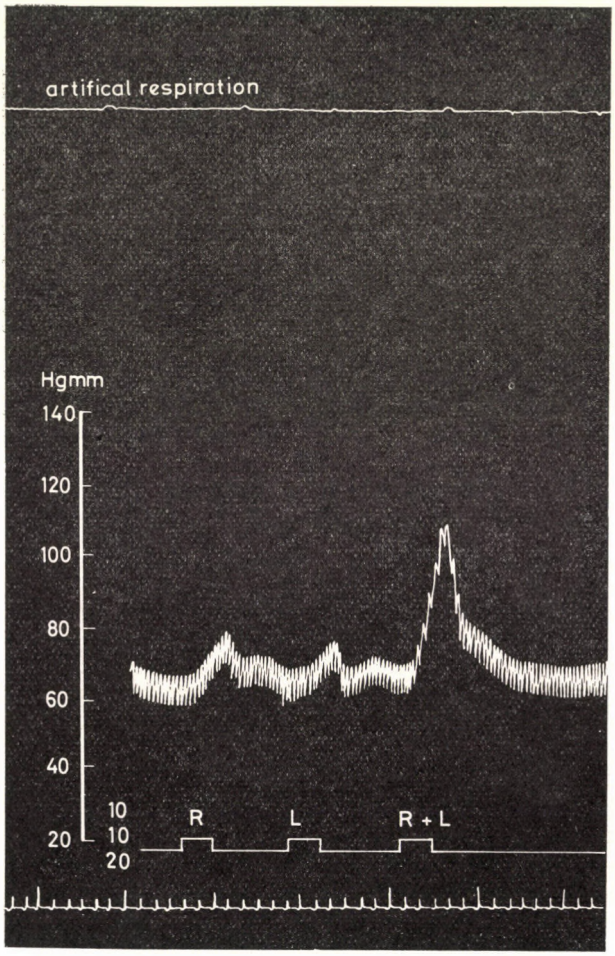



*Fig. 9a*

A particular manifestation of the interrelationship of opposite reactions is facilitation. Figure 7 demonstrates the augmentation of the pressor, whereas Fig. 8 that of the depressor response when combined with simultaneous contralateral opposite vasomotor responses.

### *Bilateral interaction of vago-pressor reflex mechanisms*

It has been shown earlier (PÓRSZÁSZ and SUCH 1958) that the administration of additional doses of chloralose to a cat already under medium depth anaesthesia results in a complete blocking of the depressor mechanisms, and vagal stimulation results in a pressor response. The bilateral interaction of

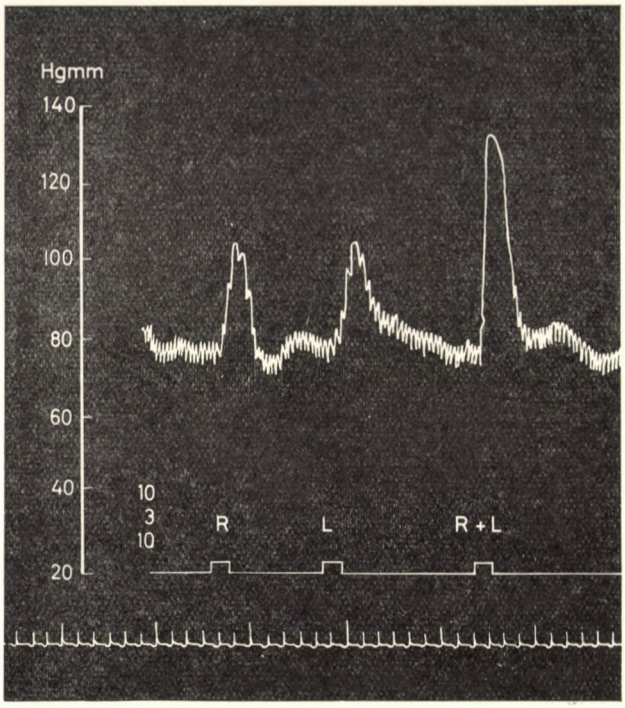



*Fig. 9.* Cat weighing 3.6 kg, anaesthetized with 70 mg/kg of thialbarbital intraperitoneally, and additional intravenous chloralose, 4 times 20 mg/kg. Vagopressor reflexes show facilitation and addition. Stimulus parameters: *A*, 10 c/s, 10 msec, 20 V; *B*, 10 c/s, 3 msec, 10 V

vagal pressor reflexes is more likely to occur in the form of addition and facilitation than in the form of depressor responses (Fig. 9). Occlusion could be observed only with marked pressor responses, the increase in blood pressure being more than 30 mm Hg. The same holds for rebound-like pressor response.

The inversion of the reflex response, which can be attained not only by chloralose but also by an overdose of pentobarbital (DOUGLAS et al. 1960), does not occur immediately, and in the period of transition opposite responses may be elicited from the two vagi the interaction of which shows characteristics similar to those discussed above. In this state, however, the unstable side is always that from which the depressor response was elicited.

### *Experiments in unanaesthetized cats*

In cats immobilized with gallamine and respirated arteficially, stimulation of the vagus nerves 2 to 3 hours after the initial ether anaesthesia caused invariably a depressor response. A variety of combinations of the stimulus parameters failed to give rise to a pressor reaction. The interaction of bilateral

depressor reflex mechanisms, on the other hand, manifested itself with marked occlusion.

These observations are inconsistent with the earlier observations of GRASTYÁN et al. (1952) who showed that separate stimulation of the two vagal stumps causes opposite reflex responses particularly in the alert state. However, these authors applied only local anaesthesia and thus the inconsistency in the results may have been due to differences in the experimental conditions.

## Discussion

Data concerning the central interaction of symmetrical vegetative afferentation are scarce. GRASTYÁN et al. (1952) studied the effect of separate and simultaneous vagal stimulation on blood pressure, cortically evoked motor reactions, and cortical electrical activity. In these experiments, however, the effect of separate and simultaneous stimulation were compared only in a few cases, therefore, the manifestations and variations of bilateral interrelations could unfrequently be established. In a study devoted to the cardioinhibitory reflex originating from the carotid sinus of the dog SCOTT and REED (1955) succeeded in demonstrating that the response obtained after bilateral simultaneous stimulation was usually greater than the sum of the responses after separate stimulation of the carotid sinuses, and occlusion was frequent. Acute unilateral vagotomy reduce only by about 50% the intensity of the response to ipsilateral carotid sinus stimulation. From these findings the occurrence of convergence and functional crossing in the medullary reflex centres was assumed. FROLKIS (1954) found that after adaptation of the oculocardiac reflex elicited from one eye, stimulation of the contralateral eye did not substantially affect the heart rate; this two may point to a marked central overlapping.

Arterial blood pressure, the only parameter studied in the present experiments, constitutes an indirect measure of the activity of the vasomotor centres, and the obtained reflex vasomotor responses may be regarded as the net effect of the changes in peripheral resistance. The results, however, unequivocally demonstrated that, in the case of homogeneous reflex responses in the same direction, separate stimulation of the vagus nerves activates only a fraction of the total bilateral reflex capacity. It has been shown that the identical central neuronal areas innervated by the two vagi act synergetically (Fig. 3), and it is most likely that in the bilateral organization of the vagal vasomotor centres and the neurones participating in the vagal vasomotor reflexes the principles of convergence and divergence are both effective. The occlusion of the reflex mechanisms may be regarded as a functional phenomenon as clearly indicated by the results obtained with simultaneous stimulation. The partial

central decussation of vagal afferentation has been proved also by electrophysiological studies (HARRISON and BRUESCH 1945; ANDERSON and BERRY 1956; PORTER 1963; STEFANTSOV and GONCHAROVA 1968).

As already mentioned, there is some minor difference in the bilateral interrelation of the pressor and depressor responses, since depressor reactions show a tendency to occlusion whereas pressor ones to facilitation and addition. The difference allows the assumption that the two vasomotor mechanisms are integrated on the basis of different principles. Most likely, the greatest differences are in the central organization. This is supported by morphological data concerning the structure of the reticular formation (OLSEWSKI 1956; BRODAL 1958), as also by the observation of OBERHOLZER (1960) in that the central transmissions of the depressor afferents originating in the carotid sinus and the aortic arch can easily be distinguished, despite abundant vertical overlapping. The findings of SCOTT and REED (1955) mentioned before also support this concept; these authors have shown that, on bilateral stimulation, the carotid sinus cardioinhibitory reflex results in facilitation.

The bilateral interaction of responses of different character and direction can be interpreted as a manifestation of the algebraic summation of heteronymous nervous processes, although the prevalence of inhibition and mutual facilitation in some cases indicates that other factors may also be at play.

The interpretation of "bilateral antagonism" presented by GRASTYÁN et al (1952) according to which stimulation of the vagus nerve causes sympathetic predominance by exerting an anelectronic effect on the contralateral centre, seems to be contradicted by the present results. In the case of bilateral antagonism it is not the symmetrical and identical centres which act antagonistically, for the two vagi do not activate analogous mechanisms; consequently, the phenomenon is a simple reflex antagonism. Electrical stimulation of the vagus stumps invariably causes an inhomogeneous afferentation, and the actual functional state of the centres and the intensity of the two opposite nervous processes will determine which of the inputs becomes predominant. The possibility cannot be excluded that in the transitory phases of anaesthesia the equilibrium of the bilateral central processes is disturbed and, in turn, reactions may manifest themselves in opposite directions. For this assumption speaks that opposite responses appeared in the so-called transitory phase only, and only under such conditions was it possible to cause a reversal of the response by changing the stimulus intensity on the side which functionally seemed to be more labile. In the alert state as well as in moderate or deep anaesthesia heterogeneous reactions are absent.

As with alternate stimulation no bilateral interaction could be demonstrated even when the interval between the stimuli was gradually decreased, we are inclined to believe that the bilateral interference does not play any definite role in the production of opposite and uneven blood pressure responses.

## LITERATURE

ANDERSON, F. D., BERRY, C. M.: An oscillographic study of the central pathways of the vagus nerve in the cat. J. comp. Neurol., **1956,** 106: 163—182.

BRODAL, A.: The reticular formation of the brain stem. Anatomical aspects and functional correlations. Oliver and Boyd, Edinburgh **1958.**

DOUGLAS, W. W., INNES, I. R., KOSTERLITZ, H. W.: The vasomotor responses due to electrical stimulation of the sinus and vagus nerves of the cat and their modification by large doses of sodium pentobarbital (Nembutal). J. Physiol. (Lond.), **1950,** 111: 215—230.

FROLKIS, V. V. (Фролькис, В. В.): Анализ механизмов адаптации безусловных рефлексов на сердце по изменению условно-рефлекторной деятельности. Журн. высш. нерв. деят. **1954,** 4 : 705—711.

GRASTYÁN, E., HASZNOS, T., LISSÁK, K., MOLNÁR, L., RUZSONYI, Z.: Activation of the brain stem activating system by vegetative afferents. Acta physiol. Acad. Sci. hung. **1952,** 3: 103—122.

HARRISON, F., BRUESCH, S. R.: Intramedullary potentials following stimulation of the cervical vagus. Anat. Rec. **1945,** 91: 280.

OBERHOLZER, R. J. H.: Circulatory centers in the medulla and midbrain. Physiol. Rev. **1960,** 40 Suppl. 4, 179—195.

OLSEWSKI, J.: The cytoarchitecture of the human reticular formation. In: DELAFRESNAYE, J. F. (Ed.): Brain Mechanisms and Consciousness. Blackwell, Oxford **1956.**

PÓRSZÁSZ, J., SUCH, G.: The reflectory chronaxie of vasomotor reflexes. Acta physiol. Acad. Sci. hung. **1958,** 14: 13—26.

PORTER, R.: Unit responses evoked in the medulla oblongata by vagus nerve stimulation. J. Physiol. (Lond.) **1963,** 168: 717—735.

SCOTT, J. C., REED, E. A.: Comparation of unilateral with bilateral stimulation of the carotid sinus cardioinhibitory reflex. Amer. J. Physiol. **1955,** 181: 27—29.

STEFANTSOV, B. D., GONTCHAROVA, L. S. (Стефанцов, Б. Д., Гончарова, Л. С.): К вопросу о надежности в деятельности некоторых парных образований продолговатого мозга животных. Физиолог. Ж. СССР. **1968,** 54 : 398—405.

SUCH, G., VICSAY, M., PÓRSZÁSZ, J.: The problem of symmetry in the vasomotor reflex activities. Acta physiol. Acad. Sci. hung. **1958,** 14: 27—38.

György SUCH, Ferenc OBÁL
Orvostudományi Egyetem Élettani Intézete,
Szeged, Dóm tér 10, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 271—283 (1969)*

# STOPPING ELICITED BY CONDITIONED STIMULI AS COMPARED WITH THE THALAMIC INDUCED ARREST REACTION

By

L. ÁNGYÁN, MARGIT RÓZSAVÖLGYI and P. KOLTA

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received September 13, 1968)

A stopping, resembling the arrest reaction elicited by electrical stimulation of midline thalamic nuclei, has been regularly produced by the simultaneous or successive presentation of approach and avoidance conditioned stimuli. Both appearance and duration of the immobility showed a close correlation with the strength of the conditioned responses as measured by their corresponding latencies. Small and great differences in strength between the conditioned responses brought into collision seemed to determine the short or long duration of the stoppings.

A similarity was found between the accompanying neocortical and hippocampal electrical activities of the thalamic induced arrest reaction and those of the stopping elicited by incompatible conditioned stimuli, both activities exhibiting desynchronization regularly intermingled with, and/or superimposed on, slow waves.

On the basis of the findings the possible mechanisms of stopping and of the arrest reaction are discussed. It is suggested that the different types of cessation of spontaneous motor activity might primarily be related to different intensities of the central excitation, producing a collision between incompatible tendencies to move.

The sudden arrest of movement produced by stimulation of midline thalamic nuclei with definite intensity was described by HUNTER and JASPER (1949) as arrest reaction and subsequently observed by several authors (cf. KAADA 1960; DELGADO 1964). On the basis of previous studies (ÁNGYÁN and GRASTYÁN 1965; GRASTYÁN and ÁNGYÁN 1967a) it has been suggested that the arrest reaction is produced by a collision between antagonistic movement-patterns and appears at a moment when stimulation has induced approximately identical excitement in the two hemispheres, as reflected by their cortical electrical activity (ÁNGYÁN et al. 1967). Since both in our own studies and in those of others the arrest reaction was elicited by electrical or chemical stimulation of brain structures, the question arose whether there was any similarity between the electrically elicited arrest reaction and the naturally induced stopping, or if the arrest reaction was an arteficial phenomenon without any physiological significance. The aim of the present experiments was, first, to study the conditions of stopping by natural (sound) conditioned stimuli and, second, to compare the arrest reaction elicited by electrical stimulation of midline thalamic nuclei with the stopping produced by conditioned stimuli.

## Methods

The experiments were carried out on six adult cats with chronically implanted electrodes. Epidural silver ball electrodes were placed on symmetrical frontal points of the two hemispheres, and bipolar stainless steel electrodes were implanted by the conventional stereotaxic technique in the dorsal hippocampus and the midline thalamic nuclei.

After allowing the cats to recover from the operation, approach and avoidance conditioned reflexes were elaborated simultaneously in a suitable experimental cage (CZOPF et al. 1964). To one side of the cage (Fig. 1) an automatic feeding device was fixed from which

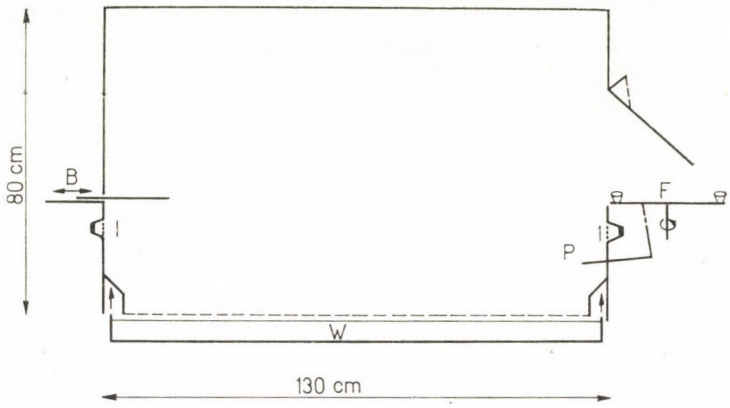



*Fig. 1.* Diagram of the experimental cage. Abbreviations: *B* — bench; *F* — feeding device; *l* — loudspeaker; *P* — pedal; *W* — water

the animal could take a piece of meat through a window opened by pressing a pedal. As conditioned stimulus 10/sec clicks were given through a loudspeaker built into the cage wall under the window. On the opposite side a mobile bench served as a place of flight, and cold water flooding the floor of the cage through a metal grid as the unconditioned stimulus, for avoidance conditioning. In two cats electric shock was also applied as unconditioned stimulus because their reaction to water was inadequate. The harmful effects of a painful electric shock delivered through a metal grid make it difficult or impossible simultaneously to elaborate an approach conditioned reflex in the same situation. Therefore the electric shock was presented through the water flooding the cage floor, by connecting one of the poles of a 70—100 $\mu$A power supply to the water and the other pole to a wound clip fixed to the back skin of the cat. In this arrangement the electric shock did not hinder the simultaneous elaboration of an approach conditioned reflex. The avoidance conditioned stimulus was a 1000/sec tone given through the loudspeaker built into the cage wall under the bench.

Both during the elaboration of conditioned reflexes and in further trials the avoidance and approach conditioned stimuli were presented in a random sequence. Care was taken to deliver the conditioned stimuli when the animal was staying in the centre of the cage. The latency of the conditioned responses was used for comparing the response strengths of the conditioned reflexes. The animal's movements were recorded by a special device fixed to its head (SZABÓ et al. 1965). After completion of the experiments the animals were sacrified and the location of the electrodes was verified histologically.

## Results

After the two conditioned reflexes had been established in the experimental situation, two movement tendencies, incompatible in their direction, were regularly elicitable with the appropriate conditioned stimuli. It is assumed

that any conflict produced by two or more incompatible reaction tendencies may result in a stop of the animal's movement (MILLER 1959; DIAMOND et al. 1963). On the basis of this assumption and our previous hypothesis that the arrest reaction is produced by a collision between antagonistic movement patterns it was expected to produce immobility by bringing the two conditioned stimuli into collision.

### *I. Simultaneous presentation of the conditioned stimuli*

In response to simultaneously presented conditioned stimuli the animal became, either immediately or after some searching movements, temporarily motionless, after which it started in the direction of one or the other goal. In most cases the start was rapid and definite, but occasionally it was accompanied by searching or hesitation. In some cases, after starting in the direction of one of the goals, the cat stopped, remained motionless for a short time, then turned towards the other goal. In its overt manifestation this stopping and motionless posture resembled the arrest reaction elicited by electrical stimulation of brain structures.

Appearance and duration of the immobility seemed to be related to the latencies of the conditioned reflexes. The appearance of immobility was also influenced by the actual environmental stimuli especially in the case of short latencies. If the animal was looking at one of the goals, it was impossible to arrest it by the simultaneous presentation of the opposite conditioned stimulus. On the other hand, if the animal was not influenced by any of the goals the immobility regularly appeared also with short latencies. The mean duration of immobility was 6.7 ($\pm$2.1) seconds. The longest duration of immobility measured in this series of experiments was 35 seconds. When the immobility ceased the animal most frequently (76%) performed that conditioned reflex which had the shorter latency. If the animal was looking at one of the goals at the onset of sound stimulation it immediately ran there with a shorter latency than earlier. Occasionally, mainly after immobility of longer duration, the animal sat down instead of performing any of the conditioned reflexes.

### *II. Successive presentation of the conditioned stimuli*

The duration of immobility was easy to measure when the conditioned stimuli were presented in succession. The first conditioned stimulus elicited the appropriate locomotion during which the second conditioned stimulus was presented; simultaneously the first one was switched off. By the onset of the second conditioned stimulus the animal was halted and remained motionless in its existing position. Then it either continued the interrupted movement or turned in the opposite direction and performed the conditioned reflex

corresponding to the second stimulus. The fact alone that the second stimulus was an avoidance or an approach conditioned stimulus did not affect the induction of stopping.

It is obvious from the graphic representation of a daily training session that the appearance of stopping varied with the latency of the conditioned response (Fig. 2).

For a more detailed analysis, 200 collisions between incompatible movement tendencies elicited by successive presentation at random of the two con-

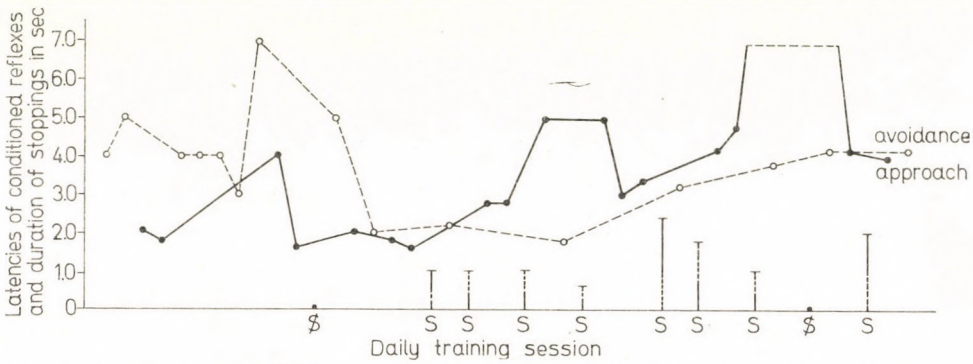



*Fig. 2.* Curves constructed from the latencies of approach and avoidance (dotted line) conditioned reflexes of a daily training session. The duration of the stopping *(S)* elicited by successive presentation of the conditioned stimuli is shown by vertical lines the lower part of which represents the first, and the upper one the second, conditioned stimulus. When the curves diverge, no stopping occurs

ditioned stimuli were chosen from a material of about 500 such cases. The only requirement in sampling was that both avoidance and approach reinforcements should have occurred among three reinforcements given in random sequence before the collision, the difference in latency between the members of a pair of two different reactions nearest to the collison was used for further analysis. Table I summarizes some of the characteristics of the evaluated cases.

Taking into consideration the limitation of measuring time, the latency differences were ranged in 1 second intervals. The distribution of stoppings in these intervals is shown in Fig. 3, which indicates that the stopping appeared most frequently when the difference between the latencies of the approach and avoidance conditioned responses preceding the collision was below 1.0 second.

Besides the absolute value of the latency difference the appearance of a stopping was also influenced by the time sequence of the conditioned stimuli. It was decisive whether before the collision the conditioned response corresponding to the second conditioned stimulus had the shorter or longer latency. The intervals between the latency differences were therefore ranged according

**Table I**

| | | Number of cases |
|---|---|---|
| Total evaluated | | 200 |
| The second conditioned stimulus was | approach | 56 |
| | avoidance | 144 |
| Measurable stopping | | 149 |
| The performed response corresponded to | the first stimulus | 17 |
| | the second stimulus | 146 |
| | both stimuli (in succession) | 37 |

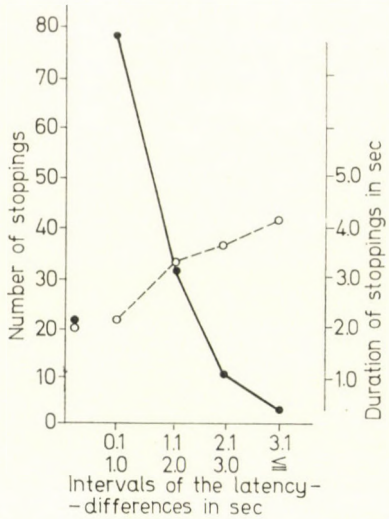



*Fig. 3.* Distribution of stoppings and their duration (dotted line) in the intervals of the latency differences. 22 stoppings with a mean duration of 2.1 seconds were preceded by equal latencies of approach and avoidance conditioned reflexes

to the latency of the conditioned reaction corresponding to the second conditioned stimulus minus the latency of the conditioned reaction corresponding to the first conditioned stimulus ($LCR_2 - LCR_1$); the distribution of stoppings in the intervals is shown in Fig. 4. On the basis of this distribution it could be concluded that the appearance of a stopping is most probable when the latency of the conditioned response corresponding to the second conditioned stimulus is at most 1.0 second shorter than that of the first conditioned stimulus.

The mean duration of stoppings belonging to the individual intervals of the latency differences were calculated. Fig. 3 shows that longer immobilities were linked with longer differences between the latencies.

As to the above mentioned comparison ($LCR_2 - LCR_1$) a more precise conclusion could be drawn. It was found that a longer immobility was coupled with a longer latency difference only if before the collision the conditioned

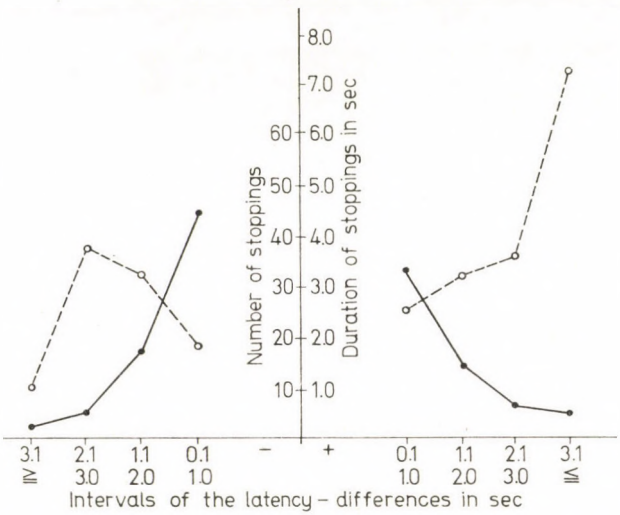



*Fig. 4.* Distribution of stoppings and their duration (dotted line) in the intervals of the latency differences, as calculated from $LCR_2$-$LCR_1$ (see in the text)

response corresponding to the second conditioned stimulus had a longer latency than the conditioned response corresponding to the first conditioned stimulus (Fig. 4), i.e. when performance of the response corresponding to the stronger tendency was arrested by the weaker tendency. In the opposite case, however, if the response corresponding to the weaker tendency was arrested by the stronger tendency, the duration of immobility decreased in parallel with the increase (above a certain level) of the latency difference.

### *III. Changing the intensity, location and duration of the conditioned stimulus*

By increasing the intensity of a conditioned stimulus the latency of the conditioned response was temporarily shortened and a stopping appeared in accordance with the above findings. When the location of the conditioned stimulus was inverted, e.g. the avoidance conditioned stimulus was applied through a loudspeaker placed under the feeding device, first an orientation reaction occurred, and then the conditioned response corresponding to the

stronger tendency was performed. If stopping appeared, its duration was generally short. Simultaneous application of the same conditioned stimulus through two loudspeakers often elicited a stopping. In the case of a great response strength, stopping was produced also by the avoidance conditioned stimulus applied at the place of flight. If the response strength was weaker, only an orientation reaction took place.

Stopping occurred also if the conditioned stimulus was switched off immediately after the animal had started for the goal. If the response strength was great, performance of the conditioned reaction was not influenced by cessation of the conditioned stimulus. In the case of a somewhat weaker response strength, however, a long-lasting stopping appeared on cessation of the conditioned stimulus, after which the appearance of the conditioned reaction depended on the response strength.

### *IV. Extinction*

Stoppings also appeared in the course of extinction. During the initial period of extinction (the first 3—5 non-rewarded trials) latency was prolonged but the animal still approached the goal without stopping on its way there. If the non-rewarded trials were continued after some searching or hesitation the animal stopped, remained motionless for a while, then either continued the interrupted response or it sat down, turned away from the goal and began to groom itself. During the last period of extinction searching or sluggish motion were only observed.

#### a) *EEG during Stopping*

The electrical activities of the dorsal hippocampus and the frontal cortical areas were recorded. The EEG recorded during conditioning corresponded to data in the literature (for reference, see GALEANO 1963).

At the appearance of a stopping, hippocampal desynchronization was periodically interrupted by groups of slow waves (Fig. 5 *b*, *c*, *d*, *e)*. If the stopping was the product of a collision between stronger incompatible movement tendencies the low amplitude fast activity was intermingled and superimposed on the slow (3—5/sec) waves. Also, cortical desynchronization was regularly intermingled with slow waves. Similar EEG changes, a decrease of the average frequency and an increase of the average amplitude, were observed in rats when in a maze they had reached a point where they had to decide which way to turn. After the decision had been taken, the frequency of the potentials increased while their amplitude diminished (YOSHII and TSUKIJAMA 1952).

As Fig. 5*e* shows, the EEG recorded during a stopping in the course of extinction resembled the above described recording. This is in agreement

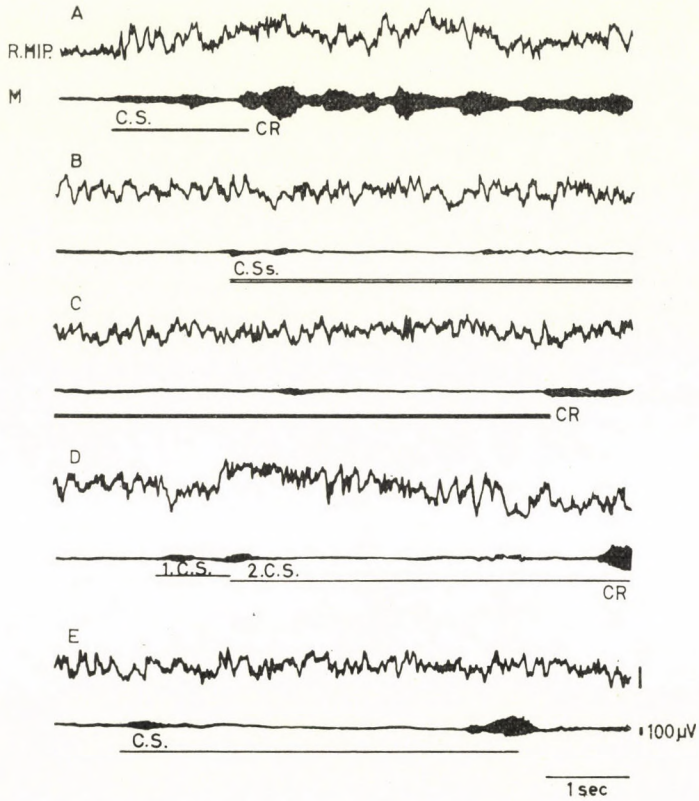



*Fig. 5.* Hippocampal electrical activity during alimentary conditioning *(A)*, simultaneous *(B* and *C* — continuous recording) and successive *(D)* presentation of conditioned stimuli, and extinction *(E)*. *CS* — conditioned stimulus; *CR* — conditioned reaction; *R Hip* — right hippocampus; *M* — recording of movements

with the observation of Roitbak (1960), who reported that during extinction of a conditioned reflex the EEG activation pattern became progressively weaker, and slow oscillations which were somehow associated with an inhibition of the corresponding pyramidal neurones appeared in the EEG.

### *b) EEG accompanying the thalamic induced arrest reaction*

During an arrest reaction elicited by high (100/sec) frequency stimulation, the accompanying hippocampal electrical activity showed 3—7/sec theta waves regularly interrupted by desynchronization (Fig. 6*b*, *c*). Low amplitude fast activity was often superimposed on the slow waves. In the frontal cortical recordings low amplitude fast activity dominated and was periodically interrupted by slow waves.

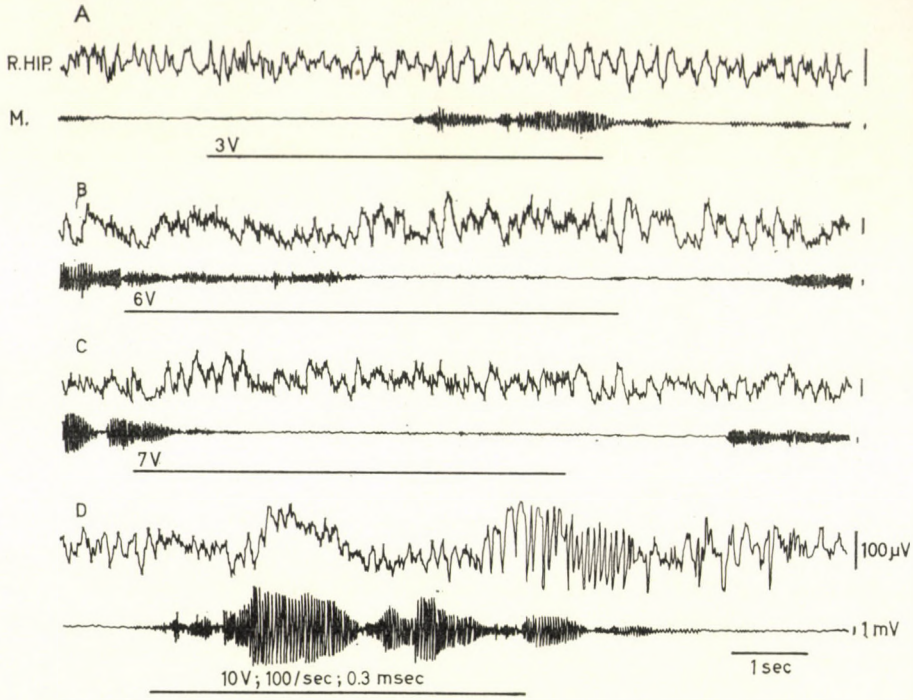



*Fig. 6.* Electrical signs of the behavioural manifestations elicited by stimulation of the same thalamic nucleus with different intensities.
*A* — orientation reaction; *B* — drinking of milk gradually brought to a stop; *C* — arrest reaction; *D* — running as direct effect and seizure as aftereffect of stimulation. *R Hip* — right hippocampus; *M* — recording of movements

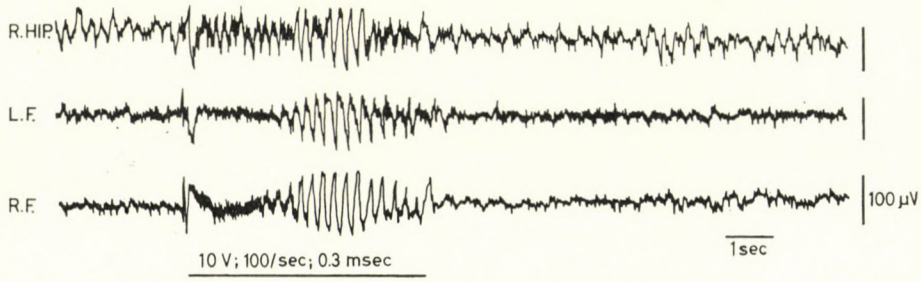



*Fig. 7.* A characteristic example of symmetrical cortical "spindles" elicited by stimulation of n. reuniens. *L F* and *R F* — left and right frontal cortex; *R Hip* — right hippocampus

In the case of suprathreshold intensity stimulation, a spindle-like activity composed of 3—7/sec waves was observed. If this spindle activity was present on both sides (Fig. 7) the arrest reaction appeared simultaneously with it, while if it occurred on one side, the arrest reaction appeared only after it, at a time when the spindle activity had been followed by desynchronization regularly interrupted by slow waves. Histological verification of the electrode

locations showed that in the former case the stimulating electrodes were in the midline, while in the latter they were situated somewhat laterally (1—3 mm) from the midline. If stimulation intensity was further increased, locomotion appeared as a direct effect, and the arrest reaction, accompanied by high amplitude slow waves (3—4/sec) intermingled with low amplitude fast activity, as an aftereffect of stimulation. Stimulation of longer duration or at somewhat higher intensity resulted in hippocampal seizure activity (Fig. 6*D*). During the hippocampal afterdischarges arrest reactions and short, quick, explosive runnings occurred, in agreement with the observations of LIBERSON and AKERT (1955). The character of the seizure activity changed in parallel with the behavioural manifestations. The arrest was accompanied by poly-spikes and waves or groups of spikes with intermittent silences, while when the spikes continuously dominated in the electrical activity, runnings or convulsions appeared. Following the suddenly terminated seizure activity, normal behaviour and electrical activity were resumed a long period.

## Discussion

The above findings show that cessation of spontaneous motor activity is regularly produced by simultaneous or successive presentation of conditioned stimuli inducing incompatible response tendencies. Both the appearance and the duration of stopping were primarily determined by the response strength. In the production of stopping, however, the relative response strength seemed to play a more important role than did the absolute strengths of the individual response tendencies.

Stopping was also elicited by one of the conditioned stimuli if its duration or location had been changed. Thus, on successive presentation of the conditioned stimuli the effect of cessation of the first conditioned stimulus was modified by the simultaneous onset of the second one, as originally observed by PAVLOV. This modifying effect of the second conditioned stimulus may offer an explanation for the case, too, when the probability and the duration of stopping change conversely. On discontinuing the conditioned stimulus eliciting a strong response tendency the animal's movements seldom ceased but if they did, their duration was increased by the simultaneous elicitation of the weaker incompatible response tendency. By reversing the original sites of the conditioned stimuli, mostly an expressed orientation reaction was observed in agreement with KARMOS et al. (1965), but occasional short stoppings also occurred.

If stopping appeared in the course of extinction it was most probably elicited by internal inhibition of the ongoing motor activity. Inhibition of an ongoing motor activity is assumed to be accompanied by disinhibition of the

opposite motor activity. So the stopping which appears in a definite phase of extinction is produced by the simultaneous persistance of, or collision between, inhibited and disinhibited movement tendencies.

DIAMOND et al. (1963) also emphasized that a conflict is essentially a failure of the inhibitory control of opposed response tendencies, leading to simultaneous disinhibition of incompatible activities.

The incompatible patterns may be firmly balanced for longer periods, as reflected by the electrical activity and by the animals behaviour, which is hesitant if the oscillation of the balance has a higher amplitude. If a change in response strength produced by external stimuli or internal inhibition upsets the balance, one of the incompatible tendencies becomes dominant. This suggestion agrees well with the observation made on birds in territorial conflict, in which they can keep their balance between attack and escape (MANNING 1967).

The stopping elicited by conditioned stimuli showed a striking similarity to the thalamic induced arrest reaction in its overt manifestation and in the accompanying electrical activity. In a previous study it was observed that the appearance of the arrest reaction elicited by low (3/sec) frequency stimulation of midline thalamic nuclei was accompanied by opposite changes in the potential complexes recorded from the symmetrical frontal cortical sites. These changes in potential complexes were supposed to reflect an ipsilateral feedback inhibition leading to contralateral disinhibition (ÁNGYÁN et al. 1967). A similar conclusion has been drawn from the appearance of the spindle-like slow waves followed by desynchronization with intermittent slow waves in the cortical activity elicited by high (100/sec) frequency stimulation (GRASTYÁN and ÁNGYÁN 1967a). Furthermore, a correlation was found between the localization of these slow wave spindles and the direction of movement (GRASTYÁN and ÁNGYÁN 1967b), since turning occurred towards the side of the slow waves. In agreement with this stimulation in the midline, eliciting slow wave spindles on each side, brought the animal to a stop.

On the basis of the above findings it was suggested that the arrest reaction appears at the moment when the excitement induced by stimulation is approximately at the same level in the two hemispheres. In other words, assuming that the two hemispheres are responsible for the organization of incompatible movement patterns, the arrest reaction is elicited by the simultaneous persistence of, or a collision between, antagonistic movement patterns of the same intensity.

The interpretation of the arrest reaction as a manifestation of collision between antagonistic movement patterns is supported by the observations of GRASTYÁN et al. (1965) who established that there are two elementary and antagonistic motor mechanisms (pull and push effects) among the apparently homogeneous motivational effects elicited by stimulation. In the accompany-

ing hippocampal electrical activity the said authors observed theta waves or desynchronization corresponding to the motor effects. The electrical activity occurring during the arrest reaction could be interpreted as a simultaneous activation of, or collision between, both (pull and push) mechanisms.

On the basis of this interpretation it seems reasonable to assume that similar mechanisms are responsible for the stopping elicited by conditioned stimuli and for the arrest reaction elicited by electrical stimulation of midline thalamic nuclei. Both of them are produced by a simultaneous activation of, or a collision between, incompatible movement tendencies of about the same intensity. The intensity of the incompatible tendencies may vary widely and, as a consequence, so may the behavioural manifestation of a collision between them. At a low intensity level only an orientation reaction appears. As the intensities of the incompatible tendencies increase and approximate each other, the animal stops in a tonic posture. In case of highly activated incompatible patterns brought into collision the induced central excitation may become generalized and a seizure occurs. MAIER (1961) also assumed that tension and cortical excitation may become acute and generalized during a conflict because the animal has no choice and few opportunities of expressing its frustrations. Thus, the different types of cessation of spontaneous motor activity seem to be primarily dependent on different intensities of central excitation producing a collision between incompatible movement tendencies.

On the basis of the above findings it seems that the thalamic induced arrest reaction is not an artificial phenomenon. Further studies are, however, required to clarify its physiological significance.

## LITERATURE

ÁNGYÁN, L., GRASTYÁN, E.: Some electrical correlates of drive processes elicited from median thalamic structures. Acta physiol. Acad. Sci. hung. **1965,** 26: 149—155.

ÁNGYÁN, L., KAJTÁR, P., SIK, E.: Correlation between thalamic-induced cortical spike and wave activity and behaviour in unrestrained cats. Acta physiol. Acad. Sci. hung. **1967,** 32: 291—306.

CZOPF, J., KARMOS, G., BAUER, M., GRASTYÁN, E.: Simultaneous elaboration of approach and avoidance conditioning in the same experimental situation for cats. Acta physiol. Acad. Sci. hung. **1964,** 25: 53—60.

DELGADO, J. M. R.: Free behavior and brain stimulation. Int. Rev. Neurobiol. **1964,** 6: 349—449.

DIAMOND, S., BALVIN, R. S., DIAMOND, F. R.: Inhibition and Choice, Harper and Row, New York **1963.**

GALEANO, C.: Electrophysiological aspects of brain activity during conditioning. A review. Acta neurol. latinoamer. **1963,** 9: 395—413.

GRASTYÁN, E., CZOPF, J., ÁNGYÁN, L., SZABÓ, I.: The significance of subcortical motivational mechanism in the organization of conditional connections. Acta physiol. Acad. Sci. hung. **1965,** 26: 9—46.

GRASTYÁN, E., ÁNGYÁN, L.: The organization of motivation at the thalamic level of the cat. Physiol. Behav. **1967**a, 2: 5—13.

GRASTYÁN, E., ÁNGYÁN, L. (Граштьян, Э., Андьян, Л.): Электрические коррелаты направления движений вызванных стимуляцией подкорковых структур. В. кн.

В. С. Русинов (Рег.) Современные проблемы электрофизиологии центральной нервной системы. Изд. Наука, Москва, 1967b. стр. 71—78.

HUNTER, J., JASPER, H. H.: Effects of thalamic stimulation in unanaesthetized animals. Electroenceph. clin. Neurophysiol. **1949,** 1: 305—324.

KAADA, B. R.: Cingulate, posterior orbital, anterior insular and temporal pole cortex. In: J. Field, H. W, Magoun and V. E. Hall/Eds./ Handbook of Physiology, American Physiological Society, Washington, D. C. **1960.** Section 1, Vol. II, Pp. 1345—1372.

KARMOS, G., GRASTYÁN, E., LOSONCZY, H., VERECZKEY, L., GRÓSZ, J.: The possible role of the hippocampus in the organization of the orientation reaction. Acta physiol. Acad. Sci. hung. **1965,** 26: 131—141.

LIBERSON, W. T., AKERT, K.: Hippocampal seizure states in guinea pig. Electroenceph. clin. Neurophysiol. **1955,** 7: 211—222.

MAIER, N. R. F.: Frustration: The Study of Behavior Without a Goal. University of Michigan Press, Ann. Arbor **1961.**

MANNING, A.: An Introduction to Animal Behaviour. Arnold, London **1967.**

MILLER, N. E.: Liberalization of basic S-R concepts: Extensions to conflict behavior, motivation and social learning. In: S. KOCH (Ed.) Psychology: A Study of a Science. Study 1, Vol. 2, McGraw-Hill, New York **1959,** Pp. 196—292.

PAVLOV, I. P.: A központi idegrendszer működésének a feltételes reflexek tanulmányozása során feltáruló legfontosabb törvényei. I. P. Pavlov Válogatott Művei, Akadémiai Kiadó, Budapest **1953,** Pp. 326—335.

ROITBAK, A. I.: Electrical phenomena in the cerebral cortex during the extinction of orientation and conditioned reflexes. In: JASPER, H. H., SMIRNOV, G. D. (Eds.): The Moscow Colloquium on Electroencephalography of Higher Nervous Activity, Electroenceph. clin. Neurophysiol. Suppl. 13, 91—100, **1960.**

SZABÓ, I., KELLÉNYI, L., KARMOS, G.: A simple device for recording the movements of unrestrained animals. Acta physiol. Acad. Sci. hung. **1965,** 26: 345—349.

YOSHII, N., TSUKIYAMA, K.: Electroencephalographic studies on conditioned behaviour of white rat. Jap. J. Physiol. **1952,** 2: 186—193.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 285—294 (1969)*

# BIOLOGICAL ROLE OF LATERAL HYPOTHALAMIC STRUCTURES PARTICIPATING IN THE CONTROL OF MATERNAL BEHAVIOUR IN THE RAT

## MOTILITY, EXPLORATIVE BEHAVIOUR, LACTATION, AND THE EFFECT OF REDUCED FOOD INTAKE

By

Z. AVAR and E. MONOS

SECOND DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,
AND EXPERIMENTAL RESEARCH DEPARTMENT, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 7, 1968)

Parafornical hypothalamic lesions in the rat known to inhibit maternal behaviour and to reduce vitality of the offsprings have been shown to exert no significant effect on the motility and explorative activity of pregnant rats. The lesion caused a transient reduction of food and fluid intake for several days. The mechanism of lactation did not seem to be directly affected by these structures. Restriction of food and water intake in intact pregnant rats has been shown to give rise to a response significantly different from the consequences of parafornical lesion.

It has been shown previously that bilateral electrolytic lesions applied in the parafornical area of the tuberoinfundibular region in primiparous rats on the 16th to 18th day of pregnancy inhibit the development of maternal behaviour. The food and fluid intake of these rats was depressed transiently and they gave birth to offsprings of reduced vitality. As a consequence of these lesions, nearly every offspring died within 48 hours after delivery (AVAR and MONOS 1966, 1967). Hypothalamic lesions were found to have no significant effect on the duration of pregnancy and the time of delivery (AVAR and MONOS 1964), an observation confirmed by HOLLOWAY and STEVENSON (1967).

There is reason to assume that disturbances in the motility of pregnant rats, in their explorative behaviour and lactation, and the reduction of food and water intake resulting from parafornical lesions would substantially influence the maternal behaviour of the animals after delivery. If this is the case, the disturbances in maternal behaviour are not directly related to the parafornical lesion. The present investigations have been undertaken to clarify this problem. In four groups of animals an attempt was made to answer the following questions.

(1) Is there any difference in the motility and explorative behaviour between pregnant rats subjected to parafornical hypothalamic lesions and sham-operated animals;

(2) Whether few days old viable offsprings delivered by intact mothers show a normal development when bred by rats with hypothalamic lesion;

(3) Whether the mammary glands display some histological difference between normal and lesioned pregnant rats;

(4) Whether restrinction of food and water in pregnant rats has the same effect on maternal behaviour and motility as parafornical lesions have.

## Methods

The experiments were performed on Wistar rats kept on a protein-rich synthetic diet. Virgin females in oestrus were placed for a night into cage of males; after 12 to 14 days, pregnancy was established by palpation and the pregnant females were placed in separate cages where temperature was adjusted between 18 and 24 °C. The back part of each cage was separated by a horizontal shelf provided with a rim. Food and water intake, body weight and rectal temperature were measured through the experiments. Soft paper strips were placed in the front part of the cage for nest building. The animals were controlled twice or three times daily. Time and course of delivery, the number of offsprings, their gain in weight or eventual death, were recorded. Nestbuilding, retrieving and nursing activies were recorded according to criteria described elsewhere (AVAR and MONOS 1967). Aggressivity of the mothers, cleaning of the offsprings and ingestion of the placenta were also observed.

Hypothalamic lesions were carried out on the 16th or 17th day of pregnancy, under intraperitoneal pentobarbital anaesthesia (35 mg/kg) using glass insulated unipolar silver electrodes introduced bilaterally by means of a KOVÁCH—SZENTÁGOTHAI stereotaxic apparatus; details of the technique have been described elsewhere (AVAR and MONOS 1966). The size of the lesions attained 0.8 to 1.0 cu. mm. In the sham-operated controls the electrodes were introduced but no electrocoagulation was carried out. After surgery, intramuscular tetracyclic treatment was undertaken for 3 days. Localization of the lesions was checked in serial sections stained with Luxol Fast Blue and Cresyl Fast Violet (KLÜVER and BARRERA 1953).

Motility was estimated in 12 rats previously subjected to hypothalamic lesion, in 17 sham-operated rats, and in 16 rats deprived of food prior to the experiment; this study was undertaken on the 20th or 21th day of pregnancy. In the same period of the day, each animal was placed for 1 hour into a transparent plastic box measuring $13 \times 13 \times 16$ cm suspended on a spring; the weight of each box was adjusted to the same value, and oscillations were appropriately damped. On the bottom of each box electrodes were attached which of vertical movements of the box were immersed in mercury, closing thereby the circuit of a telephone counting device. The number of oscillations was estimated 10 or 15 min periods. The extent of motor activity thus measured in the first half of each period and of the decrease thereafter are a function of explorative activity.

A group of 16 pregnant rats was deprived of food but no cerebral lesion was induced in these animals. From the 16th day of pregnancy, food and water intake of the animals was regulated to imitate the pattern of transiently reduced food and fluid intake of the rats subjected to hypothalamic lesion.

Four to five offsprings delivered by intact mothers, were placed at the age of 6 to 8 days into the nest of 18 mothers subjected previously to hypothalamic lesion. The cross-fostering occured on the day of delivery.

Biopsy specimens of the mammary glands of 17 lesioned and 15 control mothers was examined several days after delivery the mothers which still had living offsprings or had had just lost their offsprings. Sections were stained with haemotoxylin-eosin. As criteria of lactation served the appearance secreting terminal glands and dilated winding excretory ducts.

## Results

### 1. *Motility, explorative activity and food intake after lateral hypothalamic lesions*

The motility of rats subjected to hypothalamic lesions was not significantly different from that of the sham-operated controls (Fig. 1, *A* and *B)*. In both groups, there was a statistically significant difference between the motility measured in the first and second half of the 1-hour period was observation ($p = 0.02$); the motor activity of rats placed in a new environment twice as much during the first half hour of the observation than in the second one. The chief manifestations of motility were the followings: the rats sniffed round the box vigorously, tried to lift the covering plate which had been secured with weights, nibbled on the edge of the airing vents searching for some

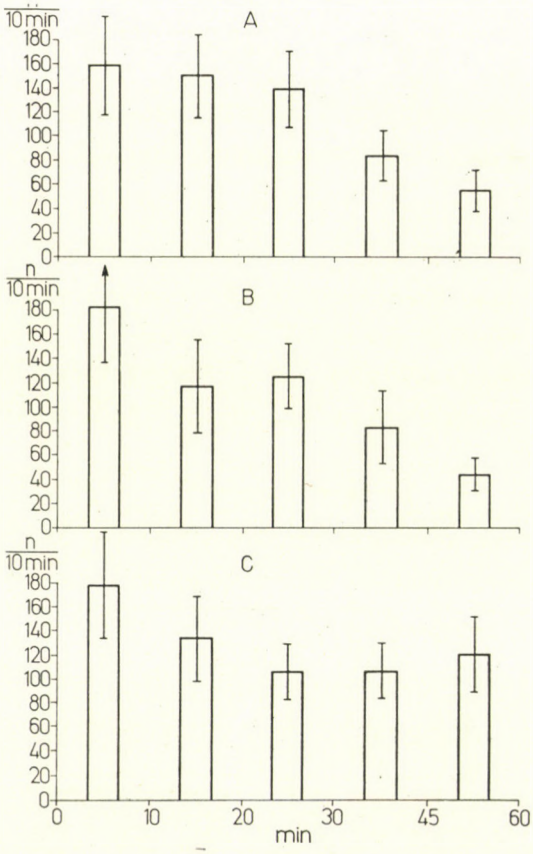



*Fig. 1.* Motility of pregnant rats placed into an unknown environment during a period of 60 min. *n*, number of closures in the motimeter circuit; *A*, sham-operated group; *B*, group subjected to lateral hypothalamic lesion; *C*, group subjected to food restriction. Above each column the standard error of the mean is shown

possibility to come out of the box; subsequently, the animals became restful, occupied themselves with cleaning and stroking down their fur.

Figure 2 summarizes the figures for food and water intake, body weight and rectal temperature in this group. On the days following the lesion, food intake was reduced to fifth of the initial value, then on the day of delivery it reached the values recorded prior to the hypothalamic lesion. The subsequent tendency of reduction seem to have been due to the circumstance that these mothers had lost their offsprings. In the control group, the reduction in food intake was considerable on the first day after the sham-operation,

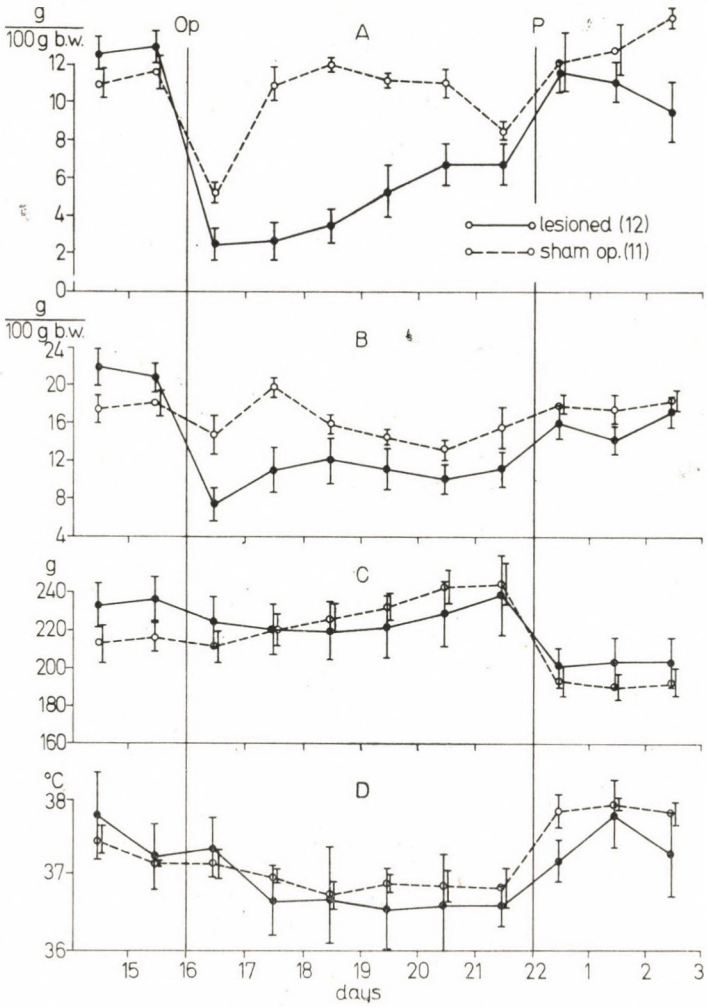



*Fig. 2.* Intake of food *(A)*, and water *(B)*, body weight *(C)*, and rectal temperature *(D)*, after lateral hypothalamic lesions and sham-operation in pregnant rats. *Op*, hypothalamic lesion or sham-operation; *P*, delivery. Mean and S. E. M.

presumably as a consequence of the pentobarbital effect. Water intake in the lesioned group was reduced to one third of the pre-operation value, and then it returned to normal until the day of delivery. In the sham-operated group no significant change in water intake was observed. Body weight slightly diminished after the hypothalamic lesion and then soon returned to initial value, whereas in the control group a more than 10% increase in body weight was observed until the day of delivery. Rectal temperature showed no significant difference in the two groups.

In accord with previous observations from this laboratory there was a marked disturbance of maternal behaviour after the lateral hypothalamic lesions; this was manifested in the absence of nest building, retrieving and nursing activities. On delivery, the mothers licked their offsprings, usually ate the placenta, and sometimes nibbled the offsprings still alive. As compared to the controls, no increase in the aggressivity of the animals was recorded. Immediately after delivery, macerated foetuses were found in a few nests. All offsprings died within 48 hours after delivery in this group; mean birth weight was 5.3 g in the experimental and 5.4 g in the control group.

2. *Survival rate of older foster offsprings*

Of the offsprings of intact mothers, when placed at the age of 6 to 8 days into the nest of rats previously subjected to hypothalamic lesion, two third survived 6 days. The mothers behaved indifferently to the whelps which, however, found the dugs by active searching. In 6 nests the exchange offsprings were still alive after 10 days.

3. *Morphology of the mammary glands of the mothers subjected to lateral hypothalamic lesion*

Forty-eight hours after delivery biopsy specimens were obtained from the mammary glands of 9 rats submitted to hypothalamic lesion and of 5 control sham-operated rats. In the former group, the histological finding revealed lactation in 5 cases, whereas in the control group signs of activity were present in 2 cases.

In specimens obtained after the first 48 hours there was finding positive in 5 rats of a total of 8 ones with hypothalamic lesions, whereas in the control group signs of lactation were observed in every specimen.

4. *Behaviour after restriction of food and fluid intake*

As mentioned previously, a group of intact rats was given the same amount of food and water that had been ingested by the rats submitted to hypothalamic lesion.

Motor activity after food restriction for 5 days was not significantly different from that of found in the control group and the rats subjected to hypothalamic lesion; during the period of observation only a slight tendency to diminution was observed (Figs 1 and 2), there being no statistically significant difference between the values found in the first and second half of the 1-hour period ($p = 0.15$).

Weight loss in the food restricted group exceeded 10% during first 4 days, which was about twice the weight loss recorded after hypothalamic lesion.

The course of pregnancy and delivery was normal in this group of rats Mean birth weight was 4.6 g. Although on the day of delivery food intake was not limited, 4 of the 16 mothers devoured their offsprings. Ten mothers bred their whelps normally even after 10 days. There was no appreciable disturbance of nest building and retrieving activity. Restlessness and increased aggressivity of these animals, however, was striking.

## Discussion

The present results indicate that hypothalamic lesions which affect maternal behaviour and reduce the vitality of offsprings have no deteriorating effect on the motility and explorative activity of pregnant rats. The parafornical lateral lesion caused a reduction in food and water intake for several days only; this observation was in agreement with previous results (Avar and Monos 1966). It seems likely that the mechanism of lactation is not directly affected by these hypothalamic structures. The effect of restricted food and water intake in otherwise normal rats was essentially different from the effect parafornical lesions.

Maternal instinct manifests itself in the rat in well differentiated and defined behavioural reactions (Rosenblatt and Lehrmann 1963). A certain level of maternal behaviour is observed in every rat, independently of the hormonal control (Rosenblatt 1967); this points to the predominating role of nervous organization in this response, although the hormonal milieu also seems to play an important part in the regulation of the maternal behavioural cycle (Riddle 1942; Beach 1951; Lehrmann 1961). Data on the role of central nervous structures in the control of maternal behaviour, however, are scarce in the literature. In earlier experiments the effect of extensive cortical ablation was studied on maternal behaviour (Davis 1939; Beach 1951). According to Stamm (1955), the median cortical (cingulate and retrosplenial) areas are of prime importance in the organization of maternal behaviour in pregnant rats. Relatively small injuries to these regions resulted in the following changes: the mothers did not build nests, did not keep all their

offsprings in the nest and did not feed them, although several-day-old foster offsprings placed into the nest induced lactation and maternal behaviour. These observations have been supported by the experiments of SLOTNICK (1967). Lesions applied in the anterior and posterior cingulate cortex resulted in retrograde degeneration of the frontal thalamic nuclei in the rat; the degree of degeneration and the disturbances in maternal behaviour were closely related. DEMPSEY and MORRISON (1952) found in cats that after bilateral thalamic lesions copulation and delivery were normal, but the mothers failed to look after their offsprings.

While copulation behaviour of rats subjected to a bilateral lesion in the dorsal hippocampal region remained essentially undisturbed, severe alteration occurred in their maternal behaviour (KIMBLE et al. 1967). Also hamsters underwent lesions in their hippocampal, septal and cingulate regions showed an altered maternal behaviour (GOODMAN et al. 1967). In contrast to this, maternal behaviour of rats remained unaffected by the lesion of the head of caudate nucleus and corpus callosum respectively (KIRKBY 1967).

In a previous study from this laboratory it has been shown that some lateral hypothalamic lesions cause severe defects in maternal behaviour: maternal care is lacking at the end of pregnancy and after delivery, the characteristic relationship between mother and offspring is absent, nest-building, retrieving and nursing are failing. The offsprings usually die within the first 48 hours after delivery (AVAR and MONOS 1967). Medial hypothalamic lesion are also associated with a high offspring mortality rate; in this case the primary cause is the severe disturbance of lactation (KURCZ 1967), but maternal behaviour is normal (KURCZ 1965; AVAR and MONOS unpublished data). Nursing activity is satisfactory when the medial hypothalamus is deafferentated (YOKOYAMA et al. 1967). HOLLOWAY and STEVENSON (1967) studied the effect of hypothalamic lesions induced on the 10th day of pregnancy and found disturbances of maternal activity only in the mothers in which the ventromedial nucleus had been damaged; except for the supraoptic region, the lateral areas of the hypothalamus were not studied in these experiments.

The present results confirm our previous data and indicate that the deterioration of maternal behaviour observed after damaging the lateral hypothalamic areas is not associated with disturbances of motility and explorative behaviour. Investigators in the Soviet Union succeeded in demonstrating that, in non-pregnant rabbits, alimentary reflexes disappeared after lateral hypothalamic lesions whereas the orientation reflex and the defensive reaction were not affected (KALUZHNI et al. 1967).

It has repeatedly been shown (ANAND and BROBECK 1951; MORGANE 1961a, b) that in non-pregnant laboratory animals the lateral hypothalamic regions in the projection of the ventromedial nucleus play an important role in the regulation of food intake. The present results have proved this in pregnant

animals; also, they indicate that maternal and alimentary behaviour are affected by lesions of lateral hypothalamic structures which are not necessarily identical although must lie close to one another. This latter assumption is supported by the finding that the regulation of food and water intake was already normal at the time of delivery while the absence of maternal activity has become apparent thereafter. The investigations so far conducted are insufficient to separate the structures responsible for the control of maternal behaviour from the structures representing the hunger motivating system described by MORGANE (1961a) in the mid-lateral hypothalamus of non-pregnant rats. It is not necessary to injure the trunk of the fornix to produce this effects. The lesions were placed immediately lateral and ventral to the fornix, partly in the substance of the medial forebrain bundle, in the tuberoinfundibular region; the area of the internal capsule is not involved in the lesion. Electrocoagulation in the medial forebrain bundle somewhat rostral to the above lesions didn't affect the maternal behaviour and offspring mortality was not significantly higher than in the control group (AVAR and MONOS unpublished data). Lesions made in the area of the so-called far-lateral feeding centre have dramatic effects in the pregnant rat; aphagia, hypodipsia, early intrauterine maceration, reduction of motility, lack of explorative activity, hypothermia are the most common consequences of such lesions, which finally lead to death of the animals (AVAR and MONOS 1969).

It is reasonable to assume that the above lesions did not affect maternal behaviour and offsprings vitality through the reduction of food and fluid intake, since the restriction of food and water to the same degree as it had been reduced spontaneously in the injured rats caused no disturbances in the nest-building and retrieving activity of rats non subjected to hypothalamic lesion. Complete deprivation of food for 5 days did not cause intrauterine death of the foetuses (AVAR and MONOS 1969). The more pronounced cannibalism observed in this group seems to have been due to fixation of the neurotic state caused by food deprivation. The enhanced motor activity could be demonstrated by direct observation by the motimeter and also by the significant weight loss. The reduction in motility characteristic of explorative activity was absent in this group; this allowed to conclude in agreement with the suggestion of other authors (CHAPMAN and LEVY 1957) that explorative activity is diminished by hunger.

The high mortality rate of the offsprings may be attributed, at least in part, to the lactation disturbance in the rats subjected to hypothalamic lesion. The comparatively higher survival rate of the older exchange offsprings, the morphology of the mammary gland, and the rapid death of the offsprings (within 2 days), however, indicate that this cannot be the primary factor. There is reason to believe that lactation is controlled mainly by medial hypothalamic structures (KURCZ 1967). Several data have recently indicated that

the afferent pathways of the milk ejection reflex reach the hypophysiotrophic area from lateral (YOKOYAMA et al. 1967). It seems on the basis of our finding that these pathways do not pass across the parafornical area. The negative energy balance due to the reduced food intake, however, may have considerably altered the secretion of milk. The slow gain in weight observed in the foster offsprings and their relatively high mortality rate supports this interpretation.

Earlier investigations from this laboratory have also indicated that foster youngs derived from normal mothers cannot induce normal maternal behaviour in mothers previously subjected to parafornical hypothalamic lesion (AVAR and MONOS 1967). In the present study, the experiments with older foster offsprings have essentially confirmed the conclusions drawn from those previous investigations.

It seems from the above data that the mid-lateral hypothalamic structures play a direct role in the regulation of maternal behaviour in the rat. It is remarkable that lesions applied to this area should cause severe disturbances in two fundamental behavioural responses: alimentary behaviour, the most important manifestation of the instinct of self-preservation, and maternal activity, the most important reaction serving species preservation. At the same time, however, an essential element of the animal behaviour, as the explorative activity, was not affected by these lesions, and the intactness of the lactation indicated a preserved balance of the hormonal system.

## LITERATURE

ANAND, B. K., BROBECK, J. R.: Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. **1951,** 24: 123—140.

AVAR, Z., MONOS, E.: The effect of hypothalamic lesion on the time of onset and course of labour, and on the development of the foetus. Acta med. Acad. Sci. hung. **1964,** 20: 395—402.

AVAR, Z., MONOS, E. Effect of lateral hypothalamic lesion on pregnant rats and foetal mortality. Acta med. Acad. Sci. hung. **1966,** 22: 259—264.

AVAR, Z., MONOS, E.: Effect of lateral hypothalamic lesion on maternal behaviour and foetal vitality in the rat. Acta med. Acad. Sci. hung. **1967,** 23: 255—261.

AVAR, Z., MONOS, E.: Behavioural changes in pregnant rats following far-lateral hypothalamic lesions. Acta physiol. Acad. Sci. hung. **1969,** 35:

BEACH, F. A.: Instinctive behavior: reproductive activities. In: Stevens, S. S. (Ed.) Handbook of experimental psychology. Wiley, New York **1951,** Pp. 387—434.

CHAPMAN, R. M., LEVY, N.: Hunger drive and reinforcing effect of novel stimuli. J. comp. physiol. Psychol. **1957,** 50: 233—238.

DAVIS, C. D.: The effect of ablations of neocortex on mating, maternal behavior and the production of pseudopregnancy in the female rat and on copulatory activity in the male. Amer. J. Physiol. **1939,** 127: 374—380.

DEMPSEY, E. W., MORRISON, R. S.: In discussion of F. A. Beach's paper "Mechanism of hormonal action upon behaviour". Ciba Found. Colloq. Endocr. **1952,** 3: 217.

GOODMAN, E. D., JANSEN, P., BUNNEL, B. N.: Four limbic lesions and maternal behavior in the hamster. Psychon. Bull. **1967,** 1: 26.

HOLLOWAY, S. A., STEVENSON, J. A. F.: Effect of various ablations in the hypothalamus on established pregnancy in the rat. Canad. Physiol. Pharmacol. **1967,** 45: 1081—1091.

Калюжный, Л. В., Некрасова, Л. И., Захарова, И. Н.: Изменения электрической активности и условнорефлекторной деятельности при разрушении латерального гипоталамуса у кроликов. Ж. высш. нерв. деятель. **1967,** 17 : 37—42.

KIMBLE, D. P., ROGERS, L., HENDRICKSON, C. W.: Hippocampal lesions disrupt maternal, not sexual behavior in the albino rat. J. comp. physiol. Psychol. **1967,** 63: 401—407.

KIRKBY, R. J.: Caudate nucleus lesions and maternal behavior in the rat. Psychon. Sci. **1967,** 9: 601.

Курц, М.: Нарушение беременности, родов и лактации у крыс при гипоталамическом ожирении. Биол. науки, **1965,** 1 : 72—77.

KURCZ, M.: Studies on the neuro-humoral regulation of lactation. In: LISSÁK, K. (Ed.) Symposium on Reproduction. Akadémiai Kiadó, Budapest **1967,** Pp. 175—211.

LEHRMAN, D. S.: Gonadal hormones and parental behavior in birds and infrahuman mammals. In: YOUNG, W. C. (Ed.) Sex and internal secretions. Williams and Wilkins, Baltimore **1961,** Vol. 2/F. Pp. 1268—1382.

MORGANE, P. J.: Distinct "feeding" and "hunger motivating" systems in the lateral hypothalamus in the rat. Science **1961a,** 133: 887—888.

MORGANE, P. J.: Alterations in feeding and drinking behavior of rat with lesions in globi pallidi. Amer. J. Physiol. **1961b,** 201: 420—428.

RIDDLE, O.: The role of hormones in the initiation of maternal behavior in rats. Amer. J. Physiol. **1942,** 137: 299—317.

ROSENBLATT, J. S., LEHRMAN, D. S.: Maternal behavior of the laboratory rat. In: RHEINGOLD H. L. (Ed.): Maternal behavior in mammals. Wiley, New York **1963,** Pp. 8—57.

ROSENBLATT, J. S.: Nonhormonal basis of maternal behavior in the rat. Science **1967,** 156: 1512—1514.

SLOTNICK, B. M.: Disturbances of maternal behavior in the rat following lesions of the cingulate cortex. Behaviour **1967,** 29: 204—236.

SZENTÁGOTHAI, J.: A központi idegrendszer mélyen fekvő részein végzett kísérleti beavatkozások módszerei. In: KOVÁCH, A. G. B. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1957,** Vol. 3/I. Pp. 19—126.

SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B., HALÁSZ, B.: Hypothalamic control of the anterior pituitary. Akadémiai Kiadó, Budapest **1962,** Pp. 330.

YOKOYAMA, A., HALÁSZ, B., SAWYER, C. H.: Effect of hypothalamic deafferentation on lactation in rats. Proc. Soc. exp. Biol. (N. Y.) **1967,** 125: 623—626.

Zoltán AVAR,
Orvostudományi Egyetem II. sz. Női Klinika
Budapest VIII. Üllői út 78/a.

Emil MONOS,
Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma
Budapest VIII. Üllői út 78/a.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 295—303 (1969)*

# BEHAVIOURAL CHANGES IN PREGNANT RATS FOLLOWING FAR-LATERAL HYPOTHALAMIC LESIONS

By

Z. AVAR and E. MONOS

SECOND DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
AND EXPERIMENTAL RESEARCH DEPARTMENT, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 7, 1968)

The effects of far-lateral hypothalamic lesion inflicted on the 16th or 17th day of pregnancy and of food restriction have been studied in pregnant rats. As a results of the lesion, aphagia and hypodipsia ensued, motor activity was diminished, and explorative activity was abolished. A certain proportion of the pregnant rats developed a terminal state as the time of delivery approached, the majority of foetuses died *in utero*. Intrauterine death was not observed in the group exposed solely to food restriction. Body weight in the hypothalamic lesion group diminished more rapidly than in the food-restricted group. As compared to sham-operated controls, both groups exhibited signs of adrenocortical hypertrophy.

The effects of far-lateral hypothalamic lesion have been compared to the consequences of parafornical lesion.

It has been shown (AVAR and MONOS 1966, 1967, 1969) that lateral parafornical areas of the hypothalamus play a prominent role in the regulation of maternal behaviour in the rat. In the course of this experiment a few animals developed aphagia. All these rats died close to the time of delivery. Histological examination revealed, in all of these rats, that the far-lateral region of the hypothalamus in the projection of the ventromedial nucleus was also damaged. Observations in non-pregnant rats have proved the prime importance of this area in the regulation of food intake (ANAND and BROBECK 1951; MORGANE 1961a). In view of these facts it seemed worth to investigate the effect of electrocoagulation of the far-lateral region on the pregnancy and on the behaviour of pregnant rats carried out on the 16th or 17th day of pregnancy.

The lesions were found to give rise to prompt and severe damage to the pregnant rats; persisting aphagia and hypodipsia ensued, motility was reduced, explorative activity abolished, the majority of the offsprings died *in utero*, and part of the mothers died near the term of delivery. Complete restriction of food in a group of intact pregnant rats in the same phase of pregnancy did not cause death of either the mother or their offsprings.

The difference between the effects of far-lateral and parafornical hypothalamic lesions was striking in view of the fact that the mean distance between the centres of the lesions was only 0.4—0.6 mm. After parafornical lesions

(AVAR and MONOS 1969), although maternal behaviour and vitality of the offsprings were seriously affected, food and water intake was reduced only transiently, and neither motility nor explorative activity showed marked differences as compared to controls.

## Methods

Wistar albino rats kept on a standardized protein-rich synthetic diet were used in the experiments. Virgin females in oestrus were placed into cages of males for one night. Twelve to 14 days later pregnancy was established by palpation, and 44 pregnant females were separated and placed into cages where temperature was adjusted between 18 and 24 °C. Food and water intake, body weight and rectal temperature were recorded. Soft paper strips were placed in the front part of the cages for nest building. The animals were observed twice or three times daily.

In 18 pregnant rats bilateral hypothalamic lesions were carried out on the 16th or 17th day of pregnancy, 1.8 to 2.0 mm laterally from the median plane, in the projection of the ventromedial nucleus. Seventeen pregnant rats subjected to sham operation served as controls. Electrocoagulation was undertaken in intraperitoneal sodium pentobarbital anaesthesia, by glass insulated unipolar silver electrodes introduced by means of a KOVÁCH—SZENTÁGOTHAI stereotaxic apparatus (SZENTÁGOTHAI 1957). The size of the lesions was 0.8 to 1.0 cu. mm. Tetracyclin was administered intramuscularly for 3 days following surgery. Location of the lesions was established in serial histological sections stained according to KLÜVER and BARRERA (1953).

Nine pregnant rats not subjected to either hypothalamic lesion or sham operation were deprived of food from the 17th day of pregnancy until the 21st or 22nd day.

In the first two groups motility of the animals was established on the 20th day of pregnancy in an unknown environment, by a method described elsewhere (AVAR and MONOS 1969).

On the 21st or 22nd day of pregnancy the rats deprived of food together with 7 sham-operated rats as well as 13 rats subjected to hypothalamic lesion were killed by decapitation, laparotomized, and the number of viable and dead offsprings was established, then the weight of several endocrine organs and the uterus was measured with an accuracy of 0.1 mg.

STUDENT's *t*-test was used for statistical analysis.

## Results

### *1. Food and water intake, body weight and rectal temperature*

Figure 1 shows that food intake of the pregnant rats with far-lateral hypothalamic lesion was reduced to 7 to 10% of the values recorded prior to the lesion. Half of the rats did not feed at all following the surgery. Water intake was depressed to 15 to 25% of the initial value. Body weight decreased continuously, a more than 22% loss in weight being recorded on the 4th days; the sham-operated rats exhibited the usual gain in weight. Rectal temperature in the group subjected to hypothalamic lesions was reduced by 2 °C on the average in the course of 5 days.

Figure 2 represents the changes in body weight and rectal temperature in the intact pregnant rats deprived of food from the 17th day of pregnancy. Remarkably enough, the loss in weight in this group was smaller than in the rats subjected to hypothalamic lesion (17%) on the 4th days; the difference

between the two groups attained statistical significance at the 0.04 level. Body temperature, after an initial rise on the 3rd day of food deprivation, showed a diminution.

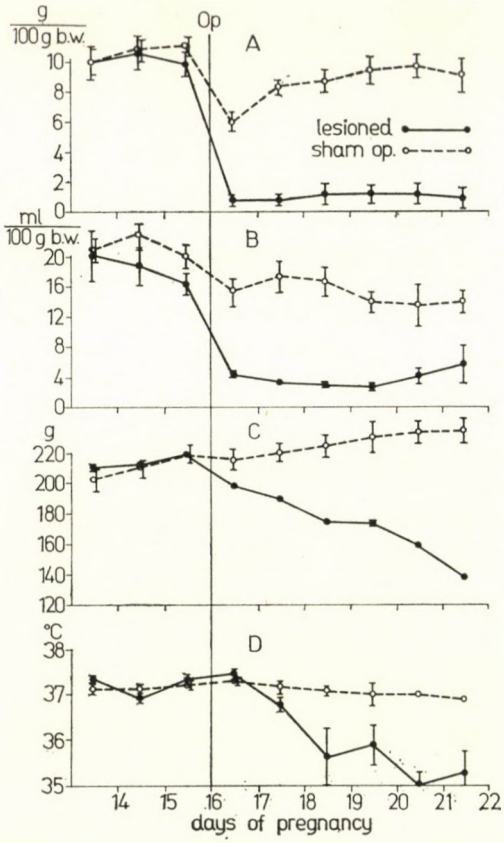



*Fig. 1.* Food intake *(A)*, water intake *(B)*, body weight *(C)* and rectal temperature *(D)* in pregnant rats following far-lateral hypothalamic lesions and sham operation. *Op*, day of hypothalamic lesion or sham-operation. Mean and S. E. M.

### 2. *Nest-building, motility and explorative activity*

Nest-building activity was apparent in the control group on the last days of pregnancy: the rats pulled the soft paper strips from the front part of the cage into a dark corner and tore them to bits. The pregnant rats subjected to hypothalamic lesions, however, showed no sign of nest-building and their motility was significantly reduced. No sign of aggressivity could be revealed in this group.

A reduction in spontaneous movements was also apparent in unknown environment (Fig. 3). Motility was about one fifth of that observed in the

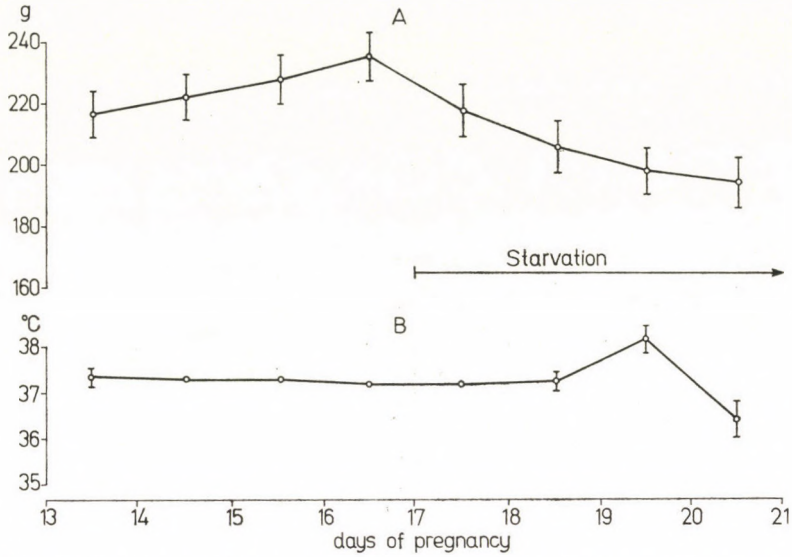



*Fig. 2.* Effect of food deprival on body weight *(A)* and rectal temperature *(B)* in pregnant rats

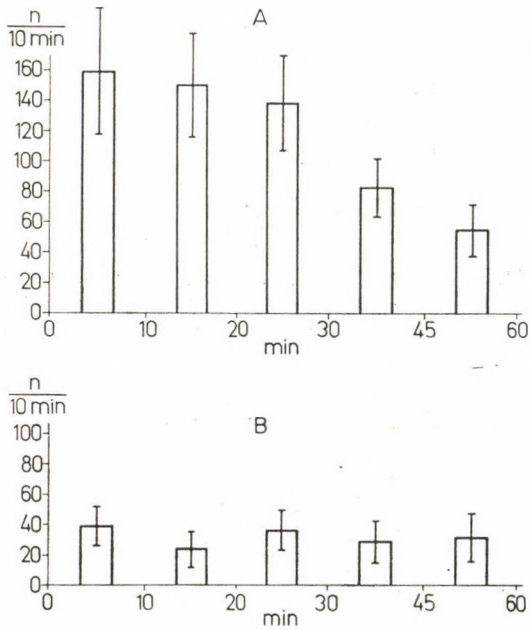



*Fig. 3.* Motility of rats placed in a new environment for 60 min.
*A*, sham-operated rats; *B*, far lateral hypothalamic lesions; *n*, number of closures recorded by motimeter. Above each column the standard error of the mean is represented by vertical lines

control rats during the first half hour of observation ($p = 0.01$). In the control group, owing to the changes in explorative behaviour, motility was reduced by more than 50% in the second half of the 1-hour period of observation ($P = 0.02$), whereas in the group subjected to hypothalamic lesions the low value obtained in the first half hour remained essentially unchanged ($p = 0.90$).

### 3. *Mortality rate*

Five of the 18 pregnant rats submitted previously to far-lateral hypothalamic lesions reached the terminal stage on the 21st to 24th days of pregnancy; autopsy carried out at this time revealed only dead, partly macerated, foetuses in the uterus.

Laparatomy was carried out on the 21st to 23rd day of pregnancy in 13 rats with hypothalamic lesions, in 7 sham-operated and 9 food-deprived rats. It was found that 87% of the offsprings of the mothers with hypothalamic lesions had died *in utero*. Multiple haemorrhages in the chorion, and in many instances shrunk and partly absorbed remnant of foetuses were found. Viable foetuses were present in one third of the mothers subjected to hypothalamic lesion, and in only 4 of these rats was no intrauterine death revealed.

None of the intact pregnant rats subjected to food deprivation and of the sham-operated ones died and in none of them occurred intrauterine death of the offsprings.

In the nests of the 10 non-laparatomized sham-operated rats dead offsprings were found in 3 cases, the number of deaths attaining only 8% of all offsprings delivered in this group.

### 4. *Weight of the endocrine organs*

The table shows the weight of the pituitary, adrenals and ovaries of the decapitated pregnant rats; all values were calculated for 100 g of body weight.

Mean adrenal weight in the rats subjected to hypothalamic lesion was twice that found in the sham-operated group; the difference was highly significant statistically ($p < 0.001$). Hypertrophy was apparent also when adrenal weight was not calculated per body weight. Adrenal hypertrophy was observed also in the rats deprived of food ($p < 0.01$).

The weight of the pituitary gland and the ovaries showed no significant difference in the three groups.

### 5. *Localization of the lesions*

Histological examination of serial sections revealed that electrocoagulation that had given rise to lasting aphagia and foetal death extended in the lateral hypothalamic area to the internal capsule, and approached or reached

**Table I**

*Weight of endocrine organs after far-lateral hypothalamic lesions and*

| Experimental procedure | Number of animals | Body weight (g) | |
|---|---|---|---|
| | | Prior to experiment | Prior to decapitation |
| Sham operation | 11 | 219.1 ± 8.3 | 226.8 ± 10.2 |
| Hypothalamic lesion | 13 | 220.4 ± 8.4 | 156.9 ± 8.5 |
| Food deprivation | 9 | 235.6 ± 8.1 | 192.2 ± 8.3 |

the ventral edge of the brain in the projection of the ventromedial nucleus. In general, the lenticular ansa and fasciculus and a substantial part of the medial forebrain bundle were also damaged (Fig. 4).

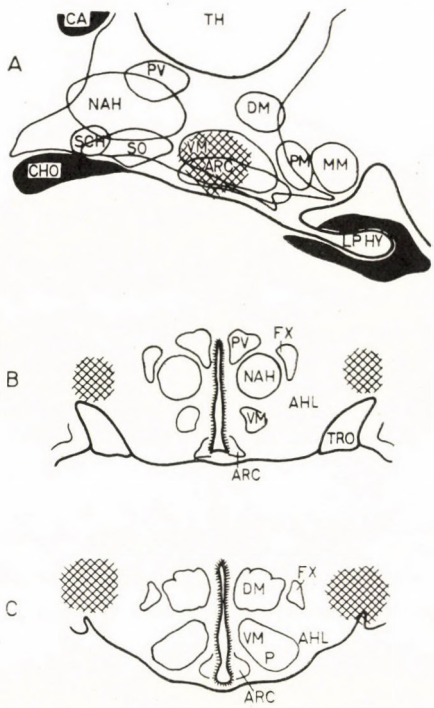



*Fig. 4.* Schematic representation of the sagittal *(A)* and frontal sections of the hypothalamus; *B*, tuberal region; *C*, infundibular region. Cross-hatched area represents the location of far-lateral lesions. *AHL*, lateral hypothalamic area; *ARC*, arcuate nucleus; *CA*, anterior commissure; *CHO*, optic chiasma; *DM*, dorsomedial nucleus; *FX*, fornix; *LP*, posterior pituitary lobe; *MM*, medial mammillary nucleus; *NAH*, anterior hypothalamic nucleus; *PM*, premammillary nucleus; *PV*, paraventricular nucleus; *SCH*, suprachiasmatic nucleus; *SO*, supraoptic nucleus; *TH*, thalamus; *TRO*, optic tract; *VM*, ventromedial nucleus; *VMP*, posterior ventromedial nucleus

*food deprivation in pregnant rats (Mean ± S.E.M.)*

| Weight of endocrine organs (g/100 g) | | | | |
|---|---|---|---|---|
| Pituitary | Adrenals | | Ovaries | |
| | right | left | right | left |
| 5.67 ± 0.52 | 15.83 ± 1.15 | 15.82 ± 1.33 | 27.01 ± 1.96 | 24.16 ± 1.65 |
| 6.13 ± 0.26 | 27.02 ± 2.35 | 32.95 ± 2.12 | 32.77 ± 2.31 | 29.23 ± 2.26 |
| 6.09 ± 0.22 | 24.63 ± 1.92 | 27.20 ± 2.26 | 31.40 ± 2.19 | 28.20 ± 1.77 |

In one of the pregnant rats the offsprings had died *in utero* and motility was substantially reduced; however, aphagia lasted for only two days after the hypothalamic lesion, and food intake was normal on the 3rd day. In this animal, the lesion did not approach the ventral edge of the hypothalamus, but the pallidofugal system was damaged on both sides.

## Discussion

The present results indicate that hypothalamic lesions placed a few tenths of a millimeter lateral from the parafornical structures the electrocoagulation of which causes significant reduction of maternal activity (Avar and Monos 1966, 1967, 1969), give rise to grave disturbances in the feeding and explorative behaviour of pregnant rats, reducing in addition their nestbulding activity and damaging their offsprings.

It is known from experiments conduced on non-pregnant animals that this far-lateral hypothalamic area plays a major part in the regulation of food intake (Anand and Brobeck 1951; Morgane 1961a). Lesions to this area, however, damage not only the alimentary reactions but also other behavioural manifestations, e.g. defensive motivation (Endrőczi et al. 1964; Balinska 1968).

The present findings support the view that the far-lateral region is of prime importance in the regulation of food intake in the pregnant rat. The severe damage to the mothers and their offsprings cannot, however, be ascribed solely to the aphagia. Food deprivation in intact pregnant rats did not cause foetal death, and the loss in body weight was more pronounced in the rats subjected to hypothalamic lesions than in the group restricted of food. Earlier observations have shown (Avar and Monos 1969) that in pregnant rats restricted in food intake for several days motility is not reduced, although explorative behaviour is inhibited; this finding is in agreement with results of Chapman and Levy (1957). In the present study, one of the rats with hypothalamic lesion displayed decreased motility and the offsprings had died *in utero*, although food intake was reduced only for 2 days and was subsequently

normalized. Laparotomy revealed that the majority of foetuses died in 2 or 3 days after the hypothalamic lesion, since at the time of laparotomy macerated and partly absorbed foetuses were found; at that time the reduced food intake must have played only a secondary role.

The present observations are insufficient to establish whether the differences in the consequences of hypothalamic lesions applied in two distinctly different lateral regions (parafornical and far-lateral) are quantitative or are due to the damage of two structures with essentially different physiological functions. Data obtained in non-pregnant rats seem to support the latter assumption; MORGANE (1961b) concluded that in the lateral hypothalamus there is a far-lateral "metabolic" system, and a mid-lateral "hunger motivating" system, the normal functioning of which depends on the intactness of two distinctly different fibre systems, in the first case of the pallidofugal tracts, and in the latter of the medial forebrain bundle.

In the present study, certain qualitative differences in the effects of the lesions could also be revealed, the parafornical lesion having been found not to alter motility and explorative behaviour in pregnant rats (AVAR and MONOS 1969), whereas the far-lateral lesions giving rise to a great reduction of spontaneous motility and fully abolishing explorative activity. Further experiments are, however, necessary to clarify the factors responsible for the observed changes in maternal behaviour and the high rate of intrauterine deaths. The pathomechanism of the damages is unknown. Disturbances in the blood supply to the uterus or in its trophic innervation might be important in this respect, and alterations in neuroendocrine function cannot be excluded either.

The obtained results allow the conclusion that far-lateral hypothalamic lesions are gravely affecting the pregnant rat and its offsprings. It is reasonable to assume that, in addition to the disturbances in motivational reactions, severe changes in the metabolism of these animals must also be considered.

## LITERATURE

ANAND, B. K., BROBECK, J. R.: Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. **1951,** 24: 123—140.

AVAR, Z., MONOS, E.: Effect of lateral hypothalamic lesion on pregnant rats and foetal vitality. Acta med. Acad. Sci. hung. **1966,** 22: 259—264.

AVAR, Z., MONOS, E.: Effect of lateral hypothalamic lesion on maternal behaviour and foetal vitality in the rat. Acta med. Acad. Sci. hung. **1967,** 23: 255—261.

AVAR, Z., MONOS, E.: Biological role of lateral hypothalamic structures participating in the control of maternal behaviour in the rat. (Motility, explorative behaviour, lactation and the effect of reduced food intake.) Acta physiol. Acad. Sci. hung. **1969,** 35: 285—294

BALINSKA, H.: The hypothalamic lesions: effects on appetitive and aversive behavior in rats. Acta Biol. exp. (Warszawa) **1968,** 28: 47—56.

CHAPMAN, R. M., LEVY, N.: Hunger drive and reinforcing effect of novel stimuli. J. comp. physiol. Psychol. **1957,** 50: 233—238.

ENDRŐCZI, E., KORÁNYI, L., LISSÁK, K., HARTMAN, G.: The role of the meso-diencephalic activating system in the EEG arousal reaction and conditioned reflex activity. Acta physiol. Acad. Sci. hung. **1964,** 24: 447—464.

MORGANE, P. J.: Distinct "feeding" and "hunger motivating" systems in the lateral hypothalamus in the rat. Science **1961a**, 133: 887—888.
MORGANE, P. J.: Medial forebrain bundle and "feeding centers" in the hypothalamus. J. comp. Neurol. **1961b**, 117: 1—25.
SZENTÁGOTHAI, J.: A központi idegrendszer mélyen fekvő részein végzett kísérleti beavatkozások módszerei. In: KOVÁCH, A. G. B. (Ed.): A kísérleti orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest **1957**, Vol. 3/I. Pp. 19—126.

Zoltán AVAR
Orvostudományi Egyetem II. sz. Női Klinika,
Budapest VIII. Üllői út 78/a.

Emil MONOS
Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma,
Budapest VIII., Üllői út 78/a.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 305—312 (1969)*

# EFFECT OF LOCAL VENOUS CONGESTION ON ADRENOCORTICAL FUNCTION

## I. CHANGES IN ADRENAL CORTICOID SECRETION AFTER STEPWISE REDUCTION OF VENOUS OUTFLOW

By

E. MONOS, EDIT KOLTAY, A. SULYOK, T. VENKEY and A. G. B. KOVÁCH

EXPERIMENTAL RESEARCH LABORATORY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 12, 1968)

To obtain a significant reduction in glycocorticoid secretion, adrenal blood flow had to be reduced at least by 30% by venous congestion. The adrenal cortex continued to secrete hormones at a rate of 40 to 50% of control values even if congestion was maintained for periods of 2 to 3 hours and was stepwise augmented to reduce venous outflow to 25% of initial values. Within 1 hour after the cessation of venous congestion, corticosteroid output was restored to control values whereas adrenal blood flow remained somewhat lower than prior to congestion.

Quantitative data concerning the effect of local venous congestion on the rate of adrenocortical hormone output are not available in the literature, although elucidation of the problem is necessary from several points of view. First, in the majority of experiments devoted to the study of adrenocortical function the adrenal vein is cannulated, a procedure frequently associated with venous congestion the degree and duration of which depends in the size of the cannula and the surgical technique. Catheterization of the adrenal vein becomes a usual technique also in the clinical practice (BUCHT 1962; WEINHEIMER 1963). Experience with adrenal transplantation in laboratory animals and with the demedullated adrenal gland have indicated that the responsiveness of the regenerating adrenal was significantly impeded by congestion due to changes in the vascular structure on the venous side (KOLTHOFF et al. 1964). Long-term administration of ACTH (KOVÁCS et al. 1968) and isoproterenol (HARRISON and BROWN 1964) were shown similarly to induce such changes in adrenal structure which were indicative of venous congestion.

The factors that may be responsible for altered adrenocortical hormone secretion under such conditions are hypoxia due to diminished blood flow, diminished supply of ACTH to the adrenocortical cells, and the increase in venous pressure. It has repeatedly been shown that the adrenal cortex is relatively insensitive to arterial hypoxia (FRANK et al. 1955; HORNBEIN 1962; HIRATI et al. 1963; DÁVID and KOVÁCS 1966; KOVÁCS et al. 1966). Experiments carried out on the adrenal perfused in situ have shown that, within the physiological range, the degree of stimulation of adrenocortical hormone output

depends on the amount of ACTH reaching the adrenal per unit of time, i.e. the function of blood ACTH concentration and of adrenal blood flow (PORTER and KLAIBER 1965; URQUHART 1965). Morphological studies by FLINT (1900) have indicated the presence of arterio-venous bypasses in the adrenal gland, thus, whenever venous outflow is impeded there must be a considerable rise in venous pressure. It should nevertheless be taken into consideration that adrenal blood supply is several times higher than what would be required for fulfilling the oxygen demand of the cells and the vascular structure of the adrenals is quite unique (HARRISON and HOEY 1960).

In the present experiments attempts have been made to inducing the degree of local congestion necessary for a significant diminution of adrenal corticoid secretion. In anaesthetized dogs, the effect of reduction of adrenal venous outflow by 25, 50 and 75% due to the constriction of the cannula inserted into the left lumboadrenal vein has been studied on hydrocortisone and corticosterone output.

## Methods

Experiments were performed on 14 mongrel dogs of both sexes, weighing 10 to 29 kg and anaesthetized with 30 mg per kg of body weight of sodium pentobarbital.

*Experimental procedure.* In the anaesthetized and tracheotomized dog the right femoral artery and vein were cannulated. Following an abdominal midline incision a cannula was inserted into the left lumboadrenal vein. Collection of blood samples was started into a graduated cylinder placed in an ice bath after the abdominal incision had been closed and heparin (300 I.U./kg bw.) had been given intravenously. The amount of blood lost through the adrenolumbar cannula was replaced by infusing heparinized blood obtained from a donor dog. After control measurements had been made, the adrenal cannula was constricted to reduce adrenal blood flow by about 25, 50 and 75% for periods of 45 to 60 min. At each level of constriction two collection periods were done the duration of which was either 10 or 15 min respectively; collection of adrenal blood for corticoid assay was made in the same way before and after the release of the constriction. The preparation of the experiments were undertaken in the morning, whereas collection of adrenal venous blood was performed in the afternoon. After the experiment, both adrenals were removed and placed into 5% formalin for histological evaluation.

*Measurement of adrenal blood flow.* A polyethylene cannula identical in diameter with the left lumboadrenal vein was inserted into the vessel and pushed forward to the point where the vein left the adrenal gland. The adrenocaval vein and the branches of the lumboadrenal vein were ligated. The cannula was passed through an incision made on the side of the abdomen. The amount of blood flowing out of the cannula was measured by an ordinate-writing droprecorder, using piezoelectric signal transformation and a Zimmermann kymograph. Venous congestion was induced by a Hoffmann clamp placed on the cannula.

*Determination of hydrocortisone and corticosterone.* Adrenal venous blood samples were centrifuged in a cold room. Corticoids, after extraction with chloroform and distillation were assayed by paper chromatography. After repeated purification, the material was run on Whatman's No. 1 paper in the BUSH $B_5$ system, then developed with tetrazolium blue; the hydrocortisone and corticosterone fractions were eluated and determined photometrically (BUSH 1961; VECSEY 1963).

*Arterial blood pressure and respiratory rate* were checked continously in each experiment.

*Histological evaluation* of the adrenals was done in 10 $\mu$ sections prepared from paraffin blocks and stained with haematoxylin-eosin.

*Statistical analysis* of the obtained results was undertaken by STUDENT's *t*-test.

## Results

Diminution of adrenal venous outflow (Table I) was not associated with a proportionate reduction of corticosteroid output (Table II). While adrenal flow was kept over longer periods at 71, 48 and 26% of the control values, the secretion rate of hydrocortisone in the respective periods attained 96, 78

**Table I**

*Values for body weight, adrenal weight, and adrenal blood flow*

| No. | Sex | Body weight (kg) | Adrenal weight (g) | Adrenal venous outflow ml/min | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Control | During constriction of the venous cannula | | | After release of constriction |
| | | | | | I | II | III | |
| 1 | ♀ | 10.0 | 0.45 | 3.1 | 2.5 | 1.7 | 0.8 | 3.3 |
| 2 | ♀ | 23.0 | 2.30 | 4.5 | 3.3 | 2.1 | 1.0 | 4.3 |
| 3 | ♂ | 12.0 | 0.87 | 3.0 | 2.6 | 1.3 | 0.9 | 3.4 |
| 4 | ♂ | 14.5 | 1.17 | 2.8 | 2.1 | 1.3 | 0.7 | 1.7 |
| 5 | ♂ | 20.0 | 1.20 | 3.3 | 2.5 | 1.6 | 0.9 | 2.5 |
| 6 | ♂ | 12.0 | 0.60 | 1.8 | 1.3 | 0.9 | 0.5 | 1.4 |
| 7 | ♂ | 10.0 | 0.45 | 1.5 | 0.9 | 0.7 | 0.5 | 1.3 |
| 8 | ♂ | 22.0 | 1.30 | 4.0 | 2.9 | 2.0 | 0.8 | 4.1 |
| 9 | ♀ | 22.0 | 1.90 | 3.9 | 2.8 | 2.1 | 0.9 | 3.2 |
| 10 | ♂ | 16.5 | 0.58 | 2.5 | 1.4 | 1.1 | 0.8 | 1.6 |
| 11 | ♀ | 29.0 | 1.56 | 6.2 | 4.4 | 2.6 | 1.6 | 5.2 |
| 12 | ♂ | 20.0 | 1.04 | 2.1 | 1.2 | 1.4 | 0.5 | 1.6 |
| 13 | ♂ | 17.0 | 0.98 | 1.5 | — | 0.6 | 0.4 | 1.2 |
| 14 | ♂ | 13.0 | 0.95 | 2.1 | — | 1.0 | — | 1.8 |
| Mean | | 17.21 | 1.10 | 3.02 | 2.33+ | 1.46+* | 0.79+* | 2.61+* |
| S.E.M. | | 1.52 | 0.14 | 0.36 | 0.30 | 0.14 | 0.08 | 0.35 |

+ The difference as compared to the controls is significant statistically ($p < 0.05$)
* The difference as compared to preceding values is significant statistically ($p < 0.05$)

and 40% of the initial readings, and that of corticosterone fell to 90, 79 and 50% (Table III). Statistical analysis has shown that a 30% reduction in adrenal flow is not associated with a statistically significant drop in hydrocortisone production ($0.20 < p < 0.50$), whereas the fall in corticosterone output (about 10%) is significant statistically within the 0.05 level. In the third phase of venous constriction, however, hydrocortisone output was more markedly reduced than the corticosterone nevertheless, the difference between the ratio of the two hormones (3.5 and 2.4) was not significant statistically ($0.05 < < p < 0.10$).

**Table II**

*Effect of local venous congestion on adrenocortical output of hydrocortisone (H) and corticosterone (C). Approximate degree of venous obstruction; I, 25%; II, 50%; III, 75%*

| No. | Control | | Congestion | | | | | | After congestion | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | | II | | III | | | |
| | H | C | H | C | H | C | H | C | H | C |
| 1 | 44.6 | 16.4 | 27.3 | 8.5 | 18.7 | 7.8 | 10.9 | 4.1 | 28.0 | 7.9 |
| 2 | 14.9 | 10.6 | 13.9 | 8.5 | 20.8 | 11.0 | 5.5 | 2.6 | 15.2 | 8.4 |
| 3 | 19.1 | 18.0 | 23.8 | 15.4 | 17.7 | 9.9 | 23.8 | 7.8 | 24.3 | 11.3 |
| 4 | 46.6 | 12.3 | 25.3 | 10.1 | 27.5 | 6.2 | 10.9 | 4.7 | 19.2 | 11.0 |
| 5 | 44.1 | 11.0 | 54.3 | 11.8 | 41.7 | 6.4 | 5.0 | 3.2 | 17.0 | 3.3 |
| 6 | 28.7 | 18.4 | 23.2 | 15.9 | 26.5 | 13.3 | 15.7 | 9.5 | 32.4 | 22.1 |
| 7 | 18.0 | 3.6 | 48.5 | 6.0 | 19.9 | 2.1 | 8.4 | 1.4 | 42.5 | 7.1 |
| 8 | 34.9 | 8.8 | 26.7 | 9.9 | 21.3 | 7.5 | 18.2 | 7.4 | 13.1 | 5.3 |
| 9 | 15.3 | 8.2 | 15.4 | 5.6 | 14.5 | 4.4 | 5.1 | 2.6 | 20.0 | 6.5 |
| 10 | 22.1 | 3.6 | 9.2 | 2.9 | 7.3 | 6.3 | 6.4 | 3.6 | 13.5 | 8.9 |
| 11 | 37.9 | 8.5 | 11.6 | 5.9 | 5.7 | 4.1 | 4.9 | 4.5 | 11.9 | 12.6 |
| 12 | 57.8 | 9.1 | 60.3 | 7.5 | 55.5 | 10.5 | 14.4 | 7.8 | — | — |
| 13 | — | — | 10.8 | 4.2 | 5.1 | 1.6 | 3.2 | 1.4 | — | — |
| 14 | 30.5 | 7.5 | — | — | 25.4 | 6.4 | — | — | 29.6 | 6.0 |
| Mean | 31.9 | 10.5 | 26.9 | 8.6+ | 22.7+ | 7.0+ | 10.2+* | 4.7+* | 22.2* | 9.2* |
| S.E.M. | 3.8 | 1.3 | 4.6 | 1.1 | 3.3 | 0.9 | 1.2 | 0.7 | 2.7 | 1.4 |

For symbols, see Table I.

**Table III**

*Percentile changes in adrenal corticoid hormone release during local venous congestion*

| | | Control | During congestion | | | After congestion |
|---|---|---|---|---|---|---|
| | | | I | II | III | |
| Adrenal blood flow | Mean<br>S.E.M. | 100 | 71.1+<br>2.7 | 47.5+*<br>1.1 | 26.4+*<br>1.0 | 84.9+*<br>7.8 |
| Arterial blood pressure (mm Hg) | Mean<br>S.E.M. | 117.4<br>6.3 | 107.0<br>5.1 | 107.3<br>5.2 | 109.3<br>4.8 | 111.6<br>5.0 |
| Hydrocortisone release | Mean<br>S.E.M. | 100 | 95.8<br>17.8 | 78.3+<br>9.7 | 39.5+*<br>8.7 | 89.8*<br>16.9 |
| Corticosterone release | Mean<br>S.E.M. | 100 | 90.4+<br>8.2 | 78.5+*<br>10.0 | 50.4+*<br>7.5 | 103.3*<br>18.5 |
| Ratio of hydrocortisone and corticosterone release | Mean<br>S.E.M. | 3.57<br>0.48 | 3.40<br>0.62 | 3.51<br>0.62 | 2.37<br>0.29 | 2.90<br>0.48 |

For symbols, see Table I.

After release of the constriction, hydrocortisone and corticosterone secretion was fully restored. Adrenal blood flow, on the other hand, remained approximately 15% under its initial value ($p < 0.01$). Arterial blood pressure was not altered significantly in the course of the experiments.

Figure 1 shows the changes in the corticosteroid hormone level of adrenal venous blood in response to varying degrees of venous congestion. As seen

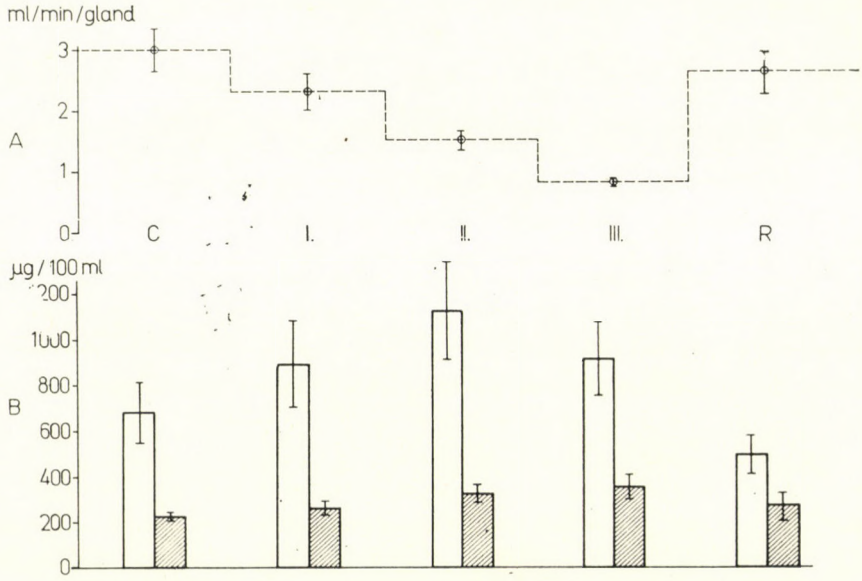



*Fig. 1.* Lumboadrenal venous outflow *(A)* and corticoid hormone concentration *(B)* during stepwise constriction of the venous cannula draining the adrenal gland. *C*, control values; I, II, III, increasing venous congestion. *R*, release of constriction. Open blocks represent hydrocortisone output, hatched blocks corticosterone output (means and standard errors)

in the diagram, the concentration of hydrocortisone and corticosterone underwent a considerable increase during congestion.

Histological observations have shown enlarged sinuses in the adrenal medulla and the reticular and fasciculate zones, and minor hemorrhages which never attained the size of a haematoma.

## Discussion

Stepwise reduction of adrenal venous outflow resulted in a disproportional diminution in corticoid output. When blood flow was reduced by 25 to 30%, the rate of corticosteroid output was essentially the same as prior to constriction, and the diminution in the hormone output did not exceed

50 to 60% when venous outflow was reduced to values 70 to 75% lower than the control readings. After cessation of the venous congestion the output rate of adrenocortical hormone secretion returned to normal values within an hour, while adrenal blood flow remained somewhat below the control readings.

Numerous data indicate that the adrenal cortex is relatively insensitive to arterial hypoxia, and occasionally an enhanced steroid secretion was observed when arterial oxygen content had diminished. Complete occlusion of all the vessels supplying the left adrenal gland in the rat for periods of 30 min to 1 hour resulted in a 50% reduction of corticosteroid output as measured 1 hour and 24 hours after release of the occlusion (DÁVID and KOVÁCS 1966). FRANK et al. (1955) found in dogs with severe haemorrhagic hypotension the values for corticoid release to vary between 60 and 100% of the control values even if blood flow through the adrenals had been reduced by more than 80%. Similar observations were made concerning adrenal medullary hormone secretion (GLAVIANO et al. 1960; FOWLER et al. 1961).

The observed reduction in adrenal corticoid secretion during venous congestion of the gland might have been the result of several factors. In addition to a possible diminution of the secretory activity of the adrenocortical cells due to elevated pressure and to diminished ACTH and oxygen supply, the fact that the lymph vessels draining the adrenal cortex may participate in the transport of the hormones has also to be considered (STARK et al. 1962), as well as the fact that part of the secreted hormone may be drained through veins not connected with the lumboadrenal system (BACHMANN and SCHARRER 1954, DONÁTH 1957). In subsequent experiments attempts will be made to clarify the role played by these factors. It is also possible that some of the adrenocortical cells are destroyed in consequence of increased venous and capillary pressure and eventual smaller haemorrhages, while the remaining cells are capable of partially compensating for the loss in secretory activity. However, several data from this laboratory (MONOS et al. 1965) speak against this possibility, for the ACTH-responsive cells of the adrenal cortex secrete corticoid hormones at a maximal rate under similar experimental conditions. Also inconsistent with the above assumption is the observation that, in the present experiments, the diminution in the secretory rate was reversible, and histological examination revealed no destructive haemorrhages.

Within an hour after release of the clamp constricting the lumboadrenal vein, adrenal venous outflow did not return to control values despite unaltered arterial blood pressure. Considering that corticosteroid secretion had fully normalized within this period it is not likely that new channels had opened which could have drained the blood deficit observed in the lumboadrenal vein. There is reason for assuming that sustained and increasing congestion caused oedema to develop in the interstitium of the adrenal cortex, resulting in a prolonged increase vascular resistance in the gland.

When evaluating the interrelationship between the degree of venous congestion and the changes in adrenocortical hormone output it has to be taken into account that adrenal blood flow was measured by determining lumboadrenal venous outflow. However, as the adrenal gland possesses an intricate vascular network, the opening of A—V shunts and changes in the distribution of blood between cortex and medulla may alter adrenocortical blood supply without measurable alterations of lumboadrenal venous outflow.

It has been concluded that the adrenal cortex is relatively resistant to local venous congestion, and the diminution in corticoid output observed during congestion lasting for several hours and augmented in a stepwise fashion is reversible after release of the venous clamp.

## Acknowledgements

The authors are indebted to Miss E. Bohdaneczky and Miss J. Kovács, and to Mr. Gy. Szilassy, for skilful technical assistance.

## LITERATURE

Bachmann, R., Scharrer, B.: Blutgefäss- und Lymphgefässapparat der innersekretorischen Drüsen. In: Möllendorff, W., Bargmann, W. (Eds.): Handbuch der mikroskopischen Anatomie des Menschen. Springer-Verlag, Berlin **1954,** Vol. 6/5, Pp. 442—470, 685—688.

Bucht, H.: Percutaneous catheterization of the left adrenal vein in man. Scand. J. clin. Lab. Invest. **1962,** 14: Suppl. 64, 27—38.

Bush, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. Biochem. J. **1952,** 50: 370—378.

Dávid, M. A., Kovács, K.: Effect of ischaemia on adrenocortical corticosterone secretion in the rat. Acta med. Acad. Sci. hung. **1966,** 23: 61—67.

Donáth, T.: Experimentelle Beiträge zum venösen Kreislauf der Nebenniere. (Fluoreszenzmikroskopische Untersuchungen.) Anat. Anz. **1957,** 104: 412—418.

Flint, J. M.: The blood-vessels, angiogenesis, reticulum, and histology of the adrenal. Johns Hopkins Hosp. Rep. **1900,** 9: 153—175.

Fowler, N. O., Shabetai, R., Holmes, J. C.: Adrenal medullary secretion during hypoxia, bleeding, and rapid intravenous infusion. Circulat. Res. **1961,** 9: 427—435.

Frank, H. A., Frank, E. D., Korman, H., Macchi, I. A., Hechter, O.: Corticosteroid output and adrenal blood flow during hemorrhagic shock in the dog. Amer. J. Physiol. **1955,** 182: 24—24.

Glaviano, V. V., Bass, N., Nykiel, F.: Adrenal medullary secretion of epinephrine and norepinephrine in dogs subjected to hemorrhagic hypotension. Circulat. Res. **1960,** 8: 564—571.

Harrison, R. G., Brown, K. N.: Suprarenal cortical degeneration following isoproterenol administration in the rat. Endocrinology **1964,** 75: 173—178.

Harrison, R. G., Hoey, M. J.: The adrenal circulation. Blackwell. Oxford **1960,** Pp. 77.

Hirai, K., Atkins, G., Marotta, S. F.: 17-hydroxycorticosteroid secretion during hypoxia in anesthetized dogs. Aerospace Med. **1963,** 34: 814—816.

Hornbein, T. F.: Adrenal cortical response to chronic hypoxia. J. appl. Physiol. **1962,** 17: 246—248.

Kolthoff, N. J., Macchi, I. A., Wyman, L. C.: Blood flow rates of intact and regenerated rat adrenals during stress. Amer. J. Physiol. **1964,** 206: 793—795.

Kovács, K., Caroll, R., Tapp, E.: Temporary ischaemia of the rat adrenal gland. J. Path. Bact. **1966,** 91: 235—240.

Kovács, K., Csernay, L., Szijj, Ilona: ACTH-val kezelt patkányok megnagyobbodott mellékveséinek vérkeringése. Kísérl. Orvostud. **1968,** 20: 422—428.

MONOS, E., KOLTAY, EDIT, KOVÁCH, A. G. B.: Adrenal blood flow and corticosteroid secretion: I. The acute effect of hypophysectomy on adrenal blood flow and corticohormone secretion in the anaesthetized dog. Acta physiol. Acad. Sci. hung. **1965,** 28: 140—153.

PORTER, J. C., KLAIBER, M. S.: Corticosterone secretion in rats as a function of ACTH input and adrenal blood flow. Amer. J. Physiol. **1965,** 209: 811—814.

STARK, E., PAPP, M., FACHET, J., MIHÁLY, KATALIN: Participation of the lymph circulation in the transport of hormones. Acta physiol. Acad. Sci. hung. **1962,** 21: 347—351.

URQUHART, J.: Adrenal blood flow and the adrenocortical response to corticotropin. Amer. J. Physiol. **1965,** 209: 1162—1168.

VECSEI, P.: A mellékvesekéreg-működés vizsgáló módszerei. In: GÓTH, E. (Ed.): Az endokrinológia újabb eredményei. Medicina Budapest **1963,** 114—150.

WEINHEIMER, B., OERTEL, G. W., LEPPLA, W., BLAISE, H., BETTE, L.: Die diagnostische Bedeutung der Nebennierenvenenkatheterisierung. Helv. Med. Acta **1963,** 30: 482—486.

Emil MONOS, Edit KOLTAY, András SULYOK, Tamás VENKEY és Arisztid KOVÁCH.

Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma.

Budapest VIII. Üllői út 78/a.

Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 313—320 (1969)*

# EFFECT OF LOCAL VENOUS CONGESTION ON ADRENOCORTICAL FUNCTION

## II. CHANGES IN CORTICOID RELEASE INTO VENOUS BLOOD AND LYMPH AND IN OXYGEN CONSUMPTION

By

A. SULYOK, E. MONOS, Edit KOLTAY, Andrea BEDE and A. G. B. KOVÁCH

EXPERIMENTAL RESEARCH LABORATORY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received January 9, 1969)

In agreement with previous results it has been established that the adrenal cortex is relatively resistant to severe local venous congestion lasting for 1 to 2 hours; reduction of adrenal blood flow to 25% of control values by elevating venous pressure to 50—60 mm Hg caused only 50% diminution of corticoid secretion.

Adrenal oxygen consumption decreased in proportion with the reduction of hormone output. The difference between arterial and venous oxygen saturation was 8% in the control period, and although the difference was considerably increased during venous congestion the obtained values did not exceed one third of those found in the resting limb.

Reduction of adrenal venous outflow resulted in enhanced hormone release into the lymph of the thoracic duct, but excess corticoid output covered only a fraction of the deficit caused by the reduced output through the adrenolumbar vein. Concentration of corticoid hormones, in arterial blood did not change significantly.

Large amounts of exogenous ACTH administered during local venous congestion had no appreciable effect on adrenal corticoid output and oxygen consumption. One hour after release of the clamp applied on the adrenal vein, corticoid output was restored to control values.

In previous experiments from this laboratory (MONOS et al. 1969) an attempt was made to establish the effect of a stepwise reduction of venous outflow on corticoid release through the lumboadrenal vein during acute stress. A 25 to 30% reduction of venous outflow had no significant effect on adrenocortical hormone output. The adrenal cortex continued to secrete at a rate of 40 to 50% of the control values even if congestion had been maintained for periods of 2 to 3 hours during which the lumboadrenal vein was gradually obstructed to reduce adrenal blood flow to 25—30% of control values. The observed diminution in adrenocortical secretion might have been due to some deterioration of the secretory capacity of the cells; if this were true, one might presume that either the reduction in oxygen supply or the diminution of available ACTH was responsible for the decreased secretion rate.

It is not certain, however, whether the observed reduction in adrenal hormone output was associated with a deterioration of adrenocortical secretory capacity. It has been demonstrated that, under pathological conditions, significant amounts of corticoid hormones are secreted into the lymph (STARK et

al. 1962). Apart from the lumboadrenal vein, the existence of other venous drainages may also be assumed (DONÁTH 1957, HARRISON and HOEY 1960).

In the present experiments the changes in adrenocortical hormone secretion accompanying severe local venous congestion and the mechanisms responsible for them have been analysed in anaesthetized dogs. Venous outflow from the adrenal gland was reduced by about 75% of the initial values and the output of hydrocortisone and corticosterone into the lumboadrenal vein and the thoracic duct lymph was estimated. The effects of venous obstruction on the arterial corticoid level, on adrenal oxygen consumption and on adrenal venous pressure were also studied and the effect of ACTH on the above parameters of adrenocortical function has been analysed.

## Methods

Fifteen mongrel dogs weighing 13 to 27 kg were used in the experiments; anaesthesia was induced by 30 mg/kg body weight of sodium pentobarbital.

The animal was tracheotomized, then the right femoral artery and vein, the left common carotid artery, and the thoracic duct were cannulated. The left lumboadrenal vein was exposed through an abdominal midline incision, and a polyethylene cannula with an internal diameter of 2.5 to 3.5 mm was pushed forward to the edge of the adrenal gland. The adrenocaval vein and the side-branches of the lumboadrenal vein were ligated. After closing the wound, 300 I.U./kg body weight of heparin was given intravenously, and transfusion of heparinized blood obtained from a donor dog was started. The cannula inserted into the lumboadrenal vein was led out through a lateral abdominal incision and the blood flowing out of the adrenal gland was allowed to drop on the membrane of a piezoelectric signal transducer of a Jacquet drop-recorder. Adrenal blood flow was recorded on a Zimmermann kymograph. For the determination of corticoids, blood and lymph were collected into graduated cylinders placed into an ice-bath.

The blood and lymph samples were centrifuged in a cold-chamber, then concentrations of hydrocortisone and corticosterone in plasma and lymph were determined. After extraction and purification in chloroform and distilled water, the samples were transferred on Whatman No. 1 paper in nitrogen stream. After repeated purification and chromatography in the BUSH $B_5$ system, the spots were developed with tetrazolium blue then eluated and hydrocortisone and corticosterone contents were determined photometrically. Corticoid concentration in arterial blood plasma was estimated similarly in samples obtained through a T-cannula from the common carotid artery.

Haematocrit value of arterial and adrenal venous blood was determined by centrifugation in capillary tubes, using a MSE haematocrit centrifuge.

Oxyhaemoglobin in blood samples withdrawn from the common carotid artery, and the femoral and lumboadrenal veins was determined by an Elema—Schönander oxymeter.

Oxygen consumption of the adrenal gland was calculated from the haemoglobin content as determined on the basis of the haematocrit value (HAWK et al. 1954), arterial and lumboadrenal venous oxygen saturation, and lumboadrenal blood flow.

After control periods, the cannula inserted into the lumboadrenal vein was constricted to reduce venous outflow by about 75% of the initial value; a manometer attached to the cannula between the site of obstruction and the adrenal gland served for continuous monitoring of adrenal venous pressure. The cannula was constricted for about one and a half hours during which four adrenal and femoral venous and arterial blood samples were withdrawn. After the second blood collection, 5 I.U. of ACTH (Organon Ltd., Oss, Netherlands) were given intravenously. Before and after constriction, blood samples were collected twice.

Arterial blood pressure in the femoral artery and respiratory rate were measured continuously throughout the experiment.

Statistical analysis of the differences between control and experimental readings was undertaken by STUDENT's *t*-test.

## Results

### *Adrenal venous blood pressure and corticoid output during congestion*

Reduction of adrenal venous outflow to about fourth of the control value caused adrenal venous blood pressure to increase to about 65 mm Hg beside a mean arterial pressure of 110 mm Hg (Table I). In the course of congestion, however, venous pressure was successively diminished, the rate of decrease attaining 10 mm Hg on the average during a period of 30 min ($p < 0.01$).

The average hydrocortisone and corticosterone output into the lumboadrenal vein was 14.1 and 6.3 μg/hour/kg body weight, respectively,

**Table I**

*Effect of severe local venous congestion on adrenal glycocorticoid secretion (Mean ± S.E.M.)*

| | | Control values | Congestion I | Congestion II | After congestion |
|---|---|---|---|---|---|
| Lumboadrenal vein | Blood flow, ml/min | 4.80 ±0.51 | 1.13+ ±0.11 | 1.11+ ±0.10 | 3.51* ±0.48 |
| | Venous pressure, mm Hg | −6.8 ±1.5 | 58.1+ ±3.2 | 43.1+* ±3.5 | −3.7* ±0.7 |
| | Hydrocortisone output, μg/hour/kg body weight | 27.1 ±6.5 | 14.1+ ±3.2 | 12.7+ ±3.1 | 28.1* ±7.1 |
| | Corticosterone output, μg/hour/kg body weight | 11.7 ±1.9 | 6.3+ ±0.8 | 5.5+ ±0.7 | 10.2* ±1.3 |
| Thoracic duct | Lymph flow, ml/min | 0.75 ±0.12 | 0.73 ±0.09 | 0.69 ±0.08 | 0.69 ±0.09 |
| | Hydrocortisone output, μg/hour/kg body weight | 0.68 ±0.20 | 1.87+ ±0.74 | 2.10+ ±0.52 | 0.50* ±0.10 |
| | Corticosterone output, μg/hour/kg body weight | 0.20 ±0.03 | 0.69+ ±0.24 | 0.45 ±0.14 | 0.26 ±0.09 |
| Arterial plasma levels | Hydrocortisone, μg/100 ml | 24.0 ±4.4 | 30.4 ±5.5 | 26.3 ±7.6 | 29.9 ±7.8 |
| | Corticosterone, μg/100 ml | 17.1 ±5.9 | 13.3 ±3.9 | 19.1 ±7.6 | 20.8 ±4.2 |
| Arterial blood pressure mm Hg | | 114.5 ±6.9 | 108.4 ±6.6 | 113.5 ±5.5 | 113.3 ±4.6 |

Between I and II, 5 I.U. ACTH was given intravenously. For symbols, see Fig. 1.

during congestion, i.e. about 40 to 50% lower than the control values (Fig. 1 and Table I). Hormone output through the thoracic duct was increased almost three times, while lymph flow was unchanged.

Administration of 5 I. U. of ACTH during congestion failed to enhance corticoid output, but somewhat increased adrenal venous blood pressure.

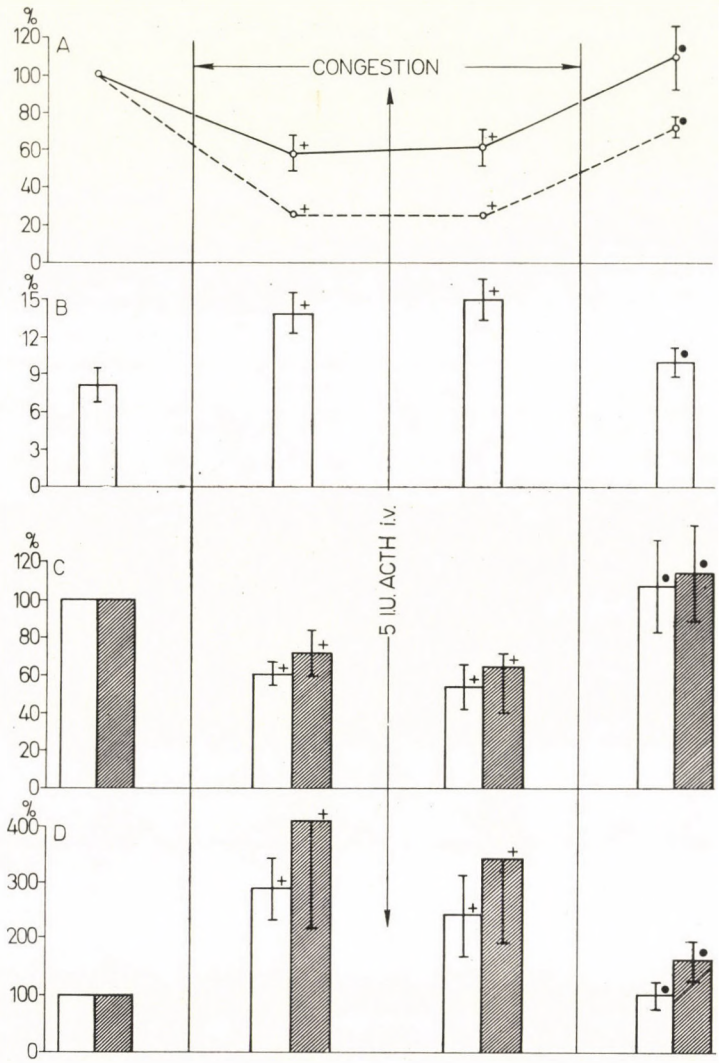



*Fig. 1.* Percentile changes in adrenal oxygen consumption (*A*, broken line), arterio-venous oxygen saturation difference *(B)*, and secretion rate of hydrocortisone (open blocks) and corticosterone (hatched blocks) in the lumboadrenal vein *(C)* and the thoracic duct *(D)*, during severe local venous congestion. Adrenal venous outflow (measured in the lumboadrenal vein) is indicated by a solid line in section A. Mean ± S.E.M.; statistically significant differences as compared to control values are indicated by +, and those compared to previous values by*

This was followed by a diminution in pressure at about the same rate as that observed prior to the administration of ACTH. Within an hour after release of the constriction, corticoid secretion returned to control values, while adrenal blood flow did not attain the preobstruction value.

Mean arterial hydrocortisone and corticosterone levels somewhat increased in the course of the experiments, but the differences were not significant statistically (Table I). Arterial hydrocortisone and corticosterone concentrations (24.0±4.4 and 17.1±5.9 μg/100 ml plasma, respectively) did not differ significantly from the control levels observed in the thoracic duct lymph (35.3±11.1 and 14.0±2.6 μg/100 ml plasma, respectively).

**Table II**

*Effect of local venous congestion on adrenal oxygen consumption, oxygen saturation and haematocrit value. Mean ± S.E.M.*

| | | Control value | Congestion I | Congestion II | After congestion |
|---|---|---|---|---|---|
| Adrenal oxygen consumption, ml/min/100 g | | 7.6 ±1.0 | 3.2+ ±0.4 | 3.3+ ±0.5 | 6.5* ±0.8 |
| Oxygen saturation (%) | Lumboadrenal vein | 79.9 ±1.6 | 73.3+ ±1.6 | 74.1+ ±1.8 | 80.3* ±1.4 |
| | Common carotid artery | 88.0 ±0.9 | 87.3 ±1.9 | 88.7 ±1.7 | 90.2 ±0.7 |
| | Femoral vein | 45.2 ±4.3 | 42.8 ±3.2 | 41.5 ±1.3 | 40.2 ±3.5 |
| Haematocrit | Lumboadrenal vein | 52.5 ±2.6 | 51.1 ±4.0 | 49.4 ±3.7 | 50.5 ±1.8 |
| | Common carotid artery | 53.5 ±3.3 | 51.6 ±4.0 | 53.3 ±4.2 | 52.6 ±3.2 |

Between I and II, 5 I.U. ACTH was given intravenously. For symbols, see Fig. 1.

### *Adrenal oxygen consumption during local congestion*

As shown in Table II, mean adrenal oxygen consumption was 7.6 ml/min/100 g in the control periods. After a 75% reduction of venous outflow, adrenal oxygen uptake was reduced, similarly to corticoid output, by 40 to 50% only (Fig. 1), and this value was not consistently altered by the intravenous administration of 5 I.U. of ACTH. After release of the constriction, oxygen consumption was restored to near the control value.

Oxygen saturation of lumboadrenal venous blood was high in the control periods, being only 8% lower than arterial saturation, whereas in the resting hindlimb venous oxygen saturation was almost 40% less than arterial saturation. The difference in oxyhaemoglobin content between arterial and adrenal venous blood was increased during local venous congestion (Fig. 1 and Table II).

The average haematocrit value in arterial and adrenal venous blood was essentially the same. Lumboadrenal venous haematocrit was not affected by local venous congestion (Table II).

## Discussion

In accordance with previous observations (MONOS et al. 1969), local venous congestion in the adrenals was associated with a disproportional diminution of adrenocortical hormone output but the decrease in corticoid secretion amounted to 40 to 50% in contrast to a 75% decrease in blood flow.

During severe venous congestion, the release of corticoid hormones into the lumboadrenal vein is not a reliable measure of the total hormone output, since the amount of steroids secreted into the lymph is considerably augmented under such conditions. Nevertheless, the excess output observed in the thoracic duct covers only about 5 to 10% of the deficit in corticoid output measured in the lumboadrenal vein. Under conditions of undisturbed adrenal venous outflow the release of corticoids into the lymph vessels is insignificant, as clearly indicated by the lack of a significant difference between arterial and thoracic duct corticosteroid levels. Similar observations were made by STARK et al. (1962) who found increased corticoid levels in the lymph after complete occlusion of adrenal venous outflow.

In congestion due to partial obstruction of the lumboadrenal vein the role played by other veins in the transport of corticoid hormones must also be considered. The progressive diminution of venous pressure during the experiment might have been due to the opening of by-passes, although the present experiments did not prove this possibility, as the diminution in venous pressure could have been the result of an increase in intra-adrenal resistance. Arterial corticoid concentrations did not change significantly during venous congestion, nevertheless, considering the degree of dilution and the many interfering factors, it is impossible to conclude from variations in peripheral hormone concentration to an altered adrenal secretion rate.

The reduction in adrenal oxygen consumption was in proportion with the diminution of corticoid output into the lumboadrenal vein, i.e. it was considerably less decreased than was venous outflow. This was due first of all to the increase in arterio-venous oxygen saturation, since the haematocrit, a reliable measure of haemoglobin content, did not change significantly.

It was striking that even the augmented arterio-venous saturation difference should have amounted to one third only of the value measured in the resting hindlimb. Thus, if some deterioration of the secretory capacity of the adrenocortical cells occurred in these experiments, it could not be the result of a deficient oxygen supply. Considering also the morphological data (FLINT 1900), it may be supposed that high capacity arterio-venous shunting exists in the adrenal gland, thus, oxygen saturation in adrenal venous blood is only an indirect measure of the extraction of oxygen in the adrenocortical capillaries. The oxygen consumption measured in the present experiments referred to the whole gland, however, in view of the finding that the weight of the cortex is about 5 times that of the medulla (MONOS, unpublished observation) it might be concluded that a substantial fraction of the oxygen is consumed by the cortical cells.

The present results indicate that the diminution of corticoid output during local venous congestion was not due to the diminution of ACTH input, since during congestion large amounts of ACTH given intravenously failed to enhance corticoid secretion and adrenal oxygen uptake. Previous observations have indicated (MONOS et al. 1965) that in majority of the experiments of this type the adrenal cortex secretes at a maximal rate owing to the surgical stress; consequently, ACTH supply to the adrenal gland might be supramaximal and the reduced adrenal blood flow due to congestion could not be associated with such a reduction of ACTH input that would have accounted for the diminution of corticoid secretion. The possibility cannot, however, be excluded that the blood supply to the adrenal cortex was diminished less than what could be inferred from the changes in lumboadrenal outflow.

Thus, it seems that the slight deterioration of adrenocortical secretory activity was due mainly to the increased venous pressure which during congestion exceeded half of the mean arterial pressure. The observed high venous pressure indicates also the presence of shunts in the adrenal circulation. It is reasonable to assume that congestion resulted in enhanced filtration of fluid into the extracapillary space which obviously increased the distance between the surface of the secreting cells and the capillary endothelium.

It is remarkable that the rate of corticosteroid secretion should so well be preserved during severe venous congestion, and the diminution of hormone output and oxygen consumption should be reversible after congestion lasting for 1 or 2 hours.

## Acknowledgements

The authors are indebted to Misses E. BOHDANECZKY and J. KOVÁCS, and to Mr. GY. SZILASSY, for skilful technical assistance.

## LITERATURE

DONÁTH, T.: Experimentelle Beiträge zum venösen Kreislauf der Nebenniere. (Fluoreszenzmikroskopische Untersuchungen.) Anat. Anz. **1957,** 104: 412—418.

FLINT, J. M.: The blood-vessels, angiogenesis, reticulum, and histology of the adrenal. Johns Hopkins Hosp. Rep. **1900,** 9: 153—175.

HARRISON, R. G., HOEY, M. J.: The adrenal circulation. Blackwell; Oxford **1960.** P. 77.

HAWK, P. B., OSER, B. L., SUMMERSON, W. H.: Practical physiological chemistry, 13th ed. Churchill, London **1954,** P. 609.

MONOS, E., KOLTAY, E., KOVÁCH, A. G. B.: Adrenal blood flow and corticosteroid secretion: I. The acute effect of hypophysectomy on adrenal blood flow and corticohormone secretion in the anaesthetized dog. Acta physiol. Acad. Sci. hung. **1965,** 28: 139—153.

MONOS, E., KOLTAY, Edit, SULYOK, A., VENKEY, T., KOVÁCH, A. G. B.: Effect of local venous congestion on adrenocortical function I. Changes in adrenal corticoid secretion after stepwise reduction of venous outflow, Acta physiol. Acad. Sci. hung. **1969,** 35: 305—312.

STARK, E., PAPP, M., FACHET, J., MIHÁLY, K.: Participation of the lymph circulation in the transport of hormones. Acta physiol. Acad. Sci. hung. **1962,** 21: 347—351.

András SULYOK, Emil MONOS, Edit KOLTAY, Andrea BEDE, Arisztid KOVÁCH
Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma.
Budapest VIII., Üllői út 78/a. Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 321—330 (1969)*

# EFFECT OF LOCAL VENOUS CONGESTION ON ADRENOCORTICAL FUNCTION

## III. ADRENAL CORTICAL AND MEDULLARY BLOOD FLOW

By

E. MONOS, ZSUZSANNA BIRÓ, A. SULYOK and A. G. B. KOVÁCH

EXPERIMENTAL RESEARCH LABORATORY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received January 22, 1969)

Adrenal cortical and medullary blood flow has been studied by the method of $H_2$ washout under basal conditions and during severe local venous congestion in dogs. Adrenal venous outflow and pressure were measured by the direct method, and oxygen consumption was determined on the basis of arterio-venous saturation differences.

Effective cortical and medullary blood flows were 0.84 and 0.92 ml/min/g, respectively, whereas total adrenal blood flow (venous outflow) was 4.21 ml/min/g. During congestion induced by constriction of the venous cannula, cortical and medullary blood flows were considerably less decreased than was venous outflow. Both cortical and medullary blood flow returned to normal values after congestion for 60 to 90 min.

The observed changes in adrenal venous pressure and oxygen consumption were in agreement with previous results.

The reliability of the obtained values is discussed. It appears that medullary flow has been underestimated by the employed method.

It has been found in previous experiments from this laboratory that the adrenal cortex is relatively insensitive to the effects of local venous congestion, as hydrocortisone and corticosterone release by the lumboadrenal vein, and oxygen consumption of the gland are diminished far less than adrenal venous outflow. If blood flow diminished by about 75% and venous pressure increased to 50 to 60 mm Hg, the secretory capacity of the adrenal cortex was reduced only by about 50%. During severe venous congestion, the lymph vessels have been shown to participate in the transport of corticoid hormones (MONOS et al. 1969 and SULYOK et al. 1969).

Since the adrenal gland has an intricate vascular structure (FLINT 1900; BACHMANN and SCHARRER 1954; HARRISON and HOEY 1960; SYMINGTON 1962) it is possible that blood flow through the cortex is much less diminished than what would be expected on the basis of the reduction of venous outflow from the adrenal gland. The aim of the present work was to test this possibility.

Owing to methodical difficulties, blood flow in the various layers of the adrenal has scarcely been studied. Several important data on adrenal medullary and cortical blood flow have recently been furnished by KRAMER and SAPIRSTEIN (1967).

In the present experiments, for the determination of adrenal blood flow a method has been chosen which is suitable for the measurement of blood supply to small regions and allows repeated estimations to be made within a short period. Local cortical and medullary blood flows were established simultaneously with adrenal venous outflow in anaesthetized dogs, under acute conditions. After control values had been determined, severe venous congestion was induced by reducing venous outflow by about 75%, and the intra-adrenal distribution of blood flow was estimated. Adrenal oxygen uptake, arterial and lumboadrenal venous mean pressures were recorded prior to and during congestion.

## Methods

Experiments were made on 11 mongrel dogs weighing 13 to 27 kg and anaesthetized intravenously with 30 mg/kg of sodium pentobarbital.

Blood flow in the adrenal cortex and medulla was estimated by the $H_2$-washout technique described by AUKLAND et al. (1964). The left adrenal gland was exposed through an abdominal midline incision, then a polyethylene cannula was inserted into the lumboadrenal vein, and platinum electrodes 0.8 mm in diameter were introduced into cortex and medulla to measure changes in tissue $H_2$ concentration (Fig. 1). The electrodes were insulated with

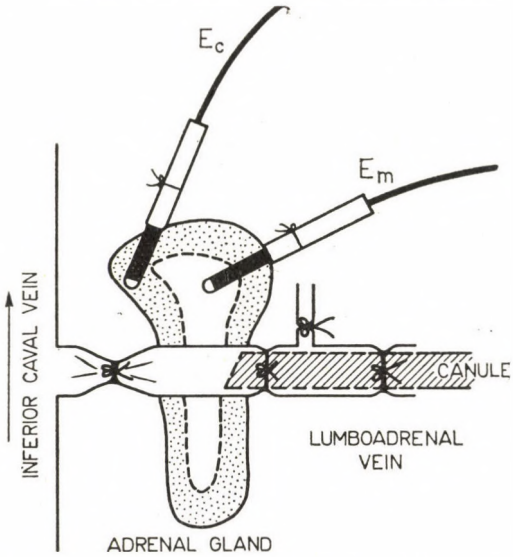



*Fig. 1.* Diagram showing the position of the platinum electrodes introduced into the adrenal to measure tissue $H_2$ concentration and of the cannula inserted into the lumboadrenal vein. $E_c$, cortical electrode; $E_m$, medullary electrode

Ins 1 = x, leaving an activated free part of 0.8 to 1.0 mm. The indifferent electrode of the circuit was a calomel electrode introduced under the skin of the femoral region via a polyethylene tubing-KCl agar bridge. The electrodes were attached to a Kipp BD-3 Micrograph, through an attenuator-compensator unit constructed in our laboratory by P. SÁNDOR, E. PÁSZTOR, GY. REISCHL and A. G. B. KOVÁCH. The unit included the variable working resistances to the electrodes, and the dry batteries and potentiometers securing the fixed positive

polarizing potential of about 250 mV supplied to the measuring electrodes. This voltage was compensated towards the Micrograph adjusted to a measuring range of 0.1 or 0.25 mV. The equipment allowed successive coupling of 3 platinum electrodes during one measurement.

$H_2$ saturation of the tissues was attained by inhalation of the gas. Optimum saturation level was obtained after $H_2$ had been introduced into the tracheal cannula at a rate of 10 to 20 ml/min for 3 to 5 min. The desaturation exponentials recorded by the Micrograph were transferred to semilogarithmic paper; the slope of the line thus obtained is proportional with blood flow which was calculated on basis of the equation,

$$k = \frac{\ln 2}{T_{1/2}}$$

and expressed in ml/min/g (KETY 1960). $H_2$ concentration of arterial blood during desaturation was considered zero, the partition coefficient ($\lambda$) of hydrogen between tissue and blood and specific gravity of the adrenal tissue were regarded as 1.

Adrenal oxygen uptake was determined on the basis of the arterio-venous (lumboadrenal) oxygen saturation difference, adrenal venous outflow, and haemoglobin content as estimated from the haematocrit value by means of the nomogram of HAWK et al. (1954). Relative oxyhaemoglobin content of blood samples obtained from the lumboadrenal and femoral veins and the common carotid artery was measured by an Elema—Schönander oxymeter. Haematocrit was determined in glass capillaries, by centrifugation for 10 min in a MSE microhaematocrit centrifuge.

Arterial and adrenal venous pressure and respiratory rate were monitored continuously throughout the experiment.

After determining two control values, the lumboadrenal cannula was constricted to reduce adrenal venous outflow to about 25% of the initial value. Duration of congestion was 1 to 1 and a half hour, during which two measurements were performed. After releasing the constriction, further two estimations were made.

Statistical analysis of the values was undertaken by STUDENT's *t*-test.

## Results

### *1. Adrenal cortical and medullary blood flow under basal conditions*

Mean blood flow in the adrenal cortex and medulla was 0.84 and 0.92 ml/min/g, respectively, the simultaneously determined venous outflow attained 4.21 ml/min/g, about 5 times the value obtained by the $H_2$-washout technique (Table I). The difference between cortical and medullary blood flow was not significant statistically.

The correlation coefficient for effective and total blood flow was 0.49 for the cortex, and 0.60 for the medulla; neither of these values was significant statistically ($p = 0.10$ and $0.10 > p > 0.05$ respectively).

In agreement with previous data (SULYOK et al. 1969) total adrenal oxygen consumption averaged 8.9 ml/min/100 g, the difference in oxygen saturation of arterial and adrenal venous blood was 8.7%; arterial and adrenal venous haematocrit values did not differ significantly (Table II).

### *2. Effect of congestion on cortical and medullary blood flow*

An about 75% reduction of venous outflow resulted in a 40% diminution of cortical and medullary tissue flow (Fig. 2). Mean blood flow in the first and second part of congestion, with an interval between the two measurements

**Table I**

*Cortical and medullary blood flow during severe adrenal venous congestion*

| | | No. | Control | Congestion I | Congestion II | After congestion |
|---|---|---|---|---|---|---|
| Adrenal blood flow, ml/min/g | Cortical | 1 | 0.85 | 0.45 | — | 0.87 |
| | | 2 | 1.24 | 0.71 | 0.95 | 1.26 |
| | | 3 | 0.54 | 0.34 | 0.33 | 0.62 |
| | | 4 | 0.31 | 0.21 | 0.19 | 0.25 |
| | | 5 | 1.22 | 1.15 | 0.83 | 1.98 |
| | | 6 | 0.60 | 0.42 | 0.22 | 0.51 |
| | | 7 | 0.34 | 0.37 | 0.35 | 0.55 |
| | | 8 | 1.16 | 0.95 | 0.98 | 1.26 |
| | | 9 | 0.91 | 0.36 | 0.38 | 0.57 |
| | | 10 | 1.20 | 0.77 | 0.79 | 1.63 |
| | | Mean | 0.837 | 0.573 | 0.558 | 0.950 |
| | | ± S.E.M. | 0.116 | 0.097 | 0.107 | 0.177 |
| | Medullary | 1 | 1.26 | 0.71 | — | 1.01 |
| | | 2 | 0.26 | 0.21 | 0.25 | 0.40 |
| | | 3 | 0.80 | 0.24 | 0.31 | 0.63 |
| | | 4 | 0.41 | 0.25 | 0.20 | 0.41 |
| | | 5 | 1.47 | 0.94 | 0.40 | 2.26 |
| | | 6 | 0.81 | 0.64 | 0.39 | 1.28 |
| | | 7 | 0.95 | 0.58 | 0.73 | 1.08 |
| | | 8 | 0.87 | 0.60 | 0.65 | 0.85 |
| | | 9 | 0.87 | 0.41 | 0.46 | 0.85 |
| | | 10 | 1.39 | 1.01 | 1.16 | 2.28 |
| | | 11 | 1.03 | 0.44 | 0.43 | 0.87 |
| | | Mean | 0.920 | 0.548 | 0.498 | 1.084 |
| | | ± S.E.M. | 0.112 | 0.082 | 0.090 | 0.194 |
| | Total | 1 | 4.55 | 1.64 | — | 4.66 |
| | | 2 | 2.97 | 0.75 | 0.71 | 2.92 |
| | | 3 | 3.11 | 0.97 | 0.84 | 3.21 |
| | | 4 | 3.01 | 0.63 | 0.60 | 2.75 |
| | | 5 | 6.76 | 1.21 | 0.86 | 6.42 |
| | | 6 | 4.96 | 1.39 | 1.51 | 4.29 |
| | | 7 | 3.61 | 0.98 | 0.91 | 3.43 |
| | | 8 | 6.46 | 1.38 | 1.46 | 5.04 |
| | | 9 | 2.55 | 0.66 | 0.63 | 2.16 |
| | | 10 | 4.90 | 1.36 | 1.16 | 5.75 |
| | | 11 | 3.45 | 0.87 | 0.80 | 3.51 |
| | | Mean | 4.212 | 1.076 | 0.948 | 4.028 |
| | | ± S.E.M. | 0.433 | 0.102 | 0.102 | 0.409 |

of about 40 to 60 min, attained 0.57 and 0.56 ml/min/g in the cortex, and 0.55 and 0.50 ml/min/g in the medulla (Table I).

As shown in Table II, mean arterial pressure did not change markedly during the congestion period. Adrenal venous pressure showed a similar increase as that observed in previous experiments; also in agreement with earlier results, there occurred a successive diminution of venous pressure during congestion, the difference between values obtained at an interval of about 40 min being significant statistically ($p < 0.05$).

The reduction of adrenal oxygen consumption was about 50 to 55% (Fig. 2), thus, in agreement with previous results, it was smaller than the reduction of venous outflow.

Adrenal arterio-venous oxygen saturation difference increased from an initial value of 8.7% to 14.1 and 17.7%. The haematocrit value did not change significantly during local congestion (Table II).

**Table II**

*Adrenal oxygen consumption, arterio-venous oxygen saturation difference, venous pressure and haematocrit during local venous congestion (Mean ± S.E.M.)*

| | | Control | Congestion I | Congestion II | After congestion |
|---|---|---|---|---|---|
| Adrenal | Oxygen consumption, ml/min/100 g | 8.88 ±1.61 | 3.66 ±0.39 | 3.60 ±0.60 | 7.81 ±0.55 |
| | Arterio-venous saturation difference, per cent | 8.73 ±1.08 | 14.09 ±1.16 | 17.67 ±1.25 | 8.18 ±0.99 |
| | Venous haematocrit | 51.9 ±1.4 | 53.7 ±1.5 | 52.7 ±2.1 | 52.6 ±2.1 |
| | Venous pressure, mm Hg | −1.8 ±2.1 | 57.3 ±4.7 | 50.1 ±4.5 | 0.9 ±1.7 |
| Arterial | Haematocrit | 51.5 ±1.5 | 51.8 ±1.9 | 51.2 ±2.0 | 52.3 ±1.5 |
| | Mean pressure | 101.8 ±4.7 | 100.0 ±4.6 | 99.0 ±5.5 | 94.1 ±4.7 |

After the constriction of the adrenal cannula had been released and congestion had subsided, blood flow and oxygen consumption were restored to control values within 30 min.

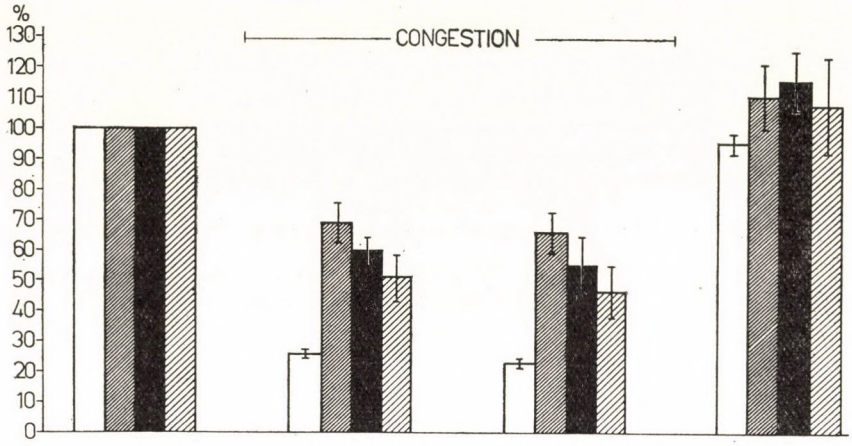



*Fig. 2.* Percentile changes in total adrenal blood flow (open blocks), effective cortical (hatched blocks) and medullary (solid blocks) blood flow, and oxygen consumption (dotted blocks) during local venous congestion. Vertical lines indicate S.E.M.

## Discussion

### *1. Adrenal blood flow under basal conditions*

Estimation of cortical and medullary blood flow by the $H_2$-washout technique yielded substantially lower values as calculated for unit of tissue weight than did the simultaneously determined venous outflow expressed in identical terms; the difference between the two methods was considerable, direct measurement revealing values 4 to 5 times those obtained by the washout technique.

It is questionable whether the discrepancy can be accounted for by certain physiological and morphological characteristics of the canine adrenal gland. There are a few data indicating, but not proving, that considerable shunting of blood flow is possible in the adrenal. According to FLINT (1900) there are about 50 medullary arteries which run from the capsular plexus directly through the cortex into the medulla and the central veins. The present investigations have shown that oxygen saturation of adrenal venous blood is high even under circumstances of maximum or nearly maximum secretory activity, the difference between arterial and venous saturation attaining only 8.7% in the present experiments, and 8.1% in a previous study (SULYOK et al. 1969). At the same time, the difference between venous saturation in the right atrium and arterial saturation, was 40.1%, about 5 times more than the adrenal arterio-venous difference. Provided that there is no significant difference in oxygen extraction between the adrenal and the other tissues, the assumption that 75 to 80% of the adrenal blood supply is diverted through

vascular shunts would provide a reliable explanation for the strikingly high oxyhaemoglobin concentration in adrenal venous blood. This assumption was further supported by the finding that the reduction of venous outflow to one fourth of the initial value by constriction of the cannula draining the adrenal caused a remarkable increase in venous pressure, up to values ranging between 50 and 60 mm Hg. Although the values for blood flow obtained by the $H_2$-washout technique were low as compared to the figures yielded by the direct measurement of venous outflow, the obtained readings are close to the values found by others in the active myocardium (AUKLAND et al. 1964). The observations of KRAMER and SAPIRSTEIN (1967) yielded 2.1 ml/min/g for adrenocortical blood flow, and 3.3 ml/min/g for medullary flow in the rat; owing to the species difference, these values cannot be compared to the present results.

On methodological reasons one may raise objections against the use of the $H_2$ desaturation technique for the measurement of tissue blood supply at high rates of flow. According to AUKLAND et al. (1964), however, under appropriate conditions the method is still suitable for the determination of blood flow up to 5 ml/min/g, but it is certainly reliable in the range below 1.5 ml/min/g; the values obtained in the present experiments were well below the latter limit.

A further methodical problem is the recirculation of $H_2$, since it has been assumed that arterial $H_2$ concentration is zero after desaturation has started. This was obviously not the case, since merely the initial (maximum 30 to 40 sec) segment of the washout curve can be distorted by arterial recirculation if saturation has been attained by $H_2$ inhalation (AUKLAND et al. 1964). In the present experiments, the washout process took part in the majority of cases in the form of a single-component exponential; occasionally, there was slight distortion of the curve in the initial segment (10 to 20 sec), therefore the slope of the lines on the semilogarothmic paper was estimated on the basis of the segment that had been obtained after 20 sec.

When determining adrenal medullary blood flow by the washout technique the problem of recirculation must be considered carefully. On basis of the intrinsic vascular structure (HARRISON and HOEY 1960) it is reasonable to assume that the blood derived from the sinusoid capillaries of the cortex comes into direct contact with the medulla, causing continuous resaturation of the medulla by the $H_2$ washed out of the cortex. Similar problems arise when adrenal blood flow is estimated by $^{86}$Rb-technique. In view of these considerations it appears that the obtained values for medullary blood flow underestimated the true blood supply to the medulla. Considering, however, that in the majority of cases the washout curves were exponential with a single component also for the medulla, it seemed worth to report these findings with the comment that their reliability has to be tested in further experiments.

Presumably, the introduction of the electrodes did not cause significant tissue destruction as the shape of the initial segment of the washout curves makes it difficult to assume that there would have been a great diffusion distance due to a poorly perfused area around the electrodes. Furthermore, total adrenal blood flow as calculated for unit of weight and oxygen consumption was not lower than the value obtained in previous experiments where no electrodes had been used (SULYOK et al. 1969).

### 2. *Effect of congestion on adrenal blood flow*

Adrenal cortical and medullary tissue flows remained relatively unaffected during severe local venous congestion; the 75% decrease in venous outflow was associated with reduction of only 40% tissue flow.

The small reduction in tissue blood supply may provide a satisfactory explanation of the earlier finding that local congestion is accompanied by a much smaller decrease in corticoid output and oxygen consumption than what would be expected in view of the grossly reduced lumboadrenal venous outflow (MONOS et al. 1969; SULYOK et al. 1969).

There is, however, no satisfactory explanation as to the mechanisms securing a satisfactory blood supply to the tissues against the considerably increased venous pressure.

It is presumable that part of the blood supplied to the adrenal is drained, instead of the usual lumboadrenal route, by other veins, e.g. the concomitant veins arising from the capsular venous plexus (FLINT 1900). If this is true, cortical hormone secretion ought to have continued at an even higher rate than what has been inferred from the measurement of corticoid release into the lumboadrenal vein and the thoracic duct. There is, however, no reason to believe that the adrenocortical cells secrete their hormones into two or more separate capillary systems.

An assumption can also be made concerning an active mechanism securing capillary flow against the high venous pressure. Although the presence of special longitudinal muscular trabeculae emerging into the lumen of the central vein and the medullary veins had been reported (BRUNN 1873; HENDERSON 1927; SYMINGTON 1962) their physiological significance has not been clarified. The possibility cannot be excluded that local venous congestion elicits a vasomotor reflex which secures a higher effective blood flow in the adrenal tissue at the expense of the by-pass. It is well known that the nerve supply to the intraglandular vessels is strikingly rich (AGARKOV 1964), and bioelectrical changes synchronous with alterations of blood pressure have been reported to occur on the afferent nerves of the adrenal gland (PINES 1962; NIIJIMA and WINTER 1968).

The present results indicate unequivocally that during venous congestion, part of the flow through the shunts is diverted towards the capillaries, since total blood flow was only twice as large as the effective tissue flow during congestion, whereas it was 4 to 5 times of that in the initial periods.

A methodological objection might be that congestion due to constriction of the venous cannula might have caused a shift in adrenal pH that could have interfered with the estimation of blood flow by the $H_2$ washout technique. To check this possibility, the baseline of the equipment was carefully observed when venous obstruction was started but $H_2$ was not yet inhaled; the reduction of venous outflow by about 75% had no appreciable effect on the electrode potential, whereas it abruptly changed on completely occluding the cannula. This observation indicates that tissue blood supply is satisfactory in the adrenals even during congestion of such a high degree.

## Acknowledgements

The authors are indebted to Misses E. Bohdaneczky and K. Galamb, and to Mr. Gy. Szilassy, for skilful technical assistance.

## LITERATURE

Agarkov, G. B. (Агарков, Г. Б.): Нервный аппарат надпочечных желез. Изд. «Медицина». Москва. **1964.** стр. 200.

Aukland, K., Bower, B. F., Berliner, R. W.: Measurement of local blood flow with hydrogen gas. Circulat. Res. **1964,** 14: 164—187.

Bachmann, R., Scharrer, B.: Blutgefäss- und Lymphgefässapparat innersekretorischer Drüsen. In: Möllendorff, W., Bargmann, W. (Eds.): Handbuch der mikroskopischen Anatomie des Menschen. Springer-Verlag, Berlin **1954.** Vol. 6/5, Pp. 442—447, 685—688.

Brunn, von, A.: Über das Vorkommen organischer Muskelfasern in den Nebennieren. Nachr. Königl. Ges. Wiss. **1873,** 421—423.

Flint, J. M.: The blood vessels, angiogenesis, reticulum, and histology of the adrenal. Johns Hopkins Hosp. Rep. **1900,** 9: 153—175.

Harrison, R. G., Hoey, M. J.: The adrenal circulation. Blackwell, Oxford **1960.** Pp. 77.

Hawk, P. B., Oser, B. L., Summerson, W. H.: Practical physiological chemistry, 13th ed. Churchill, London **1954,** P. 609.

Henderson, E. F.: The longitudinal smooth muscle of the central vein of the suprarenal gland. Anat. Rec. **1927,** 36: 69—77.

Kety, S. S.: Theory of blood tissue exchange and its application to measurements of blood flow. In: Brun, H. D. (Ed.): Methods in Medical Research. Year Book Publishers, Chicago **1960,** vol. 8, Pp. 223—227.

Kramer, R. J., Sapirstein, L. A.: Blood flow to the adrenal cortex and medulla. Endocrinology **1967,** 81: 403—408.

Monos, E., Koltay, Edit, Sulyok, A., Venkey, T., Kovách, A. G. B.: Effect of local venous congestion on adrenocortical functions. I. Changes in adrenal corticoid secretion after stepwise reduction of venous outflow. Acta physiol. Acad. Sci. hung. **1969.** 35: 305—312.

Niijima, A., Winter, D. L.: Baroreceptors in the adrenal gland. Science **1968,** 159: 434—435.

Pines, Ju. L. (Пинес, Ю. Л.): Электрофизиологическая характеристика эфферентных и афферентных связей надпочечников центральной нервной системой. Физиол. Ж. СССР. им. Сеченова. **1962,** 48 : 677—683.

SULYOK, A., MONOS, E., KOLTAY, EDIT, BEDE, ANDREA, KOVÁCH, A. G. B.: Effect of local venous congestion on adrenocortical function. II. Changes in corticoid release into venous blood and lymph, and in oxygen consumption. Acta physiol. Acad. Sci. hung. **1969.** 35: 313—320.

SYMINGTON, T.: Morphology and secretory cytology of the human adrenal cortex. Brit. med. Bull. **1962,** 18: 117—121.

Emil MONOS, Zsuzsanna BIRÓ, András SULYOK, Arisztid KOVÁCH
Orvostudományi Egyetem Kísérleti Kutató Laboratóriuma,
Budapest VIII., Üllői út 78/a.
Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 331—333 (1969)*

# CORTICOTROPHIN RELEASE INDUCED BY INJECTION OF FORMALIN IN RATS WITH HEMISECTION OF THE SPINAL CORD

By

G. B. MAKARA, E. STARK and Katalin MIHÁLY

INSTITUTE OF EXPERIMENTAL MEDICINE, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

(Received November 29, 1968)

Afferent spinal pathways of the ACTH-releasing stimulus were studied in rats with hemisection of the thoracic spinal cord. The plasma corticosterone level rose significantly less when formalin was injected into the hindlimb contralateral to the hemisection than when it was injected into the homolateral hindlimb or above the level of the lesion. It is suggested that most of the fibres which convey ACTH-releasing nociceptive impulses cross the midline and ascend the spinal cord on the contralateral side; only a minor portion of these fibres seem to be homolateral.

Some stressors are known to stimulate ACTH secretion by the hypothalamo-hypophyseal unit via somatosensory pathways. It has been shown that ACTH release induced by fracture of the femur (MATSUDA et al. 1964), electrical stimulation of the leg (REDGATE 1962) or subcutaneous injection of 3% formalin (MAKARA et al. 1967) is suppressed after transection of the spinal cord. In the present experiments we have studied the course of the ascending spinal pathways mediating the stimulus of ACTH release induced by injection of 1% formalin. As an indicator of ACTH release the plasma corticosterone level was estimated.

## Methods

Random-bred male rats of Wistar origin weighing 160—230 g were used. They were housed at 24 ± 1 °C and 65 ± 10% humidity and received commercial rat pellets and tap water ad lib.

The rats were anaesthetized with ether and laminectomy was performed at the level of the seventh cervical vertebra. The dura was incised in the midline and the left side of the cord was transected with a small sharp blade. After the operation the rats received 1.5 mg of oxytetracycline four times daily. In the case of transitory urine retention the bladder was emptied by manual pressure three times daily.

One week after operation the rats were divided at random into four groups and injected with physiological saline or 1% formalin in saline as shown in Table I. One hour later they were anaesthetized with ether and bled from the abdominal aorta within 2 minutes. Plasma corticosterone was determined by the method of GUILLEMIN et al. (1958). The spinal column was fixed in 10% formalin, and a block of the spinal cord containing the lesion was embedded in paraffin, serially cut at 10 $\mu$ and at least every tenth section was mounted and stained with haemalum-erythrosin. Results were discarded when transection had been incomplete on the left side of the cord or its medial border deviated from the midline more than about 100 micra on either side.

Plasma corticosterone values were transformed to logarithms and statistical significance was determined by analysis of variance and the multiple comparison test (SCHEFFÉ 1959).

## Results

On the day after the operation the left hindlimb was paralysed and electrical stimulation or pinching with forceps of the right paw elicited no nociceptive responses. Three to seven days later responsiveness to nociceptive stimulation improved and the rats began to use their left hindlimb.

Hemisection of the cord did not cause a significant change in the plasma corticosterone response to 1% formalin injected into the homolateral hindlimb as compared with the group injected with formalin above the level of hemisection (neck or occipital region). On the other hand, formalin given into the hindlimb contralateral to the hemisection produced only a small, although statistically significant, rise in the plasma corticosterone level (see Table I).

**Table I**

*Changes in plasma corticosterone level following injection of 1% formalin in rats with hemisected spinal cord*

| Group | Treatment* | Site of injection | No. of rats. | Corticosterone μg/100 ml plasma** | Significance |
|---|---|---|---|---|---|
| 1. | 0.9% saline | hindlimb | 13 | 11.9 ± 1.3 | 1 vs 2 $p < 0.01$ |
| 2. | 1% formalin | back of the neck | 8 | 51.4 ± 3.6 | 1 vs 3 $p < 0.01$ |
| | | | | | 2 vs 3 NS |
| 3. | 1% formalin | homolateral hindlimb | 9 | 47.8 ± 2.3 | 3 vs 4 $p < 0.01$ |
| 4. | 1% formalin | contralateral hindlimb | 13 | 19.3 ± 2.3 | 1 vs 4 $p < 0.05$ |

* 0.25 ml/100 g subcutaneously.
** Mean ± S.E.

## Discussion

Most of the pathways conveying the ACTH-releasing stimulus evoked by formalin injection seem to cross the midline and ascend the cord on the contralateral side, since hemisection of the cord strongly inhibited a rise in the plasma corticosterone level when the site of injection was the contralateral hindlimb.

The small rise after contralateral injection suggests that some of the pathways conveying nociceptive stimulation are homolateral. This would be in agreement with the persistence of some sensitivity to nociceptive stimulation on the contralateral side.

Neither sparing nor involvement of the extreme medial part of the anterior quadrants or the dorsal columns on either side were critical in inhibiting formalin-induced ACTH secretion. This suggests that most pathways are in a rather lateral position.

In other mammals pathways transmitting pain responses are either bilateral and not localized into distinct tracts as in the cat and the pig (KENNARD 1954; BREAZILE 1963), or mainly contralateral and concentrated into the ventrolateral quadrant of the cord, as in the monkey and man (see NOORDENBOS 1959).

## Acknowledgements

We thank Miss H. ÉRDI for skilful technical assistance.

## LITERATURE

BREAZILE, J. E.: A study of pain mechanisms. Thesis. **1963.** University of Minnesota.

GUILLEMIN, R., CLAYTON, G. W., SMITH, J. D., LIPSCOMB, H.: Fluorometric measurement of rat plasma and adrenal corticosterone concentration. J. Lab. clin. Med. **1959,** 53: 830—832.

KENNARD, M. A.: The course of ascending fibers in the spinal cord essential to the recognition of painful stimuli. J. comp. Neurol. **1954,** 100: 511—524.

MAKARA, G. B., STARK, E., MIHÁLY, K.: Site at which formalin and capsaicin act to stimulate corticotrophin secretion. Canad. J. Physiol. **1967,** 45: 669—674.

MATSUDA, K., DUYCK, C., KENDALL, J. W., GREER, M. A.: Pathways by which traumatic stress and ether induce increased ACTH release in the rat. Endocrinology **1964,** 74: 981—985.

NOORDENBOS, W., Pain, Elsevier, Amsterdam **1959,** 182. pp.

REDGATE, E. S.: Spinal cord and ACTH release in adrenalectomized rats following electrical stimulation. Endocrinology **1962,** 70: 263—266.

SCHEFFÉ, H.: The analysis of variance. Wiley, New York **1959.**

Gábor B. MAKARA, Ervin STARK, Katalin MIHÁLY
Kísérleti Orvostudományi Kutató Intézet,
Budapest VIII., Szigony u. 43, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 335—344 (1969)*

# PITUITARY-THYROID FUNCTION OF THE RAT AFTER SUPPRESSION OF ACTH RELEASE BY DEXAMETHASONE IMPLANTS IN THE MEDIAN EMINENCE

By

D. STRASHIMIROV*, B. BOHUS and S. KOVÁCS

INSTITUTE OF PHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received November 1, 1968)

The effect on pituitary-thyroid and adrenal function of dexamethasone implanted in the median eminence of the hypothalamus has been investigated in the rat. Dexamethasone implantation resulted in a marked inhibition of the stress-induced ACTH release for a period of 12 days. Thyroid $^{131}I$ uptake measured 2, 9 and 13 days after the implantation was similar in rats with suppressed ACTH release and in controls. Exposure to cold brought about the same $^{131}I$ uptake of both dexamethasone-implanted and control rats. Dexamethasone implants failed to affect the $^{131}I$ release rate under resting conditions and after MTU-provoked stimulation, or thyroxine-induced inhibition of TSH release. Dexamethasone implants failed to change the compensatory thyroid hypertrophy after hemithyroidectomy.

It is suggested that the prolonged inhibition of ACTH release by dexamethasone implants in the median eminence is not followed by changes in the hypothalamo-pituitary mechanism participating in the control of TSH release.

Inverse changes in the secretion of pituitary TSH and ACTH under different conditions have since long been in the focus of interest. Recently, SAKIZ and GUILLEMIN (1965) reported that the pituitary secreted more TSH in response to TRF stimulation in rats pretreated with dexamethasone and pentobarbital. It was also shown that while pretreatment with dexamethasone and pentobarbital did not affect the stress-induced inhibition of TSH secretion, it increased TSH release in response to cold exposure (DUCOMMUN et al. 1966), and it has been suggested that the increase in TSH secretion in response to cold partly depended on the concomitant inhibition of ACTH secretion. On the other hand, SALAMON (1966) observed that if ACTH release was inhibited by dexamethasone alone, a complete abolition of the TSH release in response to major stress occurred while the resting plasma level of TSH remained unchanged.

On the other hand, high amounts of glucocorticoids were found to inhibit thyroid hormone biosynthesis (KOVÁCS and VÉRTES 1962). This indicates that an increased TSH secretion induced by the feedback effect of decreased thyroid hormone output cannot be excluded when high amounts of glucocorticoids are applied systematically.

* IBRO Research Fellow on leave of absence from the Clinic of Endocrinology, Postgraduate Medical Institute (ISUL), Sofia, Bulgaria.

Recent observations have shown that minute amounts of corticoid implanted into the median eminence of the hypothalamus result in an inhibition of ACTH release (ENDRŐCZI et al. 1961; DAVIDSON and FELDMAN 1963; BOHUS and ENDRŐCZI 1964). Dexamethasone implanted in the median eminence appeared the most potent inhibitor of ACTH secretion (CORBIN et al. 1965; DAVIDSON et al. 1966; BOHUS and STRASHIMIROV in preparation).

Since the quantity of implanted steroids is ineffective systematically, a direct action on thyroid hormone synthesis may be excluded. We have therefore studied the relations of pituitary TSH and ACTH secretion in rats with dexamethasone implants in the median eminence.

## Methods

Adult male albino rats of an inbred stain were used. The animals fed on rat chow with standard iodine content were housed at constant temperature (22—24 °C), and allowed to drink water ad libitum.

Dexamethasone ($\Delta^1$-9α-fluoro-16α-methylcortisol, Organon, Oss) implants were prepared by tamping the end of a stainless steel cannula with an external diameter of 0.45 mm. The implants contained approximately 5 μg of steroid. The rats were anaesthetized with ether and the cannula mounted on a stereotaxic instrument was introduced in the median eminence region bilaterally. When the tip of cannula had reached the skull-base, it was pulled 0.5 mm back and the steroid pellet was ejected by means of a metal stylet, then the cannula was retracted. Cholesterol implants were prepared and introduced into the median eminence in a similar way. These control implants served to eliminate a possible unspecific, lesion-like or irritative effect of the implantation procedure. Dexamethasone and cholesterol were implanted also in the frontal area of the cerebral cortex to exclude systemic effects of the implants.

Thyroid $^{131}$I uptake was measured in the conscious rat as described by KOVÁCS et al. (1960). 1 μC of carrier-free Na$^{131}$I were injected intraperitoneally and activity of the thyroid gland was estimated 1, 3, 6 and 24 hours later and then after 7 or 11 days. Background activity was checked in the latter experiments prior to the intraperitoneal administration of 4 μC $^{131}$I.

The rate of thyroid $^{131}$I release was also determined in conscious rats. A dose of 10 μC $^{131}$I was injected intraperitoneally and the initial activity was recorded 24 hours later. Initial counts were expressed as 100% activity and the decay curves were plotted as the logarithm of the per cent initial activity at different points of time. Correction was made for the isotopic decay.

Compensatory thyroid hypertrophy was estimated 19 days after removal of the right thyroid lobe. The weight of the thyroid lobes was determined with an accuracy of 0.1 mg. The rate of compensatory thyroid hypertrophy was expressed in the thyroid weight difference (TWD) calculated as follows.

$$\text{TWD} = \frac{\text{left thyroid lobe weight-right lobe weight}}{\text{right thyroid lobe weight}} \times 100$$

For the estimation of pituitary-adrenal activity, the rats were exposed to ether vapour in a metal jar and 15 min later they were decapitated and the adrenals were quickly removed. Adrenal corticosteroid production in vitro was determined by the method of VAN DER VIES et al. (1960). Total corticosteroid production in vitro expressed in μg/100 mg adrenal/hour served as the index of ACTH release (VAN GOCH et al. 1963).

At the end of the experiments the brains were removed and fixed in formalin. Verification of the site of implants was carried out on 50 μ thick frozen, unstained sections, according to GUZMAN-FLORES et al. (1958).

The obtained data were analysed statistically by STUDENT's *t*-test. A probability level of 0.05 or less was accepted as a significant difference.

Experimental protocols were as follows.

*A)* Thyroid $^{131}$I uptake was investigated in two series of experiments, 2 days after dexamethasone or cholesterol implantation. $^{131}$I uptake was repeatedly determined on the

9th postimplantation day for the first series and on the 13th day for the second one. The rats were killed 48 hours after the last recording of thyroid activity and the stress-induced ACTH release was estimated.

*B)* Thyroid $^{131}$I uptake of rats exposed to cold was measured 2 days after dexamethasone or cholesterol implantation. The rats were transferred into a cold room (6 °C) immediately after the administration of $^{131}$I and thyroid uptake was determined 2, 4, 6 and 24 hours later. The rats were kept in the cold room continuously when measuring the radioactivity. Then the rats were returned to their home-cages and the ACTH release provoked by ether stress was determined 48 hours later.

*C)* Effect of suppression of ACTH release on thyroid $^{131}$I release rate. Implantation of dexamethasone or cholesterol was carried out 24 hours after the initial recording of thyroid radioactivity. To provoke TSH release, methylthiouracil (MTU) was given subcutaneously in a dose of 16 mg per rat 55 hours after the implantation, then in a dose of 32 mg per rat 16 hours later, in order to block thyroid hormone synthesis. TSH release was suppressed simultaneously by the intraperitoneal administration of DL-thyroxine (Hoffmann—La Roche, Basel) in a dose of 20 $\mu$g/rat. The rate of $^{131}$I release was followed till the 148th hour. Stress-induced ACTH release was determined 48 hours later, i.e. 8 days after the implantation.

*D)* Effect of dexamethasone implants on compensatory thyroid hypertrophy. Seven days prior to the implantation the right thyroid lobe was removed under ether anesthesia. 12 days later the rats were decapitated 15 min after exposure to ether vapour. The left thyroid lobe was weighed and TWD was calculated. Adrenal corticosteroid production in vitro was determined concomitantly.

## Results

Localization of the implants in the median eminence of the hypothalamus is shown in Fig. 1. Results obtained in rats with other placement of implants were excluded from the final evaluation.

Implantation of dexamethasone in the median eminence resulted in a marked suppression of stress-induced ACTH release (Fig. 2). The inhibition of ACTH release by dexamethasone was significant 2 ($p < 0.001$), 4 ($p < 0.001$), 8 ($p < 0.05$) and 11 ($p < 0.01$) days after the implantation. In the 16th post-implantation day ACTH release was not any more suppressed. Dexamethasone implants in the frontal cortex as compared to cholesterol implants in the cerebral cortex or the median eminence were ineffective on the 2nd and 11th postimplantation days (Table I).

### *1. Effect of dexamethasone implant in the median eminence on thyroid $^{131}$I uptake*

Fig. 3 shows the percentual changes of thyroid $^{131}$I uptake in rats bearing dexamethasone or cholesterol implants. Counts/min in the first hour in rats with cholesterol implant in the median eminence was considered as 100 per cent $^{131}$I uptake. The data of both series of experiments showed that thyroid $^{131}$I uptake in 11 rats bearing dexamethasone implants in the median eminence did not differ from that of 8 rats with cholesterol implants 2 days after the implantation. $^{131}$I uptake in 7 rats with dexamethasone and 4 rats bearing cholesterol implants in the cerebral cortex was slightly but not significantly enhanced (Fig. 3a), while 9 days after the implantation it was enhanced in rats

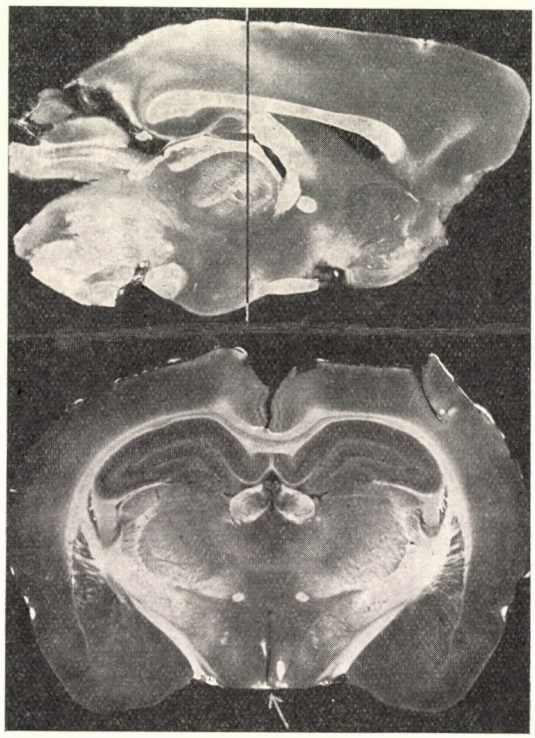


*Fig. 1.* Localization of dexamethasone implants in the median eminence of the hypothalamus in rats

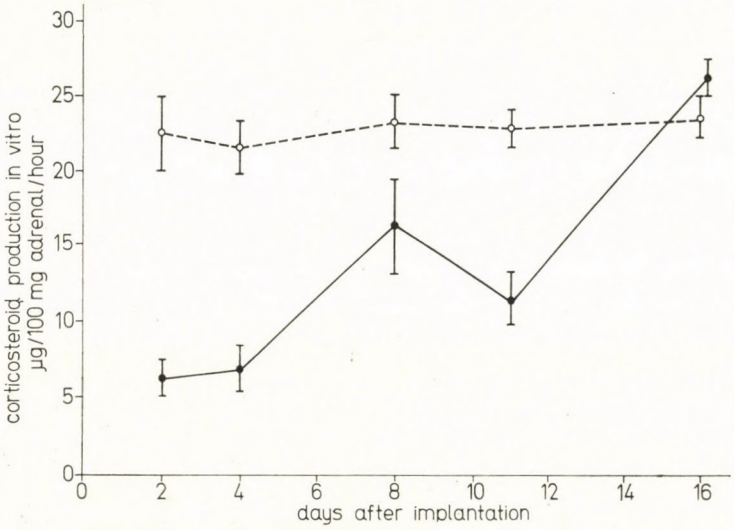



*Fig. 2.* Effect of dexamethasone (●—●) or cholesterol (○---○) implantation on stress-induced ACTH release in rats. 5—7 observations per point. Bars indicate standard error of the mean

**Table I**

*Effect of dexamethasone and cholesterol implantation in the frontal cortex or in the median eminence of the hypothalamus on stress-induced ACTH release in rats*

| Implants | Site of implants | Corticosteroid production in vitro | |
|---|---|---|---|
| | | 2 days | 11 days |
| | | after implantation | |
| Dexamethasone | Frontal cortex | 21.4 ± 2.9* (7)** | 25.9 ± 2.6 (4) |
| Cholesterol | Frontal cortex | 22.9 ± 2.6 (6) | 26.2 ± 1.6 (4) |
| Dexamethasone | Median eminence | 6.0 ± 1.2 (7) | 11.6 ± 1.7 (5) |
| Cholesterol | Median eminence | 22.6 ± 2.1 (6) | 22.8 ± 1.6 (4) |

* Mean ± Standard Error of Mean.
** Number of observations.

bearing dexamethasone implants but the differences were not significant (Fig. 3b). Similar results were obtained 13 days after implantation (Fig. 3c).

*2. Effect of dexamethasone implantation on thyroid $^{131}I$ uptake during exposure to cold*

Fig. 4 demonstrates that, during exposure to cold, the thyroid $^{131}I$ uptake in 7 rats bearing dexamethasone implants was not different from that of 7 rats with cholesterol implants in the median eminence of the hypothalamus.

*3. Effect of dexamethanose implantation on thyroid $^{131}I$ release. Action of MTU and thyroxine*

As shown in Fig. 5, the thyroid $^{131}I$ release rate was not affected by dexamethasone implanted in the median eminence 24 hours after recording the initial radioactivity. Furthermore, a marked increase in $^{131}I$ release was evident a few hours after MTU administration, while thyroxine injection resulted in an inhibition of $^{131}I$ release in 6 rats bearing dexamethasone and 5 rats with cholesterol implants in the median eminence.

*4. Compensatory thyroid hypertrophy in rats with dexamethasone implant in the median eminence*

Removal of the right thyroid lobe lead to a compensatory hypertrophy of the left thyroid lobe, as indicated by the Thyroid Weight Difference index (Fig. 6). Despite the significant ($p < 0.001$) suppression of ACTH release, compensatory hypertrophy of the left thyroid lobe developed in rats bearing a dexamethasone implant in the median eminence of the hypothalamus.

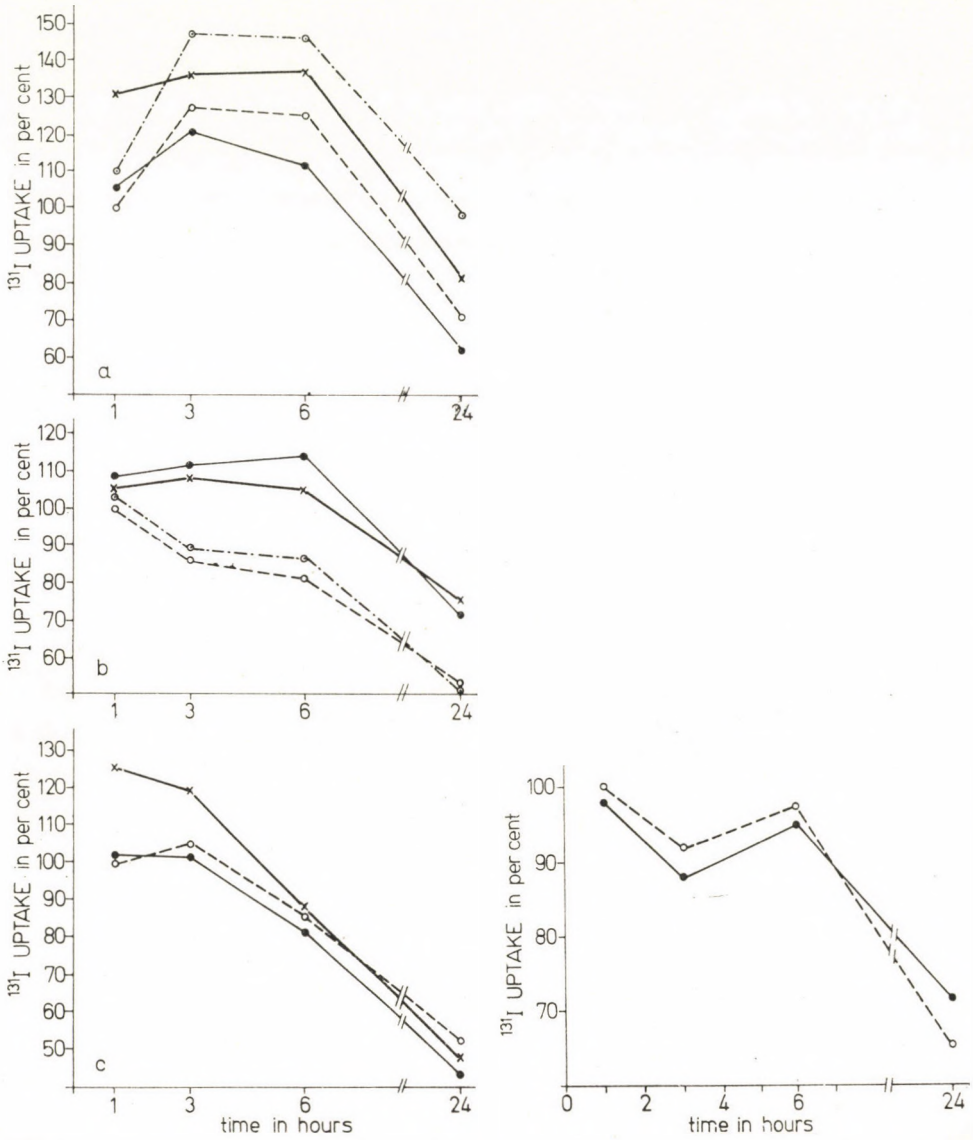



*Fig. 3.* Percentual changes in thyroid $^{131}I$ uptake of rats with dexamethasone (●——●) or cholesterol (○— —○) implants in the median eminence as well as of rats bearing dexamethasone (×——×) or cholesterol (○-·-○) implants in the frontal cortex 2 *(a)*, 9 *(b)* or 13 *(c)* days after implantation. Counts/min in the first hour of rats with cholesterol implants in the median eminence are taken as 100 per cent

*Fig. 4.* Thyroid $^{131}I$ uptake of rats bearing dexamethasone (●——●) or cholesterol (○- -○) implants in the median eminence during exposure to cold. 100 per cent uptake in rats with cholesterol implants in the median eminence 1 hour after the injection of $^{131}I$

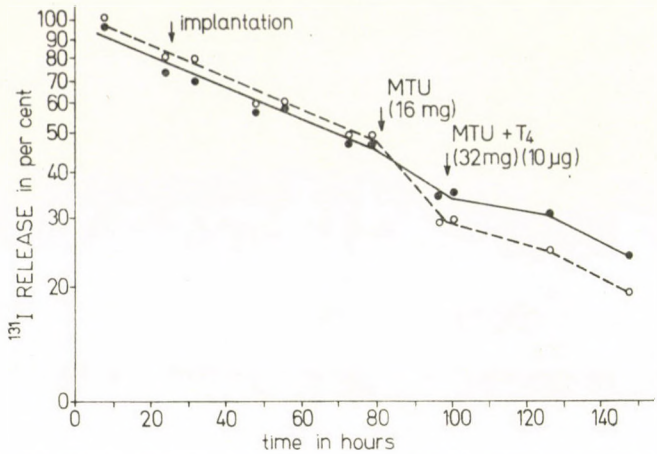



*Fig. 5.* Effect of dexamethasone (●——●) or cholesterol (○— —○) implants in the median eminence on the thyroid $^{131}I$ release

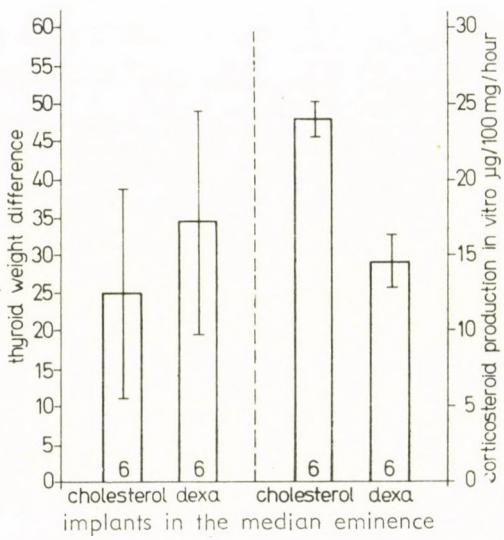



*Fig. 6.* Effect of dexamethasone implants in the hypothalamus on compensatory thyroid hypertrophy expressed in Thyroid Weight Difference as well as on ACTH release. Figures in the columns represent the number of observations. Bars indicate the standard error of mean

## Discussion

The present experiments suggested that prolonged inhibition of pituitary ACTH release does not affect pituitary-thyroid function as measured by different indices. Thus, thyroid $^{131}I$ uptake was not influenced by the implantation of dexamethasone in the median eminence despite the fact that ACTH

release was markedly depressed for a long period of time. Furthermore, enhancement of TSH release by exposure to cold or by the administration of methylthiouracil remained unchanged following the suppression of ACTH release. Observations in rats hemithyroidectomized prior to the implantation of dexamethasone in the median eminence revealed that long-lasting pituitary TSH release is similarly not affected by the diminished ACTH secretion.

Previous observations showed that chronic alterations in pituitary-adrenal function may affect the rat's pituitary-thyroid activity. Inhibition by corticosteroid of thyroid $^{131}$I uptake and release was reported by PERRY (1951), BROWN-GRANT et al. (1953) and SKEBELSKAYA (1958). It was also observed that adrenalectomy or cortisone treatment prevented the fall of thyroid $^{131}$I uptake in response to the electrical stimulation of the habenular nucleus (KOVÁCS et al. 1966). Furthermore, hypothalamic stimulation was followed by an inverse change in the thyroid function of adrenalectomized rats (VÉRTES et al. 1965). Since it is well known that corticosteroid treatment results in a suppression of pituitary ACTH release and synthesis while adrenalectomy is followed by an enhancement of ACTH release, one may suggest that the alterations in pituitary-thyroid function might be due to chronic changes in ACTH release and/or synthesis.

Several experiments have revealed that implantation of corticosteroid into the median eminence of the hypothalamus leads to a suppression of ACTH release (ENDRŐCZI et al. 1961; DAVIDSON and FELDMAN 1963; BOHUS and ENDRŐCZI 1964; SMELIK 1965), and that dexamethasone was the most potent inhibitor of both the release and the synthesis of pituitary ACTH (CORBIN et al. 1965; DAVIDSON et al. 1966; CHOWERS et al. 1967). The present findings also showed that the stress-induced ACTH release, as measured by the total adrenal corticosteroid production in vitro, is markedly depressed in rats with a dexamethasone implant in the median eminence of the hypothalamus. The suppressive effect of free crystalline dexamethasone lasted for a period of 12 days. Thus, a prolonged inhibition of ACTH release was apparent. Furthermore, it was shown that dexamethasone implants have no general effect, since implants located in the frontal cortex did not affect the stress-induced ACTH release.

In accordance with the above-quoted observations, it has been concluded that despite the prolonged inhibition of pituitary ACTH release, the pituitary-thyroid axis preserved its normal function under resting conditions, or under stimulation by exposure to cold or MTU administration. Chronic hypersecretion of TSH due to hemithyroidectomy was also unaffected by the suppression of ACTH release. It is noteworthy that unilateral adrenalectomy resulting in ACTH hypersecretion (GANONG 1954; BOHUS and ENDRŐCZI 1964) did not alter the compensatory hypertrophy of the remaining thyroid lobe after hemithyroidectomy (SHELLABARGER 1963).

Inverse changes in the release of pituitary ACTH and TSH in response to acute stress have been reported by BROWN-GRANT et al. (1954). More recent observations confirming that finding have revealed that the acute inhibition of ACTH release by systemic administration of dexamethasone and pentobarbital leads to an enhancement of TSH release in response to TRF stimulation or cold exposure (SAKIZ and GUILLEMIN 1965; DUCOMMUN et al. 1966; GUILLEMIN 1968). The present experiments have failed to confirm these observations under conditions where the ACTH release was inhibited by dexamethasone implants in the median eminence for a longer period. Since this experimental approach may exclude a possible direct influence of the steroid on thyroid hormone synthesis, one may suggest that the prolonged suppression of ACTH release does not affect the release and/or synthesis of pituitary TSH. Furthermore, it seems reasonable to assume that inverse changes in acute TSH and ACTH release are due to some interaction of the control mechanisms of the pituitary-thyroid and the adrenal systems in the central nervous system rather than to a shift between the release and/or synthesis of the two trophic hormones in the anterior pituitary.

## LITERATURE


BOHUS, B., ENDRŐCZI, E.: Effect of intracerebral implantation of hydrocortisone on adrenocortical secretion and weight after unilateral adrenalectomy. Acta physiol. Acad. Sci. hung. **1964,** 25: 11—19.

BROWN-GRANT, K., VON EULER, C., HARRIS, G. W., REICHLIN, S.: The adrenal gland and thyroid activity. J. Endocr. **1953,** 9: XLIII.

BROWN-GRANT, K., HARRIS, G. W., REICHLIN, S.: The effect of emotional and physical stress on thyroid activity in the rabbit. J. Physiol. (Lond.) **1954,** 126: 29—40.

CHOWERS, I., CONFORTI, N., FELDMAN, S.: Effects of corticosteroids on hypothalamic corticotrophin releasing factor and pituitary ACTH content. Neuroendocrinology **1967,** 2: 193—199.

CORBIN, A., MANGILI, G., MOTTA, M., MARTINI, L.: Effect of hypothalamic and mesencephalic steroid implantation on ACTH feedback mechanisms. Endocrinology **1965,** 76: 811—818.

DAVIDSON, J. M., FELDMAN, S.: Cerebral involvement in the inhibition of ACTH secretion by hydrocortisone. Endocrinology **1963,** 72: 936—946.

DAVIDSON, J. M., FELDMAN, S., JONES, L. E., LEVINE, S.: The central nervous system and pituitary-adrenocortical feedback mechanisms. In: SAYERS, G., LUNEDEI, A. (Eds.): Proc. 1st int. Symposium on Biorhythms in Clinical and Experimental Endocrinology, Universita Degli Studi, Firenze **1966,** Pp. 9—27.

DUCOMMUN, P., SAKIZ, E., GUILLEMIN, R.: Dissociation of the acute secretions of thyrotropin and adrenocorticotropin. Amer. J. Physiol. **1966,** 10: 1257—1259.

ENDRŐCZI, E., LISSÁK, K., TEKERES, M.: Hormonal "feed-back" regulation of pituitary-adrenocortical activity. Acta physiol. Acad. Sci. hung. **1961,** 18: 291—299.

GANONG, W. F.: The effect of ACTH on adrenal size in hypophysectomized rats after removal of one adrenal. Endocrinology **1954,** 55: 117—120.

VAN GOCH, J. J., DEWIED, D., SCHÖNBAUM, E.: Adrenocorticotropic activity in the rat assessed by in vivo and in vitro indices. Amer. J. Physiol. **1963,** 205: 1083—1088.

GUILLEMIN, R.: Hypothalamic control of concomitant secretion of ACTH and TSH. In: JAMES, V. H. T., LANDON, J. (Eds.): The Investigation of Hypothalamic-Pituitary Adrenal Function. Cambridge Univ. Press, Cambridge **1968,** Pp. 19—28.

GUZMAN-FLORES, C., ALCARAZ, M., FERNANDEZ-GUARDIOLA, A.: Rapid procedure to localize electrodes in experimental neurophysiology. Bol. Inst. Estud. med. biol. Mex. **1958,** 16: 29—31.

KOVÁCS, S., VÉRTES, M., KÖVESI, GY.: Effect of hypothalamic lesions on the activity of pituitary-thyroid system. Experiments with $^{131}I$ in the rat. Acta physiol. Acad. Sci. hung. **1960,** 17: 295—300.

KOVÁCS, S., VÉRTES, M.: Die Wirkung von Nebennierenrinden-Hormonen auf die Schilddrüsenfunktion in vitro Versuchen. Endokrinologie **1962,** 43: 105—109.

KOVÁCS, S., SÁNDOR, A., VÉRTES, ZS., VÉRTES, M.: The effect of stimulation of habenular nucleus on pituitary-thyroid function. Acta physiol. Acad. Sci. hung. **1966,** 30: 39—45.

PERRY, W. F.: The action of cortisone and ACTH on thyroid function. Endocrinology **1951,** 49: 284—289.

SAKIZ, E., GUILLEMIN, R.: Inverse effects of purified hypothalamic TRF on the acute secretion of TSH and ACTH. Endocrinology **1965,** 77: 797—801.

SALAMAN, D. F.: The action of stress on plasma TSH concentration in the rat. J. Physiol. (Lond.) **1966,** 186: 134 P.

SCHALLABARGER, C. J.: Compensatory hypertrophy of the thyroid gland, adrenal gland and the gonad studied singly or in combination. Endocrinology **1963,** 73: 124—126.

SKEBELSKAYA, Y. B.: (Скебельская, Ю. Б.): Влияние кортизона на щитовидную железу. Пробл. эндокрин. гормонтер. **1958,** 4/5 : 15—18.

SMELIK, P. G.: Effect of corticosterone implantation in the hypothalamus of rats on corticotrophin secretion. Acta physiol. pharmacol. neerl. **1965,** 13: 122.

VÉRTES, M., VÉRTES, ZS., KOVÁCS, S.: Effect of hypothalamic stimulation on pituitary-thyroid function. Acta physiol. Acad. Sci. hung. **1965,** 27: 229—235.

VAN DER VIES, J., BAKKER, R. F. M., DEWIED, D.: Correlated studies on plasma free corticosterone and on adrenal steroid formation in vitro. Acta endocr. (Kbh.) **1960,** 34: 513—523.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 345—350 (1969)*

# HORMONES IN LYMPH (E) CAPILLARY PERMEABILITY TO $^{131}$I-, RISA, AND Na$^{131}$I IN NEPHRITIS

By

A. A. Ismail, A. Abdel Hay, K. R. Madwar and M. Talaat

EL-MOFTY METABOLIC UNIT, BIOCHEMISTRY DEPARTMENT, FACULTY OF MEDICINE, CAIRO, U.A.R.

(Received August 22, 1968)

The rate of appearance of intravenously injected $^{131}$I-thyroxine, RISA and Na$^{131}$I in the oedema fluid of nephrotic patients was investigated by determining the radioactivity and protein content of blood and oedema fluid.

The injected substances gained access to the oedema fluid slowly and their rate of appearance was proportional to the molecular size; Na$^{131}$I was the first and RISA the last to appear. The radioactivity of thyroxine $^{131}$I and RISA was much less in oedema fluid than in blood. Also, the protein content of blood plasma was 13.8 times that of oedema fluid. Both findings indicate the presence of a high gradient between plasma and extracellular fluid.

In previous papers it has been shown that thyroxine and triiodothyronine have an extravascular circulation via the lymphatics and their concentration in lymph approximated their concentration in blood (Ismail et al. 1967a). We also claimed that lymphatics play an important role in the mobilization of these hormones after their intramuscular injection (Ismail et al. 1967b). They are carried in lymph bound to a thyroxine-binding globulin (El Ridi et al. 1967).

The presence of both hormones ($T_4$ and $T_3$) in lymph in amounts more or less similar to those in blood, would not indicate that they may be present in tissue fluids in such quantities. This is simply because the tissue fluids i.e. fluids in the vicinity of the cells, collect from the periphery and are subjected to several changes before they turn into lymph (Yoffey et al. 1956). It is, however, evident that substances of molecular weights more than 2000 are carried from the periphery to the lymph trunks without leakage (Mayerson 1963), but crystalloids and water leak freely out of the lymph glands.

Oedema fluid represents a tissue fluid which can be obtained in measurable volumes and subjected to different tests. Cases of nephrotic oedema seemed thus to offer a possibility for studying the rate of appearance of different hormones in tissue fluids and their concentration after being injected into the blood, taking into consideration that under these pathological conditions the capillary membrane might behave differently from the normal one.

In the present study we obtained oedema fluid from nephrotic patients and compared the content of intravenously injected RISA, $^{131}$I thyroxine and Na$^{131}$I in oedema fluid and blood for

(a) assessing capillary permeability in the nephrotic syndrome;

(b) finding the ratio between blood and extracellular fluid concentrations of the above substances.

## Methods

Eighteen patients between 10 and 25 years suffering from the nephrotic syndrome (Ellis type 2 nephritis) were divided into 3 comparable groups as regards age, duration of the disease and the severity of oedema. None of the patients received steroids before the test.

Each subject in group I received 50 $\mu$c of RISA,* in group II 20 $\mu$c of $^{131}$I thyroxine,** and in group III 20 $\mu$c of Na$^{131}$I.*** All substances were given intravenously and irrespective of body weight. Oxalated venous blood samples were obtained at 30, 90, 150 and 210 minutes from the time of injection.

Oedema fluid was obtained from the patients at 90, 150, 210 and 270 minutes from the time of injection. Both blood and oedema fluid samples were tested for total radioactivity in an EKCO well-type counter.

Collection of tissue fluid samples was done by a specially devised needle, similar to a lumber puncture needle but with 20 side holes one millimeter in diameter each, made along a spiral line of 5 cm from its tip with a rate of spiral twist of one turn/2.5 cm.

The patient was allowed to sit in a comfortable chair with a good back support. The legs of the chair were long enough to allow, about 80 cm distance below the patient's feet. The needle was introduced with the stilette in position, vertically upwards, just behind the medial malleolus, to a distance of 6—7 cm in the subcutaneous tissue. Care was taken to avoid a too superficial position or to pierce the deep fascia. The stilette was then withdrawn and the dripping fluid was collected in a test rube. A 15—30 minutes period was allowed for equilibration, to obtain blood-free fluid, before the test compounds were injected intravenously. With this technique 4—30 ml of extracellular fluid could be collected every hour.

## Results

Total blood radioactivity and oedema fluid radioactivity at different periods are shown in Tables I and II and Figures 1 and 2.

Total radioactivity in blood after intravenous injection of 50 $\mu$c of RISA and 20 $\mu$c of each of $^{131}$I thyroxine and Na$^{131}$I, diminished rapidly in the case of Na$^{131}$I, and much slower in the case of RISA, while $^{131}$I thyroxine was between the two (Fig. 1). Average per cent of the total radioactivity present in blood at 30 min after the injection was 77.48 for Na$^{131}$I, 85.92 for $^{131}$I thyroxine, and 90.65 for RISA.

The diminution at the end of 210 min was $51.4 \pm 4.15$ for Na$^{131}$I, $32.59 \pm 6.4$ for $^{131}$I thyroxine, and $23.06 \pm 3.3$ for RISA.

The rates of disappearance of the injected substances in nephrotics over the whole period of tracing were, 0.74% per minute for Na$^{131}$I, 0.42% per min for $^{131}$I thyroxine, and 0.216% per min for RISA. These rates differ somewhat from those in normals where they are 0.34% per min for $^{131}$I thyroxine (Ismail et al. 1967a) and 0.1% per min for RISA (El-Moufti et al. 1965).

* Obtained from the Radiochemical Centre, Amersham. England. Its activity is 566 $\mu$c/mgm.

** Thyroxine $^{131}$I was obtained from the same centre, its activity is 1 mc/16.0 $\mu$g substance.

*** Obtained from the Atomic Energy Centre, Egypt.

**Table I**

*Rate of disappearance from blood of I.V. injected RISA, $^{131}I$-$T_4$ and $Na^{131}I$ in nephrotics*

| Substance | | Time in minutes | | | | | | | | Disappearance rate % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 | | 90 | | 150 | | 210 | | |
| | | Cpm/ml** | %* I.C. | Cpm/ml** | % I.C. | Cpm/ml | % I.C. | Cpm/ml** | % I.C. | |
| RISA | Mean | 8476 | 90.65 | 7378 | 78.91 | 7048 | 75.38 | 6317 | 65.63 | 23.06 |
| | S.E.± | 780 | | 650 | | 690 | | 490 | | 3.30 |
| $^{131}I$-$T_4$ | Mean | 2363 | 85.92 | 1763 | 64.11 | 1531 | 55.67 | 1422 | 51.61 | 32.59 |
| | S.E.± | 735 | | 534 | | 526 | | 540 | | 6.40 |
| $Na^{131}I$ | Mean | 1937 | 77.48 | 1070 | 42.80 | 838 | 33.52 | 650 | 26.00 | 51.41 |
| | S.E.± | 112 | | 51 | | 46 | | 39 | | 4.15 |

* I.C. = Initial concentration.
** Count/minute per ml.

**Table II**

*Rate of appearance in oedema fluid of I.V. injected RISA, $^{131}I$-$T_4$, and $Na^{131}I$ in nephrotics*

| Substance | | Time in minutes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 90 | | 150 | | 210 | | 270 | |
| | | Cpm/ml** | % I.C.* | Cpm/ml** | % I.C. | Cpm/ml** | % I.C. | Cpm/ml** | % I.C. |
| RISA | Mean | 38 | 0.406 | 51 | 0.50 | 70 | 0.75 | 82 | 0.87 |
| | S.E.± | 9 | | 8 | | 13 | | 11 | |
| $^{131}I$-$T_4$ | Mean | 68 | 2.47 | 111 | 4.00 | 170 | 6.18 | 147 | 5.30 |
| | S.E.± | 16 | | 16 | | 35 | | 19 | |
| $Na^{131}I$ | Mean | 133 | 5.32 | 286 | 10.44 | 359 | 14.36 | 455 | 18.20 |
| | S.E.± | 27 | | 44 | | 25 | | 19 | |

* I.C. = Initial concentration.
** Count/minute per ml.

Figure 2 shows that the activity took $1\,^1/_2$ hours to appear in the oedema fluid where it was calculated to be 5.32% of the initial blood activity at zero time for $Na^{131}I$, 2.47% for $^{131}I$ thyroxine and 0.406% for RISA. The oedema fluid radioactivity then increased gradually till the end of the experimental period in all cases.

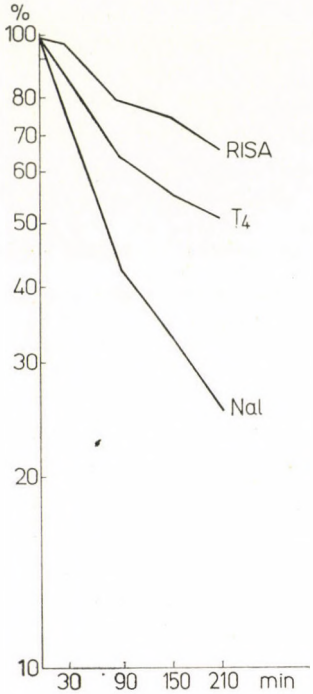



*Fig. 1*

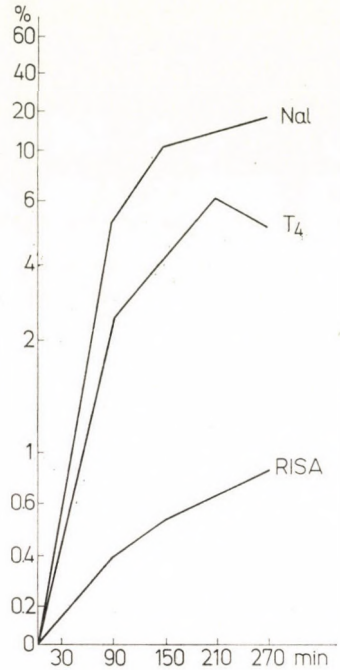



*Fig. 2*

## Discussion

As seen from the results, the concentration in blood of the substances injected intravenously decreased progressively during the next three hours $Na^{131}I$ showed the fastest rate of decrease and albumin the slowest one, while thyroxine stood between the two.

The majority of the substances that leaked out of the vascular bed must have passed into the extracellular fluid. Small amounts of RISA, thyroxine or $Na^{131}I$ might have been taken up by the tissues or cleared by the kidneys. However, in view of the short period of follow up, such pathways would account but for a limited proportion of the substances injected (LANDIS 1934). The major portion can thus be considered to have been distributed over the extracellular fluid.

Accordingly, the injected test substances were mainly distributed into two pools, an immediate one which was the blood plasma, and a slowly developing pool which was the extracellular fluid. The ratio of the size of these two pools is normally about 1 : 6. In nephrotics who may increase in weight by 7—10 kg with the development of oedema (WRIGHT 1961), this ratio may reach 1 : 7 to 1 : 10. One would thus expect that by the end of the experiment the concentration of the test substances would be much lower in the extracellular

fluid than in the blood. The results shown in Tables I and II agree well with these deductions. Thyroxine concentration in the oedema fluid was 5.30% of the initial concentration and 51.61% in the blood; RISA concentration in oedema fluid was 0.87% and 65.63% in the blood. This was not the case with $Na^{131}I$, this being a crystalloid.

These findings might not be restricted to nephrotics, as the disappearance rates of RISA, thyroxine and NaI from the blood of nephrotics follow a pattern quite comparable to normal indicating that capillary permeability is not markedly affected.

This observation was further confirmed by the protein concentration in plasma and extracellular fluid of these patients. The plasma proteins averaged 6.66 gm/100 ml while the oedema fluid proteins averaged 0.48 gm/100 ml. Thus, the plasma protein concentration was 13.8 times that in the extracellular fluid, in spite of the state of complete equilibration.

LANDIS (1934) compared the protein content of tissue fluid obtained from normals and nephrotics and found no significant difference between them. This also shows that extracellular fluid collected from nephrotics can represent the extracellular fluid in general.

The results presented can thus be regarded as evidence of the existence of a gradient between plasma and extracellular contents. This gradient is steep with large molecular substances a nd small with low molecular substances. In nephrotics it amounted to 13.87: 1 for albumin. Three hours after intravenous injection the gradient was 7 : 1, 9.6 : 1, and 1.43 : 1 for RISA, thyroxine and NaI, respectively. Results for RISA would differ much if sampling were followed for 24 hours; a period sufficient to reach equilibration.

We have failed in obtaining sufficient quantities of tissue fluid from normal subjects, but if such a great difference in albumin and thyroxine concentration between plasma and extracellular fluid proves to occur in normal persons, it would open the way to many questions, especially to what extent the estimation of a hormone in blood represents its concentration in extracellular fluid i.e. its concentration at the target organ; also, to what extent do changes in capillary permeability influence the concentration of substances in extracellular fluid.

## LITERATURE

EL MOFTY, A. H., ISMAIL, A. A., KHALIFA, K., MADWAR, K. R.: Capillary permeability to radioiodinated serum albumin. J. egypt. med. Ass. **1965,** 48: 282—292.

EL RIDI, M. S., ISMAIL, A. A., ABDEL HAY, A., KAMEL, G., TALAAT, M.: Hormones in Lymph, A. Thyroxine Carriage. J. egypt. med. Ass. **1967,** 50: 347—353.

ISMAIL, A. A., EL RIDI, M. S., BADRAN, A., KHALIFA, K., ABDEL HAY, A., TALAAT, M.: Extravascular circulation of Thyroid Hormones. Amer. J. Physiol. **1967a,** 213: 1391—1396.

ISMAIL, A. A., EL RIDI, M. S., ABDEL HAY, A., KAMEL, G., TALAAT, M., FAYEK, K. I.: Hormones in Lymph (D) Role of Lymphatic vessels in Absorption of intermuscularly injected $^{131}$I insulin and $^{131}$I thyroxine. Acta physiol. Acad. Sci. hung. **1967b,** 31: 321—330.

LANDIS, E. M.: Physiol. Rev. **1934,** 14: p. 404.

MAYERSON, H. S.: The lymphatic system with particular reference to the kidney. Surg. Gynec. Obstet. **1963,** 198: 155—159.

WRIGHT, S.: Applied Physiology, Oxford University Press, London **1961,** P. 46.

YOFFEY, J. M., COURTICE, F. C.: Lymphatics, Lymph and Lymphoid Tissue. Harvard Univ. Press, Cambridge, Mass **1956,** Pp. 53—120.

A. A. ISMAIL, A. ABDEL HAY, K. R. MADWAR, M. TALAAT

El-Mofty Metabolic Unit, The Biochemistry Department, Faculty of Medicine, Cairo University

Cairo, U. A. R.

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 351—354 (1969)*

# MELANOCYTE STIMULATING HORMONE

By

I. KAUSZ

SECOND DEPARTMENT OF SURGERY, SEMMELWEIS HOSPITAL, BUDAPEST

(Received September 12, 1968)

1. The hypophysis of carps contains melanocyte stimulating activity measurable by appropriate assay methods.

2. Observations carried out for 3 years have revealed that melanocyte stimulating activity is highest between April and June, in the period of spawning of the carp in Hungary.

3. Parallel estimations melanocyte stimulating activity in deep frozen and lyophilized preparations have shown that the hormone is not destroyed by these procedures.

4. Melanocyte stimulating activity of the carp pituitary can be augmented by about 40% during spawning by administration of pituitary extract.

It has been concluded that the carp hypophysis is best suited for transplantation in the second trimenon of the year, and particularly after pretreatment with pituitary extract.

The pars intermedia of the pituitary makes up in man about 0.5%, whereas in the cow 5%, and in the teleosts 40% of the whole gland (LANGE 1926). In view of these data KUBÁNYI (1962) introduced the transplantation of the pars intermedia of the fish pituitary into patients with intermediate lobe failure such as pigmentary degeneration of the retina, etc. The result varied according to the time of transplantation.

What are the local and overall reactions after the transplantation of the pars intermedia of the pituitary gland?

The local reactions are as follows.

(1) Stage of vascularization. Capillaries grow into the transplanted gland (1 to 2 days);

(2) Transitory stage (2 to 4 days);

(3) Stage of absorption or rejection (6 to 8 days).

These reactions show a considerable variation according to species and the kind of transplant. Endocrine glands have a low antigenicity and absorption of such grafts is therefore more common than their rejection; later the graft is replaced by connective tissue.

The overall reaction is an immunological one, the transplant behaves as an antigen. It is believed that the mechanisms governing the immunological processes have an afferent and an efferent limb. The antigenic proteins are carried from the transplant via the lymphatic and capillary networks and the

venous circulation into the reticulo-endothelial organs of the accepted organism. The antibodies formed in the latter are then transferred into the transplant via the arterial system.

These immunological events show considerable variations according to season, environmental temperature etc., and these factors may be assumed to affect the therapeutic value of the transplant. A thorough analysis of this problem is beyond the scope of the present paper whose only purpose was to establish the season most suitable for transplantation of fish pituitary into humans. The following questions had to be answered.

(1) Whether the activity of pituitary hormones is subject to seasonal variation and which season is the most suitable for transplantation;

(2) Whether lyophilized or deep frozen preparations are of equal therapeutic value as native hypophyses;

(3) It is possible to augment the hormonal activity by pretreatment of the donor animal with gonadotrophic hormone?

To assay hormonal activity in the pituitary, estimation attempts were made of gonadotrophic hormone was not considered suitable in view of the inaccuracy of the Galli—Mainini technique. Therefore preference was given to melanocyte stimulating hormone (hereafter referred to as MSH assay a simple method allowing rapid estimation of the functional state of the hypophysis to be transplanted).

Melanophoric activity in the frog is based on the expansion of pigment cells and a shift in the distribution of pigment within the cell in response to MSH, resulting in a darkening of the skin (KAUSZ 1962).

There is reason to assume that MSH activity is proportional to the overall hormone activity of the pituitary gland. Thus, the higher the MSH content of the preparation, the better must be the therapeutic effect of the transplant.

To estimate MSH activity frogs deprived of cutaneous pigmentation are required. Various methods have been described to achieve this goal. SULMANN (1952) placed *Ephyla arborea* frogs on a white ground and irradiated them with white light. FITZPATRICK et al. (1954) used hypophysectomized frogs for this purpose. The latter authors have elaborated a method employing the isolated frog skin which was immersed in solutions of varying ionic concentration, pH and temperature. Determinations were made with a photoelectric device. GYERMEK (1954) succeeded in lightening the skin of the frog by the administration of 1.5 $\mu$g/g of adrenaline. This method has been employed in the present experiments.

## Methods

Male *Rana esculenta* frogs of about the same weight were used in the experiments. Each series contained 35 frogs, 3 times 5 controls and 20 frogs for the assay.

The frogs were kept for 4 to 6 days in a dark place during this time their skin became dark enough for the evaluation of the effect of 1.5 $\mu$g/g of adrenaline.

The animals were arranged in four groups: Groups I to III (5 frogs each) served as controls, and Group IV (20 frogs) served for MSH assay.

The animals in Group I received only adrenaline; those in Group II adrenaline and about 0.1 g of fish brain emulsion;

Animals in Group III received adrenaline and about 0.1 g of frog muscle emulsion. In Group IV the frogs were given adrenaline and an extract prepared from the pooled pituitary glands obtained from 10 carps of about the same weight. After appropriate dilution in 0.65% saline, about one twentieth to one eightieth of the whole extract was injected intraperitoneally. For obvious reasons, the same procedures were undertaken with the lyophilized and deep frozen pituitary preparations.

Evaluation of the reaction. In preliminary observations, the colour of the skin was checked 30 min, 1 and 2 hours after the injection of the preparation. There being no appreciable difference between the results obtained at different intervals, evaluation was carried out uniformly, 60 min after the administration of the preparation.

Adrenaline has been found to cause lightening of the skin of the frogs.

MSH proved to prevent the adrenaline effect as the animals retained the darkness of their skin.

Frogs with a transitory skin colour were classified in 50% to the dark, and in 50% to the light group.

The results were expressed in per cent. To give an example, if from among 12 frogs 4 had become light and 4 intermediate, the result was regarded as 70% positive (14 tanned and 6 light).

The observations were repeated in every season of the year.

## Results

The skin of the control animals became invariably light, and neither fish brain extract nor frog muscle emulsion prevented the effect of adrenaline.

The effect was most marked in the period from April to June. In this period, with one fiftieth of the total volume of the pituitary extract a 70% efficiency was obtained, as from 12 frogs 4 became light, and 4 of an intermediate colour. Extracts prepared in other months were $^1/_3$ to $^4/_5$ less potent.

In another series, the pituitary glands were lyophilized and deep frozen. Subsequent bioassay has shown that these procedures had not affected the preparation's activity as of 12 dark frogs 4 became light and 4 of intermediate colour.

In the third series of experiments gonadotrophic stimulation was carried out according to the method commonly used in fish-breeding. Fish pituitary glands were suspended in physiological saline, then injected under the dorsal fin in the direction of the head; each fish received an emulsion prepared from one whole fish pituitary. The animals were then placed in water of 17 to 18 °C, through which oxygen was bubbled and 24 hours later they were killed, the pituitary gland was removed and bioassayed for MSH activity. This was about 40% higher than without pretreatment as of 10 frogs 2 became light and 8 of an intermediate skin colour.

## Discussion

The obtained results indicate that fish pituitary glands are most suitable for transplantation purposes when removed between April and June. In this period of the year, the best transplants can be obtained after pretreatment

of the donor fish with fish hypophyseal extract 24 hours prior to removal of the pituitary gland.

Our experience with fish pituitary transplants in clinical practice will be reported elsewhere.

## LITERATURE

BÁLINT, P.: Klinikai laboratóriumi diagnosztika. Medicina Budapest **1962**, Pp. 595—596.

GYERMEK, L.: Adrenocorticotrop hormon gyors biológiai meghatározása. Orv. Hetil. **1954**, 95: 100—103.

KAUSZ, I.: Seasonal observation of the effect produced by melanocyte — Stimulating hormone. Acta biol. Acad. Sci. hung. **1962**, Suppl. 5: 67.

KUBÁNYI, E.: Versuche zur Prüfung der Intermedin-Hormonwirkung von Fisch-Hypophysen. Ztsch. für Arzt. Fortb. **1962**, 56: 18—22.

LANGE, K.: Adatok néhány édesvizi halunk hypophysisének alak- és élettani ismeretéhez. Egyetemi Nyomda, Budapest **1926**.

FITZPATRICK, B., SHIZUME, K., LERNER, A. B.: In vitro bioassay for the melanocyte stimulating hormone. J. Endocr. **1954**, 54: 553—560.

SULMANN, F. G.: Mechanism of the Chromatophorotrophic effect of ACTH. J. Endocr. **1952**, 8: 275—280.

István KAUSZ
Pest megyei Tanács Semmelweis Kórháza II. sz. Sebészeti Osztály
Budapest, VIII. Gyulai P. u 2. Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 355—363 (1969)*

# THE STRUCTURE OF PROTEIN-POLYSACCHARIDES AND THEIR ROLE IN THE FUNCTION OF CONNECTIVE TISSUE FIBRES

By

Ilona BANGA

FIRST INSTITUTE OF PATHOLOGICAL ANATOMY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 23, 1968)

In addition to the known fractions PP-L (protein-polysaccharide-light) and PP-H (proteinpolysaccharide-heavy) it was possible to extract from the nasal cartilage with 40% KJ a mucopolysaccharide fraction with a protein component different from that of the mentioned fractions. The obtained fraction has been termed cartilage mucoprotein$_1$ (CMP$_1$) for it is probably identical with mucoid$_1$, a protein extracted from collagen fibres during heating and chemical contraction. The fractions PP-L and a fraction corresponding to CMP$_1$ were successfully isolated also from the fibrous cartilage of the Achilles tendon; the latter has been termed Achilles tendon-mucoid$_1$ (AT mucoid$_1$). PP-L, CMP$_1$ and AT-mucoid$_1$ altered the physical properties of the chemically contracted fibre, increasing in all age groups the degree of chemical relaxation. From this finding it has been concluded that various PP fractions are bound chemically to the contracted fibre and play a part in its function.

The mucopolysaccharide present in largest amounts in the nasal cartilage is chondroitin sulphate with which in its native form is bound to protein. This has been established by SHATTON and SCHUBERT (1954) who termed the complex chondromucoprotein (CMP). MALAWISTA and SCHUBERT (1958) elaborated a new extraction method which allowed to dissolve the majority of CMP on the first extraction. GERBER et al. (1960) named the CMP protein polysaccharide and denoted it with the abbreviation PP which has been adopted by most English and American authors (JACKSON 1964; MUIR 1964; SERAFINI-FRACASSINI and SMITH 1964; SMITH et al. 1967), although PARTRIDGE and DAVIS (1958) and PARTRIDGE et al. (1961) used the original term chondroitin sulphate-protein complex.

Fraction PP has been shown by GERBER et al. (1960) to contain two fractions, viz. PP-L (protein polysaccharide light) and PP-H (protein polysaccharide heavy). By ultracentrifugation, PAL et al. (1966) could separate in the presence of salt solutions of varying concentration a total of 6 subfractions in the nasal cartilage and termed them PP-L$_2$, PP-L$_3$, PP-L$_4$, PP-L$_5$ PP-L$_6$ and the residue, CRR; these such fractions showed differences in both polysaccharide and protein content. The amino acid composition of these subfractions was similar except for PP-L$_6$ which did not contain more than 2 to 3% collagen either, as measured on basis of the hydroxyproline (Hypro) content.

In view of our earlier observations it seemed worth to investigate the relationship between the PP fractions and the protein-containing polysaccharide liberated from collagen fibres during heat-induced and chemical contraction, which was considered their stabilizing factor (BANGA 1953; BANGA et al. 1954, 1956; BANGA 1959; BANGA and SZABÓ 1965). The substance has been termed collagenmucoid$_1$ (BANGA 1966); owing to the low concentrations (0.1 to 0.3%) in which it is present in collagen fibres the compound could not be isolated in a purified form.

Fractions PP-L and PP-H constitute about 40 to 50% of the dry substance of the nasal cartilage, therefore it is technically easy to separate and extract these fractions. The residue CR (cartilage residue) still contains polysaccharides which in part can be extracted by 1 M hydroxylamine or 1 M KCN and which have been designated by PAL and SCHUBERT (1965) as PP-LF and CRF (soluble), PP-HF (insoluble), and residue (CR) or CRR-$NH_2OH$. The latter still contained hexosamine, hexuronic acid and hexose, and as measured on the basis of the Hypro content, the majority of the collagen was also in this fraction; this seemed to contain the mucoid$_1$ described by us since previous analysis indicated that most of the protein component of mucoid$_1$ was collagen. When isolated from the nasal cartilage, this fraction has been termed CMP$_1$ (cartilage mucoprotein$_1$), whereas the similar fraction extracted from the Achilles tendon has been designated AT-mucoid$_1$.

It is characteristic of collagen fibres that they contract under the effect of heat or chemical stimuli, and this contraction is followed by relaxation (BANGA et al. 1954). The time course of chemical relaxation is a function of age. This is in agreement with the well-known differences in the function of collagen fibres in persons of various ages. This difference in function is demonstrable also in the responsiveness to chemical agents such as ascorbic acid and other organic acids which increase (BANGA 1957), and potassium permanganate which diminish or prevent, the chemical relaxation of collagen fibres (BANGA et al. 1956).

In the present experiments, the method of chemical relaxation was used to establish whether the various PP fractions isolated from the nasal cartilage and from the Achilles tendon had a part in function of the collagen fibres. It has been assumed that, if the relaxation of the contracted fibres is affected by these compounds, they must be bound to collagen fibres and, obviously, the PP fractions may closely related to the functioning connective tissue.

The present paper deals with the isolation of various PP fractions from the nasal cartilage and the cartilaginous substance of the tendons, with their chemical analysis, and their effect on the chemically induced relaxation of collagen fibres.

## Methods

*Isolation of the fractions PP-L and PP-H*

The PP fractions were extracted from the minced cartilage of the nasal septum, which was dehydrated with acetone, then dried, pulverized, and homogenized. Extraction and separation of the fractions PP-L and PP-H were undertaken by the method of MALAWISTA and SCHUBERT (1958).

*Preparation of $CMP_1$ (cartilage mucoprotein$_1$)*

After PP-L and PP-H had been extracted, the residue was dehydrated in alcohol and dried in a vacuum exsiccator. To each g of the obtained material 100 ml of 40% KJ were added, then the material was homogenized for 30 min and vigorously shaken for 4 hours by a Labor type shaking device. After centrifugation, the supernatant was dialysed against distilled water for 3 days at 0 °C, until the iodine reaction had disappeared. After the addition of 1.5% potassium acetate, the $CMP_1$ fraction was precipitated at 0 °C with the same volume of alcohol. Washing was performed twice with absolute alcohol, then the material was dried in a vacuum exsiccator.

The fraction which after the above extraction procedures remained insoluble has been termed cartilage residue (CR).

*Preparation of PP-L from Achilles tendon (AT-PPL)*

The cartilaginous portion of appropriately cleansed Achilles tendons was dehydrated by acetone and pulverized. The extraction procedure was the same as that described above for the preparation of PP-L and PP-H. In many cases instead of 0.15 M KCl potassium acetate was used which ensued a more complete precipitation with alcohol.

*Isolation of Achilles tendon-mucoid$_1$ (AT-mucoid$_1$)*

This fraction is identical with the collagen mucoprotein$_1$. After AT-PP-L had been removed, the residue was dehydrated with alcohol and extracted with 40% KJ; the material was then treated as described for $CMP_1$.

The residue obtained after the above extraction procedures has been termed AT-residue.

*Chemical relaxation of collagen fibres*

About 12 collagen fibres were isolated and loaded at one end with a lead shot weighing 80 mg and suspended at the other end; the fibre thus prepared was immersed in a 40% solution of KJ at 20 °C to produce a contraction to half of the original length, i.e. from 6 cm to 3 cm. Then the fibre was removed from the KJ solution, washed for 1 or 2 min in 50 ml distilled water, and dried. Six fibres served as controls and were incubated for 1 hour in 0.1 M phosphate buffer pH 7.0, and the other six fibres were placed in solutions containing 10 mg/ml of PP-L, $CMP_1$, or AT-mucoid$_1$. After incubation, the fibres still loaded with the shot were rinsed with distilled water, their length was measured, then they were placed in 40% KJ, and the changes in length were determined at 10 sec then later at longer intervals.

## Results

### (i) *Yield and analysis of the isolated PP fractions*

Tables I and II summarize the yield and analysis of PP fractions isolated from the nasal cartilage and the cartilaginous portion of the Achilles tendon. As shown in Table I, the fractions PP-L and PP-H extracted from the cartilage of the nasal septum showed insignificant differences in hexuronic acid, hexosa-

mine and hexose contents as compared to $CMP_1$. As regards the protein content, however, there were not only quantitative differences, but the difference found in the Hypro content allowed the conclusion that, in contrast to fractions PP-L and PP-H which did not contain the protein collagen, the protein component of $CMP_1$ consisted mainly of collagen (29.6% collagen content, corresponding to about 70% of the total protein contents). As shown in Table II,

**Table I**

*Percentual yield and analysis of protein polysaccharides isolated from bovine nasal cartilage*

| Isolated substances | Yield | Protein | Hexuronic acid | Hexosamine | Hexose | Hypro |
|---|---|---|---|---|---|---|
| PP-L + PP-H* | 30 | 25 | 25 | 30 | 5 | 0.2 |
| $CMP_1$** | 21 | 41 | 23 | 24 | 7 | 4 |
| CR*** | 39 | 85 | 1.2 | 3 | 3 | 9 |

* PP-L = protein polysaccharide light; PP-H = protein polysaccharide, heavy.
** $CMP_1$ = collagen $mucoprotein_1$ dissolved from the residue of nasal cartilage with 40% KJ after extraction of PP-L + PP-H.
*** CR = cartilage residue after extraction of PP-L + PP-H and $CMP_1$.

**Table II**

*Percentual yield and analysis of protein polysaccharides isolated from bovine Achilles tendon*

| Isolated substances | Yield | Protein | Hexuronic acid | Hexosamine | Hexose | Hypro |
|---|---|---|---|---|---|---|
| AT.-PP-L* | 23 | 70 | 2 | 3 | 4 | 11 |
| AT.-$mucoid_1$** | 15 | 70 | 5 | 9 | 6 | 11 |
| Residue | 60 | 98 | 0.3 | 0.3 | 0.2 | 14 |

* AT. PP-L = protein polysaccharides dissolved by water and 0.15 M KCl on vigorous shaking from Achilles tendon cartilaginous.
** AT. mucoid = collagen $mucoprotein_1$ dissolved by 40% KJ from the residue of cart. Achilles tendon after extraction of AT. PP-L.

the fractions At. PP-L and At.-$mucoid_1$ extracted from the cartilaginous portion of the tendon were similar in hexuronic acid, hexosamine, hexose and collagen-containing protein content and differences were only noted in solubility. They were, however, considerably different from the fractions prepared from the nasal cartilage, since their polysaccharide content was lower and the protein content significantly higher.

### (ii) *Effect of isolated PP fractions on the relaxed collagenous fibres*

Figures 1 and 2 demonstrate the effect of PP-L and $CMP_1$ on the relaxation of the tail tendon of 2 $^1/_2$-months-old rats. The contracted, dried and incubated fibres began promptly to relax when placed into a 40% solution of KJ at 20 °C, relaxation becoming complete within 120 sec (Fig. 1). This was followed by a slight contraction and a subsequent relaxation, the latter being considerably longer in duration than the first relaxation (Fig. 2). As determined in six fibre preparations, all tested PP fractions significantly enhanced the relaxation of the fibres obtained from young rats; statistical analysis revealed P values ranging between 0.001 and 0.02.

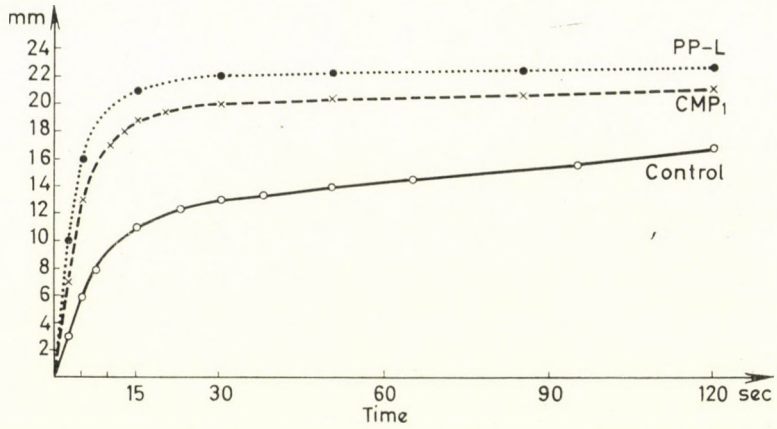



*Fig. 1.* Effect of PP-L and $CMP_1$ on the relaxation of 50% contracted in (40% KJ at 20 °C) collagen fibres tail tendon of the two and half months old rat. The contracted, washed and dried fibres were incubated for 1 hr in pH 7.4 phosphate buffer, 10 mg/ml of PP-L or $CMP_1$ solutions. After treatment, the washed fibres were put again in 40% KJ solution and the time of relaxation was observed. Ordinate: elongation in mm. Abscissa: time in sec. The increase of relaxation was at every point significant statistically (*t* test, 0.1—0.5%)

Figure 3 shows the effect of fractions PP-L, $CMP_1$ and AT.-$mucoid_1$ on the relaxation of fibres obtained from rats aged 6 and 8 months and contracted to 50% of their original length. Following initial lengthening, the fibres began to contract and only after a maximal contraction characteristic of the animals age had been reached, did they relax very slowly. The initial lengthening was enhanced by PP-L, $CMP_1$ and AT.-$mucoid_1$, if measurement was carried out on fibres previously contracted to half their original length; the greatest effect was produced by AT.-$mucoid_1$. The PP fractions reduced the contraction, the fibres having not attained within 20 min the original length measured at contraction. (The horizontal zero line indicates the length of the contracted fibre; lengthening and shortening are indicated by positive and negative figures, respectively.)

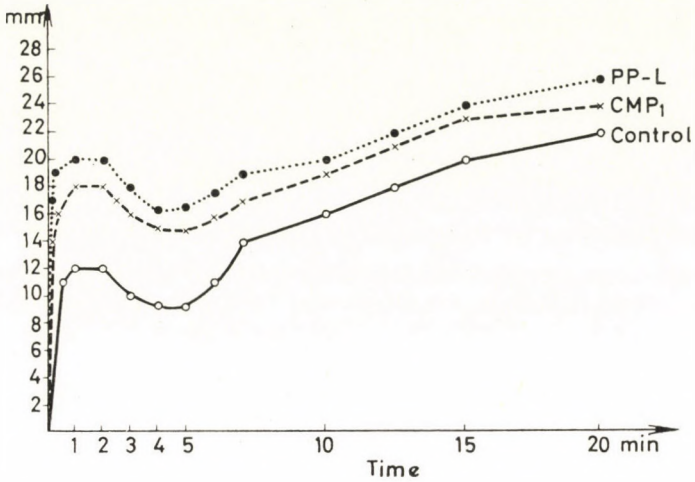



*Fig. 2.* Effect of PP-L and $CMP_1$. Treatment as in Fig. 1. The whole time-curve during relaxation. Ordinate: fibre length. Abscissa: time in min. Significance by *t* test at every point, 0.1—1%

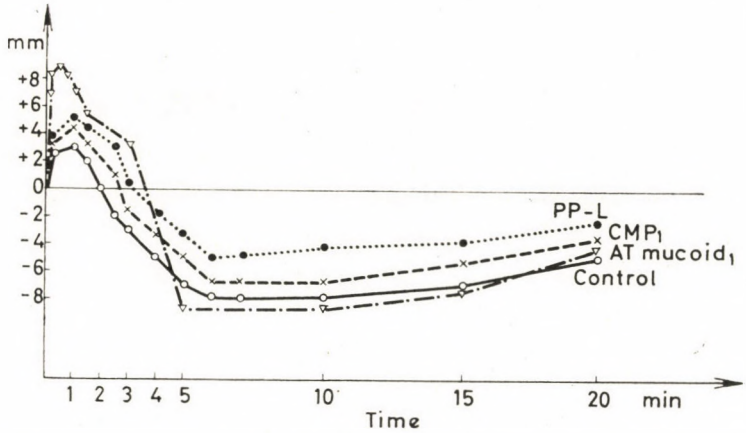



*Fig. 3.* Effect of PP-L, $CMP_1$ and AT-$mucoid_1$ on 50% contracted collagen fibres of tail tendon of six month old rat. Treatment as in Fig. 1. Ordinate: fibre length in mm. Positive values mean elongation, negative ones, contraction. Abscissa: time in min. Significance by *t* test at every point, 0.1—1%

As in rats aged 2 to 3 months a contraction to 50% of the original length means maximal contraction, whereas in 6- or 8-months old rats the maximal contraction is 66 to 70% of the original length, an additional series of experiments was carried out on 6-months-old rats the tail tendon of which was contracted maximally, i.e. to 66 to 70% of the initial length. In these experiments, the time course of the changes in length was similar to that observed in the

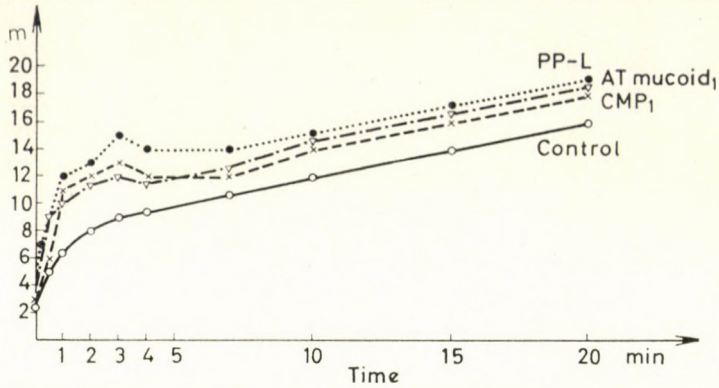



*Fig. 4.* Effect of PP-L, $CMP_1$ and AT-$mucoid_1$ of maximally contracted (from 6 cm to 1.8 cm) collagen fibres of tail tendon of six month old rat. Treatment as in Fig. 1. Ordinate: length in mm. The starting point means the length of contracted fibres after incubation in PP-fractions. Abscissa: time in min. Significance by *t* test, between 0.1 and 2%

young animals, only the degree of relaxation was smaller (Fig. 4). In all experimental series, the effect of protein polysaccharides could well be demonstrated. The differences were significantly statistically.

In general, fraction PP-L were the most potent in affecting the relaxation of collagen fibres. This might have been due to the circumstance that this is the most soluble fraction of the cartilaginous tissue and is perhaps attached to the groups on the surface of the collagen fibre. Fractions $CMP_1$ and AT.-$mucoid_1$, on the other hand, may be bound to the groups sited in more internal regions of the collagen helix which are therefore less accessible than the fractions of the surface.

## Discussion

Analysis of the extracted PP fractions revealed that, in addition to the identified and analysed protein polysaccharide fractions, there are other less soluble PP fractions in both cartilage and tendon. Consequently, after complete extraction with dilute salt solutions (0.15 M KCl or potassium acetate), it is possible to extract by 40% KJ such PP fractions which in their mucopolysaccharide content are similar while in their protein component considerably different from the known PP fractions. The protein component of PP-L and PP-HF is a soluble protein which was shown by PAL and SCHUBERT (1965) to contain high amounts of glutaminic acid and aspartic acid but only 0.1 to 0.2% of hydroxyproline.

The protein component of the fractions $CMP_1$ and AT.-$mucoid_1$ isolated by us contains 50% of collagen, i.e. an insoluble scleroprotein. The water-insoluble collagen, however, is converted by 40% KJ into soluble metacollagen (BANGA et al. 1954; BANGA 1966).

The present results indicate that about one third of the mucopolysaccharides can be isolated from the cartilage or tendon when its collagen has already been extracted by 40% KJ. It is therefore reasonable to assume that the mucopolysaccharide in question is closely associated with the protein collagen, or even bound to it chemically by ester bonds. According to SMITH et al. (1967), the PP macromolecules are located between "a" and "$b_1$" in the cross striation of the collagen fibres.

During chemical contraction, the bonds between polysaccharide and collagen are loosened; this leads to relaxation which is more complete in the young animal and is less pronounced with ageing ceasing completely in old animals. The finding that the PP fractions enhanced relaxation in both young and old animals indicates that these macromolecules belong to those constituents of the complex collagen fibre which determine its physical properties, its contraction and relaxation. These may be considered the function of the fibre, particularly because its shortening and lengthening is affected by age.

It has been claimed by SCHUBERT (1966) that, although PP fractions have been successfully isolated from cartilaginous tissue, no evidence is available indicating that these fractions are again adsorbed by the collagen fibre on its immersion into solutions containing the fractions. It appears that, in the chemically contracted fibre, chemical bonds become available for the binding of PP fractions, converting the fibre into one with new physical properties. It seems on the basis of these results that the present method is sufficiently sensitive for the demonstration of the binding of PP fractions to the collagen fibre.

## LITERATURE

BANGA, I.: Thermal contraction of collagen and its dissolution with elastase. Nature (Lond.) **1953,** 172: 1099—1100.

BANGA, I.: Der Effekt der schwachen organischen Säuren auf die Rattenschwanz-Kollagenfasern von jungen und alten Tiere. Gerontologia (Basel) **1957,** 1: 325—346.

BANGA, I.: The role of polysaccharides in connective tissue. Acta morph. Acad. Sci. hung. **1959,** Suppl. 8: 8—13.

BANGA, I.: The stabilizing factors of the collagen fibre. Acta biol. Acad. Sci. hung. **1959,** Suppl. 3: 38.

BANGA, I.: Structure and function of elastin and collagen. Akadémiai Kiadó, Budapest **1966.**

BANGA, I., SZABÓ, D.: Examination of collagen fibres following treatment with lyotropic agent. Acta morph. Acad. Sci. hung. **1965,** 13: 255—265.

BANGA, I., BALÓ, J., SZABÓ, D.: Contraction and relaxation of collagen fibres. Nature (Lond.) **1954,** 174: 788—791.

BANGA, I., BALÓ, J., SZABÓ, D.: The structure, ageing and rejuvenation of collagen fibres. Experientia (Basel) **1956,** Suppl. 4: 28—34.

BANGA, I., BALÓ, J., SZABÓ, D.: Metacollagen as the apparent elastin. J. Geront. **1956,** 11: 242—250.

GERBER, B. R., FRANKLIN, E. C., SCHUBERT, M.: Ultracentrifugal fractionation of bovine nasal chondromucoprotein. J. biol. Chem. **1960,** 235: 2870—2875.

JACKSON, S. F.: Connective tissue cells. In: BRACHET, J., MIRSKY, A. E. (Eds.): The Cell. Academic Press, New York **1964,** 6: 387—509.

MALAWISTA, J., SCHUBERT, M.: Chondromucoprotein: New extraction method and alkaline degradation. J. biol. Chem. **1958,** 230: 535—544.

PAL, S., SCHUBERT, M.: The action of hydroxylamine on the proteinpolysaccharides of cartilage. J. biol. Chem. **1965,** 240: 3245—3248.

PAL, S., DOGANGES, P. T., SCHUBERT, M.: The separation of new forms of the proteinpolysaccharides of bovine nasal cartilage. J. biol. Chem. **1966,** 241: 4261—4266.

PARTRIDGE, S. M., DAVIS, H. F.: The chemistry of connective tissues. 4. The presence of a non-collagenous protein in cartilage. Biochem. J. **1958,** 68: 298—305.

PARTRIDGE, S. M., DAVIS, H. E., ADAIR, G. S.: The chemistry of connective tissues. 6. The constitution of the chondroitin sulphate-protein complex in cartilage. Biochem. J. **1961,** 79: 15—26.

SCHUBERT, M.: Structure of connective tissues, a chemical point of view. Fed. Proc. **1966,** 25: 1047—1052.

SERAFINI-FRACASSINI, A., SMITH, J. W.: Observations on the morphology of the proteinpolysaccharide complex of bovine nasal cartilage and its relationship to collagen. Proc. roy. Soc. B. **1966,** 165: 440—449.

SHATTON, J., SCHUBERT, M.: Isolation of a mucoprotein from cartilage. J. biol. Chem. **1954,** 211: 565—573.

SMITH, J. W., PETERS, T. J., SERAFINI-FRACASSINI, A.: Observations on the distribution of the proteinpolysaccharide complex and collagen in bovine articular cartilage. J. Cell Sci. **1967,** 2: 129—136.

Ilona BANGA,
Orvostudományi Egyetem I. sz. Kórbonctani Intézete,
Budapest VIII., Üllői út 26, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 365—368 (1969)*

# CONVERTING ENZYME ACTIVITY IN EXPERIMENTAL CIRRHOSIS OF THE RAT

By

GY. DAUDA, I. DÉVÉNYI and V. SZOKOLY

INSTITUTE OF PATHOLOGICAL ANATOMY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received January 11, 1969)

Renin and angiotensinogen prepared from blood of $CCl_4$-treated cirrhotic rats were incubated, and the reaction product (angiotensin) was tested in cirrhotic rats with the aim to establish whether liver cirrhosis was associated with converting enzyme deficiency. The formation of angiotensin with the usual pressor effect was observed also in the cirrhotic animals. The response produced in these animals was smaller than that seen in the normal test animal, but this was due to the increased angiotensinase level in the cirrhotic rats. Thus, converting enzyme is neither absent nor inhibited in liver cirrhosis.

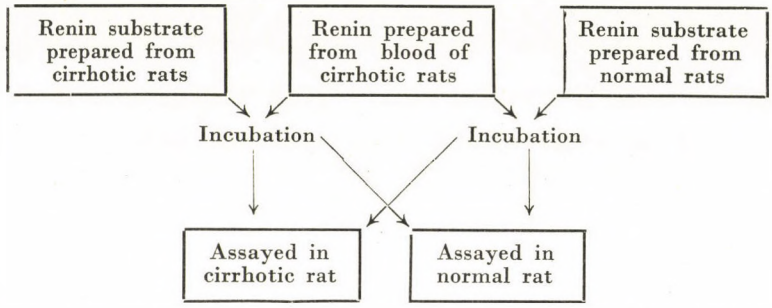
Converting enzyme is the least studied component of the renin-angiotensin system. The initial reaction product of the enzyme renin and its substrate is the decapeptide angiotensin I which does not possess pressor activity. In the plasma, it is converted by the converting enzyme into the octapeptide angiotensin II, the potent pressor substance. The conversion is associated with the splitting from the terminal carboxyl of angiotensin I of the dipeptide histidyl-leucine (SKEGGS et al. 1967). Concentrated and purified converting enzyme was first prepared by SKEGGS et al. (1956). For the action of the enzyme, which was found susceptible to alterations in pH and temperature, free $Cl^-$ ions must be present in the medium. According to PAGE and BUMPUS (1961), converting enzyme is produced in the liver.

LOYKE (1964, 1965, 1967) and LOYKE and MACKRELL (1967) carried out experiments in $CCl_4$-treated and $CCl_4$-cirrhotic rats and suggested that the injured liver produced a substance which inhibited converting enzyme. After $CCl_4$ treatment, LOYKE (1964) found normal angiotensinase activity in the plasma. On the other hand, observations from this laboratory (DÉVÉNYI et al., in preparation) revealed increased angiotensinase levels in $CCl_4$-induced liver cirrhosis of the rat, and similar results were obtained by other authors in patients with cirrhosis (ARAI 1967; LUBASH et al. 1968) and in rabbits rendered cirrhotic by $CCl_4$ treatment (KOKUBU 1967). The inconsistency between these findings has prompted us to reinvestigate the problem of converting enzyme and liver cirrhosis. The experimental conditions were such to secure maximal inhibition of converting enzyme according to the concept of LOYKE (1964, 1965, 1967).

## Methods

Renin prepared from the blood of $CCl_4$-cirrhotic rats was incubated in the presence of renin substrate derived also from cirrhotic rats; the reaction product thus formed was bioassayed in $CCl_4$-cirrhotic rats.

The experimental schedule was as follows.




Female Wistar rats weighing 140 to 150 g, kept on a standardized diet and allowing free access to water, were given subcutaneously 0.2 ml of $CCl_4$ and 0.2 ml of paraffin oil twice weekly over a period of 40 weeks. At the end of this treatment, 3 ml of blood were withdrawn from the aorta of 8 rats anaesthetized with ether, then the animals were killed, by bleeding. Blood samples obtained from 2 rats were pooled and, after sufficient inhibition of plasma angiotensinase, samples of 0.5 ml were incubated for 5 hours at 37 °C with renin substrate prepared either from nephrectomised rats (standard renin substrate; for technical details the reader is referred to earlier works from this laboratory [DAUDA 1969a, b]) or from 5 cirrhotic rats deprived of their kidneys 20 hours prior to the preparation of renin substrate. Angiotensin was bioassayed in normal and cirrhotic rats subjected to bilateral nephrectomy 16 hours prior to testing. The assay was carried out in urethane anaesthesia (1.4 g per kg body weight subcutaneously), after pretreatment with pentolinium (5 mg per kg body weight intravenously) and heparin (500 I.U. intravenously). The rats were tracheotomized and subjected to bilateral vagotomy. The pressor response was bracketed between blood pressure rises elicited by known amounts of standard angiotensin II (Hypertensin, Ciba Ltd., Basel, Switzerland). Each sample was assayed in duplicate.

## Results

The incubation of renin substrate prepared from cirrhotic rats and standard renin substrate with renin derived from cirrhotic rats yielded the results shown in Table I.

Thus, the result depends on the test animal, irrespective of whether the renin substrate has been prepared from normal rats or rats rendered cirrhotic

**Table I**

| Source of renin substrate | Source of renin | Test animal | Amount of angiotensin II formed, ng/ml |
|---|---|---|---|
| Hepatic cirrhosis | Hepatic cirrhosis | Hepatic cirrhosis | 40.0 ± 3.6 |
| Hepatic cirrhosis | Hepatic cirrhosis | Normal | 68.0 ± 5.8 |
| Normal | Hepatic cirrhosis | Hepatic cirrhosis | 36.8 ± 3.4 |
| Normal | Hepatic cirrhosis | Normal | 64.0 ± 6.1 |

by $CCl_4$ treatment. The amount of angiotensin II per ml of plasma in the cirrhotic rats was 40.0 and 36.8 ng, whereas the assay performed in normal rats yielded 68.0 and 64.0 ng/ml angiotensin II.

## Discussion

A pressor substance (angiotensin II) could be successfully demonstrated in both normal and cirrhotic test rats after incubating renin substrate prepared from normal and cirrhotic rats with renin prepared from cirrhotic rats. The pressor effect proved to be 41% lower in cirrhotic than in normal rats.

If renin prepared from the blood of cirrhotic rats was incubated with renin substrate prepared from normal rats, essentially the same results were obtained. The pressor effect produced by this incubate was 43% lower in the cirrhotic than in the normal rats.

The observations of SKEGGS et al. (1956) have unequivocally demonstrated that converting enzyme present in blood is inactivated during the preparation from blood of renin and renin substrate. Consequently, during incubation only angiotensin I a substance without pressor effect is formed. This substance when injected into the test animal, immediately yields angiotensin II owing to the action of converting enzyme present in the blood of the test rat. The high rate of angiotensin II formation is due to the high plasma concentration of converting enzyme, and to the great reaction velocity.

In view of these findings it seems reasonable to assume that converting enzyme is present in the blood of cirrhotic rats.

Thus, the difference in pressor response obtained in the normal rats and those rendered cirrhotic by the administration of $CCl_4$ cannot be attributed to the absence of converting enzyme or to its inhibition by some factor. Results obtained in another study (DÉVÉNYI et al., in preparation) indicate that the above difference in responsiveness is due to the considerably increased angiotensinase level in liver cirrhosis.

The present findings are inconsistent with the data of LOYKE (1964, 1965, 1967) indicating inhibition of the converting enzyme in liver cirrhosis.

## LITERATURE

ARAI, J.: Clinical and experimental studies on serum angiotensinase activity. Jap. Circulat. J. **1967,** 31: 911—921.

DAUDA, GY.: Standard patkány renin substratum készítése és concentratiojának meghatározása. Kísérl. Orvostud. **1969a,** In press

DAUDA, GY.: Standard patkány renin preparatum készítése és physiologiai sajátosságai. Kísérl. Orvostud. **1969b,** In press

KOKUBU, T., HIWADA, K., UEDA, E., FUJIMOTO, S., AKUTSU, H., KATO, A., YAMAMURA, Y.: Plasma angiotensinase activity in liver disease. Biochem. Med. **1967,** 1: 54—61.

LOYKE, H. F.: Experimental hypertension treated with $CCl_4$: Measurements of adrenal function, vascular responsiveness, angiotensinase and converting enzyme. Proc. Soc. exp. Biol. (N. Y) **1964,** 115: 1035—1040.

LOYKE, H. F.: Converting enzyme in experimental hypertensions: effect of $CCl_4$. Amer. J. med. Sci. **1965**, 250: 19—23.
LOYKE, H. F.: The pressor response to serum and exogenous renin in rats. J. Lab. clin. Med. **1967**, 69: 1013—1017.
LOYKE, H. F., MACKRELL, J. S.: Juxtaglomerular cells in $CCl_4$-treated experimental hypertension. Proc. Soc. exp. Biol. (N. Y.) **1967**, 125: 1032—1035.
LUBASH, G. D., HAMMEL, E. C., MEARLES, R. J.: Serum angiotensinase activity in cirrhosis. Amer. J. med. Sci. **1968**, 255: 105—112.
PAGE, I. H., BUMPUS, M.: Angiotensin. Physiol. Rev. **1961**, 41: 331—390.
SKEGGS, L. T., KAHN, J. R., SHUMWAY, N. P.: The preparation and function of the hypertensin-converting enzyme. J. exp. Med. **1956**, 103: 295—299.
SKEGGS, L. T., LENTZ, K. E., GOULD, A. B., HOCHSTRASSER, H., KAHN, J. R.: Biochemistry and kinetics of the renin-angiotensin system. Fed. Proc. **1967**, 26: 42—47.
YAMAMURA, Y.: Significance of angiotensinase on renin-angiotensin system. Jap. Circulat. J. **1966**, 30: 186—188.

György DAUDA, István DÉVÉNYI, VIKTOR SZOKOLY
Orvostudományi Egyetem Kórbonctani Intézete
Debrecen 12, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 369—374 (1969)*

# *Pharmacologia*

## EFFECT OF CAPSAICIN ON THE ISOLATED ILEUM AND AURICLE OF THE GUINEA PIG

By

J. MOLNÁR,* L. GYÖRGY, Gabriella UNYI and Judit KENYERES

DEPARTMENT OF PHARMACOLOGICAL RESEARCH, INSTITUTE OF EXPERIMENTAL MEDICINE, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

(Received October 21, 1968)

Capsaicin at concentrations from $5 \times 10^{-8}$ to $10^{-7}$ g/ml has been found to elicit contractions of the isolated ileum and to exert positive inotropic and chronotropic effects on the isolated auricle of the guinea pig. In both preparations, a rapidly ensuing, prolonged, and highly specific tachyphylaxis to capsaicin has been observed. No crossed tachyphylaxis to acetylcholine, histamine, serotonin, nicotine, DMPP, bradykinin, KCl or $BaCl_2$ could be demonstrated. The contractions produced on the ileum could not be antagonized by atropine, diphenhydramine, methysergide or hexamethonium but were abolished by papaverine, caramiphen and cinchocaine. In the isolated auricle, the positive inotropic and chronotropic effects could not be antagonized by either propranolol or cocaine.

Capsaicin exerts its most marked effect on the peripheral part of the sensory nervous system, i.e. on the nerve terminals (JANCSÓ 1955, 1960; JANCSÓ et al. 1967, 1968) and on the afferent fibres (BARAZ et al. 1968*a*). These structures are first stimulated then inhibited by capsaicin. Experiments on cats have shown the compound to have in addition a direct effect on the heart and the blood vessels, so that capsaicin seems to possess pharmacological actions on other biological systems as well. It has been shown that the compound elevates blood pressure in the decapitated cat (PÓRSZÁSZ et al. 1955) and, when administered intravenously, produces pulmonary hypertension in the anaesthetized cat (MOLNÁR and GYÖRGY 1967), diminishes and in turn increases the amplitude of heart contractions (MOLNÁR and GYÖRGY 1967), finally, when injected directly into the coronaries, it causes a marked local vasoconstriction (OSADCHII et al. 1967).

In an endeavour to clarify the mechanism of action of capsaicin a study has been made on the effects of the compound in isolated organs. Such experiments have been so far done mainly with impure extracts of paprika (for references, see MOLNÁR 1965) and in the available literature only two papers

* Present address: Department of Experimental Toxicology, State Institute of Occupational Health, Budapest.

could be found dealing with the effects of capsaicin on isolated organs. Thus, VÁRADY and KOTURNYA (1931) showed that crystalline capsaicin had a negative inotropic effect on the frog heart, and caused relaxation of the rabbit's isolated intestine while TOH et al. (1955) reported that capsaicin gave rise to contractions in the isolated ileum of the guinea pig; owing to the marked tachyphylaxis, this effect could not be repeated on the same preparation.

## Methods

In the experiments, freshly removed guinea pig ileum was used. The isolated intestine was placed in a 20-ml flask containing the bathing solution through which oxygen was bubbled at 37 °C. The composition of the bathing medium was, NaCl, 6.9 g; KCl, 0.35 g; $MgSO_4$, 0.29 g; $NaHCO_3$, 2.1 g; $KH_2PO_4$, 0.16 g; $CaCl_2$, 0.28 g; glucose, 1.00 g; for 1000 ml. Contractions of the ileum were recorded by an isotonic lever.

The medium bathing the isolated and spontaneously contracting right auricle of the guinea pig heart at temperatures ranging between 32° and 33 °C had the following composition NaCl, 7.5 g; KCl, 0.41 g; $CaCl_2$, 0.24 g; $NaHCO_3$, 0.24 g; $NaH_2PO_4$, 0.14 g; glucose, 1.00 g per 1000 ml. Recording was done by a light glass lever with a spring serving as the adjustable counterweight. In part of the experiments, frequency of the contractions was also recorded, by means of a special ordinate recorder. A total of 24 heart auricles was used in the experiments.

A stock solution of capsaicin was prepared with Tween 80 and 96% alcohol to give a concentration of 1 mg/ml; dilutions were made with physiological saline solution. Equal doses of the used solvents proved to have no effect when given by themselves. The doses of the various substances tested in this study refer to the respective salts of these substances.

## Results

### (i) *Effect of capsaicin on the isolated guinea pig ileum*

Capsaicin elicits contraction of the isolated ileum at concentrations of $5 \times 10^{-8}$ and $10^{-7}$ higher. The same dose applied a second time had a slighter or no effect. After a small initial dose, higher concentrations were effective, but after the second response no contraction could be elicited by additional doses, i.e. the preparation became completely unresponsive to capsaicin (Fig. 1). Tachyphylaxis was lasting as repeated washings over periods of 2 hours failed to restitute susceptibility of the isolated ileum to capsaicin while histamine, acetylcholine, serotonin, nicotine, dimethyl-phenyl-piperazine (DMPP), bradykinin (BRS 640, Sandoz Ltd., Basel, Switzerland), KCl and $BaCl_2$ (Fig. 1) proved invariably capable of eliciting contractions.

A series of experiments was carried out with compounds of antagonistic effect. Specific blocking agents were added to the bath after some of the compounds with stimulating effect had been added. After administration of the blocking compound, again the agonistic compound was given, and if the latter had no effect, capsaicin was added to the medium in a concentration of $10^{-7}$ g/ml. As agonistic agents, histamine, acetylcholine, serotonin, nicotine and DMPP were used. Owing to the tachyphylaxis it was impossible to evaluate

the responses quantitatively; they were therefore denoted either as "Inhibition" or "No inhibition". The response was regarded as positive ("Inhibition") if capsaicin failed to elicit contractions of the isolated ileum. Each blocking agent was tested at least on 8 isolated preparations but none of them was capable

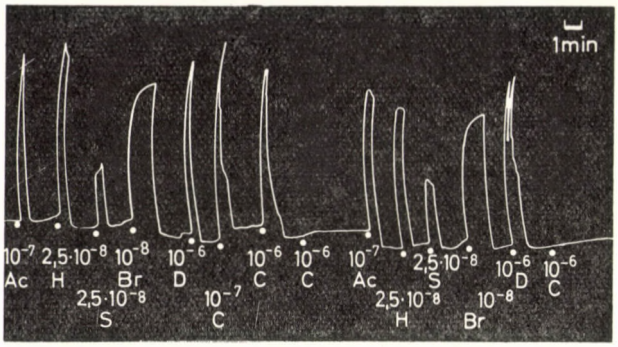



*Fig. 1.* Isolated guinea pig ileum. Ac, acetylcholine; H, histamine; S, serotonin; Br, bradykinin; D, DMPP; C, capsaicin

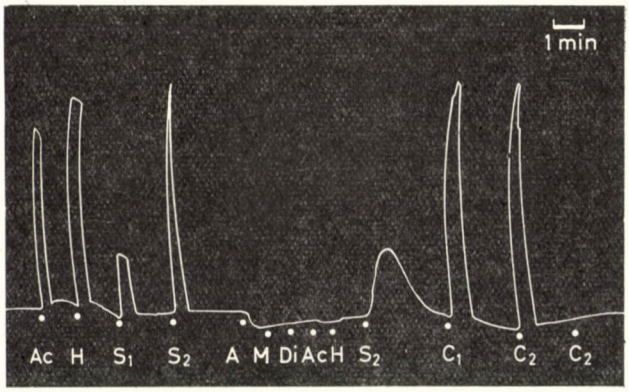



*Fig. 2.* Isolated guinea pig ileum. Ac, acetylcholine, $5\times10^{-8}$ g/ml; H, histamine, $10^{-8}$ g/ml; $S_1$, serotonin, $10^{-8}$ g/ml; $S_2$, serotonin $3.3\times10^{-8}$ g/ml; A, atropine, $10^{-6}$ g/ml; M, methysergide, $10^{-6}$ g/ml; Di, diphenhydramine, $10^{-6}$ g/ml; $C_1$, capsaicin, $10^{-7}$ g/ml; $C_2$, capsaicin, $10^{-6}$ g/ml

of antagonizing the contractions elicited by capsaicin, even at $10^{-6}$ g/ml concentration, sufficient to block the effects of the respective stimulating agent (Fig. 2). Thus, capsaicin caused the isolated ileum to contract after atropine, methysergide, diphenhydramine and hexamethonium ($10^{-5}$ g/ml), when acetylcholine, histamine, serotonin and DMPP produced no effect at all.

On the other hand, the contractions elicited by capsaicin were inhibited by papaverine, caramiphen and cinchocaine at $10^{-5}$ g/ml concentration.

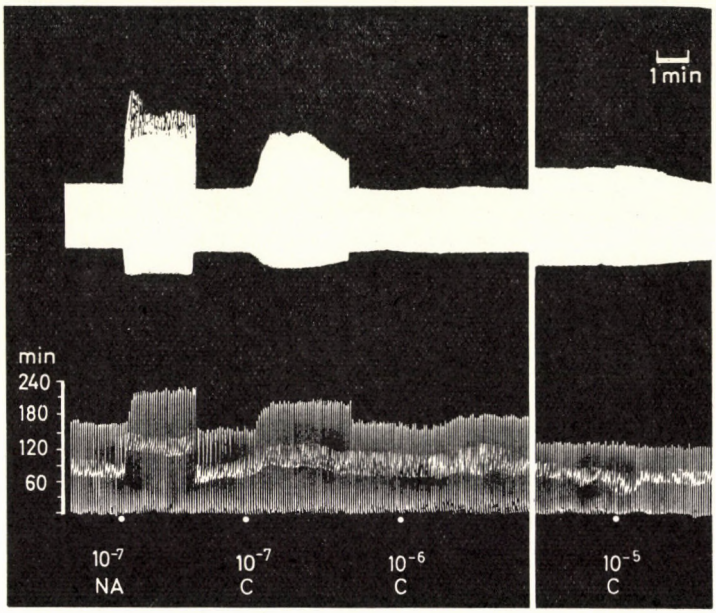



*Fig. 3.* Isolated guinea pig auricle. Top tracing, amplitude of contractions, below, frequency of contractions. NA, noradrenaline; C, capsaicin

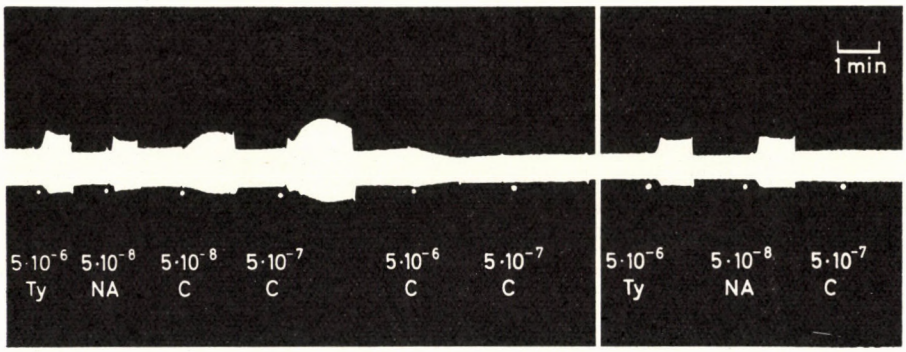



*Fig. 4.* Isolated guinea pig auricle. Ty, tyramine; NA, noradrenaline; C, capsaicin

### (ii) *Effect of capsaicin on the isolated guinea pig auricle*

Capsaicin at concentrations between $5 \times 10^{-8}$ and $10^{-7}$ g/ml, had a positive inotropic and chronotropic effect on the isolated auricle (Fig. 3) but tachyphylaxis ensued rapidly and was of considerable duration. Adrenaline, noradrenaline, isoprenaline, tyramine, DMPP and nicotine retained their stimulating effect after tachyphylaxis to capsaicin had ensued (Fig. 4). The capsaicin effect could not be abolished by the previous addition to the medium of propranolol or cocaine. These blocking agents, on the other hand, potently antagonized the effect of catecholamines and tyramine.

## Discussion

Considering that capsaicin has been denoted as the most potent peripheral sensory structure exciting agent (BARAZ et al. 1968a), it was not surprising that it exerted a stimulatory effect on other tissues, too. The most interesting point in the excitatory effects of capsaicin on isolated structures seems to be the marked tachyphylaxis occurring on both organs tested in this study.

As shown by experiments in vivo, medium doses of capsaicin do not give rise to tachyphylaxis in chemoreceptor structures, in the pulmonary vessels, or in the coronaries (PÓRSZÁSZ et al. 1955; MOLNÁR and GYÖRGY 1967, OSADCHII et al. 1967). If tachyphylaxis occurs, it usually does not last longer than 15 to 20 min, as far as this can be determined (MOLNÁR 1966). For tachyphylaxis to ensue under conditions in vivo it is necessary to give such doses which already block the response to other, e.g. mechanical, stimuli (BARAZ et al. 1968*b*). On the other hand, desensibilization carried out by repeated administration of capsaicin is associated with a reduction or complete abolition of the response given to a number of other irritating agents (JANCSÓ 1955; PÓRSZÁSZ 1966; MAKARA et al. 1967).

In the isolated organ, tachyphylaxis to capsaicin developed rapidly; it was longstanding and highly specific. All the compounds with agonistic properties could be demonstrated to have the usual effect when tested on the ileum and auricle already unresponsive to capsaicin. This also means that capsaicin had no harmful effect on the contractile elements of the isolated organ.

As to the point of action of capsaicin, our possibilities were limited, for tachyphylaxis made it impossible to carry out reliable pharmacological analysis. Informative experiments have shown that, in the isolated guinea pig ileum the site of action of capsaicin is certainly not the intramural ganglia or the receptors of acetylcholine, histamine or serotonin, at the specific blocking agents of these compounds even when applied together, failed to prevent the response to capsaicin. In addition, the present experiments indicate with great probability that in the isolated auricle capsaicin does not exert its effect directly on the adrenergic receptors.

Summing up the present results, the following conclusions can be drawn:

*(1)* capsaicin has a marked excitatory effect on the isolated intestine and auricle of the guinea pig;

*(2)* it exerts its effect on some hitherto unidentified biological structure (receptor) to which it has a high affinity and which, after an initial excitatory process, rapidly becomes insensitive to capsaicin;

*(3)* tachyphylaxis to capsaicin ensues rapidly it is lasting and highly specific. No cross tachyphylaxis to other compounds could be revealed. According to our knowledge, there is no other compound with a similarly specific tachyphylaxis in isolated organs.

## LITERATURE

BARAZ, L. A., KHAYUTIN, V. M., MOLNÁR, J.: Analysis of the stimulatory action of capsaicin on receptors and sensory fibres of the small intestine in the cat. Further contribution to the problem of pain. Acta physiol. Acad. Sci. hung. **1968a**, 33: 225—235.

BARAZ, L. A., KHAYUTIN, V. M., MOLNÁR, J.: Effects of capsaicin upon the stimulatory action of potassium chloride in the visceral branches of spinal afferents of the cat. Acta physiol. Acad. Sci. hung. **1968b**, 33: 237—246.

JANCSÓ, N.: Speicherung. — Stoffanreicherung im Reticuloendothel und in der Niere. Akademie Verlag Budapest **1955**, P. 468.

JANCSÓ, N.: Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard Fillmore Hosp. **1960**, 7: 53—77.

JANCSÓ, N., JANCSÓ-GÁBOR, A., SZOLCSÁNYI, J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicine. Brit. J. Pharmacol. **1967**, 31: 138—151.

JANCSÓ, N., JANCSÓ-GÁBOR, A., SZOLCSÁNYI, J.: The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. Brit. J. Pharmacol. **1968**, 32: 32—41.

MAKARA, G. B., GYÖRGY, L., MOLNÁR, J.: Circulatory and respiratory responses to capsaicin, 5-hydroxytryptamine and histamine in rats pretreated with capsaicin. Arch. int. Pharmacodyn. **1967**, 170: 39—45.

MOLNÁR, J.: Die pharmakologischen Wirkungen des Capsaicins, des scharf schmeckenden Wirkstoffes im Paprika. Arzneimitt.-Forsch. **1965**, 15: 718—727.

MOLNÁR, J.: Effect of capsaicin on the cat's nictitating membrane. Acta physiol. Acad. Sci. hung. **1966**, 30: 183—191.

MOLNÁR, J., GYÖRGY, L.: Pulmonary hypertensive and other haemodynamic effects of capsaicin in the cat. Europ. J. Pharmacol. **1967**, 1: 86—92.

OSADCHII, L. I., BALUEVA, T. V., MOLNÁR, J.: Reflex changes in systemic vascular resistance following intracoronary injection of capsaicin and veratrine in the cat. Acta physiol. Acad. Sci. hung. **1967**, 32: 215—219.

PÓRSZÁSZ, J.: Vasomotor és légzési reflex mechanizmusok pharmaco-physiologiája. Thesis, Szeged **1966**.

PÓRSZÁSZ, J., GYÖRGY, L., PÓRSZÁSZ-GIBISZER, K.: Cardiovascular and respiratory effects of capsaicin. Acta physiol. Acad. Sci. hung. **1955**, 8: 61—76.

TOH, C. C., LEE, T. S., KIANG, A. K.: The pharmacological actions of capsaicin and analogues. Brit. J. Pharmacol. **1955**, 10: 175—182.

VÁRADY, M., KOTURNYA, M.: A paprika hatása. A Szegedi Orvostudományi Egyetem pályamunkája. Szeged **1931**.

Jenő MOLNÁR
Országos Munkaegészségügyi Intézet, Kísérleti Toxikológiai Osztály,
Budapest IX., Nagyvárad tér 2.

Lajos GYÖRGY, Gabriella UNYI, Judit KENYERES
Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutató
Intézete, Gyógyszerkutatási Osztály,
Budapest VIII., Szigony u. 43, Hungary

*Acta Physiologica Academiae Scientiarum Hungaricae, Tomus 35(3—4), pp. 375—385 (1969)*

# *Recensiones*

E. R. CAIANIELLO (Ed.)

## Neural Networks

Proceedings of the School on Neural Networks
Ravello, June 1967, Springer-Verlag, Berlin—Heidelberg—New York, 1968.
190 pages with 80 figures. Price DM 68, — US$ 17.00

The volume contains the seventeen lectures presented at the School of Neural Networks supported by the NATO Advanced Study Institute Programme.

The foreword of the Editor, Prof. E. R. CAIANIELLO, is followed by the presentations of F. RATLIFF: On Fields of Inhibitory Influence in Neural Network; E. F. EVANS: Upper and Lower Levels of the Auditory System; V. BRAITENBERG: On Chiasmas; M. A. ARBIB, G. F. FRANKLIN and N. NILSSON: Some Ideas on Information Processing in Cerebellum; D. M. MACKAY: The Importance of "Landmarks" for Visual Perception; W. S. MC CULLOCH: Logic and Closed Loops for a Computer Junket to Mars; E. R. CAIANIELLO, A. DE LUCA and L. M. RICCIARDI: Neural Networks: Reverberations, Constants of Motion, General Behavior; A. DE LUCA and L. M. RICCIARDI: Probabilistic Description of Neurons; J. D. APLEVICH: Models of Certain Nonlinear Systems; P. J. M. JOHANNESMA: Diffusion Models for Stochastic Activity of Neurons; J. CL. LEVY: Statistical Approach to the Study of Neuronal Networks; S. NOGUCHI, K. NAGASAWA and J. OISUMI: A Fundamental Consideration on Pattern Recognisers; A. STARSZAK: Multilayer and Multilevel Control Structures; R. E. KALMAN: On the Mathematics of Model Building; B. TOUSCHEK: A Statistical Clock; J. D. COWAN: Statistical Mechanics of Nervous Nets. After such highly interesting presentations it is unfortunate that the discussions and the conclusions have not been included in the book.

From this truly interdisciplinary course it may be concluded that only the systematic collaboration of neuroanatomists, neurophysiologists, mathematicians and logicians with chemists, physicists and engineers can develop realistic models of neuronal networks for the sake of a better understanding of nervous integration.

The book is warmly recommended first of all to neuroanatomists, neurophysiologists, mathematicians and to everybody concerned with this most interesting problem of neurobiology.

K. LISSÁK

G. C. QUARTON, T. MELNECHUK and F. O. SCHMITT (Eds)

## The Neurosciences: A Study Program

The Rockefeller University Press, New York, 1967. 962 pages. Price $ 17.50

After earlier successful publications (Neurosciences Research Symposium Summaries, vol. I—II) the Neurosciences Research Program has brought out again a book of great significance.

In the summer of 1966 the Neurosciences Research Program invited more than 150 scientists from all parts of the world to the Campus of the University of Colorado in Boulder to discuss the actualities of neurosciences in the course of a month-long Intensive Study Program. The participants represented the most different fields of neuroscience from molecular biology to psychology. This volume contains the 65 lectures delivered by outstanding experts of the fields selected for discussion.

With works of a summarizing type there is always a danger of oversimplification. The great value of this book is that it has avoided this danger. The authors not only survey their topic but give an account of their recent research work and in the light of the month-long

multidisciplinary discussions they outline the further perspectives of the given field. Thus, if one reads the volume (which is not a simple task considering its 962 pages) one gets a thorough insight into the present status of neurosciences.

The book consists of seven chapters. After a short morphological introduction (2 lectures) one chapter deals with *molecular biology* in general (17 lectures), the other with the special problems of *molecular biology of brain cells* (11 lectures). The chapter *Neuronal Physiology* (11 lectures) is devoted to problems of synaptic processes, neural coding and information processing. In the next chapter entitled *Brain Correlates of Functional Behavioral States* 9 lectures discuss the neurophysiological aspects of biological rhythms, attention and emotions. The chapter *Brain Correlates of Learning* (12 lectures) is a survey of cellular processes as well as of more complex mechanisms of learning and memory. In the last chapter headed *Interdisciplinary Topics*, three lectures deal with problems broader than those in the previous chapters. The book is completed by the list of the participants of the Intensive Study Program and by a detailed name and subject index.

The editing is first rate, the illustrations are excellent.

This volume is not only important from the scientific point of view but also a work which can be recommended as a valuable contribution to the library of all neuroscientists,

K. Lissák

N. S. Assali (Ed.)

## Biology of Gestation

Volume I. The Maternal Organism
Academic Press, New York and London, 1968. 507 pages with 155 figures.
Price $ 27.00

The aim of this work is to collect all contemporary knowledge concerning the processes of gestation. Research in the different basic sciences during the past decades yielded many new facts concerning the physiological and biochemical processes which take part in foetal growth and development, and in the adaptation to intrauterine life and the external environment.

The subject of the first volume is the maternal organism, discussed by thirteen well-known authors in eight chapters. The first chapter by Roger A. Gorski discusses the problems of the neural control of ovulation. The second chapter by Louis Fridhandler deals with gametogenesis and implantation. The third chapter by Ralph M. Wynn describes the morphology of the placenta. The fourth chapter by N. S. Assali, Preston V. Dills, Albert A. Plenth, Thomas H. Kirschbaum, and Stanley J. Gross reviews the physiology of the placenta. In chapter five Hans H. Simmer discusses the placental hormones. Chapter six deals with the regulation of myometrial composition, growth, and activity. Chapter seven reviews the pregnant uterus, the process of labour, puerperium, and lactation. Finally, in chapter eight, F. E. Hytten and A. M. Thomson are discussing the problems of maternal physiological adjustment.

The reader looks forward with anticipated expectations to the second volume, which will deal with the mechanisms assisting the foetus to birth. The first volume has accomplished its aim of presenting a complete and coordinated picture of the maternal organism during pregnancy. The book will be useful not only for the gynaecologist but also for the physiologist and the biologist. It would be desirable to compile such books in the fields of other clinical sciences also.

K. Lissák

C. von Euler, S. Skoglund and U. Söderberg (Eds)

## Structure and Function of Inhibitory Neural Mechanisms

Wenner-Gren Center International Symposium Series, Volume 10.
Pergamon Press, Oxford 1968. IX + 563 pages, with 261 figures. Price $ 24.00

The book contains the material presented by more than fifty well-known contributors to the *Wenner-Gren* symposium in September, 1966. The subject of the Symposium was the important problem of nervous inhibition. The results of the recent investigations in neurobiology have made it necessary to integrate the particular data of ultrastructure research, neurophysiology, pharmacology and neurochemistry in the problem of inhibitory mechanisms.

The papers presented were ranged in five groups: I. Morphological correlates of pre- and postsynaptic inhibitory processes. II. The mechanisms of centrifugal inhibition of sense organs. III. Physiological significance of pre- and postsynaptic inhibition. IV. Pharmacology and neurochemistry of inhibitory processes. V. Role and importance of inhibitory mechanisms as links in neural circuits.

The multidisciplinary Symposium has accomplished its original programme and offers an integrated contemporary picture of inhibition in the nervous system, and a perspective for future research. The excellent book is highly recommended for every worker in neurobiology.

K. Lissák

G. A. Kerkut (Ed.)

## Experiments in Physiology and Biochemistry

Volume I. Academic Press, London and New York, 1968. XV + 408 pages, with 154 figures. Price s 120

This new series "Experiments in Physiology and Biochemistry" intends to give a detailed description of methods to enable the reader to carry out the experiments described. Twenty-four experienced contributors offer a wide range of biochemical, general and comparative physiological experimental methods in the following fields: 1. Enzymes of arginine and urea metabolism in invertebrates. 2. The distribution and pool size of free amino acids in animal tissue; the detection and estimation of α-L amino-transferase activity in animal tissue; the incorporation of carbon from radioactive substrates into the soluble intermediates of animal tissues using two-dimensional paper autoradiography; biochemical changes during muscle contraction; fermentation and respiration in yeast. 3. The relation between the amount of oxygen available to tissues and the type of enzyme present. 4. Biological oxidation; digestion by mammalian gut tissues; the measurement of enzyme activity in the digestive gland of Arion ater. 5. Snake venom peptidase activities; hydrolyses of arginine esters by snake venoms. 6. Estimation of the esterase activity of serum; starch-gel electrophoresis of mouse serum. 7. Buffering capacity of snail blood; determination of choline esterase activity in snail blood. 8. The role of contractile vacuole; ion antagonism and physiologically balanced salt solutions; some effects of radiation on cells; bioluminescence; oxygen equilibrium curve of haemocyanin; active transport through cell membranes; salinity and the volume and weight of animals; physiological colour changes of vertebrates. 9. Biological membranes in the frog and toad urinary bladder. 10. Temperature and the goldfish intestine. 11. Hunger in insects and taste threshold rise in blowflies after feeding. 12. Allectomy of the American cockroach (Periplaneta americana L.). 13. Hormonal control of excretion in an insect. 14. The use of the kymograph; the clam-heart technique of bioassay for acetylcholine; preparation of nerve extracts for bioassay of bound and free acetylcholine; the function of the cardioregulator nerves in the crayfish; pharmacology of the crayfish heart; spontaneous activity of the crayfish hindgut and its control by drugs. 15. Facilitation and other properties of nervous systems of sea anemones; autorhythmic organs; peripheral inhibition in crustaceous; slow and fast axon control of contraction in insects; properties of some insect sense organs; "satch" mechanisms of molluscs. 16. Recording from the crayfish abdominal extensor muscle preparation with microelectrodes. 17. Isolated and in situ heart preparation of the snail Helix aspersa; intracellular microelectrode recording from the brain of Helix. 18. Chromatographic demonstration of dopamine in the brain of Helix aspersa. 19. Thin-layer chromatography of amino acids and sugars; the snail nerve-muscle preparation; perfusion of the cockroach leg. 20. Physiological response to asphyxia in animals including man. 21. Intestinal absorption of glucose in vivo. 22. The consumption of oxygen by the whole animal. 23. Measurement of the respiratory rate of a small terrestrial animal.

This very useful manual will certainly find its way to the library shelf of every biochemical and physiological department, to assist both the beginner and the experienced research worker.

K. Lissák

## Computing in Medicine

**British Medical Bulletin, Ed.: C. C. Spicer. Vol. 24, No. 3, September 1968. Published by the Medical Department, The British Council, London. 273 pages, Price £ 2**

The introduction of the computer principles and technology in biology including medicine have revolutionised science. The old belief that biological sciences are non-mathematical had been based on the complexity of biological systems where measurements were inadequate for mathematical formulation. However, the attempts of the past decade at introducing computing in medicine have led to major advances.

The well selected collection of this issue of the *British Medical Bulletin* represents the present state of computing in medicine. After the introduction of J. A. B. *Gray*, Secretary of the Medical Research Council, fifteen chapters present the application of computer technology in different fields of medicine.

L. C. *Payne* presents "The basic principles and brief history of computer technology"; *John Anderson* "The computer: medical vocabulary and information"; J. A. *Bates* "Preparation of clinical data for computers"; E. D. *Acheson* "Linkage of medical records"; M. J. R. *Healy* "The disciplining of medical data"; G. B. *Hill*, C. C. *Spicer* and A. C. *Weatherell* "The computer surveillance of congenital malformations"; I. D. P. *Wootton* "Computer science in the biochemistry laboratory"; J. A. *Boyle* and J. A. *Anderson* the problems of "Computer diagnosis: clinical aspects"; J. A. *Anderson* and J. A. *Boyle* "Computer diagnostic: statistical spects"; D. N. *Baron* and P. M. *Fraser* "Medical application of taxonomic methods"; E. W. *Emery* "A brief note on computer applications in radiotherapy"; E. *William Emery* "Some aspects of computers in radiobiology"; E. J. M. *Campbell* and C. M. E. *Matthews* "The use of computers to stimulate the physiology of respiration"; A. M. *Halliday* "Computing techniques in neurological diagnosis"; finally D. *Rutovitz* "Automatic chromosome analysis". There is a glossary most useful for the beginner in computation terminology.

This excellent issue will be very useful for everybody interested in the progress of medical science.

K. Lissák

F. Erbslöh, A. Oksche, F. Seitelberger (Eds)

## Symposium on Neuroglia — Symposium concernant la neuroglie — Symposium über die Neuroglia

Papers Presented on the 12th Annual Meeting of the Vereinigung Deutscher Neuropathologen und Neuroanatomen, Berlin, October 20—22, 1966.
Springer-Verlag, Berlin, Heidelberg, New York — Springer-Verlag, Wien, New York
(Acta Neuropathologica, Supplementum IV.) Price US$ 16.25

Some of the papers dealing with the development *(Oksche, Fleischauer)* and general histopathology *(Colmant)* of glia are to be regarded as comprehensive reviews, in which the authors have included results of their own research. The writer of the report on embryology was guided by the points of view of comparative histology. Besides the results of the traditional staining methods the authors also take into account those of submicroscopical research and enzyme histochemistry. Some of the reports are essentially based on recent methods *(Hager, Friede, Wolff, Lierse)*.

The greater part of the volume consists of papers reporting on investigations into special problems partly on human material and partly on the basis of animal experimentation. Several authors concern themselves with the spongy states *(Ule, Kolkmann, Seitelberger)*. Workers of the Max Planck Research Institute in Cologne *(Sehrbundt; Kleihues* and *Schultze; Hossmann)* investigated the morphological and functional aspects of the behaviour of the glial elements in experimental brain oedema. *Peiffer* is in favour of the opinion that enzymatic glial deficiency in some hereditary metabolic disorders such as leucodystrophies and gargoylism cannot be brought under the general heading of glial insufficiency. Jacob shows that, in macroscopical examinations, the affinity of glial fibres to myelin staining haematoxylin may lead one to mistake the findings for glial-myelinic dissociation. Further problems dealt with are: enzyme patterns of acid phosphatase in various glial structures *(Blank)*, glial and mesenchymal reac-

tions in the radiologically *(Harder)* and traumatically *(Noetzel* and *Gollbach)* injured animal brain, microglial reaction in connection with axonal nerve cell change *(Kreutzberg)*, glial reaction to subarachnoidal injection of iron containing substances *(Blinzinger)*.

In contrast to the utilisation of ultramicroscopic, histochemical and autoradiographic procedures, impregnation methods do not receive enough attention. The whole volume contains only three photographs of slides impregnated with the "spanish" methods. It may be noted that it was *Cajal*'s gold sublimate impregnation which enabled *K. Schaffer* to recognize the astroglial reaction independent of parenchymal lesion (called by *Schaffer* "Eigenerkrankung der Neuroglia").

Except the communications of *Friede* (Cleveland, Ohio) and *Seitelberger* (Vienna) all contributions come from the laboratories of the German Federal Republic. The language of the papers is German, with a preceding summary in English. The international literature is extensively referred to. The papers are richly illustrated with figures of high quality.

Besides being of considerable value to everybody who wishes to gain a general survey of the results and current questions of normal and pathological morphology of glia, this stimulating work invites to the study of special problems.

ST. KÖRNYEY

W. EICKHOFF, C. HERBELHOLD

## Die Lymphbahnen der menschlichen Schilddrüse (Lymph vessels of the human thyroid)

Springer Verlag, Berlin—Heidelberg—New York, 1968. 32 figures. 111 pages. Price DM 39.60

There is a growing interest in lymphology all over the world. The authors emphasize this fact in the Introduction to this volume mentioning that the recent foundation of the International Lymphological Association symbolizes the gain in importance of this branch of science among researchers working in a variety of fields.

This excellently made-up and richly illustrated monograph contains all the important data on the lymph system of the thyroid gland, starting from the historical aspects of the problem to most recent findings. Numerous methods are described and criticized by the authors. The anatomy of the lymph vessels, the thyroid gland, the draining lymph trunks, and the location of the regional lymph nodes are dealt with. Numerous data are included on the physiological, pathophysiological and pathological aspects of the problem.

The book is most valuable to lymphologists, and it will be of interest to endocrinologists, physiologists, pathologists, and surgeons operating on the neck.

M. FÖLDI

P. L. MILLER (Ed.)

## Aspects of Cell Motility

Symposion of the Society for Experimental Biology No. 22
Cambridge University Press, Cambridge, 1968. 370 pages. Price 85 s

The central concern of this new number of the famous series is a search for the common features of all kinds of motile mechanisms encountered in living cells. In the last two decades a spectacular progress has been achieved toward the elucidation of the molecular mechanism of muscle contraction. It is only natural that the search for common aspects of cell motility should be pervaded by ideas and techniques originating in this most successful area. As it can be inferred from the wellcome diversity of themes, the organizers of the symposium were nonetheless quite unbiassed for or against the assumption of a "general contractile mechanism" underlying all cell movements.

Besides the contributions referring to muscle contraction (kept relatively restricted in space) the topics extend through ciliary, flagellary and amoeboid movements, to movements associated with cell division, to movements of nuclei and chloroplasts in the protoplasm, etc.

Regarding the contributions from the angle of possible generalizations it is quite apparent that some pattern modelled after the ATP-fuelled sliding of actin and myosin filaments as found in different types of muscle can be extended in some form or other to several types of cell motility, first of all to those based on permanent structures. There is more than sufficient evidence for the involvement of ATP in energy liberation of cilia and flagellae. A protein called with full right actin (cross-reacting with muscle myosin) can be isolated from motile plasmodia. The flagellary and ciliary microtubules seem to be built up from globular monomers with molecular weight near to this of actin. The monomers can be reversibly reassembled to microtubules in vitro in a way resembling the depolymerization-polymerization cycle of actin. The "arms" seen in cross section pictures of flagellae projecting from the outer sets of microtubules are formed by aggregation of the ATPase protein "dynein" with a molecular weight of 600 000 (myosin, 500 000).

In addition to these systems being now at a stage of detailed biochemical and biophysical analysis, interesting works are included on topics which are studied at present only by classical cytological methods such as stroboscopic filming of inclusions streaming during amoeboid movement, or pictures of mitotic spindles in living dividing cells visualized amazingly with the help of the Nomarsky interference microscope.

The cell movements not based on observable permanent structures cannot be interpreted at present on the grounds of a general mechanism. Nevertheless, the interesting speculations of Pringle going systematically through muscle types of lesser and lesser degree of structural organization, convincingly attempt to bridge the gap between visible and (supposed) invisible filamentous structures generating force. His "minimal model" of force development is based on a change of shape of a complex of two kinds of proteins, one of them being a filament of polarized structure like actin.

The volume is illustrated with figures in the text and about 60 plates, many of them representing masterpieces of modern ultrastructural research.

N. A. Biró

N. I. Lasarev (Ed.)

## Hormone Therapy of Malignant Neoplasms

(In Russian) Medicina, Moscow, 1968. 215 pages with 32 tables and 35 figures

The volume comprises the experimental and clinical work of 20 collaborators; the subject of the investigations was mammary, uterine, ovarian, thyroid and laryngeal cancer. Certain views of the authors will most probably be debated, e.g. the suggested hypothesis for the development of hormone-dependent tumours, the primary role of FSH in the development of mammary cancer, treatment of mastopathy with microdoses of iodine, etc. Undoubtedly, these assumptions have to be confirmed in the future.

Chapter 1 deals with the *theoretical grounds* of the hormone therapy of malignancies. The authors believe that dyshormonal tumours are of a multiple aetiology. Continuous stimulation of proliferative processes over longer periods of time will lead to cancer if the next phase of the physiological cycle fails to ensue, for instance the secretion of milk after a proliferation of the mammary tissue. Hormone treatment is beneficial only in the case of hormone-dependent neoplasms and is based on the inhibition of the hormones which maintain proliferation of the tumour.

Chapter 2 comprises about half of the volume and deals with the hormone therapy of breast cancer. Experimental and clinical observations have led the authors to conclude that among the pituitary hormones it is the FSH which is of prime importance in the genesis of mammary proliferation and cancer. Persisting and cystic follicles in the ovaries indicate overproduction of FSH. The authors emphasize that oestrogens are secreted not only by the ovaries and the adrenals but also by accessory adrenocortical tissue. Hormone therapy has the aim of suppressing oestrogen and/or FSH secretion. Corticosteroid treatment (instead of adrenalectomy) ensured the best therapeutic results prior to the menopause, the administration of androgens immediately before the menopause, and oestrogen therapy 10 years after menopause. Recidives are due to increased production of FSH as a consequence of adaptation to oestrogen treatment, e.g. to the administration of synoestrol. In animal experiments prolactin was found to inhibit (!) the development of mammary neoplasms.

Chapter 3 discusses the hormone therapy of *mastopathy*. In such patients oestrone and oestradiol are not excreted with the urine, and only oestriol can be demonstrated. The disease

is a consequence of disturbances in the ovarian cycle and the formation of cystic follicles. Therapy is aimed at depressing FSH secretion or luteinization of the cystic follicles. Authors succeeded in demonstrating that rats maintained in the state of permanent oestrus by subtotal castration, X-ray irradiation or by keeping them in a room where illumination is constant, administration of 500 $\mu$g of potassium iodide daily results in ovulation and restoration of the normal sexual cycle in 2 weeks. The clinical application of potassium iodide treatment (daily one spoonful of the 0.25% solution) in 6 to 10 intermenstrual periods yielded encouraging results, improvement having been observed in 120 cases of a total of 167.

Chapter 4 deals with the hormone therapy of *cancer of the uterine corpus*, whereas Chapter 5 with the treatment of *ovarian cancer*. The best results in the treatment of uterine tumours were achieved with 17-oxyprogesterone capronate. In neoplastic diseases of the ovaries authors recommend the use of synoestrol in combination with thio-TEPA.

The subject of Chapter 6 is neoplasms of the *thyroid*, while that of Chapter 7 is *laryngeal* tumours. Satisfactory results are said to have been reached in thyroid neoplastic diseases with the administration of thyroidine and tri-iodothyronine; for the management of laryngeal tumours they recommend irradiation treatment and synoestrol.

It is difficult to review in a short space all the experimental and clinical investigations included in this book, and it is even more difficult to criticize and to estimate the value of its contents. Owing to the theoretical and practical importance of the subject, the work will undoubtedly interest many experts.

M. Kurcz

H. Harders (Ed.)

## Advances in Microcirculation. Vol. 1

S. Karger, Basel, 1968, 160 pages, 89 figures, 1 table. Price $ 11.95

This book is the first volume of the most recent series of the well known sets of monographs published by *S. Karger, Ltd.* The reason why the publishers have intended to issue a whole series on microcirculation is, as indicated in the prospectus, that first of all owing to the interdisciplinary character of this branch of medical science pertaining results have been reported in a variety of periodicals and this has obviously hampered the exchange of methodical problems, ideas and views on the subject. The first step of international co-operation has been made by *S. Karger Ltd.* in 1960, with the publication of the Proceedings of the European Microcirculation Conferences held regularly every second year; the whole material of these conferences has since then been published in the periodical Bibliotheca Anatomica. The four volumes so far issued give an almost complete survey of the research in this field and on the results so far obtained. These volumes, however, contain only lectures delivered at conferences and, therefore, the papers presented are short, and neither the methods, nor the results and the problems encountered in the course of experiments could be discussed in so much detail that would have been necessary. This gap in scientific information seems to have been satisfactorily filled by the present series of monographs the next volumes of which will be published at irregular intervals, but in the near future.

The present volume is made up of four independent monographs.

The first one, by P. I. *Brånemark* of Göteborg, Sweden, bears the title "Bone Marrow. Microvascular Structure and Function" (pp. 1—65). This is a detailed survey of the data concerning the anatomy and physiology of circulation of the bone marrow; the author has reviewed a total of 114 papers, of them 5 of his own concerning results obtained with vital microscopic methods. Particular attention is paid to the haemoplastic and haemoclastic activity of bone marrow. A detailed description is given of the vascular topography of medullary vessels, their innervation and blood flow, the structure and function of the endothelium, the pressure in bone marrow, and several problems of haematological pathology in which bone marrow microcirculation may have some role. The text is richly illustrated with microphotographs, original records, and schematic drawings summarizing the results of observations in vivo. According to the author, our present knowledge about bone marrow microcirculation is deficient and in many respects conflicting. At any rate, the view that the bone marrow is a cell suspension included in a rigid bony chamber seems to be incorrect. It appears that the bone marrow islets found in various parts of the skeleton constitute an organizational entity which responds uniformly to various impacts and which has a characteristic and well-defined microvascular system adapted to the highly differentiated function, and which is in a close

morphological and functional entity with the circulation of the bones and joints. The monograph contains valuable data not only for haematologists and physiologists dealing with circulation, but also for clinicians interested in pathological changes of the bones.

The second monograph in the volume is also from Göteborg; the authors are *G. Lundborg* and *P. I. Brånemark*, and the title is "Microvascular Structure and Function of Peripheral Nerves. Vital Microscopic Studies of the Tibial Nerve of the Rabbit" (pp. 66—88). The peripheral nerves possess two integrated but functionally considerably independent microvascular systems. The one is the intrinsic system of the vascular fascicular plexus, and the other is the extrinsic system of the nutritive arteries and epineural plexuses. The peripheral nerves are supplied along their whole length by segmentary nutritive arteries, and the intrinsic and extrinsic vascular systems have close functional connections; in the case of damage to one of the two systems, the other system may replace its function on a considerably long section of the nerve. This is perhaps the reason why the vasa nervorum are so resistant to various injuries: owing to the abundant microcirculation and connections between the two systems, blood supply may be sufficient even after 12 hour ischaemia. Further investigations are necessary to clarify the exact interrelationship between the nervous functions and the changes in the function of the circulatory system of the peripheral nerves. The monograph is illustrated with numerous microphotographs of high quality, and by well arranged schematic diagrams. In the opinion of the present reviewers, this part of the volume is the most interesting and best elaborated monograph in the book, useful for the morphologist as well as the physiologist, pharmacologist, neurologist and neurosurgeon.

The third chapter of the volume was written by N. *Heisig* under the title "Functional Analysis of the Microcirculation in the Exocrine Pancreas" (pp. 89—151). The author, by means of the transparent chamber method modified by himself, investigated the changes occurring in response to secretin and pancreozymin in the terminal circulatory system of the pancreas. In systemic circulation there was no change while the nutritive capillary system of the pancreas displayed an increased blood flow after the administration of the hormones. The monograph is devoted mainly to methodical problems which are discussed in detail. More or less well-known points are treated in too much detail and often not clearly. At any rate, research workers less experienced in the methodical problems of microcirculation will be interested also in this part of the book.

The last part of the volume deals with the biomicroscopic analysis of microangiographic methods *(J. Lundskog, P. I. Brånemark, J. Lindström:* Biomicroscopic Evaluation of Microangiographic Methods, pp. 152—160). During the past years, microangiographic methods have increasingly been employed for the clarification of problems concerning microcirculation. In this work, the authors have attempted to establish whether and to what extent the picture obtained by the microangiographic method corresponds to the structure of the capillary network in vivo. The comparative studies were undertaken on the ear of rabbits and the cheek-pouch of the hamster. The results have shown that the microangiographic technique does not reveal all vascular structures detectable by vital microscopy, and the lumina estimated on the basis of the microangiogram are usually smaller than the true size of the capillaries. When these points are considered, microangiography may provide valuable additional information about the architecture of the microvessels of certain organs. Of particular value seems to be the method of stereomicroangiography. Some recent modifications and the perfection of the technique of microangiography will presumably allow in the near future to observe capillary circulation also in the human.

A. Erdélyi and K. Thuránszky

Cl. Pfister (Ed.)

## Probleme der molekularen und allgemeinen Biologie der Zelle

Verhandlungen der Deutschen Gesellschaft für Experimentelle Medizin, No. 16, Leipzig, 20—22 Mai, 1966. VEB Verlag Volk und Gesundheit, Berlin, 1967, MDN 34.20

Several recent results of up-to-date cytology have formed the subject of this Conference of the German Association of Experimental Medicine; a total of 29 papers was given, in part reviews and in part reports on experimental results. The recent results obtained by electron microscopy and by biochemical as well as physicochemical methods, and the concepts derived from them are surveyed, involving a large field of biological and medical basic sciences. The material consists of four parts, arranged according to subjects.

The first part deals with the general morphology and biochemistry of the cell in 8 papers. The first one reviews the macromolecules, their electron microscopic appearance, the structural basis of the cell. Another paper deals with the ultrastructure of the synapsis and the extracellular compartment of the central nervous system, with special regard to the various types of synaptic junction. The third review surveys the chemical regulation of cellular metabolism including the most recent results of the regulation of enzyme synthesis at the level of the genes. Further papers deal with electron microscopic histochemistry, cell differentiation, and the structure of bacterial DNA.

The second part of the book is devoted to the physiology and pharmacology of the excitation process (3 papers). The following problems of fundamental interest are dealt with: physiology of the excitation process, pharmaco-receptors, and the molecular biology of synaptic transmission. Particularly in the last two papers, the authors succeeded in covering nearly all the important aspects of the discussed problems.

In the third section, 10 papers are devoted to the molecular and general cellular biological problems of muscle cells. Several lectures discussed the ultrastructural changes of cardiac muscle due to shortage in oxygen supply and to increased glycolysis. Other papers discussed the ATP-system of skeletal muscle, the role of the ATP-splitting enzymes, and the effects of various ions on contraction and on membrane potential.

The last section comprises papers devoted to several molecular and cytological problems of liver tissue. Attempts were made in these papers at offering a common interpretation of the ultrastructural and biochemical changes occurring in various pathological conditions. Further papers were devoted to the ribosomal RNA-turnover of regenerating liver cells, the mechanism of ergastoplasm formation, and on the iodine and copper metabolism of liver cells

A. Tigyi

## Polymorphismes Biochimiques des Animaux. X$^{e}$ Congrès Européen sur les Groupes Sanguins et le Polymorphisme Biochimique des Animaux. Paris, 5—8 juillet 1966

Institut National de la Recherche Agronomique.
Imprimerie Firmin-Didot, Paris—Mesnil—Ivry, 1967. 544 pp. Prix: 50 F

The European Society for Blood Group Research organized an international congress in the Institut Pasteur and Institut National de la Recherche Agronomique, Paris, between the 5th and 8th of July, 1966. The present book includes all the lectures delivered at this conference and the discussions attached to them, in English and French. The congress began with 4 main papers, of which the first was read by *A. Robertson* of Edinburgh on the significance of biochemical polymorphism in animal husbandry, emphasizing the importance of studying the genetic variants and the biochemical polymorphism underlying them by revealing the specific sites of the peptide chains on basis of the quantitative estimation of the possible variants; by this means it would be possible successfully to employ the obtained results in the selection program. *R. R. A. Coombs* of Cambridge discussed the serological properties of red blood cells and their practical value in general immunological methods. He analysed the "almost perfect" serological behaviour of the red blood cells, and emphasized by examples the significance of the interrelationship between specific antigenic structure and genetic determination. *M. P. Grabar* of Paris reviewed the immunochemical phenomena related to the antigen-antibody reaction, and their application in studying the structure of macromolecules. Z. *H. J. Huisman* of Georgia surveyed some more recent data concerning the type of haemoglobins occurring in domestic animals.

In addition to these main papers, there are 12 chapters in the book. The first one comprises 12 papers on the blood groups of cattle, the second one 10 papers on swine blood groups, and the third one 13 papers on blood groups of the domestic fowl. Polymorphism of the serum proteins (transferrin, serum amylase, albumin) is the subject of the following two chapters, the fourth one containing 2 papers dealing with this problem in cattle, and the fifth chapter 5 papers devoted to the ovine and canine serum proteins. Chapter VI contains lectures on the blood groups and serum protein variations in the horse, and the next chapter discusses these problems in fishes. Chapter VIII deals with the haemoglobin of various domestic species, and Chapter IX is devoted to the proteins of milk. Of particular interest are the 6 papers of the next chapter dealing with the genetical aspects of various biochemical properties. Thus, for example, *B. A. Rasmusen* and *J. G. Hall* of Edinburgh have concluded from their observations carried out in 820 sheep that in the M system of the red blood cells of sheep the alleles deter-

mining the phenotype also determine the K concentration of the erythrocytes, for the erythrocytes derived from an M negative sheep contain less K than those of an M positive animal. A similar relationship could be revealed in the goat. Chapter XI analyses the blood group chimaerism and histocompatibility in various species (9 papers), while in Chapter XII 3 papers are devoted to the antigenic properties of seminal fluid and sperm. The book is supplemented with a carefully arranged subject index.

The work which presents all the lectures and discussions in full length, deals with a variety of problems. The connection between the subjects discussed in the 12 chapters is somewhat loose, and the reader has to rely upon his previous knowledge when attempting to find such connections. Despite this lack of completeness, which of course could not be the aim of a similar work, it is valuable for everybody interested in the field, for it offers an almost complete survey of the investigations carried out in various laboratories all over the world with the purpose of establishing a connection between immunological methods, blood group research, serum protein analysis, on the one hand, and genetical studies, on the other. Thus, the book is valuable not only for workers dealing with theoretical problems of blood groups and for immunochemists, but also for biochemists interested in the structure of macromolecules, and for physicians and physiologists working in the field of molecular biology; all the important works done in this field up to 1966 are practically included in the book under review.

L. Kesztyüs

## New Aspects of Human Genetics

British Medical Bulletin, 1969. Vol. 25, No. 1. Price £ 2

This issue of British Medical Bulletin contains some very useful information for geneticists, biologists and medical practitioners. It gives an excellent survey of the most important recent advances in human genetics. Each of the sixteen papers is a complete summary of the work done in the given field and describes valuable new experimental results. Seven papers deal with biochemical genetics. H. Harris discusses enzyme and protein polymorphism in human populations. He indicates that there are twenty variants of glucose-6-phosphate dehydrogenase, which are equivalent to twenty alleles in traditional genetics. H. Lehmann and R. W. Carrell report on exceptional types of haemoglobin with structural abnormalities, which are unstable and lead to anaemic states. D. J. Weatherall outlines the structure and genetic control of normal human haemoglobin and the distribution of thalassaemia. The genetics of thalassaemia, which about ten years ago was still thought to be comparatively simple, has in the meantime been shown to be extremely complex. W. R. J. Morgan and W. M. Watkins give an account of the development during the past ten years of the chemistry of the human blood groups A, B, H and related substances. They have shown that the specificity of the large molecules characterizing the blood groups depends on carbohydrate chains, and that the primary protein products which differentiate them must be glycosyltransferase enzymes. G. Dean describes the different forms of porphyria and their treatment.

Two papers deal with aminoacidopathies. C. R. Scriver gives a survey of inborn errors of amino-acid metabolism and summarizes how diseases in the transport-system category are differentiated by recent methods. D. C. Cusworth and C. E. Dent give a detailed account of homocystinuria.

M. A. Carter treats the genetics of common disorders, emphasizing that the genetic element in most such conditions is neither a chromosome abnormality nor a mutant gene but very probably an underlying polygenically determined and continuously distributed genetic predisposition. A valuable criticism of the possible interpretations of familial disposition in multifactorial traits is given by J. H. Edwards. Among the papers on cytogenetics, J. H. Renwick deals with the linkage of genes on the autosomes; W. M. Court-Brown gives an account of human population cytogenetics, which is concerned with the frequency of chrosome aberration, its causation and the selective forces that may act on their carriers.

P. E. Polani reports on the extraordinary variety of autosomal abnormalities. The abnormalities of sex chromosomes are dealt with by P. A. Jacobs. C. E. Ford discusses mosaics and chimeras and shows how to distinguish this special group of phenomena. This issue of the Bulletin closes with a valuable contribution on reciprocal translocations by C. E. Ford and H. M. Clegg.

A. Tigyi

## Bulletin of the Veterinary Institute

Editor-in-chief: HENRIK JANOWSKY, Pulawy, Poland
Vol. 11(3—4): 79—172, 1967

This Bulletin is a periodical published by the well-known Veterinary Institute of Pulawy, Poland, under the leadership of Prof. S. KRAUSS; four issues are published annually. They report on the experimental work conducted in various departments of the Institute. Each issue contains original papers, preliminary reports, and detailed summaries of works which have already been, or are to be published, in other journals.

The Bulletin gives an excellent survey of the up-to-date basic and applied research work conducted in the Institute. Numerous original articles are dealing with Newcastle Virus and Erysipelothrix rhusiopathiae. Of considerable interest are the papers dealing with serological studies concerning pullorosis, and in the field of veterinary physiology those devoted to the analysis of abomasal and reticulo-ruminal secretion and motility of calves.

Of the morphological studies mention should be made of the excellent pathohistological works on hog cholera and Aleuti disease of minks, illustrated with first-rate microphotographs.

The many-sidedness of the Bulletin is shown by the fact that it contains a paper on the diseases of the carp. In addition, information can be gained in part from the preliminary papers and in part from the summaries about important subjects such as radiobiology, artificial insemination, diagnosis of tuberculosis, and E. coli enterotoxaemia, a disease well known by veterinary practitioners to cause substantial losses among piglets and calves. The papers are discussing not only the diagnostic and therapeutic aspects of these diseases, but provide significant data concerning their pathomechanism.

Unfortunately, this well-presented volume which complies with the international requirements does not contain abstracts of the papers. A detailed index containing the title of the cited papers would have been a considerable aid to the reader. The Bulletin is published in English; the translations are satisfactory although halting at some points.

In addition to veterinarians, pathologists, microbiologists and comparative physiologists will surely be interested in this Bulletin.

A. FORRAI

LIESELOTTE GERHARD

## Atlas des Mittel- und Zwischenhirns des Kaninchens
## Atlas of the mesencephalon and diencephalon of the rabbit

Springer-Verlag, Berlin, Heidelberg, New York, 1968. 64 figures, 184 pages. Price $ 44.50

This bilingual atlas represents a continuation of the works of H. MEESSEN and OLSZEWSKI and provides an almost complete survey of the cyto- and myelo-architecture of the rabbit's mesencephalon and diencephalon. Sagittal and horizontal sections, in addition to the frontal planes, are included to aid spatial orientation. The serial sections were stained alternately for myelin and for nerve cells and the plates were magnified 20 : 1, 40 : 1 and in some sections 100 : 1.

A great advantage of this excellent atlas is that the sections are accompanied by a short description of the anatomical structures and a special part deals with the main projections and connections of the brain stem, diencephalon and limbic system.

The last part of the atlas gives a list of references referring to the morphological and neurophysiological data and to stereotaxic measurements of the rabbit brain.

The book is beautifully produced with admirably clear print and excellent photographic technique. It will be an essential reference work for anyone engaged in the study of neurophysiology and neuroanatomy on this species.

E. ENDRŐCZI

*Printed in Hungary*

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Farkas Sándor
A kézirat nyomdába érkezett: 1969. VI. 4. — Terjedelem: 16,75 (A/5) ív, 67 ábra

69.67769 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

INDEX

PHYSIOLOGIA

*Gömöri, P., Hársing, L., Kállay, K., Bartha, J., Debreczeni, L.:* Arterio-Venous Anastomoses in the Kidney. I. Renal Blood Flow Following the Infusion of Serum Albumin ..... 201

*Takács, L., Debreczeni, L., Lulity, I.:* Pressor Activity of Renal Venous Blood in Anaesthetized Dogs ..... 211

*Forgács, I., Châtel, R., Visy Mária:* The Effect of Hypertonic Sodium Chloride Infused into the Renal Artery ..... 219

*Kovách, A. G. B., Bálint Terézia:* Comparative Study on Haemodilution after Haemorrhage in the Pigeon and the Rat ..... 231

*Molnár, L., Czopf, J.:* Études des problèmes de la régulation du débit sanguin cérébral. I. Sur le rôle de l'hypothalamus dans la régulation du débit sanguin du tronc cérébral ..... 245

*Such, Gy., Obál, F.:* Bilateral Interaction of Vagal Vasomotor Reflexes ..... 257

*Ángyán, L., Rózsavölgyi Margit, Kolta, P.:* Stopping Elicited by Conditioned Stimuli as Compared with the Thalamic Induced Arrest Reaction ..... 271

*Avar, Z., Monos, E.:* Biological Role of Lateral Hypothalamic Structures Participating in the Control of Maternal Behaviour in the Rat ..... 285

*Avar, Z., Monos, E.:* Behavioural Changes in Pregnant Rats Following Far-Lateral Hypothalamic Lesions ..... 295

*Monos, E., Koltay Edit, Sulyok, A., Venkey, T., Kovách, A. G. B.:* Effect of Local Venous Congestion in Adrenocortical Function. I. Changes in Adrenal Corticoid Secretion after Stepwise Reduction of Venous Outflow ..... 305

*Sulyok, A., Monos, E., Koltay Edit, Bede, Andrea, Kovách, A. G. B.:* Effect of Local Venous Congestion on Adrenocortical Function. II. Changes in Corticoid Release into Venous Blood and Lymph and in Oxygen Consumption ..... 313

*Monos, E., Biró Zsuzsanna, Sulyok, A., Kovách, A. G. B.:* Effect of Local Venous Congestion on Adrenocortical Function. III. Adrenal Medullary and Cortical Blood Flow ..... 321

*Makara, G. B., Stark, E., Mihály, Katalin:* Corticotrophin Release Induced by Injection of Formalin in Rats with Hemisection of the Spinal Cord ..... 331

*Strashimirov, D., Bohus, B., Kovács, S.:* Pituitary-Thyroid Function of the Rat after Suppression of ACTH Release by Dexamethasone Implants in the Median Eminence ..... 335

*Ismail, A. A., Abdel Hay, A., Madwar, K. R., Talaat, M.:* Hormones in Lymph (E) Capillary Permeability to $^{131}$I-, Risa, and Na$^{131}$I in Nephritis ..... 345

*Kausz, I.:* Melanocyte Stimulating Hormone ..... 351

*Banga Ilona:* The Structure of Protein-Polysaccharides and their Role in the Function of Connective Tissue Fibres ..... 355

*Dauda, Gy., Dévényi, I., Szokoly, V.:* Converting Enzyme Activity in Experimental Cirrhosis of the Rat ..... 365

PHARMACOLOGIA

*Molnár, J., György, L., Unyi, Gabriella, Kenyeres Judit:* Effect of Capsaicin on the Isolated Ileum and Auricle of the Guinea Pig ..... 369

RECENSIONES

*E. R. Caianiello* (Ed.): Neural Networks. Proceedings of the School on Neural Networks. Ravello, June 1967. Springer V. Berlin—Heidelberg—New York, 1968. (K. Lissák) ..... 375

*G. C. Quarton, T. Melnechuk, F. O. Schmitt* (Eds.): The Neurosciences: A Study Program. The Rockefeller University Press, New York, 1967. (K. Lissák) ..... 375

*N. S. Assali* (Ed.): Biology of Gestation. Vol. I. The Maternal Organism. Academic Press, New York and London, 1968. (K. Lissák) ..... 376

*C. von Euler, S. Skoglund, U. Söderberg:* Structure and Function of Inhibitory Neural Mechanism. Wenner-Gren Center International Symposium Series, Vol. 10. Pergamon Press, Oxford, 1968. (K. Lissák) ..... 376

*G. A. Kerkut:* Experiments in Physiology and Biochemistry. Vol. I. Academic Press, London and New York, 1968. (K. Lissák) ..... 377

*C. C. Spicer* (Ed.): Computing in Medicine. British Medical Bulletin. Vol. 24. No. 3, September 1968. Published by the Medical Department, The British Council. London. (K. Lissák) ..... 378

*F. Erblsöh, A. Oksche, F. Seitelberger* (Ed.): Symposium on Neurologia—Symposium concernant la neurologie—Symposium über die Neurologia. Berlin, October 20—22, 1966. Springer V. Berlin—Heidelberg—New York, Springer V. Wien—New York (Acta Neuropathologica, Suppl. IV). (St. Környei) ..... 378

*W. Eickhoff, C. Herbelhold:* Die Lymphbahnen der menschlichen Schilddrüse (Lymph Vessels of the Human Thyroid). Springer V. Berlin—Heidelberg—New York, 1968. (M. Földi) ..... 379

*P. L. Miller* (Ed.): Aspects of Cell Motility. Symposium of the Society for Experimental Biology. No. 22. Cambridge University Press, Cambridge, 1968. (N. A. Biró) 379

*N. I. Lasarev* (Ed.): Hormone Therapy of Malignant Neoplasma. (In Russian). Medicina, Moscow, 1968. (M. Kurcz) ..... 380

*H. Harders* (Ed.): Advances in Microcirculation. Vol. 1. S. Karger, Basel 1968. (A. Erdélyi and K. Thuránszky) ..... 381

*Cl. Pfister* (Ed.): Probleme der molekularen und allgemeinen Biologie der Zelle. Verhandlungen der Deutschen Gesellschaft für Experimentelle Medizin, No. 16. Leipzig, 20—22 Mai, 1966. VEB Verlag Volk und Gesundheit, Berlin, 1967. (A. Tigyi) ..... 382

*Polymorphismes Biochimiques des Animaux.* X$^{e}$ Congrès Européen sur les Groupes Sanguins et le Polymorphisme Biochimique des Animaux, Paris, 5—8 juillet 1966. Institut National de la Recherche Agronomique. Imprimerie Firmin-Didot, Paris—Mesnil—Ivry, 1967. (L. Kesztyüs) ..... 383

*New Aspects of Human Genetics.* British Medical Bulletin. 1969. Vol. 25. No. 1. (A. Tigyi) 384

*H. Janowski* (Ed.): Bulletin of the Veterinary Institute, Pulawy, Poland. Vol. 11 (3—4): 79—172. (A. Forrai) ..... 385

*L. Gerhard:* Atlas des Mittel- und Zwischenhirns des Kaninchens. Atlas of the Mesencephalon and Diencephalon of the Rabbit. Springer V. Berlin—Heidelberg—New York, 1968. (E. Endrőczi) ..... 385

# ITALIAN SPORTS MEDICINE FEDERATION

## *COMPETITION ANNOUNCEMENT*

In the ambit of its statute activities (Art. 2 Comma C.) set out to develop and spread the study, scientific research and the high-level improvement of sports medicine, the ITALIAN SPORTS MEDICINE FEDERATION, announces **a competition in memory of the physiologist M. Mitolo endowed with:**

**A PRIZE FOR LIT. 1,000,000 (one million)**

**awarded to a research work which may bring a valid contribution in the physiological field applied to sports, especially as regards the functional valuation of athletes,**

1) The application for the prize is open to all Medical Graduates and Surgeons, both Italian and foreign. The works must be unpublished and written in Italian, French, English or Spanish.

2) The award will be made by an Adjudicating Committee composed of three members, appointed by the F.M.S.I. The Committee's decision is final.

3) Those persons who intend to compete should forward to the F.M.S.I. – Viale Tiziano, 70 – Roma CAP 00100 – by March 31st, 1970 (postal dated) an application form, in which the candidate should declare, on his own responsibility, that he possesses the required qualifications and that his work is unpublished. The candidates will be asked at a given time, to provide documentary evidence of what has been declared. The application should be presented together with five types copies of the work and an abstract in four languages (Italian, English, French and Spanish).

4) Works by more than one author will be accepted.

5) The Adjudicating Committee reserves the right to refuse the award of the prize if the judgement should be negative, as well as to propose a sub-division of the prize ex-aequo between two or more papers.

For information apply to:

FEDERAZIONE MEDICO-SPORTIVA ITALIANA

Viale Tiziano, 70

00100 ROMA.

*Heinz Karger Prize 1969*

The prize which is awarded every year in memory of Heinz Karger, the wellknown Basle publisher, for an outstanding scientific work has in 1969 been conferred in equal parts to

Edit BEREGI, Budapest

and

Alex COMFORT, London

for their papers on the subject of "Basic Research in Gerontology".

The Heinz Karger Memorial Foundation invites the submission of papers on the following subjects:

1970: An original paper on "COMPUTERS IN MEDICAL DIAGNOSIS"

1971: An original paper on "BIOCHEMICAL ASPECTS OF IMMUNOLOGY"

*Conditions:*

Manuscripts on the subject for 1970 should not exceed 30 typewritten pages and manuscripts on the subject for 1971 should not exceed 20 typewritten pages, including illustrations, tables and bibliography. Manuscripts marked "Competition" must reach the publishers, S. Karger AG, Arnold-Böcklin-Strasse 25, CH-4000 Basle 11 (Switzerland), not later than June 30. The manuscript must be typewritten on one side only, double-spaced, and is to be submitted in duplicate, and in accordance with the instructions contained in „The Manuscript" (Rules for the preparation of manuscripts and bibliographies of scientific papers). This booklet can be obtained free of charge from the publishers if the request is marked "Competition".

Language: English, German or French.

Publication: The winning papers will be published in one of the Karger journals.

The award for the prize will be SFr. 5000.–. The Council of the Foundation will judge the papers and confer the prizes.

# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY
## and Cognate Medical Sciences

Chairman: D. WHITTERIDGE, F. R. S., B. Sc., D. M. (Oxon).
Press Editor: HUGH BEGBIE, M. A., Ph. D., M. B., Ch. B.

**Publication Dates: January, April, July, October**
**Annual Subscription: £6**
**Single Number: £1. 10s**

The *Quarterly Journal of Experimental Physiology* has always specialised in the publication of original articles with high-grade half-tone illustrations both in colour and black and white. All aspects of human and animal physiology are covered and the journal is essential for anyone wishing to keep abreast of current research in this field.

**Published by**
**E & S LIVINGSTONE LTD**

**15—17 TEVIOT PLACE**
**EDINBURGH**

## K. Tóth

# THE EPIDEMIOLOGY OF DENTAL CARIES IN HUNGARY

*In English · Approx. 320 pages · 17×25cm · Cloth*

This volume, which is unique in its field, is aimed at presenting a comprehensive survey of the history of dental caries to prehistoric ages. Results of seventeen years' investigations by the author are included. Connections between caries incidence and nutriton, pregnancy as well as the state of the central nervous and endocrine systems are dealt with, and a number of practical conclusions are drawn. The reader will find the list of the complete bibliography of the subject at the end of the book. The description of the methods of research and classification is followed by a chapter based on archaeological and palaeodontological findings tracing back the history of dental caries to prehistoric ages.

## M. Wollemann

# MÉTABOLISME DES MÉDIATEURS CHIMIQUES DU SYSTÈME NERVEUX À L'ÉTAT PHYSIOLOGIQUE ET PATHOLOGIQUE

*In French · Approx. 176 pages · 17×25cm · Cloth*

The work elucidates the metabolism of the mediators under physiological and pathological conditions in the light of the latest achievements in the fields of biochemistry, neurochemistry, physiology, pharmacology, neuropathology and histochemistry. Contrary to works published earlier, the complex enzymes and proteins participating in the synthesis and decomposition of the mediators as well as in their fixation and storage are described in full. The action of the known drugs of psychopharmacology is explained on molecular level. Apart from a complete bibliography and critical analysis of the relevant scientific literature, the experimental research work of the author completed in the last 20 years is also treated in detail.

Joint edition with **Masson et Co., Paris**

AKADÉMIAI KIADÓ
PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES BUDAPEST

ACTA PHYSIOLOGICA

ТОМ 35 — ВЫП. 3—4

# РЕЗЮМЕ

## ФУНКЦИОНАЛЬНОЕ ИССЛЕДОВАНИЕ АРТЕРИО-ВЕНОЗНЫХ АНАСТОМОЗОВ ПОЧЕК
## I. ИЗУЧЕНИЕ ВНУТРИПОЧЕЧНОГО КРОВООБРАЩЕНИЯ ПОСЛЕ ВВЕДЕНИЯ СЫВОРОТОЧНОГО АЛЬБУМИНА

П. ГЕМЕРИ, Л. ХАРШИНГ, К. КАЛЛАИ, Й. БАРТА
и Л. ДЕБРЕЦЕНИ

В прежних работах авторы уже неоднократно сообщали о своих коррозионных исследованиях, в которых с помощью морфологических методов удалось выявить артериовенозные анастомозы почек при различных шоковых состояниях. Настоящие эксперименты они проводили с целью получить функциональное доказательство о существовании артерио-венозных анастомозов. Авторы исходили из того наблюдения, согласно которому на основе собственных, а также литературных данных после введения сывороточного альбумина в почках понижается выделение ПАГК, что может быть и последствием открытия шунтов. Для измерения полного кровообращения почек они применяли электромагнитный измеритель кровотока, а для определения питательного кровообращения почек — метод вытяжки $^{86}Rb$ по Сапирштейну, модифицированный Харшинг и Пеллеи. Ради простоты они называют разницу этих двух величин «инертной» циркуляцией. В ходе экспериментов выяснилось, что в то время, как у контрольных собак «инертная» циркуляция составляет 5%, то после введения сывороточного альбумина эта величина повышается до 28%, тогда же медуллярное кровообращение не изменяется. Все эти функциональные исследования делают вероятным предположение об открытии артериовенозных анастомозов в почках.

## ПРЕССОРНАЯ АКТИВНОСТЬ ВЕНОЗНОЙ КРОВИ ПОЧЕК

Л. ТАКАЧ, Л. ДЕБРЕЦЕНИ и И. ЛУЛИТИ

В острых опытах на собаках было установлено, что после понижения кровотока в почках (до около 20% контрольной величины) существенно повышается прессорная активность нативной венозной плазмы почек. Причиной этого является повышение количества прессорных веществ, а не изменение гемодинамики (концентрация).

## ДЕЙСТВИЕ ВЛИВАНИЯ ГИПЕРТОНИЧЕСКОГО РАСТВОРА NaCl В ПОЧЕЧНУЮ АРТЕРИЮ НА ФУНКЦИЮ ПОЧЕК

И. ФОРГАЧ, Р. ШАТЕЛЬ и М. ВИШИ

У собак, предварительно содержанных на бедной солями диете, вливание в почечную артерию гипертонического раствора NaCl, повышающее количество фильтруемых $Na^+$ на 50%, вызывает очень значительное понижение RBF и GFR. Если животные получают до эксперимента богатую солями диету, то вливание такого же количества NaCl не вызывает изменения RBF и GFR. Как у животных, получавших предварительно бедный солями режим питания, так и у собак, содержавшихся на богатом солями режиме питания, под влиянием инфузии раствора NaCl многократно повышается выделение, выраженное в процентах фильтруемого Na. Это указывает на уменьшение канальцевой реабсорбции или на увеличение внутриканальцевой концентрации натрия.

Полагается, что ответственным за вызывающее почечную вазоконструкцию действие вливания гипертонического раствора NaCl в почечную артерию, является освобождение ренина, происходящее в результате повышения концентрации натрия в канальцах.

## РАЗЖИЖЕНИЕ КРОВИ ПОСЛЕ ГЕМОРРАГИИ У ГОЛУБЯ И КРЫСЫ

А. Г. Б. КОВАЧ, Т. БАЛИНТ

У 29 крыс и 27 голубей авторы проводили под наркозом стандартизированное на вес тела обескровливание и установили по времени степень разжижения крови на основе гемоглобина, гематокрита и числа эритроцитов; они определили также содержание общего белка, альбумина, глобулина и калия в плазме.

В случае быстрого обескровливания (в течение 10 мин) в отношении разжижения крови между голубями и крысами не было найдено различия.

При медленном обескровливании (в течение 60 мин) наблюдалось очень характерное отклонение, развивающееся через первые 15 мин. В то время как вначале разжижение крови происходит у обоих видов животных с приблизительно одинаковой скоростью, то через 15—20 минут после обескровливания крысы не показывают дальнейшего разжижения, тогда как у голубей оно продолжается еще с неизменной интенсивностью. Для гемодилюции используется бедная белками изотоническая жидкость.

Значительная разница выносливости в отношении потери крови, установленная раньше у летущих и плавающих птиц, объясняется с более эффективной гомеостатической емкостью, достигаемой за счет более интенсивного разжижения крови.

## РОЛЬ ГИПОТАЛАМУСА В РЕГУЛИРОВАНИИ КРОВООБРАЩЕНИЯ СТВОЛА МОЗГА

Л. МОЛЬНАР и Й. ЦОПФ

У наркотизированных собак электрическое раздражение гипоталамуса вызывает изменение понтобульбарного кровотока лишь в том случае, если этим же раздражением в существенной мере изменяется и общее кровообращение и дыхание. Между изменением кровотока, электрической активностью и изменением дыхания нет закономерной связи.

Электрическое раздражение гипоталамуса может вызвать как уменьшение, так и повышение сосудорасширяющего действия $CO_2$, оказанного на сосуды ствола мозга.

Деятельность гипоталамуса имеет большое значение в обеспечении равновесия неокортико-бульбарной связи.

## БИЛАТЕРАЛЬНОЕ ВЗАИМОДЕЙСТВИЕ СОСУДОДВИГАТЕЛЬНЫХ РЕФЛЕКСОВ, ПРОИСХОДЯЩИХ ИЗ БЛУЖДАЮЩИХ НЕРВОВ

Д. ШУХ и Ф. ОБАЛ

На кошках, в наркозе хлоралозом, проводилось сравнение изменений кровяного давления, полученных сепарированным и симультанным раздражением центральных культей блуждающих нервов, при одинаковых параметрах раздражения. Было установлено, что

а) при идентичных и гомогенных депрессорных или прессорных рефлекторных процессах билатеральное взаимодействие может проявляться и в форме окклюзии и облегчения;

б) унилатеральное раздражение активирует лишь определенную фракцию билатеральной рефлекторной способности;

в) двусторонние рефлекторные процессы одинакового направления действуют в качестве синергистов;

г) взаимодействие негомогенных рефлексов одинакового направления может вызвать и торможение;

д) так называемый «билатеральный антагонизм» представляет собой не антагонизма симметричных рефлекторных центров. В таких случаях два блуждающих нерва активируют неаналогичные механизмы и двустороннее взаимодействие в общем вызывает алгебраическое суммирование, хотя иногда наблюдается и облегчение.

## СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ОСТАНОВКИ, ВЫЗЫВАЕМОЙ УСЛОВНЫМИ ЗВУКОВЫМИ РАЗДРАЖЕНИЯМИ И РЕАКЦИИ ОСТАНОВКИ, ВЫЗЫВАЕМОЙ РАЗДРАЖЕНИЕМ ТАЛАМУСА

Л. АНДЬЯН, М. РОЖАВЕЛЬДИ и П. КОЛТА

Одновременным или последовательным применением оборонительных и пищевых условных звуковых раздражений регулярно удалось вызывать поведение, напоминающее реакции остановки, вызываемой электрическим раздражением ядер в средней линии таламуса. Появление и продолжительность неподвижности также показывали тесную корреляцию с ответной силой условных рефлексов, для измерения которой использовалась продолжительность скрытого периода. Незначительная разница в ответной силе столкнувшихся друг с другом условных рефлексов способствовала появлению неподвижности меньшей продолжительности. При большей разнице вероятность появления неподвижности меньше, однако, если она появляется, то ее продолжительность больше.

В неокортикальных и гиппокампальных сопутствующих явлениях также была найдена аналогия, поскольку как реакция остановки, вызываемая раздражением таламуса, так и неподвижность, вызываемая условными звуковыми раздражениями, сопровождались регулярно прерванной медленными волнами, или суперпонированной на последние десинхронизованной активностью.

На основе этих результатов обсуждается возможный механизм неподвижности и реакции остановки. Авторы полагают, что различные типы прекращения самопроизвольной двигательной активности можно рассматривать как манифестации центрального возбуждения различной интенсивности, вызывающего несовместимые двигательные тенденции.

## АНАЛИЗ БИОЛОГИЧЕСКОЙ РОЛИ ЛАТЕРАЛЬНЫХ СТРУКТУР ГИПОТАЛАМУСА, УЧАСТВУЮЩИХ В РЕГУЛИРОВАНИИ МАТЕРИНСКОГО ПОВЕДЕНИЯ У КРЫСЫ

(Изучение подвижности, эксплорационного поведения, лактации и действия уменьшения питания)

З. АВАР и Е. МОНОШ

После повреждения парафорникальных структур гипоталамуса, препятствующего развитию материнского поведения и вызывающего уменьшение жизнеспособности новорожденных детенышей, двигательная активность и эксплорационное поведение животных не показывает изменения. Повреждение указанных структур вызывает временное (в течение нескольких дней) уменьшение потребления пищи и воды беременными крысами. На механизм лактации эти структуры, по-видимому, не оказывают непосредственного действия. Последствия временного уменьшения снабжения пищей и водой у здоровых беременных крыс в существенной мере отличаются от последствий повреждения парафорникальных структур гипоталамуса.

## ПОВЕДЕНИЕ БЕРЕМЕННЫХ КРЫС И СУДЬБА БЕРЕМЕННОСТИ ПОСЛЕ ПОВРЕЖДЕНИЯ ОТДАЛЕННЫХ ЛАТЕРАЛЬНЫХ СТРУКТУР ГИПОТАЛАМУСА

З. АВАР и Е. МОНОШ

Действие повреждения отдаленных латеральных структур гипоталамуса, нанесенного на 16—17 день беременности, а также последствия лишения животных пищи, изучались у беременных крыс. После вмешательства наблюдалась длительная афагия и гиподипсия, двигательная активность животных значительно понижалась, и они не показали никакой эксплорационной активности. Часть беременных крыс в период около рождения попадала в терминальное состояние и большинство плодов погибло внутриутробно. У животных, заставленных голодать в подобный период, ни в одном случае не

наблюдалось внутриутробное умирание плодов. У беременных крыс с повреждением гипоталамуса уменьшение веса тела проходило быстрее, чем у животных голодающей группы. В обоих группах была установлена гипертрофия надпочечников, которая однако отсутствовала у животных контрольной группы с ложной операцией.

Последствия повреждения отдаленных латеральных структур гипоталамуса были сопоставлены с последствиями повреждения парафорникальных структур, описанного в прежней статье.

## ДЕЙСТВИЕ МЕСТНОГО ВЕНОЗНОГО ЗАСТОЯ НА КОРУ НАДПОЧЕЧНИКОВ I. ИЗМЕНЕНИЯ СЕКРЕЦИИ КОРТИКОИДОВ НАДПОЧЕЧНИКАМИ ПОСЛЕ ПОСТЕПЕННОГО ПОНИЖЕНИЯ ВЕНОЗНОГО ИСТЕЧЕНИЯ

Э. МОНОШ, Э. КОЛТАИ, А. ШУЙОК, Т. ВЕНГЕИ и А. Г. Б. КОВАЧ

В острых опытах на собаках венозный застой вызывает значительное понижение секреции гликокортикоидов надпочечниками лишь в том случае, если кровоток органа понижается на более чем 30%. Кора надпочечников сохраняет по меньшей мере 40—50%-ную функциональную способность даже тогда, когда застой продолжается в течение 2—3 часов, и за это время постепенно повышается в такой мере, что венозное истечение составляет лишь 25% контрольной величины. После прекращения сужения канюли секреция гормона возвращается к норме в течение 60 минут, тогда как кровоток надпочечников остается ниже контрольных величин.

## ДЕЙСТВИЕ МЕСТНОГО ВЕНОЗНОГО ЗАСТОЯ НА КОРУ НАДПОЧЕЧНИКОВ II. ВЫДЕЛЕНИЕ КОРТИКОИДОВ ЧЕРЕЗ ВЕНЫ И ЛИМФАТИЧЕСКИЕ ПУТИ И ИЗМЕНЕНИЯ ПОТРЕБЛЕНИЯ $O_2$ НАДПОЧЕЧНИКАМИ В СВЯЗИ СО ЗНАЧИТЕЛЬНЫМ ЗАСТОЕМ

А. ШУЙОК, Э. МОНОШ, Э. КОЛТАИ, А. БЕДЕ и А. Г. Б. КОВАЧ

В соответствии со своими прежними результатами авторы устанавливают, что кора надпочечников сравнительно устойчива в отношении 1—2-часового местного застоя: при понижении кровотока до 25% контрольной величины и при венозном давлении примерно в 50—60 мм рт. ст., кора надпочечников способна к около 50%-ой функции.

Потребление $O_2$ понижается во время застоя в такой же мере, как и выделение кортикоидов. Контрольная величина разности артерио-венозного кислородного насыщения в надпочечниках была лишь примерно 8%; в течение значительного застоя эта величина повысилась, однако она не превысила одну треть артерио-венозной разности в конечностях при покое.

Понижение венозного оттока надпочечников привело к повышению выделения кортикоидов через грудной проток. Однако, это лишнее выделение составляет лишь небольшую долю понижения секреции гормонов через крестцово-надпочечниковую вену. Концентрация кортикоидов в артериальной плазме не показывает достоверных изменений.

Во время застоя дача большой дозы экзогенного АКТГ не изменяет выделение кортикоидов и потребление $O_2$. После суживания канлюли функция коры надпочечников в пределах 60 минут возвратилась к норме.

## ДЕЙСТВИЕ МЕСТНОГО ВЕНОЗНОГО ЗАСТОЯ НА КОРУ НАДПОЧЕЧНИКОВ III. ИЗУЧЕНИЕ КРОВОСНАБЖЕНИЯ КОРЫ И МОЗГОВОГО СЛОЯ НАДПОЧЕЧНИКОВ

Э. МОНОШ, Ж. БИРО, А. ШУЙОК и А. Г. Б. КОВАЧ

Авторы изучали местное кровообращение в коре и мозговом слое надпочечников у собак с помощью метода вымывания $H_2$, в основных условиях, а также в остром опыте в связи со значительным местным застоем. Они установили непосредственный венозный отток и венозное давление, а также потребление $O_2$ в надпочечниках.

В коре и мозговом слое эффективный кровоток был в среднем 0,84 или 0,92 мл на мин. на г, в то время как величина общего кровотока, на основе измерения непосредственного оттока, составила 4,21 мл на мин. на г. Под влиянием застоя, вызванного сужением венозной канюли, местное кровообращение коры и мозгового слоя надпочечников понизилось в существенно меньшей мере, чем венозный отток. После 1—1,5-часового застоя кровоснабжение коры и мозгового слоя совершенно нормализовалось.

Изменения венозного давления надпочечников и потребления $O_2$ надпочечниками соответствуют результатам прежней работы авторов.

Дается подробное обсуждение надежности величин кровотока в коре и мозговом слое надпочечников. Можно предполагать, что до сих пор кровообращение в мозговом лое было недооценено. .

## ВЫДЕЛЕНИЕ КОРТИКОТРОПИНА, ВЫЗВАННОЕ ВВЕДЕНИЕМ ФОРМАЛИНА, ПОСЛЕ ОДНОСТОРОННЕГО ПЕРЕСЕЧЕНИЯ СПИННОГО МОЗГА

Г. Б. МАКАРА, Э. ШТАРК и К. МИХАЙ

Изучались афферентные спинномозговые пути выделения кортикотропина (АКТГ) у крыс. После одностороннего торакального пересечения спинного мозга, введение 1%-ого формалина в контралатеральную заднюю конечность достоверно менее повышает уровень кортикостерона в плазме, чем введение формалина в гомолатеральную заднюю конечность или его введение над уровнем пересечения. Большая часть волокон, передающих ноцицептивные импульсы, стимулирующие секрецию АКТГ, перекрещаясь идет вверх в контралатеральной половине спинного мозга. Лишь незначительная часть волокон идет гомолатерально.

## СИСТЕМА ГИПОФИЗ-ЩИТОВИДНАЯ ЖЕЛЕЗА У КРЫС ПОСЛЕ ТОРМОЖЕНИЯ ВЫДЕЛЕНИЯ АКТГ ДЕКСАМЕТАЗОНОМ, ИМПЛАНТИРОВАННЫМ В ОБЛАСТЬ МЕДИАЛЬНОГО ВОЗВЫШЕНИЯ

Д. СТРАШИМИРОВ, Б. БОХУШ, Ш. КОВАЧ

У крыс было исследовано влияние имплантации дексаметазона в область медиальной эминенции на гипофизарно-щитовидную и надпочечниковую функции. Имплантация дексаметазона привела к значительному торможению вызванной стрессом секреции АКТГ, которое длилось 12 дней. Определение поглощения радиойода щитовидной железы, проведенное 2,9 и 12 дней после имплантации не показало существенных изменений. Реакция щитовидной железы на холодовое раздражение, судя по поглощении радиойода, не менялась под влиянием имплантированного дексаметазона. Скорость выделения меченого йода из щитовидной железы как в покое, так и в условиях торможения тироксином или стимуляции метилтиоурацилом тоже не изменилась после имплатации дексаметазона. Далее, имплантация дексаметазона не оказала действия на компенсаторную гипертрофию щитовидной железы после односторонной тиреоидэктомии.

На основании этих результатов можно предполагать, что длительное торможение секреции АКТГ дексаметазоном имплантированним в область медиального возвышения не влияет на гипоталамо-гипофизарные механизмы, участвующие в регуляции щитовидной железы.

## ГОРМОНЫ В ЛИМФЕ. ПРОНИЦАЕМОСТЬ КАПИЛЛЯРНЫХ СОСУДОВ ДЛЯ ТИРОКСИНА С $J^{131}$, RISA И $NaJ^{131}$ ПРИ НЕФРИТЕ

В отечной жидкости у больных с нефритом изучалось появление внутривенно введенного тироксина с $J^{131}$, RISA и $NaJ^{131}$. В крови и в отечной жидкости проводились определения радиоактивности и концентрации белков.

З отечной жидкости отдельные вещества появляются в зависимости от величины молекул в различном порядке: вначале появляется $NaJ^{131}$ а позже всего — RISA. В отечной жидкости содержится гораздо меньше тироксина с $J^{131}$ и RISA, чем в крови. Концентрация белков в плазме на 13,8 раз больше, чем в отечной жидкости. Полученные данные указывают на значительный градиент, существующий между плазмой и внеклеточной жидкостью.

## ИССЛЕДОВАНИЕ ДЕЙСТВИЯ ГОРМОНА, СТИМУЛИРУЮЩЕГО МЕЛАНОЦИТЫ

И. КАУС

Автор изучает действие гормона, стимулирующего меланоциты, присутствующего в гипофизе карпа, исследуя также оптимальный срок для его применения. Он ищет также ответ и дает ответ на вопрос, равноценны ли лиофилизованные и замороженные гипофизы с нативными, и обсуждает внедренную в ихтиологию «гипофизацию» при подробном описании методики.

Между нативными, замороженными и лиофизированными гипофизами в отношении терапевтического действия нельзя выявить различия. Наиболее благоприятные результаты получаются так называемым «гипофизированным веществом», если гипофизация производится в период от апреля до июня. Поэтому автор рекомендует провести трансплантацию в эти месяцы.

## ПОСТРОЕНИЕ ПРОТЕИНПОЛИСАХАРИДОВ И ИХ РОЛЬ В ФУНКЦИИ СОЕДИНИТЕЛЬНОТКАННЫХ ВОЛОКОН

И. БАНГА

Из хряща носовой перегородки, с помощью 40%-ого раствора KJ, наряду с известными фракциями PP—L (proteinpolysaccharide-light) и PP—H (proteinpolysaccharide-heavy) удалось получить и такую фракцию мукополисахаридов, белковый компонент которой отличается от белкового компонента первых двух фракций. Авторы называют эту фракцию cartilage mucoprotein$_1$ (CMP$_1$), так как она по всей вероятности тождественна с мукоидом$_1$, освобождающимся из коллагенных волокон при тепловой и химической контракции. Кроме хряща носовой перегородки из волокнистой хрящевой ткани ахиллового сухожилия также удалось получить фракцию PP—L и фракцию, соответствующую CMP$_1$, которую в целях диференциации авторы называют Achilles tendon-mucoid$_1$ [AT mucoid$_1$]. Эти фракции PP, CMP$_1$ и AT-mucoid$_1$ изменяют физические свойства химически-сокращенных волокон и в присутствии 40%-ого раствора KJ они у животных всех возрастных групп повышают степень химического расслабления, вызываемого при температуре 20° C. Из этого можно заключить, что различные протеинполисахариды входят с сокращенными волокнами, в функции которых они участвуют, в химическую связь.

## АКТИВНОСТЬ ФЕРМЕНТА «CONVERTING ENZYM» У КРЫС С ЦИРРОЗОМ ПЕЧЕНИ

Д. ДАУДА, И. ДЕВЕНЬИ и С. СОКОЛИ

Авторы инкубировали препараты ренина и ангиотенсиногена крови, взятой у крыс с циррозом печени, вызванном введением $CCl_4$ и изучали действие продукта реакции (ангиотенсина) на подопытных животных с циррозом печени. На этой экспериментальной модели они изучали вопрос о том, вызывает ли недостаток фермента «converting enzym» цирроз печени. Образование ангиотенсина II прессорного действия наблюдалось и у животных с циррозом печени. Его количество меньше, чем прессорное действие, определяемое у нормальных подопытных животных, однако это можно приписать повышенному уровню ангиотенсиназы в плазме животных с циррозом печени. Следовательно фермент «converting enzym» не отсутствует и не является уменьшенным у животных с циррозом печени.

# ДЕЙСТВИЕ КАПСАИЦИНА В ПРЕПАРАТАХ ИЗОЛИРОВАННОЙ ТОНКОЙ КИШКИ И СЕРДЕЧНОГО УШКА МОРСКОЙ СВИНКИ

Й. МОЛЬНАР, Л. ДЬЁРДЬ, Г. УНЬИ и Ю. КЕНЕРЕШ

При концентрации в $5.10^{-8} - 10^{-7}$ г на мл, капсаицин вызывает сокращение тонкой кишки и оказывает положительное инотропное и хронотропное действие на предсердие. На обеих препаратах в отношении капсаицина развивалась скоро проявляющаяся, прочная и сильно специфическая тахифилаксия. В отношении изученных веществ [ацетилхолин, гистамин, серотонин, никотин, ДМРР, брадикинин, KCl и $BaCl_2$] перекрестной тахифилаксии не было отмечено. На препарате тонкой кишки специфические антагонисты капсаицина (атропин, дифенгидрамин ,метизергид, гексаметоний) не препятствовали сокращению, в то время как папаверин, карамифен и цинхокаин оказались эффективными антагонистами капсаицина. На препарате сердечного ушка пропранолол и кокаин не влияли на положительное инотропное и хронотропное действие капсаицина.

The *Acta Physiologica* publish papers on experimental medical science in English, French German or Russian.

The *Acta Physiologica* appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the *Acta Physiologica* is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. — Account No. 43-790-057-181) or with representatives abroad.

---

Les *Acta Physiologica* paraissent en français, anglais, allemand et russe et publient des mémoires du domaine des sciences médico-expérimentales.

Les *Acta Physiologica* sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à la rédaction à l'adresse suivante:

*Acta Physiologica, Pécs, Rákóczi út 80.*

Toute correspondance doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forint par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. — Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

---

«*Acta Physiologica*» публикуют трактаты из области экспериментальной медицинской науки на русском, немецком, английском и французском языках.

«*Acta Physiologica*» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации рукописи следует направлять по адресу:

*Acta Physiologica, Pécs, Rákóczi út 80.*

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «*Acta Physiologica*» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «*Kultúra*» (Budapest I., Fő utca 32. — Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

## Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA

Ndermarja Shtetnore e Botimev
*Tirana*

AUSTRALIA

A. Keesing
Box 4886, GPO
*Sydney*

AUSTRIA

Globus Buchvertrieb
Salzgries 16
*Wien I*

BELGIUM

Office International de Librairie
30, Avenue Marnix
*Bruxelles 5*

Du Monde Entier
5, Place St. Jean
*Bruxelles*

BULGARIA

Raznoiznos
1, Tzar Assen
*Sofia*

CANADA

Pannonia Books
2, Spadina Road
*Toronto 4, Ont.*

CHINA

Waiwen Shudian
*Peking*
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Směčkách 30
*Praha 2*

Poštová Novinová Služba
Dovoz tisku
Vinohradská 46
*Praha 2*

Mad'arská Kultura
Václavské nám. 2
*Praha I*

Poštová Novinová Služba
Dovoz tlače
Leningradská 14
*Bratislava*

DENMARK

Ejnar Munksgaard
Nörregade 6
*Copenhagen*

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
*Helsinki*

FRANCE

Office International de Documentation et Librairie
48, rue Gay-Lussac
*Paris 5*

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
*Leipzig 701*

Zeitungsvertriebsamt
Fruchtstrasse 3—4
*1004 Berlin*

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
*7 Stuttgart S.*

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
*Wellingborough. Northants.*

Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
*Oxford*

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
*Amsterdam C.*

Martinus Nijhof
Lange Voorhout 9
*The Hague*

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP
GPO Post Box 1374
*Bombay I*

ITALY

Santo Vanasia
Via M. Macchi 71
*Milano*

Libreria Commissionaria Sansoni
Via La Marmora 45
*Firenze*

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
*Tokyo*

Maruzen and Co. Ltd.
P. O. Box 605
*Tokyo-Central*

Far Eastern Booksellers
Kanda P. O. Box 72
*Tokyo*

KOREA

Chulpanmul
*Phenjan*

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
*Oslo*

POLAND

RUCH
ul. Wronia 23
*Warszawa*

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
*Bucureşti*

SOVIET UNION

Mezhdunarodnaya Kniga
*Moscow G—200*

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
*Stockholm*

USA

Stechert Hafner Inc.
31, East 10th Street
*New York, N. Y. 10003*

Walter J. Johnson
111, Fifth Avenue
*New York, N. Y. 10003*

VIETNAM

Xunhasaba
19, Tran Quoc Toan
*Hanoi*

YUGOSLAVIA

Forum
Vojvode Mišića broj 1
*Novi Sad*

Jugoslovenska Knjiga
Terazije 27
*Beograd*