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

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Tomus XXXVIII

INDEX

PHYSIOLOGIA

Balázsi I : Insulin Uptake by Isolated Rat Fat Cells 51

Banga Ilona, Mayláth-Palágyi Jolanda: Cytochrome Oxidase Determination Using a Manganese Citrate Complex 117

Barankay T., Jancsó T., Nagy S., Petri G.: Cardiac Output Estimation by a Thermodilution Method Involving Intravascular Heating and Thermistor Recording 167

Bartha J., Szőcs Éva: ^{24}Na Uptake and PAH Accumulation of Renal Cortical Slices in the Presence of Angiotensin 151

Bohus B.: The Medial Thalamus and the Opposite Effect of Corticosteroids and Adrenocorticotrophic Hormone on Avoidance Extinction in the Rat 217

Bohus B., Grubits J., Kovács G., Lissák K.: Effect of Corticosteroids on Passive Avoidance Behaviour of Rats 381

Fazekas I. Gy., Fazekas A. T.: Seasonal Variations of the Corticosteroid Fractions in Guinea Pig Tissue and Organs 33

Fenyvesi T., Kállay K.: The Presence of β-Adrenergic Receptors in the Renal Vascular Bed 159

Fischer E., Varga F.: Hepatic Transport of Iodipamide in Various Animal Species 135

Gesztelyi I., Kovács L.: New Method for the Electrophysiological Analysis of the Separated Neural and Aneural Parts of Frog Sartorius Muscle 343

Hantos Z., Lázár Z.: The Flow of Fluid through the Wall of Capillary Systems Studied by a Mathematical Model 265

Heiner L., Domonkos J., Vargha M. jr.: Effect of Denervation on the Composition of Phospholipids in Tonic and Tetanic Muscles 317

Kállay K., Debreczeni L. A.: Effect of Human Serum Albumin on Renal Blood Flow Autoregulation in the Anaesthetized Dog 9

Kenedi I., Dávid G.: Increased Sympathetic Innervation of the Rabbit Heart. Explanation of the Lability of the ECG in the Rabbit 175

Kopa J., Poór Gy., Molnár L.: Études des problèmes de la régulation du débit sanguin cérébral. IV. Le rôle les nerfs pneumogastriques et sympathiques cervicaux dans la régulation du débit cérébral 247

Kovách A. G. B., Monos E., Koltay E., Desrius A.: Effect of Hypothalamic Stimulation on Adrenal Blood Flow and Glycocorticoid Release prior to and after Acute Hypophysectomy 205

Kovács T., Szabó B., Vissy Ágnes: Some Effects of Hypertonic NaCl Solution on the Mechanical and Electrical Responses of Slow and Fast Muscles of the Cat 183

Lázár G.: Species Specificity of Fibrinogen 1

Makara G. B., Stark E., Mihály Katalin: Corticotrophin Release Induced by Traumatic Stress in Rats with Unilateral Spinal Cord Lesion 199

Makara G.B.: Superficial and Deep Chemonociception: Differential Inhibition by Pretreatment with Capsaicin 393

Мархсина И., Оранский И.: Географическое изучение соотношения церебральной гемодинамики с периферическим кровотоком у людей, занятых умственным трудом 67

Nagy I., Kurcz M., Halmy L., Mosonyi L., Baranyai P., Kiss Cs.: Effect of Stress, Drugs and Hypothalamic Extract on Anterior Pituitary Somatotropin Content in the Rat 357

Nagy I., Kurcz M., Kiss Cs., Baranyai P., Mosonyi L., Halmy L.: The Effect of Suckling, Stress and Drugs on Pituitary Prolactin Content in the Rat 371

Nyakas C., Endrőczi E.: Olfaction Guided Approaching Behaviour of Infantile Rats to the Mother in Maze Box 59

Selmeci L., Pósch E., Balogh E.: Effect of Tourniquet on Total Lactate Dehydrogenase (LDH) Activity and Isoenzyme Pattern of Rat Serum 125

Shkhinek E. K., Nyakas C., Endrőczi E.: Frequency-Specific Response of the Pituitary Adrenocortical System on Pontine Stimulation 51

Stark E., Makara G.B.,'Palkovits M., Mihály Katalin: Afferent Pathways of Stressful Stimuli: their Dependence on Strength and the Time Elapsed after the Onset of Stimulation 43

Stark E., Varga B., Medzihradszky K., Bajusz S., Hajtman B.: Relationship between the Structure and the Adrenal and Extra-Adrenal Effects of ACTH Fragments 193

Schmidt P., Andik I.: Relationship between Water and Food Intake in the Rat ... 291

Szilágyi G.: Effect of Acidification and Alkalinisation on Calcium and Citrate Metabolism in Normal and Parathyroidectomized Dogs 281

Varga F., Fischer E.: Biliary Secretion of Rose Bengal in the Dog 143

Varga E., Kovács L., Gesztelyi I.: Analysis of the Depolarizing Effect of Nicotine on Frog's Skeletal Muscle 325

Várnai Ilona, Farkas Mária, Donhoffer Sz.: Thermoregulatory Effects of Hypoxia in New-Born Rats and their Correlation to Body Temperature and Heat Production Prior to and after Exposure to Hypoxia 19

Várnai Ilona, Farkas Mária, Donhoffer Sz.: Thermoregulatory Effects of Hypercapnia in the New-Born Rat. Comparison with the Effect of Hypoxia 225

Várnai Ilona, Farkas Mária, Donhoffer Sz.: Thermoregulatory Heat Production and the Regulation of Body Temperature in the New-Born Rabbit 299

Zwiener U.: Hintergrundaktivität und Informationsverarbeitung Dienzephaler Einzelneurone unter Hyperthermie 237

PATHOPHYSIOLOGIA

Gábor M., Antal A., Lipták Klára, Zoltán Ö. T., Győri I., Benkő S.: Capillary Resistance in the Skin of Rats Fed Flavone-Free and Atherogenic Diets, and their Response to Hesperidin-Methyl-Chalcone 71

Huszák I., Durkó Irene: Disturbances of Porphyrin Metabolism in Schizophrenia ... 77

Nelkin W.: Diabetogenicity of Ketoheptoses 401

Szilágyi A. Katalin: Bestimmung der Guanidylessigsäurekonzentration im Blutserum und Liquor cerebrospinalis 405

PHARMACOLOGIA

Hadházy P., Fenyvesi T., Illés P., Seres J.: Comparison of the Cardiodepressant and Antiarrhythmic Effects of 2-Piperidinyl-1-Naphthyl-Propanol HCl (S-1043) and Propranolol 93

Soltï F., Krasznai I., Rév Judit, Nagy Júlia: Effect of Propranolol on Circulatory Regulation 85

Szilágyi T., Kávia Mária: The Effect of Chloroquine on the Antigen-Antibody Reaction .. 411

COMMUNICATIO BREVIS

Székely M., Kellermayer M., Sümegi Irene, Cholnoky Gabriella: Morphology and Function of Interscapular Brown Adipose Tissue in the Non-Cold-Adapted Adult Guiena Pig 419

RECENSIONES

Adamson K. (Ed.): Diagnosis and Treatment of Fetal Disorders. Springer Verlag, Berlin, Heidelberg, New York 1968. (G. Illei) 113

Bálint P.: Normale und pathologische Physiologie der Nieren. VEB Verlag Volk und Gesundheit, Berlin 1969. (F. Balogh) 260

Bechtereva N. P. (Ed.): Physiologie und Pathologie der tiefen Hirnstrukturen des Menschen. VEB Verlag Volk und Gesundheit, Berlin 1969. (K. Lissák) 105

Birke G., Norberg R., Plantin L. O. (Eds): Physiology and Pathophysiology of Plasma Protein Metabolism. Pergamon Press, Oxford 1969. (K. Lissák) 106

Burkhardt D.: Wörterbuch der Neurophysiologie. VEB Gustav-Fischer-Verlag, Jena 1969. (K. Lissák) 105

Cereijido M., Rotunno C. A.: Introduction to the Study of Biological Membranes. Gordon and Breach Science Publishers, New York, London, Paris 1970. (K. Lissák) .. 425

Chestok L. (Ed.): Psychophysiological Mechanisms of Hypnosis, Springer Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 429

Danziger K. (Ed.): Readings in Child Socialization. Pergamon Press, Oxford 1970. (Stefanie Lissák) 429

Diethelm I., Olson O., Strnad F., Vieten H., Zuppinger A.: Handbuch der medizinischen Radiologie. Band X. Teil 1. Springer Verlag, Berlin, Heidelberg, New York 1968. (G. Benkő) 262

Doerr W., Seifert G., Uehlinger E. (Eds): Spezielle pathologische Anatomia. Band 4. Springer Verlag, Berlin, Heidelberg, New York 1969. (G. Romhányi) 113

Doerr W.: Spezielle pathologische Anatomie I. Springer Verlag, Berlin, Heidelberg, New York 1970. (G. Romhányi) 430

Doerr W., Quadbeck G.: Allgemeine Pathologie. Springer Verlag, Berlin, Heidelberg, New York 1970. (G. Romhányi) 432

Gray C. H., Bacharach A. L. (Eds): Hormones in Blood. Academic Press, London 1967 and 1968. (G. Telegdy) 108

Grosse-Brockhoff F. (Ed.): Pathologische Physiologie. Springer Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 106

Hafferl A.: Lehrbuch der topographischen Anatomie. Springer Verlag, Berlin, Heidelberg, New York 1969. (B. Flerkó) 112

Hill A. V.: First and Last Experiments in Muscle Mechanics. Cambridge University Press, 1970. (K. Lissák) 424

Hollinshead W. H.: Functional Anatomy of the Limbs and Back. W. B. Saunders Co. Ltd., London 1969. (M. Frogon) 429

Hort W. (Ed.): Herzinfrakt. Grundlagen und Probleme. Springer Verlag, Berlin, Heidelberg, New York 1969. (T. Jávor) 260

Hudlická O. (Ed.): Circulation in Skeletal Muscle. Pergamon Press, London, New York 1968. (K. Lissák) 424

Janzen R.: Elemente der Neurologie auf der Grundlage von Physiologie und Klinik. Springer Verlag, Berlin, Heidelberg, New York 1969. (St. Környey) 261

Kerkut G., Norberg R., Plantin L. O. (Eds): Physiology and Pathophysiology of Plasma Protein Metabolism. Pergamon Press, Oxford 1969. (K. Lissák) 106

Kerkut G. A. (Ed.): Experiments in Physiology and Biochemistry. Vol. 2. Academic Press, London, New York 1969 (K. Lissák) 105

Kerkut G. A. (Ed.): Experiments in Physiology and Biochemistry. Vol. 3. Academic Press, London, New York 1970. (K. Lissák) 423

Kunze K.: Das Sauerstoff-Druckfeld im normalen und pathologisch veränderten Muskel. Springer Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 259

Lakshminarayanaiah N.: Transport Phenomena in Membranes. Academic Press, New York, London 1969 (K. Lissák) 425

Leibovic K. N. (Ed.): Information Processing in the Nervous System. Springer Verlag, Berlin, Heidelberg, New York 1969 (K. Lissák) 428

Lichtlen P.: Klinische Vektor-Elektrokardiographie. Springer Verlag, Berlin, Heidelberg, New York 1969. (G. Bohenszky) 259

Ludwig W.: Das Rechts-Links-Merkmale der Pflanzen. Springer Verlag, Berlin, Heidelberg, New York 1970. (K. Lissák) 427

Milcu St. M., Maximilian C., Ionescu B.: Endocrinopatiile genetice. Editure Academiei Republicii Socialiste Romania, Bucuresti 1968. (F. Obál) 112

Peters G., Roch-Ramel F. (Eds): Progress in Nephrology. Springer Verlag, Berlin, Heidelberg, New York 1969. (F. Balogh) 111

Pilz H.: Die Lipide des normalen und pathologischen Liquor cerebrospinalis. Springer Verlag, Berlin, Heidelberg, New York 1970. (K. Lissák) 427

Porter R. (Ed.): Breathing: Hering-Breuer Centenary Symposium. J. and A. Churchill, London 1970. (K. Lissák) 426

Rüdiger W. (Ed.): Lehrbuch der Physiologie. VEB Verlag Volk und Gesundheit, Berlin 1969. (K. Lissák) 423

Snell F., Wolken J., Iverson G., Lani J. (Eds): Physical Principles of Biological Membranes. Gordon and Breach Science Publishers, New York, London, Paris 1970. (K. Lissák) 426

Schubert E.: Praktikum der Physiologie. VEB Gustav-Fischer Verlag, Jena 1969. (K. Lissák) 423

Sterba G. (Ed.): Zirkumventrikuläre Organe und Liquor. VEB Gustav-Fischer Verlag, Jena 1969. (K. Fendler) 110

Swyer G. I. M. (Ed.): Control of Human Fertility. British Medica Bulletin, 1970. British Council, London. (F. Szontagh) 114

Van Cauwenberge H., Franchimont P.: Assay of Protein and Polypeptide Hormones. Vol. 33. Pergamon Press, London, New York 1970. (K. Lissák) 425

Wolstenholme G. E. W., Knight Julie (Eds): Circulatory and Respiratory Mass Transport. J. and A. Churchill, London 1970. (K. Lissák) 107

Wolstenholme G. E. W., Knight Julie (Eds): Progesterone: Its Regulatory Effect on the Myometrium. J. and A. Churchill, London 1969. (G. Telegdy) 108

Wolstenholme G. E. W., Knight Julie (Eds): Taste and Smell in Vertebrates. J. and A. Churchill, London 1970. (K. Lissák) 427

Wolstenholme G. E. W., Knight Julie (Eds): Control Processes in Multicellular Organisms. J. A. Churchill, London 1970. (K. Lissák) 107

INDEX AUTORUM

A

Andik I. vide Schmidt P. 291
Antal A. vide Gábor M. 71

B

Balázsi I.: Insulin Uptake by Isolated Rat Fat Cells 351
Balogh E. vide Selmeci L. 125
Banga I., Majláth-Palágyi J.: Cytochrome Oxidase Determination Using a Manganese Citrate Complex 117
Barankay T., Jancsó T., Nagy S., Petri G.: Cardiac Output Estimation by a Thermodilution Method Involving Intravascular Heating and Thermistor Recording 167
Baranyai P. vide Nagy I. 357, 371
Bartha J., Szőcs É.: ^{24}Na Uptake and PAH Accumulation of Renal Cortical Slices in the Presence of Angiotensin 151
Benkő S. vide Gábor M. 71
Bohus B.: The Medical Thalamus and the Opposite Effect of Corticosteroids and Adrenocorticotrophic Hormone on Avoidance Extinction in the Rat 217
Bohus B., Grubits J., Kovács G., Lissák K.: Effect of Corticosteroids on Passive Avoidance Behaviour of Rats 381

C

Cholnoky G. vide Székely M. 419

D

Dávid G. vide Kenedi I. 175
Debreczeni L. A. vide Kállay K. 9
Desrius A. vide Kovách A. G. B. 205
Donhoffer Sz. vide Várnai I. 19, 225, 299
Domonkos J. vide Heiner L. 317
Durkó I. vide Huszák I. 77

E

Endrőczi E. vide Nyakas Cs. 59
— vide Shkhinek E. K. 51

F

Farkas M. vide Várnai I. 19, 225, 299
Fazekas I. Gy., Fazekas A. T.: Seasonal Variations of the Corticosteroid Fractions in Guinea Pig Tissue and Organs 33
Fenyvesi T., Kállay K.: The Presence of β-Adrenergic Receptors in the Renal Vascular Bed 159
— vide Hadházy P. 93
Fischer E., Varga F.: Hepatic Transport of Iodipamide in Various Animal Species 135

G

Gábor M., Antal A., Lipták K., Zoltán Ö. T., Győri I., Benkő S.: Capillary Resistance in the Skin of Rats Fed Flavone-Free and Atherogenic Diets, and their Response to Hesperidin-Methyl-Chalcone 71
Gesztelyi I., Kovács L.: New Method for the Electrophysiological Analysis of the Separated Neural and Aneural Parts of Frog Sartorius Muscle 343
— vide Varga E. 325
Grubits J. vide Bohus B. 381
Győri I. vide Gábor M. 71

H

Hadházy P., Fenyvesi T., Illés P., Seres J.: Comparison of the Cardiodepressant and Antiarrhythmic Effects of 2-Piperidinyl-Naphthyl-Propanol HCl (S-1043) and Propranolol 93
Hajtman B. vide Stark E. 193
Halmy L. vide Nagy I. 357, 371
Hantos Z., Lázár Z.: The Flow of Fluid through the Wall of Capillary Systems Studied by a Mathematical Model 265
Heiner L., Domonkos J., Vargha M. jr.: Effect of Denervation on the Composition of Phospholipids in Tonic and Tetanic Muscles 317
Huszák I., Durkó I.: Disturbances of Porphyrin Metabolism in Schizophrenia 77

I

Illés P. vide Hadházy P. 93

J

Jancsó T. vide Barankay T. 167

K

Kállay K., Debreczeni L.: Effect of Human Serum Albumin on Renal Blood Flow Autoregulation in the Anaesthetized Dog 9
— vide Fenyvesi T. 159
Kávai M. vide Szilágyi T. 411
Krasznai I. vide Solti F. 85
Kellermayer M. vide Székely M. 419

Kenedi I., Dávid G.: Increased Sympathetic Innervation of the Rabbit Heart. Explanation of the Lability of the ECG in the Rabbit 175
Kiss Cs. vide Nagy I. 357, 371
Koltay E. vide Kovách A. G. B. 205
Kopa J., Poór Gy., Molnár L.: Études des problèmes de la régulation du débit sanguin cérébral. IV. Le rôle les nerfs pneumogastriques et sympathiques cervicaux dans la régulation du débit cérébral 247
Kovách A. G. B., Monos E., Koltay E., Desrius A.: Effect of Hypothalamic Stimulation on Adrenal Blood Flow and Glycocorticoid Release prior to and after Acute Hypophysectomy 205
Kovács G. vide Bohus B. 381
Kovács L. vide Gesztelyi I. 343
Kovács L. vide Varga E. 325
Kovács T., Szabó B., Vissy Á.: Some Effects of Hypertonic NaCl Solution on the Mechanical and Electrical Responses of Slow and Fast Muscles of the Cat 183
Kurcz M. vide Nagy I. 357, 371

L

Lázár G.: Species Specificity of Fibrinogen 1
Lázár Z. vide Hantos Z. 265
Lipták K. vide Gábor M. 71
Lissák K. vide Bohus B. 381

M

Makara G. B., Stark E., Mihály K.: Corticotrophin Release Induced by Traumatic Stress in Rats with Unilateral Spinal Cord Lesion 199
— vide Stark E. 43
Makara G. B.: Superficial and Deep Chemonociception: Differential Inhibition by Pretreatment with Capsaicin 393
Mayláth-Palágyi J. vide Banga I. 117
Medzihradszky K. vide Stark E. 193
Mihály K. vide Makara G. B. 199
— vide Stark E. 43
Molnár L. vide Kopa J. 247
Monos E. vide Kovách A. G. B. 205
Mosonyi L. vide Nagy I. 357, 371
Мархасина И., Оранский И.: Географическое изучение соотношения церебральной гемодинамики с периферическим кровотоком у людей, занятых умственным трудом 67

N

Nagy I., Kurcz M., Halmy L., Mosonyi L., Baranyai P., Kiss Cs.: Effect of Stress, Drugs and Hypothalamic Extract on Anterior Pituitary Somatotropin Content in the Rat 357
Nagy I., Kurcz M., Kiss Cs., Baranyai P., Mosonyi L., Halmy L.: The Effect of Suckling, Stress and Drugs on Pituitary Prolactin Content in the Rat 371
Nagy J. vide Solti F. 85
Nagy S. vide Barankay T. 176
Nelkin W.: Diabetogenicity of Ketoheptoses 401
Nyakas C., Endrőczi E.: Olfaction Guided Approaching Behaviour of Infantile Rats to the Mother in Maze Box 59
— vide Shkhinek E. K. 51

O

Оранский И. vide Мархасина И. 67

P

Palkovits M. vide Stark E. 43
Petri G. vide Barankay T. 167
Poór Gy. vide Kopa J. 247
Pósch E. vide Selmeci L. 125

R

Rév J. vide Solti F. 85

S

Schmidt P., Andik I.: Relationship between Water and Food Intake in the Rat 291
Selmeci L., Pósch E., Balogh E.: Effect of Tourniquet on Total Lactate Dehydrogenase (LDH) Activity and Isoenzyme Pattern of Rat Serum 125
Seres J. vide Hadházy P. 93
Shkhinek E. K., Nyakas C., Endrőczi E.: Frequency-Specific Response of the Pituitary Adrenocortical System on Pontine Stimulation 51
Solti F., Krasznai I., Rév J., Nagy J.: Effect of Propranolol on Circulatory Regulation 85
Stark E., Varga B., Medzihradszky K., Bajusz S., Hajtman B.: Relationship between the Structure and the Adrenal and Extra-Adrenal Effects of ACTH Fragments 193
Stark E., Makara G. B., Palkovits M., Mihály K.: Afferent Pathways of Stressful Stimuli: their Dependence on Strength and the Time Elapsed after the onset of Stimulation 43
— vide Makara G. B. 199
Sümegi O. vide Székely M. 419

SZ

Szabó B. vide Kovács T. 183
Székely M., Kellermayer M., Sümegi I., Cholnoky G.: Morphology and Function of Interscapular Brown Adipose Tissue in the Non-Cold-Adapted Adult Guinea Pig 419

Szilágyi G.: Effect of Acidification and Alkalinisation on Calcium and Citrate Metabolism in Normal and Parathyroidectomized Dogs 281
Szilágyi A. K.: Bestimmung der Guanidylessigsäurekonzentration im Blutserum und Liquor cerebrospinalis 405
Szilágyi T., Kávai M.: The Effect of Chloroquine on the Antigen-Antibody Reaction 411
Szőcs É. vide Bartha J. 151

V

Varga B. vide Stark E. 193
Varga E., Kovács L., Gesztelyi I.: Analysis of the Depolarizing Effect of Nicotine on Frog's Skeletal Muscle 325
Varga F., Fischer E.: Biliary Secretion of Rose Bengal in the Dog 143
— vide Fischer E. 135
Vargha M. jr. vide Heiner L. 317
Várnai I., Farkas M., Donhoffer Sz.: Thermoregulatory Effects of Hypoxia in New-Born Rats and their Correlation to Body Temperature and Heat Production prior to and after Exposure to Hypoxia 19
Várnai I., Farkas M., Donhoffer Sz.: Thermoregulatory Effects of Hypercapnia in the New-Born Rat. Comparison with the Effect of Hypoxia 225
Várnai L., Farkas M., Donhoffer Sz.: Thermoregulatory Heat Production and the Regulation of Body Temperature in the New-Born Rabbit 299
Vissy Á. vide Kovács T. 183

Z

Zoltán Ö. T. vide Gábor M. 71
Zwiener U.: Hintergrundaktivität und Informationsverarbeitung Dienzephaler Einzelneurone unter Hypertermie 237

Acetylcholine 95, 343, 346, 396—398
—, its effect on RBF autoregulation 14
— sensitivity of end-plates 348
acetylsalicylic acid 396
acidification, its effect on calcium and citrate metabolism 281
— with ammonium chloride 287
acidosis, metabolic 282
acoustic analyzers 59, 64
ACTH 213, 217, 222, 390
—, adipokinetic activity of 193
— administration 218, 220
— concentration 212
— depots, extrahypophyseal 213
— fragments 46, 194, 195
— —, adrenal and extraadrenal effects of 193
— —, their effect on corticoid secretion 194—197
—, hypoglycaemic activity of 193
—, lipolytic effect of 194
—, native, chemical modification of 193, 197
— pretreatment 43
— release 43, 45, 47, 48, 51, 53, 199, 207
— —, mesencephally-induced 55
— releasing spinal pathways 199, 203
ACTH-adrenal system 205
ACTH-like peptides 217, 390
action potential, effect of sodium at high concentration on 189
— —, in muscle 183, 186—188
— —, propagation of 346, 348
activity of diencephalic neurons during hyperthermia 237, 239, 241, 244
adenosintriphosphate, its effect on RBF autoregulation 14
adenyl cyclase enzyme system 355
adipose tissue, brown 419, 420
adipsia 291
adrenal 205
— activity 215
— blood flow 193, 194, 197, 205, 206, 208, 209, 212, 215
— — supply 214
— circulation 212
— cortex, diurnal variations in 33
— effect of ACTH 193
— vascular resistance 207
— venous outflow 205, 210
adrenalectomy 214, 215, 401
adrenaline 95, 163, 165, 244, 366
β-adrenergic excitation 181
— receptor 164
— —, in the renal vascular bed 159
— —, its role in cardiac function 159
— —, — — in muscle flow 159
β-adrenergic receptor, its role in fat and carbohydrate metabolism 159
— —, renal localization of 165
— stimulation 162, 164, 165
adrenocortical activation 205
— hormone secretion 214
— secretion, frequency-specific activation of 56
afferent pathways of stressful stimuli 43
after-potentials 330
aldosteron 151
alkalinisation, its effect on calcium and citrate metabolism 281
alkalosis, metabolic 282—284, 287, 288
alpha blocking effect of S-1043 95
— receptor blockade 163—165
amigdaloid nuclei 292
ε-aminocaproic acid 416
ammonium chloride, acidification with 287
anaphylactic reaction 5, 414
— shock 413
angiotensin 151, 153—157
—, direct tubular action of 152
—, hypertensive effect of 151
angiotensin II, effect on RBF autoregulation 14
angiotensinase, nonspecific 154
antigen-antibody complex 412, 414, 416
— reaction 411, 417
antigenic structure of fibrinogen 1
aphagia 291
approaching behaviour, olfaction-guided 59, 61
arginin 406, 409, 410
arrhythmia, ouabain induced 99, 101, 102
arterenol, its effect on RBF autoregulation 14
ascites tumours 118
ascorbic acid 194
atherogenic diet 71—74
atherosclerosis 71, 74
ATPase activity 149
—, Na—K 157
atrium, right 173
atropine 178, 179, 181
atropine-like effect of S-1043 95
avoidance response, conditioned 218
— —, extinction of 194, 217, 218, 220
— —, in shuttle-box 222

Behaviour, approaching, olfaction-guided 59, 61
—, avoidance, passive 381, 384, 386, 388, 390
—, infantile 64
beta blockade 88, 89, 95, 99, 102
— blocking effect, importance of chemical properties in 101

beta receptors 85, 89, 90
bicarbonate diuresis 288
bile flow 146, 148
— production 138, 139, 141, 149
blepharospasm 394
blood cells 411
— fibrinogen level 1
— flow 195, 196
— —, adrenal 193, 194, 197, 205, 206, 208, 209, 212, 215
— —, hepatic 88
— —, in renal medulla 165
— —, intrarenal 166
— —, muscle 88
— —, ovarian 193, 194
— —, renal 9, 11, 15, 88, 160, 162—165
— —, —, autoregulation of 9, 14, 15
— plasma, guanidil acetic acid concentration in 405, 408—410
— pressure 9, 10, 85, 89, 139, 144, 149, 151, 155, 206, 208, 209, 211, 212, 215, 248
— protein level 10
body temperature 19, 26—28, 31, 225, 230, 233, 234, 239, 244
— —, after the termination of hypoxia 28
— —, during hypercapnia 233
— —, effect of hypoxia on 20—23, 25—27, 29—31
— —, in newborn rabbit 303, 304, 306, 309, 311, 313
— —, its effect on the response to hypoxia 28
— —, post hypercapnic 232, 234
— —, regulation of 299
bradycardia 89
bradykinin 398
brain haemodynamic 67—70
— stem 247
— —, lower 51
— —, —, synchronizing influence of 51
— —, electric stimulation of 248, 251, 256
bromsulphothalein 143, 148
brown adipose tissue, interscapular 419, 420

Calcium level, serum 281—283, 288
— metabolism, effect of acidification on 281
— —, — of alkalinisation on 281
—, urinary excretion of 282, 283, 287
calciuria 284, 288
capillary, filtration coefficient of 279
— flow intensity 271, 272
— hydrostatic pressure 266, 272
— permeability 279, 280
— — in the kidney 16
— porosity 274
— pressure 278
— resistance in the skin 71—74
— wall 265, 268
— —, permeability of 265, 267, 277
capsaicin 393, 398
— treatment 394—397
carbachol 343, 346
carbohydrate metabolism 366
— —, role of β-adrenergic receptors in 159
carbon dioxide breathing 225—227, 229, 230, 233
7-6-5-carboxylic porphyrins 80, 82, 83
cardiac acceleration 398
— activity, role of beta receptors in 85, 159
— muscle, contractility of 96
— output 85, 89, 90, 167, 171, 173, 209
— —, its decrease after propranolol treatment 88
— —, organ fractions of 89, 90
cardiazol 256
carotid sinus baroreceptors 89
cephalins 320, 322, 323
—, fatty acid ester content of 321
—, — aldehyde content of 321
chemonociception 393, 394, 397
chloroquine 411—414, 417
— pretreatment 416
cholecystography 135
cholesterol 38, 218, 220
— feeding 71—74
choline phosphatides 318, 319, 322, 323
— —, fatty acid ester content of 320
— —, — aldehyde content of 320
cirdulation, adrenal 212
—, cerebral, regulation of 247, 248, 251
circulatory transit time 88, 90
citrate level, serum 281, 282, 283, 288
— metabolism, effect of acidification on 281
— —, — of alkalinisation on 281
—, urinary excretion of 282—284, 287
citraturia 284, 287
clearance (C_{in}) 165
— (C_{PAH}) 10, 15
cocaine 348
colonic temperature 25, 27—31, 226, 227, 330, 232, 233
— — after hypercapnia 234
— —, effect of hypoxia on 21—24, 26, 28, 30
— — in new-born rabbit 304, 306, 308, 309, 311—313
compound X_0 35, 38, 39
— X_1 35, 38, 39
— X_2 35, 38, 39
conditioned reflex, avoidance, extinction of 217, 218, 220
contractile protein of tonic muscle 191
— — of tetanic muscle 191
coproporphyrin 79, 82
cortical blood flow, renal 15
— evoked potential 222
corticoid release 208, 210, 212, 213
— secretion, effect of ACTH fragments on 194—197
corticosteroids 217, 221, 381, 388—390
—, chemoreceptive field for 55
—, fear suppression by 389
— fractions 33, 35
— in the organs and tissues, effect of alcohol on 34

corticosteroids in the organs and tissues, effect of formal stress on 34
—, intracerebral implantation of 217
—, their effect on passive avoidance behaviour 381
corticosterone 38, 39
— level of plasma 33, 45, 46, 201—203
— production 53
— release 208
cortisol 37—39
cortisone 37—39, 383, 388
— acetate 218, 220, 222
— — administration 383, 384, 386, 389
cross precipitation 2, 5
curare 330, 334, 339, 340, 348
cutaneous sensory function, early maturation of 59
cytochrome c 117, 119, 120, 124
cytochrome oxidase 117, 119
— — activity assay 118

11-Dehydrocorticosterone 38
dehydrogenase, tissue 119
delta-aminolaevulinic acid synthesis 79, 83
— — excretion, urinary 79
denervation atrophy in muscle 317
4-deoxypyridoxine loading 77—81, 83
depolarization, slowly developing 325, 330
—, — ceasing 325
α-2-D-desoxyglucose 366
desynchronization, EEG 56
dexamethasone 386—388
diethyl-p-phenylene-diamine sulphate 118, 120
diuresis 162
—, bicarbonate 288
draining resistance 267, 273
drive, thirst 389

ECG of the rabbit 175, 179, 181
EEG 244
— activity 53
— desynchronization 56, 251, 256
effective filtration pressure 15
Ehrlich tumours 118
EKG 240
electrical activity of subcortical structures 238
endocardium 173
end-plate area of muscle 325, 343, 346
— potential 335, 341
epinephrine
—, its effect on RBF autoregulation 14
erythema 416
E_{in} 15, 162
E_{PAH} 10, 162, 165
—, effect of human serum albumin on 15
extracellular fluid, NaCl concentration of 188
evoked potentials, cortical 222

facilitatory mechanism, ascending reticular 221
— —, diencephalic 217
facilitatory mechanism, mesencephalic 217
fat cells, insulin uptake by 351
— —, metabolic activity of 353
— metabolism, role of β-adrenergic receptors in 389
—, white 419
fear, suppression by corticosteroids 389
femur 199
fibrin precipitation 173
fibrinogen, antigenic relation of 1
—, — structure of 2
—, avian 6
—, —, structure of 2, 3
—, degradation products of 1
—, denaturation of 1
— level in blood 1
—, mammalian 6
—, —, antigenic elements of 5
—, —, structure of 2, 3
—, native 2
—, species specifity of 1
fibrinolysis 1
Fick principle 171
filtration 265, 267, 269, 276
— flow intensity 269, 271, 278
flavone administration 71
flavone-deficient diet 71, 73
flavone-free diet 72, 74
fluid intake, total, in rat 292
food intake in rat 291—293, 295—297
— —, osmotic regulation of 296
forebrain 390
— area, cortisone implants in 222
— inhibition 222
— inhibitory mechanism 217, 221
—, implantation of cortisone in 222
—, regulation of sleep and wakeful states of 51
— structures 381
formaldehyde, subcutaneous injection of 199, 202

Galactoheptuloze 403
gamma globulin 412
gastrointestinal tract 396
glomerular filtration rate (GFR) 10, 151, 165
— — —, effect of human serum albumin on 15
glomerules 287
glucoheptuloze 401
gluconeogenesis 401
glucose 401
— infusion 9
— metabolism 355
glucose-1-^{14}C 353
glucosuria 401
glycocorticoid(s) 282
— mobilization 213
— release 205
glycogen production 351
goal-directed activity 389
— behaviour 59, 64
— motor pattern 64

guanidyl acetic acid concentration in blood plasma 405, 408—410

Haematocrit 10, 146
haematuria 288
haem-synthesis, normal intermediates of 82
heart, papillary muscle of 348
—, rabbit, sympathetic innervation of 175, 180
— rate 180
— — at different body temperature 240
heat capacitance of the body 173
— production 19, 21, 27, 31, 225, 226, 229, 233, 234
— — after the termination of hypoxia 28
— —, effect of hypoxia on 22—24, 30
— — in new-born rabbit 299, 303, 309
— —, post-hypercapnic 234
— —, post-hypoxic 234
hesperidin-methyl-chalcone 71
— treatment 72, 74
hexahydrocortisol 39
hydrochlorothiazide 281
hydrocortisone 401
— release 207
hydrostatic pressure 268
— —, capillary 266, 272
— — difference 265
— —, interstitial 266
5-hydroxy-indole acetic acid 77
hypercalciuria 287, 288
—, idiopathic 287
hypercapnia 19, 226, 227, 230, 232—234
—, body temperature during 233
—, colonic temperature after 234
—, its effect on O_2 consumption 230, 233
—, thermoregulatory effect of 225, 226
hyperthermia 237
hyperventilation 89
hypoglycaemia 360, 365, 371, 372
—, insulin-induced 357
hypophysectomy, acute 205, 209, 210, 213, 214
hypophyseotropic area, medial 215
hypothalamic extract, rat 357, 358, 365, 370, 380
— region, anterior 218, 220
— stimulation 205, 206, 208, 210—214
hypothalamo-hypophyseal connections 365
— — unit 43
hypothalamus 55, 205
—, lateral, bilateral electrolytic lesion of 291
—, medial basal, partial deafferentation of 45—47
hypoxia 226, 229, 230, 234, 314
—, body temperature after the termination of 28
—, heat production after the termination of 28
—, its effect on beta receptors 89
—, its effect on body temperature 28
—, its effect on colonic temperature 21—24, 26, 28, 30
—, its effect on heat production 22—24, 30
—, its effect on O_2 consumption 20, 25—28, 230
—, thermoregulatory effect of 19, 225, 226
Immune serum 2
— —, antifibrin 2, 3
immunoreactive insulin 351, 353
indocyanine green excretion 141
indol 77
infantile behaviour 74
inhibition 56
inhibitory influences, forebrain 390
— processes, internal 381, 388
insulin 351, 352, 354, 355, 358, 365, 372
—, immunoreactive 351, 353
— treatment 360, 365
— uptake by fat cells 351
insulin-induced hypoglycaemia 357
interstitial flow 268
— hydrostatic pressure 266
— pressure 266, 267, 274, 276, 277
intertrial activity 388
intravascular heating 167, 168
inulin clearance 165
— space in fast and slow muscle 189
iodipamide 141
— concentration in liver and plasma 137, 140
— —, biliary 138, 139
—, excreted with bile 140
—, — with urine 140
— excretion 136—139, 142
—, hepatic transport of 135
—, transfer maximum of 135, 141, 142
isometric contraction in fast muscle 191
— — in slow muscle 191
isoniazid (INH) 77, 82
isoprenaline 95, 160—162, 164, 165
isoproterenol 163, 179—181
intracranial pressure 14

KCl 325
K efflux, effect of nicotine on 340
ketoheptoses 401, 403
ketosis 401
kreatin 405, 409
kreatinin 49
Krebs—Szentgyörgyi cycle 287
kwashiorkor 409

Lactate dehydrogenase (LDH) activity 125
— — —, effect of limb tourniquet on 126
— — —, effect of nephrectomy on 128
— — — in heart 128
— — —, total 126, 131
— — isozymes 125, 128, 131, 133
Laewen-Trendelenburg frog preparation 183
learning ability, retarding by destruction of olfactory bulb 64
— processes 59
limb tourniquet, its effect on LDH activity 126

lipid-P content in muscles 318—324
liquor, guanidyl acetic acid concentration in 405, 406, 409, 410
lymphatic system 265, 266
lymph flow 277
lysosome 417

Magnocellular nuclei of the pontine reticular formation 53, 56
manganese citrate complex 118—120, 122
mannitol infusion 9
mannoheptulose administration 401, 403
marasmus 409
mechanonociceptors 393
medical basal hypothalamus, partial deafferentation of 45—47
membrane potential 334, 344, 345
metabolic acidosis 282
— alkalosis 282—284, 287, 288
methionine 194
methyl guanidin 406
mitochondria 287
morphine sulphate 396
motor activity 23
— function after spinal cord hemisection 201, 202
α-MSH 193, 217, 222
muscle, action potential in 183, 186—188
— blood flow 88, 183, 184, 191
— cell 183, 190
— —, sodium concentration in 190
— —, potassium concentration in 190
—, denervation atrophy of 317
—, direct electric stimulation of 346
—, end-plate area of 325, 343, 346
—, fast, isometric contraction in 191
—, gastrocnemius 186, 187, 189, 321
—, —, intracellular water in 190
—, —, inulin space in 189
—, lipid-P content in 318—324
—, papillary, of the heart 348
—, rectus 325
—, red 131
—, resting potential of 329, 331, 339, 344, 346, 348
—, sartorius 183, 327, 329, 330, 332—334, 339, 343—346, 348
—, slow 183, 184, 189, 191
—, —, isometric contraction in 191
—, solens 184, 186, 187
—, —, intracellular water in 190
—, —, inulin space in 190
—, tetanic 317, 319, 320, 322, 323
—, —, denervation of 317
— tremor 176
—, tonic 317, 320, 322, 323
—, —, denervation of 317
—, vascular resistance of 88
— viscosity 183, 191
—, white 131
myocardial excitability, effect of S-1043 on 97, 102
myocardium 172

Na influx 333—335, 339—341
Na permeability 334
NAD 149
$NaHCO_3$ infusion 284
naphthylalkylamine derivatives, antiarrhythmic effects of 93
naphthylalkylamine derivatives, inotropic effects of 93
natriuresis 151
neocortex 247
nephrectomy, its effect on LDH activity 128
nephrolithiasis 287
nerve, saphenous 394
neuromuscular block 341
— junction 186, 339, 341, 348
nikotine 329—332, 335, 339—341, 343, 346, 348
— depolarization, mechanism of 332
—, depolarizing effect of 325, 327, 328, 334, 339
—, its effect on K efflux 340
—, repolarization in presence of 332
non-collagenous protein 321, 323
noradrenalin 244

Oedema 416
oestrus 371
olfaction-guided approaching behaviour 59, 61
olfactory bulb destruction 64
— function, early maturation of 59, 64
oncotic pressure 266, 268, 270—272, 276, 279
— — difference 265, 267, 270
ouabain 102
— arrhythmia 97, 99, 101, 102
ovarian blood flow 193, 194
oxalate, excretion of 83
— infusion 282—284, 287, 288
oxygen consumption 19, 28, 30, 31, 227, 229, 233, 300, 302, 303, 309, 311—314, 419
— —, absolute reduction in 28
— —, effect of human serum albumin on 15
— —, effect of hypercapnia on 230, 233
— —, effect of hypoxia on 20, 25—28, 230
— — of kidney slices 153, 157
— —, renal 11
ovalbumin 411, 412
oxytocin 358, 360, 365—367, 371, 376—378

Pacemaker activity of S-1043 96
PAH accumulation of renal cortical slices 151, 154, 157
— clearance 10, 15
— extraction 157
pain 393, 397, 398
—, deep 393
—, superficial 393
papaverine, effect on RBF autoregulation 14
parafascicular area, thalamic 217
paraphenylene diamine 117—119
parathormone 281, 288
parathyroid function 281

— gland 281, 282
parathyroidectomy 281, 283, 284, 288
passive avoidance behaviour 381, 384, 386, 388, 390
— — task, retention of 383, 386
— — —, long term retention of 383, 386
permeability of the capillary wall 265, 267, 277
phenol red extraction 157
phenoxybenzamine 165
— pretreatment 162—164
phospholipids 321—323
—, in muscle, composition of 317
2-piperidinyl-1-α-naphthyl-1-propanol HCl (S-1043) 93, 95, 97, 99
—, alpha blocking effect of 95
—, atropine-like effect of 95
—, inotropic effect of 96, 101
—, its effect on ouabain induced arrhythmia 99, 101, 102
—, pacemaker activity of 96
pituitary 205
—, anterior, ACTH release from 51
—, intact 206
— peptide hormones 352
pituitary-adrenal axis 390
pituitary-adrenocortical system, activation of 55
— — —, frequency-specific response of 51
plasma osmolality 15
— potassium concentration 190
— protein concentration 279
— sodium concentration 190
plasmalogen 323, 324
pontine stimulation 51
porphyria 77, 83
—, chemically-induced 79
— cutanea tarda 77, 82
— erythropoietica 82
—, hepatic 79
porphyrin metabolism in schizophrenia 77, 83
potassium concentration in muscle cells 190
— — in plasma 190
pregnanetriol 40
procaine, its effect on RBF autoregulation 14
prolactin content, pituitary 371, 372, 376—378, 380
— release 377
propranolol 93, 95—97, 99, 101, 102, 164, 165, 179—181
— administration, effect of l-isoprenaline before and after 161, 162
— —, decrease of cardiac output 88
—, its effect on circulatory regulation 85
protein coagulation 173
— concentration of plasma 266, 267, 270, 272, 279
— level in blood 10
—, non-collagenous 321, 323
—, permeability for 280
protoporphyrin content of erythrocytes 78, 79, 83
— synthesis, hepatic 83
pyridoxal enzymes 79, 83
— — disturbance of 77
— metabolism 83
pulse rate 210
— — at different body temperature 240

Red nucleus 210
redox potential 119
refractory period, functional 96, 101, 102
— — in gastrocnemius 191
regulation of cerebral circulation 247, 248, 251
renal A—V oxygen difference 11
— blood flow (RBF) 9, 11, 15, 88, 160, 162—165
— — — autoregulation 9, 14, 15
— — — —, effect of acetylcholine on 14
— — — —, — of adenosintriphosphate on 14
— — — —, — of angiotension II on 14
— — — —, — of arterenol on 14
— — — —, — of epinephrine on 14
— — — —, — of papaverine on 14
— — — —, — of procaine on 14
— — — —, — of renin on 14
— — — —, — of theophylline on 14
— — —, cortical 15
— — —, elevation of 15
— — —, increase in 9, 14
— cortical shunts 16
— medulla, blood flow in 165
— oxygen consumption 11, 287
— sodium excretion 151
— tubular acidosis 282
— — cell 287
— vascular bed, β-adrenergic receptors in 159
— — resistance 160, 162—165
— vasoconstriction 164
— vasodilation 164
— vessels, dilatation of 14
renin, its effect on RBF autoregulation 14
renin-angiotensin system 213
repolarization, in presence of nicotine 332
respiratory rate 210, 212, 248
resorption 265, 267, 269, 271, 276
— flow intensity 278
resting potential of the muscle 329, 331, 339, 344, 346, 348
reticular activating system 381, 390
— areas, diencephalic 390
— —, mesencephalic 390
— formation, mesencephalic 51, 55, 222
— —, pontine 53, 55
riboflavin deficiency 83
rose bengal 144
— —, amount in urine 145
— —, biliary secretion of 143, 146
— — concentration in the liver 148
— — — in the kidney 144
— — — in the muscle 144
— — — in the skin 144

— — — in the small intestine 144
— —, hepatic transfer of 141
— — storage capacity of liver 147
— — transfer maximum of 146, 148

Schizophrenia, porphyrin metabolism in 77, 83
sciatic nerve stimulation 222
sedoheptuloze 401, 403
sensory analyzers 59
— function 201
— —, cutaneous 59
septal nuclei, 218, 220
serotonin 178, 179, 181, 398
serum albumin, human 9
— —, —, infusion of 12, 15
— —, —, its effect on blood constituents 10
— —, —, — — on blood pressure 10
— —, —, — — on E_{PAH} 15
— —, —, — — on GFR 15
— —, —, — — on oxygen consumption 15
— —, —, — — on renal blood flow 10
— —, —, — — on RBF autoregulation 9, 11, 14, 16
— —, —, mode of action of 15
— Ca level 281—283, 288
sham-adrenalectomy, than surgical stress 47, 48
shivering 225
shuttle-box conditioning 389
sodium concentration in muscle cells 190
— — in plasma 190
— —, intracellular, in soleus muscle 191
— —, —, in gastrocnemius 191
— content of kidney slices 153, 156, 157
—, electrochemical potential gradient of 156
— excretion, renal 151
—, extracellular, exchange rate of 156
—, high concentration, its effect on action potential 189
— reabsorption, tubular 151
— uptake in gastrocnemius 191
— — in soleus muscle 191
— — of renal cortical slices 152, 157
somato-motor pattern 383, 388, 389
somatotropin 368
—, blood level 367
— content in anterior pituitary 357, 360, 365—367
— production, normal 365
— releasing factor 357, 367
specific sensory pathways 55
spinal cord lesion, unilateral 199, 201, 202
— pathways, ACTH releasing 199, 203
spinothalamic tract 203
splenectomy 366
Starling equilibrium 265
steroid content, total, of the adrenals in guinea pig 40
— —, —, of the blood 40
— metabolism, tissular 38
stress 357, 371
—, blood loss 358, 360, 366, 372
—, formalin administration 366
—, laparotomy 358, 366, 376
— reaction, in two phases 48
—, traumatic 199, 202, 203
stressful stimuli 43
strichnin 256
subcortical structures, electrical activity of 238
subthalamic projections 51
succinylcholine 343, 346, 348
suckling 371, 372, 377
— stimulus 358, 360, 365, 376, 380
swimming 358, 360, 365, 377
sympathetic activity 215
— innervation of heart 175, 180
— vasodilatator relay 215
sympathic nerve transection 247, 256, 257
sympathin I 163

Tachycardia 180, 181
tetanic contraction 184
tetanus 187—189
tetracaine 64
tetrahydrocortisol 35
tetrahydrocortisone 36, 39
tetrodotoxin 331, 334, 335, 341
thalamic nuclei, non-specific 51
— parafascicular area 217
— reticular mechanism 220
thalamus, centromedian nuclei of 218
—, medial 217, 220, 222
—, parafascicular nuclei of 218, 222
—, paraventricular nuclei of 218
theophylline, its effect on RBF autoregulation 14
thermogenesis 419
thermoregulation, chemical 235
—, physical 235
thermoregulatory system 234
thirst-drive 389
thyrocalcitonin 282, 288
thyroid gland destruction 71
thyroidectomy 72, 73, 74
thyro-parathyroidectomy 283, 284
thyroxine deficiency 288
transection of sympathic nerve 247, 256, 257
tissular steroid metabolism 38
T_{PAH} 11, 15
transmural flow intensity 265—268, 271, 272, 278
— fluid movement 274, 276, 279
— pressure difference 279
tryptophan 77, 79, 83
tuberoinfundibular region 51

Urea infusion 9
urine excretion 10
— flow 160, 163—165

Vagus 178, 181
vascular resistance 85, 88

— —, adrenal 207
— — of the kidney 88
— — of the liver 88
— — of the muscle 88
vascular resistance of the, renal 160, 162—165
— —, systemic 89
— wall 172
vasoconstriction 90
—, renal 164
vasodilatation 90, 164
— in the kidney 14
vasopressin 215, 358, 360, 366, 367, 371, 376
venous resistance in kidney 16
ventricle, right 173
visual analyzers 59, 64
vitamin B_6 77
— — deficiency 83
vitamin D 287
vocalization 396

Water intake 291—293, 295—297, 388

Xanthurenic acid, excretion of 83

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SPECIES SPECIFICITY OF FIBRINOGEN

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The antigenic structure of fibrinogen in taxonomically remote animals is determined primarily by the species proper, since the fibrinogen of the two classes of vertebrates, the mammals and the birds, is not in antigenic relation. On the other hand, an extensive antigenic relation can be demonstrated in the fibrinogen of animals within the same systemic class, i.e. closely related taxonomically. However, antigenic relation revealed by the radial double diffusion technique does not show a complete identity of the reaction type, only partial identical types. According to our investigations, the anti-fibrin immune sera do not react with the fibrinogen of the animal used for the preparation of the immune serum despite the fact that with an anti-fibrin serum prepared in some other animal, a close antigenic relation can be demonstrated between the fibrinogens of the animals in question.

Fibrinogen exerts its specific action by a characteristic reaction, viz., upon the effect of thrombin it changes from the sol state into a gel. Considering that the function and the characteristic reaction of fibrinogen are similar in different animal species, the question of the antigenic relation of fibrinogen has been raised long ago. Earlier findings concerning the problem were not unequivocal, due probably to differences in the methods applied for the preparation of the fibrinogen used in the experiments and which methods may have led to denaturation of the fibrinogen. The most comprehensive study in this field was performed in the early fifties by Hungarian authors: KESZTYŰS et al. (1950), SZILÁGYI et al. (1952) as well as BAGDY and SZILÁGYI (1953). Their statements have been fully corroborated by our own investigations.

Since the basic publications of SALMON (1959), BARTA and BARTOVA (1960) as well as NUSSENZWEIG et al. (1961) immune diffusion procedures are extensively used for the study of the degradation products of fibrinogen and fibrinogen-fibrin and it has become possible to demonstrate the participation of fibrinolysis in pathological events accompanied by a decrease of the blood fibrinogen level and to differentiate intravascular clotting from excessive fibrinolysis (MERSKY et al. 1964; LEWIS and WILSON 1965; LÁZÁR 1966). The modern immunological methods such as OUCHTERLONY's radial double immune diffusion technique allowed the elucidation of antigenic relations. Since this method has not yet been used for investigations into the antigenic relation of fibrinogens of various origin, it seemed worth-while to re-investigate the problem with the aid of the procedure. In order to avoid structural changes in

the antigenic structure of the fibrinogen molecule, native fibrinogen, i.e. plasma, was used in our experiments as an antigen for the gel diffusion. Immune serum was prepared by means of fibrin which, according to SALMON (1959), ensures an immune serum identical in value with anti-fibrinogen serum.

Methods

Blood clotting was prevented with sodium citrate, and the plasma obtained from various animal species was stored in the deep freezer.

Immune serum was prepared against human, rat and fowl fibrin in rabbits; anti-rabbit fibrin serum was prepared in guinea pigs. Human, rat and fowl fibrin was carefully washed, dried, pulverized and mixed with physiological saline and aluminium hydroxide gel. The preparation was administered in a dose of 0.02 g dry weight to rabbits at weekly intervals over a period of 5 weeks by the intramuscular route. The animals were bled two weeks after the last injection, and the anti-fibrin serum, which also contained antibodies against some other serum constituents mainly belonging to the β-globulin fraction, was absorbed by the corresponding serum. This was followed by conservation with merthiolate and streptomycin and storage in a deep freezer. For absorption an amount of serum corresponding to 1/5 part of the immune serum was sufficient. In order to approach the optimum precipitation zone of the individual antigens, this amount of serum was added to the immune serum in several doses. The preparation of anti-rabbit fibrin serum differed from the above procedure inasmuch as the rabbit fibrin absorbed to aluminium hydroxide was injected into guinea pigs in a dose of 0.02 g once a week by the intraperitoneal route.

The radial double diffusion procedure (OUCHTERLONY 1958) was carried out partly on 5×5 cm glass plates with a 2.5 mm agar layer with 5 mm holes placed 5 mm apart, and partly by a modified technique described previously (LÁZÁR 1963). This modification makes the original method more sensitive, for the plexiglass tanks above the thin agar layer allow the diffusion of a larger amount of antigen and immune serum, and thus present a reliable picture of systems giving a weak precipitation (LÁZÁR 1967). The investigations were carried out with both methods in parallel at least in triplicate. The results obtained with the two procedures agreed well. Immune electrophoresis was performed according to the micro-scale method of SCHEIDEGGER (1955). One per cent Difco-agar in physiological saline was used for the radial double immune diffusion procedure, and one per cent Difco-agar in veronal buffer of 0.05 ionic strength, pH 8.6, for immune electrophoresis. The agar plates were photographed according to ALMEIDA et al. (1965), or the stained preparations were used as negatives.

Results

After absorption with homologous serum, the immune sera form one sole precipitation band with the homologous plasma both with the radial double diffusion method and with immune electrophoresis. The precipitation band after immune electrophoresis was located at the site corresponding to the fibrinogen, around the starting point of the antigen, somewhat moved towards the cathode.

Mammalian anti-fibrin sera did not give a precipitation band with fowl, duck, turkey, pigeon and sparrow plasma. Similarly, anti-fowl fibrin serum did not react with the plasma of the mammals investigated. Thus, the antigenic structure of mammalian fibrinogen is remote from that of avian fibrinogen. On the other hand, a close antigenic relation was found to exist between the various mammalian fibrinogens. Several degrees of relation could be shown, from spike formation up to a just demonstrable cross-precipitation. However, an identity reaction could not be demonstrated in any of the cases.

The anti-fibrin immune sera did not react with the fibrinogen of the animal species used for the preparation of the immune serum. Thus, the anti-rabbit fibrin serum prepared in guinea pig did not react with guinea pig plasma. Similarly, rabbit immune sera prepared against human or rat fibrin

Table I

Antigenic relation of fibrinogens of various origin

Class	Order		Anti-human fibrin rabbit serum	Anti-rat fibrin rabbit serum	Anti-rabbit fibrin guinea pig serum	Anti-fowl fibrin rabbit serum
Mammals	Rodents	Rabbit	—	—	×	—
		Rat	r.	×	r.	—
		Mouse	r.	r.	r.	—
		Guinea pig	r.	r.	—	—
	Carnivores	Dog	r.	r.	r.	—
		Cat	r.	r.	r.	—
	Ungulates	Horse	r.	r.	r.	—
		Pig	r.	r.	×	—
		Ox	r.	—	×	—
		Sheep	r.	—	r.	—
		Goat	r.	—	r.	—
	Primate	Human	×	r.	r.	—
Birds	Lamellirostres	Duck	—	—	—	r.
	Gallinaceae	Fowl	—	—	—	×
		Turkey	—	—	—	r.
	Columbidea	Pigeon	—	—	—	r.
	Passeridae	Sparrow	—	—	—	r.

r. = related — = alien × = not investigated

did not show precipitation with rabbit fibrinogen, even though a close antigenic relation could be demonstrated to exist between rabbit, human and rat fibrinogen as tested with anti-rabbit fibrin serum prepared in the guinea pig.

As judged on the basis of immune diffusion, the avian fibrinogens are closely related, even though an identity reaction could not be demonstrated in any of the cases.

The results are shown in Table I and Figs 1 to 6.

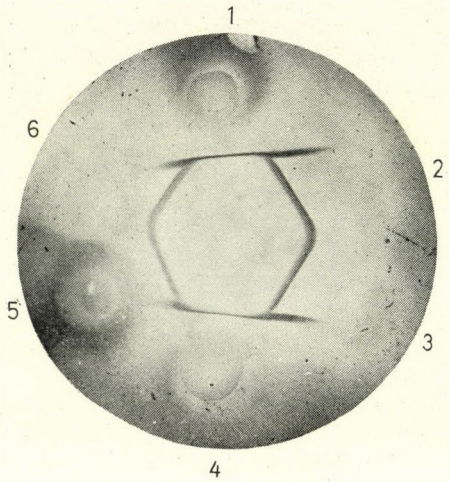

Fig. 1. Centre: anti-rabbit fibrin guinea pig serum. 1: rabbit plasma; 2: sheep plasma; 3: goat plasma; 4: rabbit plasma; 5: dog plasma; 6: cat plasma

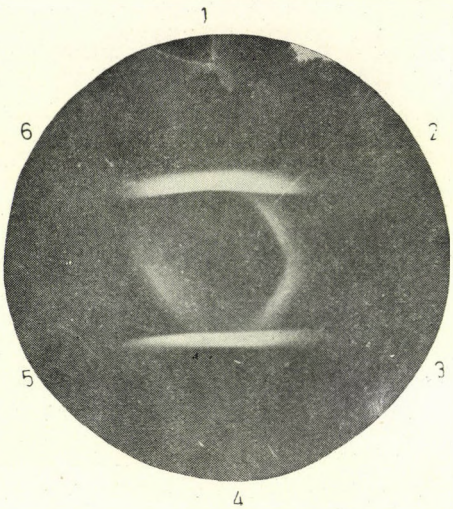

Fig. 2. Centre: anti-human fibrin rabbit serum. 1: human plasma; 2: rat plasma; 3: guinea pig plasma; 4: human plasma; 5: mouse plasma; 6: rabbit plasma

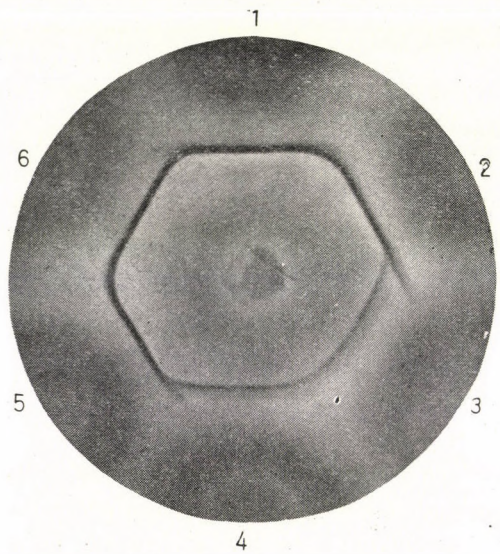

Fig. 3. Centre: anti-fowl fibrin rabbit serum. 1: fowl plasma; 2: fowl plasma; 3: pigeon plasma; 4: pigeon plasma; 5: turkey plasma; 6: turkey plasma

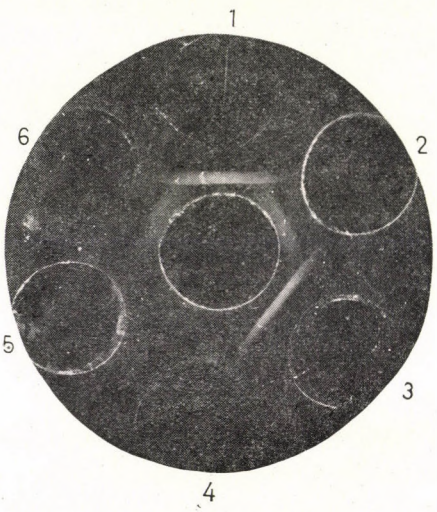

Fig. 4. Centre: anti-rat fibrin rabbit serum. 1: rat plasma; 2: guinea pig plasma; 3: rat plasma; 4: blank; 5: rabbit plasma; 6: human plasma

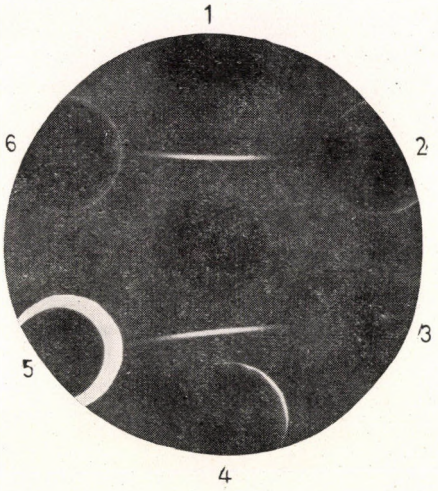

Fig. 5. Centre: anti-human fibrin rabbit serum. 1: human plasma; 2: rat plasma; 3: guinea pig plasma; 4: human plasma; 5: blank; 6: rabbit plasma

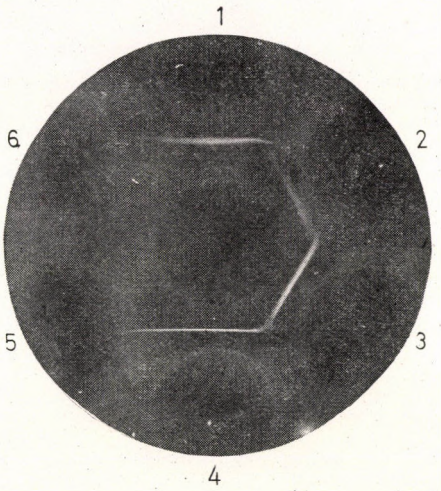

Fig. 6. Centre: anti-rabbit fibrin guinea pig serum. 1: rabbit plasma; 2: rat plasma; 3: human plasma; 4: rabbit plasma; 5: blank; 6: guinea pig plasma

Discussion

BAUER and ENGEL (1912) described that antiox fibrinogen serum reacted with ox fibrinogen and, in a much lower titre, with pig fibrinogen. HEKTOEN and WELKER in 1927 showed that the antigenic elements of mammalian fibrinogen are more or less common, and this is the case also with the fibrinogens of the birds, but mammalian fibrinogen differs immunologically from that of the birds. Similar conclusions were drawn by KYES and PORTER in 1931; according to their investigations an anti-avian fibrinogen rabbit serum readily precipitated avian fibrinogen but failed to precipitate sheep and horse fibrinogen. SAEKI (1931) studied the question by means of the anaphylactic reaction and found that, in guinea pigs sensitized with goat fibrinogen, an anaphylactic shock could be elicited by goat, ox or sheep fibrinogen while dog fibrinogen caused a slight reaction only. In 1936 KOMATU, using the precipitation method, established antigenic relations between the fibrinogens of mammals. KESZTYŰS et al. (1950), SZILÁGYI et al. (1952) as well as BAGDY and SZILÁGYI (1953) demonstrated that mammalian fibrinogen was not related to the avian fibrinogen, and that the various mammalian fibrinogens give cross-precipitation with one another and homologous fibrinogen acts in a higher titre than does the heterologous one. After absorption with heterologous fibrinogen, anti-mammalian fibrinogen rabbit serum shows precipitation with homologous fibrinogen.

According to our investigations, mammalian fibrinogen is not in antigenic relation with avian fibrinogen. However, within the same taxonomic class, a close antigenic relation could be demonstrated between mammalian and avian fibrinogens. Several degrees of the relation reaction type could be observed but an identity reaction was never demonstrated. The anti-fibrin immune sera did not react with the fibrinogen of the animals used for the preparation of the immune serum in spite of the finding that, with an antifibrin serum produced in some other animal, a close relation could be demonstrated between the fibrinogens of the animals in question.

The various mammalian albumins are known to give a cross-precipitation (WEIGLE 1961). Similarly, an antigenic relation was demonstrated between the immune globulins of several animal species (KORNGOLD and LEEUWEN 1960). According to our investigations, the antigenic structure of the fibrinogen of taxonomically remote animals is determined primarily by the species proper, since the fibrinogen of mammals and birds shows no antigenic relation. Among animals taxonomically near to each other, the functional identity already leaves its mark on the molecule's antigenic structure. Within the class of the mammals, the fibrinogens of rodents, carnivores, ungulates and primates are in antigenic relation and also within the class of the birds, the fibrinogens of the Lamellirostres, Gallinaceae, Columbidae and Passeridae are closely related in antigenic structure.

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EFFECT OF HUMAN SERUM ALBUMIN ON RENAL BLOOD FLOW AUTOREGULATION IN THE ANAESTHETIZED DOG

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Following the infusion of 3 g/kg human serum albumin in anaesthetized dogs, renal blood flow increases and the autoregulation of flow deteriorates. Taking into account the effect of HSA on E_{PAH} and on E_{in}, the diminished autoregulation can be explained by the opening of renal shunts.

In recent years several papers have dealt with the effect of human serum albumin on renal haemodynamics and function in anaesthetized dogs (GÖMÖRI et al. 1964, 1969). In these experiments, an increase in renal blood flow was observed together with unchanged or even diminished blood pressure. Recently, NAVAR et al. (1966) have reported on elevated renal blood flow and deteriorated autoregulation following the infusion of hypertonic NaCl, glucose, mannitol or urea. These findings led us to presume that human serum albumin (HSA), by increasing renal blood flow, would have an influence on renal blood flow autoregulation as well.

Methods

The experiments were performed on 12 mongrel dogs of both sexes with a body weight of 12 to 25 kg (mean 16.6 kg), anaesthetized with 0.10 g/kg chloralose. Urine was collected from the ureters exposed by laparotomy. Carotid and aortic (at the level of the renal arteries) blood pressure (BP) was measured by means of polyethylene catheters with Elema-Schönander EMT 35 electromanometers, mean pressures were obtained by electric integration. The left renal artery was exposed through a lumbar incision and flow (RBF) was measured by means of a Nycotron non-cannulating electromagnetic blood flowmeter. The pressure and flow values were registered on a Mingograf 81 polygraph, at a paper speed of 2.5—5.0 mm/sec.

Blood samples for the determination of renal extraction of para-amino-hippuric acid (E_{PAH}), inulin (E_{in}), and of the arteriovenous oxygen difference ($A\text{-}V_{rcn}\ O_2$ diff.) were taken through catheters placed in the left renal vein under fluoroscopic control. The left testicular or ovarian vein had previously been ligated. The proper place of the catheter in the renal vein was controlled throughout the experiment.

For the study of RBF autoregulation at higher pressure ranges the carotic sinuses were denervated in six dogs.

Serum PAH and inulin levels were estimated according to BRATTON and MARSHALL (1939) and HARRISON (1942), respectively. Serum total protein, albumin and globulin were determined by the biuret method. Tubular PAH transport (T_{PAH}) and tubular load of PAH (L_{PAH}) were calculated according to HARVEY (1966). Glomerular filtration rate (GFR) was expressed as the product of E_{in} and renal plasma flow (RPF).

Oxygen saturation of blood samples was measured by the Kipp hemoreflector (Typ MO 1). Arterial haematocrit values (Htc) were determined in the Hawskley microhaematocrit

centrifuge (12 000 g, 5 min). From the haematocrit value the haemoglobin concentration was read by means of a nomogram. In the knowledge of haemoglobin concentration and oxygen saturation, the oxygen content of the blood samples was expressed in volume per cent. Oxygen consumption of the left kidney was calculated as $Qo_2 = RBF \cdot A-V_{ren}\ O_2$ diff.

The course of the experiment was as follows. Having completed the necessary preparations and cannulations, an infusion of PAH in physiological saline was started at a rate of 2 ml/min. For the investigation of RBF autoregulation, renal perfusion pressure was diminished in 10—20 mm Hg steps by means of a Blalock clamp placed on the aorta above the renal arteries. Each step was taken only after the flow had stabilized. Renal perfusion pressure was usually diminished to 25—30 mm Hg. After the sudden release of the clamp the ensuing maximal flow was registered, then following a pause of 10—15 minutes, the pressure-flow relationship was registered again. This was followed by 2—3 periods lasting 5 minutes each, during which urine was collected and blood samples were taken for $A-V_{ren}\ O_2$ diff. from six animals, for E_{PAH} from five animals, and for E_{in} from four animals.

Thereafter a 25% solution of HSA in physiological saline was rapidly (4—8 min) infused in a total amount of 3 g/kg body weight. The infusion was followed by two 5 min periods during which the aforementioned extraction rates were estimated. Then the pressure-flow relationships were again registered while reducing aortic BP stepwise.

The data appearing in Table I were recalculated for 100 g kidney weight, and were drawn from the averages of control and experimental periods, respectively. The changes were evaluated by Student's *t*-test.

For calculating renal vessel conductance, the $C_{ren} = RBF/BP$ equation was used; renal venous blood pressure was considered zero.

In the evaluation of pressure-flow relationships (Fig. 2), the individual data above and under 90 mm Hg were treated separately. Statistical correlation was calculated as well as regression lines drawn according to BLISS (1967). To delineate the near-maximal dilatation of the renal vasculature, flow values belonging to the lowest perfusion pressures were treated together with the flow maxima registered after the sudden release of the clamp. Correlation coefficients and the level of probability of distribution were evaluated according to FISHER and YATES (1963).

It should be noted that data obtained in 5 other animals have been omitted, because we could not detect autoregulation even under control conditions.

Results

Data referring to renal haemodynamics and function are shown in Table I.

1) Effect of HSA on the blood constituents. Administration of 3 g/kg body weight of HSA resulted in an increase of the total blood protein level; this was mainly due to the elevation of serum albumin. The haematocrit value decreased.

2) Effect of HSA on renal haemodynamics. Blood pressure. Soon after the beginning of the HSA infusion BP decreased by 20—30 mm Hg, later to approach the control levels.

RBF. The early decrease in BP was accompanied by an increase in RBF which persisted for a long time. Thus, the elevated blood flow in face of a diminished perfusion pressure indicated renal vasodilatation, i.e. better conductance.

3) Effect of HSA on certain parameters of renal function. In spite of the augmented RBF, urine excretion diminished on both sides, and in some experiments it ceased for several minutes. The decrease of urine flow was greater on the left side, presumably due to previous manipulation. The reduction of GFR was proportional to that of urine excretion, but at the same time C_{PAH} (E_{PAH} ·

Table I

	n	Control	After HSA infusion	Within sample standard deviation
Blood pressure (BP) mm Hg	6	143	119**	19.25
Renal blood flow (RBF) ml/min/100 g kidney	6	356	473**	151.6
Renal conductance (C_{ren}) PCU = RBF : BP	6	2.47	3.91***	0.955
Renal extraction of PAH (E_{PAH})	5	0.687	0.434**	0.115
Renal extraction of inulin (E_{in})	4	0.311	0.091***	0.08
Renal arteriovenous O_2 diff. (A—$V_{ren}O_2$ diff.) vol percent	6	2.43	1.78	0.73
Urine flow, left side (V_{left}) ml/min/100 g kidney	6	1.28	0.57*	1.16
Urine flow, right side (V_{right}) /ml/min/100 g kidney	6	1.42	0.92*	1.31
Arterial haematocrit (Htc)	6	0.465	0.397**	0.099
Serum albumin concentration g per 100 ml	5	2.22	4.20***	0.60
Total protein concentration g per 100 ml	5	4.72	6.58**	0.55
Glomerular filtration rate (GFR) ml/min/100 g kidney	4	47.5	24.2***	21.15
PAH clearance (C_{PAH}) ml/min/100 g kidney	5	125.96	123.70	56.78
Tubular transport of PAH (T_{PAH}) mg/min/100 g kidney	4	2.67	3.22	1.10
Tubular load of PAH (L_{PAH}) mg/min/100 g kidney	4	3.72	7.04*	2.19
Renal oxygen consumption ml/min/100 g kidney	6	8.24	8.15	3.64

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

· RPF + V) did not change. The increase in RBF did not result in an appropriate increase of renal O_2 consumption. Nevertheless, A—V_{ren} O_2 diff. virtually showed no change, the 27% decrease failed to reach statistical significance.

According to the change in RBF, the tubular PAH load increased. The amount of PAH in mg/min transported by the tubular cells (T_{PAH}) was not altered.

4) Effect of HSA on RBF autoregulation. As mentioned above it was in 12 out of 17 dogs where complete autoregulation was manifest. One typical experiment appears in Fig. 1 which clearly shows that up to a perfusion pressure of 90 mm Hg there is no difference in the pressure-flow relationship between control and experimental conditions. On the contrary, after HSA infusion and above 90 mm Hg BP higher flow values belong to identical perfusion pressures. Thus, these results point to a diminished flow autoregulation.

Under control conditions in the pressure range above 90 mm Hg perfusion pressure, the mean increase in flow to each 1 mm Hg increment of perfusion

pressure was 1.10 ± 0.72 ml/min (n = 19). On this basis, blood flow autoregulation was considered diminished or absent, when the increase of flow was higher than 1.82 ml/min (mean + 1 SD of the control). Among the 26 pressure flow curves following HSA infusion 14 exhibited an increase in flow of more than 1.82 ml/min/mm Hg, with a mean of 2.78 ± 1.04 ml/min/mm Hg. The remaining 12 curves showed a good autoregulation, the mean increase having been 1.14 ± 0.51 ml/min/mm Hg. It should be noted, however, that

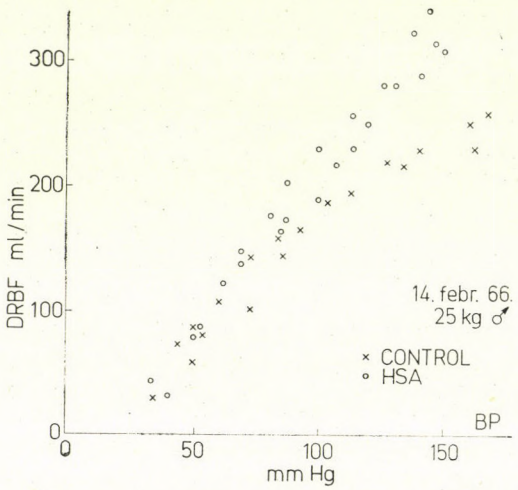

Fig. 1. Effect of HSA on renal blood flow autoregulation. A typical experiment

the calculations of the regression lines were based on all available data in order to gain a better approach to the events expected to follow HSA infusion.

The regression lines (Fig. 2) and equations shown in Table II represent the individual pressure-flow data of 19 control and 26 HSA autoregulatory "steps" carried out on 12 animals.

Under control conditions (Y'_c) in the pressure range of 90—180 mm Hg (n = 98) RBF is fully autoregulated. Flow through the kidney is independent of perfusion pressure, the regression coefficient does not differ from 0 ($p > 0.10$).

Following HSA infusion (Y'_{HSA}) higher pressures are accompanied by increasing flow values (n = 124); flow is correlated to pressure at the 5% level of significance.

In the pressure range below 90 mm Hg, both control (Y''_c) (n = 58) and HSA flow values (Y''_{HSA}) (n = 67) are highly correlated with pressure ($p = 0.001$), there is no difference between the two correlation coefficients ($p > 0.90$).

The Y^0 regression lines connect flow values belonging to the lowest perfusion pressures (n = 37) with the highest ones after the sudden release of

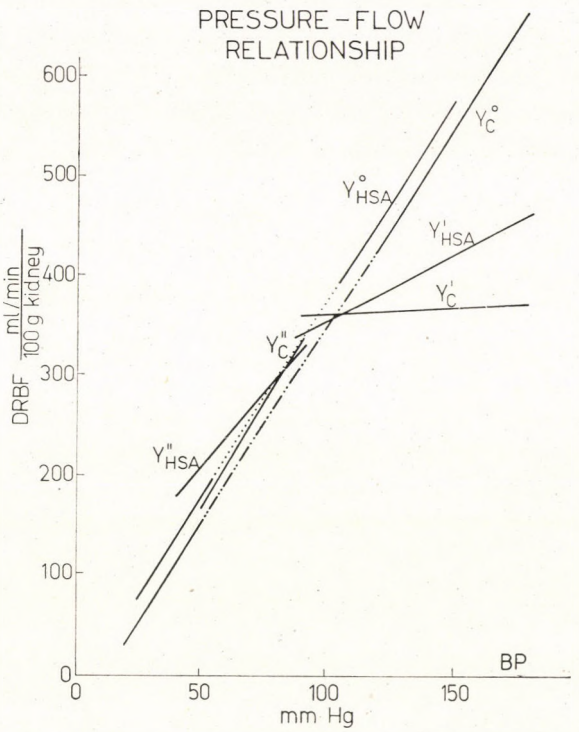

Fig. 2. Regression lines based on equations of Table II

Table II

Pressure-flow relationship

	n	Correlation	$Y = a + b\,(\pm s_b)x$	$\bar{x} \pm s_{\bar{x}}$	$\bar{y} \pm s_{\bar{y}}$
Blood pressure > 90 mm Hg					
Control	98	0.0374	$Y'_C = 339.19 + 0.19\ (\pm 0.51)x$	128.1±2.5	363.4±12.5
After albumin infusion	124	0.2270*	$Y'_{HSA} = 219.66 + 1.34\ (\pm 0.52)x^*$	125.9±2.2	389.0±13.3
Blood pressure < 90 mm Hg					
Control	58	0.5699***	$Y''_C = -44.40 + 4.22\ (\pm 0.81)x^{***}$	68.7±1.8	245.5±20.6
After albumin infusion	67	0.3983***	$Y''_{HSA} = 49.10 + 3.15\ (\pm 0.91)x^{**}$	67.7+1.7	262.4±4.13
Reactive hyperaemia					
Control	37	0.4178**	$Y^0_C = -50.87 + 3.94\ (\pm 0.45)x^{***}$	90.9±9.6	307.0±45.7
After albumin infusion	52	0.3954**	$Y^0_{HSA} = -27.73 + 3.98\ (\pm 0.44)x^{***}$	83.3±6.9	303.8±34.6

Signs of statistical significance regarding correlations and coefficients of regression:

No sign = $p > 0.05$
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

the clamp ($n = 52$). The Y^0 lines are practically parallel and the corresponding regression coefficients are nearly identical ($p > 0.80$), a fact suggesting that HSA has no effect on the maximal dilatation of the renal vessels.

Discussion

The data obtained in this experiment seem to prove that HSA has a deleterious effect on RBF autoregulation. It should, however, be emphasized that HSA deteriorated the autoregulation but did not abolish it. This is clearly seen on the slope of regression line Y'_{HSA} (Fig.2) which is between the control and Y^0 lines, the latter representing the completely passive pressure-flow relationship. The position of regression line Y'_{HSA} indicates a certain degree of vasodilatation in the kidney at least in the pressure range above 90 mm Hg. It is noteworthy that the vasodilator action of HSA is not specific to the kidney, as indicated by previous experiments (GÖMÖRI et al. 1964).

It is well-known that RBF autoregulation is influenced by certain drugs. Concerning the effect of vasoconstrictive materials, the experimental results are highly controversial. According to WAUGH (1964) angiotensin II impairs the autoregulatory ability but BELLEAU and EARLEY (1967) demonstrated unchanged autoregulation while infusing angiotensin or renin. In NAHMOD and LANARI's experiments arterenol diminished RBF without interfering with autoregulation. On the contrary, WINTON (1964) showed a deterioration of the autoregulatory tendency following epinephrine and cooling. Epinephrine was observed completely to abolish autoregulation by FOLKOW and LANGSTON (1964) and by ONO et al. (1966). In contrast, SCHMID et al. (1964) were able to restore autoregulation which had disappeared in the course of the experiments by the application of pressor agents. On the other hand, according to ONO et al. (1966), in the spontaneously non-autoregulating kidney, flow autoregulation can be restored by vasodilating agents. Autoregulation could not be demonstrated when sympathetic tone increased following the elevation of intracranial pressure (FOLKOW and LANGSTON 1964).

While the findings concerning the effect of vasoconstrictor drugs on RBF autoregulation are contradictory, they are uniform as regards the vasodilating agents. Increased RBF and lessened or abolished autoregulation of flow can be achieved by the administration of papaverine (THURAU and KRAMER 1959; ONO et al. 1966), theophyilline (ONO et al. 1966), acetylcholine (NAHMOD and LANARI 1964; ONO et al. 1966), procaine (WAUGH and SHANKS 1960), or adenosinetriphosphate (HARVEY 1964). The connection between augmented RBF and weakened autoregulation was clearly shown by NAVAR et al. (1966) who have studied the initiating feed-back mechanism of autoregulation; in their experiments the infusion of hyperosmotic solutions was followed by an increase in RBF and diminished autoregulation. The progressive

disappearance or autoregulation was proportional to the increase of plasma osmolality.

In the present experiments the infusion of HSA resulted in a better conductance of renal vessels and, in the majority of cases, in the elevation of RBF together with a decrease in blood flow autoregulation. Considering the aforementioned data on the effect of vasodilator substances on RBF and renal autoregulation, these results were not surprising. The question of the mode of action of HSA, however, remained unanswered. To solve the problem the following should be kept in mind.

1) Effect of HSA on E_{PAH}. HSA in sufficient dose and concentration leads to a decrease of E_{PAH} (CARGILL 1948; ELPERS and SELKURT 1963; GÖMÖRI et al. 1964). In the deterioration of E_{PAH}, tubular damage has no role since Tm_{PAH} was normal (ELPERS and SELKURT 1963). In the present experiments, too, a tubular damage seemed improbable as T_{PAH} did not change following the infusion of HSA.

2) Effect of HSA on glomerular filtration. In our experiments, E_{in} as well as diuresis diminished, the latter even ceased in some cases. There is one factor which cannot be neglected in the interpretation of these findings, namely the pronounced change in plasma colloid osmotic pressure. Following HSA infusion the serum albumin concentration increased to twofold, therefore it seems justified to presume a marked fall in effective filtration pressure leading to the decrease of E_{in}.

3) Effect of HSA on renal O_2 *consumption.* In the group mean value no change could be observed in Q_{O_2}. Since, according to DEETJEN and KRAMER (1961) the most important factor determining renal O_2 consumption is the reabsorption of sodium, in the present experiments two factors acting in opposite directions must be considered. One factor is the decreased amount of filtered sodium which would lead to a decrease of O_2 consumption, the other is the peculiar effect of the albumin itself, which increases sodium reabsorption (NIZET 1968).

4) Effect of HSA on the intrarenal distribution of blood flow. If, as proposed by REUBI (1958), C_{PAH} is accepted as a measure of cortical blood flow, in the present experiments no intrarenal flow redistribution could be observed.

All the above results are compatible with the hypothesis that following HSA infusion part of RBF passes through functionally inert tissue, i.e. through shunts. This possibility is supported by some recent experiments in which GÖMÖRI et al. (1969) have shown that following HSA infusion RBF is augmented (electromagnetic flowmeter) without affecting the nutritive flow (^{86}Rb uptake).

Several reports have stressed the role of plasma osmotic pressure in the regulation of the microcirculation (STAINSBY 1964; FROHLICH 1966; NAVAR et al. 1966; MELLANDER et al. 1967). The increase in plasma osmolality causes

dilatation of resistance vessels and precapillary sphincters, while venous resistance and capillary permeability changes slightly.

Thus, we think it justified to postulate that the flow increase together with the deterioration of renal autoregulation following HSA infusion is connected with vasodilatation and/or opening of renal cortical shunts.

Acknowledgement

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THERMOREGULATORY EFFECTS OF HYPOXIA IN NEW-BORN RATS AND THEIR CORRELATION TO BODY TEMPERATURE AND HEAT PRODUCTION PRIOR TO AND AFTER EXPOSURE TO HYPOXIA

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Rats aged less than 12 hours, 1—2 days, 4—10 days and 11—20 days were subjected at an ambient temperature below the thermoneutral zone to hypoxia by breathing 12% O_2 in N_2 for 30 min. Oxygen consumption and colonic temperature were recorded continuously for 30 min prior to, during, and after, the termination of hypoxia. At an ambient temperature of 30°C no correlation could be detected between body temperature prior to hypoxia and the subsequent fall in heat production during hypoxia, whereas a significant correlation was found between colonic temperature before exposure to hypoxia and the response of body temperature to hypoxia. In rats aged less than 12 hours, 1—2 days, and 11—20 days, no correlation could be detected between oxygen consumption after the termination of hypoxia and the concomitant increase in body temperature, whereas in the group aged 4—10 days a clearcut correlation was established. In rats aged 11—20 days with colonic temperatures above 34°C prior to exposure to hypoxia, no correlation could be found between the fall in body temperature during hypoxia and the reduction of heat production in hypoxia or the level of heat production during hypoxia. The results are interpreted to indicate that, in the new-born rat, the fall in body temperature in response to hypoxia and the increase in body temperature after the termination of hypoxia are not a simple consequence of changes in heat production, but also of variations in the activity of the mechanisms of physical regulation.

Exposure of new-born rats to ambient temperatures below their thermoneutral zone led to the conclusion that the profound fall in body temperature observed under these conditions is not a simple consequence of a failure to increase heat production further, but must be attributed to the operation of a regulatory mechanism characteristic of the new-born rat (VÁRNAI 1970a; VÁRNAI and DONHOFFER 1970). For the sake of further analysis it was deemed advisable to investigate thermoregulatory changes evoked without changing the thermal environment. At temperatures below the thermoneutral zone, both hypoxia and hypercapnia comply with this requirement.

LINTZEL (1931) demonstrated conclusively that, at temperatures below the neutral zone a reduction of barometric pressure is followed in the adult rat by a fall in heat production and body temperature. This observation has been confirmed many times and extended to adults and new-borns of other species. Reduction of the oxygen content of the respired air has been shown to produce in the appropriate thermal environment a fall in oxygen consumption and body

temperature in the human neonate (CROSS et al. 1955, 1958; ENGELSON et al. 1956; BRODIE et al. 1957), in the new-born monkey (DAWES et al. 1960), in the new-born dog (MOORE 1956a), in the new-born kitten (HILL 1958, 1959; MOORE 1956b, 1959), in the new-born rabbit (ADAMSONS 1959), and in the new-born rat (MOUREK 1959; TAYLOR 1960).

The effect of hypoxia on oxygen consumption and on body temperature is closely linked to thermoregulatory heat production since the response is confined to ambient temperatures below the thermoneutral zone. A detailed investigation of these responses in the new-born rat appeared, therefore, a promising tool for gaining further insight into thermoregulatory mechanisms.

Methods

New-born rats from the Institute's colony, aged from a few hours up to 20 days, were used. Oxygen consumption and CO_2 production were measured with a Noyons—Kipp diaferometer as modified by DONHOFFER et al. (1958) with the further modification of VÁRNAI (1970b) allowing to reduce air flow to 300—700 ml per min. Oxygen consumption was calculated for unit body surface, according to the formula $10\sqrt[3]{kg^2}$. The 12% O_2 in N_2 gas mixture was prepared in double-walled plastic bags, the outlet of which could be attached to the inlet of the apparatus. Since the volume of the chamber and that of the connecting tubing was considerably greater than the volume of the tubing leading directly to the measuring block, any change at the inlet reached the measuring block through the chamber with a delay as compared to that flowing directly to the measuring block. Therefore, measurements had to be suspended whenever the composition of the supplied gas had been changed until about four times as much of the new gas mixture had passed through the chamber as the volume of the chamber plus the connecting tubing. Even with air-flows as small as 300 or 400 ml per min, measurement had to be suspended only for 2 to 3 min. Measurements were discontinued also for a few minutes at intervals of 30 to 60 min, to establish the base-line. In many instances this was done immediately after and/or just prior to a change from air to 12% oxygen, or from 12% O_2 to air. Colonic temperature was measured continuously by copper-constantan thermocouples at a depth of 0.5 to 2.0 cm from the anus, according to age.

About an hour before starting the measurements the small cage with the rat(s) was introduced into the metabolic chamber (VÁRNAI 1970b) and the latter was submerged into a water bath of appropriate temperature. According to age, one to seven rats were introduced simultaneously into the metabolic chamber, usually, however, not more than 4 or 5 animals were used even in the two younger age-groups. When more than one animal was used simultaneously, for technical reasons colonic temperature was measured in one animal only. The effect of hypoxia was studied at ambient temperatures of 30 and 25°C, the same animals being subjected to hypoxia only at one of the two temperatures. Rats exposed to hypoxia at an ambient temperature of 30°C, were, however, often exposed previously to an ambient temperature of 25°C, breathing air. Oxygen consumption and colonic temperature were recorded in every experiment for 30 min supplying room air to the metabolic chamber. This was followed by supplying 12% oxygen for 30 min, then room air was supplied for another 30 min. For statistical evaluation, STUDENT's *t*-test was used. In part, PEARSON's product-moment coefficient of correlation *(r)* and significance of the regression coefficient was also calculated (LACEY 1953).

Results

Effect of hypoxia at an ambient temperature of 30°C. In response to hypoxia, oxygen consumption fell significantly ($p < 0.01$) during the first 12 hours of extrauterine life at an ambient temperature of 30°C, a temperature well below the neutral one of the new-born rat (35—36°C). The reduction in heat

production was even more impressive in the second 24 hours ($p < 0.001$) and remained of the same order of magnitude up to the end of the third week of life. Switching from 12% oxygen to room air was followed by an equally significant increase in heat production in all age-groups (Fig. 1). In some experiments, especially in older animals, heat production rose well above the level observed prior to exposure to hypoxia.

Essentially the same can be said of the effect of hypoxia on colonic temperature (Fig. 2), albeit in the youngest age-group neither the decrease

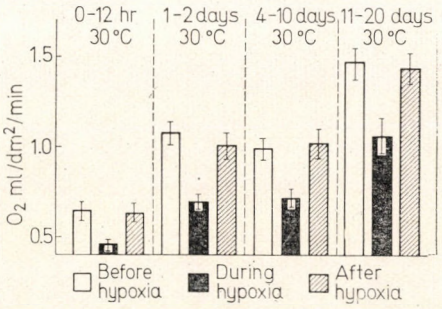

Fig. 1. Oxygen consumption of rats aged less than 12 hours (n = 14), 1—2 days (n = 25), 4—10 days (n = 16), and 11—20 days (n = 19), before, during and after breathing 12% O_2 in N_2 at an ambient temperature of 30°C. For details, see text

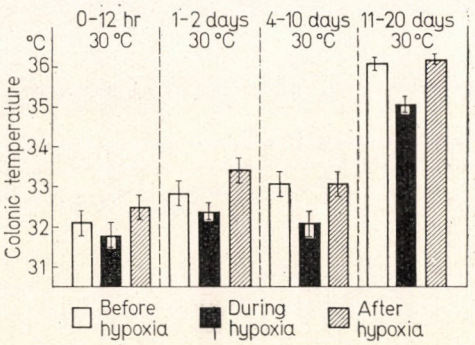

Fig. 2. Colonic temperature of rats aged less than 12 hours (n = 14), 1—2 days (n = 25), 4—10 days (n = 19), and 11—20 days (n = 19), at an ambient temperature of 30°C before changing from breathing room air to 12% O_2 in N_2, after breathing 12% O_2 for 30 min, and 30 min after restoration of room air. For details, see text

during hypoxia, nor the increase after reverting from 12% oxygen to room air was significant statistically. This was due, in part, to a greater variability in colonic temperature and, even more important, to the fact that in the youngest age-group colonic temperature exceeded ambient temperature on the average by slightly more than 2°C, and so a considerable fall in colonic temperature could not be expected. In the second age-group, the fall in colonic temperature failed to reach statistical significance, the increase after reverting to air breathing was, however, significant statistically ($p < 0.01$). Analysis of the individual experiments showed convincingly that both the decrease in colonic temperature during hypoxia and the increase after the termination of hypoxia were real also in the majority of the youngest animals. In the two older age-groups the decrease in body temperature during hypoxia was significant statistically ($p < 0.05$ and $p < 0.001$, respectively) and so was the increase after restoration of air breathing.

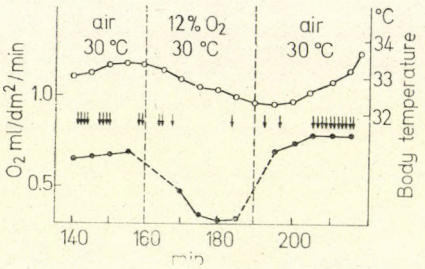

Fig. 3. Response of a litter of 4 rats aged less than 12 hours to breathing 12% O_2 in N_2. Colonic temperature was measured in one rat only. Oxygen consumption represents the mean of the four rats. Arrows indicate motor activity

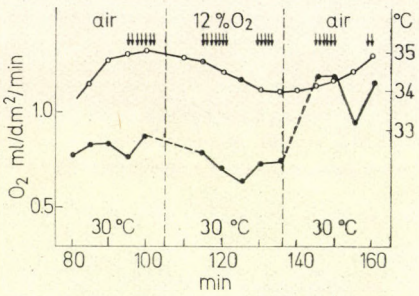

Fig. 4. Response to breathing 12% O_2 in N_2 of a rat aged 13 days. Arrows indicate motor activity

Fig. 3 originates from an observation on a litter of four rats aged somewhat less than 12 hours. There can be no doubt that both heat production and body temperature decreased during hypoxia and increased to a higher level after restoring room air than that observed prior to exposure to hypoxia.

Fig. 4 demonstrates an observation on a single rat aged 13 days. The fall in colonic temperature during hypoxia was of the same magnitude as in the younger animal in Fig. 3; the reduction of heat production was relatively small, the increase in heat production after reverting to air breathing was,

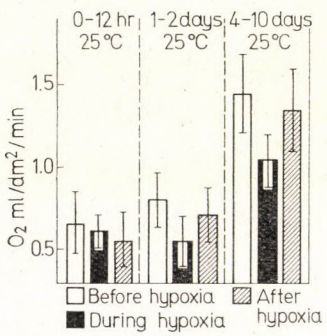

Fig. 5. Oxygen consumption of rats aged less than 12 hours (n = 6), 1—2 days (n = 4), and 4—10 days (n = 5) before, during, and after breathing 12% O_2 in N_2 at an ambient temperature of 25°C. For details, see text

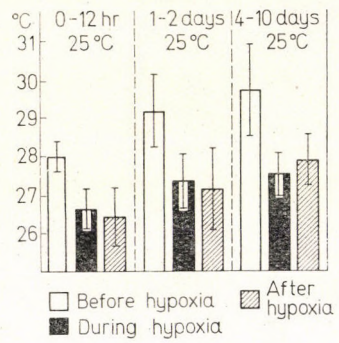

Fig. 6. Colonic temperature of rats aged less than 12 hours (n = 6), 1—2 days (n = 4), and 4—10 days (n = 5), before exposure to 12% O_2 in N_2, after breathing 12% O_2 for 30 min, and 30 min after switching back to room air, at an ambient temperature of 25°C. For details, see text

however, impressive. It should be noted that motor activity during this period was, if anything, less intense than during hypoxia.

Effect of hypoxia at an ambient temperature of 25°C. Figs 5 and 6 demonstrate results obtained at an ambient temperature of 25°C. The scatter of the results of different experiments was much greater within each age-group than at the ambient temperature of 30°C. This, together with the small number of the observations, accounts for the lack of statistically significant differences between the means. Statistical significance had actually been reached only by the difference between colonic temperature before and during hypoxia in the youngest age group. The results were, however, considerably more uniform than the means and standard errors would have suggested. Nevertheless,

Table I

Changes in oxygen consumption and colonic temperature after reverting from 12% oxygen to air breathing at an ambient temperature of 25°C
(unchanged = change in oxygen consumption attaining not more than 5%, in colonic temperature not more than 0.1°C)

Age	Oxygen consumption			Colonic temperature		
	Increased	Unchanged	Decreased	Increased	Unchanged	Decreased
< 12 hrs n = 6	1	1	4	∅	1	5
1—2 days n = 4	4	∅	∅	∅	2	2
4—10 days n = 5	5	∅	∅	4	1	∅

since colonic temperature was still declining before exposure to hypoxia, the results were not easy to interpret; however, the decline in body temperature was evidently accelerated by the exposure to hypoxia also in these cases. Since at an ambient temperature of 25°C, heat production and body temperature decreased during hypoxia in all observations, only the changes after reverting from 12% oxygen to air are recorded in Table I.

The increase in heat production after reverting to air breathing, consistently observed in all age-groups at an ambient temperature of 30°C, was observed in the youngest age-group in one experiment only. In another experiment, heat production remained unchanged, and in four it declined even further. In the two older age-groups oxygen consumption increased in all experiments and in the oldest age-group approached or had reached the level observed prior to exposure to hypoxia. In contrast to the observations at an ambient temperature of 30°C, colonic temperature failed to increase after the termination of hypoxia in the two younger age-groups. In rats aged less than 12 hours, body temperature continued to decline after restoration of air breathing in 5 observations, and stayed at the level reached during hypoxia in one. Essentially similar results were obtained up to the age of two days. In the age-group

of 4 to 10 days, colonic temperature increased after hypoxia in four, remained unchanged in one experiment, and declined further in none. The increase in body temperature after hypoxia was, however, small and colonic temperature was after 30 min well below the level observed prior to hypoxia. Figs 7 and 8 demonstrate experiments from the two older age-groups. In the experiment with one day old rats (Fig. 7), heat production increased after reverting to air breathing, while colonic temperature continued to decline slowly until it

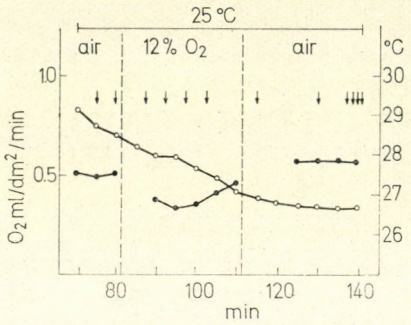

Fig. 7. Response to breathing 12% O_2 at an ambient temperature of 25°C of a litter of 7 one day old rats. Colonic temperature was measured in one rat only; oxygen consumption represents the mean of the 7 rats. Arrows indicate motor activity

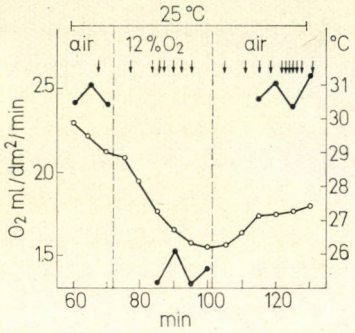

Fig. 8. Response to breathing 12% O_2 at an ambient temperature of 25°C of 2 seven days old rats. Colonic temperature was measured in one animal only; oxygen consumption represents the mean of the two rats. Arrows indicate motor activity

had stabilized about 1.5°C above the ambient temperature. In the 7 day old rats (Fig. 8) both heat production and colonic temperature increased after changing the 12% oxygen for room air.

At an ambient temperature of 25°C, the effect of hypoxia on heat production and body temperature was, therefore, essentially the same as at the environmental temperature of 30°C. The explanation for the absence of an increase in body temperature and, in the youngest age-group, in heat production after termination of hypoxia can be sought in the profound fall in body temperature. Prolonged exposure to an ambient temperature of 25°C in itself may reduce body temperature to a level approaching that below which heat production diminishes in the adult animal; hypoxia may reduce body temperature below this level. At colonic temperatures of less than 28°C, few adult animals are able to raise their body temperature without transfer to a warmer environment and, apparently, the same applies to the new-born, the level being here probably lower. For instance, in the rat represented in Fig. 8 colonic temperature had fallen almost to 26°C before the termination of hypoxia and the animal was still able to increase its body temperature and heat production thereafter at the same ambient temperature.

Effect of body temperature on the response to hypoxia. Many of the rats exposed to hypoxia at an ambient temperature of 30°C had been exposed earlier to an environmental temperature of 25°C, albeit in no instance to hypoxia at this temperature. It has, however, been shown that after re-transfer from 25°C to 30°C, body temperature recovered at a variable rate and had in 30 min usually not reached the level observed at the same ambient temperature before exposure to 25°C (VÁRNAI and DONHOFFER 1970). Considering the observation that a number of animals with very low colonic temperatures before exposure to hypoxia failed to respond to hypoxia with a fall in body temperature and that in some of them colonic temperature even increased during hypoxia, as well as the fairly wide variation of colonic temperature prior to hypoxia, it was deemed advisable to analyse the effects of hypoxia on oxygen consumption and colonic temperature also according to body temperature before exposure to hypoxia.

No significant correlation between colonic temperature and oxygen consumption could be demonstrated in any of the groups by simply dividing in each group the observations into two samples according to colonic temperature prior to exposure to hypoxia.

As the arbitrary dividing line a colonic temperature of 32°C was chosen for the rats aged less than 12 hours and 33°C for other age-groups (Table II, Part A). By calculation of the coefficient of correlation (r) and of the signifi-

Table II

Response of oxygen consumption to exposure to hypoxia according to colonic temperature before the exposure at the same ambient temperature (30°C)
In brackets, number of observations

A

Age	Colonic temperature	Oxygen consumption %	Colonic temperature	Oxygen consumption %	p
< 12 hrs	> 32°C (9)	−34.7 ± 4.0	< 32°C (7)	−22.4 ± 6.0	n.s.
1—2 days	> 33°C (11)	−39.0 ± 3.6	< 33°C (11)	−34.0 ± 3.0	n.s.
4—10 days	> 33°C (17)	−28.3 ± 2.6	< 33°C (12)	−32.0 ± 3.0	n.s.
11—20 days	> 33°C (23)	−27.3 ± 2.7	< 33°C (2)	−29; − 14	n.s.

B

	Coefficient of correlation (r)	Significance of coefficient of regression
<12 hrs (16)	−0.690 $p < 0.01$	$t = 3.20$ $p < 0.01$
1— 2 days (22)	−0.328 n.s.	$t = 1.54$ $p > 0.1$
4—10 days (29)	+0.169 n.s.	$t = 1.23$ $p > 0.2$
11—20 days (25)	−0.236 n.s.	$t = 1.15$ $p > 0.2$

cance of the coefficient of regression, correlation of body temperature prior to hypoxia and the reduction in oxygen consumption during exposure to hypoxia could, however, be established in the group aged less than 12 hours, but in none of the older age-groups (Table II, Part B). In some observations on rats aged less than 12 hours, however, both body temperature and oxygen consumption were low already before exposure to hypoxia.

In contrast, a marked correlation of the response of body temperature to hypoxia and of the colonic temperature before exposure to hypoxia was evident both by the simple comparison employing a dividing line (Table III),

Table III

Response of colonic temperature to exposure to hypoxia according to body temperature before exposure at the same ambient temperature (30°C)

In brackets, number of observations

Age	Colonic temperature before hypoxia	Change in hypoxia in °C	Colonic temperature before hypoxia	Change in hypoxia	p
< 12 hrs	> 32°C (9)	−0.52±0.17	< 32°C (7)	−0.03±0.07	< 0.02
1—2 days	> 33°C (11)	−0.86±0.16	< 33°C (11)	+0.23±0.17	< 0.001
4—10 days	> 33°C (17)	−0.65±0.13	< 33°C (12)	−0.01±0.19	< 0.01
11—20 days	> 33°C (23)	−1.00±0.09	< 33°C (2)	+0.65; +0.65	—

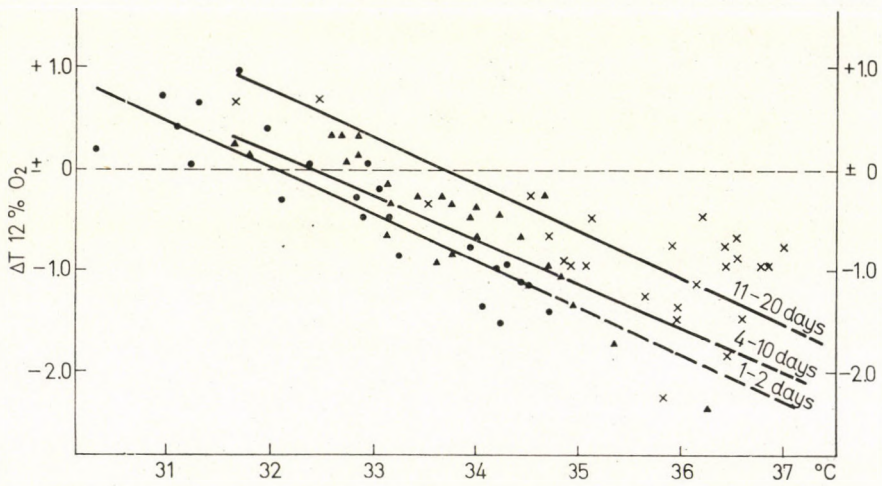

Fig. 9. Scatter-diagram of the response of colonic temperature to breathing 12% O_2 at an ambient temperature of 30°C according to body temperature before exposure to hypoxia. Coefficients of correlation *(r)* were −0.89 ($p < 0.001$), −0.74 ($p < 0.001$), and −0.42 ($p < 0.05$) for rats aged 1—2 days (●), 4—10 days (▲), and 11—20 days (×), respectively. The respective coefficients of regression were significant at the same level ($t = 8.6$; 4.1; 2.27, $p < 0.001$; < 0.001; < 0.05)

and by calculating the coefficient of correlation (r) and the significance of the coefficient of regression.

Fig. 9 shows the scatter diagram and the regression lines for the three older age-groups. The results obtained in rats aged less than 12 hours were similar, but neither the coefficient of correlation ($r = -0.407$), nor the coefficient of regression reached statistical significance ($t = 1.23$, $p > 0.2$).

Rats with a low body temperature before exposure to hypoxia, i.e. 30 min after having been re-transferred to an ambient temperature of 30°C following exposure to 25°C, had also a significantly lower body temperature at an ambient temperature of 30°C prior to exposure to 25°C (Table IV), i.e.

Table IV

Colonic temperature of rats at an ambient temperature of 30°C before exposure to 25°C, according to colonic temperature 30 min after re-transfer to 30°C (before exposure to hypoxia)

In brackets, number of observations

Age	Colonic temperature at an ambient temperature of 30°C, 30 min after re-transfer from 25°C	Colonic temperature at an ambient temperature of 30°C prior to exposure to 25°C
Less than 12 hours	> 32°C (6)	33.69 ± 0.29
	< 32°C (7)	33.00 ± 0.18 $0.1 > p > 0.05$
1—2 days	> 33°C (11)	34.39 ± 0.23
	< 33°C (11)	33.51 ± 0.20 $p < 0.01$
4—10 days	> 33°C (17)	34.84 ± 0.20
	< 33°C (12)	33.69 ± 0.16 $p < 0.001$

30 min after transfer to an ambient temperature of 30°C from the thermoneutral environment of 35°C.

Albeit the response of oxygen consumption to hypoxia has been shown not to depend on body temperature, it appeared necessary to examine whether the animals with a low colonic temperature at an ambient temperature of 30°C both before exposure to 25°C and 30 min after re-transfer to 30°C differed in heat production from those with higher body temperatures. At the age of 1—2 days, heat production was significantly lower at an ambient temperature of 30°C both before and after exposure to 25°C in animals with a low colonic temperature than in those with higher body temperatures ($p < 0.05$). In rats aged 4—10 days, the difference in heat production was, however, small and statistically not significant.

Relationship of oxygen consumption and the reduction in body temperature in hypoxia. The fall in body temperature in response to hypoxia is usually interpreted to be the simple consequence of the reduction in heat production. Observations with wide variations in colonic temperature are not well suited for the analysis of the relationship of heat production and the body temperature

response to hypoxia. Evidently, animals displaying a low colonic temperature before exposure to hypoxia could be expected to show a lesser fall in response to hypoxia, if for no other reason than simply because of the considerably smaller difference between the colonic and the ambient temperature. The problem was, therefore, approached by analysing the responses of a more homogeneous group of animals, i.e. rats aged between 11 and 20 days with colonic temperatures above 34°C before exposure to hypoxia. Correlation between heat production and the response of body temperature was tested by comparing *a)* the percentual reduction in oxygen consumption during hypoxia, *b)* the absolute reduction in oxygen consumption, and *c)* the level of oxygen

Table V

Relationship between a) *percentual reduction in oxygen consumption;* b) *absolute reduction in heat production, and* c) *level of heat production during hypoxia, on the one hand, and the concomitant reduction in body temperature, on the other hand, in rats aged 11 to 20 days with a colonic temperature above 34°C prior to exposure to hypoxia (n = 22)*

	Coefficient of correlation	Significance of coefficient of regression
Percentual reduction in oxygen consumption and the concomitant fall in body temperature	$r = +0.391$ n.s.	$t = 1.95$ $p > 0.05$
Reduction in oxygen consumption in ml/dm²/min and reduction in body temperature	$r = +0.298$ n.s.	$t = 1.41$ $p > 0.1$
Level of oxygen consumption in hypoxia and the fall in colonic temperature	$r = +0.191$ n.s.	$t = 0.86$ $p > 0.2$

consumption in hypoxia with the concomitant fall in body temperature (Table V), but no correlation could be demonstrated.

Correlation of oxygen consumption and the increase in colonic temperature after the termination of hypoxia. By using as basis of comparison the dividing line of a colonic temperature of 33°C before exposure to hypoxia it could be shown that rats with a colonic temperature below 33°C before exposure to hypoxia consume less oxygen after the termination of hypoxia than those with higher body temperatures prior to hypoxia (Table VI). However, the increase in body temperature differed significantly only in the age-group of 4 to 10 days. Considering the limits of the procedure of taking an arbitrary dividing line, the results were subjected to a more searching analysis. Fig. 10 shows the scatter-diagram of the changes in body temperature 30 min after the termination of hypoxia according to oxygen consumption during the 30 min period after restoration of air breathing. In the age-group of 4 to 10 days the correlation between oxygen consumption and the increase in body temperature, already suggested by the data in Table VI, was confirmed, whereas no correlation

Table VI

Oxygen consumption and change in body temperature after termination of hypoxia according to colonic temperature before exposure to hypoxia.

Figures for oxygen consumption represent the means of the 30 min period following termination of hypoxia, colonic temperatures represent those reached at the end of the 30 min. In brackets, number of observations

Age	Colonic temperature before exposure to hypoxia	Oxygen consumption after termination of hypoxia		Colonic temperature 30 min after hypoxia	
1—2 days	> 33°C (11)	1.10 ± 0.11	$p < 0.05$	+1.09 ± 0.17	$p > 0.3$
	< 33°C (11)	0.81 ± 0.04		+0.86 ± 0.13	
4—10 days	> 33°C (17)	0.92 ± 0.05	$p < 0.05$	+1.33 ± 0.17	$p < 0.01$
	< 33°C (12)	0.74 ± 0.05		+0.69 ± 0.13	

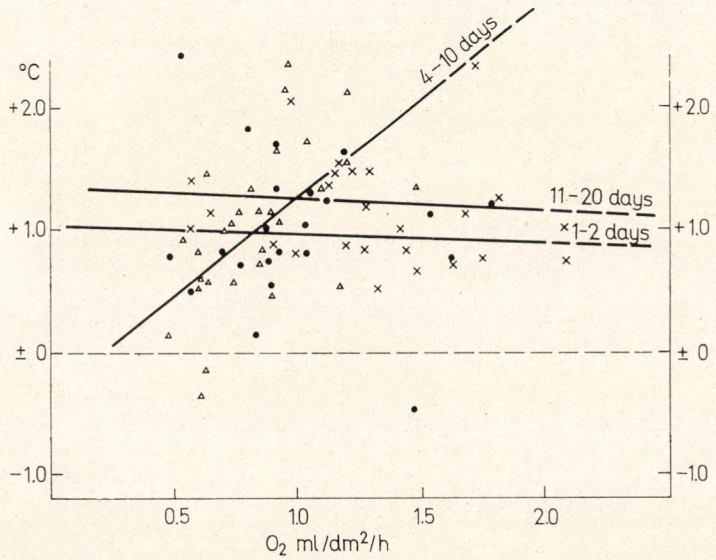

Fig. 10. Scatter-diagram of the increase in colonic temperature according to oxygen consumption after the termination of hypoxia. Coefficients of correlation *(r)* were for the age-groups of 1—2 days (●), 4—10 days (▲), and 11—20 days (×) −0.125 (n.s.), +0.554 ($p < 0.01$), and −0.088 (n.s.), respectively. The respective coefficients of regression showed the same, $t = 1.24$ ($p > 0.2$), $t = 3.66$ ($p < 0.01$), and $t = 0.47$ ($p > 0.6$), respectively

whatever could be detected in the two other age-groups. The same applied to rats aged less than 12 hours (coefficient of correlation: $r = 0.20$, n.s.; coefficient of regression: $t = 0.61$, $p > 0.5$).

Correlation between colonic temperature before the termination of exposure to hypoxia and the subsequent increase in body temperature. The marked correlation between the colonic temperature prior to exposure to hypoxia and the decrease in body temperature in the course of hypoxia (Fig. 9) suggested that

a correlation could exist also between the body temperature level reached in the course of hypoxia and the subsequent rise in colonic temperature after switching from 12% O_2 in N_2 to room air. However, such a correlation was found in none of the four age-groups. Therefore, only the results of the relevant calculations are presented (Table VII).

Table VII

Correlation between body temperature at the end of exposure to hypoxia and the subsequent increase in colonic temperature after breathing room air for 30 min

In brackets, number of observations

	Coefficient of correlation (r)	Significance of coefficient of regression
< 12 hrs (16)	$r = -0.073$ (n.s.)	$t = 0.263$ $p > 0.7$
1— 2 days (22)	$r = 0.167$ (n.s.)	$t = 0.384$ $p > 0.7$
4—10 days (29)	$r = 0.304$ (n.s.)	$t = 1.67$ $p > 0.2$
11—20 days (25)	$r = 0.267$ (n.s.)	$t = 0.685$ $p = 0.5$

Discussion

The results have confirmed, on the one hand, the observation of others (MOUREK 1959; TAYLOR 1960) that at ambient temperatures below the thermoneutral zone, hypoxia reduced heat production and body temperature in the new-born rat soon after birth and, on the other hand, pointed to new aspects of the response, some of which do not seem to fit the explanation that the response of body temperature was simply a consequence of the reduction in heat production.

The observation that animals with low colonic temperatures prior to exposure to hypoxia often failed to respond with a fall in colonic temperature to hypoxia, suggested that body temperature could be an important determinant of the response to hypoxia. Correlation between colonic temperature prior to exposure to hypoxia and the reduction in heat production could be demonstrated only in the group aged less than 12 hours, but in none of the others (Table II, Part B). In some rats of this age-group with low body temperatures, oxygen consumption at an ambient temperature of 30°C even before hypoxia hardly exceeded the basal level observed at an ambient temperature of 35—36°C and, therefore, no further significant fall in heat production could be expected in response to hypoxia. Animals with a colonic temperature above 32°C and a metabolic rate well above the basal level responded to hypoxia with a fall in heat production of a similar magnitude than did the other age-groups (Table II, Part A).

In contrast, a significant correlation was found between colonic temperature before exposure to hypoxia and the response of body temperature to hypoxia (Table III and Fig. 9), viz. the higher the body temperature before exposure to hypoxia, the greater the fall in response to hypoxia. In animals with the lowest body temperatures colonic temperature actually increased during hypoxia despite a marked fall in heat production. These animals had earlier been exposed for 30 min to an ambient temperature of 25°C and not only failed in regaining the body temperature observed prior to exposure to 25°C 30 min after having been re-transferred to an ambient temperature of 30°C, but had significantly lower colonic temperatures already before exposure to an ambient temperature of 25°C, i.e. 30 min after having been transferred from the thermoneutral environment to an ambient temperature of 30°C (Table IV). In contrast to the highly significant correlation between body temperature prior to hypoxia and the change in hypoxia, no correlation could be detected between the colonic temperature at the end of exposure to hypoxia and the subsequent increase after the termination of hypoxia (Table VII).

The variation of colonic temperature within wide limits before exposure to hypoxia complicated the analysis of the relationship between the reduction in heat production and the response of body temperature. In individual experiments, discrepancies similar to those demonstrated by Figs 3 and 4 could be found in many instances in all age-groups. In the experiment of Fig. 3, breathing 12% oxygen was followed by a profound fall in heat production, while in that of Fig. 4, oxygen consumption suffered a slight reduction, and still body temperature was reduced to about the same extent in both cases. The analysis of a more homogeneous group, i.e. rats aged 11 to 20 days with colonic temperatures above 34°C prior to exposure to hypoxia, showed convincingly the absence of any significant correlation between the fall in body temperature during hypoxia on the one hand, and the reduction in heat production, whether calculated as per cent of the oxygen consumption prior to hypoxia or in absolute figures, or with the level of heat production during hypoxia, on the other hand (Table V). These results are not consistent with the usual assumption that, in the new-born, the fall in body temperature during exposure to hypoxia is simply a consequence of the reduction in heat production, and point out the importance and the variability of so-called physical regulation in the new-born rat.

The same conclusion appears to emerge from the comparison of heat production and the increase in body temperature after the termination of hypoxia (Table VI and Fig. 10). Over a three- to fourfold range of heat production no correlation could be detected with the concomitant increase in body temperature in three of the four age-groups, clearly indicating that in addition to heat production other factors, i.e. mechanisms of physical regulation, play a part in the determination of the changes in body temperature. In the group aged

4 to 10 days, however, a definite correlation between heat production after the termination of hypoxia and the concomitant increase in colonic temperature could be established ($p < 0.01$, almost reaching 0.001). No plausible explanation of this phenomenon can be offered. Chance, pure and simple, appears to be hardly probable; it could, of course, be characteristic for this limited period of postnatal age, but the observations have to be repeated before a definite statement can be made.

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SEASONAL VARIATIONS OF THE CORTICOSTEROID FRACTIONS IN GUINEA PIG TISSUE AND ORGANS

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Corticosteroid fractions of the extracts of various organs were determined by paper chromatography in 48 male and female guinea pigs of the same breed, kept on the same diet and divided in six groups of 8 animals each. Three groups were killed in the month of March and the rest in October. It was found that the adrenals, organs and tissues of the spring animals contained more corticosteroid than did those of the autumn animals. Certain fractions were absent in some organs of the autumn animals while others were not found in certain organs of spring animals. Some fractions were absent in certain organs of both spring and autumn guinea pigs; thus, compound X_1 could not be found in the spleen, lungs, heart and brain while compound X_2 could not be found in the spleen, heart and brain of either spring or autumn animals.

Seasonal changes are known to be accompanied by a series of cyclic manifestations in the functions of plants and animals. There are many examples of diurnal variations in the functioning of various organs of animals. Such diurnal variations have been demonstrated in the adrenal cortex, too (Barter et al. 1959; Peterson 1959; Yales et al. 1961). Agid et al. (1961) studied vipers while Saint Girons et al. (1961) dormice, and found an increased functional state of the adrenal cortex and medulla during the awake state in spring in comparison with winter sleep.

Sicart et al. (1963) investigated the corticosterone content of the blood plasma of dormice during sleep and found 14 μg per 100 g (11.5—17 μg) in December—January, 32 μg (27—34 μg) in March—April and 24 μg per 100 g (20—27 μg) in May, June, July and September. One hour after waking the values were 33 μg per 100 g (27—32 μg) in December—January, 55 μg (44—76 μg) in March—April, 37 μg per 100 g (34—48 μg) in May, June, July and September. The plasma corticosterone level in the dormouse was determined with the fluorometric method of Zenker and Bernstein (1958). In spite of the great accuracy of the method, the values displayed a wide scatter, a fact to be accounted for by the different individual characteristics.

In previous experiments we have demonstrated various corticosteroid fractions in all organs and tissues of rats (Fazekas and Fazekas 1964), guinea pigs (Fazekas and Fazekas 1965), rabbits (Fazekas and Fazekas 1966a) and humans died from various causes. These corticosteroids in the organs and tissues were found to undergo qualitative and quantitative changes upon the

effect of alcohol (FAZEKAS and FAZEKAS 1966b) or formolstress (FAZEKAS and FAZEKAS 1968). Differences were also found depending on the cause of death (FAZEKAS and FAZEKAS 1967). Since these changes were worthy of attention not only from the physiological and pathological but even from the therapeutic point of view, it seemed desirable to elucidate whether the corticosteroid fractions (thus not only corticosterone) would show qualitative or quantitative differences during the various seasons.

The present paper reports on the results of investigations into the corticosteroid fractions of normal (intact) guinea pig organs and tissues in spring (March) and autumn (October).

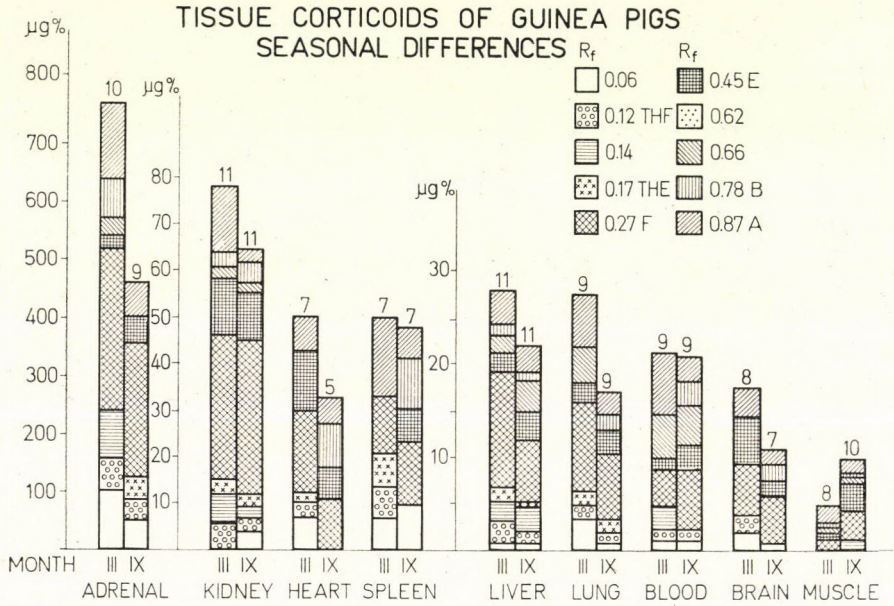

Fig. 1. Seasonal variations of the corticosteroid fractions in guinea pig tissue and organs. III = March, X = October

Material

The investigations were carried out on the organs of 48 male and female guinea pigs of the same breed. The animals weighed 500 to 600 g and were kept on a standard diet in a bright room of 20 to 22°C, looking south. The animals were divided in six groups of 4 males and 4 females each. Of these, a total of 24 animals of the first three groups were decapitated and bled in the middle of March, 1967, and the remaining animals were killed in the middle of October, 1967, at 9 a.m. Their brain, heart, lungs, spleen, liver, kidneys, skeletal muscles, adrenals and blood were immediately put into a refrigerator of —10°C for two hours. Thereafter, the organs were homogenized. The homogenates of the animals belonging to the same group were weighed and extracted. To prevent clotting, the blood samples of animals of the same group were collected in 10 ml of distilled water containing 0.5 ml of heparin.

Methods

Extraction of corticosteroids from the pooled identical organs of animals from the same group was made according to ENDRŐCZI (1962), originally developed for adrenal tissue. In addition, the method of BUSH and FERGUSON (1953) originally developed for determinations in blood, was used. The procedures have been modified by us. After two purifications and elutions the extracts were subjected to paper chromatography in the $Bush_5$ system. Preparation of the extracts as well as the procedure for paper chromatography have been described previously in detail (FAZEKAS and FAZEKAS 1964; 1965; 1966a).

The chromatograms were developed according to BUSH and MAHESH (1959), as described previously (FAZEKAS and FAZEKAS 1964; 1965; 1966a). This involves the use of a 9 : 1 mixture of 2 N NaOH and of 0.1% tetrazolium blue in water which stains the corticosteroid spots a darker or brighter bluish-violet. By comparing these spots with the appropriate standards one can estimate the μg value of the spots with a $\pm 15\%$ limit of error. As standard reference substances a mixture of 0.2, 0.5, 1.0, 2.0, 3.0 and 5.0 μg of tetrahydrocortisol, tetrahydrocortisone, cortisol, cortisone, corticosterone, and 11-dehydrocorticosterone, respectively, was run together with the extracts.

Then the chromatogram was dried under an infrared bulb, a procedure resulting in the appearance of a yellow fluorescence due to the alkaline-fluorescence reaction with NaOH of steroids containing a Δ^4 binding. This fluorescence was examined under the light of an analytical quartz lamp with a U. G. 5 filter. By comparing the size and fluorescence intensity of the spots with the appropriate standards we could determine the corticosteroid value of the spots with an error of $\pm 15\%$. As the real result was regarded the mean of the two estimations. The μg values thus obtained were calculated for 100 g of fresh tissue and expressed in μg per 100 g. The determination of the corticosteroid fractions of the blood differed from that of the tissues only in the omission of the acetone-water mixture; instead of this, to the blood collected in 10 ml of distilled water containing 0.5 ml of heparin an equal amount of distilled water was given and the mixture was shaken vigorously to obtain complete haemolysis. This was followed by an extraction with 3×2 volumes of a mixture of ether and ethyl acetate (2 : 1). The following steps of the procedure were identical with those used in the case of tissues.

Results

Results are summarized in Table I showing the mean values and their standard deviations for the corticosteroid fractions in the organs and tissues of the spring and autumn groups of animals, as well as the total steroid content in μg per 100 g, together with the Rf value, tetrazolium blue reaction, NaOH fluorescence, the native blue fluorescence and the names of the fractions as identified on the basis of standards.

Six corticosteroid fractions were identified in the organs of intact male and female guinea pigs. Three further fractions were termed X_0, X_1 and X_2, since appropriate standards were not at disposal. These substances were, therefore, characterized by their Rf value, tetrazolium blue reaction and NaOH fluorescence.

Compound X_0 (Rf = 0.06) was found in various amounts in every organ except the muscle in every spring group and in the heart in every autumn group. In the heart of spring animals compound X_0 was found in considerable amounts.

Tetrahydrocortisol (Rf = 0.12) was lacking in the spleen, heart and brain in any of the three autumn groups, while it was present in these organs of the spring animals, in which it was lacking in the skeletal muscles.

Table I

Variations in μg per 100 g of the mean value for the corticosteroid fractions of the organs

Organ	Tissular corticosteroids in μg							
	0.06		0.12		0.14		0.17	
	March	October	March	October	March	October	March	October
Liver	1.3 ±0.4	1.0 ±0.3	2.1 ±0.6	1.6 ±0.4	2.2 ±0.0	2.5	1.6 ±0.4	0.8 ±0.2
Kidney	0.3 ±0.1	3.6 ±0.7	5.8 ±1.6	3.3 ±1.0	6.3 ±2.1	2.6 ±0.8	3.0 ±1.0	2.4 ±0.8
Spleen	7.0 ±1.5	10.0 ±3.1	7.0 ±2.1	—	—	—	7.0 ±2.1	—
Muscles	—	0.2 ±0.0	—	0.3 ±0.1	0.7 ±0.2	—	—	—
Lungs	2.9 ±0.9	1.1 ±0.3	1.5 ±0.4	1.1 ±0.3	—	—	1.6 ±0.4	1.0 ±0.3
Heart	6.9 ±2.1	—	3.5 ±1.1	—	—	—	1.9 ±0.6	—
Brain	2.2 ±0.7	0.9 ±0.3	1.8 ±0.6	—	—	—	0.5 ±0.2	—
Adrenals	106.6 ±35.1	52.8 ±16.2	55.4 ±15.5	38.5 ±11.3	78.9 ±22.5	—	—	19.9 ±5.2
Blood	1.0 ±0.3	1.5 ±0.5	1.0 ±0.3	0.8 ±0.2	2.5 ±0.8	—	—	0.3 ±0.1
Total	128.2	71.1	78.1	45.6	90.6	5.9	15.6	24.4
Native blue fluorescence	+		—		—		—	
Tetrazolium blue reaction	—		+		—		+	
NaOH fluorescence	+		—		+		—	
Name of steroid	X_0		Tetrahydro-cortisol		X_1		Tetrahydro-cortisone	

Compound X_1 (Rf = 0.14) was lacking in the spleen, lungs and brain of spring animals as well as in the adrenals and blood of autumn animals. It was present in the liver, kidney and skeletal muscles of both spring and autumn animals.

Tetrahydrocortisone (Rf = 0.17) was not found in the muscles, adrenals and blood of spring animals. In autumn animals it was present in the spleen, muscles, heart and brain.

and tissues of intact normal guinea pigs in the months of March and October

according to the Rf value										Total tissular steroid	
0.27		0.45		0.66		0,78		0.87			
March	October	March	October	March	October	March	October	March	October	March	October
12.4 ±4.1	6.9 ±2.1	1.9 ±0.5	3.2 ±1.0	1.9 ±0.6	2.1 ±0.7	1.5 ±0.5	0.8 ±0.2	3.6 ±1.1	2.2 ±0.7	28.5	21.1
31.8 ±10.6	32.8 ±10.1	11.8 ±3.4	10.3 ±3.2	2.4 ±0.7	2.6 ±0.8	3.0 ±1.0	5.2 ±1.5	13.1 ±3.2	3.8 ±1.1	77.5	66.6
11.9 ±3.5	13.5 ±4.2	—	5.9 ±1.8	—	—	—	11.7 ±3.5	16.6 ±5.1	6.2 ±2.0	49.5	47.3
1.3 ±0.4	3.2 ±1.0	0.7 ±0.2	3.3 ±1.0	0.6 ±0.2	0.5 ±0.1	0.6 ±0.2	0.5 ±0.1	1.9 ±0.6	1.1 ±0.3	5.8	9.9
9.3 ±3.0	8.5 ±2.5	2.3 ±0.7	2.9 ±0.8	4.2 ±1.3	—	—	1.1 ±0.0	5.6 ±1.5	2.6 ±0.8	27.4	18.4
17.3 ±5.3	11.4 ±3.0	13.4 ±3.2	6.8 ±2.1	—	—	—	9.3 ±3.1	7.2 ±2.1	3.6 ±1.1	50.2	31.1
5.1 ±1.6	4.8 ±1.5	5.2 ±1.8	1.9 ±0.6	—	—	—	2.0 ±0.6	2.7 ±0.9	1.9 ±0.6	17.5	11.5
279.1 ±69.4	238.1 ±71.5	21.9 ±7.0	50.7 ±15.1	36.2 ±12.0	0.4 ±0.2	65.6 ±20.5	—	108.7 ±32.1	63.7 ±20.9	750.4	464.1
3.5 ±1.0	13.4 ±3.2	1.0 ±0.3	2.6 ±0.8	5.2 ±1.2	—	—	0.6 ±0.2	7.0 ±2.1	2.1 ±0.7	21.2	21.3
371.7	332.6	58.2	87.6	50.5	5.6	70.7	31.2	164.4	87.2	1028.0	691.8
—		—		+		—		—			
+		+		—		+		+			
+		+		+		+		+			
Cortisol		Cortisone		X_2		Cortico-sterone		11-dehydro-cortico-sterone			

Cortisol (Rf = 0.27) could be demonstrated in all organs and tissues of both spring and autumn animals.

Cortisone (Rf = 0.45), just like cortisol, could be demonstrated in all groups of animals both in spring and autumn in all tissues except the spleen, where it was lacking in the spring.

Compound X_2 (Rf = 0.66) could not be demonstrated in the spleen, heart and brain of spring animals. In autumn animals no compound X_2 was found in the adrenals, spleen, lungs, heart and brain.

Corticosterone (Rf = 0.78) was lacking in the spleen, lungs, heart, brain and blood of spring animals. In contrast, corticosterone was demonstrated in all organs of the autumn animals.

11-dehydrocorticosterone (Rf = 0.87) was found in all organs of both spring and autumn animals.

As to quantitative aspects, in spring the adrenals mainly contained cortisol, 11-dehydrocorticosterone, compound X_0, and tetrahydrocortisol. At the same time the peripheral tissues contained larger amounts of cortisol, 11-dehydrocorticosterone, corticosterone and cortisone.

In the adrenals of autumn animals cortisol represented the main fraction. In addition, compound X_1, 11-dehydrocorticosterone and compound X_0 occurred. In the tissues we found mainly cortisone, corticosterone and 11-dehydrocorticosterone, in addition to cortisol.

Discussion

The variations in the steroid content of the adrenals and peripheral organs of guinea pigs during spring and autumn revealed that, in addition to cortisol as the main fraction, other steroids also underwent considerable quantitative and qualitative changes. Cortisol was the characteristic main steroid fraction of the guinea pig organism in both spring and autumn. In addition, 11-dehydrocorticosterone, compound X_0, and compound X_1 occurred in decreasing order during spring, and cortisone, 11-dehydrocorticosterone, compound X_0 and tetrahydrocortisol during autumn.

The above variations clearly showed the sensitivity of tissular steroid metabolism. Within the frame of quantitative alterations the qualitative changes must not be neglected either. The causes of the development of a qualitatively altered steroid state and of its consequences cannot be explained on the basis of our present knowledge, as the many-sided mode of action of the individual steroid compounds is not clearly known, although it would offer an insight into the energetical and regulatory processes or the organism's chemical system. We cannot give any explanation of the finding that corticosterone was lacking in the adrenals of autumn animals, though it was present in other tissues. This contrasts with the general view that the corticosteroid content of the tissues originates from the adrenals, even though one cannot preclude the possibility of corticosteroid formation in other organs, the more so as cholesterol, the basic material of steroids, is present in every tissue. However, there are no proofs of corticosteroid synthesis in other tissues and organs.

Table I shows that the organs of normal intact guinea pigs generally contain the various corticosteroid fractions in higher amounts in spring than in autumn. Still, slightly higher amounts of the following steroids were found in autumn than in spring: compound X_0 in kidney, spleen and blood; cortisol in kidney, spleen, muscles and blood; cortisone in liver, muscles, lungs, adrenals and blood; compound X_2 in liver and, finally, corticosterone in the kidney.

The total steroid content of the organs and tissues was also higher in spring than in autumn. The decrease in autumn was 1.6-fold in the adrenals and heart, 1.5-fold in the lungs, 1.4-fold in the liver and 1.2-fold in the kidney. An exception was the skeletal musculature which exhibited a total steroid content 1.7-times higher in autumn than in spring.

The sum of identical corticosteroid fractions present in all the organs was also higher in spring than in autumn. An exception were tetrahydrocortisone and cortisone which exhibited somewhat higher values in the autumn animals, but only because in this season both compounds could be found in the adrenals (tetrahydrocortisone) or because they were present in higher amounts in autumn than in spring (cortisone).

Finally, it should be mentioned that certain fractions were lacking in some organs of either spring or autumn animals.

The following compounds were not demonstrable in autumn: compound X_0 in the heart; tetrahydrocortisol in the spleen, heart and brain; compound X_1 in the spleen, lungs, muscles, adrenals and blood; tetrahydrocortisone in the spleen, muscles, heart and brain; corticosterone in the adrenals. On the other hand, the following compounds were not demonstrable in spring: compound X_0, tetrahydrocortisol and tetrahydrocortisone in the muscles; compound X_1, cortisone, compound X_2 and corticosterone in the spleen; compound X_1 and corticosterone in the lungs; compound X_1 and corticosterone in the heart; compound X_1, compound X_2 and corticosterone in the brain; tetrahydrocortisone in the adrenals; tetrahydrocortisone and corticosterone in blood.

No standard preparation of known chemical structure was at our disposal which would have exhibited physico-chemical properties identical with those of the two compounds found in the organ extracts with Rf values of 0.06 and 0.66, respectively. Thus, these substances were termed compound X_0 and compound X_2. However, since under identical conditions these compounds were always detected in the adrenals together with the other steroids, it seems justified to consider them corticosteroids, in spite of the fact that their native fluorescence and NaOH fluorescence in UV light were by themselves of no conclusive value. However, for their corticosteroid character speaks not only their solubility but also the finding that they occurred in overwhelming amounts consistently in the adrenals. In additions, they exhibited the above-mentioned Rf values in the $Bush_5$ system in which, according to BUSH, hexahydrocortisol

(cortol) runs with an Rf of 0.05 and pregnanetriol with an Rf of 0.65. Similar considerations apply to compound X_1, too.

The total steroid content of the adrenals in spring guinea pigs was 750.4 μg per 100 g while that in autumn guinea pigs, 464.1 μg per 100 g. This means that in the spring the adrenals contained 286.3 μg per 100 g, i.e. 61.6%, more total steroid than in autumn. The peripheral organs (except the adrenals) of spring animals contained an average of 33.9 μg per 100 g, those of autumn animals an average of 28.4 μg per 100 g of total steroid. This means that more total corticosteroid was demonstrated in the organs of the spring animals than in those of autumn animals. The total steroid content of blood was the same in spring and autumn animals, in spite of the fact that the latter exhibited 61.6% less total adrenal steroid than did the spring animals. This is well explained by our previous finding according to which under conditions of adrenalectomy, stress, etc., the corticosteroid fractions of the tissues are mobilized and passing into the blood they maintain or augment the plasma corticosteroid level for a certain period of time (FAZEKAS 1964).

The above results may have some physiological importance and help in choosing the time of animal experiments, and in the evaluation and comparison of their results.

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AFFERENT PATHWAYS OF STRESSFUL STIMULI: THEIR DEPENDENCE ON STRENGTH AND THE TIME ELAPSED AFTER THE ONSET OF STIMULATION

By

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The pathways through which a subcutaneous injection of different doses of formaldehyde and surgical trauma release corticotrophin (ACTH) have been studied in rats with anterolateral deafferentation of the medial basal hypothalamus (MBH) and denervation of a hind limb. Plasma corticosterone concentration was taken the index of ACTH release.

A low dose of formaldehyde raised the plasma corticosterone level in normal rats but failed to do so when given to rats with deafferented MBH or into a denervated hind limb. A large dose resulted in the same rise when injected into controls, into rats with anterolateral deafferentation, or into a denervated hind limb.

In rats with anterolateral deafferentation of the MBH, unilateral sham adrenalectomy failed to change the plasma corticosterone level at the end of the first hour but raised it at 2 and 4 hours. This shows that the anterior, lateral and dorsal neural pathways to the MBH are not essential for the 2 and 4 hour response to surgical trauma. This stimulus activates different pathways at different times after onset. Anterolateral deafferentation of the MBH, however, prevented ACTH release at all points of time after low doses of formaldehyde.

It is concluded that a change in the intensity of a stressful stimulus can involve another pathway; with time, different pathways may be at work after exposure to some stressful stimuli but not to others.

Several lines of evidence suggest that the mechanism of stress-induced corticotrophin (ACTH) release depends on the nature of the stressful stimulus. The ACTH-releasing effect of various stimuli is differentially inhibited by neurohypophysectomy (DE WIED et al. 1964), glycocorticoid blockade (YATES 1967), and long-term ACTH pretreatment (STARK et al. 1968a). Some authors hold that different stressful stimuli activate different hypothalamic structures (SMELIK 1959), others propose that they stimulate the hypothalamo-hypophysial unit through different pathways (FORTIER 1951; FELDMAN et al. 1968; STARK et al. 1968b; MAKARA et al. 1969b). Some workers classify the stimuli on the basis of differences in intensity (SAYERS et al. 1958; MANGILI et al. 1966).

The first problem here is whether a change in the intensity of a stimulus is accompanied by a change in the pathway through which ACTH release is brought about; the second problem is whether a given stimulus would release ACTH through the same afferent pathway if the time interval between the onset of the stressor stimulus and the estimation of ACTH release is varied.

Methods

The experiments were performed on male Wistar rats of 160—250 g body weight. The rats were kept in a room of constant (24±1°C) temperature and 55—70% relative humidity, and given a standard diet and tap water ad libitum.

Anterolateral deafferentation of the medial basal hypothalamus (MBH) was performed by the method of HALÁSZ and PUPP (1965), as described earlier (MAKARA et al. 1969b).

The partially deafferented MBH is shown in sagittal section in Fig. 1. The cut began just behind the optic chiasma, passed below the paraventricular nuclei, backwards to the level of premammillary nuclei; the posterior connections and the stalk were left intact. Seven to eight days after the operation the rats were subjected to one of the following stimuli: unilateral sham-adrenalectomy on the left side under intraperitoneal pentobarbital anaesthe-

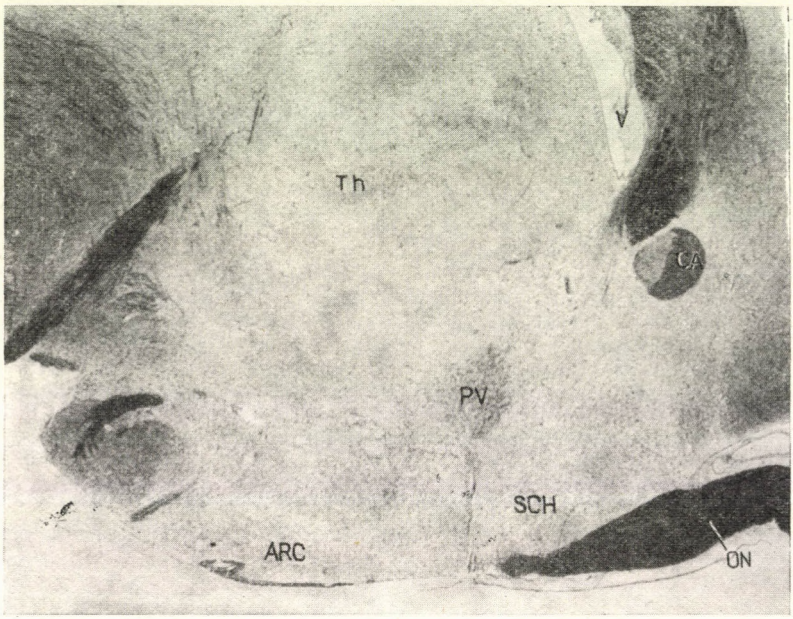

Fig. 1. Parasaggital section through the hypothalamus. Anterolateral deafferentation of MBH. Luxol Fast Blue - cresyl violet stain, ×18. ARC: arcuate nucleus; CA: commissura anterior; ON: optic nerve; PV: paraventricular nucleus; SCH: suprachiasmatic nucleus; Th: thalamus; V: lateral ventricle

sia (Mebubarbital, Rhône-Poulenc, Paris; 3 mg/100 g); subcutaneous injections of formaldehyde in saline, and for control purposes, of physiological saline as listed in the Tables. The sensitivity of the adrenals to ACTH was tested under ether anaesthesia 4 hours after the intraperitoneal injection of 250 μg/100 g of dexamethasone (Oradexon, Organon, Oss) and an intravenous injection of 20 ng/100 g of β1—24 ACTH (Synacthen, CIBA) dissolved in acidified saline containing 0.1% bovine albumin. Blood was withdrawn 15 minutes later.

The right hind limb was denervated by resecting the femoral and sciatic nerves under ether anesthesia. One week later the rats were injected subcutaneously with formaldehyde in saline and, for control purposes, with physiological saline, as listed in Table III.

Blood samples were collected between 9 and 11 a. m. Blood was withdrawn from the abdominal aorta under ether anaesthesia within 2.5 minutes after removal from the cage. As an indicator of ACTH release, plasma corticosterone concentration was used; it was determined by the method of GUILLEMIN et al. (1959).

Plasma corticosterone values were transformed to logarithms, and analysis of variance and paired comparisons were performed according to the method of SCHEFFÉ (1959).

The hypothalamus was fixed in 10% formalin, embedded in paraffin and serially sectioned at 10 μ thickness, mostly in the frontal plane. Every section was mounted and stained with haemalum-erythrosine or Luxol Fast Blue-cresyl violet (KLÜVER and BARRERA 1953).

Results

The rats with partial deafferentation of the MBH failed to respond to injections of 2.5 and 7.5 mg/100 g b.wt. of formaldehyde but showed the same rise of plasma corticosterone as their controls when injected with a 25 mg/100 g b.wt. dose (Table I). This indicates that the nerve fibres coming to

Table I

Effect of formaldehyde injection on plasma corticosterone level in rats with anterolateral deafferentation of the MBH

Treatment*	Time** (min)	Plasma corticosterone (μg/100 ml)			
		sham-operation		anterolateral deafferentation	
Vehicle	30	11.8±1.9***	(12)****	11.2±1.1	(10)
2.5 mg/100 g formaldehyde	30	37.8±5.3□	(9)	13.7±1.2□□	(9)
2.5 mg/100 g formaldehyde	120	15.4±4.0	(10)	16.2±2.6	(11)
7.5 mg/100 g formaldehyde	30	43.1±3.3□	(6)	18.9±4.2□□	(6)
12.5 mg/100 g formaldehyde	30	42.9±2.9□	(3)	24.6±2.6	(2)
25 mg/100 g formaldehyde	30	45.2±2.5□	(7)	42.8±4.2□	(11)

* in 0.25 ml/100 g of physiological saline, subcutaneously
** from injection to bleeding
*** mean ± S.E.
**** number of rats
□ significantly different from the saline treated controls in the same column ($p < 0.05$)
□□ significantly different from the sham-operated group within the same row ($p < 0.05$)

the MBH from an anterior, lateral, or dorsal direction are essential for responses to arise after low doses of formaldehyde, but not after high doses; in addition, the lack of ACTH release after certain stimuli cannot be explained by a general non-specific block of rapid responses due to hypothalamic damage.

ACTH was found to elicit an identical adrenocortical response in the partially deafferented and sham-operated rats, despite the significant decrease in adrenal weight in the former group (Table II). Therefore, the failure of the rats to respond to 7.5 mg/100 g formaldehyde cannot be explained by a difference in the adrenocortical sensitivity to ACTH.

A dose of 2.5 mg/100 g of formaldehyde injected into an innervated hind paw raised the plasma corticosterone level within one hour, but failed to act in that way if injected into a denervated hind paw. A dose of 25 mg/100 g b.wt.

Table II

Effect of β 1—24 ACTH on plasma corticosterone level in rats with anterolateral deafferentation of the MBH

Pretreatment	Treatment*	No of rats	Body weight g	Adrenals mg	Plasma corticosterone μg/100 ml
Sham-operation	vehicle	12	213±6**	41.8±2.1	7.2±0.8
	20 ng ACTH	14	215±4	42.2±1.2	38.4±1.6□
Anterolateral	vehicle	9	228±11	36.1±1.5□□	7.3±0.9
deafferentation	20 ng ACTH	11	215±10	30.0±1.9□□	33.9±4.4□

* 0.25 ml/100 g; all rats pretreated with dexamethasone.
** mean ± S.E.
□ significantly different from its vehicle treated control ($p < 0.05$)
□□ significantly different from the sham-operated groups ($p < 0.05$)

induced, however, the same rise after injection into either a denervated or an innervated hind limb (Table III).

A 2.5 mg/100 g dose of formaldehyde did not change the plasma corticosterone level at 1, 2, 3, 5 or 7 hours after injection into a denervated hind paw (Table III). A dose of 2.5 mg/100 b.wt. caused a rise which passed off

Table III

Effect on plasma corticosterone level of formaldehyde injected into innervated or denervated hind limb

Treatment*	Site of injection (hind limb)	Time from injection to bleeding (hour)	Plasma corticosterone (μg/100 ml)	
0.1 ml/100 g physiological saline	denervated	1	16.4±2.2**	(8)***
2.5 mg/100 g formaldehyde○	innervated	1	52.4±3.1□	(10)
2.5 mg/100 g formaldehyde○	denervated	1	17.6±2.5	(8)
2.5 mg/100 g formaldehyde○	denervated	2	18.9±2.5	(8)
2.5 mg/100 g formaldehyde○	denervated	3	13.4±1.4	(8)
2.5 mg/100 g formaldehyde○	denervated	5	17.6±1.2	(8)
2.5 mg/100 g formaldehyde○	denervated	7	21.5±3.9	(8)
0.25 ml/100 g physiological saline	denervated	1	12.9±1.4	(12)
25 mg/100 g formaldehyde○○	innervated	1	46.3±2.3□	(3)
25 mg/100 g formaldehyde○○	denervated	1	39.4±2.9□	(12)

* subcutaneously
** mean ± S.E.
*** number of rats
□ significantly different from controls treated with physiological saline ($p < 0.05$)
○ 0.1 ml/100 g
○○ 0.25 ml/100 g

within two hours in the controls and failed to affect adrenocortical function in rats with partially deafferented MBH, at every point of time (Table I).

Unilateral sham-adrenalectomy induced a significant rise in the controls, with a peak at one hour after the operation. In deafferented rats, the plasma level remained unchanged for one hour, but later reached control stress values; the rise in the second hour was significant statistically (Table IV).

Table IV

Effect of surgical stress on plasma corticosterone level in rats with anterolateral deafferentation of the MBH

Group	Treatment*	Time to bleeding (hour)	Plasma corticosterone (μg/100 ml)		Significance
			sham-operated (S)	deafferented (D)	
1	none	1	11.3±2.3** (15)***	13.4±1.5 (14)	1D—2D NS
					2S—2D $p < 0.01$
					1D—3D $p < 0.10$
2	sham-adrenalectomy	1	43.2±2.5 (12)	11.3±0.7 (12)	2D—3D $p < 0.05$
3	sham-adrenalectomy	2	30.5±4.0 (17)	28.4±2.4 (15)	2D—4D $p < 0.10$
4	sham-adrenalectomy	4	27.4±4.8 (10)	28.7±4.0 (9)	1S—3S $p < 0.05$
					1S—4S $p < 0.05$

* all rats were anaesthetized with 3 mg/100 g pentobarbital i.p.
** mean ± S.E.
*** number of rats

Discussion

The results demonstrate that the pathway through which the occurrence of stressful stimulation is signalled to the MBH depends on the intensity of the stimulus. Low doses of formaldehyde, in contrast to high ones, failed to increase ACTH release both in animals with a denervated hind limb and in those with a partially deafferented MBH. Low doses release ACTH via nerve fibres ascending mainly across the contralateral spinal cord (MAKARA et al. 1967) and converging to the MBH from an anterior, lateral or dorsal direction, in agreement with our present findings and earlier data (MAKARA et al. 1969b). The above contrast proves well that at high dose levels pathways are operating which are different from those working at low dose levels. Substances liberated from tissues by a large dose of formaldehyde might activate the MBH or induce a change in the internal milieu, leading to activation of ACTH release. Accordingly, the stimulus, which had triggered ACTH release after a high dose, had travelled along a "humoral" pathway different from the "neural" one, made use of after a low dose.

BRODISH (1964) suggested that the stress reaction occurs in two phases: a rapid neural phase of ACTH release is followed by a delayed humoral phase. The present experiments have shown that in the sham-adrenalectomized rats the time course of the stress response after anterolateral deafferentation of the MBH is similar to that observed by BRODISH (1964) in unilaterally adrenalectomized rats with hypothalamic lesions. The anterior, lateral or dorsal nervous connections which are essential for the rapid phase of ACTH release are not essential for the delayed phase.

That a rapid neural phase of stress-induced ACTH release is not always followed by a delayed humoral phase is shown by the fact that small formaldehyde doses did not induce a delayed humoral phase. Moreover, the response to them passed off in the first 120 minutes and partial deafferentation of the MBH prevented ACTH release at every point of time studied.

It appears that the hypothalamo-pituitary unit can be activated through several independent pathways having different thresholds; further, that more than one pathway may operate at any given time and different pathways may be at work, with time, after exposure to a single stressful stimulus.

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FREQUENCY-SPECIFIC RESPONSE OF THE PITUITARY-ADRENOCORTICAL SYSTEM ON PONTINE STIMULATION

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Electrical stimulation of the gigantocellular reticular nucleus under chronic experimental conditions resulted in a frequency-specific response of the pituitary-adrenocortical system in the rat. Low frequency (6 to 10 cps) stimulation induced incremental responses of the neocortex and a marked increase in corticosterone production. High frequency (100 cps) stimulation of the same pontine area did not induce activation of the pituitary-adrenocortical system, although it produced a desynchronization of EEG activity.

Involvement of the lower brain stem in controlling autonomous and vegetative processes as well as in the regulation of sleep and wakeful states of the forebrain is supported by a number of data (MORUZZI 1960; JOUVET 1961; ZANCHETTI 1962; SÖDERBERG 1964; etc., as reviews). The synchronizing influence of the lower brain stem cannot be separated by a point to point stimulation of the pontine reticular core from those nervous functions which play role in ascending activation and result in a desynchronization of EEG activity. Nevertheless, a frequency-specific dissociation of the EEG response to stimulation of this part of the brain has been shown (see for reviews, ZANCHETTI 1962).

The major ascending pathways which trigger ACTH release from the anterior pituitary are conveyed at the level of the mesencephalic reticular formation and reaching the ventromedial and tuberoinfundibular region through subthalamic projections or by pathways relayed in the non-specific thalamic nuclei. Transection or lesion of the midbrain at the collicular level was followed by an impairment of ACTH release under the effect of various stressors (ANDERSON et al. 1957; ROYCE and SAYERS 1958; MARTINI et al. 1960; SLUSHER 1960; etc.). In a recent study, GIBBS (1969) has found that lesions of the pontine reticular formation prevented the effect of traumatic stress on the pituitary-adrenocortical system. In the present investigations, the effect of the electrical stimulation of the pontine reticular formation has

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been studied on the resting pituitary-adrenocortical activity and the adrenocortical response to stimulation at different frequencies of the pontine reticular formation.

Methods

The experiments were carried out on 30 male rats weighing 160—180 g. For recording of neocortical EEG activity, silver ball electrodes were implanted in the parieto-frontal cortex and electrical activity was registered with a 12-channel Hellige Neuroscript apparatus. Bipolar stainless steel, enamel insulated electrodes which served for stimulation of the gigantocellular reticular nucleus were placed by means of a stereotaxic apparatus. All surgical procedures were performed under pentobarbital anaesthesia at least 7 days before the electrophysiological and endocrinological study (for details, see NYAKAS and ENDRŐCZI 1970). Estimation of adrenocortical activity was made in vitro according to VAN DER VIES et al. (1960). The animals were placed in an experimental box for 5 min for habituation to the situation from the 3rd to 7th postoperative day. During this period the rats were divided into three groups on the basis of the EEG response to low or high frequency stimulation. Group 1 was placed in the box for 10 min and received no stimulation. The rats of Group 1 were sacrificed together with Groups 2 and 3 on the 7th to 10th postoperative day and served as operated control. Group 2 received low frequency stimulation at the optimal intensity and frequency range to induce incremental responses in cortical records (6 to 10 cps, 1—5 V, 0.1—0.4 msec). Group 3 was stimulated at a frequency and intensity range to produce desynchronization of the EEG activity for a 10 min period (0.5—2 V, 100 cps, 0.1—0.2 msec).

At the end of the experiment the brain was removed and fixed in 10% formol solution. Electrode placement was checked in 50 μ thick frozen sections.

Results

The typical electrode placements applied in the study are shown on Fig. 1. On most occasions, electrical stimulation was performed in the magnocellular

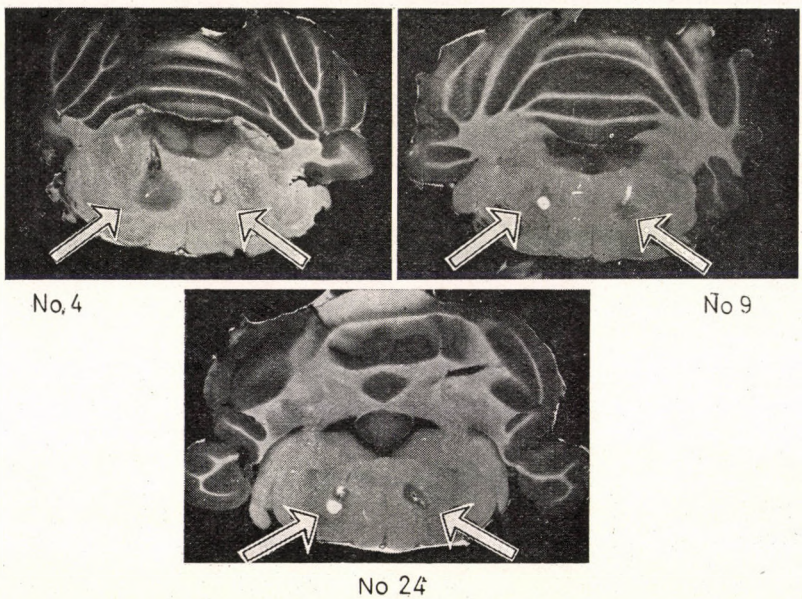
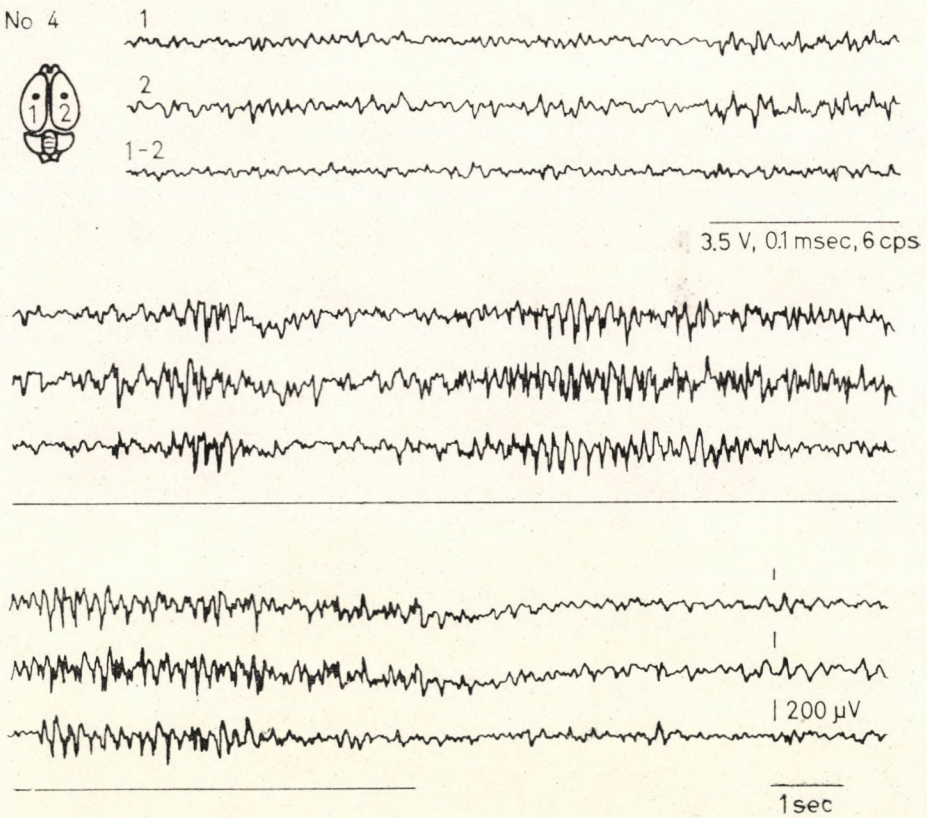


Fig. 1. Placement of stimulating electrodes in the rostral part of the gigantocellular reticular nucleus

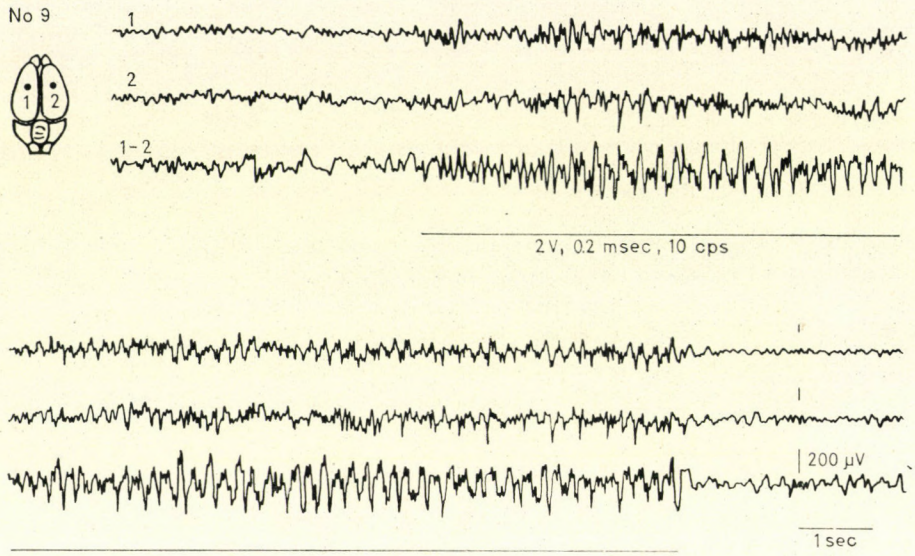
nuclei of the pontine reticular formation bilaterally; the rats with assymetrical electrode location which usually resulted in a failure of synchronized EEG activity to low frequency stimulation, served as controls.

Fig. 2 shows the appearance of incremental responses to low frequency stimulation of the pontine reticular formation and the desynchronization of EEG activity in response to stimulation at 100 cps.

Low frequency stimulation which produced slow waves and incremental electrical activity of the neocortex appeared significantly to activate pituitary ACTH release. In contrast high frequency stimulation of the same region was accompanied by a flattening of EEG activity and failed to increase corticosterone production. Fig. 3 compares the data obtained following 10 min stimulation of the pons at different frequencies and the data of operated controls and those of normal rats which had been removed from their home cage without any experimental procedure. The significant difference in corticosteroid production between the normal and operated control groups was attributed to the



a)



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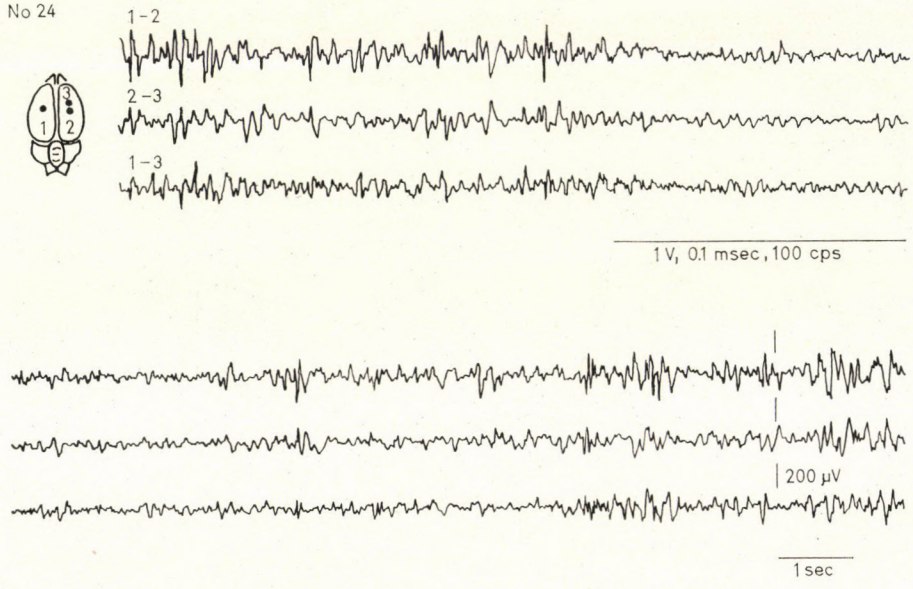

c)

Fig. 2. Cortical electrical activity during stimulation of pontine reticular formation. Incremental responses of the cortex to stimulation at lower frequencies (upper and middle records) and desynchronization on high frequency stimulation (below)

lack of habituation or to the implantation of chronic electrodes in the latter animals.

Behaviourally, the rats showed no emotional reactivity to stimulation of the pontine reticular formation either at high or low frequency; occasional forced movements and slight tremor was observed at high and low frequency stimulation, respectively, but spontaneous movement was usually not blocked during stimulation.

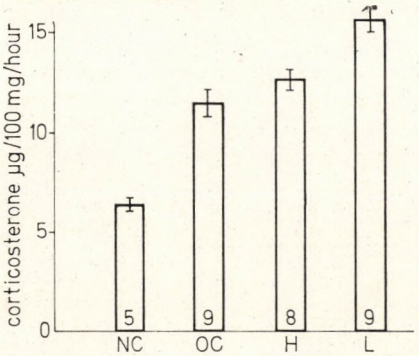

Fig. 3. Adrenal corticosterone production in vitro in response to pontine stimulation. Groups are as follows: NC — normal control; OC — operated control; H — high frequency stimulation; L — low frequency stimulation. Statistical evaluation by STUDENT's *t*-test:

	OC	H	L
NC	.001	.001	.001
OC		NS+	.001
H			.01

+NS — no significance

Discussion

An activation of the pituitary-adrenocortical system by the stimulation of the mesencephalic reticular formation has been shown earlier (ENDRŐCZI and LISSÁK 1960; SLUSHER and HYDE 1969). The pathways involved in that activation appear to be conveyed at the midline region of the brain stem reticular formation. Recent data have suggested that the reticular core should not be considered to represent solely a neural linkage between the specific sensory pathways and the hypothalamus but also a chemoreceptive field for corticosteroids (ENDRŐCZI et al. 1961; BOHUS and ENDRŐCZI 1964; CORBIN et al. 1965).

REDGATE (1964) observed that activation or inhibition of the mesencephally-induced ACTH release depends on the hormonal background; if previous corticosteroid secretion had been high, stimulation of the brain stem reticular core led to an inhibition of adrenocortical secretion and vice versa.

In the present observations, electrical stimulation of the pontine reticular formation at the level of the magnocellular nuclei resulted in a frequency-specific activation of adrenocortical secretion. Low frequency stimulation increased corticosterone production and high frequency stimulation in the same region failed to exert this effect. Taking into consideration that the appearance of slow waves and incremental responses of the cortex is characteristic of a decrease in the excitatory state of the forebrain and is interpreted as a kind of inhibition in the neurophysiological sense, an activation of the pituitary-adrenocortical system in the presence of large amplitude slow waves, and a lack of such endocrine activation during desynchronization appears to represent a dissociation of nervous and endocrine activatory mechanisms. For the elucidation of the mechanism of this frequency-specific activation of the pituitary-adrenocortical system, further studies are required.

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OLFACTION GUIDED APPROACHING BEHAVIOUR OF INFANTILE RATS TO THE MOTHER IN MAZE BOX

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Approaching behaviour of 10-day old rats to the mother has been studied in a simple U-maze. It was found that the infants went to the mother and to a less extent to nonlactating females but not to males. Application of tetracaine on the nasal mucosa of the young rats resulted in a random behaviour and suggested a guiding role of olfaction.

The role of sensory analyzers in learning processes and orientation in space were studied by a number of investigators. The early maturation of both olfactory and cutaneous sensory functions, appearing in the rat soon after birth raised the possibility that these functions had a specific role in behaviour during the postnatal days. A late development of the visual and acoustic analyzers as neocortical functions were observed in all mammals (ANDERSON and PATRICK 1934; FOX 1966; VOLOKHOV 1968; etc.).

Goal-directed behaviour of infantile animals may be acquired by experience and establishment of temporary linkages or by the guiding function of appropriate sensory analyzers. A behaviour like approaching the mother may be observed after maturation of coordinated locomotor activity (SMALL 1899) in the 6—7 postnatal days.

In the present investigations establishment of the goal-directed motor patterns of infantile rats has been studied in a simple maze on the 10th postnatal day when both acoustic and visual functions were still immature.

Methods

Ten litters of rats were used in the present study. For ensuring a homogeneous maturation of the brain and body functions the number of littermates was reduced to six in every litter. When they were 10 days old, observations were made in a plexiglass box (Fig. 1). After each experimental session the box was washed out carefully to avoid remaining odours.

The young rats were separated from their mothers 5 hours before the observations. Then the mother was placed in part A of the box, and about 15 min later the young was so put in part B, that it laid in a different axis of the box in each trial. Each young was tested in 5 consecutive trials in each experimental session. The response was considered positive if the head of the young had reached one of the goal-lines and entered one of the goal-compartments within a 2 min period. Behaviour of the infantile rat was considered negative when the animal did not touch the goal-line or when, as was mostly the case, it remained in part B, show-

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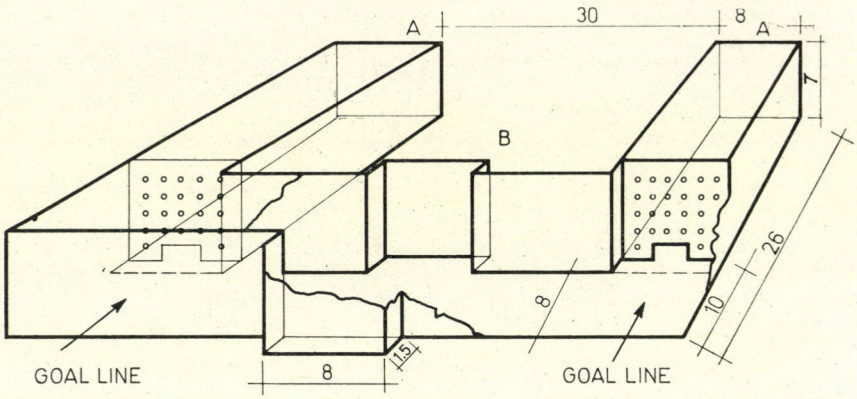

Fig. 1. U-maze box for investigation of goal-directed approaching activity of infantile rats to the mother animal. Both goal-compartments are separated by plexiglass gates having multiple holes. The young were placed in compartment B, from where they could run through the 11 cm long corridor into the goal-compartments (A)

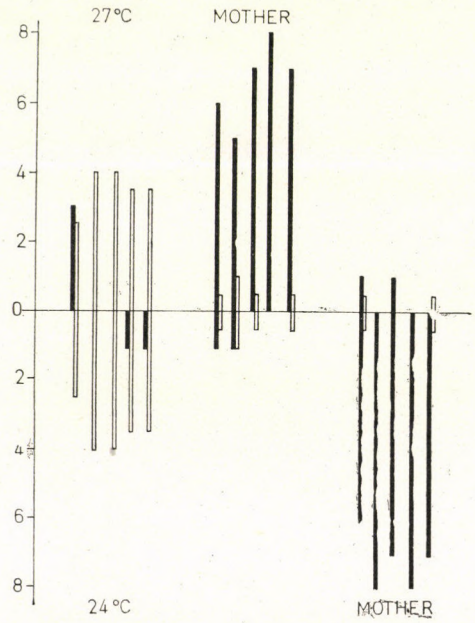

Fig. 2. Groups of colums corresponding to five trials each in three consecutive experimental sessions. 1. Influence of temperature difference on random activity of infantile rats; 2. presence of mother in one of the compartments A; 3. after placement of the mother in the contralateral compartment A. In this figures and all the following ones, dark columns represent positive responses; empty columns correspond to negative responses (see the text). On the ordinate, the numbers increasing from zero indicate the response number on one or the other side (part A), and the number of young animals

ing no approaching activity within the 2 min observation period. If the young had found their mother they were allowed to spend a 30 sec time together with her. If the attempt to find the mother had been unsuccessful, but a positive response occurred in the above mentioned sense, from the goal-compartment the young was immediately placed back into part B of the box. Animals which had displayed an entirely negative response were removed from the box for some seconds and placed back into the central part of the box. During the first experimental session in two experiments (see Figs 2 and 6) the effect of temperature was studied on random behaviour; a temperature difference was ensured by means of a bulb above one of the goal-compartments.

Results

A random behaviour of the infants to approach parts A of the box in the absence of the mother was tested whether it was depending on temperature differences. As shown in Fig. 2, the young hesitated to walk into the heated goal-compartment; in most cases they spent the whole observation time in the centre of the box. After the mother had been placed in any compartment of part A, the young approached the correct side. An olfaction-guided approaching behaviour of the young was revealed by the fact that after 30 min staying

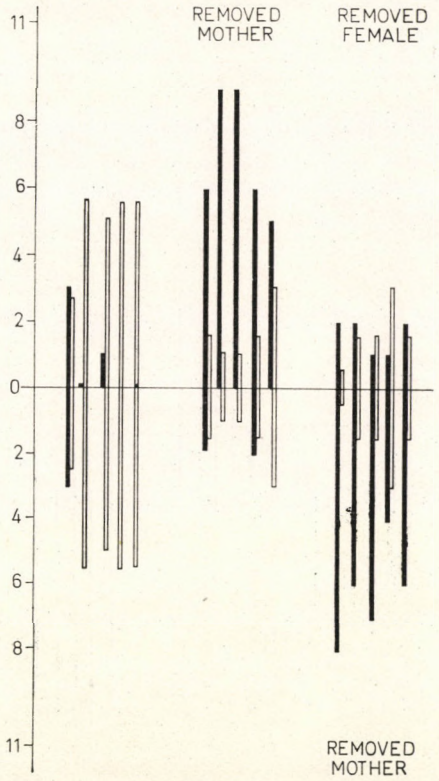

Fig. 3. Chance approaching behaviour in "empty" box (first group of columns); after removal of mother (second session); after removal of mother and of a nonlactating female rat from the two goal-compartments where they had been placed for a half hour period

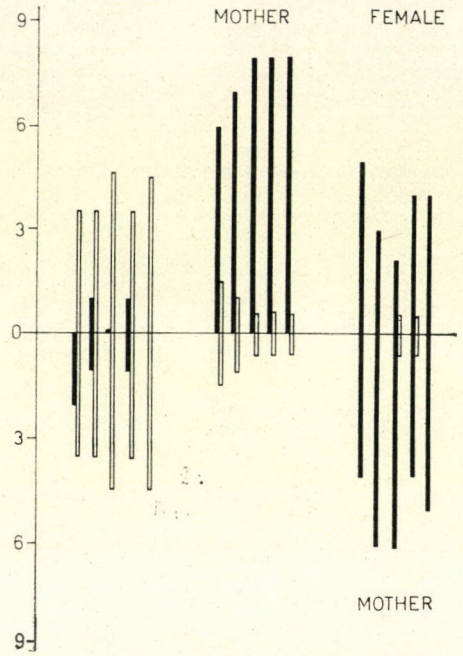

Fig. 4. Approaching behaviour to the mother (second session) and response to simultaneous presentation of mother and of nonlactating female rat (last session)

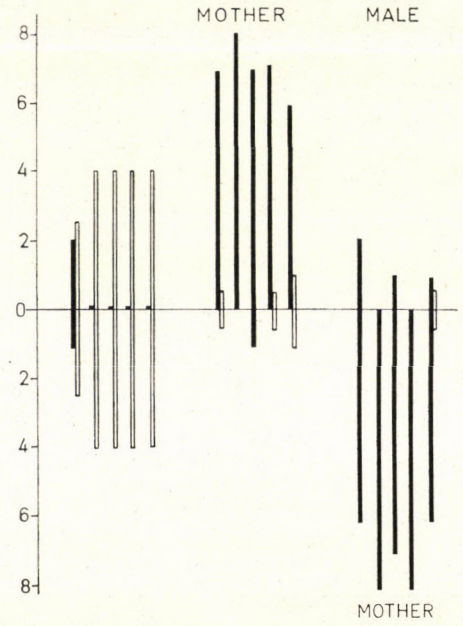

Fig. 5. Guided behaviour of infant rats in the presence of mother (second session) and of the mother and a male rat (third session)

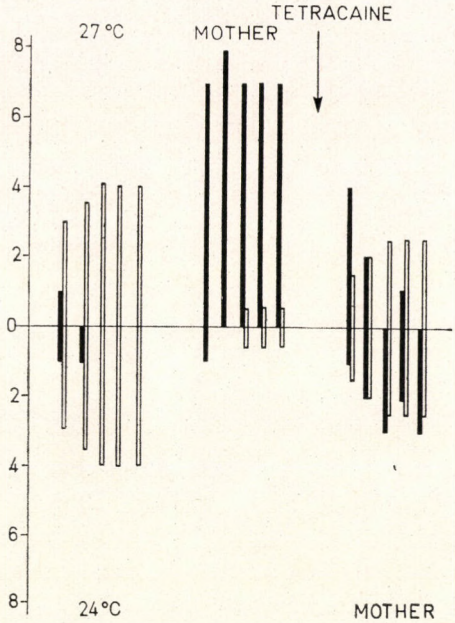

Fig. 6. Approaching behaviour after tetracaine anaesthesia of nasal mucosa

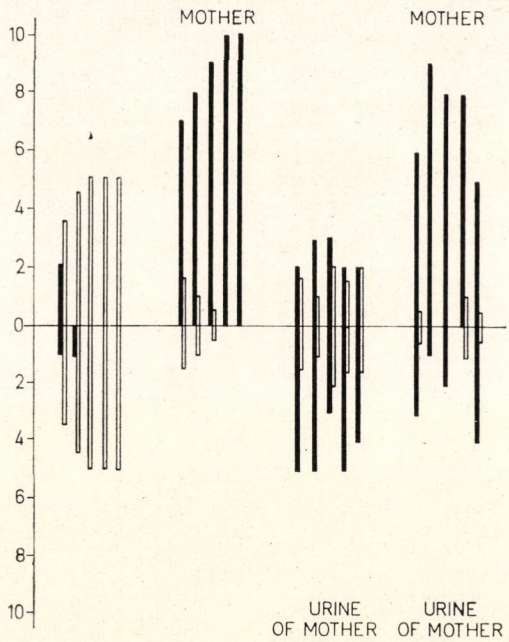

Fig. 7. Behaviour of infantile rats in four sessions under different experimental conditions. 1. Random activity in "empty" box. 2. Approaching activity to the mother. 3. Behaviour in the presence of cotton wood soaked in mother's urine. 4. Approaching behaviour to the mother if urine of mother was presented in the contralateral compartment A

of the mother in part A, the young visited the correct side even after the mother had been removed from there. If the mother was placed in parts A together with a nonlactating female, and both these rats were removed after 30 min the young approached the place of the mother and only seldom went to that of the nonlactating female (Fig. 3).

A specific guiding role of the lactating female in contrast to nonlactating ones is indicated by Fig. 4; there were, however, some young displaying a chance approaching activity.

On placing a male in one and the mother in the other goal-compartment, the young always went to the side of the mother and did not approach the male (Fig. 5).

Local application of 1% tetracaine into the nose of the young resulted in a random behaviour in the box, and the infants showed no goal-directed response to the mother (Fig. 6). Furthermore, if a cotton-wool soaked in the mother's urine was placed in part A, the smell elicited an orienting behaviour and in several cases the infants showed lateralization to the marked compartment. Simultaneous presentation of the mother on the contralateral side appeared as dominant in guidance of the litter (Fig. 7).

Discussion

The findings indicated that 10-day old rats can display a characteristic approaching behaviour to the mother in a maze and this somatomotor activity is guided by some kind of smell of lactating females. The fact that presentation of a male in the goal-compartment did not induce this goal-directed motor pattern showed the involvement in the process of specific olfactory cues. The young with inhibited olfaction did not go to the mother and showed hesitation to leave the centre of the box. This was attributed to the lack of the guiding sensory function.

The participation of olfaction in the learned behaviour of adults was studied by a number of investigators (LINDLEY 1930; CASPER 1933; HONZIK 1936; PHILLIPS 1968; WENZEL and SALZMAN 1968). Substitution of olfaction by other sensory functions in anosmic animals suggested that its role is limited after the development of visual and acoustic analyzers, but it seems that ablation or destruction of the olfactory bulb may retard the learning ability.

On the other hand, the early maturation of the olfactory analyzer in the rat and other mammals, as well as the chance of an establishment of temporary linkages to the olfactory system on the first postnatal day (VOLOKHOV 1959; KAWABE and NAKAJIMA 1963) strongly suggest that this sensory analyzer, which is one of the oldest in phylogenic differentiation, plays a specific role in infantile behaviour.

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РЕОГРАФИЧЕСКОЕ ИЗУЧЕНИЕ СООТНОШЕНИЯ ЦЕРЕБРАЛЬНОЙ ГЕМОДИНАМИКИ С ПЕРИФЕРИЧЕСКИМ КРОВОТОКОМ У ЛЮДЕЙ, ЗАНЯТЫХ УМСТВЕННЫМ ТРУДОМ

И. МАРХАСИНА и И. ОРАНСКИЙ

УРАЛЬСКИЙ ПОЛИТЕХНИЧЕСКИЙ ИНСТИТУТ ИМ. КИРОВА И СВЕРДЛОВСКИЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКИЙ ИНСТИТУТ КУРОРТОЛОГИИ К ФИЗИОТЕРАПИИ

(Статья поступила в редакцию 18. III. 1970 г.)

Состояние церебральной и периферической гемодинамики у людей, занятых умственным трудом, исследовалось методом реографии. Выявлено, что под влиянием умственной нагрузки возникают изменения в системе авторегуляции мозгового кровотока. Это сопровождается повышением кровенаполнения передних отделов, которое при нарастающей продолжительности умственного труда сменяется падением кровотока в передних отделах головного мозга.

Связь ментальных процессов с деятельностью сердечно-сосудистой системы была неоднократно обсуждена различными исследователями. Ещё в 1914 году Weber, исследуя кровенаполнение различных участков тела, показал, что при умственной работе, наряду с увеличением кровенаполнения головного мозга, возрастает кровенаполнение внутренних органов и падает кровоток в конечностях. Gillespie (1924) отметил увеличение частоты пульса и систолического артериального давления при умственной деятельности. Abramson и Ferris (1940), как и Burch (1948) наблюдали выраженную вазоконстриктивную реакцию при счёте в уме. Luria и Vinogradova (1959) показали, что при предъявлении испытуемому зрительных, слуховых или тактильных раздражителей возникает сужение сосудов конечности и расширение сосудов кожи головы у человека. Отметил изменение состояния сердечно-сосудистой системы при электрической стимуляции мозга, вызывающей поведенческие реакции Rushmer (1961). Weinman (1967) показал, что ментальные процессы оказывают существенное влияние на объём циркулирующей крови и объёмный пульс периферических сосудов.

Настоящая работа посвящена реографическому изучению связи церебральной гемодинамики с периферическим кровотоком у человека, занятого умственным трудом.

Методика

В исследовании принимало участие 40 испытуемых — практически здоровых мужчин и женщин в возрасте 28—50 лет; по профессии инженеры они в день исследования были заняты своей обычной работой — проектированием. Реографические регистрации проводились с помощью реографической приставки (30 кгц, 1,5 ма) на двухканальном чернильнопишущем электрокардиографе (модель 047) при горизонтальном положении испытаемого с приподнятым на 30° головным концом четырёхкратно в 09,00 часов (I проба), 11,30

(II), 14,00 (III) и 16,30 (IV). Длительность исследования 5—10 минут. Реоэнцефалограмма (РЭГ) записывалась при битемпоральном наложении прямоугольных свинцовых электродов площадью 2 см², реокардиограмма (РКГ) — в классическом варианте Holzer et al. (1946). Максимальные анакротические и дикротические амплитуды РЭГ и РКГ рассчитывались по отношению к калибровочному сигналу в условных единицах (усл. ед.). Статистическая обработка данных производилась на электронно-вычислительной машине Минск 22.

Результаты

Данные наблюдений представлены в таблицах I и II. Регистрация исходных значений (I проба) выявила, что между анакротическими и дикротическими амплитудами РЭГ и РКГ, отражающими соответственно состояние кровенаполнения мозга и верхней половины тела в систолической и диастолической фазах, возникает сильная корреляционная зависимость.

Таблица I

Значения анакротических и дикротических амплитуд у людей, занятых умственным трудом

Пробы	Отведения	Амплитуды	
		анакротическая	дикротическая
I	РКГ	1.42±0.07	0.80±0.04
	РЭГ	0.62±0.03	0.50±0.02
II	РКГ	1.60±0.08	0.90±0.05
	РЭГ	0.67±0.03	0.53±0.02
III	РКГ	1.50±0.07	0.95±0.05
	РЭГ	0.72±0.05	0.53±0.02
IV	РКГ	1.55±0.07	0.95±0.05
	РЭГ	0.64±0.03	0.53±0.02

Среднее значение ± стандартная ошибка средней в усл. ед.

Таблица II

Коэффициенты корреляции между анакротическими и дикротическими амплитудами РЭГ и РКГ у людей в процессе умственного труда

Пробы	Отведения	
	РКГ	РЭГ
I	+0.75	+0.87
II	+0.05	+0.75
III	+0.75	+0.80
IV	+0.73	+0.14

Спустя 2,5 часа от начала трудовой деятельности (II), когда регистрируются максимальные значения анакротических амплитуд ($p < 0{,}05$), она резко падает. Соответствующие этой пробе реоэнцефалографические амплитуды с $p < 0{,}05$ не отличаются от своих исходных значений.

III проба указывает на восстановление корреляционной зависимости амплитуд РКГ при максимальных дикротических значениях ($p < 0{,}01$), которые устанавливаются до конца исследования. Для РЭГ в этой пробе отмечаются максимальные значения анакротических амплитуд ($p < 0{,}05$) без статистически достоверных изменений дикротических величин с сохранением сильной положительной корреляционной зависимости.

В IV пробе выявляется падение корреляционной связи между анакротическими и дикротическими амплитудами РЭГ с их значениями, не отличающимися от исходных величин при $p < 0{,}05$.

Обсуждение

Полученные данные позволяют проследить динамику кровенаполнения головного мозга и верхней половины тела у человека умственого труда на протяжении рабочего дня. В физиологических условиях определяется доста-

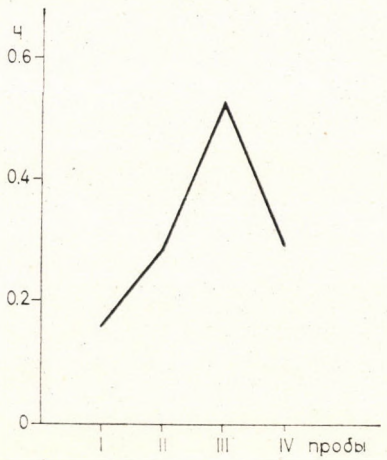

Рис. 1. Динамика значений коэффициента корреляции (ч) между анакротическими амплитудами РЭГ и РКГ у людей, занятых умственном трудом

точно строгое соответствие кровенаполнения исследуемого сегмента в систолической и диастолической фазах сердечного цикла как для церебрального, так и для периферического кровотока. Реографическая динамика изменений кровенаполнения мозга и периферических отделов позволяет считать, что при нарастающей интенсивности и продолжительности умственного труда определяется выраженное увеличение кровотока в мозге — в частности в его пе-

редних отделах. Этому предшествует периферическая реакция на предъявляемую умственную нагрузку: возникает увеличение кровенаполнения верхней половины тела, которое сопровождается явлениями дискоординации процесса, регулирующего кровенаполнение периферических отделов в систолической и диастолической фазах. Не исключено, что такая реакция может быть компенсаторной, поскольку при нарастающей умственной нагрузке в следующем этапе выявляется резкое повышение значения коэффициента корреляции между анакротическими амплитудами РЭГ и РКГ (Рис. 1). Тогда естественно предположение, что в данный период имеет место нарушение функционирования авторегулирующих систем контроля кровотока в мозге, которое проявляется в возникновении некоторого общего процесса, регулирующего периферический и церебральный кровоток. Эти изменения нивелируются к концу рабочего дня, однако на фоне возвратившихся к исходному уровню амплитудных значений РЭГ наблюдается разрыв корреляционной связи между ними. Эти данные позволяют считать, что на определённом этапе умственной нагрузки возникает снижение кровенаполнения передних отделов мозга, которое сопровождается явлениями дискоординации церебральной гемодинамики.

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Pathophysiologia

CAPILLARY RESISTANCE IN THE SKIN OF RATS FED FLAVONE-FREE AND ATHEROGENIC DIETS, AND THEIR RESPONSE TO HESPERIDIN-METHYL-CHALCONE

By

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In rats fed a flavone-deficient diet, capillary resistance decreased significantly within 10 weeks while thyroidectomy and an atherogenic diet for 10 weeks failed to produce significant changes in capillary resistance. When the flavone deficient diet was combined with thyroidectomy and atherogenic diet capillary resistance decreased excessively; in this effect, flavone deficiency had the leading role. Administration of hesperidin-methyl-chalcone greatly reduced the diminution of capillary resistance in rats fed flavone deficient and atherogenic diets.

Increased capillary fragility is a well-known feature of atherosclerosis. The phenomenon was studied experimentally by MCGRAW, on animals fed an atherogenic diet (MCGRAW 1963, 1964, 1965). Capillary resistance of the skin significantly decreases in rats on a flavone-deficient diet (RUSZNYÁK and BENKŐ 1941; RUSZNYÁK et al. 1964); it can be well controlled by flavone administration (RUSZNYÁK and SZENT-GYÖRGYI 1936; ARMENTANO et al. 1936).

The correlation between flavone deficiency and atherosclerosis is not clear. The combined action of flavone-free and atherogenic diets on capillary resistance has not been investigated. There are data as to how flavone treatment would influence the capillary changes induced by the combination of flavone-free and atherogenic diets.

The present paper describes the changes in capillary resistance induced by a combination of flavone-free and atherogenic diets and the influence exerted on these changes by the flavone compound, hesperidin-methyl-chalcone. It is well known that in rats atherosclerotic plaques will develop in the blood vessels when cholesterol feeding is combined with destruction of the thyroid gland (PAGE and BROWN 1952; WISSLER et al. 1954; FILLIOS et al. 1956), therefore several groups of our experimental animals were thyroidectomized.

Methods

A total of 81 male R-Amsterdam rats, weighing 160 to 230 g, was observed. They were divided into seven experimental groups.

6 rats of Group I were anaesthetized with ether and sham-operated. The sham-operation consisted of a transversal incision of the cervical skin, pulling apart the subcutaneous connective tissue and suturing the wound. The animals were fed a standard compressed food over 10 weeks.

16 rats of Group II were thyroidectomized under ether anaesthesia. Three weeks later they were fed the SHERMAN—LAMER—CAMPBELL (1922) vitamin C and flavone free diet of oatmeal, 59 g; heated powdered milk, 30 g; butter, 9 g, and NaCl, 1 g, ad libitum. In addition, they were given 1 g/kg body weight of cholesterol through an intragastric tube daily over 10 weeks. The animals were treated with 50 mg/kg of hesperidin-methyl-chalcone (Gee—Lawson Chemical Ltd., London) (HMC) dissolved in 1 ml physiological solution, injected subcutaneously every other day for 10 weeks.

In Group III, 16 rats were thyroidectomized. In addition to the standard diet they received 1 mg/kg of cholesterol daily over 10 weeks.

16 rats of Group IV underwent thyroidectomy, then three weeks later they were started on the Sherman—LaMer—Campbell diet and the above specified cholesterol treatment, continued for 10 weeks.

5 rats of Group V were fed exclusively the Sherman—LaMer—Campbell diet throughout the experimental period.

In Group VI 15 rats were thyroidectomized and three weeks later started on the Sherman—LaMer—Campbell diet.

8 rats of Group VII were sham-operated. Three weeks later they received the Sherman—LaMer—Campbell diet of 1 g/kg cholesterol daily over a 10 week period.

Capillary resistance was determined by the LAVOLLAY—NEUMANN (1949) method, on the depilated back skin, every week over 10 weeks, using a suction cup 8 mm in diameter The change in CR was expressed by the difference in per cent of the mean initial and fina. values. Statistical analysis was made by STUDENT's t test.

The animals were killed by decapitation at the end of the experiment.

Results

Results are presented in Table I.

The decrease of capillary resistance in Group I (normal diet, sham-operation) was significantly different only from that in Groups IV, V, VI and VII, fed the flavone-free Sherman—LaMer—Campbell diet. There was no significant difference from the animals of Group III (thyroidectomy, normal diet, 1.0 g/kg cholesterol) and from those of Group II (thyroidectomy, Sherman—LaMer—Campbell diet, plus cholesterol and HMC administration). The decrease in capillary resistance was significantly smaller in the animals fed the flavone-free diet combined with cholesterol and treated with hesperidin-methyl-chalcone following thyroidectomy than in those of the other groups fed the flavone-free diet (Groups IV, V, VI and VII). Obviously, the HMC-treated Group II showed no significant difference either from the sham-operated control Group I, or from the animals of Group III (thyroidectomy, atherogenic diet). There was a significant difference between the thyroidectomized cholesterol-fed group (Group III) and the groups fed the Sherman—LaMer—Campbell diet (Groups V, VI and VII). If the post-thyroidectomy flavone-free diet was combined with cholesterol administration (Group IV), the decrease in capillary resistance was significantly smaller than in the animals fed the flavone-free diet

Table I

Capillary resistance in thyroidectomized rats fed Sherman—LaMer—Campbell's flavone deficient diet and treated with cholesterol

No.	Groups and treatment	Number of animals	Mean CR values before treatment	Mean CR values after treatment	Mean CR decrement	Percentage CR decrement	Significance
I.	Sham-operation. Normal diet	6	31.6	23.0	8.6±1.25	27.3	$p < 1.0\%$
II.	Thyroidectomy. Sherman—LaMer—Campbell diet. Cholesterol plus HMC	16	26.2	16.0	10.2±2.89	39.0	$p < 0.1\%$
III.	Thyroidectomy plus cholesterol	16	29.0	16.0	13.0±2.33	44.9	$p < 0.1\%$
IV.	Thyroidectomy. Sherman—LaMer—Campbell diet plus cholesterol	16	23.5	7.8	15.7±1.29	66.8	$p < 0.1\%$
V.	Sherman—LaMer—Campbell diet	5	33.8	10.0	23.8±3	67.0	$p < 1.0\%$
VI.	Thyroidectomy. Sherman—LaMer—Campbell diet	15	27.2	7.3	19.9±3.28	73.2	$p < 0.1\%$
VII.	Sham-operation. Sherman—LaMer—Campbell diet plus cholesterol	8	32.0	8.0	24.2±2.02	75.0	$p < 0.1\%$

Significance of differences between groups, taking into consideration the mean decrements. Significant differences are presented exclusively.

I : IV $p < 2.0$ II : V $p < 2.0$ III : V $p < 1.0$ IV : V $p < 5.0$
I : V $p < 1.0$ II : VI $p < 1.0$ III : VI $p < 2.0$ IV : VI $p < 5.0$
I : VI $p < 5.0$ II : VII $p < 1.0$ III : VII $p < 1.0$ IV : VII $p < 0.1$
I : VII $p < 0.1$

CR = capillary resistance

alone (Group V), or those subjected to thyroidectomy and Sherman diet (Group VI), or to sham-operation and flavone deficient atherogenic diet (Group VII). In Group V the decrease in capillary resistance amounted to as much as 67%. The diet alone produced a greater change in capillary resistance than the diet combined with thyroidectomy and cholesterol administration (Group IV), and greater change than that produced by the combination of thyroidectomy and cholesterol treatment (Group III). The combination of thyroidectomy and flavone deficiency (Group VI) or of flavone deficiency and atherogenic diet following sham-operation (Group VII) induced no changes significantly different from those found in the purely flavone-deficient rats.

Discussion

The results obtained indicate that the flavone-free diet alone and in combination with an atherogenic diet (Groups IV, VI and VII) significantly reduced capillary resistance.

During the 10-week experimental period, thyroidectomy and cholesterol feeding (Group III) caused a non-significant decrease of capillary resistance in the rats.

This evidence is not in contradiction with the results obtained by McGRAW (1963, 1964, 1965) who found a major fall of capillary resistance in rabbits with experimental atherosclerosis. In addition to the difference of species the question arises whether feeding of the atherogenic diet for 10 weeks sufficed for producing significant capillary changes. When the atherogenic diet was combined with thyroidectomy and flavone deficiency (Group IV) the decrease in capillary resistance was smaller than in the flavone-deficient group (Group V).

It may be stated that the atherogenic diet, at least in the course of 10 weeks, did not enhance the capillary resistance-decreasing effect of flavone deficiency. On the other hand, flavone deficient feeding alone proved sufficient to produce a significant decrease of capillary resistance in 10 weeks.

HMC treatment significantly reduced the diminution of capillary resistance in the rats fed the atherogenic and flavone-deficient diets (Group II). There is published evidence of the favourable influence of HMC treatment on the capillary resistance of the skin in animals with damaged capillaries whether their diet was deficient or normal (MARTIN et al. 1954; GÁBOR 1960). Certain flavonoids even prolonged the survival of rats kept on an atherogenic diet (ROBBINS 1967).

In atherosclerotic rabbits and chicks LEGER et al. (1960) found that flavone treatment had a favourable influence on the elastic structural changes of arteries, and in atherosclerotic patients flavonoid treatment was reported to restore the decreased capillary resistance to normal (CHUNAKOVA et al. 1968).

The decrease of capillary resistance was prevented by HMC treatment in rats fed a combination of atherogenic and flavone-deficient diets. This effect might have been due to a relief of flavone deficiency by the active bioflavonoid ("vitamin P"). We have produced this effect with low doses (50 mg/kg), as compared to the 100, 200 or even 400 mg/kg doses employed by others (CHARLIER et al. 1963) in acute experiments.

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DISTURBANCES OF PORPHYRIN METABOLISM IN SCHIZOPHRENIA*

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In experimentally induced vitamin B_6 deficiency the porphyrin fractions of blood and urine of schizophrenics were determined with the following loads: 4-deoxypyridoxine, 4-deoxypyridoxine and tryptophan, as well as with isoniazid (INH). The protoporphyrin level of the erythrocytes and the urinary coproporphyrin content decreased only during the l-tryptophan load in both the controls and the schizophrenics.

Urinary delta-aminolaevulinic acid excretion decreased in the controls, whereas in the schizophrenics the excreted quantity fluctuated in a wide range.

The esters of 7-6-5-carboxylic porphyrins formed in uro-coproporphyrin conversion were separated by thin-layer chromatography. Experiments with INH loads showed results similar to those obtained with 4-deoxypyridoxine.

The possible role of pyridoxal enzymes in the disturbances of porphyrin metabolism of schizophrenics is discussed.

It has been shown (HUSZÁK and DURKÓ, 1962, 1968) that in the urine of schizophrenics, besides indoles, 6-carboxylic and other porphyrins could be observed in increasing amounts with and without tryptophan loading. Special interest has been added to this by the findings of PRICE et al. (1959) who observed in patients with porphyria, mainly in the acute phase like in schizophrenics, an increased urinary excretion of indole metabolites.

The psychic manifestations of these two conditions may display common symptoms. Porphyrias showing schizoform symptoms have therefore been denoted by PRICE as "porphyric-schizophrenic". In both syndromes a functional disturbance of the pyridoxal enzymes is assumed, although on vitamin B_6 administration the metabolic disorders and clinical symptoms improve only slightly or not at all. According to IPPEN (1967), in the acute phase of porphyria cutanea tarda there is a parallelism between the enhanced excretion of porphyrins and of the indole metabolites especially of 5-hydroxy-indole acetic acid. Among the substances precipitating porphyria, IPPEN emphasized the effect of isoniazid (INH). In a search for the disturbances of porphyrin metabolism in schizophrenics, we have studied the role of the pyridoxal enzymes. In experimental vitamin B_6 deficiency the porphyrin fractions of the blood and urine of schizophrenics was determined after loading with 4-deoxypyridoxine, 4-deoxypyridoxine and tryptophan, as well as INH.

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Methods

Materials

Talc; copro III tetramethylester (Calbiochem); Kieselgel-G nach Stahl (Merck); 4-deoxypyridoxine (Fluka); l-tryptophan (Reanal); ion-exchange resin (Dowex 50W-X8); delta-aminolaevulinic acid hydrochloride (Calbiochem). All the substances were of analytical grade.

Spectrophotometric determination of protoporphyrin in blood

Blood samples were handled in the dark and immediately analyzed. The determination was done from 13—18 ml of blood treated with heparin by HEILMEYER's extraction method (HEILMEYER and CLOTTEN, 1964). Protoporphyrin was calculated by the formula,

$$\text{Protoporphyrin } \mu g/100 \text{ ml red blood cells} = \frac{\mu g\%/\text{ml} \times 1.528^2 \times \text{extr. ml} \times 100}{\text{blood volume, ml} \times \text{haematocrit}}$$

Spectrophotometric determination of coproporphyrin

25 ml of urine collected over 24 hours in a dark bottle was used for the determination carried out according to RIMINGTON (1961). The amount of coproporphyrin was calculated

$$\text{in } \mu g/l = \left[2\, D_{401}^{max} - (D_{430} + D_{380})\right] \cdot 837 \cdot \frac{v}{V}$$

V = quantity of urine taken
v = quantity of 5% HCl extract

A single column method for the determination of urinary delta-aminolaevulinic acid

Urine collected over 24 hours in a dark bottle was adjusted with acetic acid to pH = 4—6. Of this, 1 ml was used for the determination. Both the determination and the preparation of Dowex 50W—X8 ion exchange resin were done by the method of MU-WAN-SUN et al. (1969).

Extraction of porphyrins

For porphyrin extraction from urine and esterification, DEZELIC and CETINIC's method (1966) was used with a slight modification. Urine was collected over 24 hours periods in glass jars shielded from light, analyzed daily, or occasionally refrigerated at —20°C. 2 g talc was added to each 100 ml of urine and the solution was shaken in a 250 ml bottle for 5 minutes. Thereafter it was washed through a folded filter paper with 2×50 ml distilled water and 1×50 ml saturated sodium acetate solution. Then the filter with talc containing the porphyrins was transferred into a 100 ml Erlenmeyer flask. 20 ml methanol and concentrated sulphuric acid (20 : 1) were added and the mixture was allowed to stand for 12 hours in the dark. The alcoholic solution together with the talc was filtered through a folded filter paper which was washed again with 50 ml ethylacetate and then the filtrate was poured into a separation funnel. Subsequently, 20 ml of a 17% solution of sodium acetate was added and the mixture was shaken again. The separated ethylacetate phase was concentrated with a rotary evaporator under reduced pressure in the dark.

Separation of methyl esters of porphyrins

Separation of methyl esters of porphyrins was done by thin-layer chromatography. 0.01—0.02 ml of the evaporated substance (final volume, 0.2—0.4 ml) was applied onto a Kieselgel-G layer. After 1 hour equilibration, chromatographic separation was carried out with the solvent isopropanol-1N ammonia (5 : 1) for about 6 hours at 22°C.

Results

The protoporphyrin content of erythrocytes was estimated by HEILMEYER's extraction method (HEILMEYER and CLOTTEN 1964). On the first and second day of the loads, 100 mg 4-deoxypyridoxine was administered orally and on the third and fourth days further 1 g tryptophan was added. Values are given in μg/100 ml red blood cells.

The protoporphyrin level of the erythrocytes — taking for normal the value of 20—50 μg/100 ml erythrocytes given by HEILMEYER — fell under the normal value only during the l-tryptophan load on the third and fourth days, as can be seen in Fig. 1. In the controls — taking into account the average of ten experiments — the rate of this fall was always greater than in the schizophrenics. After discontinuing the loads, the values for protoporphyrin rapidly returned to normal; they never attained the high values characteristic of hepatic or chemically-induced porphyria.

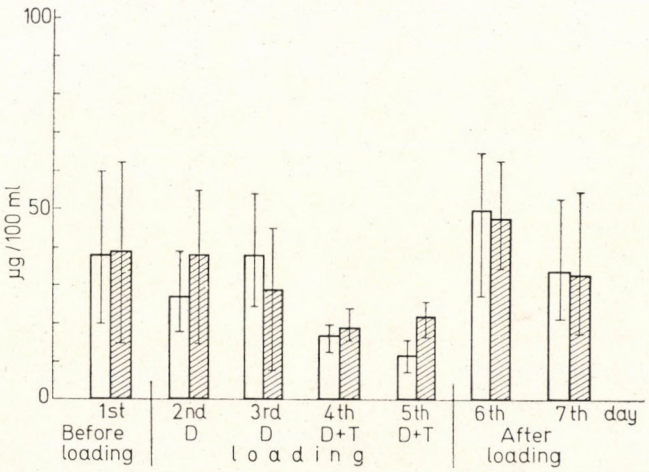

Fig. 1. Protoporphyrin content of red blood cells. The columns represent the mean value of ten experiments and the bars the minimum and the maximum values. D = 4-deoxypyridoxine; T = l-tryptophan; □ = control; ▨ = schizophrenia

Under the effect of the above loadings, the coproporphyrin values as determined spectrophotometrically, fluctuated between the normal limits of 0—100 μg/24 h. Since the data in the literature vary greatly as to the normal values for coproporphyrin, so that in the case of 4-deoxypyridoxine and especially in that of l-tryptophan all that can be stated is that they showed a decreasing tendency. In the schizophrenics, after discontinuing loading coproporphyrin excretion suddenly rose whereas the controls gradually decreased to the initial level.

Since pyridoxal enzymes are taking part in delta-aminolaevulinic acid synthesis and since in the different forms of porphyria, urinary delta-aminolaevulinic acid excretion is enhanced, this, too, has been studied.

As seen in Fig. 3, under the effect of the above loadings delta-aminolaevulinic acid excretion decreased in the controls, whereas it fluctuated in a wide range in the schizophrenics. It should be mentioned that with rare exceptions

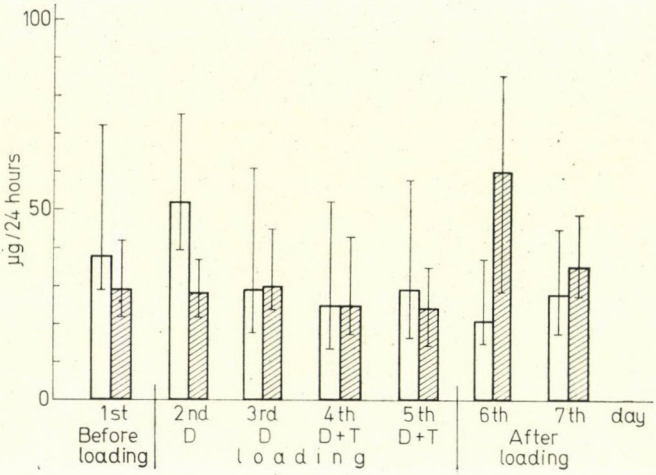

Fig. 2. Daily urinary excretion of coproporphyrin. The columns represent the mean value of ten experiments and the bars the minimum and the maximum values. D = 4-deoxypyridoxine; T = l-tryptophan. □ = control; ▨ = schizophrenia

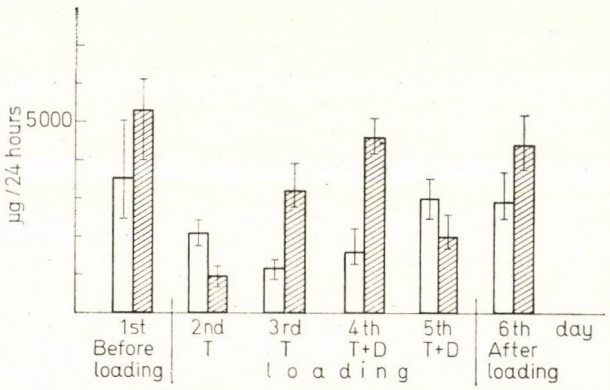

Fig. 3. Daily urinary excretion of delta-aminolaevulinic acid. The columns represent the mean value of ten experiments and the bars the minimum and the maximum values. D = 4-deoxypyridoxine; T = l-tryptophan; □ = control; ▨ = schizophrenia

the screening tests for detecting an increase in urinary porphyrins, were negative in the schizophrenics.

The esters of 7-6-5-carboxylic porphyrins formed during uro-coproporphyrin conversion were separated by thin-layer chromatography. Fig. 4 illustrates porphyrin excretion ratios before loading, Fig. 5, those under 4-deoxypyridoxine loading, and Fig. 6, those after the loading. The following deductions can be made: As shown in Fig. 4, in contrast to the controls in schizophrenics the intermediates of uro-copro conversion could be observed in the preload state, while coproporphyrin could not be detected in any case. Fig. 5 shows the

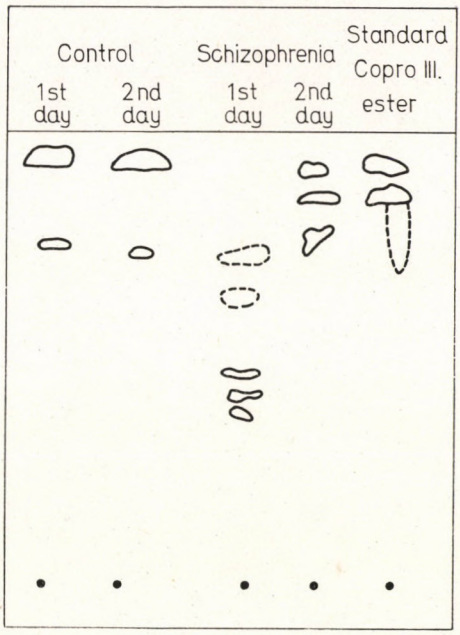

Fig. 4. Thin-layer Kieselgel-G chromatogram of urinary porphyrin methylesters indicated under U. V. light. Solvent system: iso-propanol-1 NNH_3 (5 : 1)

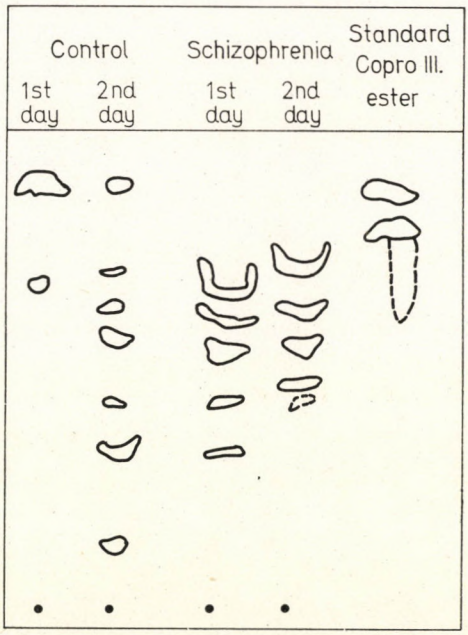

Fig. 5. Thin-layer Kieselgel-G chromatogram of urinary porphyrin methylesters under 4-deoxy-pyridoxine administration, indicated under U. V. light. Solvent system: isopropanol-1 NNH_3 (5 : 1)

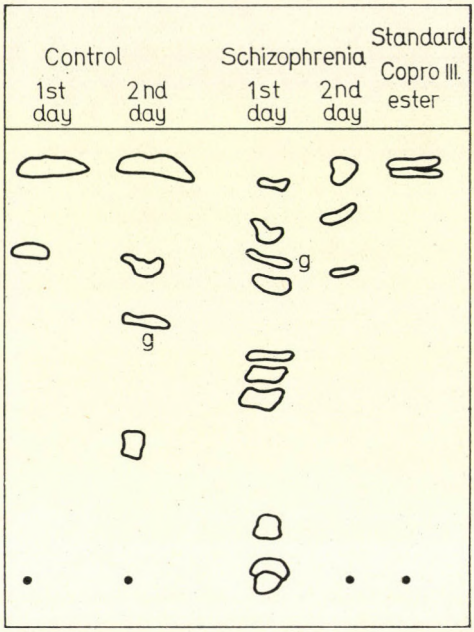

Fig. 6. Thin-layer Kieselgel-G chromatogram of urinary porphyrin methylesters after 4-deoxypyridoxine administration, indicated under U. V. light (g = green). Solvent system: isopropanol-1 NNH_3 (5 : 1)

changes occurring during 4-deoxypyridoxine administration. The most striking change was that these uro-copro intermediates appeared also in the controls.

Fig. 6 illustrates that after the loading the controls excreted only coproporphyrin, while in the urine of schizophrenics an increasing number of spots was found and the initial state was only reached in one or two days.

Experiments with INH loads yielded results similar to those obtained with 4-deoxypyridoxine.

Thus in addition to disturbances in tryptophan metabolism a certain disturbance in porphyrin metabolism can also be observed in schizophrenics. In such patients, from among the intermediates of uro- and coproporphyrin transition, urinary excretion of the 6-5-carboxylic porphyrins is enhanced.

Discussion

San Martin de Viale and Grinstein (1968) have shown that the 6-5-carboxylic porphyrinogens (III) are normal intermediates of haem-synthesis. In some porphyrias, e.g. porphyria cutanea tarda (Rimington, 1952), erythropoietica, as well as in the experimental porphyrias an increased excretion of

8-7-6-5-4-carboxylic porphyrins can be observed (CHU and CHU 1965). In schizophrenia such a considerable excretion of these intermediates cannot be detected even under different loads, and, in contrast to the porphyrias, 7-carboxylic porphyrin is not excreted.

The effect of l-tryptophan was remarkable in that, when it was administered simultaneously with pyridoxal antagonists, the disturbances of porphyrin metabolism became more pronounced. Some authors (HENDERSON et al. 1955) assumed that pyridoxal metabolism was directly influenced by tryptophan loads. MCCOY et al. (1965) stated that in experimental vitamin B_6 deficiency the excretion of oxalate, and particularly that of xanthurenic acid increases and according to them the latter is an indicator of vitamin B_6 deficiency. In the opinion of KOYANAGI et al. (1968), however, xanthurenic acid excretion is increased in riboflavin deficiency, too. Therefore, it seemed unnecessary to check the relative vitamin B_6 deficiency of the organism on the basis of oxalate and xanthurenic acid excretion, and in our opinion it is more suitable to estimate the vitamin B_6 components in the blood. These investigations are in progress.

The role of coenzyme-pyridoxal P in delta-aminolaevulinic acid synthesis is well known. With 4-deoxypyridoxine loads, a slight decrease in delta-aminolaevulinic acid could be observed and this was more expressed with simultaneous tryptophan loads. These effects were more pronounced in the controls than in the schizophrenics. The role of pyridoxal enzymes in the decarboxylation process of 8-7-6-5-4-carboxylic porphyrins has not been elucidated. According to the present assumption a "single enzyme is responsible" for the decarboxylation process (GOODWIN 1968). RIMINGTON (1959) stated that during haem-synthesis the active intermediates are uro-7-6-5-coproporphyrinogens and not the corresponding porphyrins excreted by the organism as end-products. This would explain the fact that in schizophrenics not subjected to loadings, apart from the above described increase in 6-5-carboxylic porphyrins, no disturbances in the protoporphyrin content of erythrocytes could be observed. NAKAO et al. (1967) have shown that in case of a normal erythrocytic protoporphyrin level there cannot be a considerable disturbance in hepatic protoporphyrin synthesis. In their opinion, in experimentally induced porphyrias the over-production of protoporphyrins takes place first of all in the liver and only if it had reached there a certain level can it become manifest in the erythrocytes as an excess. The decrease in the erythrocytic protoporphyrin level observed with l-tryptophan loads suggests that these may primarily influence the haem-synthesis in the liver.

In view of all this, we cannot offer a satisfactory explanation of the above effect of tryptophan on porphyrin metabolism. Nevertheless, it is assumed that the disturbances of porphyrin metabolism may be in some relation with the pathological tryptophan metabolism in schizophrenia.

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Pharmacologia

EFFECT OF PROPRANOLOL ON CIRCULATORY REGULATION

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The effect of 0.2 mg/kg of propranolol on blood flow in the brain, heart, kidneys, liver, skin and muscles has been studied in 20 dogs, by the combined administration of ^{131}I-antipyrine and ^{86}Rb. Cardiac output, circulating blood volumen and mean transit time were determined by the dye dilution method using Evans blue.

The data of 40 dogs were used as controls. As an acute effect of propranolol, beta blockade, diminished cardiac output, increase of total peripheral resistance and a significant diminution of flow velocity were observed. Blood pressure changed insignificantly.

While in the organ fractions of cardiac output an increase occurred in the brain, heart and skin, in the striated muscles vasoconstriction took place that manifested itself with a diminished fraction of cardiac output for this area. Kidney and liver blood flow decreased parallel with the diminution of cardiac output. The data indicated a basic influence of beta receptors upon circulatory regulation.

The role of beta receptors in the control of cardiac activity and vascular resistance is well-known. On their stimulation, a considerable change in cardiac output, blood pressure, systemic and pulmonary resistance occurs. On the other hand, the role of beta receptors in the regulation of the circulation as well as their effect upon the organ fractions of cardiac output is still unknown (CHAMBERLAIN and HOWARD, 1964).

The aim of the present experiments was to study the blood flow and the cardiac output's participation of the brain, heart, kidneys, liver, skin and muscles under the effect of beta blockade by propranolol.

Method

A total of 60 mongrel dogs of either sex weighing 10—15 kg was used under intravenous hexobarbital-Na anesthesia. In 20 dogs the proximal part of the external iliac artery was set free and cannulated. Blood pressure was recorded by means of a mercury manometer on the one side, whereas the other served for collecting blood samples. For beta blockade, propranolol (Inderal ® I. C. I.) was administered intravenously in a dose of 0.2 mg/kg.

The hemodynamic studies were performed at the peak of blockade, 10—15 min after drug administration. Cardiac output, circulating blood volume and mean transit time were determined according to STEWART and HAMILTON, injecting 2 mg/kg of Evans blue.

In each consecutive second samples were collected from the femoral artery. In order to determine blood volume a sample was withdrawn at the third minute after dye injection. Blood flow and the organ fractions of cardiac output were determined by SAPIRSTEIN's method in the brain, kidneys, liver, skin, and striated muscles (SAPIRSTEIN et al. 1960), two minutes after

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Table I

Effect of propranolol on circulatory control

	P_m mm Hg		CO l/min		TVR dyn sec cm^{-5} x 1332		MT sec		Brain				Heart			
									BF		VR		BF		VR	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
n	40	20	40	20	40	20	40	20	20	20	20	20	38	20	38	20
x	112	109	2.4	1.8	2.69	3.64	12	17	55	49	130	135	132	119	56	55
se	1.76	1.97	0.01	0.05	0.10	0.12	0.28	0.30	2.07	1.57	4.20	4.92	3.75	3.71	2.70	2.65
s diff	3.44		0.14		0.26		0.57		3.80		0.19		0.48		4.38	
p	>0.4		<0.001		<0.001		<0.001		<0.1		>0.5		>0.05		>0.7	

Abbreviations:

P_m = mean systemic blood pressure
CO = cardiac output per 10 kg weight
TVR = total peripheral vascular resistance
MT = mean transit time
BF = tissue blood flow (ml/min) per 100 g
VR = vascular resistance to tissue blood flow (per 100 g)
A = control group
B = propranolol treated group
n = No. of experiments
x = average

	Kidney				Liver				Muscle				Skin			
	BF		VR		BF		VR		BF		VR		BF		VR	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
n	20	20	20	20	17	19	17	19	40	20	40	20	40	20	40	20
x	555	332	14	22	84	55	85	124	18	8	485	1080	17	17	510	505
se	7.20	22.19	0.43	1.66	2.22	2.41	2.22	4.68	0.70	0.84	29.40	149.50	0.63	0.87	27.20	61.13
s diff	45.0		2.09		4.35		7.46		1.60		109.7		1.47		68.8	
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		>0.6		>0.8	

se = standard error
s diff = standard error of the difference between A and B
p = statistical probability

the intravenous injection of 30 μC ^{86}Rb and 5 μC ^{131}I-antipyrine in 5 ml physiological NaCl.

Immediately afterwards the animals were killed by the intracardiac administration of 2 g sodium hexobarbital. The brain, heart, kidneys, skin, liver and muscles were weighed on analytical scales, then boiled and dissolved in 3 N NaOH. Activity was recorded simultaneously by a spectrometer according to the different photo peaks (SOLTI et al. 1967). The data of other 40 dogs served as control.

Cardiac output was expressed per 10 kg of body weight, blood flow and vascular resistance were computed for 100 g organ weight. The organ fractions of cardiac output were calculated for 100 g organ weight.

For statistical analysis, STUDENT's *t*-test was used.

Results

Results are summarized in Table I.

As Table I reveals, mean systemic pressure, cerebral, myocardial and cutaneous flow did not change significantly after beta blockade, as compared to the controls. On the other hand, after propranolol treatment there was a considerable decrease in cardiac output, renal, hepatic and muscle blood flow, while a significant increase in total peripheral resistance and in the vascular resistance of the kidneys, liver and muscles could be observed, and mean circulatory transit time was prolonged.

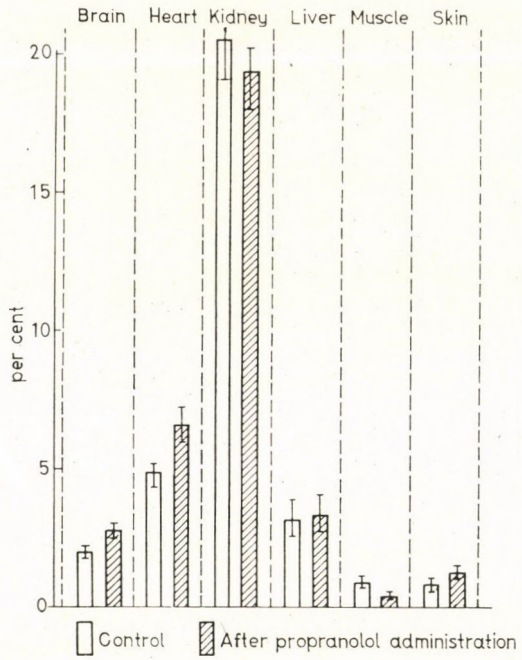

Fig. 1. The organ fractions of the cardiac output after beta blockade

The behaviour of the organ fractions of cardiac output after beta blockade is represented in Fig. 1. As a most characteristic change, a pronounced increase in the fractions for brain, myocardium and skin occurred while the striated muscle fraction diminished.

Discussion

Propranolol blocks the beta receptors selectively, without a preceding sympathomimetic effect (BLACK et al. 1967).

The cardiac and circulatory effects do not seem to be due to the bradycardia induced by the drug (BLOOMFIELD and SOWTON 1967; DONOSO et al. 1966). According to FLACKE et al. (1967) previous sympathetic blockade by means of high spinal anaesthesia is inhibiting the circulatory effects of propranolol.

The above data show that propranolol exerts its circulatory effect primarily through a blockade of beta receptors which makes it suitable for studying the effect of beta receptors on circulatory control.

In our experiments propranolol induced a considerable decrease of cardiac output, an increase of systemic resistance, and a slowing down of the whole circulation. These data are in agreement with the results of the majority of authors (CUNNING and CARR 1966; EPSTEIN et al. 1965; HARRIS and SCHOENFELD 1966; MCKENNA et al. 1966; NAKANO and KUSAKARI 1966; NAYLER et al. 1967; SAPIRSTEIN et al. 1960; SOWTON and HAMER 1966; VOGEL and BLOUNT 1967) and point to the important role of beta receptors in the control of systemic vascular resistance and cardiac output.

In contrast, CHAMBERLAIN and HOWARD (1964) and MURRAY et al. (1966) have not found beta blockade to diminish cardiac output, nor could they demonstrate an increase in peripheral resistance. This latter discrepancy in the experiments of CHAMBERLAIN and HOWARD might have been due to their using pronethalol — a not quite specific beta blocking agent —, while MURRAY et al. applied an unusually high dose of propranolol (0.5 mg/kg).

According to our results, normal blood pressure is not significantly depressed by beta blockade, though a retardation of the whole circulation occurs after propranolol administration (see Table I).

Concerning the role of beta receptors in circulatory control, few data are available. KORNER et al. (1967) ascribed the circulatory changes after arterial hypoxia to beta receptors, while COFFMAN and KELLY (1966), BRICK et al. (1967) could show their role in the circulatory reactions due the hyperventilation. LACROIX and LEUSEN (1966) could prevent the augmentation of cardiac output following stimulation of the carotid sinus baroreceptors, whereas blood pressure remained unaltered.

Studying the circulatory actions in a great number of patients with hyperkinetic circulation, thus with increased cardiac output, heart rate and shortened circulatory transit time, GRAF and STRÖM (1966) concluded to a sympatho-adrenergic preponderance in such cases. On propranolol treatment the complaints were eliminated and the circulatory parameters normalized (BLOOMFIELD and SOWTON 1967; FRÖHLICH et al. 1966; MASON and BRAUNWALD 1967). It is also probable that beta receptors may have a role in circulatory regulation during exercise (CUNNING and CARR 1966; EPSTEIN et al. 1965; MCKENNA et al. 1966; SOWTON and HAMER 1966).

The present experiments revealed also a change in the circulatory control after propranolol. The organ fractions of cardiac output for the vital organs such as the heart and the brain increased (see Table I), which allowed the flow in these organs to remain at a normal level in spite of the decreased cardiac output (see Table I).

An interesting change took place in the muscle and skin vessels after beta blockade: the skin fraction of cardiac output increased, whereas a pronounced vasoconstriction became manifest in the muscular vessels; the cardiac output fraction of muscles diminished. Since stimulation of beta receptors elicits vasodilation in the muscle vessels, their inhibition manifests itself with vasoconstriction and an increase in vascular resistance. Total vascular resistance of the extremities is chiefly a result of both muscular and cutaneous vascular resistance. After beta blocking there is only a minor change in the resulting resistance of the whole extremity (BOLLINGER and SIMON 1967; BRICK et al. 1967; COFFMAN and KELLY 1966; KAYALP and KIRAN 1966); in fact, a small decrease occurs.

In the present experiments no remarkable change could be observed in the vascular bed of the kidney and the liver; under the effect of propranolol, the flow in these organs decreased parallel with the diminished cardiac output (see Table I, and Fig. 1).

It could thus be shown that beta blockade leads to remarkable changes in the circulation and its regulatory processes also in resting condition. This stresses the importance of beta receptors in the control of systemic circulation, as well as in the local control of the vascular areas of the brain, heart, muscles and skin. Hyperkinetic circulation is characterized mainly by a low systemic vascular resistance, an increased cardiac output and the increased velocity of circulation. As another characteristic of this state, GRAF and STRÖM (1966) pointed to the decreased vascular resistance in muscle, i.e. to increased muscle flow. It is also known that in great number of cases the hyperkinetic symptoms of vasoneurotic patients are eliminated by beta blocking agents (BOLLINGER and SIMON 1967; FRÖHLICH et al. 1966). This underlines the significance of our observation that changes contrary to the hyperkinetic state could be observed after pharmacological beta blockade; cardiac output diminished, total

peripheral resistance increased, the circulation slowed down and vasoconstriction was manifest in the muscles.

Thus, a number of pathological states accompanied by a hyperkinetic circulation is based on a beta receptor preponderance.

Acknowledgement

We are indebted to Mr. J. FISCHER, Mathematical Research Institute of the Hungarian Academy of Sciences, for statistical analysis of the data.

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COMPARISON OF THE CARDIODEPRESSANT AND ANTIARRHYTHMIC EFFECTS OF 2-PIPERIDINYL-1-α-NAPHTHYL-1-PROPANOL HCl (S-1043) AND PROPRANOLOL

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It has been shown that 2-piperidinyl-1-alpha-naphthyl-1-propanol (S-1043) even at the highest concentration applied (8 μg/ml), has no beta blocking potency. The substance, like propranolol, decreased myocardial excitability and pacemaker activity; it exerted a local anaesthetic effect on the rabbit cornea and prolonged the functional refractory period in atrial muscle. This effect was found to be stronger than that of propranolol. S-1043 decreased the contractile force of the heart muscle to a lesser extent than propranolol, and was found to be twice as potent as propranolol in the reversal of ouabain-induced arrhythmia in the dog.

In the past years, a number of phenyl- and naphthylalkylamine compounds were studied in our laboratory (KNOLL et al. 1965; KNOLL et al. 1966), to clarify the relationship between their chemical structure and their pharmacological effect. As a result, several derivatives with antiarrhythmic activity have been synthetized (HADHÁZY et al. 1968) the cardiac actions of which were in many respects similar to those of propranolol, but they did not block the β-adrenergic receptors. Further studies of the naphthylalkylamine derivatives revealed that it was possible to separate the antiarrhythmic and negative inotropic actions. The importance of this kind of dissociation has been emphasized by several authors (STEPHEN 1966; WOLFSON et al. 1966; SZEKERES and PAPP 1968). S-1043 was chosen for detailed analysis since the relation between its inotropic and other depressant actions appeared to be favourable. The present paper describes some pharmacologic effects of this compound in comparison with those of propranolol. Both S-1043 and propranolol contain a naphthyl-ring and an alkyl-link with a tertiary N. This structure (aromatic ring—alkyl-link—tertiary N) is characteristic of the antiarrhythmic substances (DI PALMA and SCHULTZ 1950; SZEKERES and PAPP 1968).

Methods

Analysis of inotropic effects. Isolated left auricles from cats were mounted on platinum electrodes and placed in a 20 ml organ bath containing Tyrode's solution at 30°C of the following composition (mEq per liter): Na^+, 137.3; K^+, 2.7; Ca^{++}, 3.6; Cl^-, 133.4; $H_2PO_4^-$, 0.4; HCO_3^-, 23.8; dextrose, 5.0. The solution reservoir and the organ bath were bubbled through with a mixture of 95% O_2 and 5% CO_2. Temperature and composition of the organ bath were

the same as in our previous experiments. Constant stimulation with 60/min, 3 fold threshold voltage, 1-msec square impulses delivered from a DISA Multistim stimulator was applied. Contractions were recorded through an Isometric Microdynamometer (Ugo Basile) on a smoked drum. The resting tension of the auricles was adjusted to obtain maximal contractions.

For determination of β-adrenergic activity, isoproterenol bitartrate (Isolevin, Cilag) was added to the bath at a concentration of 2×10^{-8} g/ml, exerting a submaximal inotropic effect. After washing, 8 μg/ml S-1043 was added to the bath and after 90 min the action of isoproterenol at the above concentration was again recorded. The effect on contractile force was determined 90 min after administration of the test substances. Changes were expressed in per cent of control values. Concentrations inducing a 50% decrease in contractile force were calculated from dose-response curves.

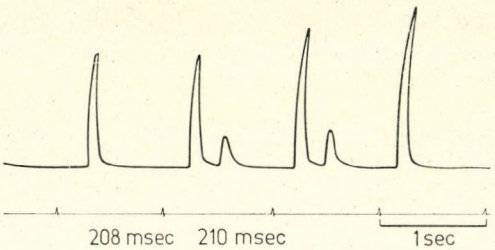


Fig. 1. Determination of the functional refractory period of the cat auricle by the paired stimulation method. Increase of the interval of the driving and test stimulus from 208 to 210 msec results in a contraction to each stimulus

Chronotropic studies. Isotonically contracting right auricles from cats were used. After a steady state in frequency had set in, the test substances were added to the organ bath and 60 min later the frequency was again determined. Changes in the number of beats/min were expressed as per cent of the initial value.

Measurement of the refractory period. The functional refractory period (RP) of the electrically driven left cat auricle was measured by the method of DAWES (1946) and by the paired stimulation method of GOVIER (1965). In the latter case the auricles were stimulated at a basic driving frequency of 60 beats per minute, at 3 times threshold voltage and 1 msec duration. RP was measured by delivering a second stimulus, identical to the first, at increasing intervals following the driving stimulus until each stimulus was followed by a contraction. The method is illustrated in Fig. 1.

The effect of S-1043 and propranolol on the refractory period was calculated from concentration-response curves. Drugs were allowed to act for 60 minutes.

Effect on myocardial excitability. The electrical threshold was determined by noting the minimum voltage required to induce contraction. The thresholds at different impulse durations (0.05 to 100 msec) were determined and the values for chronaxie and rheobase were calculated from the strength-interval curve.

Local anaesthetic effect. The local anaesthetic effect was studied by the method described by CHANCE and LOBSTEIN (1944). Drugs at different concentrations were instilled into the conjunctival sac of rabbits. Five minutes later surface anaesthesia was estimated by touching the cornea five times in rapid sequence. If no blinking reflex was elicited by this procedure for at least 10 minutes, corneal anaesthesia was regarded to be present. Measurements were carried out at intervals of 5 minutes until the return of the corneal reflex.

Ouabain-induced arrhythmia. Mongrel dogs of either sex, weighing 8 to 12 kg were used. They were anaesthetized with sodium pentobarbital (30 mg/kg), supplemental doses being administered according to need. Lead II electrocardiograms were taken. Ventricular tachycardia was elicited by ouabain in an initial dose of 40 μg/kg followed by 20 μg/kg 30 minutes later. Doses of 10 μg/kg were then given at 15 minute intervals until a persistent ventricular tachycardia had ensued (LUCCHESI and HARDMAN 1961). Test substances were administered 10—15 minutes after development of the arrhythmia. S-1043 was injected intravenously over 3 to 5 sec to five dogs in cumulative manner in order to estimate the antiarrhythmic efficacy. Propranolol and S-1043 were tested in further two groups of animals in the following way. Both compounds were infused at a rate of 5 mg/min until normal sinus rhythm had reappeared and

over an additional minute in order to prolong somewhat the duration of the effect. Reappearance of the arrhythmia was thought to be important to avoid possible experimental bias resulting from spontaneous reversal. Reappearance of even a single ventricular beat was considered the end of the antiarrhythmic effect. This semiquantitative method is widely accepted for estimating antiarrhythmic effects in digitalis-arrhythmia (LUCCHESI 1964; SOMANI and LUM 1965; SCHMID and HANNA 1967; BARRETT and CULLUM 1968).

Results

Beta-receptor activity. S-1043 at 8 μg/ml concentration did not influence the positive inotropic effect of isoprenaline in electrically driven left auricles of cats (n = 4), while under the same conditions 0.05 μg/ml of propranolol

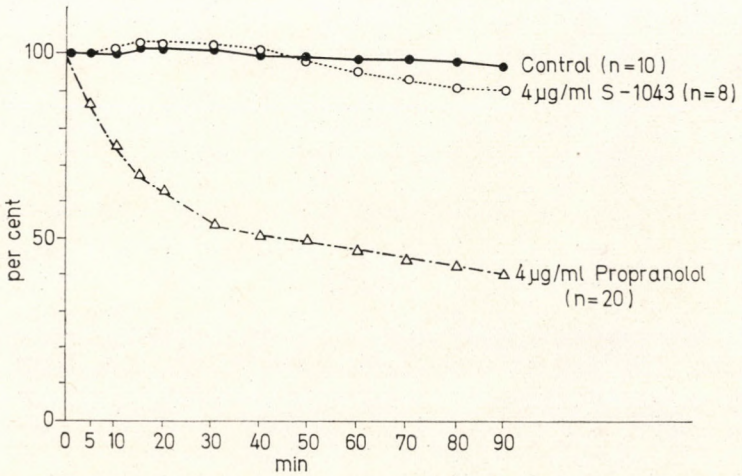
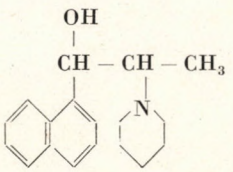
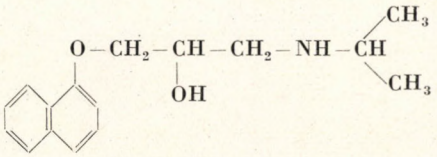


Fig. 2. Effect of S-1043 and propranolol upon the contractile force of the cat's electrically driven left auricle



S—1043



Propranolol

exerted a considerable beta blocking action. In our experiments on isolated organs — except for inotropic tests — the concentration of S-1043 did not exceed 4 μg/ml, in intact animals 5 mg/kg. Thus the beta blocking activity of S-1043 — if it, at all exerts such an action at higher concentrations — could be left out of consideration. Neither the pressor effect of adrenaline, nor the depressor action of acetylcholine was modified by 5 mg/kg of S-1043 in anaesthetized cats. Accordingly, alpha blocking as well as atropine-like effects could be excluded.

Contractility of cardiac muscle. S-1043, like naphthyl-derivatives in general, decreases the force of cardiac contractions. Nevertheless, its negative inotropic effect is surpassed by propranolol. Based on dose-response curves calculated by regression analysis, the concentration of propranolol inducing a 50% decrease in contractile force was 2.7 μg/ml, while that of S-1043 was 7.5 μg/ml. Accordingly, under our experimental conditions the negative inotropic effect of the latter drug was about 3 times weaker than that of the reference substance. While 4 μg/ml of S-1043 caused no significant change in the contractile force of cat auricles within 90 minutes, propranolol decreased the contractions

Table I

*Negative chronotropic effect of S—1043 and propranolol in the spontaneously beating cat auricle**

Treatment	n**	Heart rate before treatment	Heart rate after treatment	Decrease per cent	Significance
Propranolol 2 μg/ml	10	99.8± 3.9	79.4± 2.3	20.4± 2.3	$p < 0.001$
S—1043 2 μg/ml	7	104.0± 7.3	81.7± 5.5	22.3± 3.3	$p < 0.05$

* Mean values ± S.E.
** Number of experiments

by 60% under the same conditions (Fig. 2). The time course of the negative inotropic effect of S-1043 at higher concentrations was similar to that of propranolol as well as to those of other nonspecific cardiopressants. Contractions kept diminishing gradually. S-1043 — like other naphthyl-compounds — cannot be removed from the organs by washing, i.e. it is strongly bound to the tissues.

Pacemaker activity. Both S-1043 and propranolol at a concentration of 2 μg/ml decreased the initial frequency of the cat's right auricle approximately by 20% after 60 minutes incubation (Table I). The decrease was significantly different from the control value and the effect of the two drugs was identical. The low initial frequency was ascribed to the low temperature of the organ bath (30°C).

Refractory period. Effects on the functional refractory period are illustrated in Fig. 3. The left part of the graph shows the prolongation of the refractory period determined by the "maximal following rate" method. The slopes of the concentration-response curves did not differ significantly when tested by the parallel line assay, and S-1043 proved to be twice as potent as

propranolol. Results obtained with the paired stimulation procedure are shown in the right part of the figure. We failed in obtaining a dose-response curve for propranolol with this method, since the drug at low concentrations (1—2 μg/ml) did not influence the refractory period, while at higher concentrations (4 μg/ml) it decreased the values to such an extent that measurements became impossible. By using GOVIER's method, S-1043 was estimated to be about 3—4 times as potent as propranolol.

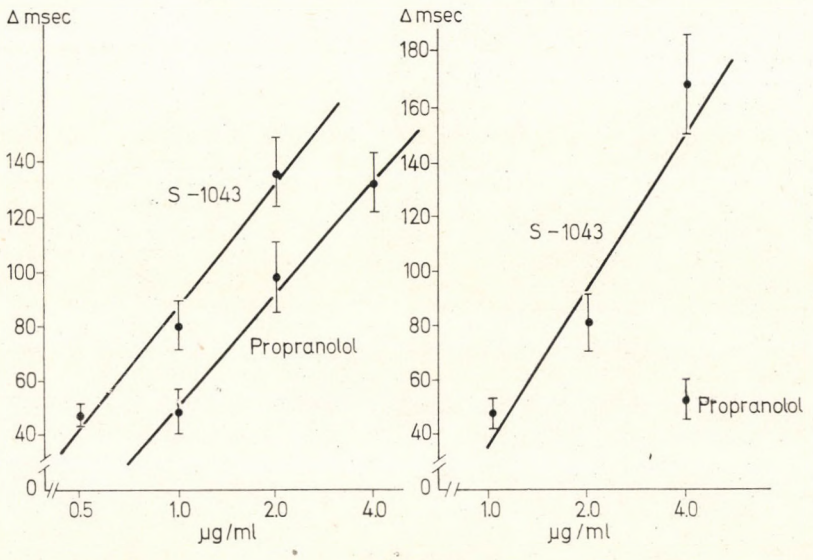


Fig. 3. Effect of S-1043 and propranolol on the functional refractory period of the isolated cat auricle. Left part: results obtained by Dawes's method. Right part: results obtained by the paired-stimulation method. Abscissa: concentrations of test substances. Ordinate: prolongation of the refractory period. Vertical bars: S. E.-values. Every point was obtained from at least five experiments

Myocardial excitability. Propranolol and S-1043 at 4 μg/ml concentration increased the threshold voltage by 50% to 100%. The strength-interval curves were shifted by both substances to the right. Although considerable inhomogeneity of the data allowed no quantitative comparison, it could be established that both S-1043 and propranolol decreased the excitability of the cat's left auricle.

Local anaesthesia. The surface anaesthetic effect of the two substances was similar (Table II). There was no correlation between the duration of effect (15—30 min) and the concentration.

Reversal of ouabain arrhythmia. S-1043 proved effective in abolishing ventricular tachycardia due to ouabain (Fig. 4). Small doses (1 mg/kg) exerted

Table II

Local anaesthetic effect of S—1043 and propranolol on the rabbit cornea

Concentration per cent	S—1043	Propranolol
0.2	2/9*	2/9
0.3	8/12	6/12
0.4	7/12	8/12

* Numerator: Number of potent treatments. Denominator: Number of all experiments

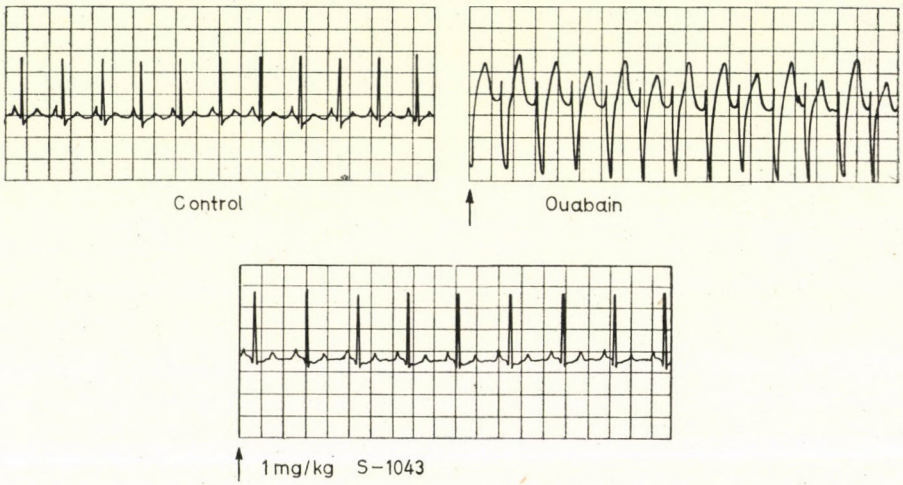


Fig. 4. Reversal of ouabain-induced ventricular tachycardia by S-1043 in the anaesthetized dog. ECG: lead II

Table III

Effect of S—1043 on ouabain

S—1043 intravenously

No. of experiment	Heart rate		after 1 mg/kg		Time min*
	basal	after ouabain	Heart rate	Duration of effect, sec	
1.	160	193	154	80	2
2.	162	208	167	60	2
3.	180	250	180	ineffective	3
4.	153	195	183	ineffective	2
7.	183	221	208	ineffective	3
Mean ± S.E.	167.6±5.9	213.4±10.4	178.4±9.0	80 and 60	2—3

some effect, 2 mg/kg terminated the arrhythmia in each animal tested (n = 5). As illustrated in Table III, there was a correlation between the doses and the negative chronotropic effect.

In order to compare the antiarrhythmic efficacy of the two test substances, 8 dogs were treated with S-1043 and another group of 8 animals with propranolol.

The drugs were administered intravenously at a rate of 5 mg/min up to the reversal of the arrhythmia and over an additional 1 min (Table IV). Since dogs weighing 8 to 12 kg were used, the dose was approximately 0.5 mg/kg per min.

The dose of ouabain required to produce ventricular tachycardia in the two groups of dogs did not differ significantly (73.8±0.3 μg/kg and 71.2± ±0.3 μg/kg). S-1043 produced reversal of the arrhythmia in a mean dose of 2.6±0.3 mg/kg, while propranolol in one of 5.2±0.5 mg/kg. The difference was significant statistically ($p < 0.001$). Thus, S-1043 proved to be twice as potent as propranolol in reversing ouabain-induced arrhythmia. The effective doses of S-1043 and propranolol lowered the high frequency of ventricular rhythm by 40 beats per min and 90 beats per min respectively; the difference was significant ($p < 0.001$). The effect of the two substances did not differ in duration ($p < 0.1$).

Five dogs were selected at random from the group treated by propranolol. They were infused S-1043 after the reappearance and persistence of ventricular tachycardia (Table V). In spite of the considerable beta-blockade, the effective dose of S-1043 was the same as in the group not pretreated with propranolol.

arrhythmia in the dog

+1 mg/kg;		total: 2 mg/kg		+1 mg/kg;		total: 3 mg/kg
Heart rate		Duration of effect, sec	Time min**	Heart rate		Duration of effect, sec
before	after			before	after	
treatment				treatment		
190	139	280	8	193	118	440
200	160	360	33	185	161	440
254	170	60	8	234	163	180
208	135	560	11	195	126	350
214	168	100	8	206	153	240
213.2±10.9	154.0±7.1	272.0±91.1	8—33	202.6±8.5	144.2±9.3	330.0±52.6

* Time elapsed between 1st and 2nd doses
** Time elapsed between 2nd and 3rd doses

Acta Physiologica Academiae Scientiarum Hungaricae 38, 1970

Table IV

*Reversal of ouabain induced arrhythmia by 5 mg/min infusion of S-1043 and of propranolol in the dog**

Treatment	n**	Body weight, kg	Total dose of ouabain	Heart rate			Potent mean dose mg/kg	Duration of effect, sec
				Control	after ouabain	after treatment		
S-1043	8	8.8±0.3	73.8±0.3	170 ±7.1	211.9±4.9	172.4±7.5	2.6±0.3	262.0± 57.6
Propranolol	8	9.5±0.5	71.2±0.3	170.8±5.1	229.8±2.2	131.1±6.1	5.2±0.5	396.3±105.3

* Mean values ± S.E.
** Number of experiments

Table V

Effect of S-1043 infusion on ouabain arrhythmia after propranolol pretreatment, in the dog

No. of experiment	Dose of propranolol mg/kg	Time min*	Potent dose of S−1043 mg/kg	Heart rate		Duration of effect, sec
				S−1043		
				before	after	
12.	7.0	50	2.8	181	90	240
13.	4.4	30	3.2	231	118	230
15.	8.0	45	2.5	197	104	270
17.	5.4	40	1.9	202	141	390
22.	4.0	32	2.6	234	159	140
Mean ± S.E.	5.8±0.8	30−50 39.4±3.8	2.6±0.2	209.0±10.2	122.4±16.0	254.0±40.4

* Time elapsed between propranolol and S−1043 treatment

Discussion

According to the above results, S-1043, beside its strong cardiodepressant action proved to be effective in terminating ouabain arrhythmia. In contrast with propranolol, S-1043 has no beta blocking activity. This might be due to the greater distance between the N-atom and the naphthyl-ring as compared to the propranolol molecule, and, perhaps, to the methyl-substitution of the C-atom next to the N. The importance of these chemical properties in the beta blocking effect was thoroughly discussed by ARIËNS (1967). The significance of $-O-CH_2-$ in the alkyl-link is supported by the fact that this constituent is common to the potent beta-blockers like propranolol, Kö-592 (ENGELHARDT 1965) and H-56/28 (BRÄNDSTRÖM et al. 1966). Moreover, several naphthyl-alkylamine derivatives similar to propranolol in structure but lacking this chemical group have no beta blocking effect (HADHÁZY et al. 1968).

The relations between the inotropic and refractory period prolonging effect of S-1043 as well as its efficacy in ouabain arrhythmia seem to be more favourable than that of propranolol. In decreasing cardiac contractions, propranolol was found to be 3 times as potent as S-1043. As regards antiarrhythmic activity, the latter proved to be twice as potent as the reference substance. It should be mentioned that S-1043 at 4 μg/ml concentration did not influence the contractile force while it considerably prolonged the functional refractory period. Concentrations higher than 4 μg/ml were needed to decrease the cardiac contractions. Propranolol exerted a negative inotropic effect in a concentration range at which it influenced the refractory period only slightly.

The negative chronotropic effect of the two drugs proved to be similar in isolated preparations. In ouabain-induced arrhythmia — taking the antiarrhythmic dose into account — propranolol was more potent than S-1043 in decreasing the frequency. As shown in Table V, the negative chronotropic effect of S-1043 (87 beats/min) was more considerable after beta blockade than in its absence (40 beats/min; Table IV). These data suggest that the inhibition of beta-receptors per se elicits a decrease of frequency in ouabain ventricular tachycardia. Our results did not permit to clarify this problem. The greater potency of propranolol is due to its higher dose (Tables IV and V). Moreover BARRETT and CULLUM (1968) reported that the chronotropic effect in the rat of the very weak beta-blocker dextro-propranolol and the potent beta-blocker laevo-propranolol was similar.

The antiarrhythmic total dose of propranolol infused at a rate of 5 mg/min practically agreed with the data reported by LUCCHESI et al. (1966) and by HOWE and SHANKS (1966). In the experiments of LUCCHESI et al. (1966), an average dose of 6.1 mg/kg of propranolol was required to reverse the ouabain-induced arrhythmia and maintain normal sinus rhythm for 30 minutes or longer. In our experiments a 5.2 mg/kg dose of propranolol restored the normal

rhythm for 7 minutes (3—17 min). The difference in duration of reversal was thought to be due in part to the lower dose of propranolol and in part to the higher dose of ouabain used for inducing ventricular tachycardia. 40—80 μg/kg of ouabain (average 60 μg/kg) was given by LUCCHESI et al.; we administered 60—80 μg/kg (average 71.2 μg/kg).

The influence of propranolol pretreatment upon the efficacy of S-1043 was studied in 5 dogs. S-1043 was infused 30—50 minutes after the administration of propranolol, where beta blockade was already considerable (SHANKS et al. 1966). Insofar beta blockade plays an important role in the abolition of the ouabain arrhythmia, propranolol is expected to potentiate the efficacy of a nonspecific antiarrhythmic agent (not taking into account the quinidine-like property of propranolol).

As shown in Table V, in spite of the presumably strong beta blockade, the antiarrhythmic dose of S-1043 was the same as without propranolol. This supports the view that beta blockade is not relevant in the reversal by propranolol of the ouabain-induced arrhythmia. In this respect the literature is conflicting (LUCCHESI and HARDMAN 1961; VAUGHAN WILLIAMS and SEYKIA 1963; LUCCHESI 1965; SOMANI and LUM 1965; FREY and NIELSEN 1966; DUCE et al. 1967; DOHADWALLA et al. 1969; PAPP and VAUGHAN WILLIAMS 1969).

The basis of the antiarrhythmic effect of S-1043 is thought to be a decrease in myocardial excitability and a prolongation of the functional refractory period. The favourable relation between the negative inotropic and other cardiodepressive effects of the drug seems to be advantageous.

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Recensiones

N. P. BECHTEREWA (Ed.)

Physiologie und Pathologie der tiefen Hirnstrukturen des Menschen

VEB Verlag Volk und Gesundheit, Berlin 1969. 220 pages with 79 figures and 5 tables. Price: M 55.70

This book dealing with the physiology and pathology of the human deep brain structures, is a translation of the first Russian edition published in 1967. N. P. BECHTEREWA in collaboration with A. N. BONDARTSHUK, W. M. SMIRNOV and A. J. TROCHATSHOV reviews the results of a many-sided brain research work performed in the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR. In the course of the diagnostic and therapeutic studies on hyperkinesis, many interesting new theoretical and clinical facts have resulted. The detailed methodological descriptions seem to be a particularly useful part of the book.

The work is interesting not only for neurologists but also for neurosurgeons and neurophysiologists.

K. LISSÁK

Dietrich BURKHARDT

Wörterbuch der Neurophysiologie

VEB Gustav-Fischer-Verlag, Jena 1969. 316 pages, 18 figures. Price: M 26.—

The great progress of contemporary neurophysiology resulted in a new terminology, which is sometimes impossible to translate correctly into other languages. During the past decades there was always some difficulty to translate correctly the special neurophysiological terminology from Russian into English or German, or vice versa. Similarly, some technical terms were not familiar to everybody, e. g. parabiosis (WEDENSKY), or dominant (UCHTOMSKY). For this reason this Dictionary is very useful; it gives a good glossary of neurophysiological technical terminology in German, and the terms in English and Russian.

It is probably a consequence of the fast progress that some terms have not been included, e.g. arrest reaction, electroretinogram, extinction, internal inhibition, reverberate circuit, startle reaction, etc.

The Dictionary of Neurophysiology will serve the better and correct understanding among neurophysiologists reading the English, German and Russian literatures and will be a useful book in every medical library.

K. LISSÁK

G. A. KERKUT (Ed.)

Experiments in Physiology and Biochemistry

Volume 2. Academic Press, London and New York 1969. XVI + 410 pages with 164 figures and 29 tables. Price: 120 s, $ 18.—

This second volume of Experiments in Physiology and Biochemistry follows the programme of the first volume of this useful series, i.e. to give sufficient practical details to enable the reader to follow and carry out the experiments described.

The thirteen chapters of the book include widely different experimental methods in various fields. J. A. M. HINKE describes "The constructions of pNa and pK microelectrodes for the measurements of aNa and aK in muscle fibres". Stewart A. MIDDLER and Charles R. KLEEMAN reviews "Methods of ureteral catheterization and hypophysectomy of the toad".

Howard L. GILLARY demonstrates "Stimulation of an insect taste receptor". Frances V. MCCANN presents "The insect heart as a model for electrophysiological studies". M. OVERTURE and R. L. DRYER are reviewing the "Experiments in the biochemistry of animal lipids". D. MENDEL and Rosemary BAKER demonstrate "The measurement of blood flow in the intestine and skeletal muscle of the rat". YOSHIAKI WASHIZU describes "Membrane potential and tension in guinea-pig ureter". Harold GAINER demonstrates "Multiple innervation of fish skeletal muscle". J. del CASTILLO and T. MORALES present "Electrophysiological experiments in *Ascaris lumbricoides*". J. E. HEATH and P. A. ADAMS are discussing methods of "Temperature regulation and heat production in insects". Steven VOGEL deals with "Low speed wind tunnels for biological investigations". C. A. MARSDEN and G. A. KERKUT present "The cellular localization of monoamines in invertebrates using the Edwards-Pearse tissue freezer dryer". Finally, Andris K. TÉBECIS and John W. PHILLIS review "The pharmacology of the isolated toad spinal cord". The book is completed with a subject index and with a Cumulative Contents.

This second volume of Experiments in Physiology and Biochemistry will be of use for both the experienced research worker and the student to become acquainted with new techniques and new experimental animals. The book is recommended to biologists, biochemists and physiologists.

K. LISSÁK

G. BIRKE, R. NORBERG and L. O. PLANTIN (Eds)

Physiology and Pathophysiology of Plasma Protein Metabolism

Proceedings of the International Symposium held in Stockholm, May, 1967. Volume 12 of the Wenner-Gren Center International Symposium Series. Pergamon Press Ltd., Oxford 1969. IX + 264 pages with 80 figures and 51 tables. Price: 95 s, $ 12.50

In a series of conferences organized on the broad theme of protein metabolism, the fourth conference held at Saltsjabaden was restricted to problems concerning the metabolism of albumin and immunoglobulins. This volume contains the papers given at the conference, including the discussions. The subject of the first part is the separation, purification, labelling and kinetics of proteins. The second part discusses protein metabolism under normal conditions. The third part deals with the protein losing disorders. Finally, the section Varia widened the aspects of the symposium with the problems of plasma protein catabolism in old people; the metabolism of vitamin B_{12}; the mechanism of action of various anti-intrinsic factor antibodies; low-density lipoprotein metabolism; colloid-osmotic pressure and the respective role of albumin and globulin in burned patients. The Symposium was closed by the concluding remarks of P. VESIN.

The nearly thirty papers presented by outstanding experts provide up-to-date information in this wide field of physiology. The book will be of great interest for biochemists, physiologists and pathophysiologists.

K. LISSÁK

F. GROSSE-BROCKHOFF (Ed.)

Pathologische Physiologie

Second edition, Springer-Verlag, Berlin, Heidelberg, New York 1969. XXXI + 797 pages, 376 figures and 112 tables. Price: DM 96.— US $ 24.—

This Textbook of Pathophysiology written by the Editor and seventeen of his former and present collaborators reflects well the tremendous progress of physiology and biochemistry during the past decades. In spite of this the book presents to the reader in an easily readable uniform style every physiological and biochemical information which is important in contemporary medicine. The main chapters of the work are, Blood, Respiration, Heart, Peripheral circulation, Pulmonary circulation, Digestive organs, Liver, Cholecyst, Kidney, Water, electrolyte and mineral household, Acid-base balance, Trace elements, Hormones, Carbohydrate metabolism, Protein metabolism, Lipid metabolism, Lipoid metabolism, Energy metabolism, Temperature regulation, Cellular metabolism, Tissue metabolism including Immunity.

The book is recommended first of all to students engaged in clinical fields; it will be useful to postgraduate students of medicine as well.

K. LISSÁK

G. E. W. WOLSTENHOLME and Julie KNIGHT (Eds)

Circulatory and Respiratory Mass Transport

A Ciba Foundation Symposium. J. and A. Churchill, London 1969. 310 pages, 103 figures.

This volume contains the papers and the discussions of an interdisciplinary Symposium held under the chairmanship of C. G. CARO and with twenty-three members, in July, 1969, in London. The problems discussed involved three fields, viz. the interstitial fluid and spaces, the physiology of blood and blood vessels, and the respiratory system.

The three sections of the contents reflect the international representation of physiologists and physical scientists. In the first section on mass transport in tissue spaces, A. C. GUYTON discussed "Interstitial fluid pressure-volume relationships and their regulation", and J. R. PHILIP "Theory of flow and transport processes in pores and porus media." In the second section, dealing with mass transport in blood vessels, the papers were: E. M. RENKIN "Exchange of substances through capillary walls"; A. C. BURTON "The mechanics of the red cell in relation to its carrier function"; M. J. LIGHTHILL "Motion in narrow capillaries from the standpoint of lubrication theory"; G. B. MASON and H. L. GOLDSMITH "The flow behaviour of particulate suspensions"; E. W. MERILL, H. J. MEISELMAN, E. R. GILLILAND, T. K. SHERWOOD, E. W. SALZMANN "Flow of human blood in glass and plastic fibres: a filmed study"; M. G. TAYLOR "The optimum elastic properties of arteries"; C. G. CARO, M. F. SUDLOW, T. H. TOBY, A. UR "Pressure-flow relations in small blood vessels"; D. L. SCHULTZ, D. S. TUNSTALL-PEDOE, G. de J. LEE, A. J. GUMMING, B. J. BELLHOUSE "Velocity distribution and transition in the arterial system". The third section, on mass transport in the lungs contains the papers: J. MEAD "The distribution of gas flow in lungs"; B. ALTSHULER "Behaviour of airborne particles in the respiratory tract"; P. R. OWEN "Turbulent flow and particle deposition in the trachea"; J. B. WEST, J. B. GLAZIER, J. M. B. HUGHES, J. E. MALONEY "Pulmonary capillary flow, diffusion ventilation and gas exchange"; L. E. FARHI "Diffusive and convertive movement of gas in the lung". Finally, in a general discussion "A mathematical treatment of dispersion in flow through a branching tree" was discussed and the Symposium finished by the Closing remarks of the Chairman.

This interdisciplinary Symposium is an excellent product of the fruitful collaboration of physiologists and physical scientists. The book will be interesting and profitable not only for physiologists but for all investigators of the medical sciences.

K. LISSÁK

G. E. W. WOLSTENHOLME and Julie KNIGHT (Eds)

Control Processes in Multicellular Organisms

A Ciba Foundation Symposium. J. and A. Churchill, London 1970. 424 pages and 107 figures. Price: 80s.

This broad titled Ciba Symposium held under the chairmanship of F. G. YOUNG, with twenty-six members from eight different countries representing four continents, was held in March, 1969, at New Delhi.

After the Chairman's opening remarks, seventeen papers were presented and discussed by the participants. Space allows only a list of the papers.

S. BOURGEOIS and J. MOND: In vitro studies of the lac operon regulatory system; F. LYNEN: Modulation of enzyme activities by metabolites; H. HARRIS: The expression of genetic information: a study with hybrid animal cells; R. W. BUTCHER, G. A. ROBISON, E. W. SUTHERLAND: The role of cyclic AMP in certain biological control systems; A. KORNER: Insulin and growth hormone control of protein biosynthesis; G. P. TALWAR, B. L. JAILKHANI, S. K. SHAROMA, M. L. SAPORI, M. R. PANDIAN, G. SUNDHARDAS, K. N. RAO: Mechanism of action of growth hormone and oestrogens; J. R. TATA: Hormonal control of metamorphosis; P. M. BHARGAVA: Intercellular control of intracellular metabolic activity; C. FORTIER, F. LABRIE, G. PELLETIER, J. P. RAYNAUD, P. DUCOMMUN, A. DELGADO, R. LABRIE, M. A. HO-KIM: Recent studies on the feedback control of ACTH secretion, with particular reference to the role of transcortin in pituitary-thyroid-adrenocortical interactions; A. WHITE, A. L. GOLDSTEIN: The role of the thymus gland in the hormonal regulation of host resistance; J. F. A. P. MILLER and G. F. MITCHELL: Interaction between thymus cells and bone marrow cells in response to

antigenic stimulation; M. COHN: Anticipatory mechanisms of individuals; G. M. EDELMAN: Antibody structure; a molecular basis for specificity and control in the immune response; M. G. DEO and V. RAMALINGASWAMI: Regulatory mechanisms of cellular proliferation in a protein-deficient organism; V. P. WHITTAKER: Subcellular fraction techniques in the study of chemical transmission in the central nervous system; B. K. ANAND: Regulation of visceral activities by the central nervous system; R. B. LIVINGSTON: Some general integrative aspects of brain function. Each paper was followed by a lively and interesting discussion of many important problems.

This Ciba Foundation Symposium will be of interest to physiologists, endocrinologists, biochemists as well as to neurologists, immunologists and other workers of biological and medical sciences.

K. LISSÁK

G. E. W. WOLSTENHOLME and Julie KNIGHT (Eds)

Progesterone: Its Regulatory Effect on the Myometrium

Ciba Foundation Study Group No. 34 J. and A. Churchill, London 1969. 193 pages, 64 illustrations. Price: 30 s.

This Study Group volume is dedicated to the effect of progesterone on myometrial function. Nine papers have dealt with the different aspects of the subject. Their full text together with the accompanying discussion and references are presented in the book.

The chairman, A. CSAPO, presented new evidence supporting the role of progesterone in the "defence mechanism" of human pregnancy. In the following paper four direct regulatory factors of human and animal myometrial function are analysed. KERÉNYI et al. showed the effect of progesterone on the nonpregnant and early pregnant human uterus, while PULKKINEN presented data on mid-trimester human pregnancy. TURNBULL et al. and WOOD discussed the role of the foetus in the regulation of uterine activity and in the initiation of parturition. The biochemical data by WIEST revealed that the uterine tissue is capable of concentrating and metabolizing progesterone. MARSHALL showed that ovarian hormones were able to modify the uterine response to hypogastric stimulation. PORTER's findings revealed that in the guinea-pig progesterone is unable to delay parturition or to suppress spontaneous and oxytocin-induced uterine activity in non-pregnant animals.

The volume offers all up-to-date knowledge concerning the action of progesterone on uterine function, approaching the subject from different aspects. It is recommended to physiologists, endocrinologists, biochemists and gynaecologists interested in uterine function and its regulating factors.

G. TELEGDY

C. H. GRAY and A. L. BACHARACH (Eds)

Hormones in Blood

Academic Press, London 1967 and 1968, 2nd edition. Vol. 1. 682 pages. Price: $ 22,50. Vol. 2. 577 pages. Price: $ 26,—

These books are the second edition of the 1961 volume which has been revised and largely rewritten to include up to date information from the different branches of endocrinology. Since 1961, many new methods have become available, thin layer chromatography and gas-liquid chromatography have opened up new possibilities in hormone determination and the immunoassay of peptides and protein hormones has greatly improved knowledge of these hormones. The present two volumes are covering practically the whole endocrinology, describing the physiological role of different hormones, their methods of determination and normal and pathological levels in body fluids. At the end of each chapter, references are given concerning the subject. Each volume is completed with an author and subject index.

The first volume is devoted mainly to protein and peptide hormones and divided into sixteen chapters. After the first introductory chapter written by the editors, the second (by

C. A. MALE) and third (by A. D. WRIGHT and K. W. TAYLOR) chapters deal with the gel filtration technique and immunoassay for the preparation and determination of peptide and protein hormones. Chapter IV written by K. W. TAYLOR describes insulin, its chemistry, determination, and the factors influencing its secretion. Chapter V (R. H. UNGER and A. M. EISENTRAUT) deals with the chemical, physical, immunological and biological properties of glucagon as well as the methods for its determination. Chapter VI, Oxytocin; Chapter VII, Vasopressin; and Chapter VIII, Melanocyte-Stimulating Hormones (by I. M. WALKER) summarize the present knowledge about these hormones, with the most recent methods of assay and the blood levels. Chapter IX, Adrenocorticotropin, by G. SAYERS offers data on the nature of ACTH, a detailed evaluation and description of the different methods for determination as well as the blood level in normal and pathological conditions. Chapter X, by F. C. GREENWOOD describes the growth hormone, its chemical nature, origin, determination, normal and pathological plasma levels. Chapter XI (I. A. FORSYTH) deals with prolactin and placental lactogen, summarizing the most important information about these two hormones, the different methods of determination and the normal and pathological levels. Chapter XII, Pituitary Gonadotropins (M. APOSTOLAKOS and J. A. LORAIN), presents data on the chemical nature, extraction and determination, the level of these hormones in normal and different pathological cases, and the effect of different hormonal treatments on gonadotropins. Chapter XIII, Human Chorionic Gonadotropin (J. A. LORAIN) deals with the chemical nature, methods of assay, levels of the hormone and its renal clearance in normal and pathological pregnancy. Chapter XIV, Pituitary Stimulating Hormone and other Thyroid Stimulating Substances (P. G. CONDLIFFE and J. ROBBINS) describes the origin, the factors controlling TSH secretion, the methods of assay and the levels of human TSH. Chapter XV, The Iodine-Containing Hormones (J. ROBBINS and J. E. RALL), deals with iodoaminoacids, iodoproteins, biosynthesis of iodine containing hormones, transport of thyroid hormones, methods of determination, distribution and concentration of thyroid hormones. Chapter XVI (A. V. SCHALLY et al.) deals with the different hypothalamic releasing and inhibiting factors, discussing in a separate subdivision each factor, its nature, secretion and synthesis, the methods of determination, and the tissue and plasma levels.

Volume II is divided into 12 chapters. The first three deal with the technical questions of hormone determination such as spectrometry, thin layer chromatography and gas chromatography. Chapter I by J. E. PAGE covers the spectrometry of steroids: ultraviolet and visible spectroscopy, fluorescence, optical rotatory dispersion, infrared and Raman spectroscopy, nuclear magnetic resonance and mass spectrometry. Chapter II written by E. J. SHELLARD gives a description of thin layer chromatography and its applicability in hormone separation and detection. Chapter III by C. J. W. BROOKS and ZABKIEWICZ describes gas chromatography; the use of gas chromatography for qualitative and quantitative analysis of chemical compounds and its application in hormone analysis. Chapter IV by V. J. O'DONNEL and J. R. K. PREEDY deals with oestrogens, their chemistry, determination of oestrogens from different sources, and the methods of assay. Data on the classical oestrogens found in blood by different authors are also given. Chapter V, Androgens (W. R. EBERLEIN, J. WINTER and R. L. ROSENFIELD) presents data on the physiological and biochemical aspects of androgens, the methods of determination, normal values and values in endocrine disorders. Chapter VI (H. J. VAN DER MOLEN and A. AAKVAAG) summarizes the characteristics of progesterone, its site of production, estimation of progesterone and its metabolites, plasma level, and the factors influencing this level. Chapter VII (P. F. DIXON, M. BOOTH and J. BUTLER) describes the corticosteroids; chemistry and physiology of corticoids, factors influencing corticoid secretion in normal and pathological conditions, estimation of corticosteroids in body fluids are dealt with. Chapter VIII written by J. P. COGHLAN and J. R. BLAIR-WEST deals with aldosterone, its biochemistry, physiology, methods of determination, physiological and pathological variations. Chapter IX by W. S. PEART gives a review of renin and angiotensin chemistry and of the physiological role of these compounds, methods of determination in blood, normal and pathological plasma levels. Chapter X (B. A. CALLINGHAM) is devoted to the catecholamines, adrenaline and noradrenaline. Chapter XI deals with parathyroid hormones and thyrocalcitonin (J. ANDERSON and R. W. S. TOMLINSON), their chemistry, origin, the methods of determination and their plasma level.

"Hormones in Blood" is one of the most comprehensive book published in the last few years. In two volumes it covers not only the most modern methodology available till the book's publication but also gives up-to-date information of the different hormones from the biochemical and physiological point of view, and of the normal and pathological levels of the different hormones. The book is highly recommended to physiologists, biochemists, clinical chemists and clinicians engaged in hormonology.

G. TELEGDY

G. STERBA (Ed.)

Zirkumventrikuläre Organe und Liquor

Bericht über das Symposium Schloß Reinhardsbrunn vom 13. bis 16. Mai 1968, VEB Gustav-Fischer-Verlag, Jena 1969. Price: M 51.50

This symposium was organized with the aim to offer a uniform aspect of the special territories of the mesencephalic ependyma including the pineal gland and neurohypophysis. The seventy-two participants gave forty-four papers accompanying the four main reviews. A separate paper was given by OLSSON on the phylogeny of the ventricular system.

The first theme "Subcommissural organ and Reissner's fibre" was introduced by STERBA's paper on the Morphology of the subcommissural organ, which was presented with excellent electronmicroscopical and fluorescence microscopical pictures. He ruled out the role of this organ in the regulation of aldosterone secretion and salt and water balance, on the basis of his own data and the data of others, and introduced an interesting theory, supported mainly by indirect evidences, about the physiological role of these territories. According to this concept the subcommissural organ and its special secretory product, the Reissner's fibre, is organized in the brain for the elimination of unwanted substances; it would thus have a function in the brain similar to that of the kidney in the body.

In the discussion, OLSSON, LUPA and NAUMANN pointed out that the lectures presented seemed to support STERBA's hypothesis. According to their opinion, it has been established that Reissner's fibre originates only in part from the cells of the subcommissural organ. For its final formation, materials present in the cerebrospinal fluid are necessary. For further analysis, X-ray diffraction structure-analysis was recommended.

The second theme was presented by HOFER's survey, "Anatomy of the circumventricular organs". He pointed out that these organs are the oldest territories of metabolism and vascularization of the central nervous system. He mentioned WEINDL's hypothesis published in 1965, according to which the organon vasculosum laminae terminalis is a thermoregulatory centre. A detailed review is offered of the identities and differences of the secretion of the subcommissural organ and hypothalamic magnocellular neurosecretory areas.

In the accompanying papers, AKER has shown that the cholinergic limbic system is part of the subfornical organ; VIGH pointed out that the paraventricular organ is functioning as a contact neuronal system of the cerebrospinal fluid, and its monoamine content depends on the compartments of the cerebrospinal fluid.

In the discussion, BARGMANN, PALKOVITS and MÜLLER raised the possibility of neurohypophyseal and pineal glandular secretion into the cerebrospinal fluid and therefore it would seem justified to consider them part of the circumventricular organs. The measurement of the nuclear-size as a functional method was criticized. HELLER criticized the different extraction procedures. The role of the subcommissural organ in salt and water balance was again brought up by PALKOVITS.

The third theme was introduced by LEONHARDT's lecture "Ependyma". He dealt in detail with the secretion processes and mentioned KNOWLES' supposition that the secretion of the ependyma plays a regulating role in the neurosecretion via cerebrospinal fluid. The present knowledge of intraventricular absorption was summarized. The so-called bouquet-shaped ependymal cells surrounding the recessus infundibularis in the rabbit were described. These cells were characterized by the author in 1968. Their function has not yet been elucidated.

In the discussion, MILINE, STERBA and ERMISCH brought up the morphological criteria of the secretion and significance of the tumorous changes of these organs. According to their opinion it is too early to determine the significance of the "bouquet-shaped" cells.

The fourth subject started with HELLER's review: Neurohypophysial hormones in the cerebrospinal fluid. The literature concerning the question was reviewed in detail. The limitations of hormone assays in cerebrospinal fluid were discussed critically. It was shown that in the cerebrospinal fluid mainly vasopressin was found, but vasopressin is able to penetrate rapidly through the blood-cerebrospinal fluid barrier. It is not justified to draw conclusions from the quantity of hormones found in the cerebrospinal fluid to the secretion of neurohypophyseal hormones directly into the cerebrospinal fluid.

LENCKER and HÄGGENDAL showed interesting data about the changes of the noradrenaline content of the cerebrospinal fluid. The theme was summarized by HELLER, GERSCH and SCHÄKER.

The material of the symposium is highly recommended to neurophysiologists, neurologists, and neuroendocrinologists; it is the first summary of present knowledge of a relatively less known field.

K. FENDLER

G. PETERS and F. ROCH-RAMEL (Eds)

Progress in Nephrology

Springer-Verlag, Berlin, Heidelberg, New York 1969. Price: DM 88.—

The 5th symposion of the "Gesellschaft für Nephrologie" was held between the 21st and 23rd September, 1967, in Lausanne, Switzerland. The papers presented there form the contents of the book.

The papers are grouped into six main sections.

I. The renal excretion of nitrogen containing metabolites

In the first section, the paper of B. SCHMIDT-NIELSEN and W. E. LASSITER was dealing with the excretion of urea. The papers of F. ROCH-RAMEL et al., B. TRUNIGER, K. J. ULLRICH and D. F. GROSSMANN discussed the intrarenal transport of urea. The role of urea in the renal concentrating function was the subject of K. KRAMER and H. W. REINHARDT. D. F. GROSSMANN et al. discussed the effect of urea overcharge on the renal function.

N. ZÖLLNER presented a paper on the excretion of uric acid. J. SCHIRMEISTER reviewed the renal effect of furosemide, A. MASBERNARD that of benziodaron. J. MOELLER presented a histological study of renal biopsy samples from uric acid excreting subjects.

R. F. PITTS dealt with the renal excretion of ammonia.

In the next section the papers of M. BRANDIS, D. RENNER, W. SCHOEPPE, V. PRÁT, H. LOSSE and D. WALTHER discussed the problems of experimental uraemia, and papers were devoted to the clinical pattern and therapy of uraemia. R. KLUTHE and H. QUIRIN reported results achieved by dietetic and dialysis treatment of chronic renal failure.

A. BLUMBERG, H. U. ZOLLINGER, A. HEIDLAND, L. CAMPANACCI, H. E. KELLER, K. KLÜTSCH and W. FRIEDRICH discussed the pathologic and metabolic aspects of uraemia. Two papers reviewed the immunological changes occurring in chronic glomerular nephritis and uraemia.

II. Toxic disturbances of renal function

In the general-toxicological part, F. GLOOR dealt with the morphological picture of acute toxic nephropathies, and D. E. OKEN presented experiments concerning acute renal failure caused by glycerol.

In the special toxicology part, M. WIEDERHOLT discussed the effect of actinomycin D on renal tissue and function; K. H. LANGER the effect of potassium cyanide, H. C. BURCK that of glycerol and G. M. EISENBACH on changes caused by dinitrophenol.

F. B. BRUNNER, K. G. THIELE, U. C. DUBACH and J. BRODEHL reviewed the toxic effects on the kidney of certain drugs or their metabolic products (penicillin G, DL-penicillamine, phenacetin, tetracycline).

III. Natriuretic and antinatriuretic agents

Two speakers discussed the diuretic effect of the ethacrynic acid series. The mode of action of the diuretic agents formed the subject of H. HOLZGREVE, W. LOSERT, A. ZESCH, H. J. MOHR. The effect of diuretics on the renal tissue and tubular function was studied by W. HERMS and J. EIGLER.

IV. Renin, angiotensin, aldosterone

In this section, A. BOHLE presented light and electronmicroscopic studies of the biopsy material obtained from patients with Conn's syndrome and J. SCHÜRHOLZ a case of pseudo-Bartter's-syndrome. D. MEYER delivered a paper on the relation of serum sodium concentration, haematocrit and juxtaglomerular granulation.

H. P. WOLFF et al. discussed primary normopotassaemic aldosteronism, and P. FAARUP et al. the relation of renal hypertension to the morphology of the juxtaglomerular apparatus. A method for the estimation of renin activity was presented by D. REGOLI and one for the purification of renin substances by H. DAHLHEIM.

M. NAGANO reported on the connection of renal hypertension and enzyme activity of the isolated rat glomerulus. G. PETERS spoke about the renal action of angiotensin and posterior pituitary peptides.

V. Urinary concentration and dilution

J. SCHNERMANN and W. THOENES gave a report on micropunction studies of renal concentration and dilution mechanisms. G. FÜLGRAFF discussed the influence of increased serum calcium on sodium reabsorption, P. SCHOLLMEYER the oxygen consumption of the human kidney, and M. STEINHAUSEN et al. the superficial blood flow of the kidney on stimulation of the renal sympathetic nerves.

VI. Miscellaneous

D. W. BEHRENBECK et al. reported about the effects on electrolyte balance of peritoneal dialysis and repeated mannitol infusions, D. GEKLE on the effect of parathyroid hormone on water reabsorption in the proximal tubule, and H. E. RENSCHLER lectured on the effect of intravenous glucose loading on renal sodium excretion.

D. P. MERTZ dealt with the effect of acute metabolic shifts on the acid-base balance on the renal excretion of sodium. Finally, D. F. GROSSMANN reported some experimental studies of the renal clearance of lissamine-green.

The book gives a wide survey of the above subjects. Of special value is the excellent reproduction of histological pictures and electronmicrograms.

F. BALOGH

St. M. MILCU, C. MAXIMILIAN, B. IONESCU

Endocrinopatiile genetice

Editura Academiei Republicii Socialiste România, Bucureşti 1968. 479 pages, including 16 pages of English and 17 pages of Russian summaries. 111 figures

The book points at the considerable frequency of endocrine diseases among the world's population and calls attention to the results of research of the past fifty years which indicate that most of these diseases are manifestations of gene mutations and chromosomal anomalies. The frequency and regularities of hereditary occurrences, characteristics of the clinical patterns and of the kariotypes are treated in eleven chapters, well supported by data in the literature, the great clinical experience of the authors, as well as the remarkable collection of clinical cases assembled by the Bucharest Institute of Endocrinology. The facts concerning disease, developing on the basis of pituitary, thyroid, parathyroid or adrenal affections, diabetes mellitus, the simple hyperglycaemia of children, obesity, constitutional weakness, sexual development and genetic disorders and finally, the syndromes caused by the gonads and the tumorous transformations occurring in association with these, are assembled in individual chapters. The book represents a valuable source of material for those who need general information and for the specialists of endocrinology and genetics.

F. OBÁL

A. HAFFERL

Lehrbuch der topographischen Anatomie

Neu bearbeitet von Walter Thiel. Dritte Auflage. Springer-Verlag, Berlin, Heidelberg, New York 1969. DM 124.—

This is the 3rd edition of HAFFERL's topographical anatomy. It is one of the most comprehensive modern textbooks in this field. On its 906 pages, the authors give a detailed description of the topographical anatomy of the human body including a reasonable amount of its systemic anatomy. The first section deals with the head. This is followed by sections on the neck, thorax, abdomen, pelvis, back, upper and lower limbs.

The 661 illustrations, many in colour, are excellent. All the figures are clearly drawn and fully labelled. Most of them represent dissections. These are of a high standard, and the size of the figures allows them to be seen clearly. When necessary, there are drawings to explain the anatomical arrangements and the development of the part or structure. The numerous radiographs are very instructive.

The terminology used is the Paris *Nomina anatomica* of 1955 (PNA). There is a comprehensive subject and author index.

On the whole, the book is an excellent and accurate piece of work which is warmly recommended to students and teachers.

B. FLERKÓ

W. DOERR, G. SEIFERT, E. UEHLINGER (Eds)

Spezielle pathologische Anatomie

Band 4. Springer-Verlag, Berlin, Heidelberg, New York 1969. 652 pages with 275 figures. Price: DM 146.—

The present book is the forth volume of the text and reference book entitled "Spezielle pathologische Anatomie", edited by W. DOERR, G. SEIFERT and E. UEHLINGER. It consists of four parts. KÖHN (Berlin) wrote two parts of the book "Pathology of the Nose and the Paranasal Sinuses" and "Pathology of the Larynx and the Trachea". WALTHARD (Bern) prepared the part on "Pathology of Thyreoidea" and FROBOESE (St. Gilgen) the one on "Pathology of the Mediastinum".

The selection of the material of this volume, as pointed out by the Editors, was made according to the topographical point of view and not according to that of systematic organ pathology. This may be of value for clinicians and pathologists who will often seek an insight into the pathological problems related to topographical regions involving different organ systems.

It is not possible to comment in detail on each part of the book. All its parts are of a high standard. Each contains a great amount of information of standard and of newly-achieved knowledge on the pathology of the respective fields. The value of each part is enhanced by a great number of illustrations. A few words will serve to illustrate the concise form of presentation. In spite of the very comprehensive treatment of the pathology of the nose and paranasal sinuses, this is presented on the whole on 100 pages including 52 figures followed by a list of references with full titles on 50 pages arranged according to the main headings of the text.

The pathology of the larynx and trachea is presented on 130 pages with 54 figures followed by a list of references with full titles on 30 pages. The pathology of the thyroid is dealt with on 100 pages with 62 figures.

The part on the pathology of the mediastinum is divided into two main chapters. The first chapter deals with the general pathology of the mediastinum with 29 figures and gives a useful description of the normal anatomy and topography of the mediastinum, and is followed by a list of references. The second chapter of this part is devoted to cysts and tumours of the mediastinum on 120 pages, with 76 figures followed by a separate list of references.

Each part is introduced by the anatomy and physiology of the organs concerned. Physiological correlations are especially extensively dealt with in the part on thyroid pathology. The general pathology of the mediastinum, and especially the chapter on its cysts and tumours will be appreciated by many pathologists and clinicians, since it is hard to find a comprehensive treatment of this special field, which has developed mainly in recent years in correlation with the advances in clinical diagnostics and surgery.

This volume, the illustrations of which are of a high technical standard and most demonstrative, will remain for long an indispensable reference book in its field for clinicians, general pathologists and oncopathologists.

G. ROMHÁNYI

K. ADAMSONS (Ed.)

Diagnosis and Treatment of Fetal Disorders

Proceedings of the International Symposium on Diagnosis and Treatment of Disorders Affecting the Intrauterine Patient. Dorado, Puerto Rico, October 29—31, 1967. Springer-Verlag, Berlin, Heidelberg, New York 1968. 304 pages, 145 figures, 38 tables. Price: DM 59.20

The last decades brought some revolutionary discoveries in the basic sciences, and medicine is also in a period of remarkable advances in many fields. One of these is the subject of this book.

Until recently the only means by which the welfare of the foetus could be determined was the auscultation of the foetal heart sounds and a recording of its rate. Otherwise, not much was known about its condition. All of this is now changing, as methods to determine the foetal welfare during pregnancy and labour have been developed. Advanced biophysical and biochemical techniques are available to study prenatal events. This new field is wide. It extends from the detection of genetic disorders in the early embryo to the correction of abnormalities in the mature foetus.

The most important points of this new and rapidly developing branch of medicine were presented at a symposium and the present volume contains the papers delivered there. Methods developed for the direct approach of the placenta by biopsy, examination of amniotic fluid obtained by amniocentesis, blood sampling from the foetal scalp, measurement of certain biochemical changes in the mother regarded as reflections of foetal metabolism, biophysical studies of the human foetus (radiography, EKG, EEG, monitoring of heart rate) formed the subject of part of the papers. Compared with the rapid evolution in diagnostic procedures, the "treatment" of the foetus advances more slowly, but is becoming more directly related to diagnosis. It was shown that analysis of the amniotic fluid obtained by amniocentesis has led to surgical treatment of erythroblastosis, while hormone analysis in the mother's urine and studies of the foetal acid-base balance provide indications for early delivery. The concept of using passive anti-Rh antibody resulted in the prevention of immunization in mothers.

The book is stimulating and fascinating, especially for those who are interested in the events of prenatal life.

G. ILLEI

G. I. M. SWYER (Ed.)

Control of Human Fertility

British Medical Bulletin, 1970, Vol. 26, No. 1. British Council, London. Price: £ 2

The volume is introduced by the paper on the "Control of Human Fertility" by SIR ALAN PARKES; the 15 subsequent papers deal with recent data concerning the reproduction processes.

The paper "Hypothalamus and Control of Ovulation" by G. W. HARRIS and F. NAFTOLIN discusses the extero- and interoceptive stimuli, the role of the central nervous system, the function of the releasing factors controlling gonadotrophin release and peripheral feed-back mechanisms, and gives a concise summary of present-day knowledge in this field.

H. M. BRUCE's paper "Pheromons" sums up the facts concerning the materials controlling the sexual development and behaviour of the different animal species. Several kinds of material seem to be produced which through the olfactory nerve exert an important effect on sexual activity, mating behaviour and the reproductive activity of the contrasexual being. As yet only the presence and some biological effects of these pheromons are known. In connection with primates and human beings there are scarcely any facts. In his paper "The Evidence for Luteolysin" L. E. A. ROWSON concentrates on the question of the factor or impulse governing the duration of corpus luteum activity. Why is the corpus luteum functioning if there has been conception, and why does it stop functioning at the end of the cycle if there has been no conception? The possibility and mode of this response are discussed. According to the biological effects in certain animal species (e.g. the cow) it must be the uterus which reacts upon the duration of corpus luteum activity. This factor might be the endometrium or the material of embryonic cells.

The paper "Induction of Ovulation with Gonadotrophins" by A. C. CROOKE discusses the effect of the various gonadotrophic hormones, the control of ovulation induced by gonadotrophin treatment, and its complications.

In his paper "Clomiphene" P. M. F. BISHOP writes on the chemistry, the pharmacology and the mechanism of action of the agent producing ovulation. The mode of clinical application, the control of its effectiveness and its side-effects are discussed.

The paper "Effectiveness and Risks of Birth Control Methods" by D. M. POTTS and G. I. M. SWYER surveys the various birth control methods, from Knaus and Ogino's rule to the intrauterine devices. Numerous, hitherto unknown and surprising facts are given on the methods which until now have been considered harmless. For instance, with the rhythm method, extrauterine gravidity, placenta praevia and developmental abnormalities are more frequent. On the other hand, national statistics have proved that oral contraception and intrauterine devices are less harmful than they were supposed. For instance, one oral contraceptive pill is not more dangerous than smoking one-third of a cigarette once daily for three weeks out of four.

"Evaluation of Rare and Adverse Effects of Systemic Contraceptives" by R. DOLL and M. P. VESSEY discusses steroid-icterus and the frequency of thromboembolism and hypertension. There are certain cause-and-effect relationships but their significance is negligible in view of their low frequency. Oral contraception does not increase the risk of endometrial and breast cancer, nor the frequency of anovulation subsequent to discontinuing.

The paper "Developments in Steroidal Hormonal Contraception" by A. KLOPPER surveys the development from C 19 norsteroids to the application of C 21 steroids (the 17-OH progesteron derivatives). The supposed mechanisms of action, the toxic effects and the future possibilities of these agents are summarized.

C. W. EMMENS: "Postcoital Contraception". After discussing the modes of action, the problem of postcoital contraception by steroid and non-steroid compounds is dealt with. Future development must depend on human testing of the results of animal experiments. A "once-a-week" or "once-a-month" pill or injection would be ideal.

The paper by P. ECKSTEIN "Mechanisms of Action of Intra-Uterine Contraceptive Devices in Women and Other Mammals" discusses the changes caused by the application of IUD in various species of animals, and the probable mechanism of action. The truth is that we are far from knowing the mechanism of this action, though several kinds such as a foreign body reaction, luteolytic effect, cytotoxic reaction, etc. have been observed in the various animal species.

P. C. STEPTOE: "Recent Advances in Surgical Methods of Control of Fertility and Infertility". The paper deals with the termination of pregnancy combined with laparoscopic sterilization and the indications and methods of male sterilization.

D. M. POTTS in his paper "Termination of Pregnancy" discusses the non-surgical methods (agents destroying the ovule, ionizing radiation, antimitotics, anti-metabolites, agents inhibiting enzymes e.g. monoamine oxidase inhibitors, diphenylalkenes), the surgical methods (abortifacient pastes, intraamniotic injections, vacuum aspiration) and their side-effects. The psychological effects of these methods are also mentioned. It is suggested that after termination of the pregnancy, women should be offered contraceptive advice.

In the paper "Immunology of Conception and Pregnancy" R. G. EDWARDS discusses the questions of autoimmune aspermatogenesis and sperm agglutination, immunity against egg and embryo, against foetus and placenta, and against the secretions of the reproductive tract. Finally, the antibodies against proteo- and steroid- hormones are dealt with. The question is raised of the application of immunological methods in fertility control.

H. JACKSON: "Antispermatogenic Agents". The synthetic materials acting upon spermatogonia and spermatocytes, and their mechanism of action is discussed. In connection with their human application first the problem must be decided whether or not these agents have any harmful genetic effects.

Under the title "Sites of Hormone Production in the Mammalian Testis and their Significance in the Control of Male Fertility" D. LACY and A. JULIET PETTITT write on the lipid droplets in Sertoli's cells and their biological characteristics. Sertoli's cells which earlier were believed to be "residual bodies", now seem to have active hormonal functions. A short summary of biosynthesis in the testicle and of the effect of androgens on spermatogenesis is given. Two sites of androgen production are supposed to exist in the testicle: Sertoli's cells and Leydig's cells, which have different biological functions.

F. SZONTÁGH

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INDEX

PHYSIOLOGIA

Lázár G.: Species Specificity of Fibrinogen 1
Kállay K., Debreczeni L. A.: Effect of Human Serum Albumin on Renal Blood Flow Autoregulation in the Anaesthetized Dog 9
Várnai Ilona, Farkas Mária, Donhoffer Sz.: Thermoregulatory Effects of Hypoxia in New-Born Rats and their Correlation to Body Temperature and Heat Production Prior to and after Exposure to Hypoxia 19
Fazekas I. Gy., Fazekas A. T.: Seasonal Variations of the Corticosteroid Fractions in Guinea Pig Tissue and Organs 33
Stark E., Makara G. B., Palkovits M., Mihály Katalin: Afferent Pathways of Stressful Stimuli: their Dependence on Strength and the Time Elapsed after the Onset of Stimulation 43
Shkhinek E. K., Nyakas C., Endrőczi E.: Frequency-Specific Response of the Pituitary-Adrenocortical System on Pontine Stimulation 51
Nyakas C., Endrőczi E.: Olfaction Guided Approaching Behaviour of Infantile Rats to the Mother in Maze Box 59
Мархасина И., Оранский И.: Реографическое изучение соотношения церебральной гемодинамики с периферическим кровотоком у людей, занятых умственным трудом 67

PATHOPHYSIOLOGIA

Gábor M., Antal A., Lipták Klára, Zoltán Ö. T., Győri I., Benkő S.: Capillary Resistance in the Skin of Rats Fed Flavone-Free and Atherogenic Diets, and their Response to Hesperidin-Methyl-Chalcone 71
Huszák I., Durkó Irene: Disturbances of Porphyrin Metabolism in Schizophrenia 77

PHARMACOLOGIA

Solti F., Krasznai I., Rév Judit, Nagy Júlia: Effect of Propranolol on Circulatory Regulation 85
Hadházy P., Fenyvesi T., Illés P., Seres J.: Comparison of the Cardiodepressant and Antiarrhythmic Effects of 2-Piperidinyl-1-α-Naphthyl-1-Propanol HCl (S-1043) and Propranolol 93

RECENSIONES

Bechterewa N. P. (Ed.): Physiologie und Pathologie der tiefen Hirnstrukturen des Menschen. VEB Verlag Volk und Gesundheit, Berlin 1969. (K. Lissák) 105
Burkhardt D.: Wörterbuch der Neurophysiologie. VEB Gustav-Fischer-Verlag, Jena 1969. (K. Lissák) 105
Kerkut G. A. (Ed.): Experiments in Physiology and Biochemistry. Volume 2. Academic Press, London, New York 1969. (K. Lissák) 105
Birke G., Norberg R., Plantin L. O. (Eds): Physiology and Pathophysiology of Plasma Protein Metabolism. Pergamon Press, Oxford 1969. (K. Lissák) 106

Grosse-Brockhoff F. (Ed.): Pathologische Physiologie. Springer-Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 106
Wolstenholme G. E. W., Knight Julie (Eds): Circulatory and Respiratory Mass Transport. J. and A. Churchill, London 1969. (K. Lissák) 107
Wolstenholme G. E. W., Knight Julie (Eds): Control Processes in Multicellular Organisms. J. and A. Churchill, London 1970. (K. Lissák) 107
Wolstenholme G. E. W., Knight Julie (Eds): Progesterone: Its Regulatory Effect on the Myometrium. J. and A. Churchill, London 1969. (G. Telegdy) 108
Gray C. H., Bacharach A. L. (Eds): Hormones in Blood. 2nd Edition. Academic Press, London 1967 and 1968. (G. Telegdy) 108
Sterba G. (Ed.): Zirkumventrikuläre Organe und Liquor. VEB Gustav-Fischer-Verlag, Jena 1969. (K. Fendler) 110
Peters G., Roch-Ramel F. (Eds): Progress in Nephrology. Springer-Verlag, Berlin, Heidelberg, New York 1969. (F. Balogh) 111
Milcu St. M., Maximilian C., Ionescu B.: Endocrinopatiile genetice. Editura Academiei Republicii Socialiste România, Bucureşti 1968. (F. Obál) 112
Hafferl A.: Lehrbuch der topographischen Anatomie. Springer-Verlag, Berlin, Heidelberg, New York 1969. (B. Flerkó) 112
Doerr W., Seifert G., Uehlinger E. (Eds): Spezielle pathologische Anatomie. Band 4. Springer-Verlag, Berlin, Heidelberg, New York 1969. (G. Romhányi) 113
Adamsons K. (Ed.): Diagnosis and Treatment of Fetal Disorders. Springer-Verlag, Berlin, Heidelberg, New York 1968. (G. Illei) 113
Swyer G. I. M. (Ed.): Control of Human Fertility. British Medical Bulletin, 1970. British Council, London. (F. Szontágh) 114

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VERKALUNGSSTUDIEN AM ENTMINERALISIERTEN ZAHN- UND KNOCHENGEWEBE

Von Dr. Dr. HANS SCHMIDT, Halle/S.

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Nach einer einführenden Darstellung der Molekularstruktur des Zahn- und Knochengewebes aus histochemischer, elektronenmikroskopischer und biochemischer Sicht werden die verschiedenen bisher entwickelten Vorstellungen über die Biochemie der Verkalkungen ausführlich besprochen. Im experimentellen Teil wird der im entkalkten Zahn- und Knochengewebe nach Behandlung mit verschiedenen Mineralsalzlösungen ablaufende Remineralisationsprozeß histologisch und quantitativ verfolgt. Die Ergebnisse werden diskutiert.

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ACTA PHYSIOLOGICA

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РЕЗЮМЕ

ВИДОВАЯ СПЕЦИФИЧНОСТЬ ФИБРИНОГЕНА

Д. ЛАЗАР

Согласно исследованиям автора у животных, систематологически отдаленных друг от друга, антигенная структура фибриногена определяется в первую очередь их видовой принадлежностью, так как в пределах штамма позвоночных фибриноген двух классов: млекопитающих и птиц, не показывает родства антигенов. В противоположность этому, между фибриногеном систематологически близких друг к другу животных, в пределах систематологического класса наблюдается очень распространенное родство антигенов. Однако при определении с радиальным двойным иммунодиффузионным методом родство антигенов ни в одном случае не дало полной (реакции идентичности), а показало только различные степени реакции частичной идентичности (родства). На основе исследований автора противофибриновые иммунные сыворотки не реагируют с фибриногеном животных, использованных для получения иммунной сыворотки, вопреки тому, что с противофибриновой сывороткой, полученной у другого животного, можно выявить очень тесное антигеновое родство между фибриногенами этих животных.

ДЕЙСТВИЕ АЛЬБУМИНА ЧЕЛОВЕЧЕСКОЙ ПЛАЗМЫ НА АВТОРЕГУЛЯЦИЮ КРОВОТЕЧЕНИЯ ПОЧКИ У НАРКОТИЗИРОВАННОЙ СОБАКИ

К. КАЛЛАИ и Л. ДЕБРЕЦЕНИ

Авторы исследовали у 12 собак, наркотизированных хлоралозой 0,10 г/кг, с помощью электромагнитного измерителя кровоток в левой почке при постепенном сужении аорты с помощью зажима. Измерения были повторены на этих же собаках после инфузии 3 г/кг альбумина человеческой плазмы. После инфузии альбумина величина кровоснабжения почки увеличилась, авторегуляция почечной циркуляции ухудшилась, и была обнаружена положительная корреляция между давлением и кровотоком. Учитывая то, что после инфузии альбумина нарушилась экскреция PAH и inulin, нарушение авторегуляции можно объяснить открытием шунтов.

ДЕЙСТВИЕ ГИПОКСИИ НА ТЕРМОРЕГУЛЯЦИЮ НОВОРОЖДЕННОЙ КРЫСЫ И КОРРЕЛЯЦИЯ ТЕМПЕРАТУРЫ ТЕЛА И ТЕПЛОПРОДУКЦИИ ПОД ГИПОКСИЕЙ И ПОСЛЕ ГИПОКСИИ

ИЛОНА ВАРНАИ, МАРИЯ ФАРКАШ и С. ДОНХОФФЕР

1—2 дневные, 4—10 дневные, 11—20 дневные крысы, и крысы, которым от рождения было меньше 12 часов, дышали кислородом 12%-ым 30 минут при температуре окружающей среды ниже, чем термонейтральная. Потребление кислорода и температура ободочной кишки непрерывно измерялись 30 минут перед гипоксией, в течение гипоксии, и 30 минут после гипоксии. При температуре среды 30°C не могли обнаружить корреляцию между температурой тела перед гипоксией и снижением теплопродукции в течение гипоксии. Наоборот, значительная корреляция была установлена между температурой тела перед гипоксией и изменением температуры тела под гипоксией. У крыс, которым от рождения было меньше

12 часов, 1—2 дневных и 11—20 дневных крыс не оказалось корреляции между теплопродукцией после гипоксии и увеличением температуры тела, против того, у 4—10 дневных крыс была установлена определенная корреляция в этом отношении. У 11—20 дневных крыс, у которых температура тела перед гипоксией была высше 34°C, не нашли корреляцию между снижением температуры тела в течение гипоксии и снижением теплопродукции или уровнем теплопродукции под гипоксией.

Результаты ясно показывают, что у новорожденных крыс снижение температуры тела в гипоксии не является простым следствием снижения теплопродукции, но, так называемая физическая терморегуляция тоже принимает участие в реакции. На это же указывает и то, что нет тесной связи между теплопродукцией и увеличением температуры тела даже после гипоксии.

СЕЗОННЫЕ ИЗМЕНЕНИЯ ФРАКЦИЙ КОРТИКОСТЕРОИДОВ В ОРГАНАХ И ТКАНЯХ МОРСКИХ СВИНОК

И. ФАЗЕКАШ и А. ФАЗЕКАШ

Из всех органов 48 морских свинок обоего пола, одинаковой породы и содержащихся при одинаковом питании, авторы изготовили вытяжки весной (III месяц) и осенью (X. месяц). Животные были распределены в три группы (по 8 животных в каждой группе, 24 животных). С помощью метода хроматографии на бумаге в вытяжках отдельных органов были определены кортикостероидные фракции. Было найдено, что надпочечники, органы и ткани животных содержат *весной* больше общих кортикостероидов, чем *осенью*. В определенных органах отдельные фракции отсутствовали осенью, а другие весной. Некоторые фракции в определенных органах животных не удалось выявить ни весной ни осенью. Так например в селезенке, легких, сердце и мозге соединение X_1 отсутствовало и весной и осенью а соединение X_2 ни весной ни осенью не было обнаружено в селезенке, сердце и мозге.

ДАЛЬНЕЙШЕЕ ИЗУЧЕНИЕ АФФЕРЕНТНЫХ ПУТЕЙ СТРЕССОРНЫХ РАЗДРАЖЕНИЙ; ИЗУЧЕНИЕ РОЛИ ИНТЕНСИВНОСТИ РАЗДРАЖИТЕЛЯ И ВРЕМЕНИ, ПРОХОДЯЩЕГО ПОСЛЕ НЕГО

Э. ШТАРК, Г. Б. МАКАРА, М. ПАЛКОВИЧ и К. МИХАЛЬ

Изучалось, увеличивается ли секреция АКТГ, если изменяется интенсивность стрессора, данного по тем же афферентным путям, или время, проходящее между применением стресса и измерением. У экспериментальных крыс медиальный базальный гипоталамус (МВН) антеролатерально был деафферентирован или их задняя нога, где стрессор был применен, была денервирована. Как показатель АКТГ секреции, был измерен уровень кортикостерона плазмы. Маленькая доза формальдегида (2,5 мг/100 г подкожно), которая значительно увеличивает уровень кортикостерона плазмы у нормальных животных, является неактивной после деафферентации МВН, или, если была дана в денервированную заднюю ногу. Большая доза формалина вызвала такое же увеличение уровня кортикостерона плазмы у всех экспериментальных животных, также как и у контрольных. У животных, у которых МВН был антеролатерально деафферентирован, ложная адреналектомия не влияла на уровень кортикостерона плазмы через час, в то время, как он значительно повышался у контрольной крысы, а через 2—4 часа увеличение было аналогично контрольному. Это показывает, что нервные волокна, восходящие к МВН снизу, латерально и сверху, которые являются необходимыми для повышенной секреции АКТГ, наступающей сразу после хирургической травмы, существенны для повышенной АКТГ секреции, наблюдаемой через 2—4 часа; другими словами, операционная травма в разное время после нее активирует различные афферентные волокна. Антеролатеральная деафферентация МВН в любое время подавляет влияние маленькой дозы формальдегида увеличивать АКТГ секрецию.

Вывод: каждому примененному стресс-раздражителю соответствует свой афферентный путь, через который стрессор влияет на АКТГ секрецию; и после применения одних стрессоров в различное время различные афферентные пути активируются, а при применении других стрессоров — нет.

СПЕЦИФИЧЕСКИЙ ОТВЕТ СИСТЕМЫ ГИПОФИЗ — КОРА НАДПОЧЕЧНИКОВ НА ЧАСТОТУ РАЗДРАЖЕНИЯ МОСТА

Е. К. ШКИНЕК, Ч. НЬЯКАШ и Э. ЭНДРЁЦИ

В условиях хронического опыта электрическое раздражение nucleus reticularis gigantocellularis pontis приводило у крыс к специфическому ответу системы гипофиз-кора надпочечников в отношении частоты раздражения. При раздражении с низкой частотой в 6—10 гц наблюдается повышение ответа электрической активности коры головного мозга и существенное повышение производства кортикостерона надпочечниками in vitro. Раздражение той же области моста высокой частотой (100 гц) не вызывает активации системы гипофиз-кора надпочечников, хотя деятельность коры головного мозга характеризуется десинхронизацией.

ОБОНЯТЕЛЬНОЕ РЕГУЛИРОВАНИЕ ПРИБЛИЖЕНИЯ ИНФАНТИЛЬНЫХ КРЫС К МАТЕРЯМ В ЛАБИРИНТЕ

Ч. НЬЯКАШ и Э. ЭНДРЁЦИ

Авторы изучали приближение 10 дневных крыс к матерям в простом, U-образном лабиринте. Молодые крысы легко находили своих матерей в лабиринте, они значительно реже приближались к нелактирующим самкам и ни в одном случае не подошли к самцам. Местное применение тетракаина на слизистую носа вызвало такое самопроизвольное поведение, которое доказало ведущую роль обоняния в поведении молодых крыс.

РЕОГРАФИЧЕСКОЕ ИЗУЧЕНИЕ СООТНОШЕНИЯ ЦЕРЕБРАЛЬНОЙ ГЕМОДИНАМИКИ С ПЕРИФЕРИЧЕСКИМ КРОВОТОКОМ У ЛЮДЕЙ, ЗАНЯТЫХ УМСТВЕННЫМ ТРУДОМ

И. МАРХАСИНА и И. ОРАНСКИЙ

На русском языке.

ИЗМЕНЕНИЯ РЕЗИСТЕНТНОСТИ КАПИЛЛЯРОВ КОЖИ КРЫС, СОДЕРЖАННЫХ НА АТЕРОГЕННОЙ ДИЕТЕ И НА ДИЕТЕ С ОТСУТСТВИЕМ ФЛАВОНА, И ВОЗДЕЙСТВИЕ НА ЭТИ ИЗМЕНЕНИЯ ДАЧЕЙ ГЕСПЕРИДИН-МЕТИЛ-ХАЛКОНА

М. ГАБОР, А. АНТАЛ, К. ЛИПТАК, Т. ЗОЛЬТАН, И. ДЬЁРИ и Ш. БЕНКЁ

Капиллярная резистентность крыс, содержащихся на протяжении 10 недель на режиме питания с отсутствием флавона, в существенной мере понижается. Тиреоидектомия и 10 недельная дача атерогенной диеты не вызывают значительного изменения капиллярной резистентности у крыс. При комбинации диеты с отсутствием флавона, атерогенной диеты и тиреоидектомии наблюдается очень значительное понижение капиллярной резистентности, причем ответственным фактором за возникновение этого изменения является состояние недостатка флавона. Дача гесперидин-метил-халкона крысам, содержащимся на атерогенной диете с недостатком флавона, в значительной мере уменьшало понижение капиллярной резистентности.

ДАЛЬНЕЙШИЕ ЭКСПЕРИМЕНТАЛЬНЫЕ ДАННЫЕ К ПАТОЛОГИЧЕСКОМУ МЕТАБОЛИЗМУ ПОРФИРИНА У ШИЗОФРЕНИКОВ

И. ХУСАК и ИРЕНЕ ДУРКО

В состояниях дефицита витамина $Б_6$, вызванных введением INH, 4-деоксипиридоксина, или совместным введением 4-деоксипиридоксина и триптофана, были определены фракции крови и мочи у шизофреников. Протопорфирин в эритроцытах и копропорфирин в моче только при нагрузке с l-триптофаном снижаются в равной мере, как у контрольной группы, так у шизофреников. Содержание дельта-аминолевулинкислоты в

моче только у контрольной группы уменьшается одинаково, в то время как количества ее у шизофреников изменяются в широких пределах.

Было проведено разделение порфирин-эстеров, содержащих 7-6-5 карбоксил, и появляющихся в течение преобразования уро-копропорфирина с помощью тонкослойной хроматографии.

Эксперименты с INH дали результаты, похожие на эксперименты с 4-деоксипиридоксином. Авторы обсуждают предположительную роль пиридоксалных энзимов в связи с нарушением метаболизма порфирина у шизофреников.

ДЕЙСТВИЕ ПРОПРАНОЛОЛА НА РЕГУЛИРОВАНИЕ КРОВООБРАЩЕНИЯ

Ф. ШОЛЬТИ, И. КРАСНАИ, Ю. РЕВ и Ю. НАДЬ

Авторы изучали в опытах на собаках действие пропранолола, блокатора симпатических β-рецепторов, на кровообращение.

У 20 собак, после внутривенного введения 0,2 мг пропранолола на кг веса тела авторы проводили фракционированное измерение кровотока в отдельных органах (головной мозг, сердце, почки, печень, кожа, мышца) методом одновременного введения ^{131}йод-антипирина и рубидия^{86}. Прочие параметры кровообращения (минутное выбрасывание, сосудистое сопротивление в большом кругу кровообращения, mean transit time) авторы определяли на основе принципа разведения красителя дачей эвансблау, и в качестве контроля они использовали соответствующие данные кровообращения у 40 собак. При остром действии пропранолола — торможение β-рецепторов — наблюдалось пониженеи минутного выбрасывания, повышение общего сосудистого сопротивления в большом кругу кровообращения и значительное уменьшение скорости кровотока. Кровяное давление не показало существенных изменений. Мозговая, сердечная и кожная фракции минутного объема увеличились, в то время как в области мышц удалось выявить выраженное сужение сосудов и понижение фракции минутного объема мышечного кровообращения. Кровообращение почек и печени уменьшались соразмерно понижению минутного объема.

Экспериментальные данные авторов указывают на то, что симпатические β-рецепторы играют также базально значительную роль в регулировании кровообращения.

КАРДИОДЕПРЕССИВНОЕ И АНТИАРИТМИЧЕСКОЕ ДЕЙСТВИЕ 2-ПИПЕРИДИНИЛ-L-АЛФА-НАФТИЛ-L-ПРОПАНОЛА HCl (S-1043) И ПРОПРАНОЛОЛА

П. ХАДХАЗИ, Т. ФЕНЬВЕШИ, П. ИЛЛЕИ и Й. ШЕРЕШ

Авторы показали, что 2-пиперидинил-l-алфа-нафтил-l-пропанол (S-1043) даже и в самой высокой примененной концентрации (8 μг/мл) не блокирует бета-рецепторы. Также как и пропранолол, он снижает раздражительность сердечной мышцы и выработку импульса синусного узла; на роговице кролика он имеет местное обезболивающее влияние. Он удлиняет функциональный рефрактерный период в предсердечной мышце — в этом отношении он является сильнее пропранолола. Меньше, чем пропранолол, снижает контрактивность сердечной мышцы, а у собаки при аритмии, вызванной оуабаином, он действует в два раза сильнее, чем пропранолол.

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Physiologia

CYTOCHROME OXIDASE DETERMINATION USING A MANGANESE CITRATE COMPLEX

By

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(Received February 23, 1970)

A rapid and simple colorimetric method has been developed for the assay of cytochrome oxidase activity of tissues and ascites tumours. The essence of the method is that in the terminal oxidative chain an electron transport system is introduced, by interposing between cytochrome c and cytochrome a_3 a manganese citrate-diethyl-paraphenylene diamine complex. Under the effect of the respiratory enzyme (cytochrome oxidase) the complex is oxidized and turns mauve in colour. The change in colour intensity (increase of extinction) is proportionate to the enzyme activity. The method has been developed for the Pulfrich photometer, but may be adjusted to any kind of spectrophotometer. The test requires a small quantity of tissue and takes 15 minutes. Enzyme activity is expressed as related to protein content.

Cytochrome oxidase (in the following: CYO) is usually assayed for activity on the basis of that characteristic property of reduced cytochrome c that its absorption maximum at 550 mμ decreases proportionately with the enzyme's oxidative power. Several spectrophotometric methods are based on this principle (ALBAUM et al. 1946; COOPERSTEIN and LAZAROW 1951; HOGEBOOM and SCHNEIDER 1952; SMITH 1954); they differ mainly in the manner of cytochrome c reduction and in the concentration of cytochrome c employed. The most widely accepted among them is the method developed by COOPERSTEIN and LAZAROW (1951). However, the difference in absorption between the reduced and the oxidized forms of the commercial preparation was found so small at 0.3 to 0.5 mg/ml concentrations that the test proved unreliable. At higher concentration (1 mg/ml) the test, if carried out in series, is too expensive.

Another known property of CYO is that it oxidizes paraphenylene diamine and its derivatives, as well as alpha naphthol so that a coloured complex compound is produced. This so-called Nadi's reaction, which was used by STRAUS for the determination of enzyme activity (1954), was found by us cumbersome and time-consuming in view of the shaking out with chloroform.

The method of SMITH and STOTZ (1949) is based on the oxidation of a reduced dye. However, owing to the excessive autooxidation of the dye the method could not gain any wider acceptance.

The enzyme may be assayed also by the classical manometric Warburg method, using paraphenylene diamine derivatives. As commonly known, this is a most cumbersome and complicated method, requiring a great amount (40 to 50 mg) of tissue suspension and the procedure is time-consuming.

These considerations have induced us to develop a method for CYO activity assay, which is easy to carry out, requires small quantities of substances and tissues, is sensitive and fast. This has been made particularly desirable as we wish to study CYO activity in ascites tumours, where only small amounts of a substance of low activity are at disposal.

Our method is based on the earlier observation that certain metal complexes are capable of inhibiting the growth of transplanted mouse Ehrlich tumours. It has been demonstrated experimentally how these complexes influence (activate or inhibit) the oxidation of paraphenylene diamine (BANGA et al. 1954). As a metal complex we have chosen manganese citrate and added it during the reaction to a paraphenylene diamine derivative. The enzyme oxidizes the manganese citrate-paraphenylene diamine complex so formed and turns mauve in colour. The changes in colour intensity (increase in extinction) are proportionate to enzyme activity.

Methods

Preparation of liver homogenate. For each test, the liver from 3 animals was used. After weighing and mincing, the liver tissue was homogenized in a China mortar, adding to it in small portions M/30 phosphate buffer to get a 1 : 1 dilution. After filtering through gauze the suspension was further diluted with phosphate buffer. Each step was carried out under cooling. (Experience has shown that 80 to 100-fold dilutions are optimal.)

Preparation of the manganese citrate complex (MnCC). Of $MnCl_2 \cdot 4\,H_2O$, 198 mg are dissolved in 2 ml of distilled water, then 630 mg of citric acid is added and enough water to dissolve it. Then, under chilling in ice, the solution is neutralized with *N* NaOH, precipitated by 96% alcohol of 4°C, centrifuged under refrigeration, washed with alcohol and dried in vacuum. The yield usually ranged from 960 to 970 mg. Of this, 10 mg is dissolved in each ml of distilled water immediately before use.

Diethyl-p-phenylene-diamine sulphate (DFD) solution (Reanal, Budapest); 10 mg is dissolved in each ml of distilled water, immediately before use.

Cytochrome c solution (Cyt. c.) (Reanal or Calbiochem). Five mg/ml are dissolved in buffer; the solution may be stored in the refrigerator for a few days.

M/30 pH 7.4 phosphate buffer.

Procedure. The test is made at room temperature. To 0.1 ml of Cyt. c solution in a Wasserman tube 0.5 ml buffer, then 0.5 ml distilled water are added. Then 0.5 ml of MnCC solution and 0.2 ml of the tissue homogenate are added. After thorough shaking the mixture is transferred into the cuvette of the Pulfrich photometer. The control cuvette contains distilled water. Then 0.2 ml of DFD solution are added to the test cuvette, which is turned over once and the extinction is read without delay using an S 55 filter (Ext_0). Fifteen minutes later the cuvette is turned over again and extinction is read (Ext_{15}). The difference between the two extinctions corresponds to the enzyme activity. We present the so-called specific enzyme activity, i.e. that related to protein. To avoid fractions, the value obtained is multiplied by 100.

$$\text{Specific activity} = \frac{Ext_{15} - Ext_0}{P} \times 100$$

where Ext_0 is the extinction read at 0 min, and Ext_{15} that read 15 minutes later, P the mg of protein contained in the added 0.2 ml volume.

Protein content was estimated according to LOWRY et al. (1951).

Modified Warburg method. The test is made at 30°C. The manometer vessel contains the tissue homogenate (40 to 50 mg of protein) and the MnCC solution, the side bulb the DFD solution, which is added after equilibration of temperature (preliminary shaking) to the manometer vessel. Into the central, sealed well 20% NaOH is added. Readings are taken every 10 minutes, for 60 minutes; we calculated with the 60-minute value.

Modified Cooperstein–Lazarow method. Cyt. c. (Calbiochem) is completely reduced by sodium hydrosulfite ($Na_2S_2O_4$). Cyt. c concentration ranged from 0.8 to 1.0 mg/ml. Complete oxidation of Cyt. c was carried out with a saturated solution of $K_3[Fe(CN)_6]$.

The measurements were made with a Spektromom 201 spectrophotometer at 550 mμ. The difference in extinction between reduced and oxidized Cyt. c was regarded as 100% enzyme activity. The results are related to 1 mg protein. In the test, 0.2 to 0.3 ml of the 80 to 100-fold diluted tissue homogenates are used.

Results and discussion

Oxidation of reduced Cyt. c with MnCC. One of the fundamental problems was whether MnCC would oxidize the reduced Cyt. c, i.e. whether its redox potential was satisfactorily high. This has been studied by the following direct technique. Three ml of 1 mg/ml Cyt. c (Calbiochem) solution were maximally reduced with $Na_2S_2O_4$ and extinction was read at 550 mμ; its value amounted to 0.86. The same solution was maximally oxidized with a few crystals of $K_3[Fe(CN)_6]$, then the extinction was 0.62.

In another experiment MnCC was added to reduced Cyt. c. At five minutes the 0.86 extinction decreased to 0.66, thus, almost complete oxidation had taken place. Similar results have been reported by DAVISON and HAMILTON (1968) who found that the copper citrate complex prepared by them also oxidized the reduced Cyt. c.

Proof that MnCC reacts with cytochrome oxidase. When diethylparaphenylene diamine is added to the complex, a compound pink in colour is formed. After the addition of tissue homogenate to the system, it becomes mauve in colour. On adding KCN, no increase in colour intensity results, because the metabolites activated by the tissue dehydrogenases reduce the mauve-coloured complex. When the tissue homogenate is placed in a water bath of 60°C for 15 minutes to inactivate the enzyme, the colour intensity will not increase. Inhibition by cyanide and the sensitivity to heat of the reaction indicate that the oxidation of the complex takes place in response to CYO.

The problem to be solved was whether MnCC increased the activity of CYO. To test this, we added to one vessel of the Warburg apparatus DFD only, and to the other DFD and MnCC, and compared the CYO activity of 10 normal rat livers. The results are presented in Figs 1 and 2. As visible, the columns in Fig. 2 are usually higher, indicating that the Q_{O_2} values were higher ($p < 0.001$). Thus, MnCC does activate the oxidation of DFD.

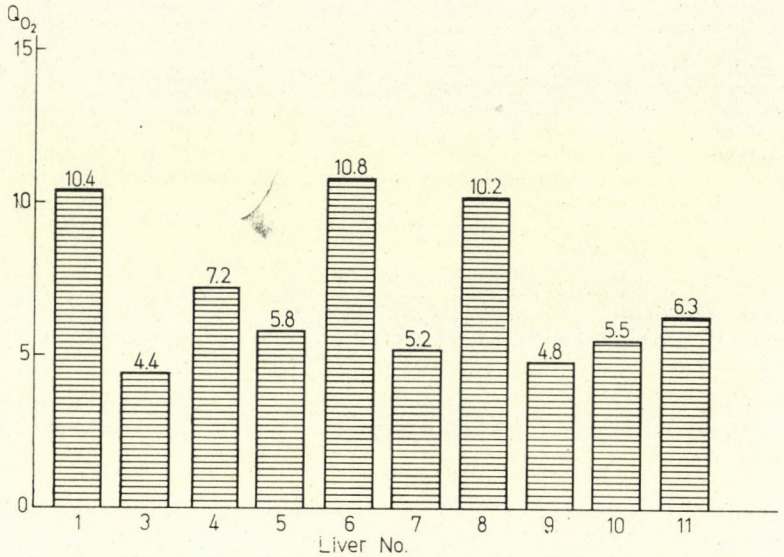

Fig. 1. Cytochrome oxidase activity of the liver of normal mice by the Warburg method (DFD alone)

The role of Cyt. c in the reaction. It was found that the complex is oxidized also when no Cyt. c is added to the reaction mixture. But when there is a small amount of Cyt. c in it (see Methods), the change in colour intensity is enhanced.

Influence of temperature on the reaction. Enzyme activity values were practically the same in the 4°C to 25°C (room temperature) range. There was an about 25% increase at 37°C. These results are comparable to those reported by COOPERSTEIN and LAZAROW (1951), who found a 45% difference in enzyme activity between the assays made at room temperature and at 37°C. Therefore, as generally accepted, we performed the test at room temperature.

Reaction time. In several experiments the reaction was followed for 30 minutes. In harmony with SMITH and STOTZ (1949) we found that 30 minutes of incubation at 37°C led to a considerable fall in enzyme activity and a decline resulted even at room temperature. As for 15 minutes there was a constant rise therefore the assays were continued for 15 minutes. Under such conditions the change in enzyme activity (rise in extinction) is proportionate to the concentration of enzyme in tissue homogenate.

Autooxidation of the complex. The compound formed by MnCC and DFD shows little autooxidation the ingredients are added in the proportions specified under Methods. Autooxidation is correlated with the relative amounts of MnCC and DFD. In our method MnCC is in excess and under such circumstances the increase of extinction caused by autooxidation does not exceed 0.00 to 0.07.

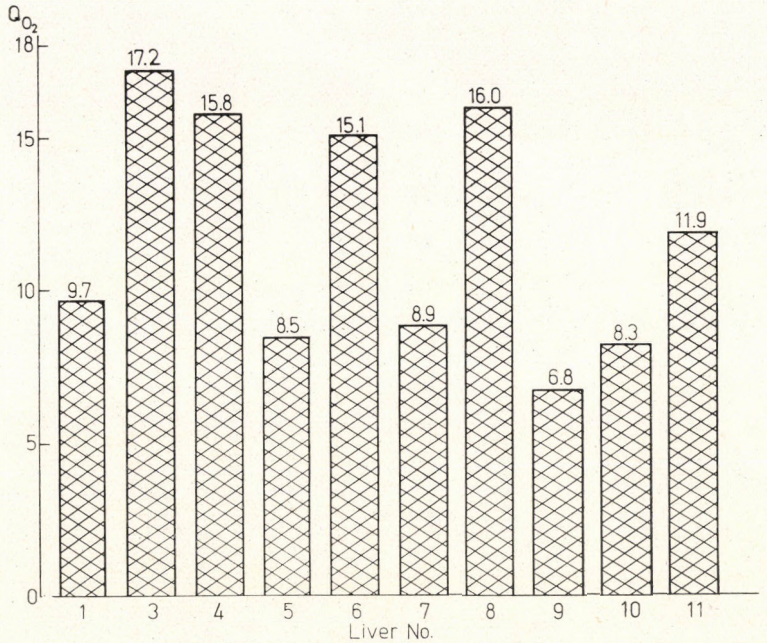

Fig. 2. Cytochrome oxidase activity of the liver of normal mice by the Warburg method (DFD + MnCC)

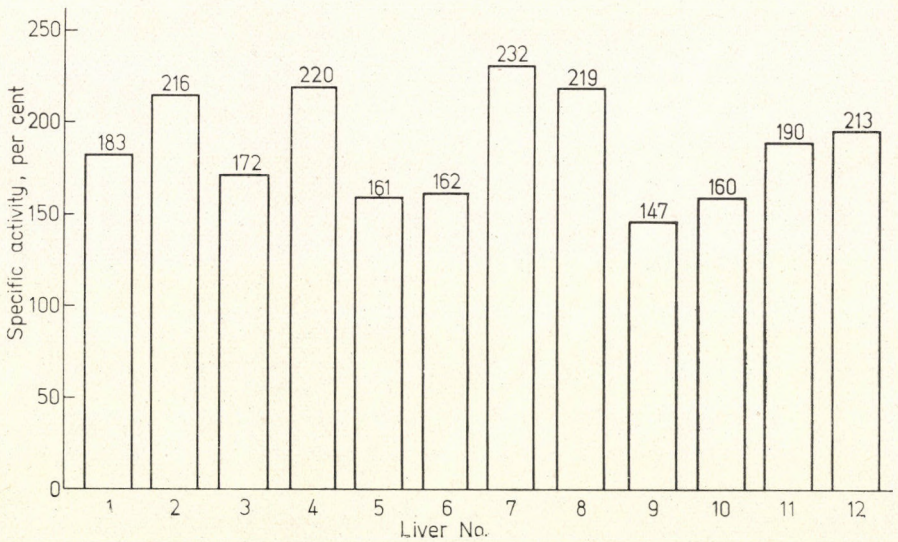

Fig. 3. Cytochrome oxidase activity of the liver of normal mice by the Cooperstein–Lazarow method

Reproduction of tests. Performing the assays in duplicate, liver homogenates and ascites were tested 70 times. The mean deviation of the parallels was 7.9%. The difference between parallels was smaller in the case of ascites than in the liver tissue assays.

Comparison of the results with those obtained by the Cooperstein–Lazarow and the Warburg methods. Twelve normal and 4 ascites mouse livers were assayed for enzyme activity. The results for the normal mouse livers are presented in Figs 2, 3 and 4.

With the Warburg method (Fig. 2) the value of Q_{O_2} is given and with the two other methods enzyme activity is computed for 1 mg of protein. To facilitate comparison, the highest enzyme activities were accepted to represent 100% and these were plotted on the same scale. A detailed comparison of the results obtained for individual livers allowed the conclusion that the results obtained by the MnCC method (Fig. 4) agree to almost 100% with those of the Cooperstein–Lazarow one (Fig. 3), while in the case of the Warburg method agreement was about 60% only (Fig. 2). The means and SD values for each liver obtained by the three methods were also calculated. The SD value in the case of the Cooperstein–Lazarow methods was 189.5 ± 29.3. In this range were found 75% of all the values, i.e. there was a satisfactory agreement between the calculated and the estimated values. With our method the SD was 233.4 ± 37.8, and 70% of all the values obtained were in this range. The Warburg method showed the widest variation, the SD had a value of 11.82 ± 3.88, and 60% of all found values fell into this range. Considering, however, that there were only 10 animals involved, even this agreement is apparently satisfactory. As determined by our method, the mean difference between parallel assays was 6% for the same livers.

Every mouse liver tested was subjected to histological examination. A slight inflammation could be demonstrated in two animals but the change was not correlated with the diminution of CYO activity.

The CYO activities of 4 ascitic mouse livers as determined by the Cooperstein–Lazarow and our own method are shown in Fig. 5. The assays were made at 7, 8, 9 and 11 days following inoculation. The results are in good agreement. Thus, enzyme activity in the liver of mice with ascites tumour is not lower than that in the liver of normal mice (Figs 3 and 4).

Advantages of the MnCC method. The Warburg method, as modified by us, requires 40 to 50 mg of protein per test. The results obtained with lower amounts of protein are not reliable. Besides, the prescribed duration of the reaction is 60 minutes and readings have to be taken at 10-minute intervals. Thus, the 15-minute reaction time employed in our method simplifies the assay and makes it quicker.

The Straus reaction, too, takes only a few minutes to carry out and is more sensitive than the Warburg method, but the extraction with chloroform

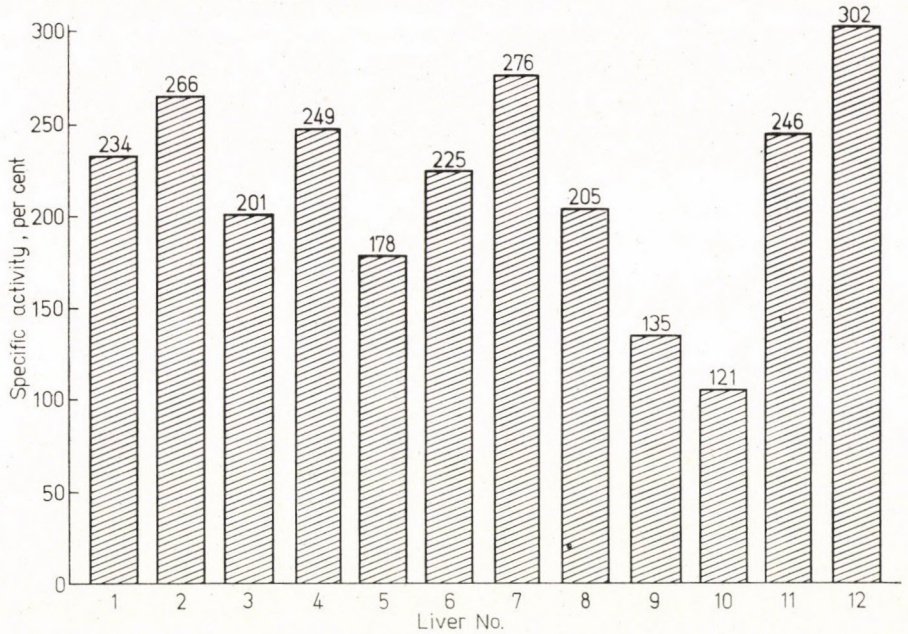

Fig. 4. Cytochrome oxidase activity of the liver of normal mice by our own method

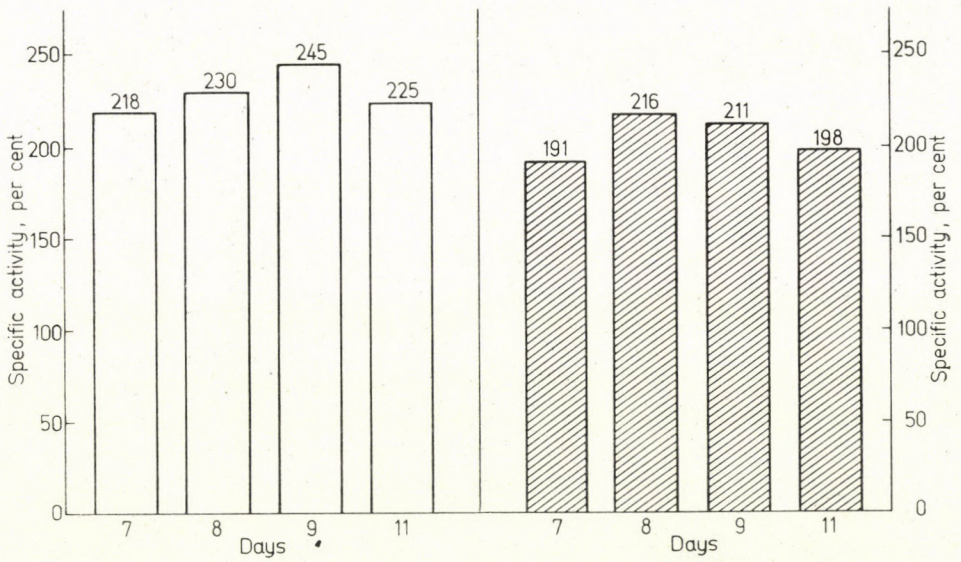

Fig. 5. Cytochrome oxidase activity of the liver from mice with ascites tumour, by two methods. On the left: Cooperstein–Lazarow's method. On the right: our own method

before the assay makes the test protracted and cumbersome. Moreover, experience has shown that if the prescribed conditions are adhered to, it is difficult to shake out quantitatively the coloured complex compounds formed.

Thus, the Cooperstein–Lazarow method may be applied successfully only when a suitable active preparation is available (such as the one produced by Calbiochem). As with our method Cyt. c is merely a catalyst, in comparative experiments it has been demonstrated that it allows the use of less active preparations. Another advantage of our method is that the materials required are inexpensive and easy to purchase, making it possible to use the test as a routine procedure.

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EFFECT OF TOURNIQUET ON TOTAL LACTATE DEHYDROGENASE (LDH) ACTIVITY AND ISOZYME PATTERN OF RAT SERUM

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Muscle anoxia was induced in rats by applying a tourniquet on one of the hind limbs. After removal of the tourniquet total lactate dehydrogenase (LDH) activity of the serum was estimated at various intervals, and the LDH isozymes were separated by means of agar-gel electrophoresis. Total LDH activity reached the maximum 3 hours after removal of the tourniquet. Thereafter, it gradually decreased, but was significantly above the control value even after 48 hours, and returned to the normal level in about 72 hours. The changes in the isozyme pattern were most marked 24 hours after removal of the tourniquet. Extracts of normal rat and rabbit skeletal and heart muscle were administered by the intraperitoneal route and the time-course of the changes in the isozyme pattern was followed. The results support the assumption that the tourniquet-induced alterations in the isozyme pattern are to be accounted for by the LDH isozymes released into the circulation by the injured muscle.

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, E.C.1.1.1.27) can be separated into five isoenzymes by means of electrophoresis (Wieland and Pfleiderer 1957; Markert and Möller 1959). Each of these isoenzymes represents a tetramer which, under the effect of various treatments, decomposes in four subunits (Apella and Markert 1961). Two types of subunit participate in the construction of the enzyme molecule. Subunit A is mainly characteristic of skeletal muscles and is called therefore also M (muscle) subunit. Subunit B can be found in the heart in large amounts and is called therefore also H (heart) subunit. The synthesis of the two subunits is directed by two genes (Boyer et al. 1963). The association of the subunits occurs at random (Markert 1963). After electrophoresis the LDH 1 isozyme is situated most closely to the anode (HHHH), and LDH 5 (MMMM) most closely to the cathode, while LDH 2 (HHHM), LDH 3 (HHMM) and LDH 4 (HMMM), also called hybrids, are situated between LDH 1 and LDH 5. The isozyme pattern of a given organ is primarily determined by the condition whether M- or H type subunits dominate in it. The isozyme pattern of a given organ is rather stable and, in general, characteristic; this is important in human diagnostics when attempts are made to conclude to the injury of certain organs from the total LDH activity and isozyme pattern of the blood plasma. The procedure is mainly used in the diagnosis of injuries of the heart muscle and of the liver (Wroblewski et al. 1960; Cohen et al. 1966; Maklári et al. 1968) but may

give valuable help also in the diseases of the skeletal musculature (WIEME and HERPOL 1962).

In the present experiments the changes caused by limb tourniquet in the total-LDH activity and isozyme pattern of the rat blood serum were investigated at various intervals after removal of the tourniquet.

Methods

Inbred albino rats of both sexes weighing 120 to 160 g were used in the experiments. One of the hind limbs was compressed by means of a rubber ring applied as proximal as possible. In part of the experiments, compression was maintained for 90 minutes and in the rest for 4 hours. The animals were kept at room temperature throughout and bled at various intervals after removal of the tourniquet, and the total-LDH activity in the blood serum was determined by the UV spectrophotometric method of WROBLEWSKI and LADUE (1955). In the case of serum the results were expressed in terms of μM/ml/min, in the case of heart muscle homogenized in a phosphate buffer pH 7.4, in terms of μM/mg protein/min. Protein determination was performed according to BÜRGI et al. (1967).

The isozymes were separated by agar-gel electrophoresis on microscope slides (WIEME 1959). Running lasted 50 to 60 minutes, at 10 V/cm, in a veronal-sodium acetate buffer medium pH 8.6, at 0.05 ionic strength. For demonstrating the isozymes, the method of VAN DER HELM (1961) was used. After an incubation period of 60 minutes the slides were fixed in 5% acetic acid for 30 minutes, then left in distilled water overnight and finally dried at room temperature. The relative percentages of the isozymes were determined by means of an ERI 65 (Zeiss, Jena, GDR) type electrophoresis-evaluating apparatus. Knowing the isozyme values we calculated the percentage ratio of H and M subunits in the various samples according to the formula of THORLING and JENSEN (1966),

$$\text{M subunit } \% \frac{(\text{MMMM}) \cdot 4 + (\text{HMMM}) \cdot 3 + (\text{HHMM}) \cdot 2 + (\text{HHHM})}{4}$$

$$\text{H subunit}\% = 100 - \text{M}\%$$

Thereafter, the ratio of H/M was also calculated.

The kidneys were removed from the animals under ether anaesthesia.

Standard deviation (S. D.) was calculated for each group. In the case of the total activity, significance was computed according to Student's *t*-test.

In further experiments we used untreated animals, namely the hind limb musculature and cardiac muscle of rats, and the heart muscle and white and red muscle, respectively, of rabbits. After previous homogenization a 10% extract was prepared with physiological saline at room temperature. This was followed by centrifuging at low speed. The supernatant was separated and, after determining its isozyme pattern, it was injected into rats in a dose of 1 ml/100 g of body weight by the intraperitoneal route. At various intervals after the injection of the organ extract the animals were bled and LDH isozyme determination was made in the serum. No total-LDH determination was performed in these groups.

Results

Total-LDH activity. LDH activity considerably increased under the effect of a 90 min tourniquet. The increase reached the maximum three hours after removal of the tourniquet. This was then followed by a gradual diminution; however, after 48 hours the activity was still significantly higher than the control value. At 72 hours there was no significant difference from the control (Table I).

Table I

Effect of 90-min tourniquet on total-LDH activity and isozyme pattern of rat serum at various intervals after removal of the tourniquet

Group	Time of blood withdrawal after removal of tourniquet	Total-LDH activity, μM/ml/min	Relative percentages of LDH isozymes					Subunit H %	Subunit M %	Ratio of H/M
			LDH 1	LDH 2	LDH 3	LDH 4	LDH 5			
1	Control (7)	0.528±0.247	6.3±2.4	3.0±3.0	0—1	0—1	90.7±8.0	8.9	91.1	0.099±0.005
2.	3 hours (5)	1.825±0.238 $p < 0.001$	14.7±7.3	18.3±4.4	3.0±2.0	1.8±1.8	62.2±7.3	30.3±8.7	69.7±8.7	0.456±0.205
3.	24 hours (6)	0.948±0.136 $p < 0.01$	20.6±3.6	18.0±4.0	4.6±1.4	2.6±1.1	54.2±7.7	38.0±6.5	62.0±6.5	0.629±0.176
4.	48 hours (9)	0.897±0.164 $p < 0.01$	11.1±4.3	4.7±0.1	1.1±1.1	0—1	83.1±4.3	14.8±4.2	85.2±4.2	0.177±0.063
5.	72 hours (8)	0.772±0.188 $p > 0.05$	9.0±2.8	5.9±1.7	0—1	0—1	85.1±3.9	14.5±3.3	85.5±3.3	0.171±0.048

In brackets, number of animals

LDH-isozymes. One strong (LDH 5) and two weak (LDH 1 and LDH 2) bands could be demonstrated in the serum of control animals. This finding agreed with the observation of ALTLAND et al. (1964). LDH 3 and LDH 4 appeared occasionally only. Upon the effect of the tourniquet the LDH isozyme pattern underwent marked alterations as compared with the control. The most striking finding was an augmentation of the relative activity of LDH 1 and partly also of LDH 2. The maximum of these changes occurred 24 hours after removal of the tourniquet. At the same time the ratio of H/M increased. At 48 and 72 hours the alteration of the isozyme pattern was slight (Table I).

Effect of nephrectomy. It could be assumed that, in addition to a direct injury of the musculature, we have to count with the indirect damage of organs in which LDH 1 and LDH 2 dominate, such as the kidney and the heart. Therefore, in an experimental group bilateral nephrectomy was performed with a sham-operation in the corresponding control rats and the tourniquet was immediately applied after the intervention. Both the increase in total-LDH activity and the isozyme alterations were the same as in the control group.

Sham-operation + tourniquet (8): total-LDH activity = 0.933 ± 0.422 μM/ml/min; LDH 1 = 23.5 ± 7.1; LDH 2 = 21.2 ± 4.6; LDH 3 = 5.7 ± 1.1; LDH 4 = 7.2 ± 4.1; LDH 5 = 42.4 ± 9.0

Nephrectomy + tourniquet (7): total-LDH activity = 1.255 ± 0.545 μM/ml/min; LDH 1 = 21.9 ± 5.6; LDH 2 = 21.8 ± 4.4; LDH 3 = 9.2 ± 2.0; LDH 4 = 8.0 ± 3.4; LDH 5 = 39.1 ± 5.1

LDH activity in heart muscle. As to the occasional role of the heart we have but indirect data. When we determined the myocardial total-LDH activity and isozyme pattern 24 hours after the tourniquet we obtained a slight, statistically non significant diminution of total-LDH activity while the isozyme pattern and the ratio of H/M remained practically unchanged as compared with the control.

Effect of rat muscle and heart extracts. In a further series of experiments completely intact animals were bled and those limb muscles were excised which anatomically corresponded to the muscles situated in the region under compression. The serum isozyme pattern of animals bled 3 and 24 hours after the injection of the extract prepared in the above way showed practically the alterations as after the tourniquet. At 48 hours there was hardly any difference from the control values. Three hours after the injection of the rat heart extract the fractions LDH 1 and LDH 2 were in prevalence. The relative percentage of the two fractions was roughly the same. 24 hours later, LDH 2 markedly diminished while LDH 1 remained high. The plus activity of LDH 1 persisted after 48 hours (Table II).

Effect of rabbit muscle and heart extracts. Three hours after the intraperitoneal injection of an extract made from the white muscle taken from the lumbal region of the rabbit a new band appeared between LDH 4 and LDH 5 in the rat serum (Fig. 1). This rabbit muscle LDH 5 which showed an R_f differ-

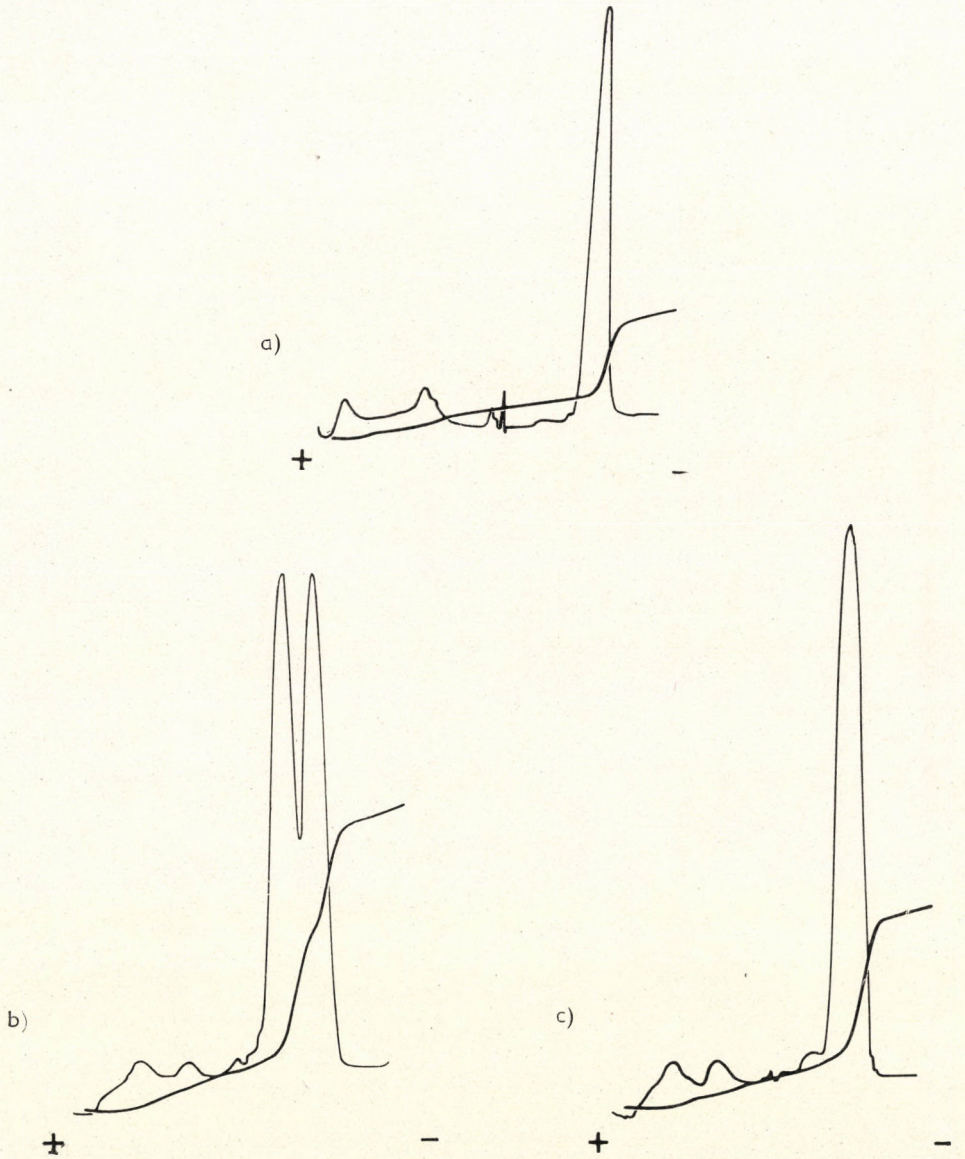

Fig. 1. a) LDH isozyme pattern of untreated rat serum. b) LDH isozyme pattern of rat serum 3 hours and c) 24 hours after the intraperitoneal injection of rabbit white muscle extract. The figures show the extinction curves and the integration curves

Table II

Effect of rat skeletal and heart muscle extracts on relative activity of LDH 1 and LDH 2 isozymes in serum, at various intervals after the injection

Treatment		3 hours	24 hours	48 hours
Limb muscle extract (10%), 1.0 ml/100 g, intraperitoneally (9)	LDH 1	25.0%	17.3%	11.7%
	LDH 2	31.7%	5.0%	6.7%
Heart muscle extract (10%), 1.0 ml/100 g, intraperitoneally (9)	LDH 1	38.3%	34.0%	19.0%
	LDH 2	45.0%	13.0%	9.6%

In brackets, number of animals

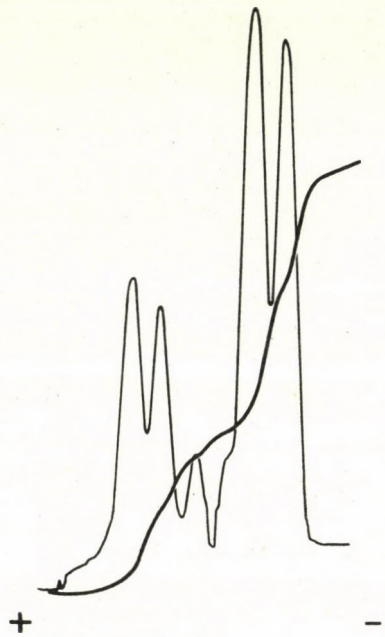

Fig. 2. LDH isozyme pattern of rat serum 3 hours after the intraperitoneal injection of rabbit red muscle extract. The figure shows the extinction curve and the integration curve

ing from that of the rat could not be demonstrated after 24 hours (Fig. 1), while LDH 1 and LDH 2 activities were unaltered. After the injection of rabbit red muscle extract, LDH 1 and LDH 2 activities were augmented (Fig. 2). After the injection of rabbit heart muscle extract (containing practically only LDH 1) the LDH 1 characteristic of rabbit situated between rat LDH 1 and LDH 2 could be demonstrated in the rat serum even after 48 hours.

Discussion

In humans, limb compression for a few minutes is already capable of eliciting a well-measurable augmentation of serum LDH activity (HESS and RAFTOPOULO 1957; STARKWEATHER et al. 1966). The changes found by us in total-LDH activity and isozyme pattern can be accounted for by the tourniquet-induced anoxia of the muscle. As a consequence of the anoxia, part of the LDH of low molecular weight enters the blood stream, causing thereby a well-measurable increase in activity, the time-course of which can easily be followed. The maximum augmentation of total-LDH activity occurred three hours following removal of the tourniquet. Then came a gradual decline but after 48 hours the value was still significantly above the control. Of the isozymes LDH 1 showed the peak 24 hours after the removal of the tourniquet, and an increase persisted after 48 hours, too, while the 72-hour values were practically normal. We originally expected the LDH released from the muscle primarily to increase the activity of the slow-running isozyme fraction of the serum, since skeletal muscles are mainly characterized by the presence of such isozymes. In contrast, it was the activity of the fast-running LDH 1 and LDH 2 which exhibited a relative increase. This was the reason why we thought that, in addition to the muscle damage, we had to count with the reactive injury of such an organ in which mainly LDH 1 and LDH 2 dominate, such as the kidney and the heart. Since, however, we obtained the same post-tourniquet LDH isozyme changes in nephrectomized animals as in normal ones, the kidney could not be the source of the increased LDH 1 and LDH 2 activities.

On the basis of our data for the heart muscle the possibility of its participation in the events could neither be supported nor excluded.

As known, skeletal muscles can be divided into white and red muscles, and a mixed type containing both red and white fibres. In white muscle, LDH 5 dominates and the activity of all other LDH isozymes is low. On the contrary, red muscles exhibit not only LDH 5 but also high LDH 1 and LDH 2 activities (BLANCHAER and VAN WIJKE 1962). The difference between the two types of muscle is caused by differences in their metabolism, the red muscle being characterized mainly by aerobic and white muscle by anaerobic metabolism (WILSON et al. 1963). The extract of rat limb muscles containing mainly mixed fibres induced upon intraperitoneal injection to rats, a plasma isozyme pattern similar to that seen after the application of a tourniquet. On the other hand, the injection of white muscle failed to increae LDH 1 and LDH 2 activity. This proves clearly that after the injection of the muscle extract, the isozymes present in the fibres had appeared in the blood since, in the case of a mere reactive action, the white muscle extract, too, should have had the same effect. On this basis it is justified to assume that the isozymes released from the limb musculature of the rat could by themselves be responsible for the post-tourni-

quet alterations. According to some authors, the increased enzyme activity in the serum may originate from many different sources simultaneously (HAUSS et al. 1958; RILEY et al. 1963); our data neither corroborated nor excluded this assumption.

The fact that the post-tourniquet enzymic changes persisted for a certain time, can be explained in various ways. One possibility is that the fast efflux

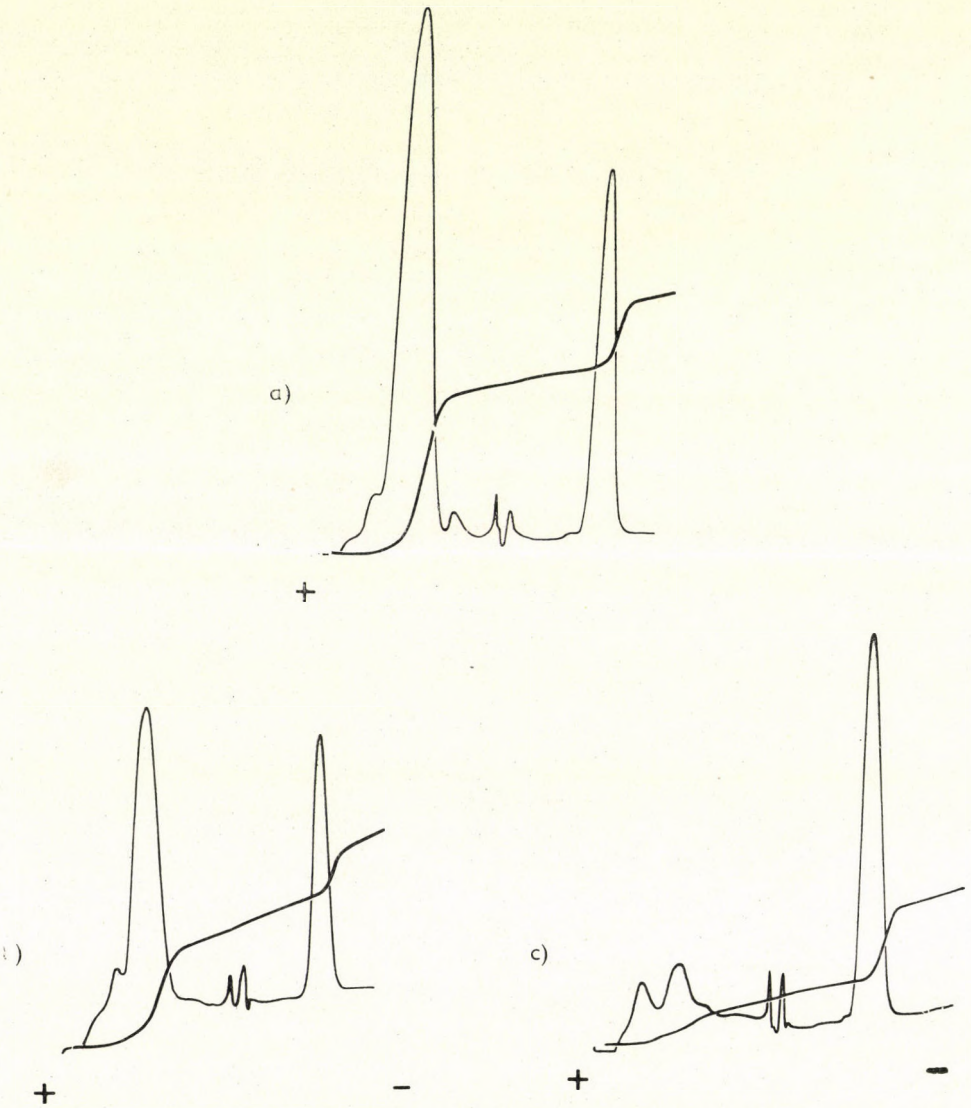

Fig. 3. LDH isozyme pattern of rat serum. a) 3 hours; b) 24 hours; c) 48 hours, after the intraperitoneal injection of rabbit heart muscle extract. The figures show the extinction curves and the integration curves

after tourniquet release is followed by a slow leaking lasting until complete restitution. It is also possible that the isozymes remain in the serum for a long time. This latter mechanism is certainly to be taken into account, since in the blood LDH 1 activity was elevated even 48 hours following the administration of rat or rabbit heart extract. LDH 2 could not be demonstrated in the serum after 24 hours, and the same was the case with LDH 5. Thus, the elimination of these fractions was more rapid than that of LDH 1. This phenomenon may undoubtedly play a part in the finding that the relative percentage ratio of LDH 1 and H-subunits was the highest 24 hours after the removal of the tourniquet and was still well measurable 48 hours later. BOYD (1966) observed in lambs an uneven elimination rate of the various LDH isozymes; elimination of LDH 5 was the fastest and that of LDH 1 the slowest. MAHY and ROWSON (1965) also observed differences in the elimination of LDH 1 and LDH 5 in the mouse. DAWSON et al. (1969) investigated human muscle necrosis but failed to demonstrate in the serum the LDH isozymes characteristic of muscle, presumably because of the rapid elimination of these fractions. The differences in the elimination of LDH 1 and LDH 5 isozymes may be explained by their different sensitivity to physical and other influences: for instance, LDH 1 is heat-stable while LDH 5 is heat-labile, and in vitro LDH 1 proved to be resistant to bacterial peptidase while LDH 5 did not (VESSEL and YIELDING 1968).

Our experiments support DAWSON's (1966) view according to which the serum enzyme pattern is determined by the enzyme activity of the organ injured, the normal enzyme activity of the serum and by the elimination rate of the enzymes in question.

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HEPATIC TRANSPORT OF IODIPAMIDE IN VARIOUS ANIMAL SPECIES

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In anaesthetized animals the hepatic transfer maximum (Tm) of iodipamide was determined by the single-injection method. It was found that the Tm was 0.23 mg/min/kg in the cat, 0.84 in the dog, 1.48 in the rat, 2.61 in the rabbit and 2.89 mg/min/kg in the guinea pig. On increasing the dose, the iodipamide concentration of bile tended to reach a maximum, lowest in the cat and guinea pig (25 mg/ml), and highest in the dog (56 mg/ml). The Tm mainly changed parallel with the bile producing capacity of the liver of the various species in the first place: cat, 9 μl/min/kg; dog, 14; rat, 38; rabbit, 72; guinea pig, 115 μl/min/kg. To obtain the Tm, iodipamide must be injected intravenously in doses as high as 400 to 800 mg/kg. This is explained, among others, by the fact that 86% of the iodipamide is retained outside the liver and to ensure maximum transfer, the hepatic concentration of iodipamide must reach 1 mg/g.

Iodipamide [N,N-adipyl-bis-(3-amino-2,4,6-triiodobenzoic acid)] is one of the most commonly used materials in intravenous cholecystography (FROMMHOLD and BRABAND 1960; KNOEFEL 1965). The concentration of iodipamide in bile may be 30 to 100 times that of plasma (LANGECKER et al. 1953) indicating that it is excreted into the bile by active transport. The process of hepatic transfer of iodipamide and of the other media used for cholecystography is not fully elucidated; the pertinent literature has been surveyed in detail by HECHT (1939), KNOEFEL (1965), WILLIAMS et al. (1965), as well as SMITH (1966). Iodipamide is of low toxicity, the intravenous LD_{50} being 3.8 g/kg in the rat and 1.9 g/kg in the dog (LANGECKER et al. 1964). In the dog's bile iodipamide may reach a concentration as high as 40 mMol/l and in the organism it is only slightly metabolized (LANGECKER et al. 1953 and 1964). These properties make iodipamide highly suitable for studies concerned with the hepatic elimination of materials alien to the organism.

We have determined in some animal species the iodipamide transfer maximum (Tm) of the liver and made an attempt at explaining the observed differences.

Methods

Rats of our own stock, weighing 200 to 280 g, guinea pigs weighing 500 to 700 g, Chinchilla rabbits weighing 2.2 to 3.5 kg, cats weighing 1.8 to 2.7 kg, and mongrel dogs weighing 8.5 to 17.5 kg were used. Anaesthesia was induced by the intraperitoneal injection of 1.2 g/kg of urethane in rats, guinea pigs and rabbits, and by administration through the same route of an allobarbital-urethane mixture (60 mg/kg and 240 mg/kg, respectively) in cats and dogs. The abdomen was opened by an upper median incision, and a polyethylene cannula was fixed

in the common duct; the cystic duct was ligated in the guinea pigs, rabbits, cats and dogs. Bile was collected in 20-minute periods. Iodipamide (Biligrafin®, Schering AG, Berlin) was administered as a single injection into the femoral vein after one or two control periods. Bile collection was continued after administration for two hours in the case of rats, rabbits and guinea pigs and for three hours in that of cats and dogs. Every bile fraction was tested for iodipamide concentration and the group means were calculated from the highest 1-hour values. The highest excretion values were obtained almost invariably in the second, third and fourth periods in the case of rabbits, and in the sixth, seventh and eighth periods in that of cats. At the end of the experiments, the liver was weighed.

Rectal temperature was recorded continuously and the animals were warmed if necessary to prevent body temperature from falling below 36°C (as measured rectally). In the dogs, blood pressure was recorded from the cannulated carotid artery by means of a mercury-manometer.

Iodipamide concentration in plasma, bile, urine and liver was determined according to LANGECKER et al. (1953), on the basis of the iodine content. The dose, and the plasma, bile and liver concentrations of iodipamide were expressed in terms of iopanoic acid.

Plasma volume was determined by the Evans blue dilution method (BÁLINT 1957); the volume for ten rats weighing 200 to 230 g proved to be 38.0 ± 3.2 ml/kg, and for dogs 51.6 ± 2.9 ml/kg ($n = 8$).

In the text, Tables and Figures the mean values and the standard deviations are shown, except in Fig. 3, where the standard error is presented. Significance was calculated by the *t*-test.

Results

Rat

In the dose range 50 to 400 mg/kg, iodipamide excretion increased with the dose, tending to reach a maximum (Fig. 1). There was no significant difference in the amounts eliminated following administration of 400 and 800

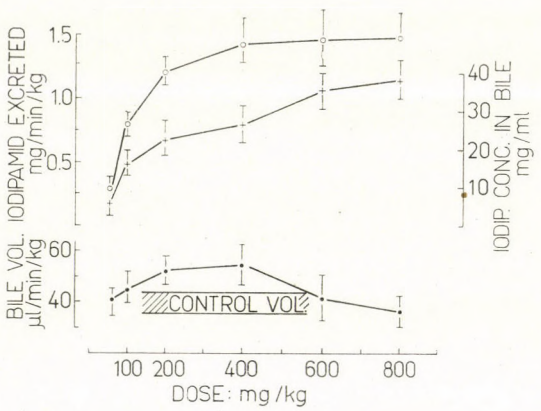

Fig. 1. Biliary excretion of iodipamide in the rat, in response to different doses. ●——● = bile volume, +——+ = biliary concentration, ○——○ = amount excreted. Every point represents the mean of values obtained in 7 to 10 animals

mg/kg, respectively. Three groups of four rats each were given 1.0, 1.2 and 1.5 g/kg iodipamide, respectively. The concentration of iodipamide in bile did not increase over 40 mg/ml, while bile flow decreased below the control level, therefore the amounts excreted (1.25, 1.31 and 1.02 mg/min/kg, respectively) were smaller than following the 800 mg/kg dose. It was attempted to enhance

iodipamide excretion by administering it after a priming dose of 600 mg/kg in the form of infusion at a rate of 3 mg/min/kg over a 2-hour period. In the period showing maximum excretion the iodipamide concentration of bile was 37.3 ± 2.7 mg/ml, and the excreted amount was 1.65 ± 0.25 mg/min/kg (= 10). These values were not significantly different from those obtained in response to a 800 mg/kg dose ($p > 0.05$).

In the next step attempts were made to estimate iodipamide concentration in liver and plasma at the time of maximum excretion. At 40 or 60 minutes following injection the animals were exsanguinated and the liver, bile and plasma were assayed for iodipamide concentration. As shown in Table I, the

Table I

Liver and plasma concentrations and biliary excretion of iodipamide in rats after administration of the drug

Dose mg/kg i.v.	Number of animals	Time (min)	Iodipamide concentration μg/g		Iodipamide excretion mg/min/kg
			Liver	Plasma	
200	5	40	765±152*	320± 75	0.99±0.18**
400	4	40	1180±210	524± 61	1.45±0.22
	5	60	1090±156	470± 55	1.47±0.15
800	5	40	2480±264	1702±165	1.35±0.23
	5	60	1990±188	1425±193	1.62±0.30

* Mean value ± standard deviation
** Iodipamide excreted in 20 minutes before sacrifice

concentration in plasma and liver increased parallel with the dose. As judged from the iodipamide excretion value estimated 20 minutes before exsanguination, a dose of 200 mg/kg was too low to produce maximum excretion. At the point of time tested there was 765 μg/g iodipamide in the liver. Thus, to make the hepatic excretory system to work at full capacity, the liver must contain at least 1.0 mg/g of iodipamide. However, the liver did not excrete more iodipamide even at twice that concentration (2.48 mg/g).

The animals treated with a 400 mg/kg dose and exsanguinated at 60 minutes excreted an average of 16.8 mg iodipamide in the bile and 1.1 mg in the urine. The average weight was 224 g and the liver weight averaged 9.18 g. Assuming that the plasma volume was 8.51 ml (38.0 ml/kg), the percentage distribution of iodipamide in plasma, liver and other organs could be calculated. The results indicate that of the iodipamide remaining in the organism 14.0 ± 3.2% were in the liver, 5.5 ± 1.1% in the plasma, and 80.5 ± 6.0% in other organs. Although most of the iodipamide is excreted with the bile, only a small fraction was in the liver, and 86% outside that organ.

Guinea pig

In response to the 600 mg/kg dose, iodipamide excretion rate was 2.72 ± 0.44 mg/min/kg (n = 5), and after a 800 mg/kg dose it was 2.89 ± 0.57 mg/min/kg (n = 10). Of the five species examined the guinea pig showed the highest rate of bile production and iodipamide transfer and the lowest biliary iodipamide concentration (Table II).

Table II

Biliary excretion of iodipamide in various species following the intravenous administration of a single dose of 800 mg/kg iodipamide

Species	Number of animals	Liver weight g/kg	Bile flow µl/min/kg	Iodipamide	
				conc. in bile mg/ml	excreted mg/min/kg
Cat	8	30.1±5.9*	9.1±4.2	25.6±7.9	0.23±0.14
Dog	6	24.0±4.6	14.9±2.0	56.8±6.6	0.84±0.09
Rat	10	40.1±3.9	38.0±6.1	39.1±5.1	1.48±0.18
Rabbit	6	29.9±5.7	72.3±8.5	36.1±3.7	2.61±0.45
Guinea pig	10	32.6±4.5	115±13	25.2±3.5	2.89±0.57

* Mean value ± standard deviation

Rabbit

The results are presented in Fig. 2. Maximum iodipamide excretion (2.61 ± 0.45 mg/min/kg) was recored after the 800 mg/kg dose. In five rabbits the dose was 1.2 g/kg; in these animals iodipamide excretion amounted to 2.48 ± 0.36 mg/min/kg. In four animals treated with 1.5 g/kg, excretion continued to decrease (2.02 ± 0.20 mg/min/kg).

Cat

In response to 400 mg/kg iodipamide, excretion was 0.19 ± 0.09 mg/min/kg (n = 4); to the 800 mg/kg dose it was 0.23 ± 0.14 mg/min/kg (n = 8). The 1.2 g/kg dose was toxic. There were significant individual differences in Tm (Table II). This was due in the first place to the varying bile volume and in the second place only to the iodipamide concentration in the bile of individual animals. Unlike other species, cats showed maximum iodipamide excretion as late as 2 or 3 hours after administration. This was explained by the low rate of bile production in the cat, as well as by the fact that the dead space of the biliary tree is about equal to the volume of bile produced in 1 hour.

Dog

During the 3.5 to 4.0 hours of the experiment, blood pressure varied from 110 to 160 mm Hg, respiration was steady, there was no need of artificial breathing. The 800 mg/kg dose induced no toxic symptoms.

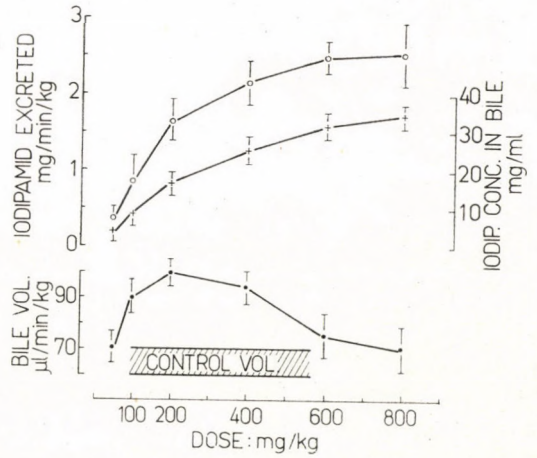

Fig. 2. Biliary excretion of iodipamide in the rabbit, in response to different doses. Signs as in Fig. 1. Each point represents the mean for 5 to 6 animals

Fig. 3 shows iodipamide excretion plotted against time. Maximum excretion usually occurred in the periods 2, 3 or 4. Subsequently, iodipamide excretion decreased rapidly, faster after the lower doses than after the high ones. After a 800 mg/kg dose, excretion rate was continuously high for about two hours, then, with the iodipamide concentration unchanged, bile volume gradually decreased.

The mean values for periods 2, 3 and 4 are shown in Fig. 4. Like in the rat and rabbit, in the dog, too, there was a tendency to reach a maximum both as regards iodipamide concentration in bile and the amount of iodipamide excreted.

Three dogs each were given a 1.2 g/kg dose. Their blood pressure decreased step by step in the following hours. One dog died 2 1/2 hours after injection. In every one of these dogs biliary excretion was lower than the means found in response to the 400 and 800 mg/kg doses.

After a priming dose of 600 mg/kg followed by infusion at a rate of 2 mg/min/kg over 3 hours ($n = 3$), biliary iodipamide concentration was 45.0 ± 4.7 mg/ml, and the maximum excretion rate reached 0.77 ± 0.14 mg/min/kg; this was not significantly different from the 0.84 ± 0.09 mg/min/kg value obtained in response to a 800 mg/kg dose ($p > 0.05$).

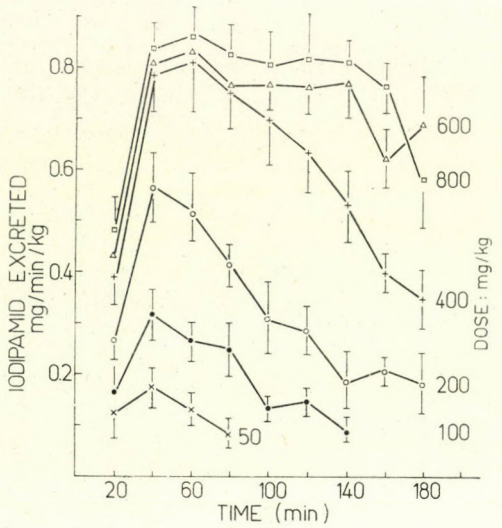

Fig. 3. Biliary excretion of iodipamide in the dog, in response to different doses, plotted against time. The curves represent the means obtained in 4 dogs (50 to 200 mg/kg) and 6 dogs (400 to 800 mg/kg), respectively

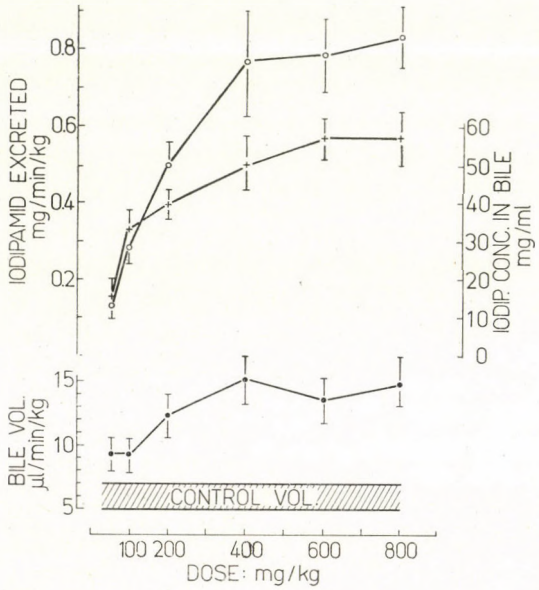

Fig. 4. Biliary excretion of iodipamide in the dog, in response to different doses. ●——● = bile volume, +——+ = biliary concentration, ○——○ = amount excreted. Each point represents the mean obtained in 4 to 6 animals

Four dogs each were treated with 600 mg/kg iodipamide. Two hours later they were exsanguinated and their plasma, liver, bile and urine collected during the experiment were tested for iodipamide concentration. During the second hour following administration the mean values were: excretion with bile, 0.758 ± 0.065 mg/min/kg; plasma level, 1.4 ± 0.25 mg/ml; liver concentration, 1.95 ± 0.2 mg/g. Iodipamide distribution was calculated in a fashion similar to that applied in the rats. In two hours, 13.4 ± 2.1% of the administered dose was excreted with bile; 10.3 ± 2.8% with the urine; 11.3 ± 2.0% were in the plasma and 7.5% in the liver. Thus, 23.7% of the iodipamide administered left the organism, and 13.0% of the remaining amount was in the liver at the time of maximal excretion.

Discussion

In studies concerned with the hepatic transfer of rose bengal in dogs it was found that at the time of maximum excretion 30 to 40% of the dye was in the liver (VARGA and FISCHER 1970). The present data showed that of the amount of iodipamide remaining in the organism only 14% were in the liver at the time of maximum excretion. To attain maximum excretion in the rat and dog, the liver had to contain at least 1 mg/g of iodipamide. Apart from that, the Tm of iodipamide is much higher than that of the other drugs excreted with bile. These three factors explain why so high doses of iodipamide had to be given to attain the Tm. As about 86% of the iodipamide was localized outside the liver, there was a copious store behind this organ, from where supplies could arrive continuously to replace the excreted amounts of iodipamide leaving the liver. In this way high and lasting excretion is provided for (see in Fig. 3 the 600 and 800 mg/kg doses) as high and lasting as cannot be observed in the case of other substances excreted with the bile.

In response to doses exceeding 800 mg/kg, iodipamide excretion decreased slightly but gradually in the rats, rabbits and dogs. In the dog this was due to a toxic effect. The rats and rabbits tolerated well the doses of 1.0 to 1.5 g/kg, there were no losses due to high dosage. The diminution of excretion following overdosage was presumably due to an inhibition of bile production by the high hepatic iodipamide concentration. A similar phenomenon has been observed in the case of indocyanine green excretion (KETTERER et al. 1960; KLAASSEN and PLAA 1969).

In every species other than the rat, the amount excreted in response to a 800 mg/kg dose corresponded to the hepatic Tm of iodipamide. In this respect there were significant differences between the various species (Table II), which could be explained neither by the liver mass per kg body weight, nor by the hepatic iodipamide concentration. The dog's liver contained iodipamide at a

higher concentration than did the rat's liver, but Tm in the dog was lower than that in the rat. The biliary concentration of the substances excreted by active transport tends to reach a maximum (COOK et al. 1952; SPERBER 1959; SMITH 1966). This has been observed also in the case of iodipamide excretion. The various species unquestionably differed in their maximum biliary iodipamide concentration. However, the iodipamide Tm in the various species is not determined primarily by the peak level but by the bile producing capacity of the liver.

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BILIARY SECRETION OF ROSE BENGAL IN THE DOG

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Rose bengal (RB) was infused to anaesthetized dogs in doses between 0.1 and 1.2 mg/kg/min for three hours. The changes in RB concentration in plasma, bile, liver and some other organs were investigated. It was established that 35.8% of the RB administered could be found in the liver after the infusion of a dose of 0.1 mg/kg/min but only 6.4% in the case of an infusion rate of 1.2 mg/kg/min. The reason for this is the liver's limited RB-storage capacity which amounts to 571 μg/g. RB concentration in kidney, small intestine and muscle increased proportionally with increasing doses. When the transport maximum (Tm) of RB (26.4 μg/kg/min) is reached, the liver's storage capacity is saturated to half. RB considerably decreased bile flow, a finding to be accounted for probably by the inhibition of active secretory function.

Drug excretion by the liver involves three steps, viz. intracellular accumulation, biotransformation, and transfer into the biliary capillaries. These processes are interconnected, and at present we cannot yet exactly determine the relative significance of these factors in the process of secretion.

MENDELOFF (1949) as well as TAPLIN et al. (1955) demonstrated rose bengal (RB) to accumulate in the parenchymal cells of the liver and to be secreted with bile through an active transport process. This is why RB is used in clinical practice for checking liver function. Unlike bromosulphophthalein, RB is metabolized but to a small extent (JIRSA and RABAN 1962); thus, its secretion only depends on its intracellular accumulation and on the activity of the secretory system. Thus RB seems suitable for studying the relationship between the storage capacity of the liver on the one hand and the transfer process on the other. The present paper describes investigations into this direction.

Methods

Mongrel dogs of both sexes weighing 8 to 18 kg were used. They were fasted 16 to 18 hours prior to the experiment, with water given ad libitum. Under allobarbital-urethane anaesthesia (60 mg/kg, and 240 mg/kg, respectively, by the intraperitoneal route) a median laparotomy was performed, the cystic duct ligated, the common bile duct cannulated with a polyethylene tube, and the bile collected in 20 min periods. After one or two control periods, RB infusion was started. RB (rose bengal, Gurr, London) was dissolved in physiological saline and infused by means of an infusion pump for three hours. The total volume of solution given during the experiments lay between 2.4 and 5.4% of the animal's weight. Blood samples were taken from the femoral artery at 20 min intervals. Tissue samples from the liver (0.5 to 1 g) were excised 60 and 120 minutes after starting the infusion. Bleeding was controlled with

gelatin sponge. After the experiment the animals were bled and RB concentration in the various organs was determined.

In some experiments, plasma volume was determined with Evans blue according to BÁLINT (1957); the value for eight dogs proved to be 48.5±3 ml/kg.

Blood pressure was measured in the carotid artery by means of a mercury manometer.

Determination of RB. Plasma and bile were diluted with physiological saline, and absorption was estimated spectrophotometrically at 540 mμ. Tissue samples were rinsed in order to remove blood, blotted with filter paper and weighed. Skin, muscle and small intestine were cut into pieces with a razor blade before homogenization. Homogenization was carried out in 70% ethanol using an "Ultra-Turrax" apparatus (Janke and Kunkel, Staufen). This was followed by centrifugation at 5000 for 15 min. The supernatant was collected, the residue re-homogenized in 70% ethanol and centrifuged again. The two supernatants were combined and diluted if necessary. The standard RB solution used for determination of tissue RB concentration was prepared in 70% ethanol. Using the above procedure we succeeded in recovering 96% of 100 to 500 μg of RB added to 5 ml of 20% liver homogenate in water.

Blood content of liver samples was determined according to the method of HOLZER et al. (1956).

Determination of RB binding to plasma proteins. 0.5 ml plasma was put on a Sephadex-25 (Molselect G-25; Reanal, Budapest) gel column — 1.2×18 cm — sorbed with physiological saline containing phosphate buffer (0.05 M, pH 7.4). Elution was made with buffered saline, and the eluates were collected in 2 ml fractions. The protein-containing fractions were identified by means of precipitation with trichloroacetic acid, adding 0.1 ml from each tube to 1 ml of 20% trichloroacetic acid. Knowing the RB concentration of the plasma and the amount of RB in the protein-containing fractions we calculated the percentage ratio of the protein-binding of RB.

The capacity of the biliary tree was determined according to the method of BARBER-RILEY (1965).

Results

The 4—4.5-hour experimental procedure was tolerated well by the animals; their blood pressure was between 100 and 160 mm Hg, with regular respiration and without any need for artificial breathing. In two dogs which had received RB at a rate of 1.2 mg/kg/min, blood pressure fell under 100 mm Hg in the second hour. Results obtained in these two animals have not been included into the tables.

Table I shows the tissue and plasma concentration of RB at the end of the 3-hour infusion. Dye concentration in the plasma increased with increasing doses. Gel-filtration revealed more than 95% of RB to be bound to plasma protein at both low and high concentrations. Of the organs investigated, it was the liver which exhibited the highest RB level. However, RB concentration in the liver did not increase proportionally to the dose administered, the ratio of liver concentration to plasma concentration being 3.87 at a dose of 0.1 mg/kg/min, but only 0.44 at an infusion rate of 1.2 mg/kg/min. In other organs the ratio tissue concentration/plasma concentration was relatively constant. The plasma RB level was high as compared with that in muscle or skin. In these two organs some 1.5 to 2.5% of blood always remain after complete bleeding; for this reason the muscle and skin RB concentrations shown in Table I are higher than the real values. After the liver, the highest RB concentrations were found in the kidney and the small intestine. When a dose of 1.2 mg/kg/min was given for 4 hours (n = 2) the RB level in these two organs

Table I

Tissue concentration of rose bengal in dogs after 3-hour infusion of the drug

Dose mg/kg/min	Number of animals	Tissue concentration (μg/g)*						
		Tissue/plasma concentration ratio						
		Plasma	Liver	Kidney	Muscle	Intestine	Skin	Fat
0.1	3	74±9.0	287±61 3.87	26±6 0.35	< 5	21±8 0.28	< 5	< 5
0.2	6	302±45	448±101 1.47	85±29 0.28	14±2.8 0.05	70±23 0.23	14±3 0.05	20±6 0.07
0.6	4	789±58	526±83 0.66	232±40 0.29	32±2.5 0.03	185±42 0.23	32±3 0.03	49±19 0.06
1.2	3	1360±36	597±73 0.44	430±44 0.31	55±14 0.04	332±18 0.24	55±14 0.04	118±51 0.09

* Mean value ± standard deviation. Values are based on wet weight of tissue

Table II

Percentage of total dose of rose bengal in plasma, liver and bile

Dose mg/kg/min	Number of animals	Percentage of total dose*								
		Plasma			Liver			Bile		
		1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
0.1	3	42.1±6.1	35.6±2.0	41.6±5.2	54.5±2.0	31.4±4.4	35.8±8.7	4.0±1.3	9.7±5.4	9.1±5.1
0.2	6	42.3±8.2	35.7±7.6	40.9±5.3	31.8±6.5	34.0±12	27.1±7.8	3.7±2.8	7.8±3.8	9.0±3.2
0.6	4	39.9±8.4	42.6±7.0	36.5±1.6	22.4±5.7	17.3±7.3	11.2±3.4	1.7±0.7	2.4±0.8	2.1±1.0
1.2	3	41.8±5.2	38.0±5.3	35.6±1.4	14.9±1.3	9.6±1.6	6.4±1.6	1.1±0.3	1.68±0.5	1.47±0.6

* Mean value ± standard deviation

approached that in the liver. The amount of RB in urine did not reach more than 0.05% of the total dose in this case either.

Table II shows the percentage of the administered dose found in plasma, liver and bile, 60,.120 and 180 min after starting the infusion. The plasma contained roughly the same percentage (35.6 to 42.6%) of the dose administered after the infusion of both 0.1 mg/kg/min and 1.2 mg/kg/min. With increasing doses the liver contained less and less of the administered amount. Similar results have been obtained concerning the biliary secretion of RB. After a 3-hour infusion of RB at a rate of 0.1 mg/kg/min, 44. 9% of the total administered dose was found in the liver and in the bile secreted during the experimental period; after an infusion at a rate of 1.2 mg/kg/min the value was only 7.87%. These findings suggest that both the RB storage capacity and the RB secreting capacity of the liver are limited.

Table III shows bile flow, biliary concentration of RB and the amount of RB secreted during the experimental period. These data show the mean values for the 1-hour period between 80 and 140 min after the infusion had

Table III

Biliary excretion of rose bengal in dogs

Dose mg/kg/min	Number of animals	Bile flow µl/kg/min	Bile RB level mg/m	Excreted RB µg/kg/min
0.1	3	3.18±0.98*	2.90±0.27	9.24±1.9
0.2	6	2.77±0.50	9.54±1.10	26.4±2.8
0.6	4	2.50±0.52	9.00±0.85	22.5±2.3
1.2	3	2.46±0.17	9.12±1.13	22.6±3.1

* Mean value (± S.D.) for period between 80—140 min

been started; this was the period of time when the maximum secretion occurred. The transfer maximum (Tm) of RB amounted to 26.4 µg/kg/min. This could be reached by a dose of 0.2 mg/kg/min and a further increase in the dose did not essentially alter either the biliary concentration of RB or the bile flow.

When determining the storage capacity of the liver one has to take into account the blood and bile content of the liver specimen. The average amount of bile per one g of liver was 3.1 µl, while the total capacity of the biliary tree, 1.06 ml (n = 7). From the haematocrit and the blood content of the liver one can calculate the blood plasma content of the liver specimen. One g of liver tissue contained an average of 32 µl of plasma (n = 7). Subtracting the amount of RB in blood plasma and bile from the amount actually measured in the liver we obtain the corrected RB concentration per one g of liver; for instance, after infusing 1.2 mg/kg/min of RB, a corrected (intracellular) liver RB con-

centration of 529 μg/g instead of the actually measured 578 μg/g was found (Table I). Calculation from the data corrected in the above way according to Lineweaver-Burk's formula (DAWES 1956) gives 571 μg/g as the liver's RB storage capacity (Fig. 1).

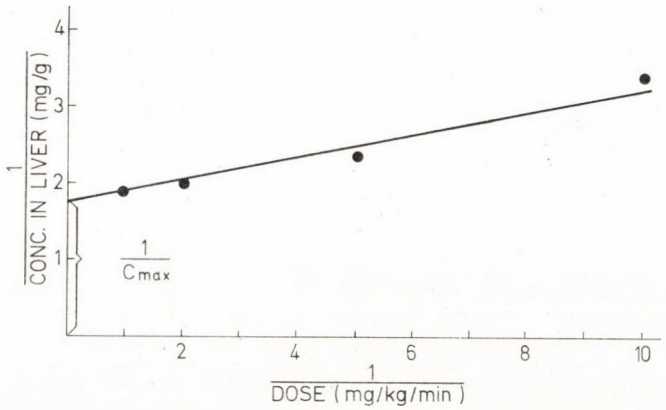

Fig. 1. Determination of the liver's rose bengal storage capacity (C_{max}) according to the graphic method of Lineweaver and Burk. $1/C_{max} = 1.75$; $C_{max} = 0.571$ mg/g

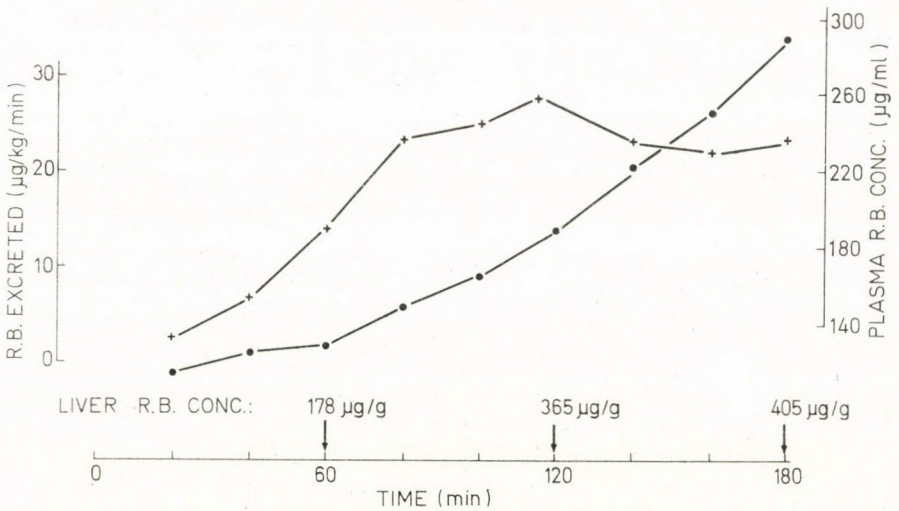

Fig. 2. Plasma level (●——●) and liver concentration of rose bengal as well as amount of rose bengal secreted with bile (+——+) in a female dog (14.6 kg) receiving rose bengal infusion of 0.2 mg/kg/min

The above data supply information concerning the relationship between the liver's RB storage capacity and the Tm of RB. Fig. 2 shows the results obtained in a dog which received 0.2 mg/kg/min of RB. RB secretion reached the maximum in the 6th period (100—120 min). Considering the bile flow

(35 μl/min between 80 and 120 min) and the capacity of the biliary tree (1.1 ml), it is evident that this value shows the cell-secretory activity corresponding to a time some 30 min earlier (80th min). At this point of time the RB level in the liver was between 178 and 365 μg/g and averaged some 240 μg/g. Interpolation of the data of all five dogs receiving RB in a dose of 0.2 mg/kg/min yielded in mean value 280 μg/g. Thus, the Tm seems to be reached at about half-saturation of the liver's RB storage.

Discussion

In a previous paper it has been shown that the distribution of chloroquine in the organism depends on the dose used (VARGA 1968). According to the present data this statement is valid for RB, too. In the case of the infusion of 0.1 mg/kg/min of RB, 35.8% of the dose administered were in the liver, while only 6.4% in the case of infusion of 1.2 mg/kg/min. In other organs the concentration of RB increased in proportion with the increasing dose, and no sign of saturation could be seen.

It is known from the investigations of LOKEN et al. (1960) that the calcium-binding capacity of human plasma proteins is 0.1 mM/g of protein. One molecule of bovine albumin can bind a maximum of 5 molecules of thiopental (YOSHIKAWA and LOEHNING 1965). It can be assumed that the capacity of the liver to store foreign organic compounds is not inexhaustible. Much work has been devoted to the study of the liver's bromsulphophthalein storage capacity (see the review of HARGREAVES 1968). *In vitro*, rat liver slices can bind a maximum of 8 mg per g of bromsulphophthalein (BRAUER and PESOTTI 1949), while under *in vivo* conditions, determination of the bromsulphophthalein storage capacity of the liver has not yet been achieved. In our experiments, the bromsulphophthalein concentration of the liver continued to increase even after a 3-hour infusion of 3 mg/kg/min of bromsulphophthalein, though at this time the liver already contained more than 1.5 mg/g of the compound (unpublished data). To our best knowledge, RB is the first material the hepatic storage of which could be determined quantitatively also *in vivo*. This value was 571 μg/g. The data at disposal do not allow to answer the question whether the intracellular binding sites for RB or its uptake into hepatic cells or perhaps some other factors determine the RB storage capacity of the liver.

In the dog, the Tm of RB is much lower than that of bromsulphophthalein, and is of a similar order of magnitude as the Tm of indocyanine green (32 μg/kg/min in our own experiments). RB has yet another characteristic common with the latter dye: it markedly inhibits bile flow. In control periods prior to the infusion of RB, bile flow was 6.23 μl/kg/min (n = 18). A dose as low as 0.1 mg/kg/min of RB diminished bile flow by 50%. In two dogs receiv-

ing 1.2 mg/kg/min of RB, bile flow nearly ceased (0.5 μl/kg/min) in the 120th and 160th minute. The blood pressure of the animals at this time was between 80 and 100 mm Hg, a finding undoubtedly to be ascribed to the toxic effect of RB. The diminution of bile flow is, however, not a consequence of the low blood pressure since in experiments with other drugs the bile flow remained unchanged at similar blood pressure levels. In experiments *in vitro* RB is bound to the active centre of the alcohol dehydrogenase and antagonizes, thereby, the NAD in a competitive way (BRAND et al. 1967). In addition, it inhibits the transport ATPase activity of erythrocyte ghosts (DASTOLI and GREEN 1965). It may therefore be assumed that in the liver RB inhibits some phase of the active secretion processes and this results in a decrease of bile production.

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^{24}Na UPTAKE AND PAH ACCUMULATION OF RENAL CORTICAL SLICES IN THE PRESENCE OF ANGIOTENSIN

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Rat kidney slices were incubated in the presence and absence of angiotensin, in Krebs-Ringer's solution at 28°C. It has been found that:

1. Angiotensin did not influence either the oxygen consumption or the PAH accumulation of kidney slices.

2. The Na content of tissues incubated for one hour in the presence of angiotensin was lower than in the control experiments.

3. The tissue Na is located in two domains. One is exchanged rapidly and the rate of exchange of this fraction is not influenced by angiotensin. This Na fraction is believed to be decisively extracellular. The other, a cellular fraction, is exchanged significantly less rapidly in the presence of angiotensin.

It is suggested that angiotensin diminishes passive Na transport across the membrane.

Depending on the dose administered, exogenous angiotensin reduces or increases renal sodium excretion (LAMEIJER et al. 1966; BONJOUR and MALVIN 1969; LOUIS and DOYLE 1965; HEALY et al. 1965; LANGFORD and PICKERING 1965; MALVIN and VANDER 1967 and others). Natriuresis increases only in response to the infusion of large amounts of angiotensin. As the increased sodium output is accompanied by no or moderate reduction of glomerular filtration rate (GFR), PETERS (1963), as well as HEALY et al. (1965) claim that angiotensin would inhibit tubular sodium reabsorption.

This view is rejected by other authors (GILL et al. 1964; CANNON et al. 1966) who claim that sodium output would increase as a result of a systemic and renal circulatory effect of angiotensin.

Both hypotheses rely upon experiments involving the clearance technique, thus they should be evaluated with caution, because such methods help only to determine changes in the net Na transport without allowing conclusions as to the localizations where the changes take place. The inaccuracy of the determination of GFR makes it difficult to judge whether the increased natriuresis is due to a vasoactive effect of angiotensin or to its direct tubular action. It is also well-known that an increase of blood pressure increases Na excretion (SELKURT et al. 1965). In the natriuretic effect of high angiotensin doses the hypertensive effect may also play a role. In experiments in vivo angiotensin exerts an indirect effect on the tubules through aldosterone and this must not be ignored, either.

The aim of the present experiments was to study the direct tubular action of angiotensin, therefore the experiments were performed in vitro. In addition to the sodium uptake of renal cortical slices we studied the uptake of paraaminohippuric acid, another substance transported by active enzyme function in the presence of angiotensin.

Methods

Inbred rats of either sex were used. Until experimented upon, the animals were allowed food and fluids ad libitum.

I. *In the first series of experiments* we studied the influence of angiotensin on oxygen consumption, PAH accumulation, Na and K contents of renal cortical slices.

The animals were killed by decapitation and the kidneys after removal were placed into Krebs—Ringer's solution of 2°C. The solution was composed of

Na^+	141 mEq/L
K^+	10.2 mEq/L
Ca^{++}	1.7 mEq/L
PO_4	7.4 mM/L
acetate	10.0 mM/L

The renal cortical slices were cut to 0.1 to 0.2 mm thickness. Of these, 200 to 300 mg amounts were placed into Warburg dishes containing 2.2 ml of Krebs-Ringer's and 0.1 ml of a 0.02 M PAH solution. In the control experiments the side bulb of the Warburg dish contained 0.5 ml of the Krebs-Ringer solution, in the angiotensin series the solution measured into the side bulb contained 50 μg of angiotensin. The CO_2 produced by the slices was bound with 0.2 ml 20% KOH. The interval between decapitation and onset of incubation did not exceed 30 minutes.

After 5 minutes of preincubation in oxygen atmosphere at 28°C, O_2 consumption of the kidney slices was measured over a period of 60 minutes. The angiotensin in the side bulb was added to the kidney slices after the preincubation.

At 60 minutes the slices were quickly removed from the incubating fluid, blotted with filter paper, weighed by torsion scales and placed into test tubes containing 5 ml of bisdistilled water. After soaking for 2 to 3 hours the tissue proteins were precipitated with 5 ml of a 20% solution of trichloroacetic acid, the precipitate was centrifuged off and PAH, Na and K were estimated in the supernatant.

When estimating the PAH content of the Krebs-Ringer solution two ml of the fluid were treated in the same way as the kidney slices.

The PAH content of the kidney slices (S) is given in μg/g wet weight, that of the incubation fluid (M) in μg/ml. PAH accumulation by the kidney slices is expressed by the quotient S/M.

K and Na were estimated with a Zeiss flame photometer and the results were calculated for μEq/g tissue.

O_2 consumption is presented in terms of μl O_2/h/mg wet weight.

II. *In a second series of experiments* the dynamics of Na uptake by kidney slices was studied. The slices prepared in the same way as in the first series were incubated at 28°C in Krebs-Ringer's solution in oxygen flow for 30 minutes to ensure a state of equilibrium. The slices were then transferred into Krebs-Ringer containing ^{24}Na and incubated for periods of 0.5, 1, 2, 4, 8, 10, 15, 20, 30 and 40 minutes. After incubation the slices were removed, rinsed with inactive Krebs-Ringer's solution and the surface was blotted with filter paper. After weighing, the kidney slices were placed in test tubes containing bisdistilled water.

The activity of the kidney slices and of 1 ml aliquots of the incubation fluid, was determined in a well-type scintillation crystal. Na and K contents were established by flame photometry.

The same procedure was employed in the angiotensin experiments, except that here both the preincubating fluid and the fluid containing ^{24}Na contained 2 μg of angiotensin per ml. When the incubation period of the slices exceeded 10 minutes, angiotensin was added repeatedly to the incubation fluid, at 5-minute intervals.

In 5 series of both the first and second type of experiment the vasopressor activity of the incubation fluid was tested in nephrectomized, ganglion blocked and vagotomized rats.

Computations

The specific activity of renal cortical slices and incubation fluids, i.e. the c.p.m. per 1 μEq of Na was computed from the activity of the tissue slices and incubation medium (cpm/g and cpm/ml, respectively) and from the Na content (μEq Na/g and μEq Na/ml, respectively). The specific activity of tissue and its relation to that of the incubation fluid (Q) is the indicator of the amount of Na exchanged during time specified ($0 \leqq Q \leqq 1$). When $Q = 1$, in the kidney slices the ^{24}Na is evenly mixed with the inactive Na, i.e. the tissue Na has completely been exchanged. The value of $1 - Q$ shows the amount of Na not yet exchanged.

The percentage of Na not yet exchanged in the kidney slices $[100(1 - Q)]$ is plotted against time in a semilogarithmic coordinate system. The curves obtained could be broken up into two monoexponential components, thus the process may be represented by the equation

$$y = A_0 e^{-\lambda t} + A_0' e^{-\lambda t} \tag{1}$$

The amount of Na in the single compartments may be expressed in percentage of the total amount of Na contained in tissue by extrapolating the given components to the y axis (A_0 and A_0', respectively). Tissue Na concentration multiplied by A_0 or A_0' gives in μEq/g the Na contained in the single compartments. An approximate estimate of the amount of Na exchanging per min. in the compartments may be obtained when the Na content of the compartments is multiplied by the exchange coefficient (λ) in Equation (1). The distribution space of Na in the single compartments is the quotient of the Na content of the compartment and the Na concentration of the incubation fluid. Half exchange time (T) of the Na in the single compartments has been calculated by the formula

$$T = \frac{0.693}{\lambda}$$

The difference between the means of the two types of experiment was calculated by Student's two-sample t-test.

Results

The results obtained in the first series of experiments are presented in Table I. The Na content of kidney slices incubated for 1 hour in the presence of angiotensin was significantly lower than the control value (control, 103.9 ± 13.5; in the presence of angiotensin, 97.6 ± 8.1 μEq/g; $p < 0.02$). Angiotensin had

Table I

Kidney cortex slices incubated in Krebs-Ringer at 28°C for 60 minutes in oxygen atmosphere. Na and K content, oxygen consumption and PAH accumulation.

		Dimension	Control	In presence of angiotensin
Unincubated tissue	Na	μEq/g	96.7 ± 20.4 (62)	—
	K	μEq/g	70.7 ± 10.8 (62)	—
Incubated tissue	Na	μEq/g	103.9 ± 13.5 (28)	97.6 ± 8.1 (40) $p < 0.02$
	K	μEq/g	55.9 ± 9.5 (28)	59.7 ± 5.9 (40)
Oxygen consumption		μl/mg/min	2.2 ± 0.35 (39)	2.3 ± 0.35 (39)
PAH accumulation		S/M	9.41 ± 1.33 (27)	8.62 ± 1.85 (41)

The data represent means, SD and, in brackets, the number of estimations.

no influence either on their oxygen consumption, or on their PAH accumulation.

The results of the second type of experiment are presented in Table II. The amount of Na not yet exchanged (expressed in per cent of tissue sodium

Table II

Certain parameters of ^{24}Na exchange in kidney cortex slices incubated in Krebs-Ringer under O_2 flow at 28°C

	Dimension	Control	In presence of angiotensin
Tissue K			
a) at end of preincubation	μEq/g	52.2±5.5	50.8±7.5
b) at end of experiment	μEq/g	50.9±8.8	53.2±9.1
Tissue Na at end of experiment	μEq/g	112.7	112.3
Na contained in			
Compartment I	μEq/g	36.6	48.2
Compartment II	μEq/g	64.7	58.4
Na exchange in			
Compartment I	%/min	24.6	20.4
Compartment II	%/min	1.55	0.45
Amount of Na exchanging in			
Compartment I	μEq/g/min	9.01	9.63
Compartment II	μEq/g/min	1.00	0.26
Distribution space of Na in			
Compartment I	ml/g tissue	0.24	0.32
Compartment II	ml/g tissue	0.43	0.39
Half exchange time in			
Compartment I	min	2.81	3.46
Compartment II	min	44.70	151.6

The data have been computed on grounds of the mean tissue Na contents and the equations (2 and 3) presented in the section on "Results". Number of experiments was 10 in both the control and the angiotensin series

content) as a function of time is plotted on Fig. 1. The curve obtained for control may be expressed by the equation:

$$y = 32.5\ e^{-0.246t} + 57.4\ e^{-0.0155t} \qquad (2)$$

while that for experiments with angiotensin is described by the equation:

$$y = 42.9\ e^{-0.20t} + 52.0\ e^{-0.0045t} \qquad (3)$$

The regression coefficients characterizing the exchange rate were practically the same in the case of the first component, while significantly different ($p < 0.05$) in the case of the second. The amount of Na exchanging from the second compartment was reduced to about 1/4 in the presence of angiotensin. The sodium content of the second compartment showed a 50% exchange in 44 minutes, in the presence of angiotensin the same process took 151 minutes.

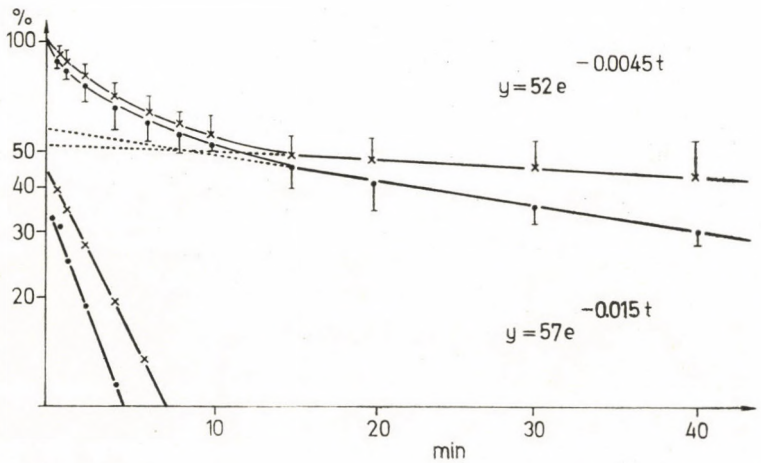

Fig. 1. Amount of Na not exchanged till time *t* is shown in percentage of the total tissue Na content. Control: —●—●—. In the presence of angiotensin: —x—x—. The results represent averages of 10 experiments each, the vertical parts signify the standard deviation (SD). The amount of Na not exchanged at 20, 30 and 40 minutes is significantly different in the two series ($p < 0.05$; < 0.01; < 0.05, respectively)

Samples of incubation fluid taken from both types of angiotensin series, when injected intravenously into rats in single doses of 0.1 ml, had a significant vasopressor effect, while the incubation fluids from the control experiments had no influence on blood pressure.

Discussion

When cutting slices of tissue, cells on the surface are damaged and release into the incubation medium proteins that may lyse angiotensin.

In order to diminish the activity of these nonspecific angiotensinases incubation was carried out at 28°C. The efficiency of this procedure was illustrated by the fact that the incubation fluid samples taken at the end of the experiment showed pressor activity, indicating that the tissue slices had been soaked throughout in media containing angiotensin.

In the second type of experiment, i.e. in which the dynamics of sodium exchange was studied, the curves representing the not yet exchanged Na could

be broken up into a fast (I) and a slow component (II), as plotted against time (see Fig. 1 and Equations 2 and 3). There was a close agreement between the control and angiotensin series in both the exchange rates of Na in the first compartment ($\lambda_c = 0.24$ min^{-1} and $\lambda_a = 0.20$ min^{-1}, respectively) and the exchange values for Na per minute (9.0 and 9.6 μEq/g min^{-1}, respectively). The distribution space calculated on grounds of the Na content of Compartment I was 0.24 in the control and 0.32 ml/g tissue in the presence of angiotensin. These data are closely similar to those published by ROBINSON (1950) who estimated the inulin space of the renal cortex at 26 per cent. All these seem to justify the assumption that the first component indicates the exchange of extracellular Na. This cannot be definitely stated, however, because the half-time of the exchange of the Na content of Compartment I is relatively long (control, 2.8, angiotensin, 3.4 min). WHITTAM and DAVIS (1954) found in the guinea pig's renal cortex at 37°C the 50% Na turnover of Compartment I to take 0.26 min. HARZA et al. (1966) found a total exchange of Na in 2 minutes in the extracellular space of the frog heart. The significantly faster extracellular Na exchange observed by the above authors cannot be explained by differences in species or the experimental conditions (e.g. temperature), because these cannot so excessively influence the exchange rate of extracellular Na. It may be assumed that in our experiments the rapidly exchanging component represents the exchange processes of Na situated extracellularly and those within the cells that had been damaged mechanically; the method we have employed is unsuitable for distinguishing between these exchange processes so similar in rate. Angiotensin influences neither processes.

The Na in the second compartment was exchanged less fast in the presence of angiotensin ($\lambda_c = 0.015$ min^{-1}, $\lambda_a = 0.0045$ min^{-1}, $p < 0.05$). In the control experiments the amount of Na exchanged per minute was fourfold of that found in the presence of angiotensin (Table II). At the same time, the Na contained by kidney slices incubated for one hour in the presence of angiotensin was significantly lower than the control value (Table I). The control and the angiotensin experiments were made under closely similar conditions, therefore it may be assumed that the extracellular space of kidney slices and the identity of the incubation fluid mean an identity of extracellular Na contents, thus, the lower Na content of tissues found in the presence of angiotensin seems to indicate also a lower intracellular Na content. This assumption was supported by the fact that the amount of slowly exchanging Na in the presence of angiotensin was less than in the control experiments (58.4 and 64.7 μEq/g tissue, respectively).

The amount of Na inside the tubular cells is determined by the transmembranous in and outflow of Na. Corresponding to its electrochemical potential gradient, Na enters the tubular cells by passive transport (or facilitated diffusion) through the luminar membrane, whence it is carried by active

transport through the basement membrane into the interstitium. Thus, the decrease in the cell's Na content may be due to a diminished passive and/or to an increased active transport. In many species and several kinds of tissue it has been demonstrated that in active Na transport a central role is played by the Na—K ATPase. Renal tissue seems to be no exception, because the Na—K ATPase content of the renal homogenate increases in response to chronic Na loading of the tubules and decreases when the loading is diminished (KATZ and EPSTEIN 1967). At the same time, angiotensin influences neither the Na—K ATPase activity of the renal cortex (BONTING et al. 1964), nor, as indicated by our experiments, the oxygen consumption by kidney slices. All this indirect evidence seems to justify the assumption that the diminution of Na exchange and the decrease of cellular Na content in the presence of angiotensin are due to a decrease of the permeability of the tubular cell membrane (presumably on the luminal side), without any influence on active transport.

The question is whether this inhibitory effect of angiotensin is exerted over the whole nephron. The stop-flow experiments of VANDER (1963) and the micropuncture findings of LOWITZ et al. (1969) suggest that this direct tubular inhibition is located to the distal tubules.

The exchange rate measured on grounds of the Na uptake of renal slices is merely a gross estimate of the Na flux taking place on the membrane of individual cells. The rate of intracellular ^{24}Na equilibration is determined, among others, by the conditions of diffusion, by the specific activity of the fluid surrounding the cell. The kidney slice consists of several layers, it is therefore obvious that the surface cells in touch with a medium of high specific activity will become equilibrated faster than the cells inside the slice, which, especially at the onset of incubation, are surrounded by interstitial fluid of low specific activity. The superficial cell layer may considerably hinder the interstitial exchange of labelled material (ROSENBERG et al. 1962). Thus, the rate of Na exchange determined by the tissue slice technique is lower than the actual exchange across the membrane. On grounds of mathematical considerations, BURG and ORLOFF (1963) arrived at the conclusion that at a given temperature the actual rate of K exchange of tubular cells would be about 2 to 4-fold of the values measured by the tissue slice technique. Thus, our experiments permit merely qualitative conclusions as to the true in vivo Na exchange rate, among others because we used excessive doses of angiotensin; under physiologic conditions only fractions of these may occur.

During angiotensin infusion an increase in phenol red extraction was found by CORCORAN and PAGE (1940) and an increase in renal PAH extraction by KÖVÉR et al. (1965), in the dog in vivo experiments. In our experiments angiotensin did not influence the PAH accumulation of kidney slices, thus it seems likely that the increase in renal phenol red and PAH extraction caused by angiotensin is due to a readjustment of intrarenal blood flow.

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THE PRESENCE OF β-ADRENERGIC RECEPTORS IN THE RENAL VASCULAR BED

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The effect of β-adrenergic stimulation and blockade on renal haemodynamics has been studied in 17 dogs.

Infusion of l-isoprenaline into the renal artery caused a decrease in renal vascular resistance and augmentation of urine flow. These effects were eliminated by β-adrenergic blockade with propranolol. Propranolol infusion itself showed a tendency to increase renal vascular resistance.

The effect of l-isoprenaline on renal haemodynamics was not influenced by previous α-adrenergic blockade by phenoxybenzamine.

Thus, β-adrenergic receptors seem to be present in the renal vascular bed; their localization and physiological role is still unclear.

The concept of β-adrenergic receptors was introduced by AHLQUIST in 1948, but detailed study only became possible a decade later when specific blocking agents had become available (MORAN and PERKINS 1958; BLACK and STEPHENSON 1962).

By now, the role of the β-receptor mechanism in cardiac function, muscle flow, and in fat and carbohydrate metabolism has been clarified. Concerning renal circulation, research has scarcely been done, and the existence of β-adrenergic receptors in the kidney is questioned. Thus, we have made a study of the problem using reliable means (FENYVESI and KÁLLAY 1967).

Methods

Seventeen dogs weighing 12 to 26 kg were anaesthetized with 0.1 g/kg of chloralose. Systemic blood pressure was measured by a cannula inserted into the carotid artery. Renal venous pressure was measured by a catheter inserted into the left renal vein under fluoroscopic control. Pressure was measured by a Statham PH 23 transducer. Blood flow in the left renal artery was measured by a non-cannulating electromagnetic blood flowmeter (Nycotron). An 8-channel Elema-mingograf served for registration. Urine was collected through ureteral cannulas. In order to maintain sufficient urine flow the animals were given 10% mannitol in infusion at a rate of 0.2 ml/kg/min. Blood samples from the femoral artery and renal vein were obtained for the determination of the renal extraction rate of para-amino-hippuric acid (E_{PAH}) and inulin (E_{in}), respectively. Haematocrit values were estimated by Hawskley's centrifuge.

After three minute control periods, doses of 1.0 to 5.0 μg/kg/min of l-isoprenaline (Isolevin, Cilag-Chemie) dissolved in 10 ml saline were infused directly in the left renal artery over 3-min periods. In the control 3-min period 10 ml of saline was infused. Generally, 3 control and test periods alternated at 10-min intervals. Urine samples were collected throughout the test and control periods, blood samples for E_{PAH} and E_{in} determination were taken in the middle of each period.

The whole procedure was repeated after intravenous administration of 300 μg/kg propranolol (Inderal, ICI). In the second series of experiments, after an initial control and 1-isoprenaline infusion period the above mentioned procedure was repeated with 5 mg/kg phenoxybenzamine (Dibenzyline, Smith, Kline and French) pretreatment. The blocking drugs were given 15—20 min before the first 1-isoprenaline infusion.

In the first 10 to 15 seconds of 1-isoprenaline infusion no systemic effect of the drug could be detected, e.g. no tachycardia or change in mean arterial pressure, except for a slight but definite increase in heart rate. Thus, we considered the change in renal blood flow (RBF) and renal vascular resistance in this period an acute primary renal vascular effect of the drug. A steady state ensued usually one minute after beginning the infusion.

In the tables, RBF values are given for 100 g kidney weight. Renal vascular resistance was calculated by the formula: renal pressure gradient in mm Hg (mean systemic blood pressure minus mean renal vein pressure) divided by RBF, in ml/min/100 g kidney weight. Statistical analysis was carried out by Student's *t*-test.

Results

Table I shows the effect of 5 μg/kg of 1-isoprenaline in five dogs.

Table I

Effect of infusion of 5.0 μg/kg/min 1-isoprenaline on renal haemodynamics
(Means and standard deviations)

	n	Control	Change during infusion of 1-isoprenaline	
			acute	steady state
RBF ml/100g kidney weight/min	11	218±84.6	+89±47.3***	+21±51.7 ∅
Renal pressure gradient, mm Hg	11	140±28.9	+1.5±5.7 ∅	−32.6±16.6***
R_{ren} mm Hg/ml/100g kidney weight/min	11	0.74±0.38	−0.26±0.26***	−0.25±0.25***
Heart rate/min	11	170±36.7	+13.0±17.9*	+72.5±38.0***
Urine flow ml/min right kidney	11	1.15±0.34		−0.11±0.23 ∅
left kidney	11	0.82±0.27		−0.04±0.34 ∅
E_{PAH} %	7	69.0±6.4		−0.5

RBF = renal blood flow
Renal pressure gradient = mean femoral arterial pressure − mean renal venous pressure
R_{ren} = renal vascular resistance
E_{PAH} = extraction of para-amino-hippurate
$p < 0.001$ = ***
$p < 0.01$ = **
$p < 0.05$ = *
$p > 0.05$ = ∅

Even though RBF values in this group were low, they were not discarded in view of the uniform character of the response. The diminution of renal vascular resistance was evident throughout, while RBF was augmented only in the acute phase but not in the steady state. Obviously, the pronounced hypotensive effect of isoprenaline in the steady state prevented the augmentation of RBF. Urine flow was unchanged throughout.

To avoid the gross systemic circulatory effects, 1.0 μg/kg/min of l-isoprenaline was infused into six dogs. The effect of the drug before and after propranolol administration is shown in Table II/A and II/B, respectively.

Table II

Effect of infusion of 1.0 μg/kg/min l-isoprenaline on renal haemodynamics
(means and standard deviations)

	A			
	Before administration of propranolol			
	n	control	change during infusion of l-isoprenaline	
			acute	steady state
RBF ml/100g kidney weight/min	18	320±146	+77.3±33.4***	+64.5±42.6***
Renal pressure gradient, mm Hg	18	140± 34	−1.3± 3.9∅	−7.3± 8.8**
R_{ren} mm Hg/ml/100g kidney weight/min	18	0.49± 0.17	−0.13± 0.11***	−0.13± 0.11***
Heart rate/min	18	155±24.9	−0.5± 6.3∅	+28.3±17.2***
Urine flow ml/min right kidney	18	0.67± 0.25		−0.04± 0.13∅
left kidney	18	0.66± 0.34		+0.19± 0.23**
E_{PAH} %	8	64.9±13.6		+0.2
E_{in} %	11	26.6± 7.4		−0.6
C_{in}, ml/min/100g kidney weight	9	54.2±23.0		+5.4±11.0

	B			
	After administration of 300 μg/kg propranolol			
	n	control	change during infusion of l-isoprenaline	
			acute	steady state
RBF ml/100g kidney weight/min	17	275±138	+12.5±27.9∅	+21.3±27.5**
Renal pressure gradient, mm Hg	17	134±29.5	+0.3± 3.4∅	+0.6± 3.8∅
R_{ren} mm Hg/ml/100g kidney weight/min	17	0.57± 0.21	+0.003± 0.09∅	−0.027± 0.059∅
Heart rate/min	17	115±15.5	+1.4± 2.6	+3.5± 3.6
Urine flow ml/min right kidney	16	0.62± 0.24		−0.01± 0.12
left kidney	16	0.69± 0.41		+0.04± 0.19
E_{PAH} %	7	69.2± 8.9		−1.2
E_{in} %	10	27.6± 7.9		−0.7
C_{in} ml/min/100g kidney weight	9	43.4±26		+5.1±13.0

E_{in} = extraction of inulin
C_{in} = clearance of inulin
Other abbreviations: see footnote in Table I

Before propranolol pretreatment, renal vascular resistance decreased in both the acute phase and the steady state. Renal perfusion pressure decreased only slightly in the steady state, though the increase in RBF was maintained during the whole infusion period.

Diuresis following l-isoprenaline infusion was significantly augmented in the left kidney, while in the right kidney it was unchanged. E_{PAH} and E_{in} did not change, nor was the clearance of inulin influenced.

Table II/B shows the data obtained on infusion of 1.0 μg/kg/min of l-isoprenaline following 300 μg/kg propranolol pretreatment. Renal pressure gradient, renal vascular resistance and diuresis did not change. RBF did not change in the acute phase, but a definite increase was observed in the steady state. The mean increase by 21.3 ml/min/100 g was small compared with the mean increase by 64.5 ml/min/100 g before propranolol administration; the difference was significant ($p < 0.02$).

For a more detailed analysis of β-adrenergic stimulation, pretreatment with α-receptor blocking agents has been suggested (FURCHGOTT 1967). Therefore, we have pretreated six dogs with 5.0 mg/kg of phenoxybenzamine. The effects of the infusion of 1 μg/kg/min l-isoprenaline into the renal artery of these dogs, before and after 300 μg/kg propranolol administration are shown in Table III/A and III/B.

Table III

Effect of infusion of 1.0 μg/kg/min l-isoprenaline on renal haemodynamics after 5.0 mg/kg phenoxybenzamine treatment

	A Before administration of propranolol			B After administration of 300 μg/kg propranolol		
	n	control	change during infusion of l-isoprenaline (steady state)	n	control	change during infusion of l-isoprenaline (steady state)
RBF ml/100g kidney weight/min	12	548±314	+13.8±85.7Ø	10	554±295	−3.4±31Ø
Renal pressure gradient mm Hg	12	104±28	−33.1±16.6***	10	109±19.5	−1.0± 4.0
R_{ren} mm Hg/ml 100g kidney weight/min	12	0.23±0.11	−0.07± 0.06**	10	0.25± 0.14	−0.01± 0.02Ø
Heart rate/min	12	209.5±49.5	+44.0±53.0*	10	152.0±19.6	+4.2±10.2Ø
Urine flow ml/min right kidney	11	1.0± 0.91	−0.33± 0.28**	9	0.99± 0.67	+0.05± 0.29Ø
left kidney	11	0.72± 0.73	−0.13± 0.33Ø	9	0.52± 0.43	+0.12± 0.2Ø
E_{PAH} %	11	66.9± 5.3	+0.2	9	63.4± 8.3	+1.2
E_{in} %	10	29.1±10.1	−1.8	8	31.0±10.0	−5.7***
C_{in} ml/min/100g kidney weight	10	102.1±67.9	−4.0	8	95.8±53.7	−21.8±21.3*

Abbreviations: see footnotes in Tables I and II.

The effect demonstrated in Table III/A resembles those in Table I. During steady state there was a significant decrease in renal vascular resistance but the augmentation of RBF was hindered by the systemic circulatory effect of l-isoprenaline resulting in very marked decrease in the renal pressure gradient. Urine flow of the infused left kidney did not change, while that of the right kidney diminished.

Propranolol pretreatment suspended all the above demonstrated renal circulatory effects of l-isoprenaline, as seen in Table III/B.

Test doses of adrenaline (3 μg) administered into the left renal artery before and after phenoxybenzamine pretreatment served to indicate the efficiency of α-receptor blockade. Phenoxybenzamine itself led to the diminution of renal vascular resistance from 0.29 ± 0.14 by 0.09 ± 0.07 mm Hg/ml/min/100 g. The change was significant statistically ($p < 0.05$) (Table IV).

Table IV

Effect of propranolol on renal vascular resistance before and after 5 mg/kg phenoxybenzamine pretreatment

	Before phenoxybenzamine		After phenoxybenzamine	
	control	change after propranolol	control	change after propranolol
R_{ren} mm Hg/ml/100g kidney weight/min	0.43 ± 0.12	$+0.05 \pm 0.027$	0.25 ± 0.13	0.01 ± 0.03
n	6		6	
p	< 0.01		< 0.1	

The effect of 300 μg/kg propranolol on renal vascular resistance is demonstrated in Table IV. In the first series of experiments, propranolol caused a significant increase in vascular resistance. Such an increase did not appear after phenoxybenzamine pretreatment.

Discussion

As early as 1947, CORCORAN and PAGE described a decrease in renal vascular resistance following the administration of isoproterenol. On the basis of CANNON's hypothesis they considered this a "sympathin I" effect. Their observation was confirmed by HANDLEY and HUGGINS (1954), and AVIADO et al. (1958). SPENCER (1954, 1956), on the other hand, found renal vasoconstriction following isoproterenol administration. GREEN and KEPCHAR (1959) denied the existence of β-adrenergic receptors in the renal vasculature. More

recently, investigators incline to accept their existence (McNAY and GOLDBERG 1966; MARK et al. 1968); nevertheless, SCHIRMEISTER et al. (1966) and ROSENBLUM et al. (1968) failed to register renal vasodilatation after β-adrenergic stimulation.

In our experiments, infusion of l-isoprenaline into the renal artery led to a decrease in renal vascular resistance. This effect was found to be prevented by propranolol premedication. Some other authors using clearance methods (SCHIRMEISTER et al. 1966; ROSENBLUM et al. 1968) were unable to detect this renal blood flow response because of its transient character. Thus, blood flow elevation soon masked by the systemic effects of isoprenaline can only be observed by continuous direct flow measurement. This might have been the cause why no significant renal vascular effect of isoproterenol was recorded by CHÂTEL et al. (1968).

In our experiments, 1.0 μg/kg/min of l-isoprenaline caused a marked elevation of urine flow in the infused kidney. ANIKIN (1968) observed this elevation only after giving significantly lower doses, while other authors observed a decrease of water excretion after isoproterenol administration in the rat (LEES and LOCKETT 1963, 1965; LEES 1968; HEINTZE and HEIDENREICH 1968). The controversy can be explained by the much shorter infusion period used in our experiments, and probably by species differences.

In the second series of experiments, α-adrenergic blocking pretreatment served for a more detailed analysis of the possible β-adrenergic activity in the kidney. A 5.0 mg/kg dose of phenoxybenzamine was used. The drug reduced renal vascular resistance, but, in contradiction to MANDELBAUM's (1967) recent data, there was no diminution of RBF. The renal vascular effects of isoprenaline were slightly modified by the blockade. In this case a lower dose of l-isoprenaline (1.0 μg/kg/min) caused effects very similar to those observed after a higher dose (5.0 μg/kg/min) administered to dogs with intact α-receptor activity. There was a marked reduction of systemic blood pressure and thus, despite the diminution of renal vascular resistance, no RBF elevation was produced.

Phenoxybenzamine pretreatment altered the urine flow changes as well. In the infused kidney there was no change in urine flow while in the other kidney it diminished.

Propranolol prevented the renal vascular effects of isoprenaline after α-adrenergic blockade too, and caused an increase in renal vascular resistance in contrast with the observation of SHANKS (1967).

Our data would seem to point to the presence of β-adrenergic tone in the renal vasculature, but after phenoxybenzamine pretreatment propranolol caused no increase in renal vascular resistance. In the first series of experiments renal vascular resistance underwent a gradual increase and this phenomenon failed to appear after blocking. Thus, there is a possibility that in the first

series of experiments the elevation of renal vascular resistance was not caused by the propranolol but by a deterioration of renal circulation.

Elimination of the renal vasconstrictor effect of adrenaline by phenoxybenzamine served as an adequate proof of α-adrenergic blockade.

The localization of renal β-adrenergic receptors and their possible physiological role has not yet been determined. The double action of the β-adrenergic mechanism seems to consist in (POISNER 1964; LEES and LOCKETT 1965; LEES 1968) (1) the participation in the postganglionic sympathetic innervation of the proximal tubuli which may be responsible for the salt and water retention observed by some authors; (2) a vascular dilator effect localized in the vas efferens which may be the cause of the augmented RBF while urine flow and GFR are diminished. The effect of β-adrenergic stimulation or blockade on these actions was not uniform. As mentioned above, we did not observe a diminution of either urine flow or GFR.

The question whether β-adrenergic stimulation affects the entire renal vascular system uniformly, or there are areas particularly susceptible to it, is still unclear. The constancy of E_{PAH} and C_{in} speaks for the uniform effect of l-isoprenaline on the renal cortex, but the increase in urine flow may indicate a disproportionally elevated blood flow in the renal medulla. Further studies are needed to answer this question.

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CARDIAC OUTPUT ESTIMATION BY A THERMODILUTION METHOD INVOLVING INTRAVASCULAR HEATING AND THERMISTOR RECORDING

By

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A method has been developed for the estimation of cardiac output based on the thermodilution technique. Heaters placed in the superior and inferior vena cava, operated with a time-switch direct current power supply unit ensured intravascular heating lasting 0.7 to 1.5 seconds. The thermodilution curves were plotted by means of a direct-writer and the thermistor was placed in the pulmonary artery.

The cardiac output values obtained by this method have been compared with those obtained on the basis of the direct Fick principle.

The method proved suitable for the estimation of cardiac output without the introduction of foreign material or blood withdrawal; the test may be repeated as many times as desirable.

Knowledge of the value for cardiac output may be important in both research and clinical practice. Most of the known methods for cardiac output estimation are based on the principle of indicator dilution where into the cir-

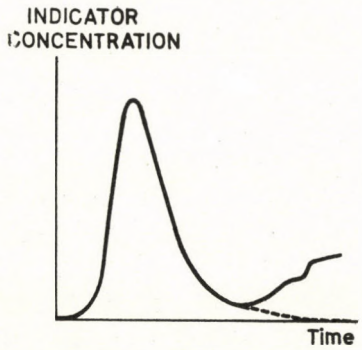

Fig. 1. Thermodilution curve obtained at the site of monitoring following rapid injection of indicator

culation a known amount of indicator is introduced and its concentration is plotted against time. A precondition of the test is that the indicator administered must completely be mixed with the blood at some cross section of blood flow. If the indicator is injected within a few seconds, the well-known indicator dilution curve will be obtained (Fig. 1).

Among others, dyes and isotopes may be used as indicators, but their application, administration, estimation of their concentration in the blood entail many sources of error, not to speak about the complexity of the procedure. When heat is used as the indicator (FEGLER 1954; FEGLER 1957; HOSIE 1962; SZEKERES et al. 1968) the determination of dilution is a simple temperature measurement. The indicator may be Ringer's solution, physiologic saline or dextran with a temperature different from that of the body, which, especially when testing is repeated, may alter circulatory parameters.

It seemed therefore important to develop a method of intravascular heating without the administration of foreign materials. Such a method of cardiac output assay is the one described by KHALIL et al. (1966), where continuous heating is ensured by high-frequency current and temperature is measured by thermocouples.

In our method, in analogy to the one injection principle we use brief DC heating and temperature is measured by means of thermistors, which are more sensitive than thermocouples.

Methods

The thermal sensor (Kőbánya China Factory, TH 2.5 type) is connected through a Wheatstone bridge to the Honeywell Brown Electronic direct writer. The thermistor and its cable were pulled into a polyethylene tube 1 mm in diameter, then this was placed into a KIFA 17,887 — I type radiopaque catheter so that the thermistor was in the lumen of the tube 0.5 mm from the tip of the catheter which was isolated by a thin layer of collodium. In anaesthetized dogs under X-ray control the catheter was passed through the right jugular vein, right atrium and right ventricle into the initial part of the pulmonary artery (Fig. 2).

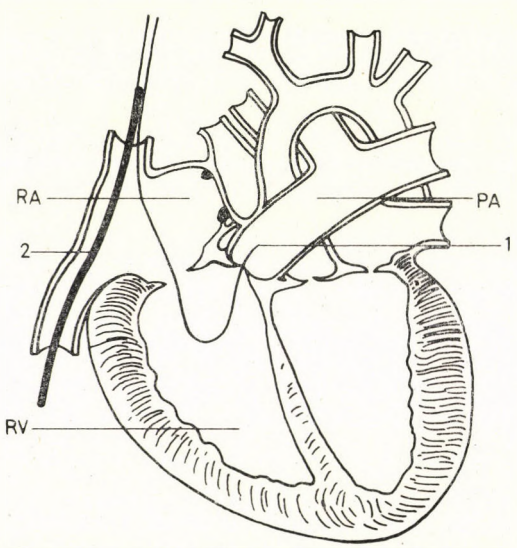

Fig. 2. Thermistorized heat sensor (1) and heat unit (2) in pulmonary artery, superior vena cava, right atrium and inferior vena cava. PA: pulmonary artery; RA: right atrium; RV: right ventricle

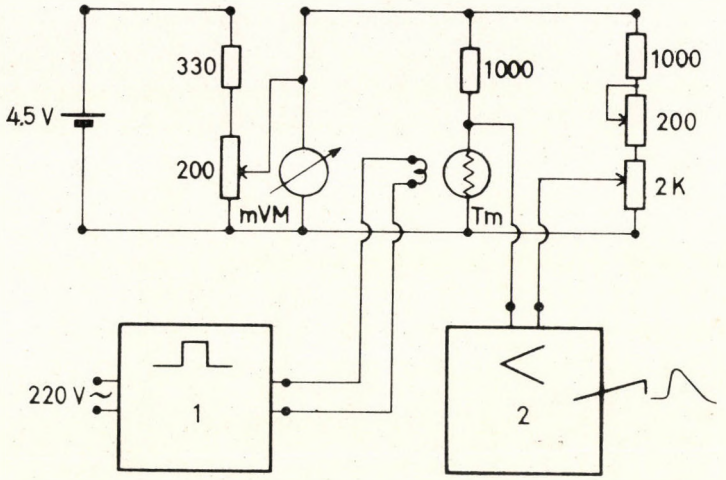

Fig. 3. Block diagram of thermodilution setup. 1: DC time-switch input unit. 2: Honeywel recorder

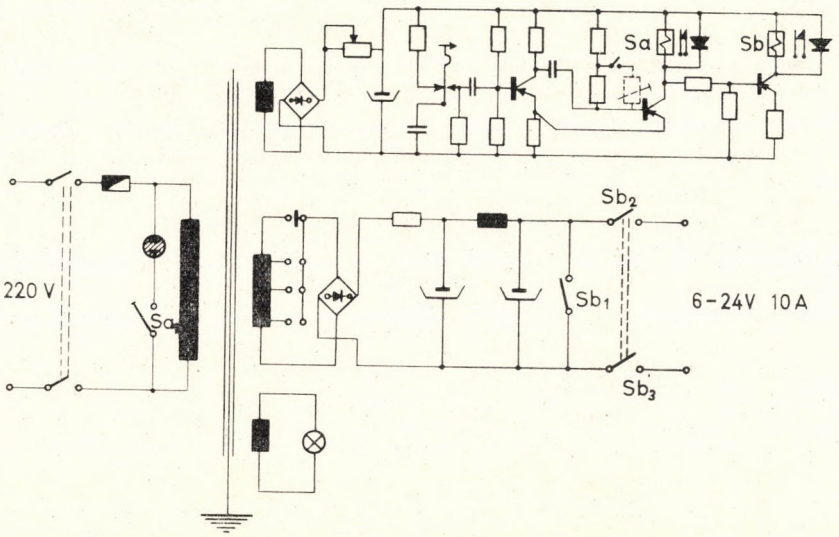

Fig. 4. DC time-switch input unit: diagrammatic representation

Intravascular heating was effected by means of a 1 cm long coil of enamel-coated copper wire 0.01 mm in diameter, placed at the tip of a polyethylene cannula 2.5 mm in diameter. The resistance of the coil was 5 to 10 Ohm. Momentary heating was induced by means of a home-made DC power supply unit, the duration of heating was controlled by a transistorized time-switch. In this way it was possible to introduce well-defined amounts of heat in 0.7 to 1.5 seconds, in the form of nearly square pulses. The block diagram of the experimental setup is shown in Fig. 3, the principle of operation of the DC time-switch power supply unit is represented diagrammatically in Fig. 4.

The spherically-tipped heater was placed in the superior vena cava, right atrium and inferior vena cava (Fig. 2). With this arrangement the heat is perfectly distributed over the entire cross section of flow between the sites of heating and recording, mainly as a result of right ventricular action, and thus the thermal dilution curve may be plotted.

Before the experiment, the output of the heater was determined several times, partly on grounds of the electrical data, partly by means of a calorimeter. The same heater produced in a calorimeter heat varying by not more than 2%. The amount of heat per heating was 30 to 50 cal, with 24 V input in 1.5 sec.

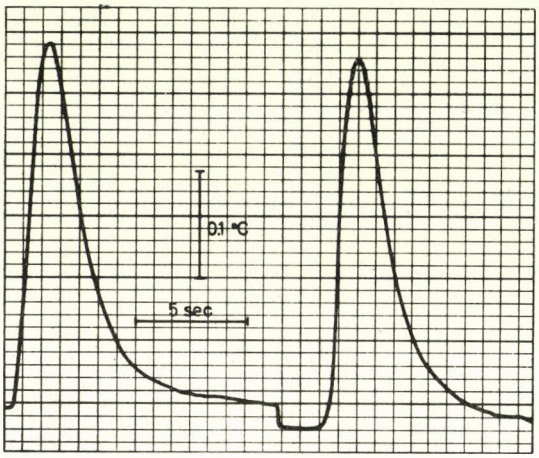

Fig. 5. Thermodilution curves obtained by the use of the setup

Cardiac output determinations were made in dogs weighing 9 to 16 kg anaesthetized with pentobarbital. Thermodilution curves obtained in such an experiment are shown in Fig. 5. From the thermal dilution curve, cardiac output is calculated on the basis of the general formula

$$F = \frac{I}{\int_0^\infty C \cdot dt} \tag{1}$$

(where I is the amount of indicator administered, C the indicator concentration at the site of observation) by means of the equation,

$$F = \frac{W \cdot t_f \cdot 0.2388 \cdot 60}{T \cdot c \cdot s \cdot (t_e - t_m)} \tag{2}$$

where

F is the cardiac output in ml/min; W the heating in W; t_f is the duration of heating in sec; 0.2388 is the conversion factor from joule to cal; T the change in temperature in °C, calculated from the average height of the corrected extrapolated curve; c is the specific heat of blood in $\frac{\text{cal}}{g \cdot °C}$; s the density of blood in g/ml; t_m the time of appearance of the curve; and t_e the time of disappearance of the corrected curve.

When the thermodilution curves after the initial exponential decrease were protracted they were corrected by logarithmic extrapolation of the initial part.

T was computed on the basis of the area under the curve, the paper spead and the excursion in response to 1°C. The area under the curve was determined planimetrically.

Results

Comparison of cardiac output values obtained by the thermal dilution method and by application of the direct Fick principle

In experiments involving dogs of either sex, weighing 9 to 16 kg, the results obtained by the thermal dilution technique were compared with those obtained by applying the direct Fick principle. O_2 consumption was determin-

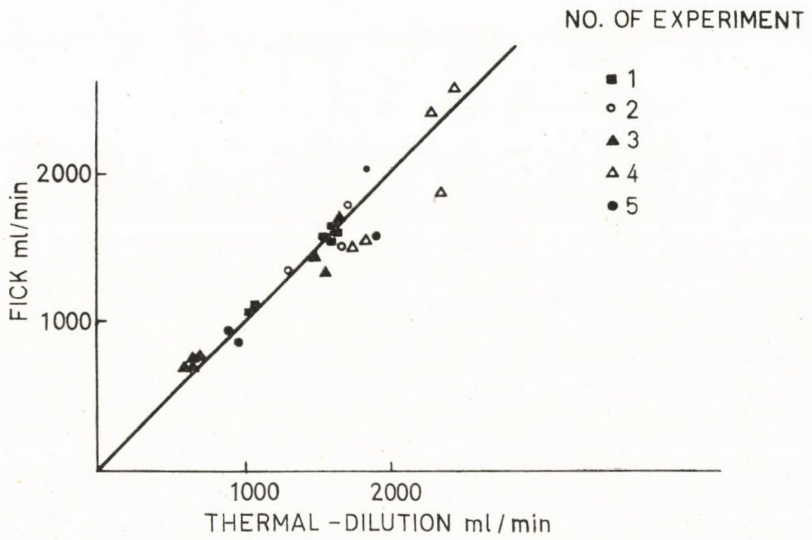

Fig. 6. Comparison of cardiac output values estimated by the thermodilution and the Fick methods

ed by means of a Kipp diapherometer, the O_2 contents of the mixed venous blood from the right atrium and of the arterial blood from the femoral artery were according to MAYERS and FORSTER (1966) and computed according to LAVER et al. (1965).

The mean values of blood samples taken before and after measurement of O_2 consumption were used for calculating cardiac output. The thermal dilution curves were likewise plotted before and after measurement of O_2 consumption.

The mean values yielded by the thermodilution technique and the Fick principle in 5 dogs are presented in Table I and Fig. 6, whence it is clear that the values were closely comparable.

Discussion

The thermodilution technique for cardiac output measurement entails the following potential sources of error.

Table I

Values for cardiac output as estimated by the thermodilution technique and by the direct Fick method

Experiment No.	Cardiac output, ml/min		Experiment No.	Cardiac output ml/min	
	Thermodilution	Fick		Thermodilution	Fick
1	1614	1785	4	1774	1541
	1563	1579		1852	1595
	1543	1497		2465	2592
	1525	1516		2271	1862
	1084	1116		2300	2416
	1076	1078			
2	1326	1363	5	1818	2012
	1636	1558		1861	1661
	1722	1801		947	846
				929	957
3	1624	1636			
	1589	1369			
	637	685			
	695	685			
	658	755			
	667	755			
	1501	1458			

1. Loss of heat between the place of heating and site of observation.
2. Inaccuracy of sensing heat.
3. Damage caused by the heat.
4. Imperfect mixing.
5. Recirculation.

1. The heater transfers heat first of all to the blood around it. Secondarily, however, adjacent tissues (vascular wall, myocardium, valves, etc.) are also heated by conduction. The studies of GOODYER et al. (1959) show that the heat loss is insignificant and during the increase or decrease of the temperature curve the heat lost is brought back to the colder or warmer blood flowing in the meantime. This applies to 1 or 2°C changes at the site of monitoring, thus, it is particularly true in our case, where the change in temperature does not amount to more than 0.2 or 0.3°C, and as a result of the small difference in temperature little heat is lost by conduction.

2. As reported by JANCSÓ et al. (1965), between 36 and 42°C the changes in resistance are linear with the thermistors used by us. The reliability of the method is indicated also by the fact that in model experiments the thermodilution curves plotted at constant flow rate were repeatedly of the same size.

3. In the necropsied dogs no thermal damage could be demonstrated in the endocardium or in the wall of the large vessels. Furthermore, in heparinized animals there was neither fibrin precipitation, nor protein coagulation on the surface of the heating unit. Thus, the surface temperature did not exceed 56°C.

4. Mixing must have been perfect as between the site of heating and that of monitoring are lying the right atrium and the right ventricle which mix blood most efficiently. Furthermore, the descending slope of the curves was exponential, which would not have been so had mixing been imperfect, a fact ascertained in model experiments.

5. As a result of the great heat capacitance of the body, there was no recirculation in some cases, while in others it manifested itself with a protraction of the curve instead of a separate wave. In this case, recirculation can be corrected by extrapolating back the descending slope of the curve.

Thus, the method seems suitable for the estimation of cardiac output without necessitating the introduction of foreign material or blood sampling and the test may be repeated as many times as necessary.

Acknowledgements

We are indebted to Mrs. K. TÁRNOKY for blood O_2 estimations and to Mr. L. TUTSEK for the power supply unit.

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INCREASED SYMPATHETIC INNERVATION OF THE RABBIT HEART

EXPLANATION OF THE LABILITY OF THE ECG IN THE RABBIT

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In the ECG recorded with needle electrodes from alert rabbits in the natural sitting posture a lability of the various deflexions was observed. Under urethan anaesthesia the lability was slighter but still demonstrable at slow paper velocity, or in long recordings.

Serotonin and isoproterenol enhanced, atropine did not influence the ECG lability of the rabbit. After propranolol administration the ECG became geometrically regular. It is concluded that the lability of the various deflections is caused by an increased sympathetic innervation.

The rabbit is one of the most extensively used experimental animals. It is therefore only natural that there is ample evidence in the literature concerning the ECG of the normal rabbit and its ECG changes under experimental conditions (BLACK 1960; FIORI 1960; etc.).

The ECG tracings were recorded by some authors with needle electrodes in anaesthetized supine rabbits (SLAPAK and HERMANEK 1957; SAYTANOV 1960), while others used plate electrodes applied to the defurred limbs (LEVINE 1942; NELSON et al. 1964). The ECG was found to display excessive individual variations such as a broad spectrum of the T axis (PETELENZ and KOSMIDER 1961; NELSON et al. 1964; etc.). Since reported (LEVINE 1942; SLAPAK and HERMANEK 1957) the pattern may be different in different recordings made on the same day, the rabbit was not considered suitable for ECG studies. As regards the ST segment, according to MAYEDA (1934) this does not appear in the rabbit, while according to BÜCHNER and LUCADOU (1934) the segment would originate high, and according to SLAPAK and HERMANEK (1957) it is similar to the human ST. The frequency dependence of the QT distance is considered a general law (MASSMANN and OPITZ 1954; SAYTANOV 1960; NELSON et al. 1964).

The authors concerned with the ECG of the rabbit have ignored its excessive lability in the unanaesthetized animal, though this is evident also from the three deflections to be seen in the figures accompanying the papers by BÜCHNER and LUCADOU 1934; STOCKER 1940; LEVINE 1942; or SAYTANOV 1960. On the other hand, it has been claimed that the 3000/min oscillation in

the rabbit ECG would be specific, although it is obviously an artefact caused by muscle tremor (STOCKER 1940).

In the present paper the lability of the ECG of the alert and of the anaesthetized rabbit is dealt with, and an attempt is made to present an explanation of the phenomenon.

Methods

Twenty-five rabbits weighing 2800 to 3500 g were used. The ECG was recorded in unrestrained animals in the natural sitting posture with needle electrodes, a one-channel direct writing apparatus, at a paper velocity of 25 and 50 mm/sec in the standard and unipolar limb leads. The rabbits sometimes were trying to escape and so parts of the tracings became unusable, but in general it was easy to make the recordings and even from alert animals over prolonged periods.

ECG was recorded in lead II before, during and for 10 minutes after the administration of drugs.

In 7 rabbits the ECG was made during and after the injection of 1 mg/kg serotonin-creatinine sulphate (Reanal) and in 5 rabbits also during intraperitoneal urethan (1.3 g/kg) anaesthesia; under such conditions the effect of 1 mg/kg of atropine was studied in 4, and that of 0.1 mg/kg isoproterenol (Isuprel®, Winthrop) in 3 rabbits. In seven further experiments involving alert rabbits the effect on the ECG of 10 to 50 μg/kg of intravenous isoproterenol and in 6 experiments that of 1 mg/kg of atropine were analysed. Thus, there were 10 isoproterenol and 10 atropine experiments altogether.In the alert rabbits, in the 7 serotonin-treated and the 4 anaesthetized and atropine-treated animals as well as in the 10 pretreated with isoproterenol the experiment was concluded by the intravenous injection of 0.4 to 2.0 mg/kg of propranolol (Inderal®, ICI).

Results

In the alert rabbits, the ECG showed a certain lability in every case. The QRS complex was usually stable, the changes in the size of R and S did not exceed those caused by respiration in man in lead III. However, the ST segment and the T wave showed remarkable changes in every case, though these differed from animal to animal. One of the most illustrative examples is shown in Fig. 1; here, in all three limb leads the R and S varied in size. The three main types of the repolarization segment (ST—T) may be seen in lead II recorded at 50 mm/sec velocity. In the first 3 and the last 4 complexes of the tracing the T is positive and tall, the ST rises steeply. In complexes 5 and 6 the T is isoelectric, the ST is slightly depressed or isoelectric. Type 3 is represented by the next two ST segments, where the tall, dome-like ST goes over into a small negative T wave.

In the rabbits anaesthetized with urethan the ST – T segments are much less irregular, there are relatively long sections with insignificant differences in the deflections. When recording is made at low paper speed or when it is continued over longer periods of time, difference in size of the positive or negative T wave may be demonstrated (Fig. 2, Fig. 3 first row).

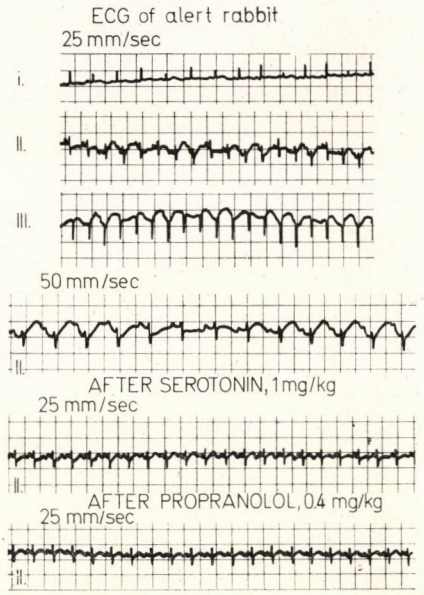

Fig. 1. Labile ECG of alert rabbit and its changes in response to serotonin and propranolol

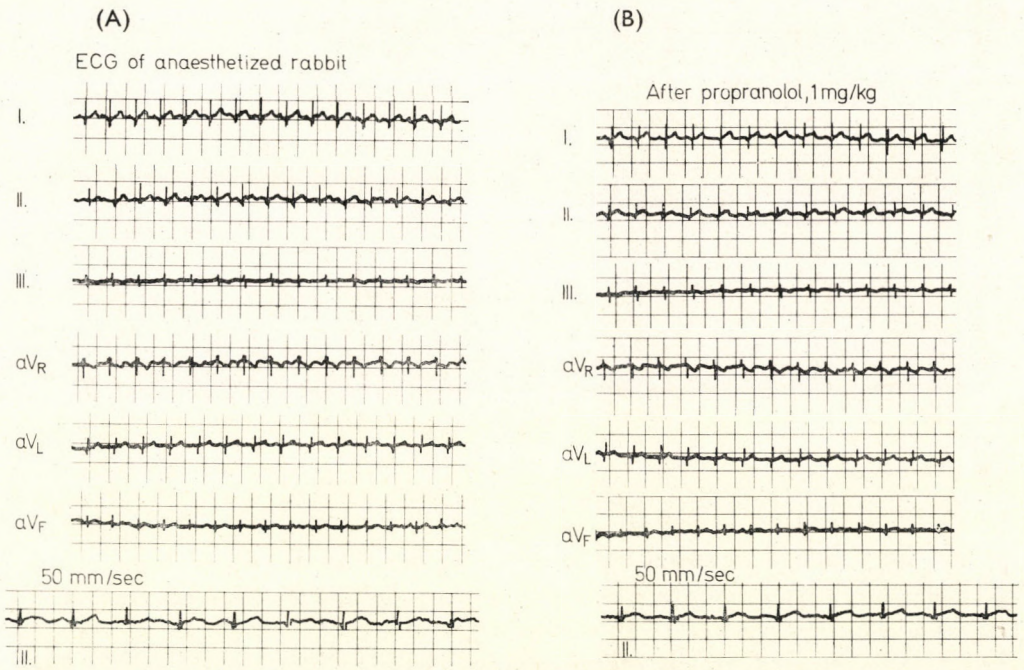

Fig. 2. Less marked lability of ECG in anaesthetized rabbit (A) and regularity in response to 1 mg/kg propranolol (B)

Thus, general anaesthesia definitely diminishes the variability of the rabbit's ECG. Experiments with drugs were then made to find the mediator responsible for lability. In four anaesthetized rabbit the vagus was blocked by the intravenous injection of 1 mg/kg, then 2 mg/kg of atropine. Frequency

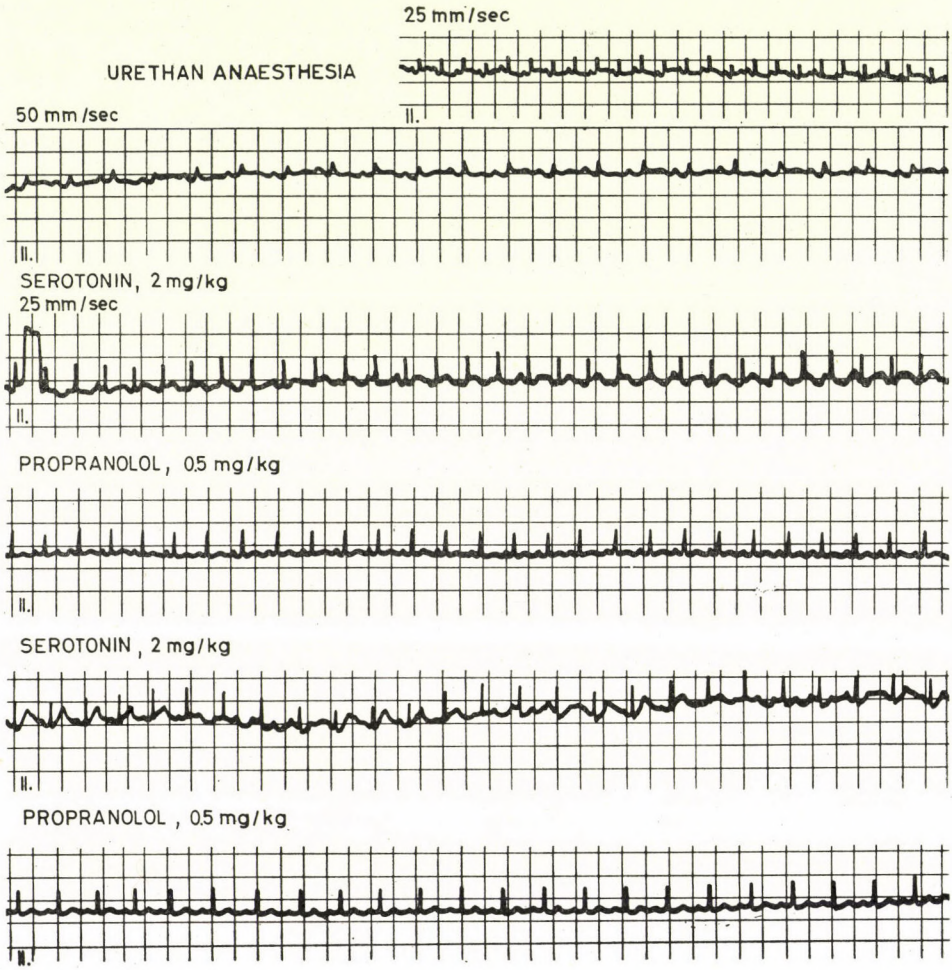

Fig. 3. Changes in the ECG of anaesthetized rabbit in response to repeated doses of serotonin and propranolol

increased from 210 to 300/min, but the changes in the size of the T wave persisted. In 6 alert rabbits 1 mg/kg of atropine was injected intraperitoneally, in response to which the irregularity of ECG increased rather than decreased.

Subsequently we reproduced our previous serotonin experiments (KENEDI and DÁVID 1967) with a 2 mg/kg dose under urethan anaesthesia. The lability of the repolarization phase was markedly enhanced (Figs 1 and 3),

the ST started from different heights from tracing to tracing (point J), and the height of T changed likewise.

The mediator of the lability was clarified by intravenous propranolol. In response to this drug in both alert and anaesthetized rabbits (Fig. 2 B), following serotonin administration (Fig. 1 and Fig. 3), as well as in atropinized animals the ECG became absolutely uniform, and series of regular ventricular complexes were recorded.

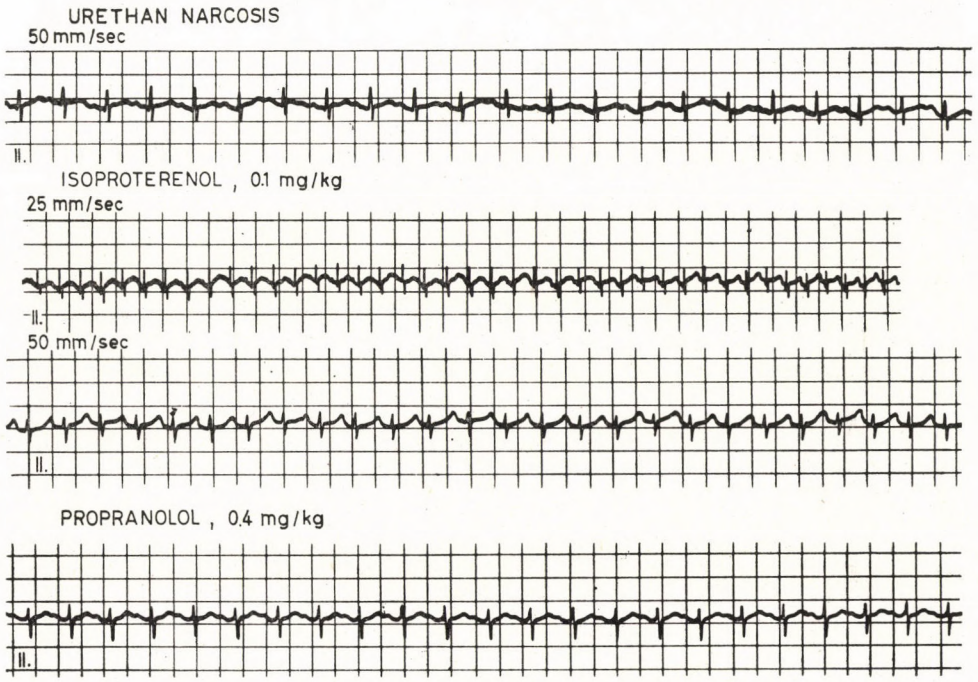

Fig. 4. Tachycardia and increased irregularity of ECG in anaesthetized rabbit in response to isoproterenol. Following intravenous propranolol the ECG becomes regular

This regular ECG turned irregular under the effect of high doses of serotonin, tall and flat T waves and elevated and depressed ST segments were again seen to alternate in rapid sequence (Fig. 3). Repeated administration of 0.5 mg/kg of propranolol, however, again normalized the irregular ECG (last row in Fig. 3).

Finally, the effect of isoproterenol was studied in both alert and anaesthetized rabbits. In one of the three urethan and atropine-treated rabbits in response to 0.1 mg/kg isoproterenol the 310/min frequency (first row in Fig. 4) increased to 390/min, the P wave increased in height and merged with the T wave of the preceding ventricular complex. It is clearly visible in the

tracings that despite the tachycardia the site of origin of the ST segment and the height of the T wave were variable. Following the administration of propranolol the heart rate decreased the P wave became flatter, and the ventricular deflexions regular and uniform (fourth row in Fig. 4).

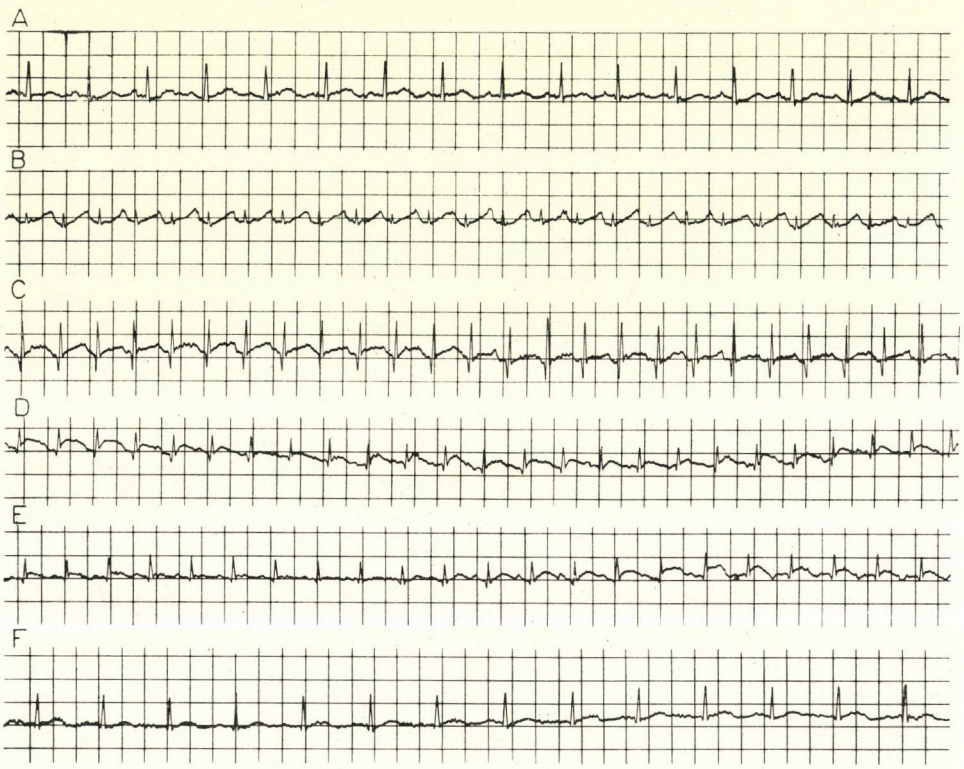

Fig. 5. Influence of 50 μg/kg of isoproterenol then of 2 mg/kg of propranolol on the ECG of the alert rabbit. Tracing A: Alert rabbit, lead II. Lability of T wave. Frequency, 230/min. Tracing B: In response to 50 μg/kg isoproterenol, lability of P wave and ST segment. Frequency, 400/min. Tracing C: Two minutes later. Q deflexion, excessive lability of T wave. Frequency, 340/min. Tracing D: Six minutes later. Excessive lability of ST and T. Frequency, 330/min. Tracing E: At 8 minutes there is still marked lability. Note elevation of ST. Frequency, 330/min. Tracing F: Normal ECG 3 minutes after the administration of 2 mg/kg propranolol. Frequency, 200/min

Even more marked was the influence of isoproterenol on the ECG of the alert rabbit, as shown in Fig. 5. In the alert state 10 to 50 μg/kg of isoproterenol accelerated the heart rate to 400/min and the ECG was labile and variable even 8 minutes later (rows A—F in Fig. 5).

Discussion

The lability of the ECG in the alert rabbit seems to have been ignored in the literature; at the most, it was described as a diurnal change. The cause of this appears to be that most of the experiments were made in deep anaesthesia, to prevent the rabbits from escaping. In deep anaesthesia the lability of the ECG is greatly diminished and may be recognized only at high speed or prolonged recordings. When a variability of the ST—T was detected, it was ascribed to defective depolarization caused by the inadequate application of the plate electrodes.

Our present studies seem to have clarified that the vagus plays no role in the lability of the rabbit's ECG, as atropine in both the alert state and under anaesthesia failed to influence it. Serotonin enhanced lability. In response to propranolol the ECG turned regular in both the alert and the anaesthetized rabbits while isoproterenol made the ECG irregular and labile despite the excessive tachycardia.

The permanent beta adrenergic excitation varied not only individually, but also diurnally in the same animal. Propranolol eliminated the ECG lability caused by the sympathetic tone. As administered in a dose as high as 2 mg/kg, serotonin broke through this sympathetic inhibition, making the rabbit's ECG labile and another dose of propranolol was required to make the ECG regular again.

It is beyond doubt that the excessive sympathetic preponderance in the innervation of the rabbit's heart ensures a permanent state of preparedness and ensures the survival of the animal, which has no other ways and means to protect its life but to run away.

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SOME EFFECTS OF HYPERTONIC NaCl SOLUTION ON THE MECHANICAL AND ELECTRICAL RESPONSES OF SLOW AND FAST MUSCLES OF THE CAT

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The effect of hypertonic NaCl solution on amplitude and time course of twitch and tetanic contraction was studied on gastrocnemius and soleus muscles of the nephrectomized cat. Infusion of 20% NaCl solution into the aorta through one of the renal arteries apparently influenced contractibility by disconnecting the link between excitation and contraction. Twitches and tetanic shortenings in response to electrical stimuli persisted in the slow muscle at a time when tension development was almost absent in the fast muscle. There was no change in excitability and impulse conduction as long as the tension could be elicited. This indicates that in spite of the higher increase in the concentration of intracellular electrolytes, the link between excitation and contraction is more resistant against the hypertonic medium in the soleus than in the gastrocnemius muscle. Thus, the coupling of excitation and contraction seems to be less developed in slow than in fast muscles.

Hodgkin and Horowicz (1957) found that contraction ceased but impulse conduction persisted when a single muscle fibre from the frog's semitendinosus muscle had been immersed in hypertonic Ringer's solution. Hill (1957) suggested that the link between energy release and contraction was disconnected but a connection between excitation and energy release was maintained during this experiment. It has been shown by Howarth (1958) that the duration of the active state and the action potentials were only slightly affected by hypertonic solutions. He supposed that there was not a real uncoupling between excitation and contraction but as a result of a considerable loss of water, the muscle viscosity rose to such an extent that just a slight shortening of the contractile elements could occur during an unaltered period of active state. On the other hand, the contractile proteins were also affected by the change in the ion concentration within the muscle cell.

Although the effect of hypertonic solutions upon excitation-contraction coupling in the frog's sartorius muscle (Pauschinger and Brecht 1959) and in the gastrocnemius muscle of the Laewen-Trendelenburg frog preparation (Varga-Mányi and Tigyi 1962) has been described, little is known of the response of mammalian muscles *in vivo* to extensive changes in extracellular sodium concentration. The aim of the experiments reported here was to study the behaviour of the cat's slow and fast muscles under such conditions.

Methods

The soleus and gastrocnemius muscles from male and female cats ranging in weight from 1800 to 4000 g served as representative slow and fast muscle, respectively. The animals were anaesthetized with 0.08 g/kg body weight chloralose. Through a dorsal incision both renal pedicles were ligated and a polyethylene cannula was introduced into a renal artery.

The appropriate muscles were freed from the surrounding tissues to avoid distorsion of the mechanical response. The limbs were fixed by two screws drilled into the femur and tibia near the distal end. The screws were secured to clamps mounted on an iron frame which supported the prone animal, so that the muscles could move in the horizontal plane.

The appropriate nerve, with all unused branches cut off, was separated from the sciatic nerve trunk and silver electrodes placed on it. For direct stimulation a pair of needle electrodes was inserted into the tendon and the belly of the muscle. A DISA Multistim or a square wave pulse generator of our own construction was used for stimulation.

Isotonic contractions were recorded on a smoked drum or by a DISA Universal Indicator; the reactance converter amplifier fed the signal of the capacitive transducer to the double beam cathode ray tube. Concentric needle electrodes were used for extracellular recording of action potentials, they were connected over a DISA a. c. amplifier to the input of the Universal Indicator.

Infusion of 20% NaCl solution at 1 ml/min rate through the cannula implanted into a renal artery was carried out by an infusion pump. Inulin was used for extracellular space determination. Before the infusion, specimens were obtained from the unused left soleus and right gastrocnemius and at the end of the experiment from the stimulated right soleus and left gastrocnemius. Blood was taken from jugular vein at the beginning and the end of the experiment.

Muscle specimens were weighed, dried overnight at 80°C and then reweighed. The next day fat was extracted and the dry muscle was ground to a homogeneous powder. Approximately 10—20 mg of muscle powder was put into a 15 ml calibrated test tube for inulin determination using the diphenylamine reagent of DISCHE (1953). The remaining muscle powder was dissolved in 0.5 ml HNO_3 (50 v/v) under an infrared lamp and then diluted to 10 ml with ion free distilled water. Determination of Na and K was carried out in diluted samples by a Zeiss flame-photometer. Calculation of intracellular concentrations of Na or K was based on the assumption that for each muscle sample the intracellular water space = (water content) — (inulin space); this was expressed in ml/kg wet wt. A second assumption was that the monovalent cation concentration in the inulin space was equal to that in the blood plasma. The amount of Na or K within the cell could then be estimated by subtracting the amount calculated to be in the inulin space from that found in the whole muscle. The intracellular concentration of Na or K was then determined by dividing this amount by the calculated value for intracellular water.

Results

A typical experiment illustrated in Fig. 1/A shows that the twitches and the tetanic contractions decreased in the fast muscle, and that no mechanical response could be elicited one hour after the infusion. The soleus muscle practically maintained its original contractibility; the tetanus was well maintained and showed no changes in the amplitude of twitch. However, if a higher dose of NaCl was infused, the response of the soleus decreased with time, as shown in Fig. 2. Thus, the electromechanical coupling in the fast muscle dissociated more quickly than in the slow muscle under the same conditions. The twitch response of fast muscle appreciably slowed, both the contraction and the relaxation periods were prolonged (Fig. 3).

It is well known that in the case of an entire muscle the tension of contraction depends on the strength of the stimulus applied. As an effect of hyper-

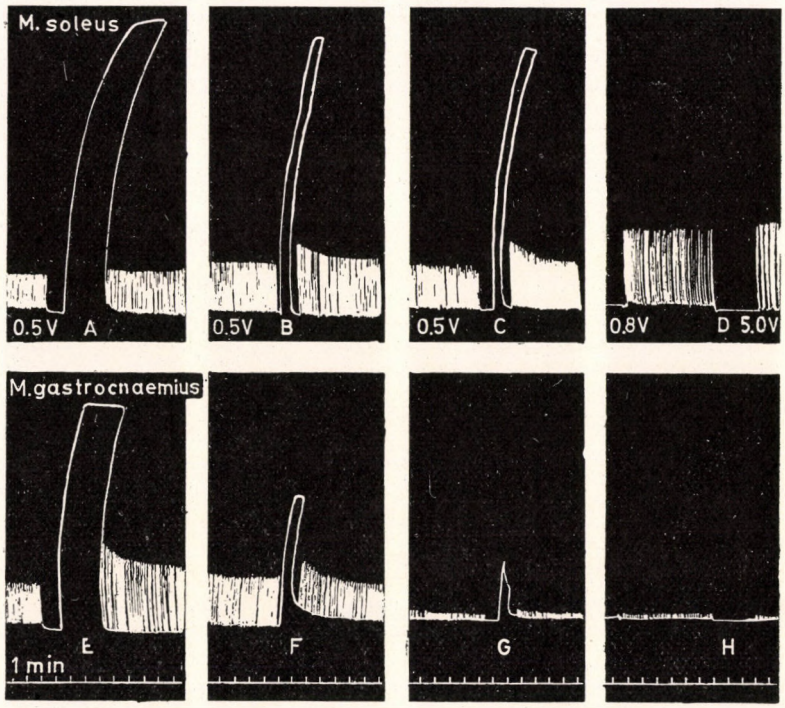

Fig. 1A. Effect of hypertonic NaCl infusion on twitch and tetanus responses of soleus (A—D) and gastrocnemius (E—H) muscles. Cat, 2.5 kg, chloralose anaesthesia. Twitches were elicited by 1 V rectangular pulses of 0.5 msec duration applied to the sciatic nerve every second. Tetanus was evoked by 25 shocks/sec. Between A and B, as well as E and F, 12 ml of 20% NaCl solution was infused into the external jugular vein. Records B and F were taken 5 min after the infusion; C and G 35 min after it. The responses in D and H were obtained by direct stimulation 55 min after the salt load

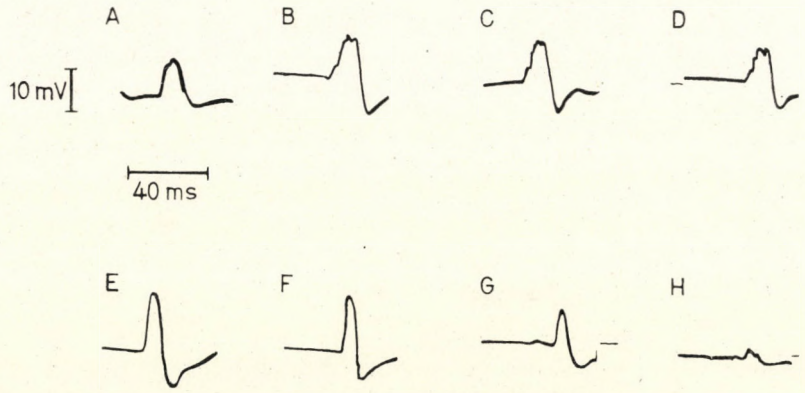

Fig. 1B. Effect of hypertonic NaCl on diphasic action potentials. Simultaneous recordings from both muscle (A—D soleus; E—H gastrocnemius)

tonic saline it was observed that the gradation in the tension disappeared and the entire muscle whether gastrocnemius or soleus obeyed an all or none law in the majority of experiments. The mechanical response could be elicited with the same threshold stimulus as before the infusion, but increasing the strength of stimuli 5—15 times above the normal threshold failed to enhance the am-

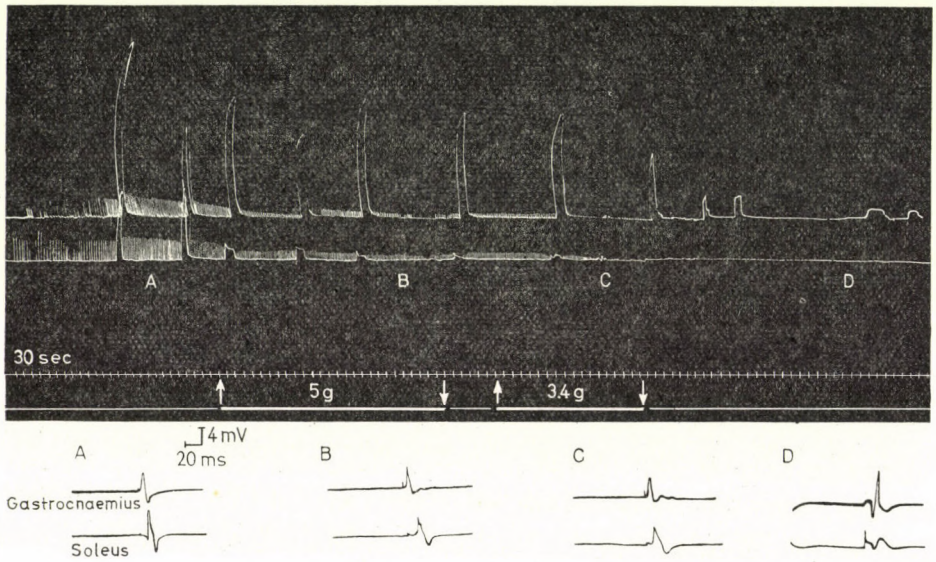

Fig. 2. Cat, 4.0 kg, chloralose anaesthesia. Upper tracing, soleus muscle; lower tracing, gastrocnemius. Saline infusion into aorta through one of the renal arteries started at the upward arrow and stopped at downward arrow. Figures between them indicate the amount of NaCl added. Muscle contraction excited by 2 V rectangular pulses of 1 msec duration, every 4 sec. The action potentials (A—D) were recorded simultaneously during the period indicated by the corresponding letters on the upper two records

plitudes. This was not due to a block of transmission in the neuromuscular junction since the phenomenon was equally observed on direct or indirect stimulation. This is clearly proved by Figs 1 B and 2 where shape and duration of the action potentials elicited by indirect stimulation after the infusion were similar to those obtained before the infusion. It can be seen in Fig. 4, which was recorded before a complete uncoupling of the electro-mechanical connection had taken place, that the gastrocnemius did not respond to each shock and contracted in irregular periods in spite of the fact that each stimulus had produced a propagated action potential.

The effect of hypertonic saline on tetanic shortening was also investigated. Muscles were stimulated directly or through their nerves at 25—50 shocks/sec for 20 sec and during this time complete tetanus at maximal tension was recorded. Figs 1 and 2 illustrate that there was a sudden drop in

the contraction amplitude of the gastrocnemius after saline infusion and a pronounced fatigue of tetanus could be observed. The slow muscle did not show any sign of fatigue during short tetanic stimulation, moreover tetanus could still be elicited when the muscle had already failed to respond to single test stimuli. The fatigue of tension following hypertonic NaCl infusion was accom-

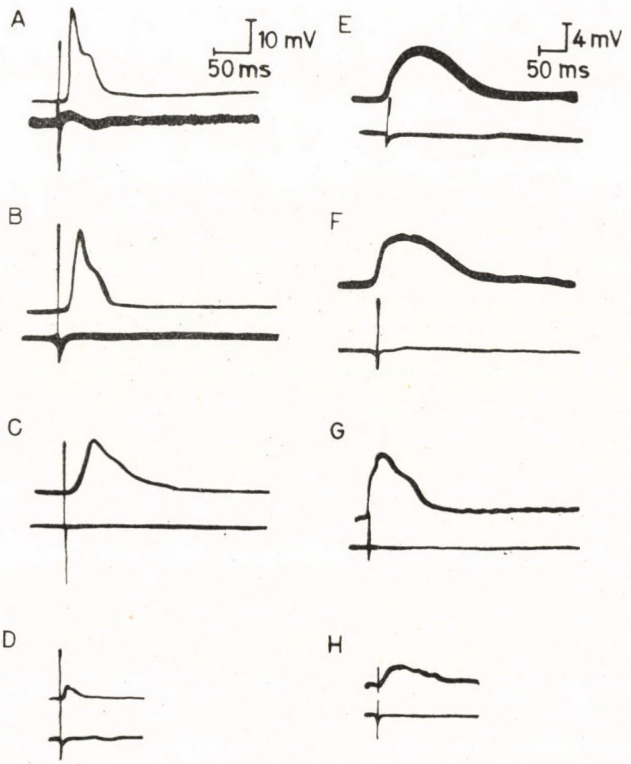

Fig. 3. Twitches (upper tracing) and action potentials (lower tracing) recorded synchronously from gastrocnemius (A—D) and soleus (E—H) muscles. Records A and E were taken before the hypertonic saline infusion, B and F after a tetanus of 30 sec duration. Between B and C, as well as F and G, 22 ml of hypertonic NaCl solution was infused into the aorta. D and H were taken 30 min after a second infusion. Indirect stimulation with 0.2 V rectangular pulses of 1 msec duration. The speed of sweep and gain were unchanged during the experiment

panied by a decrease in the amplitude of the action potentials in the gastrocnemius. An example of this is shown in Fig. 5 where the action potentials recorded with external electrodes from the gastrocnemius and soleus muscles in response to stimulation of their respective motor nerve are seen. The tetanic stimulation produced a progressive drop in the height of the action potential, and a lengthening in the rate of rise and fall of the spike in the gastrocnemius muscle. However, in the soleus muscle the action potential's height showed

a remarkable increase in response to a tetanic stimulation of 50 shocks/sec. Fig. 5 clearly demonstrates that the conditioning tetanus was followed by rapid recovery and the height of the action potential was the same after as before the tetanus.

Increasing the concentration of NaCl in the extracellular fluid by repeated infusions induced a rapid fatigue of both electrical and mechanical

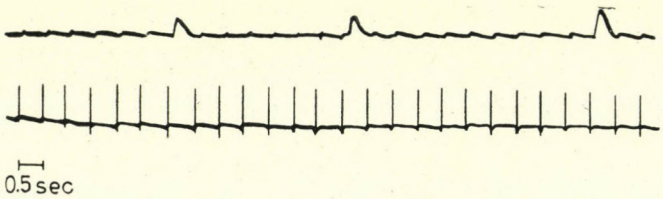

Fig. 4. Incomplete uncoupling of electro-mechanical connection in gastrocnemius. Upper tracing, mechanical responses; lower tracing, action potentials. Record taken 70 min after injecting 4 g NaCl intravenously. Stimulation of sciatic nerve with 1 V rectangular pulses every 2 sec

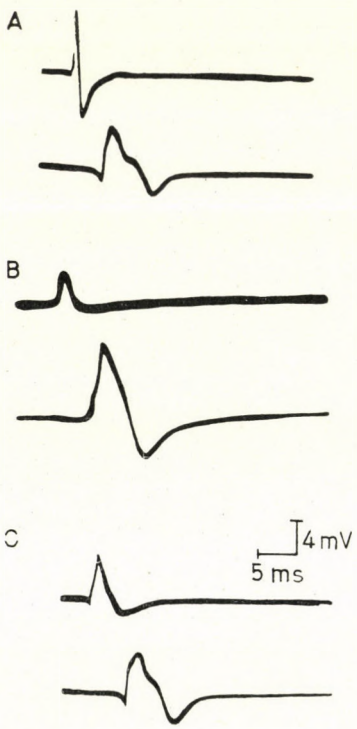

Fig. 5. Effect of tetanic stimulation and hypertonic infusion on action potentials of gastrocnemius (upper tracing) and soleus (lower tracing). Records taken 40 min after a saline infusion. A, before tetanic stimulation; B, during tetanic stimulation; C, after tetanus. Indirect stimulation with 0.3 V, 0.5 msec impulse duration

activity. A typical effect of sodium at high concentration on the action potentials and tetanic shortening of the gastrocnemius induced by a train of 30 shocks/sec lasting for 25 sec are illustrated in Fig. 6. This shows a sudden drop in potential height after the first few potentials followed by a short plateau indicating no appreciable decline in spike amplitude. The decrease of tension developed during the plateau of action potentials indicates that the

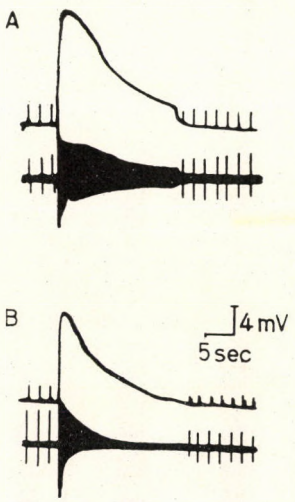

Fig. 6. Fatigue of tetanic tension and action potentials. Cat, 3.8 kg. Records taken from gastrocnemius. A, 60 min after administration of 10 ml of 20% NaCl solution; B, 10 min after a second infusion of 10 ml hypertonic saline. Twitches were evoked by direct stimulation with 0.4 V rectangular pulses of 1 msec duration, every 2.5 sec. Tetanic frequency, 30 shocks/sec

failure of tension cannot be due solely to the fatigue of spikes. After a repeated infusion the short plateau disappeared and only a rapid fatigue was observed in both mechanical and electrical activity. With the soleus muscle, the increase in height of the action potentials caused no change in the tension of tetanus.

In our experiments the electro-mechanical connection of the slow muscle proved more stable against the effect of hypertonic solution than that of fast muscle. A simple explanation for this behaviour could be a difference in ion content and water loss due to the hypertonic solution. It was therefore of interest to study the effect of hypertonic saline infusion on the intracellular concentration of sodium and potassium ions. Estimation of inulin space, water content and ion concentration were done for each muscle type before and after the infusion, as described under Methods.

It was found that the inulin space was lower in the control fast muscle than in the control slow one. The mean inulin space in the gastrocnemius was

78 ml/kg wet weight (S. E. = 3.9) as compared to the mean 123 ml/kg wt. w. (S. E. = 5.7) for the soleus. The mean control values for Na were, 148 mM/l in plasma; 22 mM/kg intracellular water (S. E. = 3.5) in the soleus; and 14.5 mM/kg i. c. w. (S. E. = 3.0) in the gastrocnemius. Changes in sodium and potassium concentration in muscle cells and plasma are presented in Fig. 7. Hypertonic NaCl solution caused a marked increase in Na^+ and K^+ concentration.

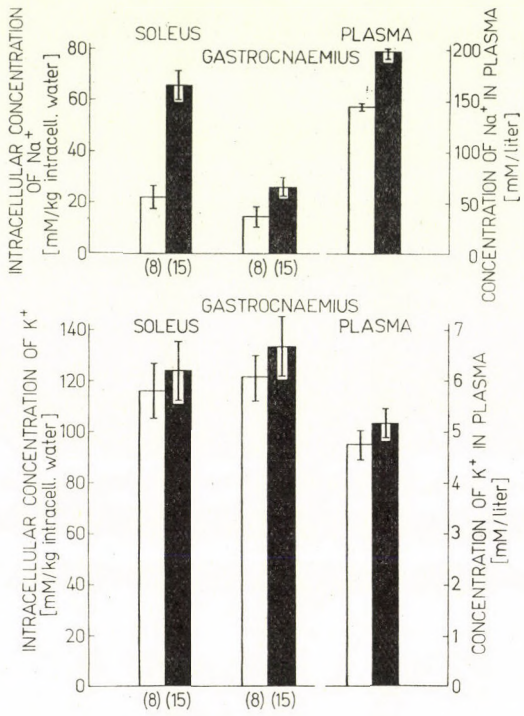

Fig. 7. Effect of infusion of hypertonic NaCl solution on intracellular sodium and potassium concentration in soleus and gastrocnemius muscles as well as on plasma Na^+ and K^+ concentration. Open columns, mean values $\pm$S. E. of cation concentration in control muscles or plasma before the infusion. Black columns, after the infusion, at end of experiments. Numbers in brackets at the bottom of columns denote number of muscles used

A comparison of intracellular sodium concentration in slow and fast muscles showed that there was a threefold increase in the soleus as compared to a 1.8 fold on in the gastrocnemius following hypertonic saline treatment. As can be seen in Fig. 7, there was a slight increase in intracellular potassium concentration in both fast and slow muscles.

Discussion

In agreement with previous reports (HODGKIN and HOROWICZ 1957; PAUSCHINGER and BRECHT 1959; VARGA-MÁNYI and TIGYI 1962), our experiments indicated that the electro-mechanical coupling of mammalian skeletal muscle is disconnected by hypertonic salt solution. A remarkable finding was a more prolonged excitation-contraction coupling in the soleus than in the gastrocnemius. A simple explanation for this behaviour could be a difference in muscle viscosity due to the water loss induced by the excess NaCl (HOWARTH 1958). However, the fact that hypertonic saline had little effect on the contraction of slow muscle and induced a higher sodium uptake in this muscle than in the fast one is inconsistent with any role of viscosity in the uncoupling process.

The effect of hypertonic solution seemed to be mediated in a complex way. It affected the refractory period as well as the propagated action potentials in the gastrocnemius, especially during tetanic stimulation. No attempt has therefore been made to relate the dynamic properties of the muscle with the changes which take place in the muscle fibre under the effect of hypertonic saline.

The present results indicate that the mammalian muscle is composed of fibres distinctly sensitive to environmental changes. This sensitivity seemed to be in connection with intracellular sodium concentration and sodium uptake. In the soleus muscle intracellular sodium concentration was higher and sodium uptake twice as much than in the gastrocnemius, and electromechanical coupling was more stable against the hypertonic medium in the slow than in the fast muscle.

It seems therefore that the excitation-contraction coupling is less developed in the cat's slow than in its fast muscle. This is supported by the observation of CLOSE (1964) showing that at birth isometric contraction is similar in fast and slow muscles and subsequent development leads in the gastrocnemius to a decrease in the duration of the active state and the force velocity properties. All this agrees well with the findings of VARGA et al. (1962) in that the contractile protein of tonic muscles proved to be less differentiated than that of tetanic muscles.

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RELATIONSHIPS BETWEEN THE STRUCTURE AND THE ADRENAL AND EXTRA-ADRENAL EFFECTS OF ACTH FRAGMENTS

By

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It has been shown earlier that ACTH augments adrenal and, extra-adrenally, ovarian blood flow. It has therefore been investigated in the dog whether these effects might change with changes in the length of the peptide chain. Organ blood flows were determined by the thermocouple method. Corticosteroid secretion was measured in hypophysectomized animals. Natural ACTH and fragment activities were compared on a weight basis with the activity of fragment 1—24. Fragment 1—14 was found to increase blood flow in both organs, though to a level which is several times lower than that of fragment 1—24. The extra-adrenal effect, however, was several orders higher than the adrenal effect. Natural porcine and synthetic human ACTH augmented blood flow much less than did the fragments 1—24 and 1—28. Fragment 5—14 and fragment 1—14 oxidized in position 4 were ineffective, even in tenfold the effectiv amount of fragment 1—14. Identical doses of fragment 5—14 induced a greater increase in corticoid secretion than did fragment 1—14. Thus, changing the length of the ACTH fragment influences the blood flow-raising adrenal effect in a measure different from the extra-adrenal one; moreover, it may affect in different degrees also the action of the same fragment on adrenal blood flow and corticoid secretion.

Recognition of the extra-adrenal adipokinetic and hypoglycaemic activities of ACTH (ENGEL and ENGEL 1954; ENGEL 1961) brought in its train the question whether structural changes in the molecule would be followed by changes in the adrenal and extra-adrenal effects. The first attempts to find the answer were undertaken by means of chemical modifications of native ACTH preparation (STEELMAN and SMITH 1960; BORIGHT et al. 1962) before the hormone had been synthetized.

With the successful synthesis of ACTH-like peptides culminating in the synthesis of the complete ACTH molecule, studies of structure-activity relationships have come to the fore. Such studies were mainly concerned with the role played by chain-length and by individual amino acids in the adrenal effect of ACTH and in the activity of MSH (LI 1962; KAPPELER 1964; BLAKE and LI 1967; for others, see SCHRÖDER and LÜBKE 1966).

The relationships between chain-length and extra-adrenal effect of synthetic ACTH fragments were studied by LEBOVITZ (1964).

It has been shown earlier (STARK et al. 1967, 1968; VARGA et al. 1969) that ACTH raised both adrenal and ovarian blood flow. The latter being an extra-adrenal effect, it seemed interesting to study if these effects would

change with changes in the length of the peptide chain. SCHWYZER (1963) holds that for its corticoid-stimulating activity ACTH depends on the presence of the 1—3 N-terminal amino acid sequence and the 4—10 heptapeptide, and that decisive for it is the 15—18 basic sequence. BOHUS and DE WIED (1966) and GREVEN and DE WIED (1967) found that the 1—10 and even the 4—10 fragments inhibited the extinction of the avoidance reflex extraadrenally. Accordingly, we studied the adrenal and ovarian flow-raising effect of the 5—14 and 1—14 fragments, of the 1—28 fragment which corresponds to the sequence of porcine ACTH, and of natural porcine and synthetic human ACTH. Oxidation with hydrogen peroxide of the methionine in position 4 is known greatly to reduce the adrenal effect of ACTH, as estimated on the basis of ascorbic acid depletion, and the extra-adrenal lipolytic effect (LEBOVITZ 1964). Our experiments were therefore extended to include a 1—14 fragment with oxidized methionine in position 4.

Methods

The effects of the ACTH fragments on corticosteroid secretion were studied in dogs weighing 10—20 kg, anaesthetized with chloralose-urethane. Organ blood flow was determined by the method of HENSEL et al. (1954). In characterizing blood-flow changes, peak and duration of the effect were considered (Figs 1 and 2). The corticoid secretion stimulating effect of the peptides was investigated within two hours after hypophysectomy. The peak effect of the peptides presented itself five minutes after their intravenous administration, and so the increment measured at that time was taken to be the characteristic of the effect. The effects the individual peptides exerted on blood flow and on corticosteroid secretion were compared on the basis of the regression straights yielded by the dose-effect curve. The effects of the ACTH fragments were compared on a weight basis with the activity of fragment 1—24 (Synacthen, CIBA); since their regression straights were significant and adequately parallel, they allowed a reliable comparison.

Results and discussion

Figs 1 and 2 show that natural porcine and synthetic human ACTH induced a considerably lower rise in blood flow than did the fragments 1—24 and 1—28. Flow was also increased by fragment 1—14 although its activity was lower than that of the entire molecule. However, by this peptide ovarian blood flow was raised several times more than was adrenal blood flow. Fragment 5—14 and the oxidized fragment 1—14 were ineffective, even in tenfold the amount of fragment 1—14.

The peptide effects on corticoid secretion are shown in Fig. 3. The regression straights, particularly those for fragment 5—14, did not completely fulfil the criterion of parallelism. This does give cause for reservations, but hardly justifies one to visualize a straight of another direction. All the regression straights but the one for the oxidized fragment 1—14, were significant statistically (Table I). The exception may be due to the inefficacy of the smallest dose applied. More exact determination of the corticoid-secretory activity of this fragment requires further investigations.

It thus seems that the 5—14 and 1—14 peptides increased corticoid secretion, though their activity was several orders lower than that of either the 1—24 or the 1—28 fragment.

Our results appear to justify the following conclusions.

(i) When an ACTH fragment is increased in length within limits, its effect on blood flow and corticoid secretion will follow essentially the same

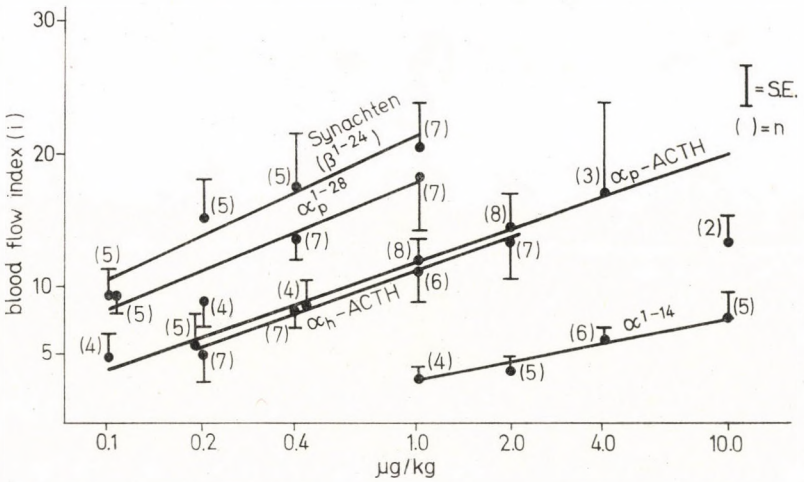

Fig. 1. Effect of synthetic ACTH fragments on adrenal blood flow

$I = \sqrt{(\text{maximum increase, per cent}) \cdot (\text{duration of increase, min.})}$

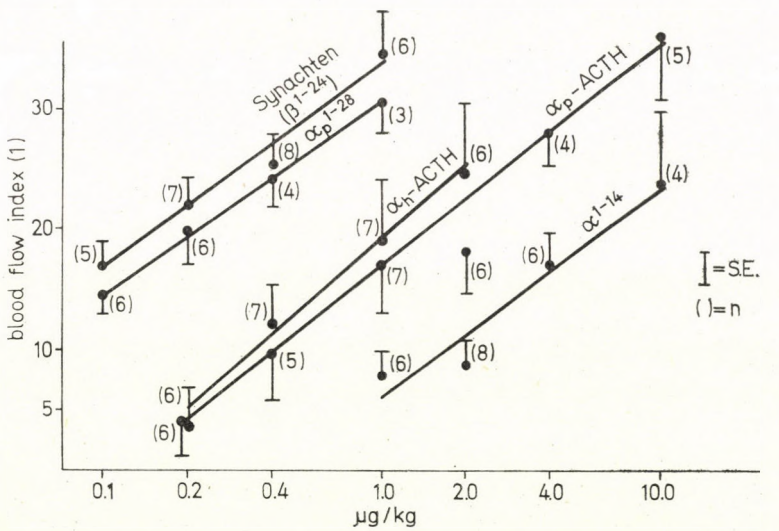

Fig. 2. Effect of synthetic ACTH fragments on ovarian blood flow

$I = \sqrt{(\text{maximum increase, per cent}) \cdot (\text{duration of increase, min.})}$

trend: both these effects of the 1—24 fragment, which contains the 15—18 partial basic sequence essential for corticoid secretion, will be several orders greater than those of the 1—14 fragment.

(ii) To lengthen the 1—24 fragment seems to offer no advantage as regards blood flow since in this respect the fragment is several times as effective as is the entire molecule. This is presumably due to the presence of the acid C-terminal molecular part. The effectiveness of the 1—28 fragment evidently falls between the two.

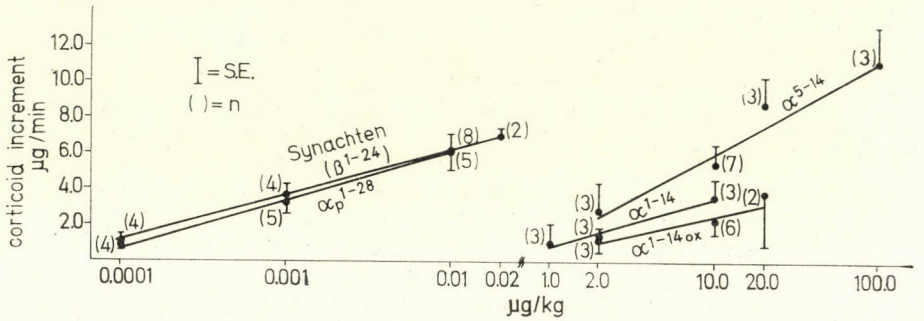

Fig. 3. Effect of synthetic ACTH fragments on corticosteroid secretion

Table I

Effectiveness of differently long synthetic ACTH fragments

✳	Relative potency			Regression straights significance		
	corticoid secretion	blood flow		corticoid secretion	blood flow	
		adrenal	ovary		adrenal	ovary
β^{1-24}	100	100	100	$p < 0.001$	$p < 0.005$	$p < 0.001$
α_h-ACTH		14	19		$p < 0.01$	$p < 0.001$
α_p-ACTH		10	12		$p < 0.001$	$p < 0.001$
α^{1-28}	77	56	70	$p < 0.001$	$p < 0.05$	$p < 0.01$
α^{1-14}	0.008	0.1	2.4	$p < 0.05$	$p < 0.05$	$p < 0.001$
$\alpha^{1-14\,ox}$	0.003	<0.1	<0.1	$p < 0.10$		
α^{5-14}	0.02	<0.1	<0.1	$p < 0.01$		

(iii) As regards the increasing effect on blood flow, a major importance seems to attach to the N-terminal 1—4 partial sequence since many times the amount of the 5—14 fragment that increases corticoid secretion is ineffective on flow.

(iv) Lengthening the ACTH fragment influences the blood flow-raising adrenal effect to an extent different from the extra-adrenal one; moreover,

it may affect in a different degree also the action of the same fragment on adrenal blood flow and corticoid secretion.

Our findings provide conclusive evidence that a chemical modification of the ACTH molecule and an alteration in its chain-length may separate the adrenal from the extra-adrenal effect.

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CORTICOTROPHIN RELEASE INDUCED BY TRAUMATIC STRESS IN RATS WITH UNILATERAL SPINAL CORD LESION

By

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Corticotrophin release induced by the traumatic stress of a leg fracture has been studied in the rat. Hemisection of the cervical spinal cord induced a lasting inhibition of the ACTH releasing effect of a contralateral leg fracture, showing that the pathways conveying ACTH releasing information are crossing the midline. Since lateral column lesions on the contralateral side also induced a significant inhibition of the response, the pathways of traumatic stress seem to be localized within, or in the vicinity of the spinothalamic tract.

Corticotrophin (ACTH) release normally induced by breaking the femur failed to occur in rats with their spinal cord transected (MATSUDA et al. 1964). Spinal cord transection blocks the ACTH releasing effect of other stressful stimuli such as surgical incision (EGDAHL 1959), electrical stimulation (REDGATE 1962) or subcutaneous injection of a small dose of formaldehyde (MAKARA et al. 1967, 1969). The present study was designed to locate the spinal pathways by which traumatic stress is triggering ACTH release.

Methods

Male rats of CFE strain weighing initially 160 to 250 g were used. The rats were housed in a constant temperature room (24°C and 55—75% humidity) and given commercial rat pellets and tap water ad libitum. They were habituated to handling and receiving injections at least 10 days prior to the stressing procedure.

Under ether anaesthesia, the spinal pathways were transected with a fine sharp wire at the level of the 7th cervical vertebra. The spinal cord was transected on the left side in most animals; in one series only the lateral column was lesioned. After the operation the rats were given 1.5 mg of oxytetracyclin subcutaneously daily for four days. Seven to eight days or 3 months after the operation the rats were given 3 mg/100 g pentobarbital (Mebubarbital, Rhône-Poulenc, Paris) intraperitoneally and 20 minutes later one femur was broken with forceps. Blood was withdrawn 30 minutes later. As an index of ACTH release, the plasma corticosterone level was estimated by the method of GUILLEMIN et al. (1958). The spinal column was fixed in 10% formaldehyde or Bouin's solution and a piece of the cord containing the lesion was embedded in paraffin and serially sectioned at a thickness of 10 μ. Sections were stained with haemalum-eosin or Luxol Fast Blue-cresyl violet (KLÜVER and BARRERA 1953). The hemisections did not deviate from the midline more than about 100 μ (Figs 1, 2). The lateral column lesions had divided all the fibres lateral to the dorsal horn (Fig. 3); sometimes a small lateral portion of the anterior column was also lesioned.

Values for plasma corticosterone concentration were transformed to logarithms and analyses of variance were carried out. Comparisons were made by the method of SCHEFFÉ (1959).

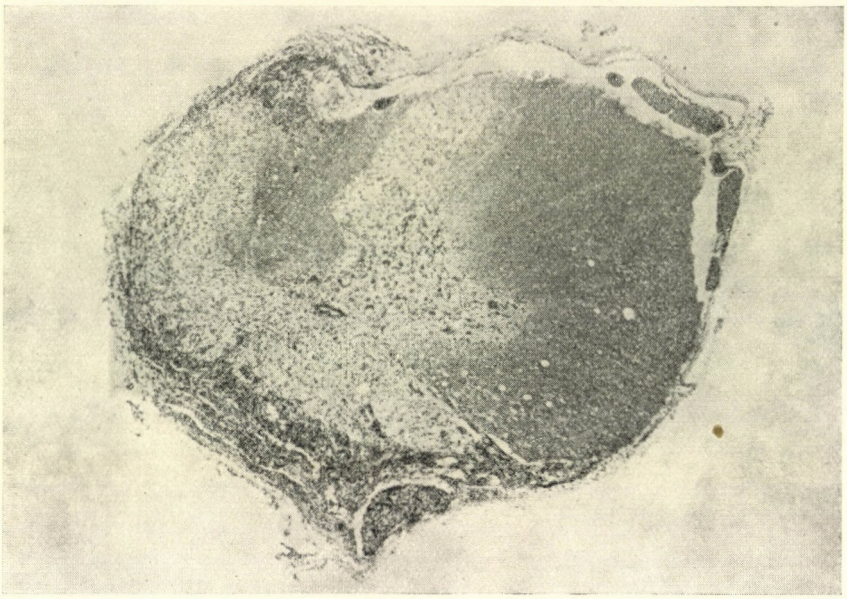

Fig. 1. Frontal section through the cervical spinal cord. Luxol Fast Blue-cresyl violet stain. One week after left hemisection of the spinal cord

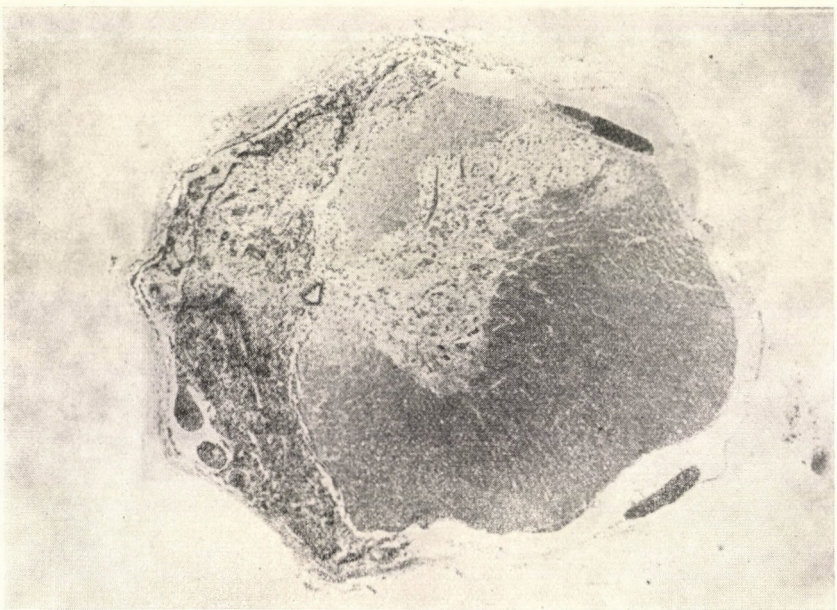

Fig. 2. Frontal section through the cervical spinal cord. Luxol Fast Blue-cresyl violet stain. 3 months after left hemisection of the spinal cord



Fig. 3. Frontal section through the cervical spinal cord. Luxol Fast Blue-cresyl violet stain
One week after lateral column lesion on the left side

Results

In the rats with spinal cord hemisection the contralateral hind leg was paralysed and nociceptive responses to mechanical stimuli were absent for a few days after the operation. Sensory and motor functions returned gradually; on the 7th to 8th day there was still some functional impairment. In the rats with lateral column transection no overt motor disturbance was found but nociceptive responses to mechanical stimuli were absent on the contralateral side for a few days after operation. One to three months after spinal cord hemisection the behaviour of the rats was practically normal.

After hind leg fracture, the plasma corticosterone level of sham-operated rats rose significantly [44.3 ± 1.8 μg/100 ml (12)]. When in the rats with spinal cord hemisection the homolateral hind leg was fractured, the plasma corticosterone level rose as in the former case (Table I). On the other hand, it was significantly lower in the group with contralateral fracture; in these animals the level was not significantly above that recorded in the non-stressed controls (Table I). The response to the traumatic stress of a fractured leg was similarly decreased in rats with a contralateral lesion of the lateral column. However, there was a small but significant rise above the level displayed by the non-stressed controls (Table II).

Table I

Effect of hind leg fracture on the plasma corticosterone level in rats after spinal cord hemisection

Group	Time elapsed after hemisection	Treatment*	Plasma corticosterone (μg/100ml)	Significance
1	7 to 8 days	no trauma	15.2±3.4 (13)**	1—2 $p < 0.01$
2	7 to 8 days	homolateral fracture	50.2±3.7 (11)	2—3 $p < 0.01$
3	7 to 8 days	contralateral fracture	23.8±5.7 (11)	
4	three months	no trauma	11.1±1.7 (3)	
5	three months	homolateral fracture	39.3±3.4 (9)	5—6 $p < 0.01$
6	three months	contralateral fracture	18.5±3.2 (5)	

* The rats were anaesthetized with pentobarbital
** $\overline{X} \pm S_{\overline{x}}$ (number of rats)

Table II

Effect of hind leg fracture on plasma corticosterone level in rats with transection of the lateral column

Group	Treatment*	Plasma corticosterone, μg/100 ml	Significance
1	no trauma	6.9±0.5 (7)**	1—3 $0.10 > p > 0.05$
2	homolateral leg fracture	49.6±4.3 (6)	2—3 $p < 0.01$
3	contralateral leg fracture	17.4±4.5 (10)	

* The rats were anaesthetized with pentobarbital
** $\overline{X} \pm S_{\overline{x}}$ (number of rats)

As the motor impairment caused by the hemisection disappeared almost completely after a period of 1 to 3 months, it was studied whether the impairment of the response to traumatic stress applied contralaterally would also disappear. This was not the case as the contralateral fracture induced no change in the plasma corticosterone level (Table I).

Discussion

In a previous study of ACTH release induced by a small subcutaneous dose of formaldehyde we have found evidence indicating that the nerve fibres conveying the ACTH releasing impulses are crossing the midline, to ascend in the spinal cord on the contralateral side (MAKARA et al. 1969). GIBBS (1969) studied the effect of traumatic stress on ACTH release in rats with spinal cord hemisection and concluded that the pathways of this stimulus ascended on

the contralateral side. In the present experiments we repeated our previous study with traumatic stress as a stimulus. Our findings have confirmed those of GIBBS. Moreover, the findings in animals with lateral column section indicated that the main pathways of the traumatic stress of a leg fracture are located in the lateral column on the contralateral side, i.e. in the vicinity of, or within, the spinothalamic tract. Some fibres carrying ACTH releasing information must, however, be outside the contralateral lateral column, since a contralateral fracture induced a small but significant rise of plasma corticosterone over the non-stressed control level. In contrast to the relatively short-lived impairment of motor function by cord hemisection, the block of ACTH releasing spinal pathways was protracted and showed no important functional reserve.

Acknowledgement

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EFFECT OF HYPOTHALAMIC STIMULATION ON ADRENAL BLOOD FLOW AND GLYCOCORTICOID RELEASE PRIOR TO AND AFTER ACUTE HYPOPHYSECTOMY

By

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The effect of medial hypothalamic stimulation on adrenal venous outflow, adrenocortical hormone release, and systemic circulation has been studied prior to and after hypophysectomy in anaesthetized dogs.

Hypothalamic stimulation increased adrenal blood flow and arterial pressure in most cases, but circulation may change independently from pressure. There was no correlation between corticoid release and blood flow on stimulation prior to hypophysectomy, while after hypophysectomy a positive correlation of the responses was observed. In the latter case an increase in corticoid release was frequent. The hormonal background may influence the effect of hypothalamic stimulation.

It is concluded that adrenal blood flow is one of the factors of extrahypophyseal corticoid mobilization. Their coordination is probably organized at the hypothalamic level.

A number of data obtained by electrical stimulation shows that certain parts of the hypothalamus, especially the central and posterior medial regions may play an important role in the activation of the ACTH-adrenal system (De Groot and Harris 1950; Hume 1953; Porter 1953; Mason 1958; Endrőczi and Lissák 1960, 1964; Suzuki et al. 1960; Slusher and Hyde 1966; etc.). Despite the fact that with an increase in adrenocortical function the tasks of the blood flow are greatly augmented (ACTH transport from the pituitary to the adrenal, continuous transport of the hormones produced, covering of the metabolic needs), it is not known whether the hypothalamus has a role in the regulation of adrenal blood flow, the coordination of the organ's function and of its blood flow.

In the present study the effect of electrical stimulation of the medial hypothalamic region on the adrenal venous outflow and glycocorticoid release has been investigated in anaesthetized dogs. Part of the study was carried out 1—2 hours after hypophysectomy in order to obtain data on the role of extrapituitary factors in adrenocortical activation. The effect of the hypo-

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thalamic stimulation on the adrenals was compared with the systemic haemodynamic changes also.

Some details of the results have been reported earlier (KOVÁCH 1965; MONOS et al. 1967).

Methods

The experiments were carried out on 24 dogs under pentobarbital (30—35 mg/kg body weight) anaesthesia.

On the day of the experiment, in six animals unipolar, glass isolated stainless steel electrodes (free tip, 0.4—0.5 mm; diameter, 0.2 mm) were implanted into the antero- and postero-medial hypothalamus bilaterally by the stereotaxic technique (LIM et al. 1960). The hypothalamus were stimulated by means of square wave pulses bilaterally (5 V, 1 msec, 45 cps.) and 1—1.5 hours after hypophysectomy, stimulation with the same parameters was repeated. Prior to and during each stimulation, adrenal glycocorticoid release, adrenal venous outflow, arterial pressure, cardiac output, regional blood flow, pulse rate and frequency of breathing were recorded by means of an Alvar Polygraph.

Corticoid content of adrenal venous plasma was determined by paper chromatography. After chromatography, carried out in ligroin, the plasma extracts were chromatographed twice in the Bush B_5 system. After purification and separation, hydrocortisone and corticosterone were detected with alkaline tetrazolium blue, eluted with ethylacetate-methanol and the extinctions were measured at 560 mμ wavelength with a Beckmann spectrophotometer. Corticoid release was estimated in 10 min samples and expressed in μg/h/kg body weight. The hypothalamus was stimulated within 5 minutes after taking the control blood sample.

To determine adrenal blood flow, after an abdominal midline incision a polyethylene cannula was inserted into the left lumboadrenal vein, then the side branches and the adrenocaval vein were ligated. The blood flowing from the cannula was dropping on the piezoelectric detector of a Jacquet drop counter and from here it was collected in a graded cylinder kept in ice. The measurements were carried out after carefully closing the abdominal cavity.

Arterial blood pressure was measured by means of a Sanborn electromanometer connected with the left femoral artery. Pulse rate was registered with the ECG.

Cardiac output and regional blood flow were determined by the thermodilution method. Physiological saline of room temperature was used as an indicator; it was injected at a rapid rate into the ascending ("A"), thoracic ("V") and abdominal ("P") aorta. The changes in temperature were detected above the aortic bifurcation by means of a thermocouple. Blood flow values were expressed in ml/min/kg body weight. Respiratory rate was estimated by a thermistor placed into the tracheal cannula. Details of these methods were published earlier (MONOS et al. 1965, 1967).

Ten further dogs with intact hypophysis were used for studying adrenal blood flow and arterial blood pressure after unilateral stimulation. In 8 hypophysectomized animals the infundibular region was stimulated with concentric bipolar electrodes through an aperture of the base of the skull.

The location of the electrodes was determined after sacrifice in the formalin fixed brains with 1 mm accuracy and described by the coordinates of the atlas of LIM et al. (1960).

At evaluation of the data, for the changing factors a time average was calculated from the half minute representative samples and this served for expressing the values obtained prior to and during hypothalamic stimulation. Correlation coefficients (r) were calculated for the per cent changes in the parameters.

Results

Altogether 41 different hypothalamic sites were stimulated in 16 dogs with intact pituitary (Fig. 1). In 30 cases (73%) was an increase of more than 5% (range, 5—45%) observed in adrenal blood flow; of these, in 15 cases arterial blood pressure was unchanged or decreased (Table I). The correlation coef-

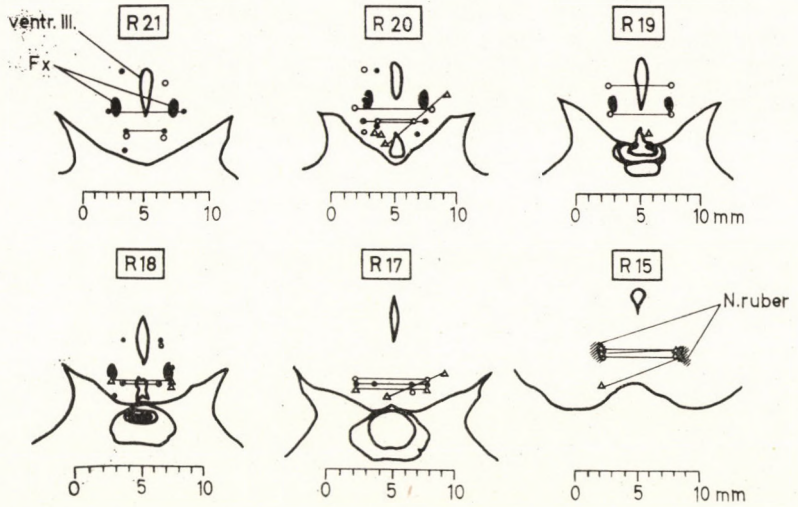

Fig. 1. Localization of hypothalamic stimulation with intact hypophysis. ●, ○: the sites of stimulation which caused more than 5% increase in adrenal blood flow. (●: the decrease in adrenal vascular resistance was more than 5%). △ ▲: the sites of stimulation from where no increase in flow was elicited. (▲: the decrease in adrenal vascular resistance was more than 5%). The sites of bilateral stimulation are connected with lines

Table I

Distribution of the direction of changes induced by hypothalamic stimulation in non-hypophysectomized dogs

Arterial pressure	Adrenal blood flow ↑	Adrenal blood flow —	Adrenal blood flow ↓	Adrenal blood flow Σ
↑	15 (36.6)	2 (4.9)	3 (7.3)	20 (48.8)
—	9 (22.0)	2 (4.9)	2 (4.9)	13 (31.8)
↓	6 (14.6)	1 (2.4)	1 (2.4)	8 (19.4)
Σ	30 (73.2)	5 (12.2)	6 (14.6)	41 (100.0)

Abbreviations: ↑ increase (⩾ 5%); — no change; ↓ decrease (⩾ 5%); upper figures: number of stimulations; lower figures: value in per cents of the total number of stimulations

ficient calculated on the basis of relative changes was 0.42 ($0.01 > p > 0.001$). Under the effect of stimulation, the decrease in adrenal vascular resistance attained more than 5% in 41%, and more than 10% in 37% of the cases. Hydro-

cortisone and corticosterone release changed in the same direction as the blood flow in 11 out of 20 cases investigated ($r = 0.09$). The correlation of blood pressure and blood flow ($r = 0.61$; $0.01 > p > 0.001$) was similar as previously (Table II).

Table II

Distribution of the direction of changes induced by hypothalamic stimulation in non-hypophysectomized dogs

Corticoid output	Adrenal blood flow ↑	Adrenal blood flow –	Adrenal blood flow ↓	Adrenal blood flow Σ
↑	7 (35.0)	1 (5.0)	1 (5.0)	9 (45.0)
–	1 (5.0)	2 (10.0)	0 (0)	3 (15.0)
↓	6 (30.0)	0 (0)	2 (10.0)	8 (40.0)
Σ	14 (70.0)	3 (15.0)	3 (15.0)	20 (100.0)

Arterial pressure	Adrenal blood flow ↑	Adrenal blood flow –	Adrenal blood flow ↓	Adrenal blood flow Σ
↑	7 (35.0)	1 (5.0)	2 (10.0)	10 (50.0)
–	4 (20.0)	2 (10.0)	1 (5.0)	7 (35.0)
↓	3 (15.0)	0 (0)	0 (0)	3 (15.0)
Σ	14 (70.0)	3 (15.0)	3 (15.0)	20 (100.0)

Abbreviations: ↑ increase (⩾ 5%); – no change; ↓ decrease (⩾ 5%); upper figures: number of stimulations; lower figures: value in per cents of the total number of stimulations

In 14 hypophysectomized dogs, 37 hypothalamic regions were stimulated (Fig. 2). In 62% of these there occurred a 5 to 170% increase in corticoid release which in 18 out of 23 cases was associated with a 5 to 45% increase in adrenal flow (Table III). The correlation coefficient for the relative change of adrenal blood flow and corticoid release was 0.34 ($0.05 > p > 0.02$). The change in adrenal blood flow showed the same trend as did the arterial blood

Table III

Distribution of the direction of changes induced by hypothalamic stimulation in hypophysectomized dogs

Corticoid output	Adrenal blood flow ↑	Adrenal blood flow —	Adrenal blood flow ↓	Adrenal blood flow Σ
↑	18 (48.7)	3 (8.1)	2 (5.4)	23 (62.2)
—	1 (2.7)	0 (0)	0 (0)	1 (2.7)
↓	8 (21.6)	3 (8.1)	2 (5.4)	13 (35.1)
Σ	27 (73.0)	6 (16.2)	4 (10.8)	37 (100.0)

Arterial pressure	Adrenal blood flow ↑	Adrenal blood flow —	Adrenal blood flow ↓	Adrenal blood flow Σ
↑	20 (54.1)	2 (5.4)	2 (5.4)	24 (64.9)
—	3 (8.1)	3 (8.1)	0 (0)	6 (16.2)
↓	4 (10.8)	1 (2.7)	2 (5.4)	7 (18.9)
Σ	27 (73.0)	6 (16.2)	4 (10.8)	37 (100.0)

Abbreviations: ↑ increase ($\geqslant 5\%$); — no change; ↓ decrease (5%); upper figures: number of stimulations; lower figures: value in per cents of the total number of stimulations

pressure in 68%; in 7 cases it was unchanged or associated with a decrease in blood pressure; their correlation was significant ($r = 0.49; 0.01 > p > 0.001$). Adrenal vascular resistance decreased by more than 5% in 38%, and by more than 10% in 27%, of the cases.

In 6 animals stimulation was carried out in 11 cases under the same circumstances prior to and after hypophysectomy. Two such cases are shown in detail in Figs 3 and 4. As it can be seen in Table IV also, hypophysectomy significantly influenced the responses; sometimes their direction changed, for instance the adrenal blood flow response in cases 2a, b, 4a and 5b. Cardiac

Table IV

Effect of hypothalamic stimulation prior to hypophysecotmy

No. of exp.			Adrenal blood flow	Corticoid release µg/h/kg		Mean arterial pressure mmHg	"A" cardiac output ml / min kg	"P" ml / min kg	Pulse rate	Respiratory rate	"R"
				Hydrocortisone	Corticosterone						
1	a	B	4.2	9.7	6.9	132	208	36	223	12	19
		D	5.0	18.7	4.8	177	415	97	219	83	
	b	B	4.6	9.5	2.4	154	197	31	236	20	15
		D	3.9	14.5	2.5	144	249	21	202	113	
2	a	B	4.0	10.5	6.6	130	110	22	170	18	19
		D	5.8	12.6	5.9	202	238	79	198	26	
	b	B	4.8	10.4	5.4	118	127	22	168	14	15
		D	5.6	12.5	5.2	185	214	21	164	74	
3	a	B	2.0	6.0	1.6	137	79	23	114	12	18
		D	1.9	9.4	1.7	135	79	29	114	14	
	b	B	2.5	6.0	1.3	141	62	23	101	12	15
		D	2.6	5.1	1.3	146	108	33	131	15	
4	a	B	4.2	18.9	7.4	146	137	34	161	17	20
		D	5.3	13.7	5.1	161	240	103	175	41	
	b	B	4.8	15.8	2.6	137	165	40	188	27	15
		D	4.4	12.5	2.6	150	148	38	175	32	
5	a	B	2.3	—	—	150	95	33	190	19	20
		D	2.1	—	—	171	148	50	191	52	
	b	B	2.3	—	—	132	121	32	194	22	17
		D	2.2	—	—	217	910	47	238	52	
6	a	B	4.5	12.3	4.4	136	72	23	161	30	20
		D	5.1	23.5	6.5	135	83	23	161	30	

Abbreviations: a anteromedial, b posteromedial stimulation; B before, D during stimu-

output, pulse rate and respiratory rate behaved similarly. On stimulation carried out after hypophysectomy, total steroid release increased in 50% of the cases.

At autopsy, 4 electrode pairs were located in the region of the red nucleus (R : 15); stimulation of this region resulted in a negative response of adrenal venous outflow and corticoid release more often than did hypothalamic stimulation.

Effect of hypothalamic stimulation after hypophysectomy

No. of exp.			Adrenal blood flow	Corticoid release μg/h/kg		Mean arterial pressure mmHg	"A" cardiac output ml / min kg	"P" ml / min kg	Pulse rate	Respiratory rate	"R"
				Hydrocortisone	Corticosterone						
1	a	B	4.8	5.8	2.9	105	139	19	208	16	19
		D	5.0	11.3	4.1	125	269	34	182	18	
	b	B	4.7	13.9	5.8	101	133	19	193	15	15
		D	4.3	18.9	6.7	109	319	27	185	78	
2	a	B	3.6	2.8	1.0	113	101	25	185	21	19
		D	4.1	1.5	1.1	150	163	63	204	32	
	b	B	2.9	2.4	1.6	114	99	26	162	9	15
		D	2.3	2.2	0.63	85	90	25	148	24	
3	a	B	3.1	0.82	0.73	134	52	19	128	24	18
		D	3.3	1.2	0.73	137	76	28	164	22	
	b	B	3.5	0.58	0.15	122	81	20	139	21	15
		D	3.9	0.58	0.06	134	81	24	178	25	
4	a	B	4.4	1.2	0.31	121	170	55	216	24	20
		D	4.4	0.94	0.31	126	192	63	189	48	
	b	B	4.4	0.65	0.26	117	164	60	200	24	15
		D	4.3	0.62	0.36	115	174	70	195	34	
5	a	B	3.6	3.5	0.64	140	121	19	205	30	20
		D	4.2	3.7	0.84	143	99	24	204	64	
	b	B	3.8	1.9	1.2	97	114	17	189	32	17
		D	4.1	1.2	0.99	162	394	38	257	78	
6	a	B	2.9	2.6	0.15	102	40	16	193	12	20
		D	3.7	3.9	0.33	133	40	13	127	4	

lation; R stereotaxic coordinate.

Discussion

From the data obtained it is supposed that in the neural regulation of the reaction of adrenal blood vessels the hypothalamus plays a role, because after hypothalamic stimulation adrenal blood flow was often changing independently from the blood pressure. The positive correlation between the response of pressure and flow shows that the change in the same direction is the more important one. In the experiment described, on the stimulation of the medial

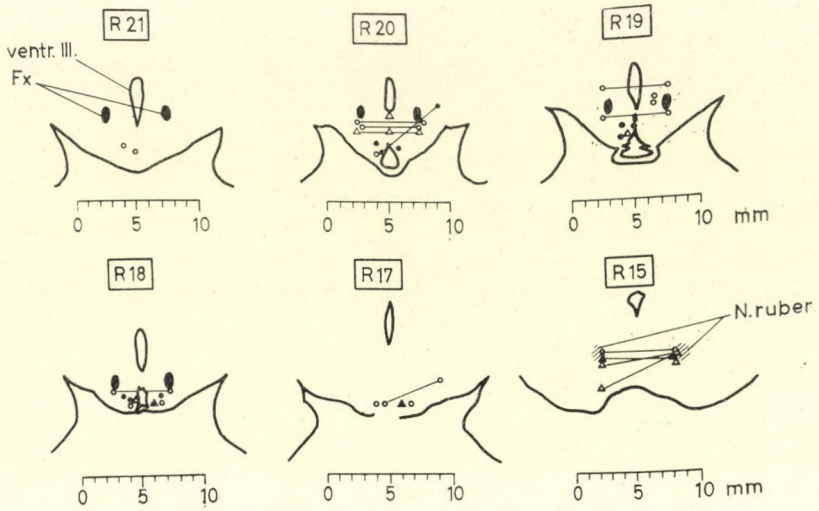

Fig. 2. Localization of hypothalamic stimulations after hypophysectomy. Abbreviations as in Fig. 1

hypothalamic structures an increase was dominating in adrenal circulation, blood pressure, hind leg blood flow and respiratory rate. Of these, the increase in adrenal blood flow might have been part of the adaptation to the strain. It has been reported by SMITH et al. (1960) that in the conscious dog after hypothalamic stimulation the circulatory responses are similar to those observed during intensive muscular activity. Similar results were obtained in cats by MANNING and PEISS (1960) as well as by ROSÉN (1961).

The increase in adrenal blood flow may play a role in the increase of hormone release due to prompt accommodation to strain. This is supported by the positive correlation between blood flow and corticoid release after hypothalamic stimulation of hypophysectomized animals. It is known that, with ACTH concentrations lower than the physiological maximum, corticoid secretion is changing in dependence on blood flow (URQUHART 1965). In animals with intact hypophysis there is no correlation between these parameters; this might be due that in part of the cases the adrenals were maximally or near maximally activated under the acute experimental conditions. The evaluation of the interrelationship between adrenal flow and corticoid release is, however, complicated by further factors. Our recent investigations have shown that the venous outflow does not reflect unambiguously the actual blood supply of the cortex and the medulla. It has also been shown that the effective cortical blood flow is four times smaller than the total flow and does not always change parallel with the latter (MONOS et al. 1969a, b). In cases in which there was no correlation between adrenal flow and corticoid release, after a high dose of

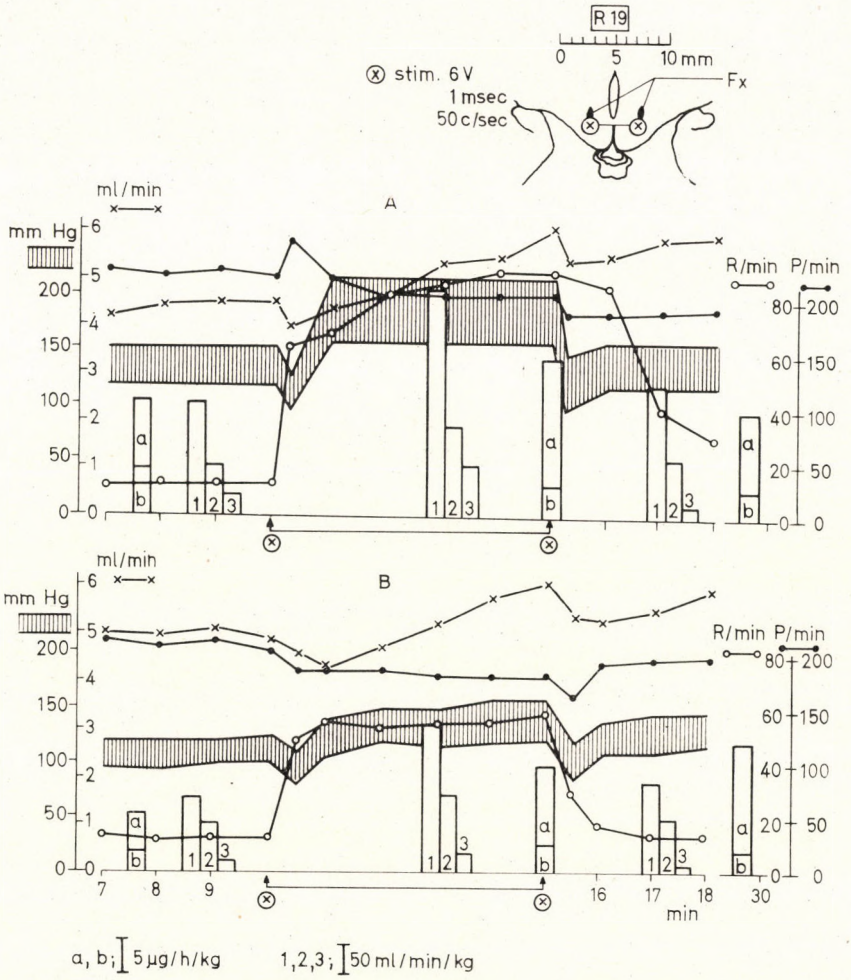

Fig. 3. Effect of hypothalamic stimulation prior to (upper) and after (lower) hypophysectomy. Dog No 5a, 17 kg, ♂; left adrenal weight 1.01 g. ×——×: adrenal blood flow; ▨: systolic and diastolic blood pressure (femoral artery); ●——●: pulse rate; ○——○: respiratory rate; a, b: hydrocortisone and corticosterone release. 1, 2, 3: blood flow in ascending aorta, thoracic aorta and abdominal aorta (between bifurcation and renal artery)

ACTH the two parameters may become correlated ($r = 0.48$; $p < 0.05$; SANZARI et al. 1965).

In glycocorticoid mobilization during hypothalamic activation, extrahypophyseal factors may also play a role. Hypothalamic stimulation carried out after hypophysectomy resulted in an increase of corticoid release in most of the cases. To explain this phenomenon, a number of factors might be taken into consideration such as the renin-angiotensin system, a neural trophic effect, or mobilization of extrahypophyseal ACTH depots. An essential factor

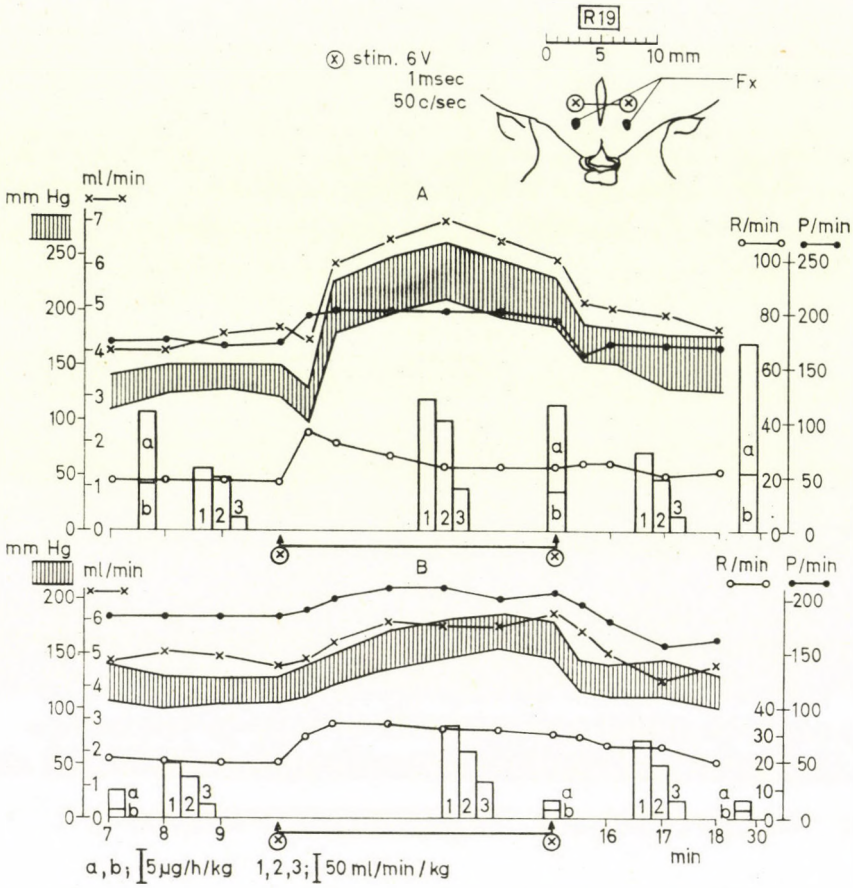

Fig. 4. Effect of hypothalamic stimulation prior to (upper) and after (lower) hypophysectomy. Dog No 6a, 26 kg ♂; left adrenal weight: 1.01 g. Abbreviations as in Fig. 3

might be the increase in blood supply, since in most cases the increase of flow was associated with an increase of corticoid release. PANKOV (1956) has shown that acute peripheral neural influences are exerting their effect through the adrenal blood supply; the autonomic nervous system does not have a direct influence on adrenocortical hormone secretion (PANKOV 1961; YUDAYEV and PANKOV 1961; SCHAPIRO and ARAGONAS 1963).

Hypophysectomy influenced significantly the effect of the hypothalamic stimulation. In the self-control experiments not only the amplitude of the response decreased or sometimes increased, but often it was reversed after hypophysectomy. These changes may have been connected with the decrease of corticoid level. SECKER (1949) has shown that in the cat after acute adrenalectomy the pressor response to somatic afferent stimulation gradually

disappears but can be restored with cortical extract. After adrenalectomy myocardial contractions become weak in the dog (LEFER and LINTZ 1965). The lack of vasopressin may also play a role in the changed haemodynamical reactions after hypophysectomy (MONOS et al. 1967).

Topographical analysis did not reveal any qualitative difference in the responses elicited from the different hypothalamic regions. A similar conclusion was reached by PITTS et al. (1941) who studied the changes in blood pressure and efferent sympathetic activity occurring after hypothalamic stimulation. An increase in adrenal blood flow can be elicited from both the ventromedial hypophyseotrophic area described by HALÁSZ et al. (1962) and the dorsomedial sympathetic vasodilatator relay (FOLKOW et al. 1959).

Thus, changes in adrenal blood flow are part of the complex circulatory reactions integrated at the hypothalamic level, and the increase in blood flow might be a factor in eliciting adrenal activity.

Acknowledgement

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THE MEDIAL THALAMUS AND THE OPPOSITE EFFECT OF CORTICOSTEROIDS AND ADRENOCORTICOTROPHIC HORMONE ON AVOIDANCE EXTINCTION IN THE RAT

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The site of the opposite effect of ACTH and corticosteroids on the extinction of a conditioned avoidance response has been studied in rats. Administration of ACTH resulted in a delay of extinction in rats bearing cortisone acetate implants in various forebrain areas. However, ACTH failed to affect the facilitated extinction in rats with cortisone implants in the centromedian-parafascicular region of the medial thalamus. It is supposed that the medial thalamus may be but one locus of the opposite action of ACTH and corticosteroids, while the influence of ACTH becomes dominant upon the forebrain inhibitory influences evoked by corticosteroid implants.

Adrenocorticotrophic hormone and corticosteroids have been shown to affect oppositely the extinction of avoidance responses in the rat. While corticosteroids facilitated, ACTH or ACTH-like peptides through extraadrenal mechanisms delayed the extinction (MURPHY and MILLER 1955; MILLER and OGAWA 1962; DE WIED 1965, 1966, 1967; BOHUS and DE WIED 1966; BOHUS and LISSÁK 1968; BOHUS et al. 1968). Experiments with intracerebral implantation of corticosteroids demonstrated that the behavioural action of corticoids involves forebrain inhibitory and mesencephalic and diencephalic facilitatory mechanisms (BOHUS 1968; VAN WIMERSMA GREIDANUS and DE WIED 1969; BOHUS 1970). Furthermore, involvement of the medial thalamus in the mediation of the behavioural effect of α-MSH, an ACTH-like peptide, was also demonstrated in rats with bilateral lesions in the thalamic parafascicular area (BOHUS and DE WIED 1967a).

In order to study the locus or loci of the opposite action of ACTH and corticosteroids, experiments were made to determine whether the influence of these hormones involved the same mechanisms or they affected different CNS areas. Therefore, the effect of ACTH treatment on extinction of a conditioned avoidance response was studied in rats with corticosteroid implants in forebrain and diencephalic areas.

Methods

Adult male albino rats of an inbred strain (R-Amsterdam Wistar) were used. The animals were fed on rat purina chow and allowed water ad libitum.

Avoidance conditioning was performed in a pole-jumping avoidance situation. The conditional stimulus (CS) was the light of a 45 W bulb while an A. C. electric shock of 0.56 mA on the paw was used as unconditional stimulus (US). Ten conditioning trials were given each

day with a predetermined sequence of intertrial intervals varying from 50—70 sec. The CS was presented 5 sec prior to the US. The CS was immediately terminated when the rats had jumped onto the pole. The rats were conditioned till the learning criterion had been achieved (24 or more avoidances from 30 trials in three consecutive sessions).

In the animals which had fulfilled the criterion, crystalline cortisone-acetate was implanted in medial thalamic and forebrain areas. Cortisone acetate was chosen for implantation because of its long life-time in brain tissue. Implants were prepared by tamping the crystalline steroid into the end of a stainless steel cannula 0.55 mm in external diameter. The rats were anaesthetized by ether and the cannula was introduced intracerebrally according to DE GROOT's (1959) stereotaxic coordinates. The implants were ejected by means of a metal stylet, the cannula then being retracted. Cholesterol was used as the control implant.

On the day following the implantation, reconditioning of the conditioned avoidance response (CAR) was begun. The rats were reconditioned to reach the learning criterion again. The rats which had fulfilled the criterion were subjected to the extinction procedure. Ten non-reinforced trials were given each day for 7 days, with the same schedule as used during acquisition except that the CS was terminated after 5 sec when avoidances had not occurred. The response latency was measured by an electric timer with a precisity of 0.01 sec throughout the extinction period. The total number of CAR's and the mean response latency scored during extinction served as indices of avoidance behaviour of each rat.

Rats bearing cortisone acetate or cholesterol implants in medial thalamic or forebrain areas were randomly treated either with ACTH or a placebo. Immediately after the last reconditioning session, 10 μg of a long-acting zinc phosphate preparation of ACTH (purified ACTH, Organon, Nv, Oss, Holland) or, as placebo, zinc phosphate complex (DE WIED 1966) was administered subcutaneously. This treatment was repeated every other day during the extinction period.

At the end of the experiments the rats were decapitated, the brain was fixed in Bouin's solution and embedded in paraffin. The site of the implants was verified in 10 μ-thick sections stained with haematoxylin and eosin.

Statistical analysis was carried out by means of MANN and WHITNEY's (1947) nonparametric ranking test. A probability level of 0.05 or less was accepted as significant difference.

Results

Cortisone acetate implants in 51 rats were seem to be located in the parafascicular, paraventricular and centromedian nuclei of the thalamus (medial thalamic implants), in the anterior hypothalamic region (area preoptica medialis and area hypothalamica anterior) or in the rostral medial and lateral septal nuclei (Fig. 1). Cholesterol implants in 24 rats were found in the same regions. Cortisone implants of 16 rats were outside of these regions. Observations in these animals are referred to as controls with cortisone acetate implants.

Rapid extinction of CAR took place in rats bearing cortisone acetate implants in the medial thalamic nuclei when a placebo was administered during extinction. Administration of ACTH failed to delay the extinction of the avoidance response in the rats with cortisone acetate implants in the same region. Response latencies in both ACTH and placebo treated rats followed the same sequence (Fig. 2). The total number of CARs and the mean response latency were of the same order of magnitude in both groups (Table I).

Rapid extinction of the CAR was also observed in rats with cortisone implants in the anterior hypothalamic region or in the rostral septal nuclei when compared to cholesterol implanted controls ($p < 0.01$) ACTH treatment, however, resulted in a delay of extinction of the avoidance response in

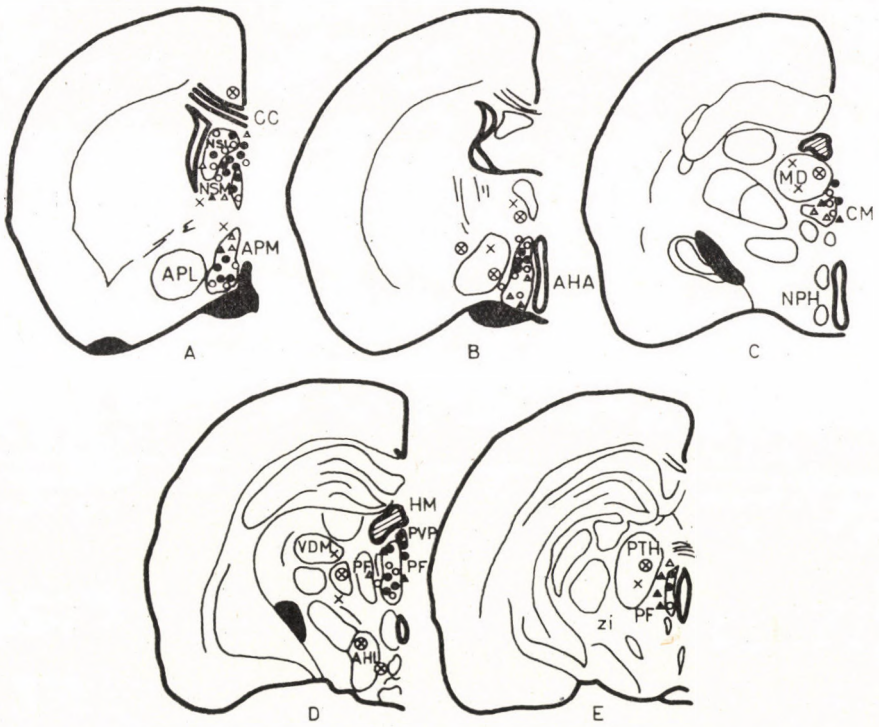

Fig. 1. Reconstruction of the site of implants at septal-anterior hypothalamic (A), anterior hypothamic (B), centro-median (C), parafascicular (D) and posterior (E) thalamic levels. ● = cortisone acetate implants + ACTH treatment; ○ = cortisone implants + placebo treatment; ⊕ = ineffective cortisone implants + ACTH treatment; × = ineffective cortisone implants + placebo treatment; ▲ = cholesterol implants + ACTH treatment: △ = cholesterol implants + placebo treatment

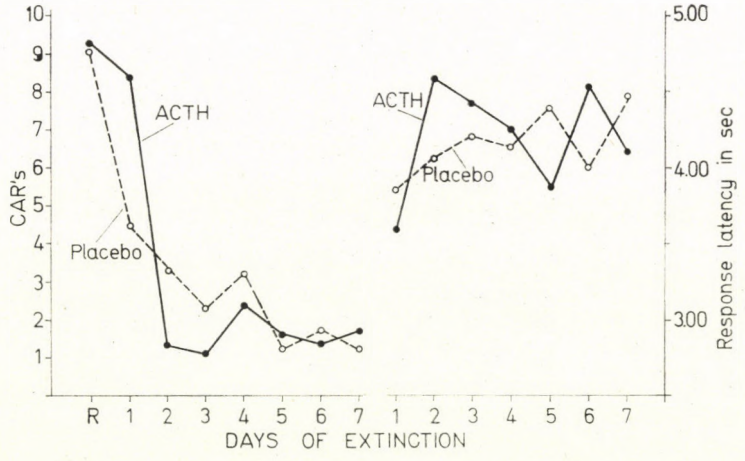

Fig. 2. Effect of ACTH treatment on conditioned avoidance response performance and on response latency during extinction in rats with bilateral cortisone acetate implants in the medial thalamus

Table I

Effect of ACTH on extinction of a conditioned avoidance response in rats with cortisone acetate implants in the diencephalon and the forebrain

Site of implant	Treatment	No. of rats	CAR's	p*	Mean response latency**	p*
Medial thalamus	ACTH	11	16.6±4.0***		3.91±0.24	N.S.
	Placebo	7	17.6±3.1	N.S.	4.32±0.18	
Anterior hypothalamus	ACTH	8	61.6±1.0	<0.01	3.94±0.09	N.S.
	Placebo	9	21.8±3.6		3.74±0.17	
Rostral septum	ACTH	8	59.2±2.9	<0.01	3.90±0.17	N.S.
	Placebo	8	22.0±3.6		4.16±0.11	
Anterior, lateral thalamus, lateral hypothalamus, etc. (Cortisone implanted controls)	ACTH	8	62.6±1.4	<0.01	2.52±0.25	<0.01
	Placebo	8	45.8±1.6		3.67±0.21	
Cholesterol-implanted controls	ACTH	13	65.0±1.0	<0.01	2.43±0.15	<0.01
	Placebo	11	47.0±1.8		3.76±0.20	

* Mann and Whitney's non-parametric test
** In seconds
*** Mean ± S. E.

rats bearing cortisone implants in these regions (Fig. 3). The total number of CARs scored by cortisone-implanted rats treated with ACTH was significantly higher in both groups (Table I). Like in the cholesterol-implanted controls, ACTH treatment resulted in a significant delay of extinction in the controls bearing cortisone implants. The total number of CAR's scored by the ACTH-treated rats was significantly higher in both control groups (Table I). ACTH treatment led to significantly shorter response latencies in cholesterol or cortisone-implanted controls. The response latencies, however, were of the same order of magnitude in ACTH and placebo-treated rats bearing cortisone implants in the anterior hypothalamus or the rostral septal nuclei (Fig. 3, Table I). The mean response latency of the ACTH-treated controls was significantly shorter than of the ACTH-treated rats with cortisone acetate implants in the medial thalamus ($p < 0.01$), in the anterior hypothalamus ($p < 0.01$), or in the rostral septal nuclei ($p < 0.01$). Similar differences were observed in placebo-treated rats. The probability levels were 0.01.

Discussion

The experiments seemed to indicate that the opposite effect of ACTH and corticosteroids on the extinction of an avoidance response presupposes thalamic reticular mechanisms. Administration of ACTH failed to delay the extinction of the CAR in rats bearing cortisone acetate implants in the medial thalamic nuclei while ACTH was effective when cortisone was implanted in the forebrain areas.

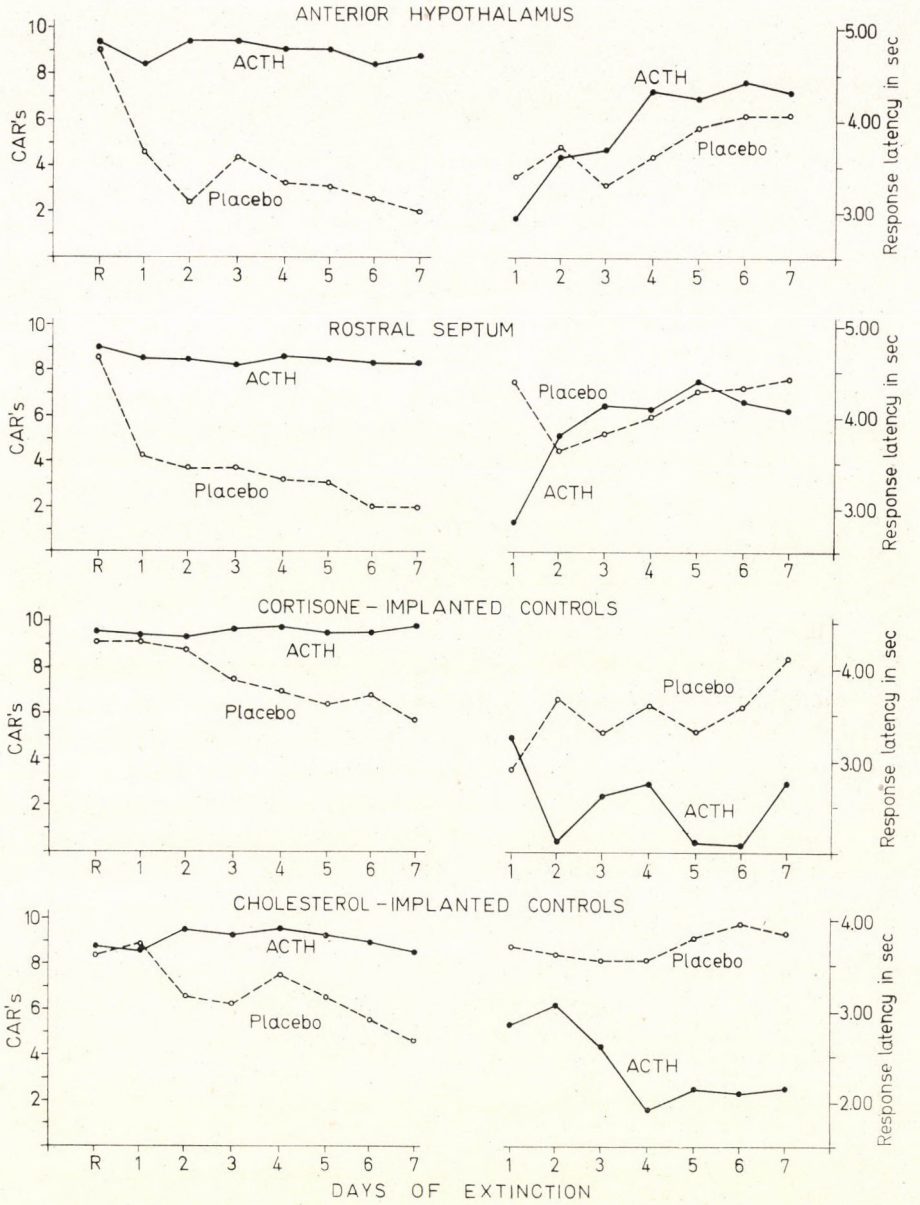

Fig. 3. Influence of ACTH treatment on avoidance extinction in rats with cortisone implants in rostral septal or anterior hypothalamic areas and in those with control implants

Behavioural and electrophysiological experiments revealed that corticosteroids may affect both the descending forebrain inhibitory and the ascending reticular facilitatory mechanisms; it was shown that forebrain-evoked inhibitory influences on reticular function are enhanced by corticosteroids

(ENDRŐCZI et al. 1968). Implantation of cortisone in the forebrain brought about an enhancement of extinction of a conditioned avoidance response, presumably due to the enhancement of forebrain inhibition, as it was shown in the present and in previous experiments (BOHUS, in press). On the other hand, inhibition of cortical potentials evoked by sciatic nerve stimulation was observed following local application of cortisol to the reticular core (SLUSHER et al. 1966). Furthermore, implantation of corticosteroids into the mesencephalic reticular formation (BOHUS 1968) or the medial thalamic nuclei including the parafascicular area (VAN WIMERSMA GREIDANUS and DE WIED 1969; BOHUS 1970) also resulted in a rapid extinction of CAR in the rat. Accordingly, the failure of ACTH to affect extinction in rats bearing medial thalamic cortisone implants might be interpreted as an inhibition of ascending facilitation triggered by ACTH. This interpretation is supported by the finding that rapid extinction of a shuttle-box avoidance response took place in rats with bilateral lesions in the thalamic parafascicular region (BOHUS and DE WIED 1967b) and this rapid extinction could not be prevented by the administration of α-MSH, an ACTH-like peptide (BOHUS and DE WIED 1967a). Both ACTH and corticosteroids might act on the same receptive field in the medial nonspecific thalamic nuclei and if this is the case, the opposite behavioural effect of ACTH and corticosteroids may be a competitive antagonism between the two hormones. This hypothesis remains, however, to be proved.

The influence of ACTH upon forebrain inhibitory function had become dominant since a delay of extinction was observed in rats bearing cortisone implants in forebrain areas. However, an analysis of the response latencies revealed that certain forebrain inhibitory influences still persisted, resulting in a longer response performance time.

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THERMOREGULATORY EFFECTS OF HYPERCAPNIA IN THE NEW-BORN RAT. COMPARISON WITH THE EFFECT OF HYPOXIA

By

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New-born rats aged less than 12 hours, 1—2 days, 4—10 days, and 11—20 days, were subjected at an ambient temperature of 30°C, i.e. below their thermoneutral zone, to hypercapnia by breathing 6% CO_2 for 30 min. Oxygen consumption and colonic temperature were recorded continuously for 30 min prior to, and after termination of, hypercapnia, as well as during breathing 6% CO_2. Most rats were subjected either before or after these observations under similar conditions to hypoxia by breathing 12% O_2 for 30 min. In contrast to hypoxia and to adult rats, hypercapnia failed to reduce heat production and body temperature in most observations in the younger age-groups, and only after the age of 10 days was there a gradual increase in the frequency of the adult-type response. By the age of 6 weeks, however, the response to hypercapnia was identical with that observed in older animals. The differences between the effect of hypoxia and hypercapnia during the postnatal period are interpreted to indicate that the mechanisms involved are not completely identical. Comparison between heat production and changes in body temperature after hypoxia and hypercapnia, respectively, led to the conclusion that not only chemical thermoregulation but physical regulation mechanisms are also active immediately after birth.

In contrast to the abundance of data on the thermoregulatory effects of hypoxia, relatively few observations were concerned with the effect of hypercapnia on heat production and body temperature even in the adult animal, and none are available on the new-born. Despite the early observations of WESTENRYK (1901) and MAGYARY-KOSSA (1920) that hypercapnia reduced both the normal and the febrile body temperature in the rabbit, the subject remained controversial for many years. For instance, in man hypercapnia was found both to elicit shivering (HENSEL 1949) and to inhibit shivering (BULLARD and CRISE 1961). Some of the discrepancies were certainly due to differences in ambient temperature, others could have been caused by differences in carbon dioxide concentration. More recent work, however, showed unequivocally that at ambient temperatures below the thermoneutral zone breathing of carbon dioxide at concentrations of 4% or more diminishes heat production and reduces body temperature in the rat (STUPFEL 1960a, b; SZEGVÁRI and VÁRNAI 1962; SZEGVÁRI et al. 1963).

An investigation concerned with the thermoregulatory response of the new-born rat during the first three weeks of life (VÁRNAI 1970b; VÁRNAI and DONHOFFER 1970) led to the conclusion that the profound fall in body temper-

ature observed at ambient temperatures a few degrees below the thermoneutral zone, was not just the consequence of simple incapability to increase heat production further, but has to be attributed to a regulatory mechanism. Thermoregulatory changes elicited without a change in the ambient temperature seemed to offer an alternative approach to the problem. Both hypoxia and hypercapnia comply with the requirement to evoke marked thermoregulatory responses without any change in the ambient temperature. The thermoregulatory response obtained by exposure of the new-born rat to hypoxia has been published (VÁRNAI and FARKAS 1970; VÁRNAI et al. 1970). An investigation into the effects of hypercapnia during the first weeks of life had the additional interest that no earlier data were available.

Methods

New-born rats from the Institute's colony, aged from a few hours up to 20 days, were used. Oxygen consumption and CO_2 production were measured with a Noyons-Kipp diaferometer as modified by DONHOFFER et al. (1958) with the further modification of VÁRNAI (1970a) allowing to reduce air-flow to 300 ml per min. Oxygen consumption was calculated for unit body surface, according to the formula $10/\sqrt[3]{kg^2}$. The gas mixture (6% CO_2 in air) was prepared in double-walled plastic bags the outlet of which could be attached to the inlet of the apparatus. Since the volume of the chamber and that of the connecting tubing is considerably greater than the volume of the tubing leading directly to the measuring block, any change at the inlet will reach the measuring block through the chamber with a delay as compared to that flowing directly to the measuring block. Therefore, measurements have to be suspended whenever the composition of the supplied gas is changed until about four times as much of the new gas mixture has passed through the chamber as the volume of the chamber plus the connecting tubing. Even with air-flows as small as 300 to 400 ml per min, measurement has to be suspended only for 2 to 3 min. Measurements were discontinued also for a few minutes at intervals of 30 to 60 min to establish the base-line. In many instances this was done immediately after and/or just prior to a change from air to 6% CO_2 or from this to air. Colonic temperature was measured continuously by copper-constantan thermocouples at depths of 0.5 to 2.0 cm from the anus, according to age.

Ambient temperature was kept throughout the observations at 30 ± 0.5 C. According to age, one to seven rats were introduced into the metabolic chamber, usually, however, not more than 4 or 5 animals were used simultaneously even in the two youngest age-groups. Colonic temperature was always measured in one animal only, for technical reasons. Most animals were previously subjected to hypoxia, in others exposure to hypercapnia preceded the exposure to hypoxia. After breathing room air for at least 30 min, 6% CO_2 in air was supplied for 30 min, then room air for another 30 min. Data for oxygen consumption refer to averages of the whole 30 min period, figures for body temperature reflect the averages of colonic temperatures reached at the end of the relevant 30 min period. The same applies to data representing the effect of hypoxia (VÁRNAI et al. 1970). Student's two-tailed *t*-test was used for statistical evaluation.

Results

In contrast to the adult rat in which, at an ambient temperature below the thermoneutral zone, breathing 6% CO_2 invariably led to a marked reduction in heat production and a concomitant fall in colonic temperature, these responses were absent in most new-born animals. In fact, responses resembling those observed regularly in the adult animal, i.e. reduction in heat production and a simultaneous fall in body temperature, were seen in only 1 of 11 experi-

ments with rats aged less than 12 hours (Fig. 1), in 3 of 22 observations on animals aged 1 to 2 days, in 3 of 33 aged 4 to 10 days, and their number increased only in the group aged 11 to 20 days to 11 out of 24. Fig. 2 represents a typical result obtained with a litter of 5 rats aged less than 12 hours: neither

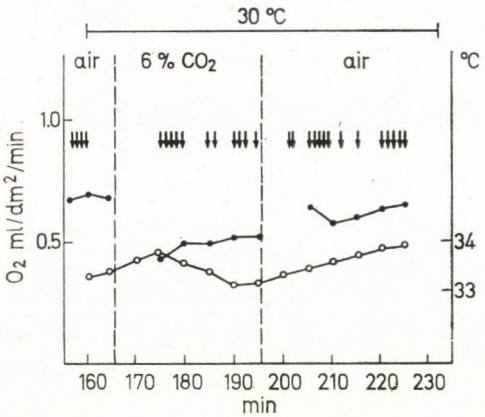

Fig. 1. January 19, 1968. Five new-born rats aged less than 12 hours. Combined body weight 35 g. Response to hypercapnia of the adult type. Arrows indicate motor activity. The abscissa shows the time after measurement of oxygen consumption and colonic temperature had been started. Dots: oxygen consumption; circles: colonic temperature

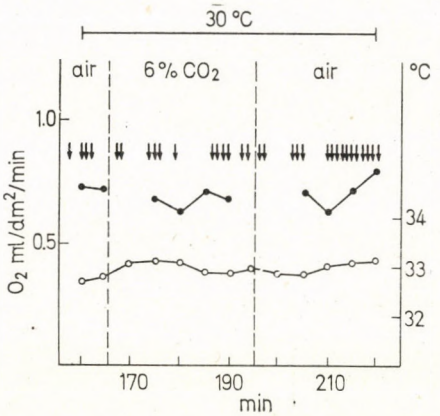

Fig. 2. February 27, 1968. Five new-born rats aged less than 12 hours. Combined body weight 38 g. No response of heat production and body temperature to hypercapnia. Arrows, dots, circles, and time scale like in Fig. 1

oxygen consumption nor colonic temperature responded with a change to breathing 6% CO_2. Fig. 3 demonstrates an adult-type response in two rats aged 2 days. Fig. 4 shows the complete absence of a response to hypercapnia in three rats aged 4 days.

Acta Physiologica Academiae Scientiarum Hungaricae 38, 1970

Table I

Effect of breathing 6% CO_2 and 12% O_2, respectively, on oxygen consumption at 30°C ambient temperature ($M \pm S.E.$)

Oxygen consumption in ml/dm²/min.

Age	Number of observations	Gas mixture	Breathing air before exposure	During exposure	After restoration of air breathing
Less than 12 hours	n = 11	6% CO_2	0.60±0.05	→ n.s. ← 0.56±0.04 → n.s. ←	0.59±0.05
	n = 14	12% O_2	0.66±0.04	→ p < 0.001 ← 0.46±0.03 → p < 0.01 ←	0.64±0.05
1—2 days	n = 22	6% CO_2	0.86±0.06	→ n.s. ← 0.83±0.05 → n.s. →	0.85±0.06
	n = 22	12% O_2	0.96±0.07	→ p < 0.001 ← 0.60±0.04 → p < 0.001 ←	0.93±0.07
4—10 days	n = 33	6% CO_2	0.84±0.04	→ n.s. ← 0.79±0.04 → n.s. ←	0.84±0.04
	n = 26	12% O_2	0.80±0.04	→ p < 0.001 ← 0.51±0.03 → p < 0.001 ←	0.84±0.04
11—20 days	n = 24	6% CO_2	1.33±0.08	→ 0.1 > p > 0.05 ← 1.16±0.06 → n.s. ←	1.27±0.09
	n = 24	12% O_2	1.43±0.08	→ p < 0.001 ← 1.04±0.08 → p < 0.001 ←	1.41±0.07

Table I summarizes the response of oxygen consumption to breathing 6% CO_2 and serves to compare it with those obtained in response to breathing 12% O_2. In marked contrast to the response to hypoxia, no significant change in oxygen consumption could be observed in the three younger age-groups

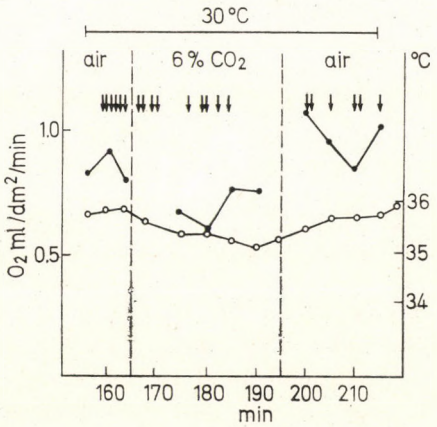

Fig. 3. February 7, 1968. Two rats aged 2 days. Combined body weight 22 g. Adult type of response to hypercapnia. Arrows, dots, circles, and time scale like in Fig. 1

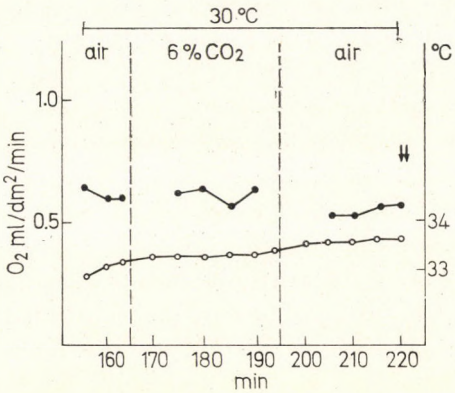

Fig. 4. October 20, 1967. Three rats aged 4 days. Combined body weight 28 g. Both heat production and colonic temperature failed to respond to hypercapnia. Arrows, dots, circles, and time scale like in Fig. 1

as a whole, and even in rats aged 11—20 days the reduction in heat production only approached statistical significance.

Table II compares the percentual change in oxygen consumption in response to breathing 6% CO_2 and 12% O_2, respectively. Whereas in the adult animal, both 6% CO_2 and 12% O_2 cause a reduction in heat production of

Table II

Percentual change in oxygen consumption in hypercapnia (breathing 6% CO_2) and hypoxia (breathing 12% O_2). ($M \pm S.E.$)

Age	Number of observations	Change in O_2-consumption in hypercapnia per cent	p between hypercapnia and hypoxia	Change in O_2 consumption in hypoxia per cent	Number of observations
Less than 12 hours	n = 11	−3.7±3.1	$p < 0.001$	−28.6±3.8	n = 16
1—2 days	n = 22	−2.6±2.8	$p < 0.001$	−36.1±2.3	n = 22
4—10 days	n = 33	−5.4±2.2	$p < 0.001$	−30.2±2.0	n = 29
11—20 days	n = 24	−11.0±2.7	$p < 0.001$	−26.7±2.9	n = 22

approximately equal magnitude, in the new-born, hypoxia reduced oxygen consumption to about the same extent as in the adult animal under comparable conditions, while hypercapnia failed to reduce oxygen consumption significantly in the three younger groups, and the reduction was small compared to that induced by hypoxia even in the oldest age-group.

Since body temperature levels at which the rats were exposed to hypoxia have been shown to influence the changes in colonic temperature during hypoxia (VÁRNAI et al. 1970), only the responses of animals with a body temperature above 33°C before exposure to hypoxia in the three older age-groups, and above 32°C in rats aged less than 12 hours, were compared in Table III. In contrast to hypoxia, breathing of 6% CO_2 failed to cause any decrease in colonic temperature in any of the three younger age-groups. Only in the animals aged 11 to 20 days was a fall in mean colonic temperature recorded, which approached statistical significance. This statement might be misleading, since it applies to the difference between the means of the temperatures actually recorded before exposure to hypercapnia and at the termination of hypercapnia. Taking as the basis of calculation the temperature differences of the individual observations and the mean of these differences, the reduction in body temperature during hypercapnia proves to be highly significant in the oldest age-group ($M \pm S.E. = -0.46 \pm 0.08$°C, $t = 5.00$, $p < 0.001$).

Table IV compares the changes in body temperature in response to hypoxia and hypercapnia in the same animals and contains additional data for rats aged 6 weeks and 6 months. Observations in the two latter age-groups have, however, been performed at an ambient temperature of 20°C, at these ages 30°C being the thermoneutral temperature for the rat. The results showed unequivocally that the striking difference between the responses to hypoxia and hypercapnia observed in the new-born rat, tended to diminish during the third week of life and had completely disappeared by the age of six weeks.

Table III

Effect of breathing 6% CO_2 and 12% O_2, respectively, on colonic temperature at an ambient temperature of 30°C (M ± S.E.) in new-born rats with a colonic temperature above 32°C in the group aged less than 12 hours, and above 33°C in the others

Age	Number of observations	Gas mixture	Colonic temperature		
			Breathing air before exposure	At the end of of exposure	30 min after restoration of air breathing
Less than 12 hours	n = 9	6% CO_2	32.90±0.20 → n.s. ←	32.85±0.20 → n.s. ←	33.19±0.24
	n = 9	12% O_2	32.81±0.18 → p < 0.02 ←	32.29±0.13 → p < 0.02 ←	32.95±0.25
1–2 days	n = 15	6% CO_2	34.18±0.26 → n.s. ←	34.20±0.20 → 0.1 > p > 0.05 ←	34.61±0.25
	n = 11	12% O_2	34.13±0.22 → p < 0.01 ←	33.27±0.13 → p < 0.001 ←	34.54±0.28
4–10 days	n = 24	6% CO_2	34.42±0.15 → n.s. ←	34.37±0.15 → p > 0.1 ←	34.76±0.20
	n = 17	12% O_2	34.09±0.14 → p < 0.001 ←	33.31±0.11 → p < 0.001 ←	34.64±0.20
11–20 days	n = 24	6% CO_2	35.90±0.15 → 0.1 > p > 0.05 ←	35.46±0.16 → p = 0.05 ←	35.97±0.18
	n = 23	12% O_2	35.93±0.16 → p < 0.001 ←	34.92±0.17 → p < 0.001 ←	36.09±0.13

Table IV

Changes in colonic temperature in hypercapnia (breathing 6% CO_2) and in hypoxia (breathing 12% O_2). Ambient temperature 30°C for the four younger age-groups and 20°C for the rats aged 6 weeks and 6 months. Initial body temperature was above 32°C in rats aged less than 12 hours, and above 33°C in the groups aged 1 to 2, 4 to 10, and 11 to 20 days. (M ± S.E.)

Age	Number of observations	Change in colonic temperature in hypercapnia	p between changes in hypercapnia and hypoxia	Change in colonic temperature in hypoxia	Number of observations
Less than 12 hours	n = 9	−0.05±0.06	p < 0.001	−0.53±0.17	n = 9
1—2 days	n = 15	−0.06±0.06	p < 0.001	−0.86±0.16	n = 11
4—10 days	n = 24	−0.05±0.06	p < 0.001	−0.65±0.13	n = 17
		↓ p < 0.001 ↑			
11—20 days	n = 24	−0.50±0.08	p < 0.001	−1.00±0.09	n = 23
6 weeks	n = 18	−1.04±0.14	p > 0.8	−1.08±0.11	n = 18
6 months	n = 17	−1.00±0.12	p > 0.3	−1.16±0.12	n = 17

Attention has to be paid to the increase in colonic temperature after the termination of hypercapnia, especially in the three younger age-groups in which it was not preceded by a fall in body temperature. Albeit none of the increases in colonic temperature reached statistical significance in the three younger age-groups when the means of the temperatures recorded at the termination of hypercapnia and 30 min thereafter were compared (Table III), a different picture emerged when the calculations were based on the temperature difference observed in the individual experiments and on the mean of these differences. As shown in Table V, the post-hypercapnic increase in body temperature proved to be statistically highly significant in all age-groups.

Table V

The post-hypercapnic increase in body temperature calculated on the basis of the temperature differences in the individual observations and the mean of these differences

Age	Number of observations	Temperature difference M ± S.E. °C	"t"	p
Less than 12 hours	n = 9	+0.36±0.08	4.375	<0.01
1— 2 days	n = 15	+0.41±0.11	3.453	<0.01
4—10 days	n = 24	+0.39±0.09	3.731	<0.01
11—20 days	n = 24	+0.49±0.10	3.900	<0.001

Discussion

In interpreting the results it has to be considered that oxygen consumption represents, with the exception of the oldest animals, average figures for more than one animal, whereas body temperature data refer always to one animal only. Results like those in Fig. 1 necessarily imply that oxygen consumption was reduced by hypercapnia in the majority of the animals, and by inference, therefore, indicate that body temperature had decreased during hypercapnia also in the majority of the five rats involved in the observation. It cannot, however, be concluded that none of the five animals failed to respond to hypercapnia by a reduction in heat production and a fall in body temperature. The same applies to observations like those recorded in Fig. 2 in which there was no reduction in heat production and no fall in colonic temperature. Heat production certainly failed to respond to hypercapnia with a decrease in the majority of the animals, but it cannot be excluded that some animals responded with a decrease in oxygen consumption either too small appreciably to affect the average, or which had been compensated by an increase in heat production in one or some of the other rats. Similarly, body temperature also could have declined in one or the other of the rats in which colonic temperature had not been recorded.

It has therefore to be assumed that more animals responded to hypercapnia with an adult-type reaction than would appear were only those observations taken into account in which colonic temperature fell in the one rat in which it was measured and, simultaneously, average heat production was reduced. This inference is born out by the observation that in some experiments colonic temperature fell in response to hypercapnia whereas average heat production was not reduced or, on the other hand, that in some instances, average heat production was reduced during hypercapnia while colonic temperature in the one animal it had been measured failed to decline or even increased.

In spite of these limitations, the results demonstrate clearly the fundamental difference in the response to hypercapnia and to hypoxia. The response to hypoxia of the new-born rat is already in the first few hours after birth essentially the same as that seen under comparable conditions in the adult animal. In contrast, only few animals respond to hypercapnia with an adult-type of reaction during the first ten days of life, and even during the third week a number of animals fail to respond to breathing 6% CO_2 with a reduction in heat production and a fall in body temperature. By the age of 6 weeks, however, the adult-type response is fully developed and, like in older animals, the effects of breathing 12% O_2 and 6% CO_2 on heat production and body temperature are practically identical. Evidently, the genetically determined response to hypercapnia of heat production and body temperature appears

at a later stage of development than the response to hypoxia. The variability in the time of appearance of the response, covering approximately the first three weeks of postnatal life, may also be considered to be due to genetic differences. Further observations on various highly inbred strains would be valuable.

The time lag between the appearance of the response of heat production and body temperature to hypoxia and to hypercapnia constitutes the first piece of evidence that these responses of the thermoregulatory system are not the manifestations of a completely identical mechanism.

The changes in colonic temperature after the termination of hypercapnia also require comment, especially those recorded in the three younger age-groups. Albeit the increase in mean colonic temperature after switching from 6% CO_2 to air had failed to reach statistical significance (Table III), it has been demonstrated in Table V by basing the calculation on the temperature differences observed in the individual experiments and the mean of these differences that the post-hypercapnic increase in body temperature is highly significant statistically. Nevertheless, the results are not easy to interpret. In some cases the colonic temperature was lower before hypercapnia than 30 min after the animal had been transferred to an ambient temperature of 30°C from a thermoneutral environment of 35—36°C, and therefore may have been approaching or regaining that temperature. Such a simple explanation seems improbable. On the one hand, in a number of instances 30 min after the termination of hypercapnia colonic temperature exceeded that observed 30 min after the transfer from a thermoneutral environment to an ambient temperature of 30°C and, on the other hand, body temperature 30 min after termination of hypercapnia was practically identical with that observed 30 min after termination of hypoxia despite that in the latter case body temperature was about one centigrade lower at the end of exposure. It is therefore tentatively concluded that in these age-groups hypercapnia tends to be followed by an increase in body temperature although it had suffered no prior reduction by hypercapnia. Similar increases in body temperature have been observed after exposure to hypoxia; in these, however, the considerable fall in body temperature during hypoxia might have served after restoration of air breathing as a stimulus for an overshoot reaction.

One other aspect of our results deserves to be pointed out. As mentioned, 30 min after the termination of hypoxia or hypercapnia body temperature had reached practically the same level albeit at the termination of hypoxia colonic temperatures were about one centrigrade lower (Table III). In other words, considerably more heat had to be stored in the body during the 30 min following the termination of hypoxia than during the same period after hypercapnia. Table I shows that there was practically no difference in heat production between the post-hypoxic and the post-hypercapnic period. This confirms

the conclusion arrived at in other experiments (VÁRNAI and DONHOFFER 1970) that, in the new-born rat, not only chemical thermoregulation but physical regulation mechanisms are also active immediately after birth.

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HINTERGRUNDAKTIVITÄT UND INFORMATIONSVERARBEITUNG DIENZEPHALER EINZELNEURONE UNTER HYPERTHERMIE

von

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Die Hintergrundaktivität und das aufsummierte Antwortmuster thalamischer Einzelneurone der wachen Ratte nach Gabe physikalisch identischer Stimuli wurden auf den einzelnen Temperaturniveaus von normaler Körpertemperatur bis 43°C untersucht. Die Hintergrundaktivität zeigt bis 40°C einen nahezu linearen Anstieg bei gleichfalls ansteigender Streuung, erreicht bei 41.5° bis 42.3°C ein Maximum und fällt wieder ab.

Die Antwortintensitäten sind mäßig negativ korreliert mit steigender Körpertemperatur, wobei ab 39°C ein großer Teil der neuronalen Antworten häufiger das Signifikanzniveau unterschreitet. Die Antwortmuster, wie näherungsweise exponentieller und exzitatorisch-inhibitorischer Responsivitätstyp, werden mit zunehmender Körpertemperatur deutlich schwächer und verschwinden bei 42°C vollständig. Diese Veränderungen werden als Ausdruck gestörter Informationsverarbeitung im neuronalen Netz diskutiert und auf psychopathologische Parallelen hingewiesen.

Pathophysiologische Modelle zum Studium gestörter neuronaler Informationsprozesse müssen reproduzierbar sein. Dies ist nicht immer gewährleistet. So können bei natürlichem und künstlichem Fieber zusätzlich zum Wärmeeffekt auftretende unkontrollierbare toxische Nebenwirkungen auf die Hirnneuronen nicht ausgeschlossen werden.

Im Gegensatz zur Wirkung des Fiebers waren unter künstlicher Hyperthermie sowohl psychische als auch elektroenzephalographische Veränderungen definierten Körpertemperaturen zuzuordnen (KOELLA und BALLIN 1954; SCHAPS und SCHMIDT 1967). Außerdem gestattete letzteres Verfahren exakt steuerbare Temperaturzeitverläufe und erschien uns insgesamt als hinreichend reproduzierbares pathophysiologisches Modell.

Von therapeutischen Ganzkörperhyperthermien am Menschen (SCHAPS und SCHMIDT 1967) ist bekannt, daß hier — ähnlich wie beim Fieber — verschiedene psychopathologische Bilder auftreten, wie Bewußtseinsstörungen, Halluzinationen, Delirien u. a. Während tierexperimenteller Hyperthermien wurden Veränderungen in der elektrischen Summenaktivität des Gehirns bei definierten Körpertemperaturen beobachtet (KOELLA und BALLIN 1954; GÄNSHIRT 1954). Dies kann als Ausdruck der allgemein gestörten Hirntätigkeit angesehen werden, ohne daß hiermit direkte Aussagen über gestörte neuronale Informationsprozesse möglich sind.

Nach GIDLÖF und SÖDERBERG (1964) reagieren die Summenpotentiale der vom Subkortex abgetrennten Hirnrinde weniger empfindlich auf Erwärmung als die des nicht isolierten Kortex. Somit wären insbesondere die elektrischen Aktivitäten subkortikaler Strukturen temperaturabhängig. Es wurde hier das dienzephale Niveau untersucht. Da die Beurteilung der Responsivität und insbesondere der einzelnen Antwortmuster nach dem Ergebnis einer einmaligen Reizgabe auf diesen Codierungsstufen unzureichend erscheint, wurde ein statistisches Kriterium der Nervenzellantwort in Form der meistens 50fachen Aufsummierung der poststimulatorischen Aktionspotentialfolgen gewählt.

Aus Wahrscheinlichkeitsgründen können Störungen in den Informationsprozessen bei hohen Körpertemperaturen bzw. Fieber eher auf höheren synaptischen Codierungsstufen vermutet werden als jenen der direkten „spezifischen" Signaltransmission mit relativ wenigen synaptischen Umschaltungen. Deshalb wurde die neuronale Impulsaktivität nicht aus den direkten afferenten Projektionssystemen abgeleitet, sondern aus thalamischen Kerngebieten unterschiedlicher funktionell-anatomischer Bedeutung. Obwohl diese Regionen nicht in die direkte Signaltransmission eingeschlossen sind, antworten deren Zellen ebenfalls auf Clickreize (ZWIENER et al. 1969).

Im Mittelpunkt unserer Untersuchungen stand die Frage, welches statistische Antwortmuster dienzephale Einzelneurone nach Gabe physikalisch identischer Stimuli auf einzelnen Körpertemperaturniveaus bis 43°C liefern. Außerdem wurde die jeweilige „background activity" untersucht.

Material und Methode

An 71 Wistar Ratten (Gewicht 200—300 g; Alter 2—4 Monate) wurden über Glasmikroelektroden stereotaktisch (nach FIFKOVÁ und MARŠALÁ) aus den weiter unten angeführten thalamischen Kerngebieten extrazellulär Aktionspotentialfolgen abgeleitet.

Die Spikes wurden 10 dekadischen Zählern nacheinander in Schritten von 0.3 sec Abstand zugeleitet, entsprechend der Methode von SMITH und BURNS (1960) und TUMA und BUREŠ (1963). Nach Aufnahme der sog. Spontanaktivität wurden über beiderseits an den hohlen Ohrstiften des stereotaktischen Gerätes angebrachte Ohrhörer automatisch Clickreize von 70 dB (gemessen an den Stiftkanälen) gegeben. Stochastische Pausen zwischen 5 bis 8 sec folgten nach jeder Reizgabe. Nach dem „averaging" -Prinzip wurden die neuronalen Einzelantworten addiert, wodurch ein dem poststimulatorischen Zeithistogramm (PSH, GERSTEIN und KIANG 1960) ähnliches Säulendiagramm entsteht.

Nach Aufnahme der Spontanaktivität und der Säulendiagramme der aufsummierten Aktionspotentialfolgen bei Normaltemperatur wurde durch Veränderung des Arbeitspunktes des Thermostaten die Körpertemperatur kontinuierlich um 1°C/10 Min. erhöht und Säulendiagramme auf den jeweiligen Temperaturstufen gewonnen. Während der Aufnahme der Säulendiagramme wurde der Thermostat ausgeschaltet. Dadurch konnte die Körpertemperatur während einer Summierungsperiode bis auf ± 0.15°C konstant gehalten werden. Die statistische Analyse der Säulendiagrammergebnisse erfolgte nach einem bei BUREŠ und BUREŠOVÁ (1965) angegeben, dem *t*-Test ähnlichen Verfahren. Weitere methodische Einzelheiten wurden an anderer Stelle beschrieben (ZWIENER et al. 1969).

Ergebnisse

Von 71 untersuchten Nervenzellen konnten 37 von Normaltemperatur bis zu 43°C Körpertemperatur in ihrer Aktivität (über mindestens 90 Min.) verfolgt werden. Davon reagierten 22 (13 aus dem nucl. ventr. pars dorsomedialis, 6 aus dem nucl. mediodorsalis und 3 aus dem nucl. lateralis anterior thalami) bei Normaltemperatur statistisch signifikant auf Clickreizung. Wie in

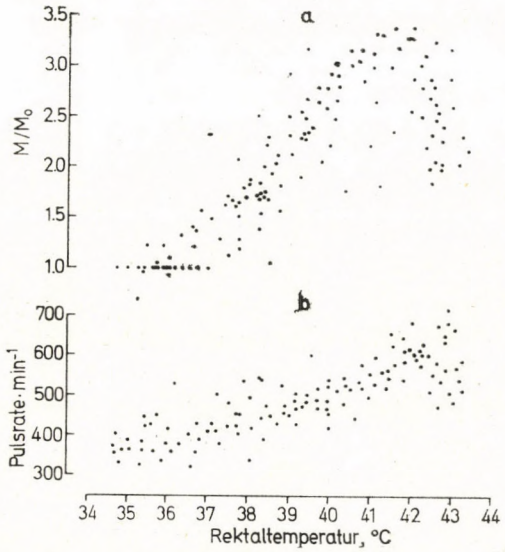

Abb. 1. a) Relativer Anstieg der Spontanentladungsrate (M/Mo — Quotient aus durchschnittlicher Entladungsrate auf dem jeweiligen Temperaturniveau und der Ausgangsentladungsrate) mit steigender Körpertemperatur von 14 thalamischen Einzelneuronen; b) Absoluter Anstieg der Herzfrequenz mit steigender Körpertemperatur bei 12 Versuchstieren

früheren Untersuchungen (ZWIENER et al. 1969; ZWIENER und DAVIDOWA 1969) waren kernspezifische Unterschiede nicht festzustellen.

Um auf jeder Temperaturstufe eine repräsentative Angabe über die mittlere Entladungsrate bei den oft starken Aktivitätsschwankungen zu gewinnen, wurden die Spikes in Zähler-Intervallen nach 50facher Summierung ohne Reizgabe gezählt und davon der Durchschnitt errechnet. Hierdurch blieb der Einfluß nicht-temperaturbedingter Veränderungen auf die Spontanaktivität minimal. Beim größten Teil der Neurone konnte ein charakteristischer Verlauf der Spontanaktivität in Abhängigkeit von der Körpertemperatur beobachtet werden. Zwischen 36° und 40°C steigt die mittlere Entladungsrate nahezu linear an. Die Steilheit des Anstiegs ist von der Ausgangsentladungsrate abhängig, so daß fast alle Neurone einen ähnlichen relativen Anstieg ihrer mittleren Entladungsrate aufweisen (Anstieg $\frac{5}{3}x > y > \frac{3}{4}x$; Abb. 1).

Parallel hierzu geht eine Zunahme der Herzfrequenz, die um das 1.3—1.6-fache ihrer Ausgangslage ansteigt. Ab 41°C wird der Anstieg in der Spontanentladungsrate schwächer, um bei allen Zellen zwischen 41.5°—42.3°C sein Maximum zu erreichen. Dieses beträgt unter 14 Neuronen das 2.5 bis 3.5-fache (Mittelwert 3.0 ± 0.3) der Ausgangsentladungsrate bei normaler Körpertemperatur. Bei den restlichen nicht derart reagierenden Einheiten kamen häufig Schwankungen in der Pulsrate und im ECoG-Bild vor. Zwischen 42.6° und 43.5°C wurden Anzeichnen eines beginnenden Kreislaufkollapses beobachtet, wobei ab 42°C die Amplituden der Aktionspotentiale häufig kleiner werden, manchmal "bursts" mit zunehmend kleineren Amplituden der sukzessiven Spikes auftraten und ab 42°—43°C neben einem Absinken der Spontanentladungsrate und der Herzfrequenz auch qualitative Veränderungen im EKG-Bild sichtbar wurden. Das ECoG zeigte zu diesem Zeitpunkt hochfrequente spannungsniedrige Wellen.

Antwortmuster

Unter den 22 auf Clickreizung responsiblen Einheiten fallen individuell große Schwankungen im Antwortverhalten auf physikalisch identische Stimuli auf. Trotzdem läßt sich am Gesamtmaterial mit zunehmender Körpertemperatur ein mäßiger Trend zu geringeren Antwortintensitäten feststellen, sowohl gemessen an den *t*-Werten [Korrelationskoeffizient (r) = —0.40] als auch an einem einfachen Antwortmaß $\Delta F/M$ (Quotient aus stimulatorischer Zunahme der Entladung und praestimulatorischem Mittelwert) mit einem r von —0.36 (Abb. 2a, b). Die stimulatorische Zuwachsrate allein (ΔF) verhält sich uneinheitlich (r = —0.14).

Um 39°C Körpertemperatur unterschreitet der größte Teil der Zellantworten (16) häufiger das Signifikanzniveau. Auffallend ist bei 14 von 22 Zellen ein vor dem Kreislaufkollaps gesteigertes Antwortvermögen, obwohl die Spontanaktivität schon abgesunken ist (Abb. 3). In keinem Verlaufe wurde jedoch das Ausgangsniveau der Antwortstärken bei normaler Körpertemperatur wieder erreicht.

Ein typisches Beispiel des Antwortverhaltens gibt Abb. 3. Die exzitatorische Antwort bleibt im wesentlichen auf 2 Zähldekaden beschränkt, wenn auch die folgenden Zähler nicht unbeeinflußt bleiben. Mit zunehmender Körpertemperatur — dieser Befund war häufig — tritt das Maximum der Antwort früher nach der Reizgabe auf.

In 2 Fällen, wie in Abb. 4a, wurde bei hohen Temperaturen ein Umschlagen der exzitatorischen in eine inhibitorische Antwort beobachtet. Die zur jeweiligen Temperaturstufe gehörigen Antwortmuster ohne Reizgabe zeigen keine Schwankungen dieser Größenordnung.

Die Hälfte der Click-responsiblen Neurone (11) weist spezifische Antwortmuster auf, die bei Normaltemperatur recht konstant nachzuweisen sind. Wie schon an früherer Stelle berichtet (ZWIENER et al. 1969), zeigen eine Reihe von Neuronen einen verzögerten Rückgang nach Reizung zur praestimulato-

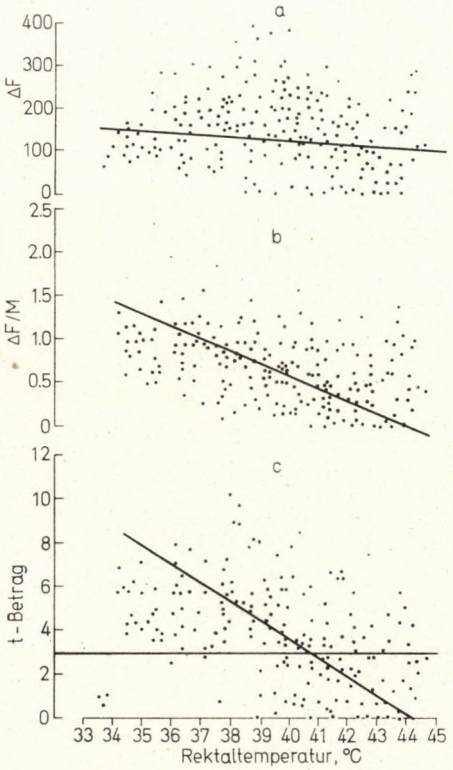

Abb. 2. Verhalten des a) stimulatorischen Entladungszuwachses (ΔF) des b) Quotienten aus Entladungszuwachs und praestimulatorischer Durchschnittsentladungsrate (ΔF/M) und c) der *t*-Werte des Signifikanztestes nach BUREŠ und BUREŠOVÁ von 22 auf Click responsiblen thalamischen Einheiten bei Körpertemperatursteigerung. a) Korrelationskoeffizient (r) = = —0.14; b) r = —0.36; c) r = —0.40. (Zusätzlich eingezeichnet: Korrelationsgeraden. Gerade parallel zur x-Achse: Signifikanzniveau)

rischen Entladungsrate, wobei sich ein näherungsweise exponentieller Verlauf ergibt (Abb. 4a). Bei allen 5 derart reagierenden Einheiten wird dieses Antwortmuster mit zunehmender Temperatur kontinuierlich undeutlicher, um meistens bei 41.5°—42°C gänzlich zu verschwinden, ohne daß sich das exzitatorische Maximum gleichermaßen verändert. Selbst bei den praekollaptischen Antwortintensivierungen wird dieses quasi-exponentielle Antwortmuster nicht wieder sichtbar. Als zweiter Typus von Antwortmustern konnten 6 Zellen mit exzitatorisch-inhibitorischem Antwortkomplex abgetrennt werden,

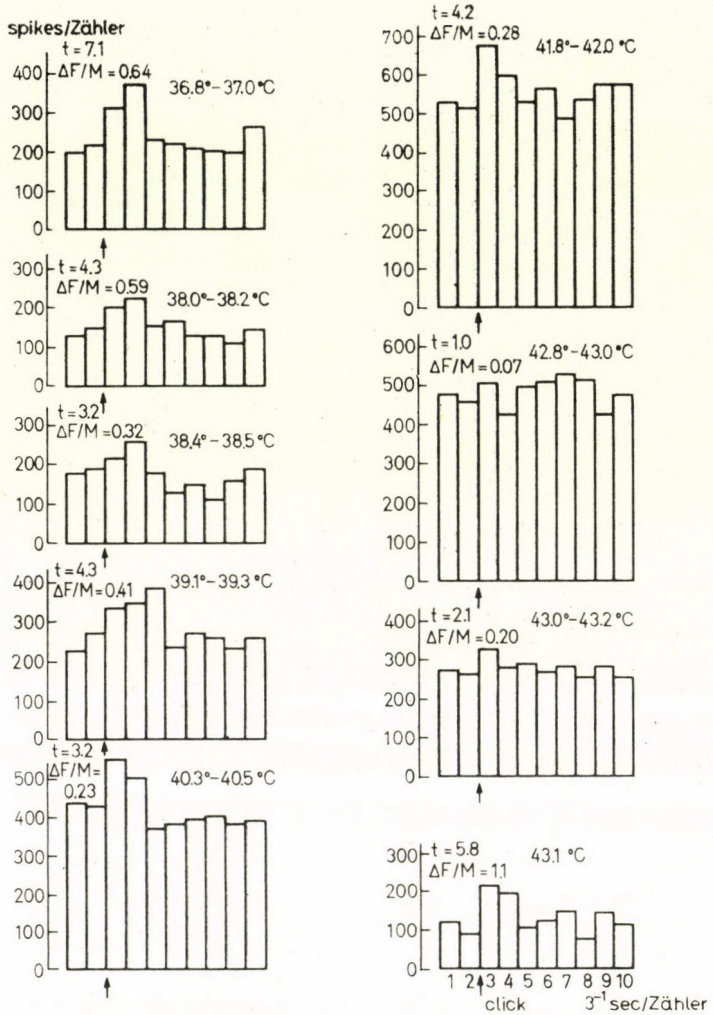

Abb. 3. Verhalten des Antwortmusters eines Neurons aus dem nucl. ventralis pars dorsomedialis thalami bei steigender Körpertemperatur auf Click-Reizung (Zählerstände: Ergebnis nach 50facher Aufsummierung der Einzelantworten; t = Signifikanzwert nach BUREŠ und BUREŠOVÁ)

wobei in 4 Fällen der Hemmung noch eine zweite geringere exzitatorisch-inhibitorische Oszillation folgte (Abb. 4b). An den dazugehörigen Säulendiagramm-Ergebnissen ohne Reizgabe waren keine derartigen Schwankungen sichtbar. Auch dieses Antwortmuster verschwand mit zunehmender Temperatur größtenteils.

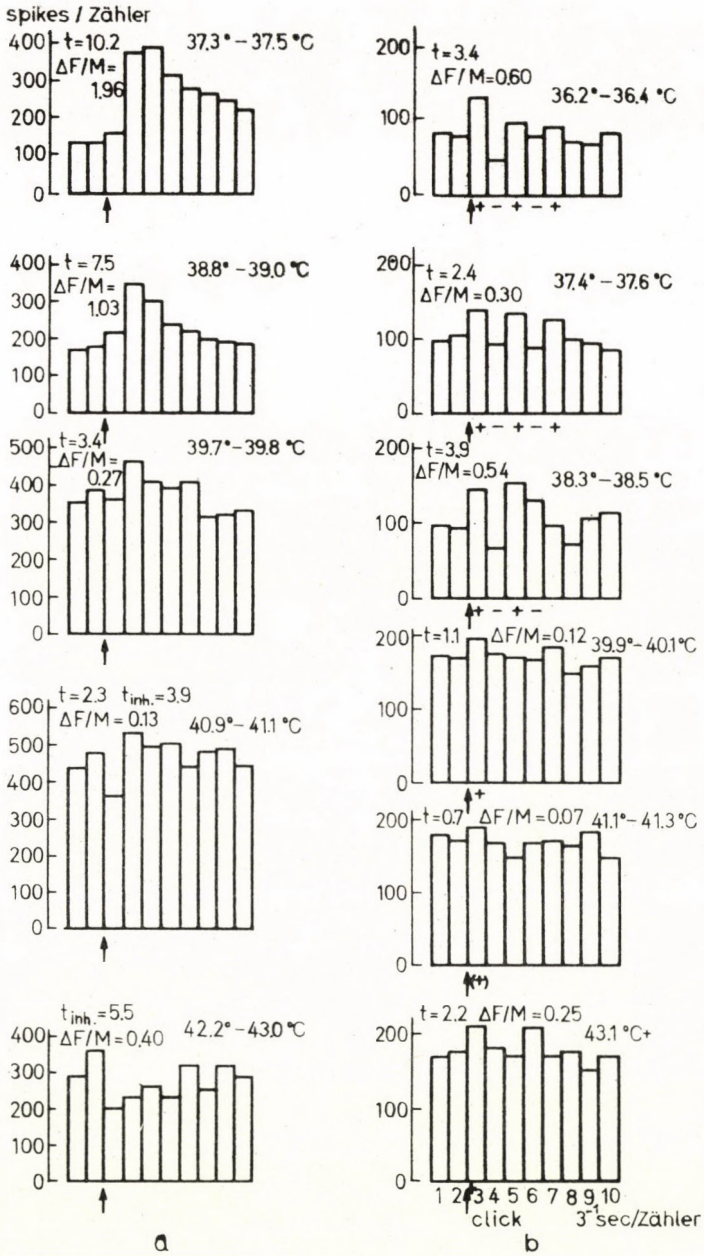

Abb. 4. Verhalten eines a) näherungsweise exponentiellen und b) exzitatorisch-inhibitorischen Antwortmusters auf Click-Reizung bei steigender Körpertemperatur (Zählerstände; Spikeanzahl nach 50facher Aufsummierung der Einzelantworten)

Diskussion

Die insgesamt nur mäßige negative Korrelation zwischen Zellantwort-Intensitäten und steigender Körpertemperatur trotz erheblicher Veränderung der Spontanaktivitätsrate und eindeutigen Veränderungen im EEG der Ratte (TEN CATE et al. 1949; eigene Ergebnisse) ist überraschend. Neuronale Kanäle können also auf mehreren Niveaus ihrer Spontanaktivität ähnliche oder vielleicht gleiche Informationsleistungen vollbringen. Ab 39°C unterschreitet allerdings ein größerer Teil der Antworten das Signifikanzniveau. Zu diesem Zeitpunkt werden die ersten temperaturbedingten EEG-Veränderungen sichtbar (TEN CATE et al. 1949).

Das charakteristische Verhalten der Hintergrundaktivität, wie der näherungsweise lineare Anstieg bis 40°C Körpertemperatur, dürfte in erster Linie durch die nach der van t'Hoff'schen Regel beschleunigten Stoffwechselprozesse verursacht sein. Daß dabei noch höhere Temperaturkoeffizienten als für andere, elementare elektrophysiologische Ereignisse an der Nervenzelle (HAAS 1962) erreicht werden, könnte durch die multisynaptischen Interferenzen und die Ausschüttung von Adrenalin bzw. Noradrenalin erklärt werden (TRAYSTMAN und KRAUS 1967; COLLINS und WEINER 1968).

Da das näherungsweise exponentielle und exzitatorisch-inhibitorische Antwortmuster die Reizeinwirkung größenordnungsmäßig überdauert, können diese Antwortstrukturen als Ergebnis von Interaktionen in neuronalen Netz angesehen werden. Die anfängliche Maximalantwort dürfte dagegen mehr das Ergebnis einer direkten synaptischen Transmission sein. Bei einer gleichmäßigen Einwirkung auf alle Neuronen des Netzes, wie durch steigende Körpertemperatur, müßte das Raum-Zeit-Muster des Netzwerkes aus Wahrscheinlichkeitsgründen störanfälliger sein als die direkte Signaltransmission zu einem Einzelneuron; denn die Netzwerkfunktion ist das Ergebnis der normalen Arbeit aller seiner Elemente. Die aufgezeigten Ergebnisse stützen diese Hypothese. Mit großer Regelmäßigkeit verschwindet bei stärkerem Anstieg der Körpertemperatur die näherungsweise exponentielle Charakteristik des poststimulatorischen Entladungsmusters und ist bei 42° bis 43°C nicht mehr nachzuweisen, ohne daß die initiale Maximalantwort gleichermaßen verändert würde. Auch die exzitatorisch-inhibitorischen Responsitivitätstypen verlieren diese Charakteristik bei hohen Körpertemperaturen, wobei zuerst die schwache, schwellennahe dritte exzitatorische Reaktion verschwindet.

Interessant ist, daß bei den präkollaptischen Antwortsteigerungen das spezifische Muster nicht wieder erscheint.

Insgesamt müssen die Veränderungen der Antwortmuster bei hohen Körpertemperaturen als Hinweis auf Störungen der Informationsverarbeitung im neuronalen Netz angesehen werden. Da bei nahezu den gleichen Körpertemperaturen, welche beim Menschen psychopathologische Störungen

verursachen (SCHAPS und SCHMIDT 1967), die gefundenen Einzelzell-Antwortmuster auf sensorische Reizung bei der Ratte verschwinden, liegt die Vermutung nahe, daß die psychischen Veränderungen bei Hyperthermie auf ähnlichen Störungen der neuronalen Informationsverarbeitung beruhen.

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ÉTUDES DES PROBLÈMES DE LA RÉGULATION DU DÉBIT SANGUIN CÉRÉBRAL

IV LE RÔLE DES NERFS PNEUMOGASTRIQUES ET SYMPATHIQUES CERVICAUX DANS LA RÉGULATION DU DÉBIT CÉRÉBRAL

Par

J. KOPA, GY. POÓR et L. MOLNÁR

CLINIQUE DES MALADIES NERVEUSES ET MENTALES DE L'UNIVERSITÉ DE PÉCS

(Reçu le 7 avril 1970)

La transection des nerfs pneumogastriques et sympathiques cervicaux modifie l'effet des stimulations électriques du tronc cérébral sur le débit du néo-cortex et du cortex cérébelleux. La modification de l'effet des stimulations électriques de la région ponto-bulbaire sur le débit n'est pas en corrélation systématique avec celle des changements de la pression artérielle et de la respiration. Il est alors probable que cette modification n'est pas due aux fluctuations de la pCO_2 et pO_2 du sang et du tissu cérébral. Dans la conduction des influx nerveux venant du tronc cérébral et cheminant jusqu'aux vaisseaux du cerveau les sympathiques et les pneumogastriques jouent également un rôle. L'influence des pneumogastriques sur la circulation du cervelet est discutable, les influx conduits par des sympathiques jouent un rôle important et dans la régulation du débit sanguin du cervelet et dans celle du débit du cerveau.

Selon la plupart des auteurs le rôle des influx nerveux est négligeable dans la régulation du débit sanguin cérébral. Toutefois, on trouve des données dans la littérature ancienne et récente qui semblent prouver que le *pneumogastrique* et le *sympathique* influencent le calibre des vaisseaux cérébraux [KROG (1964) donne dans ses travaux une excellente bibliographie concernant l'influence du sympathique cervical].

Quelques travaux des dernières années ont attiré l'attention sur l'éventuelle action exercée par des *centres du tronc cérébral* sur la circulation du cerveau (SHALIT et coll. 1967; SCHEINBERG 1968). Nos propres observations récentes nous ont montré également que la stimulation de certaines parties du tronc cérébral influe sur le débit circulatoire du néo-cortex et sur celui de l'écorce cérébelleuse (MOLNÁR et coll. 1970). Nos expériences ne nous ont cependant pas renseigné sur les voies par lesquelles les influx venant du tronc cérébral peuvent atteindre les vaisseaux cérébraux. C'est pour cette raison que nous avons étudié *l'effet des stimulations* électriques *du tronc cérébral* sur le débit du néo-cortex et de l'écorce cérébelleuse *avant et après la transection des nerfs pneumogastriques et sympathiques.*

Méthodes

Notre expérimentation a porté sur 21 lapins endormis par administration intraveineuse d'uréthane et de chloralose [uréthane 0.5 g/kg et chloralose 70 mg/kg]. La pression artérielle a été mesurée dans l'artère fémorale. La fréquence respiratoire indiquée par les variations de résistance d'une thermistance, placée dans la canule trachéale, ainsi que l'activité électrique

du néo-cortex et celle du cortex cérébelleux, recueillies par des électrodes d'argent, ont été enregistrées à l'aide d'un oscillographe. Pour mesurer le débit sanguin des territoires dont l'activité électrique a été dérivée, nous avons utilisé la méthode élaborée par SEYLAZ (1965). Des électrodes coaxiales monofilaires ont été employées pour la stimulation électrique du bulbe et de la protubérance. La localisation anatomique de l'électrode stimulatrice a été vérifiée sur des coupes histologiques.

Résultats expérimentaux

Les stimulations électriques (de la région ventro-latérale de la calotte protuberentielle aux niveaux de la Vème et VIIème paires) produisaient soit l'arrêt de *la respiration*, soit la diminution de son amplitude en même temps que sa fréquence se ralentissait ou s'accélerait. La *pression artérielle* augmentait chez 16 animaux; elle ne diminuait que dans un seul cas. Chez 4 animaux, il y avait un abaissement faible, suivi d'une augmentation beaucoup plus importante de la pression artérielle.

Les changements du *débit sanguin du cortex cérébelleux* étaient inverses de ceux de la pression artérielle produits par la stimulation (Fig. 1A). Il n'y avait que deux animaux chez lesquels le flux sanguin suivait passivement l'augmentation de la pression artérielle, mais même dans ces cas une réduction importante du débit se produisait après la cessation de la stimulation. Cette phase de diminution était suivie d'une accroissement plus ou moins important du courant sanguin. Les stimulations n'influençaient pas l'*activité électrique* désynchronisée de l'écorce du cervelet, mais en cas, où l'activité électrique était synchronisée, elle se désynchronisait sous l'effet des excitations (Fig. 1A).

Le débit du cortex cérébelleux montait après la *transection bilatérale des nerfs pneumogastriques et sympathiques* en même temps que la pression artérielle diminuait transitoirement (Fig. 1B). En cas où *seul les sympathiques* étaient *détruits*, une fluctuation du courant sanguin survenait, à la fin de laquelle le débit restait sur un niveau élevé (Fig. 1C).

L'effet des stimulations électriques du tronc cérébral sur le débit cérébelleux changeait *après la transection simultanée des pneumogastriques et sympathiques* en tant que la diminution du flux sanguin se présentant lors de la stimulation ou après la cessation de celle-ci était moins prononcée, tandisque l'augmentation qui suivait était plus importante que chez des animaux intacts (Fig. 2).

Les stimulations du tronc cérébral modifiaient le flux sanguin du cervelet de façon identique chez des animaux intacts et chez ceux, dont *seul les pneumogastriques* étaient *sectionnés*, les sympathiques étant respectés (Fig. 3A et D). Lorsque les *pneumogastriques* était *sectionnés*, les sympathiques restant intacts, une réduction du flux sanguin se développait dans l'écorce du cervelet (Fig. 3F).

Dans des cas, où *les sympathiques* étaient *interrompus*, les pneumogastriques restant intacts, les stimulations du tronc cérébral, effectuées juste après

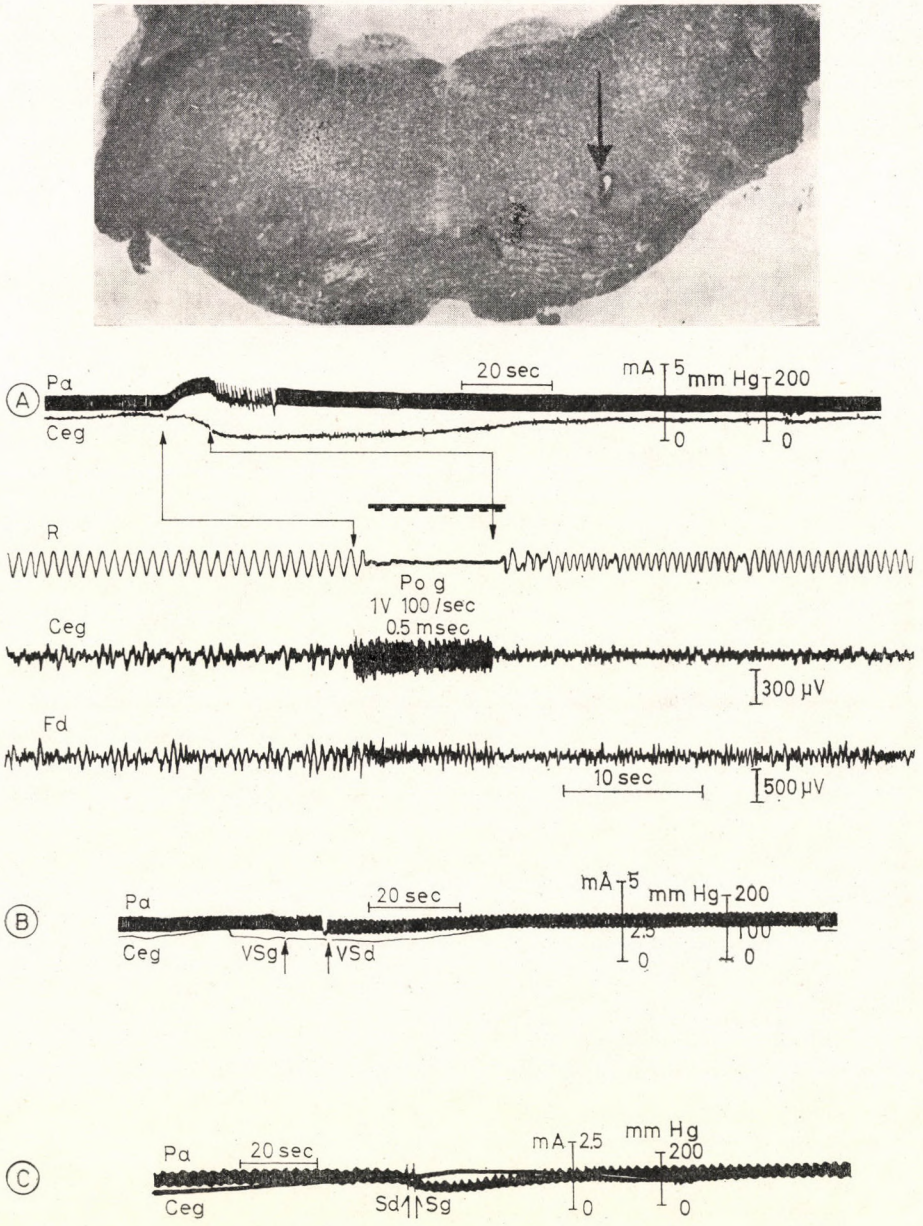
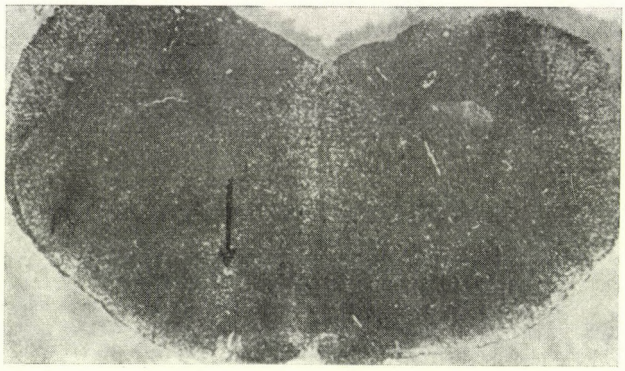


Fig. 1. A Sous l'effet d'une stimulation électrique de la partie ventro-latérale de la calotte protubérentielle (la flèche sur la microphotographie montre la localisation de l'électrode stimulatrice), on voit apparaître l'augmentation de la pression artérielle fémorale (P. a), la diminution du débit de l'écorce cérébelleuse (Ceg), l'arrêt de la respiration (R) et la désynchronisation de l'activité du cortex cérébelleux (Ceg) et cérébral (Fd). *B* Après la transection du pneumogastrique et du sympathique gauche (VSg) et droit (VSd) le débit cérébelleux monte. *C* Chez un autre animal la transection du sympathique droit (Sd) et gauche (Sg) engendre une augmentation du débit dans le cervelet



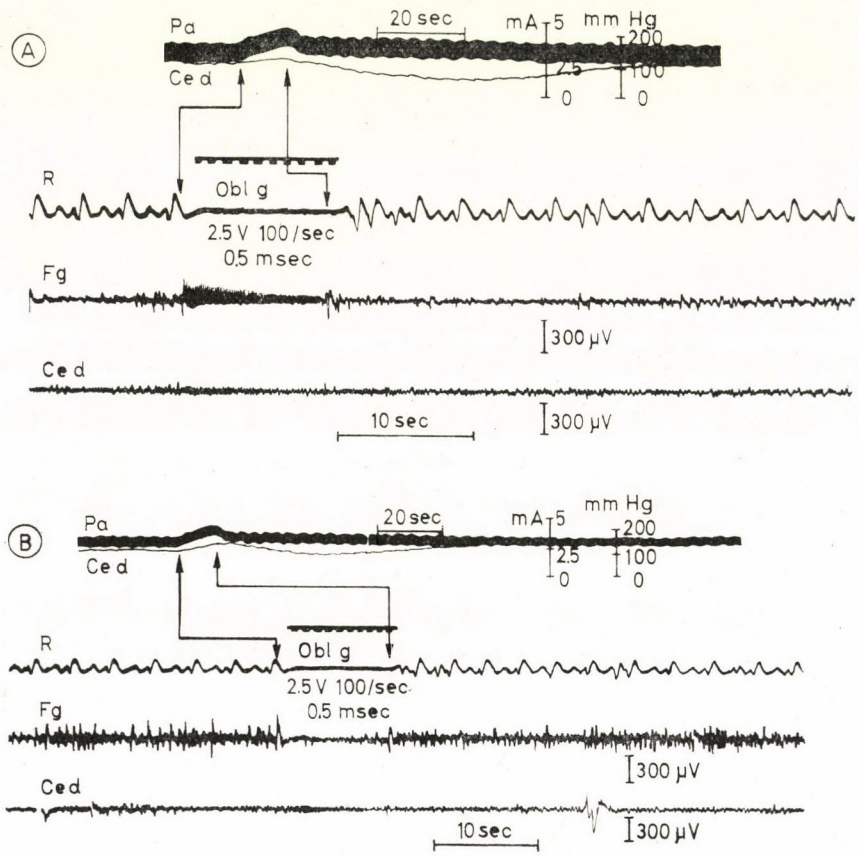


Fig. 2. A Sous l'effet d'une stimulation électrique du bulbe (le point excité est indiqué par la flèche sur la microphotographie) la pression artérielle (P. a.) monte, une augmentation faible, suivie d'une diminution plus prononcée du débit cérébelleux (Ced) apparaît, on observe un arrêt de la respiration (R); l'activité électrique de l'écorce cérébrale (Fg) et cérébelleuse (Ced) ne changent pas. *B* Après la transection des nerfs pneumogastriques et sympathiques cervicaux l'augmentation du débit pendant la stimulation est plus marquée, la diminution en est moins forte qu'elles n'étaient chez l'animal intact

la section des sympathiques, n'avait pas du tout influencé le débit du cervelet bien que les changements de la pression artérielle, de la respiration et de l'activité électriques aient été identiques à ceux observés chez des animaux intacts (Fig. 4). 5 ou 10 minutes après la transection des sympathiques des modifications du débit se produisaient de nouveau sous l'effet des stimulations du tronc cérébral, mais la diminution du flux sanguin était toujours moins prononcée que chez des animaux ayant des sympathiques intacts.

La *transection* des *pneumogastriques* était suivie d'une diminution (Fig. 3C), celle des *sympathiques* d'une augmentation du *débit néo-cortical* (Fig. 5C).

Des excitations aux niveaux ponto-bulbaires engendraient dans le *néocortex* simultanément avec la *désynchronisation de l'activité électrique*, une *diminution du débit circulatoire*. Il n'y avait que 3 cas dans lesquels le courant sanguin suivait passivement l'augmentation de la pression artérielle provoquée par la stimulation. La cessation des stimulations était suivie d'un faible accroissement prolongé du débit sanguin (Fig. 5A).

L'augmentation du débit qui se manifestait pendant la stimulation du tronc cérébral chez des animaux intacts, ne changeait pas après la *transection simultanée des pneumogastriques et sympathiques* cervicaux. Toutefois après la cessation de l'excitation l'accroissement prolongé ne se produisait plus (Fig. 5B).

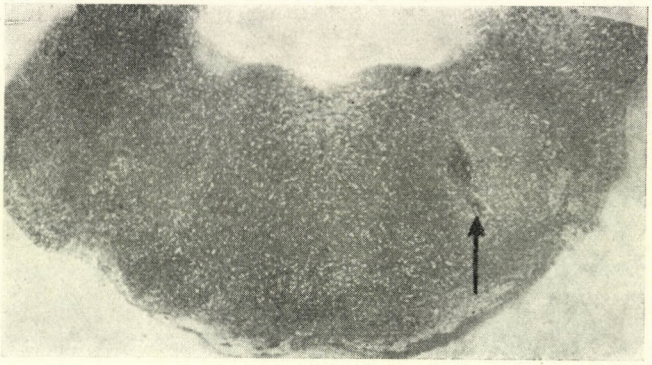
Après la transection des pneumogastriques, l'integrité des sympathiques étant respectée, les mêmes stimulations ponto-bulbaires qui n'influençaient pas le débit néo-cortical chez des animaux intacts (Fig. 3B), produisaient une diminution du flux sanguin, et ceci malgré l'arrêt de la respiration (Fig. 3E).

Dans des cas, où les pneumogastriques restaient intacts et les *sympathiques* étaient *sectionnés*, l'augmentation du débit suivant la cessation des stimulations électriques était plus importante et plus prolongée que chez des animaux intacts.

Commentaires

Le rôle décisif dans la régulation du flux sanguin cérébral est généralement attribué aux changements de la pCO_2 et de la pO_2 ainsi que ceux du pH du sang et du tissu cérébral (voir ZWETNOW 1968). Même des auteurs qui ont observé des modifications survenant sous l'effet des influx nerveux expliquaient celles-ci par des variations de la pCO_2 ou de la pO_2 produites par le fonctionnement du cerveau (voir LASSEN 1968).

Il y a cependant des données dans la littérature qui nous incitent à reconsidérer le problème de la régulation nerveuse du débit cérébral. En effet, depuis que BRACHET en 1830 a observé la constriction des vaisseaux pie-mériens sous l'effet de l'excitation des sympathiques cervicaux, de nombreux



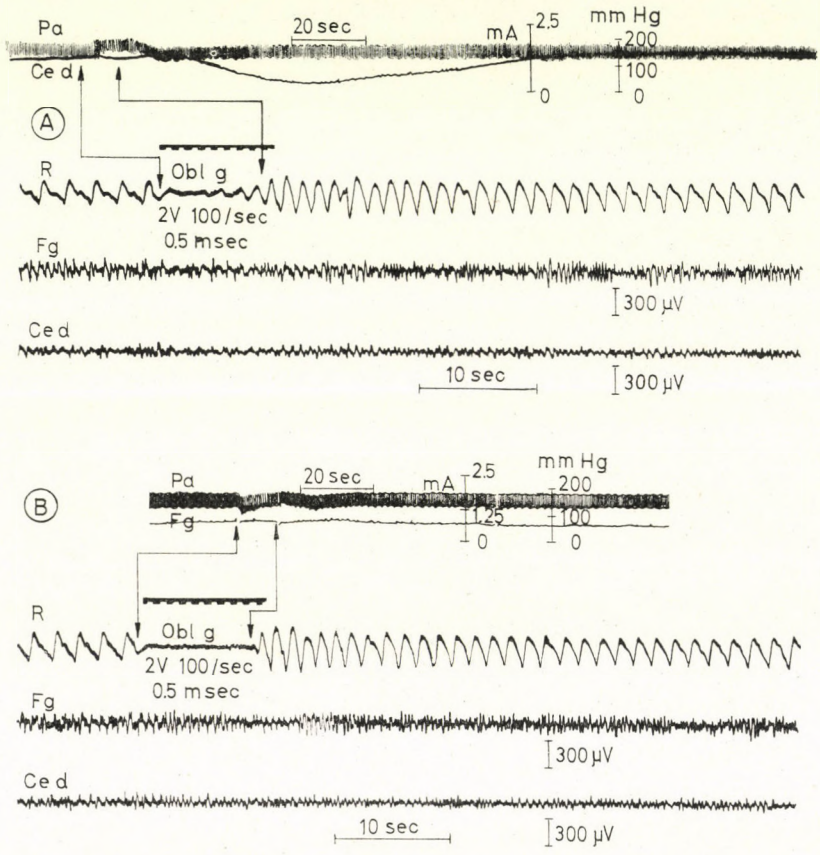


Fig. 3. A L'excitation électrique de la calotte protubérentielle (au point indiqué par la flèche sur la microphotographie) produit malgré l'augmentation de la pression artérielle (P. a.) et l'arrêt de la respiration (R) une diminution du débit du cervelet (Ced); après la cessation de l'excitation le débit cérébelleux augmente faiblement et transitoirement, puis survient une réduction du même débit; l'activité électrique du cortex cérébral (Fg) et cérébelleux (Ced) ne changent guère. *B* L'excitation des mêmes paramètres n'influence pratiquement pas le débit

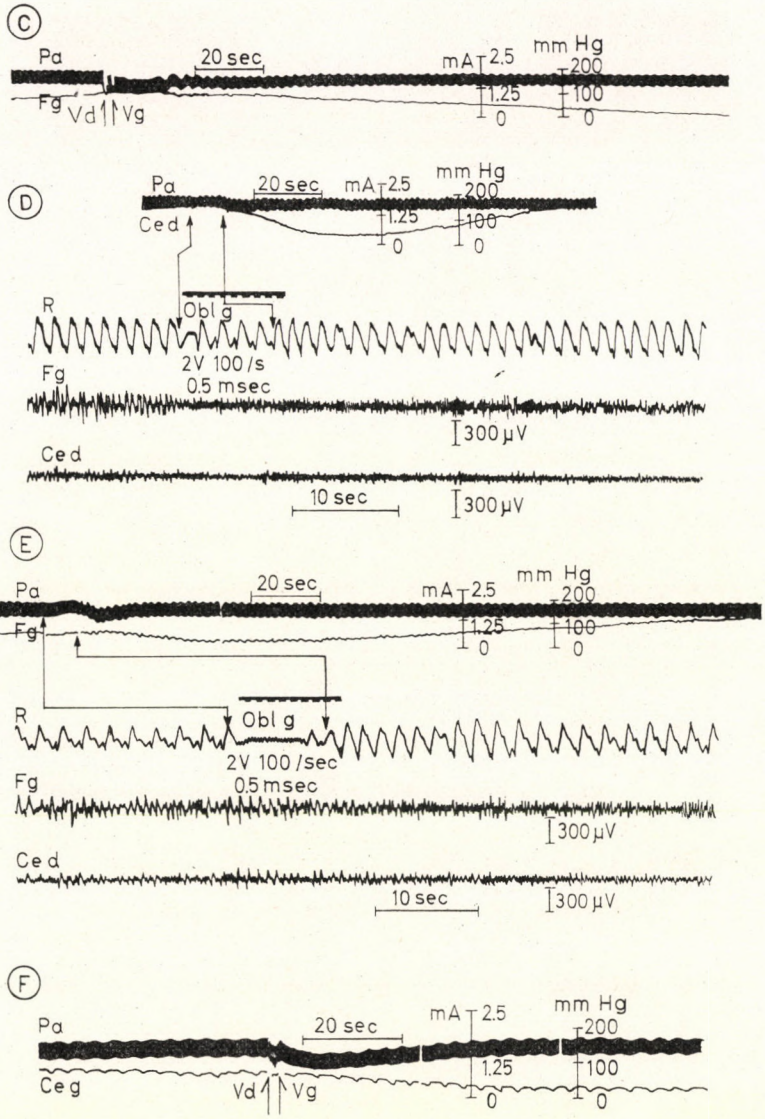


Fig. 3

du cortex cérébral (Fg). *C* Après la transection du pneumogastrique droit (Vd) et gauche (Vg) le débit du cortex frontal (Fg) diminue progressivement. *D* L'effet de l'excitation sur la débit cérébelleux après la transection des pneumogastriques est pratiquement identique à celui de l'excitation pratiquée avant cette intervention. *E* par contre, la stimulation produit une diminution du débit dans l'écorce cérébrale. *F* La transection du pneumogastrique droit (Vd) et gauche (Vg) provoque une diminution du débit cérébelleux (Ceg)

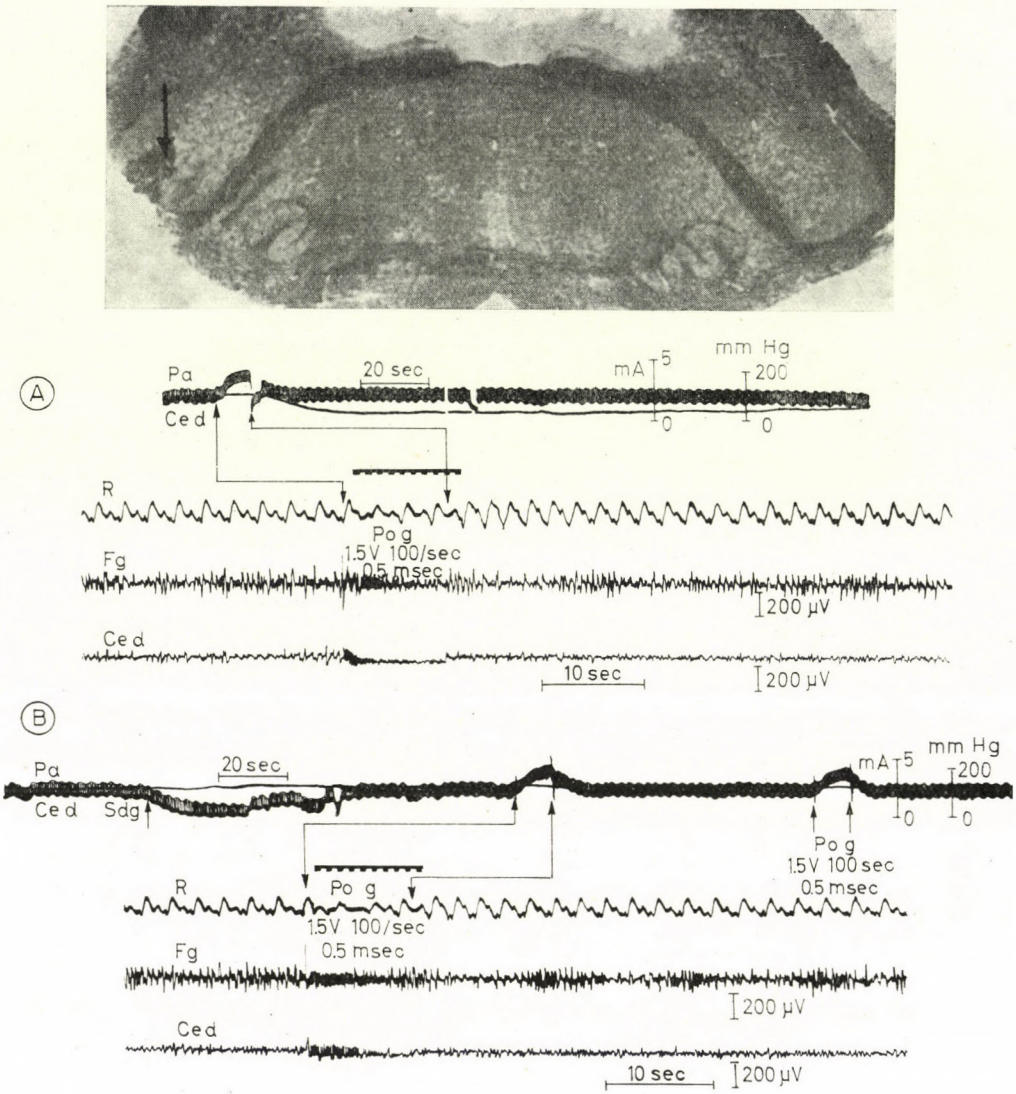


Fig. 4. A La stimulation électrique de la partie ventro-latérale de la protubérence (voir flèche sur la microphotographie) provoque une augmentation de la pression artérielle (P. a.); le débit du cervelet (Ced) ne change pratiquement pas, il diminue après la cessation de l'excitation; l'amplitude de la respiration (R) ainsi que sa fréquence diminuent, l'activité électrique de l'écorce cérébrale (Fg) et cérébelleuse (Ced) restent inchangées. *B* Après la transection des sympathiques cervicaux (Sdg) les mêmes stimulations n'ont plus d'effet sur le débit, bien quelles influencent la pression artérielle et la respiration de la même manière qu'avant cette intervention

auteurs ont pu corroborer la validité de la constatation de BRACHET et même élargir les observations concernant l'importance des influx nerveux venant des récepteurs et des centres nerveux les plus différents (sinus carotidiens, ré-

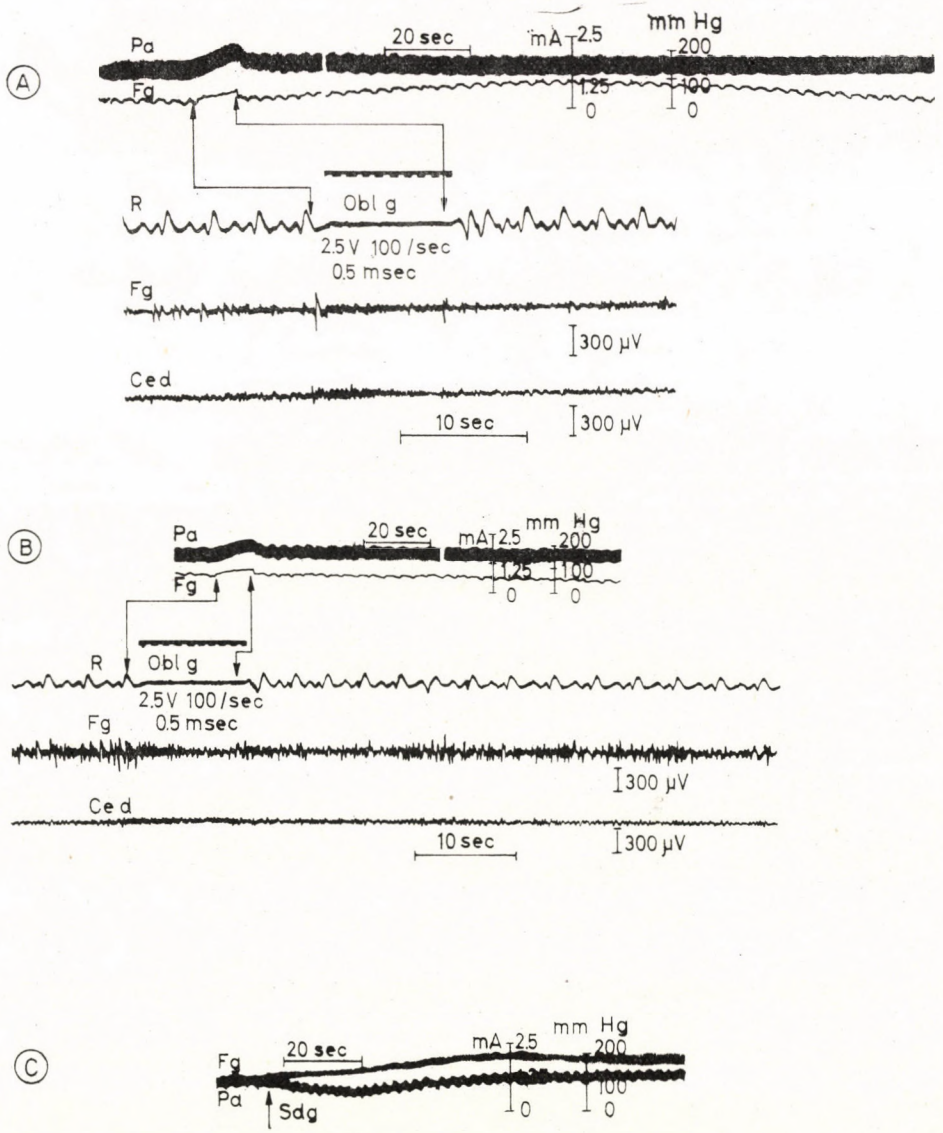


Fig. 5. Même animal que celui de la Fig. 2. *A* La cessation de la stimulation électrique est suivie d'une augmentation du débit dans l'écorce cérébrale (Fg). *B* Cette augmentation disparaît après la transection des nerfs pneumogastriques et sympathiques. *C* La transection des sympathiques (Sdg) est suivie d'une augmentation du débit dans le cortex frontal (Fg).

cepteurs de l'aorte, labyrinthes, centres du tronc cérébral; voir la bibliographie ancienne dans le travail de BOUCKAERT et JOURDAN 1949, les données plus récentes dans celui de MOLNÁR 1967; voir encore les travaux de JAMES et coll.

1968, FALCK et coll. 1968). Nous nous permettons de rappeler nos observations selon lesquelles les effets de la strychnine et du Cardiazol ainsi que ceux des excitations électriques du tronc cérébral sur le débit du cerveau et du cervelet subissent un changement essentiel lorsque le fonctionnement du néocortex est supprimé, et aussi dans le cas, où les relations anatomiques et par conséquent les relations fonctionnelles cortico-bulbaires sont interrompues (KOPA et coll. 1967, 1969; MOLNÁR et coll. 1970).

Les observations, dont les résultats sont présentés dans ce travail, montrent également que des influx nerveux peuvent modifier le débit cérébral. La transection des sympathiques affaiblit l'effet vasoconstricteur, tandis que celle des pneumogastriques diminue l'action vasodilatatrice des stimulations du tronc cérébral. Il faut cependant remarquer que le pneumogastrique semble jouer un rôle moins important dans la régulation du débit du cervelet que dans celle du débit du cerveau. Toutefois la transection des sympathiques entraîne une augmentation, celle des pneumogastriques une diminution du débit sanguin et dans le néo-cortex et dans le cortex cérébelleux. On peut alors tirer la conclusion que les nerfs pneumogastriques et les sympathiques exercent des influences antagonistes sur le tonus de vaisseaux cérébraux.

Bien que nous n'ayons pas enregistré les modifications de la pCO_2 et pO_2, nous avons tout de même de bonnes raisons de supposer qu'une accumulation de CO_2 se développait pendant l'arrêt respiratoire produit par l'excitation du tronc cérébral. Malgré cela et la désynchronisation de l'activité électrique ainsi que l'augmentation de la pression artérielle, une diminution du débit survient généralement et dans le cerveau et dans le cervelet. Comment expliquer cette diminution? Il nous semble plausible que c'est grâce à des influx venant des centres du tronc cérébral que — dans des conditions décrites — une réduction du débit se produise.

Le rôle des influx nerveux nous est prouvé aussi par le fait que pendant 5 ou 10 minutes après la transection des sympathiques cervicaux, la stimulation du tronc cérébral exerçant avant cette intervention une influence importante sur le débit cérébral et cérébelleux, devient inefficace. Ce phénomène traduit vraisemblablement la suppression de la réactivité des vaisseaux cérébraux, un phénomène fort probablement dû à la perte du tonus des vaisseaux, assuré normalement par des influx cheminant par des sympathiques. En effet, on ne peut pas imaginer que la transection des sympathiques cervicaux, manipulation qui ne touche pas directement le cerveau, produise d'éventuels changements métaboliques du cerveau engendrant de cette façon la non-réactivité des vaisseaux cérébraux. Un problème beaucoup plus délicat à expliquer est celui de la réapparition de l'action vasoconstrictrice de la stimulation du tronc cérébral 10 minutes après la transection des sympathiques. Les expériences décrites nous permettent de penser que dans ce cas c'est la proportion de la conduction extra/intracérébrale des influx prenant naissance dans le tronc

cérébral qui change. Les influx parcourant les voies intracérébrales deviennent à nouveau efficaces, mais ils n'ont plus la même intensité, car la transection des sympathiques modifie le tonus des vaisseaux cérébraux.

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Recensiones

K. KUNZE

Das Sauerstoff-Druckfeld im normalen und pathologisch veränderten Muskel

Band 3 des Schriftenreihe Neurologie. Springer-Verlag, Berlin, Heidelberg, New York 1969. 118 pages with 67 figures. Price: DM 58.—; US $ 16.00

The knowledge of local oxygen supply of normal and diseased muscles is significant in both its clinical and physiological aspects. The monograph describes a new method for measuring oxygen pressure fields. Thin platinum needle electrodes driven into the muscle by a micromanipulator allows to make step by step measurements in the resting muscle perpendicular to the muscle fibres. The author investigated 9 patients with different forms of myopathy and 13 patients with neurogenic muscle affections and detected anomalies in the local oxygen supply. The oxygen measurements were correlated with clinical, biochemical, electromyographic and histological findings.

The book will be interesting and useful for neurologists and neurobiologists.

K. LISSÁK

P. LICHTLEN

Klinische Vektor-Elektrokardiographie

Springer-Verlag, Berlin, Heidelberg, New York 1969. 235 pages, 119 figures. Price: DM 86.—

The monograph consists of nine chapters. Chapter I discusses the theoretical basis of ECG and VCG leads. Chapters II, III and IV deal with FRANK's methods, with the relation of ECG to VCG as well as with the formation of the P, QRS and T loops of the normal VCG. Thereafter (Chapters V, VI, VII) myocardial infarction, disturbances of conductivity and of the aortic valve, and auricular and ventricular hypertophy are analyzed. Chapters VIII and IX evaluate the VCG changes due to mitral and congenital disease.

FRANK's method is one of the procedures most widely used by researchers. By means of this method one can determine the spatial cardiac vectors both in the planes of projection and along the three axes. Characterization of the frontal, sagittal and horizontal planes is carried out by means of 7 electrodes. Five of these are placed on the chest in an arc corresponding to the 5th costal interspace, the 6th one is fixed on the neck while the 7th electrode on the left leg. By means of these leads as well as with the aid of resistances inserted between the electrodes one can determine the orthogonal components of the heart dipoles, i.e. the differences of potential in the individual axes.

As the most important part one has to stress the detailed description of the diagnostics of myocardial infarction and its consequences. The VCG alterations are objectivized not only with convential ECG records but also with coronarograms, angiocardiograms and, in some cases, with postmortem findings.

The important signs of anteroseptal and anterolateral infarctions are the backward and in the majority of cases, the downward dislocation of the initial vector loop. The vector loop of an anteroseptal infarction is always directed to the left and backwards while that of an anterolateral one mainly to the right. The clear lateral infarction is characterized by a normal initial running, and the loop begins to shift to the right after 10 to 20 msec. The QRS loop of a posteroseptal infarction comes pathologically forward, into the left upper quadrant; the initial part of the loop of a posterolateral infarction is pushed forward, with otherwise normal run-

ning. According to the author's experience the method offers much help in the diagnosis of infarctions accompanied by intraventricular block.

The diagnostic value of the VCG in cardiac hypertrophy is well known, especially in the case of right heart hypertrophy. With its aid the author distinguishes four types or severity grades.

The VCG alterations found in haemodynamic changes due to acquired and congenital valvular disease and in secondary myocardial lesions are brought into connection with the electropathological manifestations.

LICHTLEN's book means a great help not only in the study of the VCG but also in cardiologic diagnostics. However, it should be noted that also FRANK's VCG method is a complicated procedure. Its general and routine use is hindered by its laboursome character and by the technical difficulties involved; therefore, for the time being its use is restricted to centres of cardiology.

G. BOHENSZKY

W. HORT (Ed.)

Herzinfarkt: Grundlagen und Probleme

(Myocardial Infarction: Fundamentals and Problems) Volume 61 of the series Heidelberger Taschenbücher (Heidelberg Pocketbooks). Springer-Verlag, Berlin, Heidelberg, New York 1969. 154 + VIII pages with 26 figures. Price: DM 9.80

As promised in the title, the book deals with the fundamental aspects of myocardial infarction without any direct practical guidance in its diagnostics and therapy. Nevertheless, the book is a valuable aid for both the theoretical cardiologist and the general practitioner or clinician; the more important aspects of coronary sclerosis and infarction are dealt with in excellent style by the renowned authors.

Of the ten brief, clear-cut chapters mention should be made of Chapter III on the coronaries in myocardial infarction (D. SINAPIUS). The haemorrhage theory of plaque formation is discussed and corroborated in detail. Chapters V and VI are concerned with the conditions of development of coronary collaterals in animals and man, as well as the pertinent pharmacological and surgical results. Chapter VIII, written by H. JUST, bears the title Clinical Physiology and discusses, among others, the cardiac and haemodynamic sequelae as well as the results of intensive treatment. The possibilities of prevention are dealt with in Chapter IX (Epidemiology, by S. HEYDEN), listing the factors which may increase the inclination to the disease (hereditary factors, nicotine, blood cholesterol, overfeeding and nutrition, hypertension, diabetes, physical activities, psychic stress).

There are excellent illustrations and a list of references at the end of every chapter.

T. JÁVOR

P. BÁLINT

Normale und pathologische Physiologie der Nieren

VEB Verlag Volk und Gesundheit, Berlin 1969. 354 pages with 32 figures and 17 tables. Price: M 71.—

This monograph was first published in Hungarian in 1966. Its present German edition is not a simple translation but represents a work widened with the results of investigations made in recent years, and deals with the pathophysiology of the kidney even more exhaustively than did the first edition.

The book fills a gap not only in the German-speaking countries but also elsewhere as since the classical monographs of HOMER SMITH (1951, 1956) no similar review of renal physiology and pathophysiology has appeared.

The monograph consists of 18 chapters with more than 4000 references and with a subject index.

In Chapter 1 the anatomy, circulation and innervation of the kidney are treated. The clearance methods and their evaluation as well as the possibility of RBF and RPF determinations are discussed in detail.

The data concerning normal renal function in various animal species are discussed separately and summarized in tables. The characteristics of intrarenal pressure and circulation as well as the mechanism of autoregulation are followed by the description of tubular reabsorption and secretion processes together with the methods used for their assay.

Separate chapters are discussing electrolyte and water excretion and the role of the kidney in the maintenance of isovolaemia, isotonicity and isoosmosis (dilution-concentration, counter-flow mechanism).

Thereafter, the author describes in detail the part played by the kidney in the defense against acidosis and alkalosis, the mechanism of acidification and its location in various parts of the nephron.

Separate chapters are dealing with the effects of renal function of nervous stimulation, surgical or pharmacological (ganglioplegics) denervation. The functional characteristics of the transplanted kidney are briefly touched upon, as well as the influence on renal function of the neuro-endocrine organs.

The reflex processes connected with the maintenance of isoosmosis and isovolaemia are discussed in great detail, together with the effect of body posture on renal function.

A separate chapter is devoted to the changes in renal function after external influences and stress (emotion, anaesthesia, temperature, nephrectomy, etc.).

The electrolyte disturbances and changes of renal function in acute and chronic renal failure are discussed in detail, together with experimental acute and chronic renal failure.

Finally, even if not with all its particulars but certainly with details sufficient for the clinical researcher, the author discusses the part played by renal circulation in the reflex mechanisms which regulate blood pressure, as well as the role of the kidney in the regulation of erythropoiesis.

In summary, the monograph will be of great help to physicians and research workers interested in any aspect of renal function. Any page of the book offers such detailed data which may add to everyone's knowledge.

F. BALOGH

R. JANZEN

Elemente der Neurologie auf der Grundlage von Physiologie und Klinik

Springer-Verlag, Berlin, Heidelberg, New York 1969. 299 Seiten, 49 Abbildungen.
Preis: DM 48.—

Stoff und Problematik der Neurologie haben sich während der letzten Jahrzehnte erfreulich erweitert, ihre Grundwissenschaften sind in den Besitz von Errungenschaften gelangt, welche unsere Vorstellungen über die physiologischen und pathologischen Vorgänge in den anatomischen und chemischen Strukturen radikal umgestaltet haben.

So ist zweifellos die Zeit für eine Revision der Systematik der Neurologie und ihres Unterrichts gekommen. Versuche, die modernen submikroskopischen und neurophysiologischen Erkenntnisse sowie die auf den Fortschritten der Neurohistologie und -physiologie aufgebauten neuen Methoden der klinischen Neurologie für den Unterricht zu verwerten, sind zu begrüßen. Ein derartiger Versuch muß nicht nur neue Tatsachen und geänderte Ansichten bringen, sondern — im Hinblick auf die beängstigende Vermehrung der Angaben — auch manches Alte weglassen, selbst wenn dieses zu Recht besteht.

Das vorliegende Buch dient diesen Tendenzen. Die Auswahl des Stoffes ist in beiden Richtungen zurückhaltend, aber befriedigend. Der Verfasser hat sich auch auf seinem bevorzugten Forschungsgebiet, der Elektroencephalographie, Maß auferlegt. Seine Haltung in manchen Fragen ist sogar eher etwas hyperkritisch.

Der Verzicht auf eine lehrbuchmäßige Systematisierung bringt es mit sich, daß es nicht leicht war, die Grundlagen, welche die fachliche Einstellung und Denkweise gestalten sollen, zu erörtern und zugleich Hinweise zur praktischen Handlung zu bieten. Es wäre verfehlt, sich mit der Stellungnahme des Verfassers in schwebenden Fragen auseinanderzusetzen. Gewisse Hinweise können jedoch eine Rezension nicht versäumen. Das vom Verfasser empfohlene Vorgehen bei der Liquorentnahme, so auch der Verzicht auf die Druckmessung, dürften kaum

allgemeiner Zustimmung begegnen. Wohl ebensowenig teilen breitere Fachkreise seine Skepsis gegenüber einigen neurochirurgischen Eingriffen.

Der Verfasser betont selbst, daß man das Buch nur langsam und mit Wiederholungen lesen kann. Zweifellos hätte allerdings vieles einfacher und klarer ausgedrückt werden können. Altherkömmliche Ausdrücke durch neue zu ersetzen, war nicht immer notwendig. Zur Zeit wird die deutschsprachige wissenschaftliche Literatur in den Hintergrund gedrängt, und dies auch in Ländern, in denen sie früher verbreitet war, obgleich die Alltags- und Unterrichtssprache nicht die deutsche war. Man müßte bedauern, wenn die schwierige Lektüre des Buches seine Verbreitung ungünstig beeinflussen würde.

ST. KÖRNYEY

I. DIETHELM, O. OLSON, F. STRNAD, H. VIETEN, A. ZUPPINGER (Eds)

Handbuch der medizinischen Radiologie

Band X. Röntgendiagnostik des Herzens und der Gefässe. Teil 1. (Textbook of medical radiology. Vol. 10. Roentgen diagnosis of the heart and blood vessels. Part 1.) Springer-Verlag, Berlin, Heidelberg, New York 1968. 789 pages, 423 illustrations. Price: DM 296.—; US $ 74.00

Springer's manual of radiology, which is planned to consist of 19 volumes in a total of 39 parts, has now its Volume X/1 edited by H. VIETEN of Düsseldorf. Volume X has four parts making up independent volumes. Of these parts 3 and 4 have been published earlier and part 1 is now reviewed.

The subject matter is the methods of examination, the normal and pathological radiological appearance of the heart and blood vessels. Native examination still preponderates in cardiac diagnostics; this and the use of oesophageal filling with radiopaque medium are discussed by P. THURN. There is one chapter each on measurement of the size of the heart and aorta, written by K. MUSSHOFF and H. REINDELL, as well as the former author and J. EMMRICH, respectively. In addition to the well-known methods for the measurement of the size of the heart (projection, measurement of diameter and of surface size) the methods of determination of heart volume, more reliable than those mentioned above, are discussed in detail. Correlative values obtained by comparing the heart volume with various anatomical and physiological factors are also presented. The size of the aorta is based on the measurement of its diameter, the correlative data relate in the first place to age and chest diameter and have been recorded in males mostly.

In the chapter on kymography, written by R. HAUBRICH and K. HECKMANN, both planar and electrokymography are dealt with, the latter in more detail, being more efficient. Phase analysis, i.e. the transposition of the amplitude of electrokymographic curves to the projection of the heart is also discussed.

In the past 20 years heart catheterisation and the use of radiopaque media for the study of the heart and blood vessels have gained great significance, particularly in the diagnosis of congenital and acquired heart diseases. The next two chapters deal with these problems in extenso, quite as the subject matter deserves to be treated. The methods of heart catheterisation are discussed by F. LOOGEN and U. GLEICHMANN, the representation of the heart cavities, blood vessel lumina and blood flow by H. H. LÖHR, H. GREMMEL, F. LOOGEN and H. VIETEN, in two chapters. In the previous chapter the various methods of right and left heart catheterisation (transseptal, retrograde), of the puncture of the left ventricle and atrium are dealt with. In the latter chapter the angiographic techniques are described, then all the angiocardiographic, pulmonary angiographic, thoracic aortographic and mediastinal phlebographic procedures including the various methods of coronarography, selective filling with radiopaque medium of the bronchial artery and of the internal mammary artery. The potential complications and treatment of the administration of radiopaque media are discussed in great detail. (This chapter has a list of references covering 50 pages.) Then comes a brief essay by W. SCHULTE-BRINKMANN on the radiation exposure of patient and staff.

In the chapter on representation of the outer contours of the heart and blood vessels, L. OLIVA describes the pneumomediastinum and the pneumopericardium techniques. The next chapter, written by J. SCHOENMACKERS and H. VIETEN, is devoted to the diagnostic problems of vascular morphology and pathomorphology.

The chapter on the physiology, pathophysiology and radiology of heart function and haemodynamics has been written by W. SCHOEDEL, F. GROSSE-BROCKHOFF and P. THURN. This chapter has three subdivisions, the last one being a monograph on the general symptomatology of radiological cardiac diagnostics, a detailed description of the changes in the various cavities of the heart on exposure to variations of pressure and volume, myogenic dilatation and their combinations, illustrated with many excellent radiographs.

The last chapter deals with the cardiac changes in sportsmen, written by H. REINDELL, K. MUSSHOFF and H. ROSKAMM. The problems discussed are the changes in size and shape, pulsation, performance vs. heart size, life expectancy of sportsmen (slightly better), causes of death, regulative cardiac enlargement vs. age, etc.

There are an authors' index and a bilingual (German—English, English—German) subject index. The lists of references are given at the end of each chapter.

The book contains everything the title suggests it would, including the fundamental physiological and pathophysiological data as well as the statements that can be made and the conclusions that may be drawn from them. The problems not fully resolved or not uniformly viewed are also dealt with. Therefore, this volume of the Handbuch, just like the others, is of great value in diagnostic, therapeutic and research work.

The book is beautifully executed, with clear printing and excellent illustrations. It is a pleasure to read it. This is another high-level publication of Springer's.

G. BENKŐ

Printed in Hungary

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INDEX

PHYSIOLOGIA

Banga Ilona, Mayláth-Palágyi Jolanda: Cytochrome Oxidase Determination Using a Manganese Citrate Complex 117
Selmeci L., Pósch E., Balogh E.: Effect of Tourniquet on Total Lactate Dehydrogenase (LDH) Activity and Isoenzyme Pattern of Rat Serum 125
Fischer E., Varga F.: Hepatic Transport of Iodipamide in Various Animal Species 135
Varga F., Fischer E.: Biliary Secretion of Rose Bengal in the Dog 143
Bartha J., Szőcs Éva: ^{24}Na Uptake and PAH Accumulation of Renal Cortical Slices in the Presence of Angiotensin 151
Fenyvesi T., Kállay K.: The Presence of β-Adrenergic Receptors in the Renal Vascular Bed 159
Barankay T., Jancsó T., Nagy S., Petri G.: Cardiac Output Estimation by a Thermodilution Method Involving Intravascular Heating and Thermistor Recording 167
Kenedi I., Dávid G.: Increased Sympathetic Innervation of the Rabbit Heart. Explanation of the Lability of the ECG in the Rabbit 175
Kovács T., Szabó B., Vissy Ágnes: Some Effects of Hypertonic NaCl Solution on the Mechanical and Electrical Responses of Slow and Fast Muscles of the Cat 183
Stark E., Varga B., Medzihradszky K., Bajusz S., Hajtman B.: Relationships between the Structure and the Adrenal and Extra-Adrenal Effects of ACTH Fragments 193
Makara G. B., Stark E., Mihály Katalin: Corticotrophin Release Induced by Traumatic Stress in Rats with Unilateral Spinal Cord Lesion 199
Kovách A. G. B., Monos E., Koltay E., Desrius A.: Effect of Hypothalamic Stimulation on Adrenal Blood Flow and Glycocorticoid Release Prior to and after Acute Hypophysectomy 205
Bohus B.: The Medial Thalamus and the Opposite Effect of Corticosteroids and Adrenocorticotrophic Hormone on Avoidance Extinction in the Rat 217
Várnai Ilona, Farkas Maria, Donhoffer Sz.: Thermoregulatory Effects of Hypercapnia in the New-Born Rat. Comparison with the Effect of Hypoxia 225
Zwiener U.: Hintergrundaktivität und Informationsverarbeitung Dienzephaler Einzelneurone unter Hyperthermie 237
Kopa J., Poór Gy., Molnár L.: Études des problèmes de la régulation du débit sanguin cérébral. IV. Le rôle les nerfs pneumogastriques et sympathiques cervicaux dans la régulation du débit cérébral 247

RECENSIONES

Kunze K.: Das Sauerstoff-Druckfeld im normalen und pathologisch veränderten Muskel. Springer-Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 259
Lichtlen P.: Klinische Vektor-Elektrokardiographie. Springer-Verlag, Berlin, Heidelberg, New York 1969. (G. Bohenszky) 259
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Bálint P.: Normale und pathologische Physiologie der Nieren. VEB Verlag Volk und Gesundheit, Berlin 1969. (F. Balogh) 260
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Diethelm I., Olson O., Strnad F., Vieten H., Zuppinger A.: Handbuch der medizinischen Radiologie. Band X. Teil 1. Springer-Verlag, Berlin, Heidelberg, New York 1968. (G. Benkő) 262

INTERNATIONAL COUNCIL OF SPORTS AND PHYSICAL EDUCATION (at U.N.E.S.C.O.) WORKING GROUP ON BIOMECHANICS

PRELIMINARY ANNOUNCEMENT

THIRD INTERNATIONAL SEMINAR ON BIOMECHANICS

MAIN THEME: Biomechanical Studies on Human Motion and their Applications to Sports, Physical Education, Rehabilitation and Human Labour.

DATE: September 27th – October 1st, 1971

PLACE of the SEMINAR:
Istituto di Fisiologia Umana — Università degli Studi — ROMA

ORGANIZING COMMITTEE:

S. Cerquiglini (Chairman)
A. Venerando (Vice-Chairman)
A. Dal Monte (General Secretary)
G. Venturoli-Proja (Executive Secretary)

by order of the Working Group on Biomechanics — International Council of Sport and Physical Education (UNESCO)

PROGRAM: The Seminar will include the following Sessions:

1. Methods and Techniques
2. Fundamental Research in Biomechanics
3. EMG-Kinesiology
4. Biomechanics Applied to Sports and Physical Education
5. Biomechanics Applied to Human Labour
6. Biomechanics in Rehabilitation of Human Movement

OFFICIAL LANGUAGE: English

Simultaneous translations French—English and Italian—English will be considered by the Organizing Committee after the expiration term for papers application.

INSCRIPTION: The authors should offer the titles of communications ultimately at the end of December, 1970.

Summaries as well as texts of manuscripts (in English) must be sent in before March 1, 1971.

The number of papers, which must be based on original experimental work, will be limited and communications will be submitted to the Scientific Committee.

Other participants must apply before the end of June, 1971.

II. International Congress of Psycho-Neuroendocrinology

Budapest, July 1—3, 1971

The Congress will be held in Budapest, and the program will consider the following topics:

1. Developmental neuroendocrinology
2. Biosynthesis and release of pituitary trophormones
3. Drug actions on neuroendocrine and brain mechanisms
4. Hormonal effects and brain mechanisms
5. Developments in clinical neuroendocrinology

Each of the 5 major topics will be introduced by review lectures of 20 to 30 minute duration, which will be given by invited speakers. Each session will be followed by a free discussion of 6 minute duration and presentation of new unpublished data by registered participants.

A registration fee of U.S. $ 20 or of 20 rubels (for participants from socialist countries) will be requested from participants till March 31, at the following address: Hungarian National Bank in favour of the IBUSZ account — RCS 505019.

Further informations may be obtained from the Secretariate of the Congress of International Society of Psycho-Neuroendocrinology, MOTESZ, Budapest, I. Apród u. 1—3.

Der Erste Europäische Kongress für Biophysik

organisiert von der Österreichischen biophysikalischen Gesellschaft unter Mitwirkung der Schwestergesellschaften und unter der Patronanz der Internationalen Union für Reine und Angewandte Biophysik (IUPAB), findet in Baden bei Wien, 14—17. September 1971, statt. Anfragen und Anmeldungen an das Sekretariat, Frau E. Weidenhaus, Wiener Medizinische Akademie, Stadiongasse 6—8, A-1010 Wien, Österreich.

ACTA PHYSIOLOGICA

ТОМ 38 – ВЫП. 2—3

РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ ЦИТОХРОМОКСИДАЗЫ С ПРИМЕНЕНИЕМ МАРГАНЕЦЦИТРАТ-КОМПЛЕКСА

И. БАНГА и Й. МАЙЛАТ-ПАЛАДЬИ

Авторы разработали быстрый калориметрический метод, который легко можно применять при измерении активности цитохромоксидазы в тканях и асцит-опухолях. Суть метода в том, что в терминальную окислительную цепь между цитохром С и цитохром a_3 включают одну электронтранспортную систему, марганеццитрат-диетилпарафенилен-диамин-комплекс. При действии окислительного фермента (цитохромоксидаза) вышеупомянутый комплекс окисляется мальва-цветом. Изменение интенсивности цвета пропорционально активности энзима. Этот метод был разработан на Пулфрих-фотометер, но можно использовать всякий спектрофотометр. Изучение требует небольшого количества тканей (100—500 μг белка) и для него достаточно 15 минут. Активность энзима расчитывалось по содержанию белка.

ДЕЙСТВИЕ ТУРНИКЕТА НА ОБЩУЮ АКТИВНОСТЬ ДЕГИДРОГЕНАЗЫ МОЛОЧНОЙ КИСЛОТЫ (LDH) И НА ИЗОЗИМНУЮ КАРТИНУ СЫВОРОТКИ КРОВИ У КРЫСЫ

Л. ШЕЛЬМЕЦИ, Э. ПОШ и Э. БАЛОГ

У крысы была вызвана аноксия мышц наложением турникета на заднюю конечность одной стороны и в различные сроки после снятия турникета была определена общая активность дегидрогеназы молочной кислоты в сыворотке. При помощи электрофореза на агаргеле были отделены изозимы LDH. Установлено, что общая активность LDH достигает максимальной величины спустя три часа после снятия турникета. Затем она постепенно снижается, но даже по истечении 48 часов еще достоверно превышает контрольные величины и возвращается к норме в пределах 96 часов. Изменение картины изозимов наиболее выражено через сутки после снятия турникета. Изменения по времени в картине изозимов LDH, полученные после внутрибрюшинного введения вытяжки из скелетной мышцы или миокарда нормальных крыс или кроликов, подкрепляет предположение о том, что ответственными за изменения активности изозимов, наблюдаемые под влиянием турникета, следует считать в первую очередь изозимы LDH, попадающие в кровообращение из поврежденной мышцы.

ГЕПАТИЧЕСКИЙ ТРАНСПОРТ ИОДИПАМИДА У РАЗЛИЧНЫХ ВИДОВ

Е. ФИШЕР и Ф. ВАРГА

У наркотизированных животных с помощью метода одной-инъекции была определена гепатическая максимальная передача (Tm) иодипамида. Значение Tm у кошки 0,23, у собаки 0,84, у крысы 1,84, у кролика 2,61 и у морской свинки 2,89 мл/мин/кг. Концентрация иодипамида в желчи с повышением дозы приближается к одному максимуму. Этот максимум самый низкий у кошки и морской свинки (25 мг/мл), самый высокий у собаки (56 мг/мл). Tm прежде всего с возрастанием желчеотделения печени идет паралелльно (у кошки 9, у собаки 14, у крысы 38, у кролика 72 и у морской свинки 115 μл/мин/кг). Для достижения Tm иодипамид нужно дать в большой дозе (400—800 мг/кг внутривенно). Это можно объяснить тем, что иодипамид, находящийся в организме, локализован в 86% экстрагепатически и для обеспечения максимальной передачи в печени нужна концентрация иодипамида в количестве 1 мг/г.

ВЫДЕЛЕНИЯ БЕНГАЛЬСКОГО КРАСНОГО С ЖЕЛЧЬЮ У СОБАК

Ф. ВАРГА и Е. ФИШЕР

Авторы вливали наркотизированным собакам на протяжении трех часов по 0,1—1,2 мг бенгальского красного на кг веса тела в мин и наблюдали за изменениями концентрации красителя в плазме, желчи, печени и других тканях. После введения 0,1 мг бенгальского красного на кг веса в мин. 35,8% красителя оказывается в печени. В противоположность этому после введения 1,2 мг бенгальского красного на кг веса в мин. в печени удалось выявить только 6,4% введенного количества. Причиной этого является ограниченная накопляющая емкость печени в отношении красителя. Она определялась в 571 μг на г. В других органах (почки, тонкая кишка, мышца) концентрация бенгальского красного повышается соразмерно введенному количеству. При достижении ТМ бенгальского красного (26,5 μг на кг в мин) накопляющая емкость печени наполовину насыщена. Бенгальский красный в значительной мере понижает отток желчи, предположительно посредством торможения активной секреторной деятельности.

ПОГЛОШЕНИЕ ^{24}Na И АККУМУЛЯЦИЯ РАН СЕГМЕНТАМИ КОРКОВОГО СЛОЯ ПОЧКИ В ПРИСУТСТВИИ АНГИОТЕНЗИНА

Й. БАРТА и Е. СЁЧ

Авторы инкубировали сегменты кортекса почки крысы в Кребс—Рингер растворе на 28° С в присутствии ангиотензина или без него. Установили, что

1. ангиотензин не оказывал влияния ни на потребление кислорода, ни на аккумулирование РАН сегментамы кортекса.
2. содержание Na в тканях, которые инкубировались в присутствии ангиотензина в течение часа, было меньше, чем в контрольных опытах.
3. Na находится в тканях в двух видах. Один из них быстро обменивается и ангиотензин не влияет на величину обмена этой фракции. Считают, что эта фракция Na находится главным образом во внеклеточном пространстве. Другая фракция Na, которая находится в клетке, значительно медленнее обменивается в присутствии ангиотензина.

Предполагают, что ангиотензин снижает трансмембранный пасс ивный транспорт Na

БЕТА-АДРЕНЕРГИЧЕСКИЕ РЕЦЕПТОРЫ В ОБЛАСТИ СОСУДОВ В ПОЧКЕ

Т. ФЕНЬВЕШИ и К. КАЛЛАИ

Был изучен эффект бета-адренергической возбудимости и блокада гемодинамики почки у 17 наркотизированных собак.

l-изопротеренол, инфузированный в почечную артерию, снижал резистентность сосудов почки и повышал диурез. Блокирующий бета-адренергорецепроты пропранолол повышает оба эффекта. В части случаев пропранолол сам, кажется, увеличивает почечную васкулярную резистентность.

Обработка с алфа-адренергическим блокирующим дибензилином не изменяла эффекта l-изопротеренола на гемодинамику почки.

Результаты указывают, что сосудистая область почки содержит бета-адренергические рецепторы. Локализация и физиологическое значение рецепторов до сих пор невыясненны.

ТЕРМОДИЛЮЦИОННОЕ ИЗМЕРЕНИЕ МИНУТНОГО ОБЪЕМА ПУТЕМ ВНУТРИСОСУДИСТОГО НАГРЕВА И РЕГИСТРАЦИИ ПРИ ПОМОЩ И ТЕРМИСТОРА

Т. БАРАНКАИ, Т. ЯНЧО, И. НАДЬ и Г. ПЕТРИ

Авторами был разработан метод для термодилюционного определения минутного объема, при котором приток тепла осуществляется с прямоточным питающим прибором, снабженным реле времени, в виде 0,7—1,5 секундного внутрисосудистого нагрева при помощи элемента, введенного в верхнюю и нижнюю полые вены. Кривые термодилюции

были сняты на регистрирующем приборе при помощи термистора, введенного в легочную артерию.

Величины минутного объема, полученные этим методом, были сопоставлены с величинами, полученными при непосредственном измерении по принципу Фрикка.

Описанный авторами метод кажется пригодным для повторного измерения минутного объема, без введения чужеродного вещества и без взятия крови.

УСИЛЕННАЯ СИМПАТИЧЕСКАЯ ИННЕРВАЦИЯ СЕРДЦА КРОЛИКА. ОБЪЯСНЕНИЕ ЛАБИЛЬНОСТИ ЭКГ КРОЛИКА

И. КЕНЕДИ и Г. ДАВИД

На кроликах, находящихся в естественном сидячем положении при записи ЭКГ с помощью игол-электродов наблюдалась изменяющаяся лабильность одиночных волн.

При действии уретанового наркоза эта лабильность меньше, но при записи ЭКГ с большой скоростью или при длительной записи ее можно наблюдать.

Серотонин и изопротеренол увеличивают, а атропин не влияет на лабильность ЭКГ кролика. После пропранолола ЭКГ становится геометрически регулярной. Можно сделать вывод, что лабильность одиночных волн зависит от усиленной симпатической иннервации.

ДЕЙСТВИЕ ГИПЕРТОНИЧЕСКОГО NaCl НА ЭЛЕКТРИЧЕСКУЮ И МЕХАНИЧЕСКУЮ АКТИВНОСТЬ БЫСТРОЙ И МЕДЛЕННОЙ МЫШЦЫ КОШКИ

Т. КОВАЧ, Б. САБО и А. ВИШШИ

Изучалось влияние гипертонического NaCl раствора на интенсивность и продолжительность сокращения и тетанической контрактуры на икроножной и камбаловидной мышцах нефректомированной кошки. При действии 20%-ого NaCl раствора, инфузированного через одну из почечных артерий в аорту прекращалась связь между возбуждением-контрактурой. Авторы установили, что в медленной мышце сокращение и тетаническая контрактура сохраняются и тогда, когда в быстрой мышце уже прекращается ее сокращение при последовательной инфузии гипертонической соли. Пока механический ответ мышцы сохранялся, проведение возбуждения не показывало значительного изменения.

Данные показывают, что в камбаловидной мышце — вопреки большому повышению внутриклеточной электролитной концентрации — находится значительно более стабильная связь возбуждения-сокращения, чем в икроножной мышце. Кажется вероятным, что электро-механическая связь медленной, тонической мышцы имеет больше адаптационную способность, чем скорая тетаническая мышца.

СВЯЗЬ В АДРЕНАЛЬНОЙ И ЭКСТРААДРЕНАЛЬНОЙ АКТИВНОСТИ С АКТГ И ЕГО СИНТЕТИЧЕСКИМИ ФРАГМЕНТАМИ

Э. ШТАРК, Б. ВАРГА, К. МЕДЗИХРАДСКИ, Ш. БАЮС и Б. ХАЙТМАН

Раньше было устанавлено, что АКТГ увеличивает кровоток не только в надпочечнике, но и экстраадренально, в яичнике (Штарк и сотр. 1967).

Изучался вопрос, как зависит действие АКТГ на секрецию кортикостероидов и увеличение кровотока в надпочечнике и яичнике от длины его цепи. Опыты были произведены на собаках. Протекающее в органах количество крови было измерено методом, основанным на принципе тканевой теплопродукции. Секреция кортикостероидов была определена в венозной крови надпочечника гипофизектомированного животного (μг/мин). Действие натурального АКТГ и синтетических фрагментов было сравнено с действием фрагмента 1—24 (Synachten, Ciba). Установлено, что фрагмент 1—14 увеличивает протекающее количество крови в обоих органах, хотя активность его в некоторой степени ниже по сравнению с фрагментом 1—24. Однако влияние, оказываемое на увеличение кровотока в яичнике в некоторой степени выше, чем влияние на кровоснабжение надпочечника. Естественный АКТГ свиньи и синтетический человеческий АКТГ вызывают меньшее

увеличение кровотока обоих органов, чем фрагменты 1—24 или 1—28. Фрагмент 5—14 и фрагмент 1—14, имеющий на месте 4 оксидированный метионин, были неактивны даже в дозе в 10 раз большей, чем обычный фрагмент 1—14. Аналогичное количество фрагмента 5—14 вызывает значительно более высокую секрецию кортикостероидов, чем фрагмент 1—14.

ДЕЙСТВИЕ ТРАВМАТИЧЕСКОГО СТРЕССА НА УВЕЛИЧЕНИЕ СЕКРЕЦИИ АКТГ ПОСЛЕ ОДНОСТОРОННЕЙ ПЕРЕРЕЗКИ СПИННОГО МОЗГА

Г. Б. МАКАРА, Э. ШТАРК и К. МИХАЛЬ

Увеличение секреции АКТГ, вызванное переломом бедренной кости, изучалось на крысах. Уровень кортикостероидов в плазме был измерен путем определения секреции АКТГ. Полусекция шейного спинного мозга длительно тормозила действие контралатеральной травмы, но не влияла на способность гомолатеральной травмы увеличивать секрецию АКТГ. Главные афферентные пути травматического стресса перекрещиваются в спинном мозге и входят в tractus spinothalamicus dorsalis или на его окружении.

ДЕЙСТВИЕ РАЗДРАЖЕНИЯ ГИПОТАЛАМУСА НА КРОВОТОК И ВЫДЕЛЕНИЕ ГЛЮКОКОРТИКОИДОВ НАДПОЧЕЧНИКАМИ ПЕРЕД И ПОСЛЕ ОСТРОЙ ГИПОФИЗЭКТОМИИ

А. Т. Б. КОВАЧ, Э. МОНОШ, Э. КОЛТАИ и А. ДЕШРИУШ

Было изучено действие раздражения медиального гипоталамуса на венозный кровоток и гормональную экскрецию, а так же на системное кровообращение перед и после гипофизэктомии.

Раздражение гипоталамуса в большинстве случаев увеличивает кровоток в надпочечнике и кроводавление, а циркуляция может изменяться независимо от давления. Не было корреляции между гормональным ответом при раздражении, примененном перед гипофизэктомией, а после гипофизэктомии положительная корреляция была обнаружена. В последнем, в большинстве случаев выявилось увеличение выделения кортикостероидов. Гормональный уровень значительно может влиять на действие гипоталамического раздражения.

Полагают, что кровоток надпочечников является одним из факторов экстрагипофизеарной мобилизации кортикоидов; можно предполагать, что их координированное управление осуществляется на уровне гипоталамуса.

РОЛЬ МЕДИАЛЬНЫХ ТАЛАМИЧЕСКИХ СТРУКТУР В ПРОТИВОПОЛОЖНОМ ВЛИЯНИИ АКТГ И КОРТИКОСТЕРОИДОВ В ТЕЧЕНИЕ УГАШЕНИЯ УСЛОВНОГО ОБОРОНИТЕЛЬНОГО ОТВЕТА У КРЫС

Б. БОХУШ

Автор наблюдал в своих ранних исследованиях, что влияние АКТГ и кортикостероидов на высшую нервную деятельность крыс является противоположным. АКТГ замедляет, стероиды развертывают это влияние через чувствительные к кортикостероидам структуры переднего мозга и медиального таламуса.

В настоящих опытах автор установил, что АКТГ замедляет угашение оборонительного ответа у крыс, имеющих имплантированный кортизон-ацетат в различные структуры переднего мозга. АКТГ не оказывал влияния, если имплантаты кортизона расположены в центромедиан-парафасцикулярной области медиального таламуса.

Можно предполагать, что медиальный таламус является одним из мест противоположного влияния АКТГ и кортикостероидов. Но влияние АКТГ доминирует над торможением вызываемым кортикостероидами в переднем мозге.

ВЛИЯНИЕ ГИПЕРКАПНИИ НА ТЕРМОРЕГУЛЯЦИЮ НОВОРОЖДЕННОЙ КРЫСЫ. СРАВНЕНИЕ С ДЕЙСТВИЕМ ГИПОКСИИ

И. ВАРНАИ, М. ФАРКАШ и С. ДОНХОФФЕР

Новорожденные крысы в различном возрасте (от нескольких часов после рождении до 20 дней) дышали 6%-ой углекислотой 30 минут при температуре окружающей среды 30 °С, то есть ниже, чем термонейтральная. Потребление кислорода и температура ободочной кишки непрерывно измерялись 30 минут при дыхании воздухом, потом при дыхании углекислотой и потом опять 30 минут при дыхании воздухом. У большинства крыс перед и после этого опыта при подобном обстятельстве исследовалось влияние дыхания 12%-ным кислородом. В противоположность влиянию гипоксии гиперкапния у большинства животных не снижала термопродукцию и температуру, ободочной кишки и реакция, характерная для взрослых животных участилась только после 10-ого дня жизни. Но у 6 недельных крыс гиперкапния при температуре, ниже чем термонейтральная без исключения уменьшала теплопродукцию и температуру тела. Различное качество ответа, наступающего на гипоксию и гиперкапнию в постнатальном возрасте показывает, что их механизм не совсем одинаковый. Анализ ответов, наступающих на гипоксию и гиперкапнию в теплопродукции и температуре тела показал, что не только химическая терморегуляция но и механизмы физической терморегуляции являются активными уже с рождения.

ДИЭНЦЕФАЛИЧЕСКАЯ НЕЙТРОННАЯ ФОНОВАЯ АКТИВНОСТЬ И ОБРАБОТКА ИНФОРМАЦИИ ПОД ВЛИЯНИЕМ ГИПОТЕРМИИ

У. ЗВИЕНЕР

Фоновая активность и суммарные ответы таламических нейронов были исследованы у бодрствующих крыс после применения одинаковых физических раздражителей при различных температурах: с нормальной температуры тела до 43 °С.

Фоновая активность показала почти линейное повышение с поднимающимся рассеиванием до 40 °С, между 41,5—42,3 °С достигла максимума, потом опять падала.

Интенсивность ответов показывала негативную корреляцию с поднимающейся температурой тела, и над 39 °С, большая часть ответов нейронов падала под сигнификантный уровень. Ответы выраженно слабеют с повышением температуры тела и при 42 °С совсем исчезают. Автор полагает, что эти изменения ответов говорят о неправильной реализации в нейронной сети, и он дает психопатологические параллели.

ИССЛЕДОВАНИЯ ВОПРОСОВ РЕГУЛЯЦИИ КРОВООБРАЩЕНИЯ МОЗГА IV. РОЛЬ ШЕЙНЕГО ВАГУСНОГО И СИМПАТИЧЕСКОГО НЕРВОВ В РЕГУЛЯЦИИ КРОВООБРАЩЕНИЯ МОЗГА

Й. КОПА, Д. ПООР и Л. МОЛНАР

После пересечения шейнего вагуса и симпатического нерва влияние электрического раздражения ствола мозга на кровообращение неокортекса и кортекса мозжечка изменяется. Между действием раздражения, изменениями дыхания и общего кроводавления нет закономерной, последовательной связи, так можно предполагать, что изменение влияния электрического раздражения является независимым от давления CO_2 или O_2 крови, и ткани мозга.

При проведении возбуждений, выходящих из ствола мозга и действующих на сосуды мозга, как и вагус, так и симпатический нерв играют роль. Роль вагуса в кровообращении мозжечка дискуссионна, а возбуждения, проводимие по симпатическому нерву имеют значение в кровообращении как в мозжечке, так и в большом мозге.

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Physiologia

THE FLOW OF FLUID THROUGH THE WALL OF CAPILLARY SYSTEMS STUDIED BY A MATHEMATICAL MODEL

By

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(Received May 21, 1970)

Flow conditions with special emphasis on the resultant transmural flow intensity have been studied in the theoretical hydrodynamic model of a fictive capillary system. Beside demonstrating the dependence of flow intensity in filtration on some basic parameters (mean capillary hydrostatic pressure, oncotic pressure of the plasma, draining capacity of the lymphatics), the model experiment was aimed at studying one of the mechanisms that moderates the intensity of flow in filtration. Transmural fluid movements induce changes in the oncotic pressure of the circulating plasma. These changes, in turn, affect the movement of fluid through the capillary wall. Increasing the permeability of the wall of the capillary model for water and water-dissolved substances, the effect of the reaction becomes enhanced. The system was studied by digital computer in view of the characteristics of the mathematical model.

On the basis of the sign of the difference between hydrostatic pressure difference and oncotic pressure difference, the capillary area can be divided into a range of filtration and a range of resorption. Under normal conditions the magnitude of the fluid volume passing in either of these ranges through the capillary wall within a unit of time (i.e. the flow intensity of filtration or resorption) is greater than the difference in intensity between the two flows, thus greater than the resultant transmural flow intensity of the entire capillary area. Therefore, conditions can be regarded as physiological ones, if the mean value of pressures inducing the transmural movements of fluid approximates the Starling equilibrium.

Several regulatory mechanisms are active in keeping the resultant transmural flow intensity at a low level. Oncotic pressure difference due to the semipermeability of the capillary wall can be considered a basic condition allowing the formation of a resorption range possible. The rise of interstitial pressure due to the limited draining capacity of the lymphatic system represents the mechanism restricting the increase in transmural flow intensity. By this mechanism the forces of filtration become reduced.

Changes in the permeability of the capillary wall for water and water-dissolved substances are followed by a shift of the boundary between the

range of filtration and the range of resorption. The transmural fluid transport induces this shift by changing the protein concentration of the plasma and thus also the difference of oncotic pressure. This paper describes a theoretical physical model by which, in addition to the effect of the other physical parameter of the model on transmural flow intensity and interstitial hydrostatic pressure, the efficacy of the above regulatory mechanisms was studied. As the behaviour of the theoretical hydrodynamic model comprising oncotic regulation is described in a first order non-linear differential equation the exact solution of which is unknown, in order to obtain numerical results the model was studied by approximate calculation using a Minsk-22 type digital electronic computer.

Description of the model

The hydrodynamic model of the capillary network can be simulated in a semipermeable tube the hydrodynamic resistance of which corresponds to the resistance of the capillary network under study and the value of its circumference H corresponds to the total circumference of the capillary cross sections under identical pressure. The geometrical interpretation of a model complying simultaneously with both requirements is not possible in the general case, i.e. under the prevailing relationship between circumference and hydrodynamic resistance in the real capillaries. A further requirement involved is the identity of hydrodynamic conductivity (permeability) of the surface unit of the model tube with that of the capillary wall.

The pressures at the end-points of the tube correspond to the arterial and venous pressures of the capillary network. In view of the parallel linkage of the other microcirculatory systems, the independence of these pressure values of flow conditions in the capillary network may be assumed. Similarly, the value for flow intensity of the blood entering on the arterial side can be considered independent of these conditions because of the relatively high precapillary vascular resistance.

If the axis of the tube is taken as coordinate axis x, the length of the tube as L, then the point $x = 0$ represents the arterial side and the point $x = L$ the venous end of the capillaries (Fig. 1).

Hydrostatic pressure in the capillary is denoted by p_c, that in the interstitium by p_i, oncotic pressure in the plasma by k_c, and that in the interstitial fluid by k_i (Fig. 2). Capillary flow intensity i.e. axial flow intensity of the model tube is described by the function (x), the radial (transmural) one by the function $f_t(x)$.

Assuming a rapid equalization of pressure and concentration, the interstitial pressures appearing along the model can be regarded as being independent of x. As the various steady-state conditions of capillary filtration are investigated, transient processes (e.g. formation or absorption of oedema) reflecting transitions between different states of equilibrium are not dealt with, neither has the distensibility of the interstitium a role in the calculations. Interstitial hydrostatic pressure is thus determined by the resultant of fluid movements of filtration and resorption and by the transporting capacity of the lymphatic system.

Passive factors (tone of the peripheral lymphatic network, venous pressure at the site of inflow of lymphatic trunks, etc.), quasi active factors (sucking effect caused by the fluctuation in extramural pressure) and active transport functions (vasomotion of the lymphatics) are involved in the formation of the draining characteristic of the lymphatic system. The resultant draining characteristic developing under the combined joint effect of the factors could not be determined, therefore linear relations with different slopes were posited between the interstitial pressure and flow intensity of fluid drained by the lymphatics. Thus passive conductive transport is applied in the model; it is expressed by the condition,

$$p_i = R[f(0) - f(L)] \tag{1}$$

where R denotes extravascular and extracellular draining resistance, i.e. as a parameter it indicates the slope of the supposed draining characteristic. Finally it is assumed that at any point x of the model the product of filtering cylinder surface element $H \Delta x$ and g, the permeability of surface unit is constant.

In the following, capillary (axial) and transmural flow intensities, further t he interstitial pressure, p_i, are regarded as a function of capillary hydrostatic pressure, p_c; arterial plasma protein concentration, $b_c(0)$; draining resistance, R; and the permeability of the capillary wall for water and water-dissolved substances g. In order to establish the role of changes in oncotic pressure difference due to filtration and resorption in the regulation of the men-

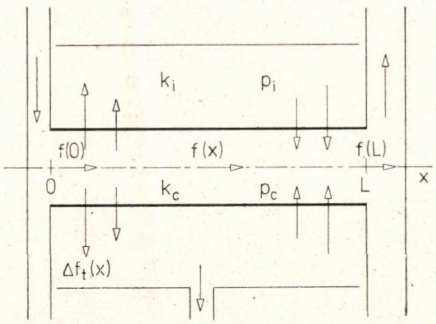

Fig. 1. Key to signs and abbreviations used in the theoretical physical model: $f(x)$, intensity of capillary blood flow; $\Delta f_t(x)$, transmural flow intensity of the surface unit of the capillary wall at point x of the model; p_c, hydrostatic pressure of intravascular fluid; k_c, oncotic pressure of the same; p_i, hydrostatic pressure of interstitial fluid; k_i, oncotic pressure of the same

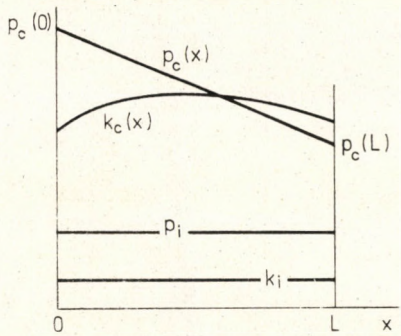

Fig. 2. Key to signs and abbreviations used in the theoretical physical model: interpretation of the character of the capillary and interstitial pressure according to our approximations along the longitudinal axis of the tube model. p_c, hydrostatic pressure of intravascular fluid; k_c, oncotic pressure of the same; p_i, hydrostatic pressure of interstitial fluid; k_i, oncotic pressure of the same

tioned quantities, flow relations are first studied with the condition $k_c(x) = k_c = \text{constant}$. Then a comparison is made with the results of the model as obtained when the changes in oncotic pressure difference are also taken into account. In both cases the effect of the other regulation, i.e. that of the change in p_i due to the draining resistance, R, is also discussed.

Data of the capillary system of a fictive organ were used in the studies. Approximate human data were applied in representing the basic parameters; one-tenth of the relevant quantities of the entire organism is taken as total surface, A (BÁLINT 1968) and flow intensity, $f(0)$ of the fictive capillary system.

$$f(0) = 600 \text{ ml/min},$$

$$A = HL = 600 \text{ m}^2$$

1. Study of capillary, transmural and interstitial flow and pressure relations under the condition $k_c = const.$

The value of the transmural flow intensity Δf_t at any part of the capillary model tube $(x; x + \Delta x)$ is determined by the resultant of hydrostatic and oncotic pressures, by filtrating surface element $H\,\Delta x$ and by g, the hydrodynamic conductivity of the surface unit of the capillary wall:

$$\Delta f_t(x) = gH[p_c(x) - p_i - k_c + k_i]\,\Delta x; \tag{2}$$

after dividing by Δx and taking the limit $\Delta x \to 0$, the following derivative is obtained:

$$\frac{df_t(x)}{dx} = gH\,[p_c(x) - p_i - k_c + k_i]\,. \tag{3}$$

The resultant value of transmural flow intensities is obtained if the elementary flow intensities are integrated. Introducing the symbol $f_t(L) = F_t$

$$F_t = \int_0^L df_t = gH \int_0^L [p_c(x) - p_i - k_c + k_i]\,dx\,. \tag{4}$$

As according to our condition (1)

$$p_i = R[f(0) - f(L)] = R\,F_t$$

assuming the linear decrease of the capillary hydrostatic pressure between the arterial and venous ends, i.e.

$$p_c(x) = p_c(0) + \frac{p_c(L) - p_c(0)}{L}\,x\,, \tag{5}$$

substituting the values of p_i and $p_c(x)$ into (4), after transposition to F_t and integration the following result is obtained:

$$F_t = g\,A\,\frac{0.5\,[p_c(0) + p_c(L)] - k_c + k_i}{1 + g\,A\,R}\,. \tag{6}$$

The value for capillary flow intensity at the point x of the model is the difference of the flow intensity on the arterial side $f(0)$ and the resultant transmural fluid movements that have taken place up to point x:

$$f(x) = f(0) - \int_0^x df_t\,. \tag{7}$$

Substituting the value df:

$$f(x) = f(0) - g\,H \int_0^x \left[p_c(0) + \frac{p_c(L) - p_c(0)}{L}\,x - k_c + k_i - F_t R\right] dx\,. \tag{8}$$

As a consequence of the conditions applied in constructing the model it has become irrelevant what values H and L are producing the total surface A. Thus in studying the x-dependent quantities, the introduction of a coordinate transformation is expedient:

$$\xi = \frac{x}{L}\,. \tag{9}$$

In this way a numerical indication of the length of the model tube becomes unnecessary; the examination is made in the interval $0 < \xi < 1$ and boundary conditions can be taken over without change in value into this model of normalized coordinate:

$$\begin{aligned} p_c(x = 0) &= p_c(\xi = 0) = p_c(0), \\ p_c(x = L) &= p_c(\xi = 1) = p_c(1), \\ f(x = 0) &= f(\xi = 0) = f(0), \\ f(x = L) &= f(\xi = 1) = f(1) \text{ etc.} \end{aligned}$$

By the transformation (9) the integration variable of Equation (8) and also the upper limit of the integration change:

$$f(\xi) = f(0) - gA \int_0^{\xi} \{ p_c(0) + [p_c(1) - p_c(0)]\, \xi - k_c + k_i - F_t R \}\, d\xi . \tag{10}$$

After integration and ordering the following equation is obtained:

$$f(\xi) = f(0) + \varepsilon \xi^2 - (\delta_1 - \delta_2)\xi \tag{11}$$

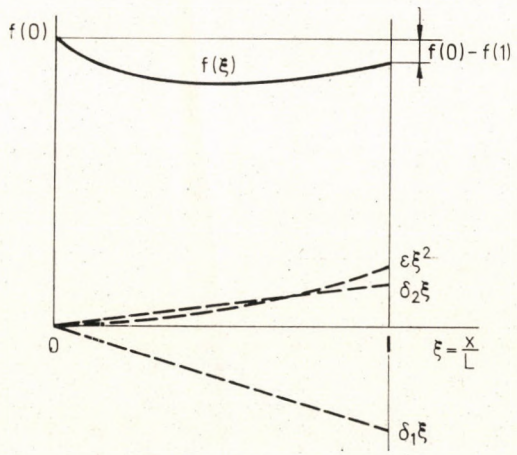

Fig. 3. Qualitative representation of the capillary flow intensity $f(\xi)$ and the components of $f(\xi)$ in the model simplified by the condition $k_c = \text{const.}$ [For the value of coefficients ε, δ_1 and δ_2 see text under relation (12).] $f(0) - f(1) = F_i$ is the resultant transmural flow intensity

where

$$\varepsilon = \frac{1}{2} gA[p_c(0) - p_c(1)],$$

$$\delta_1 = gA[p_c(0) - k_c + k_i],$$

$$\delta_2 = gA\, F_t R. \tag{12}$$

A qualitative illustration of $f(\xi)$ and its components is shown in Fig. 3.

ξ_0, the minimum point of the curve $f(\xi)$ is the boundary point between filtration and resorption ranges. In addition to the value $F_t = f(0) - f(1)$, the absolute values of flow intensity of the fluid filtered in the domain $(0; \xi_0)$ and resorbed in the domain $(\xi_0; 1)$ are of physiological importance. The filtration flow intensity, F_{t+} is obtained if transmural flow intensities are integrated to minimum point ξ_0 calculated from the condition

$$p_c(\xi_0) - k_c + k_i - p_i = 0. \tag{13}$$

According to the result as expressed in Equation (14), similarly to the value of F_t, the flow intensity of filtration F_{t+} is the linear function of gA, the permeability of the capillary total surface but it reacts more susceptibly to changes in pressure.

$$F_{t+} = \frac{1}{2} gA\, \frac{[p_c(0) - k_c + k_i - F_t R]^2}{p_c(0) - p_c(1)} = \frac{(\delta_1 - \delta_2)^2}{4\varepsilon} . \tag{14}$$

The same is valid for

$$F_{t-} = F_{t+} - F_t \tag{15}$$

flow intensity of resorption.

2. Studies taking into consideration the reaction of transmural fluid movements on oncotic pressure difference

Transmural fluid movement on the arterial side of the capillaries is usually directed towards the interstitium. As with the exception of the liver capillaries the filtered fluid is poor in protein, protein concentration and oncotic pressure of the fluid circulating in the intravascular space continuously increase until fluid movements of resorption again "dilute" the plasma.

For the qualitative study of the phenomenon let us see the elementary section of the tube model between points x and $x + \Delta x$ (Fig. 4).

From the flow intensity of the plasma, $f(x)$, the flow intensity of proteins, $f_p(x)$, is taken, i.e.

$$f(x) = f_p(x) + f_s(x), \tag{16}$$

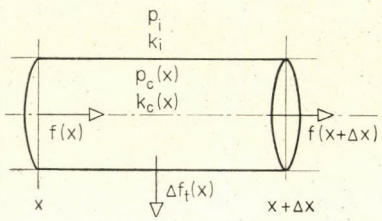

Fig. 4. Values of flow intensity and pressure at the elementary section of the tube model

where $f_s(x)$ denotes flow intensity of the protein-free fluid. Although the permeability of the capillary wall for protein generally is not zero, protein loss from the point of axial flow can be neglected with good approximation. As a consequence of this neglect actually one could not speak of interstitial oncotic pressure, still for the sake of graphic quality, k_i can be retained or else incorporated into the values p_c of an identical effect.

Consequently, the following continuity equation is valid for the section $(x; x + \Delta x)$:

$$f(x + \Delta x) = f(x) - \Delta f_t(x). \tag{17}$$

According to the above condition $\Delta f_t(x)$ originates here from $f_s(x)$ and its value is determined by the equation:

$$\Delta f_t(x) = g\, H[p_c(x) - k_c(x) + k_i - p_i]\, \Delta x. \tag{18}$$

Thus the elementary change of axial flow intensity at section Δx is:

$$\frac{f(x + \Delta x) - f(x)}{\Delta x} = -g\, H\, [p_c\,(x) - k_c\,(x) + k_i - p_i]\,. \tag{19}$$

If the value of Δx tends to zero, the following equation is obtained:

$$\lim_{\Delta x \to 0} \frac{f(x + \Delta x) - f(x)}{\Delta x} = \frac{df(x)}{dx} = -g\, H\, [p_c\,(x) - k_c\,(x) + k_i - p_i]\,. \tag{20}$$

According to LANDIS and PAPPENHEIMER (1963) protein concentration determines oncotic pressure of the plasma as follows:

$$k_c = a_1 b_c + a_2 b_c^2 + a_3 b_c^3 \quad \text{(mm Hg)}. \tag{21}$$

In Equation (21), b_c is the protein concentration of the plasma in g/100 ml (%), if the albumin/globulin concentration coefficient is $A/G = 1.1$, the value of a_1, a_2 and a_3 coefficients are

$$a_1 = 2.1$$
$$a_2 = 0.16$$
$$a_3 = 0.009.$$

As the plasma components have the same velocity in axial flow, the concentration can be expressed as the quotient of flow intensities:

$$b_c(x) = \frac{f_p}{f_p + f_s(x)} 10^2 = \frac{f_p}{f(x)} 10^2 . \tag{22}$$

Substituting Equations (1), (5), (21) and (22), Equation (20) has the following form:

$$\frac{df(x)}{dx} = -gH \left\{ p_c(0) + \frac{p_c(L) - p_c(0)}{L} x - \right.$$

$$\left. - \left[a_1 \frac{f_p \cdot 10^2}{f(x)} + a_2 \frac{f_p^2 \cdot 10^4}{f^2(x)} + a^3 \frac{f_p^3 \cdot 10^6}{f^3(x)} \right] + k_i - R[f(0) - f(L)] \right\}. \tag{23}$$

Introducing first transformation (9) by reasons mentioned under (1) and the symbols

$$g A a_1 f_p 10^2 = B_1,$$
$$g A a_2 f_p^2 10^4 = B_2,$$
$$g A a_3 f_p^3 10^6 = B_3,$$

further

$$\alpha = g A \{p_c(1) + k_i - R[f(0) - f(1)]\}$$

and

$$\beta = g A [p_c(1) - p_c(0)] \tag{24}$$

the following first order non-linear differential equation is obtained for the flow intensity $f(\xi)$:

$$\frac{df(\xi)}{d\xi} = \frac{B^1}{f(\xi)} + \frac{B_2}{f^2(\xi)} + \frac{B_3}{f^3(\xi)} - \alpha + \beta\xi . \tag{25}$$

The exact solution of the equation is unknown. After the parameters are given numerically, the computer gives the numerical values of the approximate solution of the differential equation by the help of a suitable program. Without knowing the solution $f(\xi)$ of the differential equation (25), the resultant transmural flow intensity $F_t = f(0) - f(1)$, further the filtration flow intensity

$$F_{t+} = f(0) - f(\xi_0) \tag{26}$$

and the flow intensity of resorption

$$F_{t-} = f(\xi_0) - f(1) = F_{t+} - F_t$$

cannot be expressed in explicit analytical form. Based on Equations (21) and (22), oncotic pressure of the plasma can be calculated from the following equation:

$$k_c(\xi) = a_1 \frac{f_p}{f(\xi)} 10^2 + a_2 \frac{f_p^2}{f^2(\xi)} 10^4 + a_3 \frac{f_p^3}{f^3(\xi)} 10^6 \tag{27}$$

while the interstitial pressure values from the formula

$$p_i = F_t R.$$

Results

Studying the capillary flow intensity $f(\xi)$ the typical curve seen in Fig. 5a was obtained.

Due to filtration taking place on the arterial side, capillary flow intensity decreases and reaches its minimum when the forces of filtration and resorption

are equalized. Subsequently, forces of resorption predominate and as a result capillary flow intensity continuously increases up to $\xi = 1$, the end-point of the tube. If the effect of transmural flow on the oncotic pressure of the circulating plasma is taken into consideration, the minimum point indicating the equalization is shifted towards the arterial end and thus the range of resorption is lengthened. This accounts for the fact that resultant transmural flow intensity

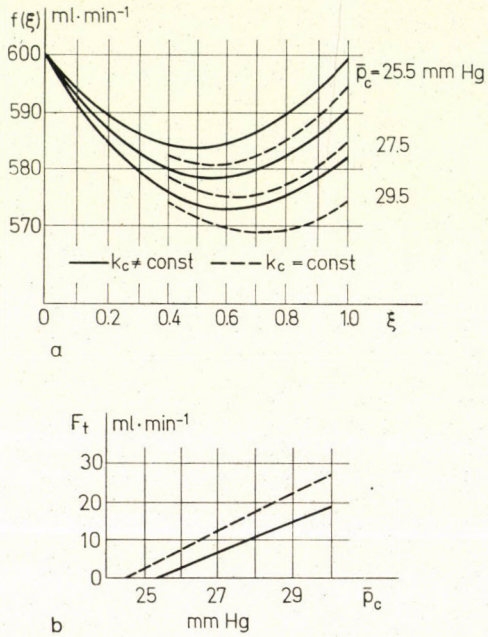

Fig. 5a. Change in $f(\xi)$ capillary flow intensity along the axis of the tube model at three values of $\bar{p}_c = 0.5\ [p_c(0) + p_c(1)]$ mean capillary hydrostatic pressure

Fig. 5b. Dependence of resultant transmural flow intensity, F_t, on mean capillary hydrostatic pressure, $\bar{p}_c$. The pressure difference $p_c(0) - p_c(1)$ is 13 mm Hg at every value of $\bar{p}_c$. Broken lines indicate the results of the k_c = const. model without oncotic pressure regulation. $b_c(0) = 7\%$; $k_i = 1$ mm Hg; $R = 0.1$ mm Hg ·min ·ml^{-1}; $gA = 10$ ml ·min^{-1} ·mm Hg^{-1}

$F_t = f(0) - f(1)$ is always lower or of a more negative value than would be obtained under the condition k_c = const. As can be seen in Fig. 5b, the equilibrium $F_t = 0$ related to the entire capillary system takes place at a higher mean capillary hydrostatic pressure, $\bar{p}_c$ (25.3 mm Hg) than in the case without regulation (24.5 mm Hg). The resultant transmural flow intensity is in both cases linearly related to the pressure $\bar{p}_c$.

Similarly, F_t shows a linear relation to the protein concentration of the blood, $b_c(0)$, entering on the arterial side. In Fig. 6 the changes of oncotic pressure k_c along the longitudinal axis of the tube model are plotted below the curves

$f(\xi)$. Naturally, the minima of the curves $f(\xi)$ are at the same point ξ_0 as the maxima of the relevant curves $k_c(\xi)$.

Draining resistance, R, of the extravascular and extracellular space affects reversely the shape and value F_t of the curves $f(\xi)$ as does $\bar{p}_c$. At an increased draining resistance, R, the same resultant filtrate would produce a higher p_i, which in turn allows a lower F_t, resultant flow intensity. In the

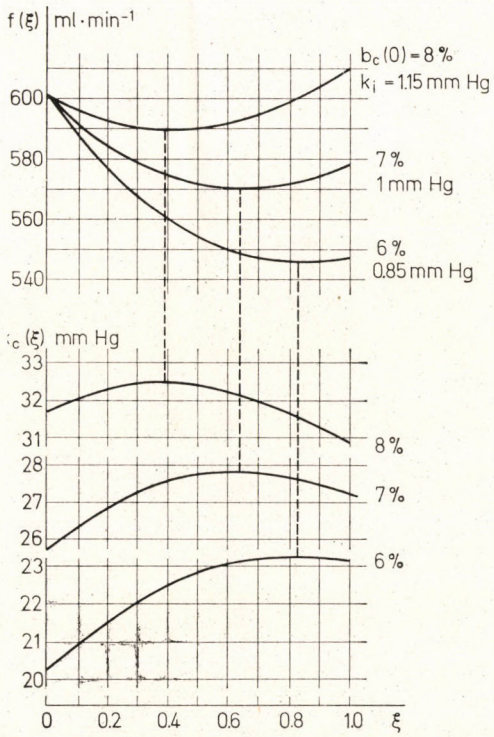

Fig. 6. The capillary flow intensity functions $f(\xi)$ and the oncotic pressure functions $k_c(\xi)$ at plasma protein concentrations of 6, 7 and 8%. $\bar{p}_c = 29.5$ mm Hg; $R = 0.05$ mm Hg ·min · ml^{-1}; $gA = 10$ ml ·mm Hg^{-1} ·min^{-1}

case when the other parameters are identical, the maximum resultant filtrate is obtained at a very low value of draining resistance and shows a hyperbolical decrease if R is increased

$$F_t = \frac{F_t\,(R=0)}{1 + g\,A\,R}$$

If R is increased the value of F_t first falls rapidly then the curve takes a gradually diminishing slope and reaches zero only in $R = \infty$. Parallel with this phenomenon the curves of filtration and resorption flow intensities approach each other (Fig. 7a).

The value of interstitial pressure p_i as the function of R is expressed in equation

$$p_i = R F_t = \frac{R F_t (R = 0)}{1 + g A R}$$

and is illustrated in Fig. 7b.

The curves $f(\xi)$ and $k_c(\xi)$ obtained in this theoretical model experiment cannot be reproduced in vivo. However, the linear dependence of resultant

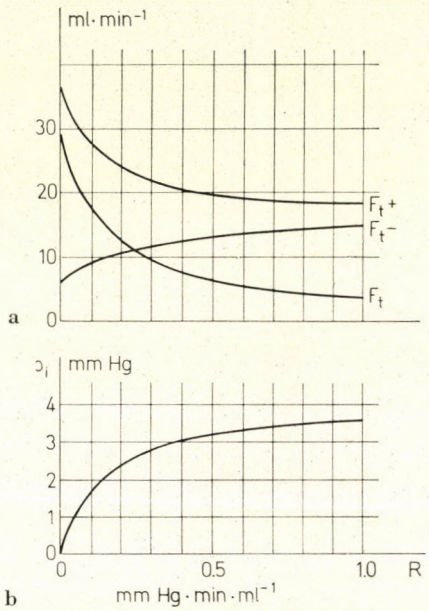

Fig. 7. Dependence of the filtration F_{t+}, the resorption F_{t-}, the resultant F_t transmural flow intensities and of the interstitial pressure p_i on the draining resistance R. $\bar{p}_c = 29.5$ mm Hg; $k_i = 1$ mm Hg; $b_c(0) = 7\%$; $gA = 10$ ml $\cdot$min^{-1} $\cdot$mm Hg^{-1}

transmural flow intensity, F_t, on $\bar{p}_c$ is in good agreement with several experimental results. LANDIS and GIBBON (1933) found that the changes in resultant transmural flow intensity had a linear dependence on venous pressure and the same was established for mean capillary hydrostatic pressure by PAPPENHEIMER and SOTO-RIVERA (1948). Based on this proportionality and also on other observations, LANDIS and PAPPENHEIMER (1963) concluded that vessels with a small lumen had a relative rigidity and that capillary surface and porosity were independent of p_c and $(p_c - p_i)$.

g, the permeability of the wall of the capillary model exerts a strange effect on the values for capillary and interstitial pressure and flow as well as on the efficacy of the relevant regulatory mechanisms. In Fig. 8, the curves $f(\xi)$ related to the same pressure and draining resistance values are shown at

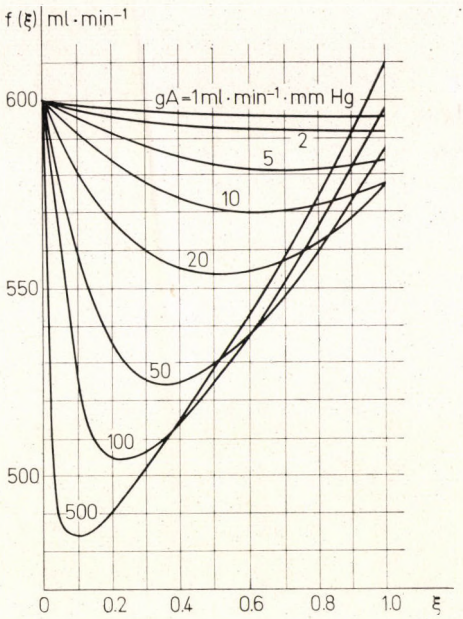

Fig. 8. The course of the flow intensity functions $f(\xi)$ related to the same capillary pressure values at different permeability values of the wall of the capillary model. $\bar{p}_c = 29.5$ mm Hg; $k_i = 1$ mm Hg; $b_c(0) = 7\%$; $R = 0.05$ mm Hg ·min ·ml^{-1}

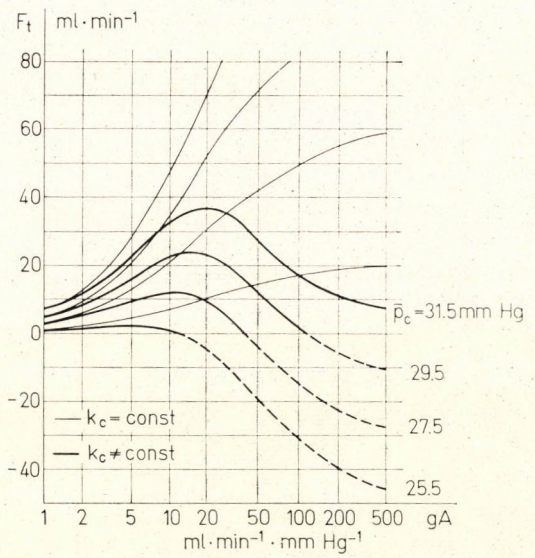

Fig. 9. The resultant transmural flow intensity, F_t taken as a function of the permeability gA at different $\bar{p}_c$, mean capillary hydrostatic pressure values. The curves obtained with the condition $k_c = $ const. are also plotted. $k_i = 1$ mm Hg; $b_c(0) = 7\%$; $R = 0.05$ mm Hg ·min · ml^{-1}; $p_c(0) - p_c(1) = 13$ mm Hg

different permeabilities. As can be seen, the boundary between the range of filtration and resorption (the minimum points) shifts towards the arterial end as values of gA are increasing.

A low permeability or a small filtrating surface results in a resultant transmural flow intensity of positive value (effective filtration) due to the predominance of filtration forces. Increasing the permeability, this value first increases but then begins to decrease and would even take a negative value (effective resorption) due to the shortening of filtration range in spite of increasing permeability. Fig. 9 illustrates more clearly this process, in the figure, the value for F_t is illustrated as the function of gA.

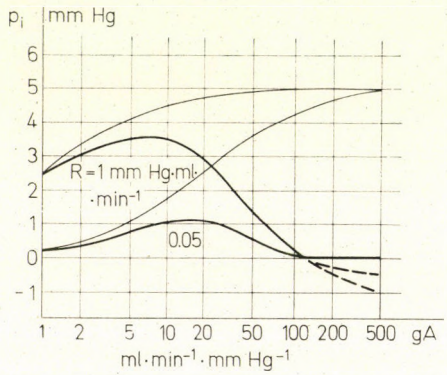

Fig. 10. Dependence of interstitial pressure values related to $\bar{p}_c = 29.5$ mm Hg mean capillary hydrostatic pressure on the permeability gA of the capillary model at two highly different values of draining resistance R. $k_i = 1$ mm Hg; $b_c(0) = 7\%$

Beside the functions $F_t(gA)$ the curves calculated by the condition $k_c = \text{const.}$ are also plotted in Fig. 9. As a regulation of oncotic pressure is not reckoned with, in the case of an initial excess of filtration, values of F_t are increasing if gA is increased.

The broken lines illustrating negative values of F_t indicate that although the draining characteristic based on the condition $p_i = RF_t$ as used in the model is valid for negative values, too, in reality the valves of the lymphatic system do not allow a "negative" drainage viz. a retrograde flow into the interstitium. If water output of tissue metabolism is left out of consideration, negative values of F_t are unreal in stationary cases: the fluid demand of the resorption range from the interstitium induces a decrease of interstitial pressure, p_i, and consequently an increased filtration. Under these circumstances resorption demand is met by the range of filtration under the congruence of F_{t+} and F_{t-}, i.e. $F_t = 0$. Finally, the excess of resorption forces brings about an equilibrium between filtration and resorption and negative (subatmospheric) values of interstitial pressure. Thus the broken-line curve illustrating negative values of p_i in Fig. 10 does not indicate that the negative sign of p

is unreal but means that its absolute value is incorrect. Interstitial pressure as the function of the permeability gA is shown with two draining resistances, Rs of highly different values and also the results calculated by the condition k_c = const. are indicated.

In contrast to the latter curves there is no considerable p_i increase in the rising part of the $k_c \neq$ const. curves either, in spite of the fact that the permeability gA has increased more than ten-fold. The worse the draining i.e. the higher the value R, the degree of increase is relatively the smaller and the

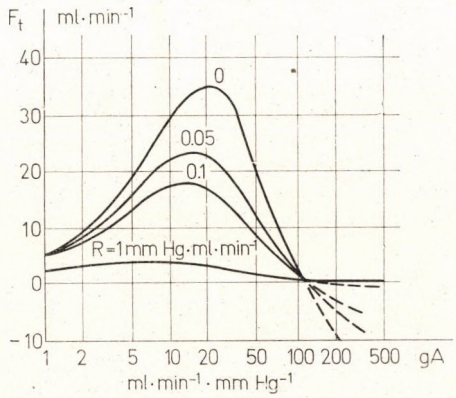

Fig. 11. The resultant transmural flow intensity F_t related to $\bar{p}_c = 29.5$ mm Hg mean capillary hydrostatic pressure taken as a function of the permeability gA of the capillary model at different values of the draining resistance R. $\bar{p}_c = 29.5$ mm Hg; $k_i = 1$ mm Hg; $b_c(0) = 7\%$

die-away part begins at lower values of gA. At exceedingly high resistances, which would be the case with a blockade of lymph flow, the increase of gA results in an early fall of interstitial pressure. Thus, the conclusion can be drawn that an isolated increase of the permeability of the capillary wall for water and water-dissolved substances cannot be made responsible for a major rise in interstitial pressure, i.e. for oedema formation.

Fig. 11 illustrates the values of F_t, resultant transmural flow intensity being taken as the function of the permeability gA of the capillary model at different values for draining resistances, R. The curves are closely related to the functions p_i in Fig. 10, i.e. maximal flow intensities belong to the extreme case of $R = 0$ when p_i is zero because of the infinitely good drainage. Increasing the draining resistance under unchanged permeability, the resultant transmural flow intensity values decrease and interstitial pressure keeps rising. In this case the curves $F_t(gA)$ similarly to the functions p_i (gA) show their minima at lower and lower values of gA. The changing maximum of the curves indicates that an increased draining resistance renders the regulatory mechanism of oncotic pressure more efficient.

The two mechanisms moderating resultant transmural flow intensity affect the values of filtration and resorption flow intensities in a smaller degree. Fig. 12 shows that the curves F_{t+} and F_{t-} related to the same hydrostatic capillary pressure taken as the function of permeability, indicate increasing values in contrast to the curves showing F_t, resultant flow intensity. Thus fluid exchange in the interstitium is improving.

Similarly, the relative and absolute values of the change caused by the draining resistance in the flow intensities of filtration and resorption are smaller than in the case of resultant flow intensity. The conclusion seems thus justified

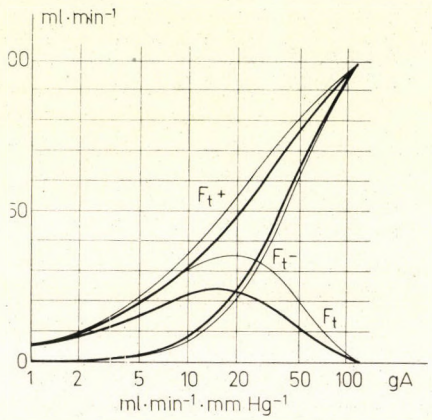

Fig. 12. Dependence of the filtration F_{t+}, the resorption F_{t-} and resultant F_t transmural flow intensities on the permeability gA. The thin lines indicate the functions related to the zero value of the draining resistance R, the thick lines the functions related to $R = 0.05$ mm Hg · min · ml^{-1}. $\bar{p}_c = 29.5$ mmHg; $k_i = 1$ mm Hg; $b_c(0) = 7\%$

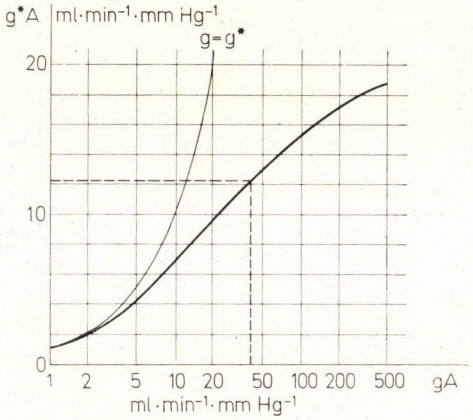

Fig. 13. Illustration of the apparent permeability g^*A of the capillary model as the function of the real permeability gA. Shown are the product of the filtration coefficient of the dog and cat muscle capillary (LANDIS and PAPPENHEIMER 1963) and of the surface A of the model (12, 24) further the real values of permeability (37) related to it

that the effect of the above two mechanisms is directed primarily at the resultant of transmural fluid movements.

It would be problematic to place the permeability of the real microcirculation systems on the axis gA of the above curves. The rate of resultant flow intensity, F_t, related to the surface unit of the wall and to the transmural pressure difference unit measured in the entire capillary system by plethysmography or isogravimetry — the "filtration coefficient" of the capillary wall (LANDIS and PAPPENHEIMER 1963) — will be smaller than the actual permeability g of the surface unit due to the regulation of oncotic pressure. Thus, oncotic pressure regulation reduces not only the values of F_t but moderates also their changes per transmural pressure difference unit. Due to the change in oncotic pressure difference induced by transmural fluid turnover, the real system of microcirculation shows, on the one hand, altered pressure conditions of a more resorptive character and behaves, on the other hand, as a system of reduced permeability. If the apparent permeability g^*A of a capillary system with surface A is taken as the function of gA, real permeability, the curve seen in Fig. 13 is obtained if arterial plasma protein concentration is $b_c(0) = = 7$ g/100 ml.

At low capillary permeability values, real and apparent permeabilities are almost identical but the apparent values lag behind if the real permeability increases and the difference between the two becomes successively greater.

If from the values of filtration coefficient indicated by LANDIS and PAPPENHEIMER (1963) the coefficient of dog and cat muscle capillaries

$$2.5 \cdot 10^{-8} \text{ ml} \cdot \text{sec}^{-1} \cdot \text{cm } H_2O^{-1} \cdot \text{cm}^{-2}$$

which presumably approach the best the human values is converted into the unit used in this experiment, the value

$$g^* = 2.04 \cdot 10^{-8} \text{ ml} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1} \cdot \text{mm}^{-2}$$

is obtained which in the case of $A = 600$ m^2 total surface means an apparent permeability of

$$g^*A = 12.24 \text{ ml} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1}.$$

According to Fig. 13 this apparent permeability is related to a real capillary permeability of about

$$gA = 37 \text{ ml} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1}$$

or per surface unit a permability of

$$g = 6.17 \cdot 10^{-8} \text{ ml} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1} \cdot \text{mm}^{-2}.$$

Finally, it should be stressed that in these studies of the values of flow intensity and pressure taken as a function of capillary permeability, the permeability of the capillary model for water and water-dissolved substances has been modified, thus our conclusions are not valid in the case of a marked change in permeability for protein.

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EFFECT OF ACIDIFICATION AND ALKALINISATION ON CALCIUM AND CITRATE METABOLISM IN NORMAL AND PARATHYROIDECTOMIZED DOGS

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1. The serum citrate level is regulated by parathyroid hormone, but not by the variations in the serum calcium level. The regulation is based on an effect on bones rather than on an effect on the kidneys.

2. Metabolic acidosis (infusion of 0.005 N HCl) and alkalosis (infusion of 0.3 N $NaHCO_3$) did not significantly alter the serum calcium and citrate levels.

3. Urinary calcium and citrate excretion decreased in response to metabolic acidosis; though the changes were not significant statistically, the tendency was obvious.

4. Urinary calcium and citrate excretion was significantly increased by metabolic alkalosis. The same applies to urine volume. Alkalosis increased the excretion of these ions by diminishing the rate of citrate oxidation by an accumulation of HCO_3^- in mitochondria.

5. Thyro-parathyroidectomy prevented metabolic alkalosis from causing hypercalciuria. It is suggested that parathyroidectomy would diminish the described effect of metabolic alkalosis by modifying the enzyme system of the tubular cell (alteration of intracellular bicarbonate level), or eventually by a loss of calcium reserves.

Experiments involving human subjects and dogs (MOSONYI et al. 1965; MOSONYI and ÁCS 1967) yielded the result that the correlation coefficient of the serum calcium and citrate concentrations was low, thus, although their interrelationship was close especially as regards the chemical bonds, it is not the change in the level of calcium ion that alters that of the citrate ion and vice versa, but their regulations are essentially independent. This view agrees well with the evidence published recently (LIÉVRE et al. 1968).

There is no doubt, however, that the functional state of the parathyroid gland influences equally the calcium and the citrate metabolism. In this a role may be played by the fact that the calcium mobilized from bones by parathormone is mostly bound to citrate (LICHTWITZ et al. 1961). The role played by the parathyroid gland (LICHTWITZ et al. 1961) was illustrated also by the different changes in citrate level in response to hydrochlorothiazide in humans (MOSONYI and ÁCS 1967) and dogs (SZILÁGYI et al. 1968), depending on the fact whether the observations had been made on human subjects with normal or diminished parathyroid function, or dogs with intact parathyroid gland or following parathyroidectomy.

Several factors may, however, interfere with the serum level and urinary excretion of calcium and citrate: the quantitative and qualitative relations of production and breakdown, physiological enzyme function, regulatory influence of hormones produced outside the parathyroid gland (glycocorticoids, thyrocalcitonin), alimentary factors, the high or low serum levels of ions (FUJITA et al. 1965), and, finally, a modification of the internal milieu. As the saluretic used by us may substantially alter the pH of the organism and MORISSEY et al. (1963) observed a decrease of citrate excretion in a patient with renal tubular acidosis, we have undertaken to study the influence of changes in pH. The serum calcium and citrate levels were determined, as well as the excretion of these ions following induction of metabolic acidosis and metabolic alkalosis.

It was also studied whether the changes were correlated with the presence of the parathyroid gland, or with a reduction of the serum calcium level induced by other methods. In this series we reproduced the experiment of FUJITA et al. (1965) and at the same time investigated the restitution tendency of the serum calcium level reduced by oxalate. In addition, the changes in serum citrate level following oxalate infusion were studied in order to obtain information concerning the interrelationship of the regulation of the serum calcium and citrate levels.

Methods

Fifty-two dogs of either sex weighing 10 to 24 kg were used. There were three groups, each consisting of three subgroups. The 6 animals of the control group were tested for *a)* changes in serum calcium and citrate level and *b)*, urinary volume, calcium and citrate excretion during the three-hour test period of infusion of physiologic NaCl solution (Group I/a).

In 6 dogs 40 mg/kg sodium oxalate was infused for 15 minutes to bind part of the circulating calcium; this was followed by the 3-hour physiologic NaCl infusion (Group I/b).

Four dogs were subjected to total thyro-parathyroidectomy. Three to four days after operation, when the serum calcium and citrate levels were mostly reduced, physiologic NaCl solution was infused (Group I/c).

Six dogs received 0.005 N HCl infusion (Group II/a).

In eight dogs the 0.005 N HCl infusion was preceded by the administration of sodium oxalate (Group II/b).

Table I

Number of animals in the different groups

	NaCl infusion	HCl infusion	Base infusion
Normal animals	6	6	6
Oxalate treatment	6	8	8
Previous parathyroidectomy	4	4	4

Four dogs received 0.005 N HCl infusion following total thyro-parathyroidectomy (Group II/c).

Six dogs received 0.3 N $NaHCO_3$ infusion (Group III/a).

In 8 dogs sodium oxalate was administered first, followed by the infusion of 0.3 N $NaHCO_3$ (Group III/b).

In four dogs the infusion of 0.3 N $NHCO_3$ was administered 3 to 4 days after total thyro-parathyroidectomy (Group III/c, Table I).

The volume infused was 50 ml/kg in every case.

Calcium was assayed according to RAPPAPORT (1933), citrate according to TAUSSKY and SHORR (1947).

During infusion the animals were anaesthetized by urethan chloralose given intraperitoneally. Urine was collected by means of catheterization. Urine volume and the amount of ions excreted were related to kg body weight, owing to the wide variation in the body weight of the dogs.

Results

1. The serum calcium level was significantly reduced by the pretreatment itself, whether oxalate infusion or thyro-parathyroidectomy (Fig. 1). The subsequently induced metabolic acidosis or alkalosis caused no significant changes (Fig. 2). On the other hand, oxalate pretreatment followed by alkaline infusion delayed the restitution of the calcium level (Table II).

2. The serum citrate level was not influenced by oxalate infusion, but was significantly reduced following parathyroidectomy (Fig. 1). Metabolic acidosis and alkalosis failed to cause further significant changes (Table II and Fig. 2).

There were significant differences in the metabolism of the two ions as regards urine output as well as calcium and citrate excretion.

1. Calcium excretion. In response to acidification calcium excretion decreased, though not significantly. Alkalinisation produced an excessive increase in calcium excretion (Fig. 3). Pretreatment with oxalate did not in-

Table II

Serum calcium and citrate levels in the different groups in response to metabolic acidosis and metabolic alkalosis

		Physiol. NaCl	Metabolic acidosis	Metabolic alkalosis
Normal	Serum Ca	no change	no change	no change
	Serum citrate	no change	no change	no change
Reduced serum Ca, following oxalate infusion	Serum Ca	no change	no change	decrease ($p < 0.1$)
	Serum citrate	no change	no change	no change
Parathormone deficiency (after parathyroidectomy)	Serum Ca	no change	no change	no change
	Serum citrate	no change	no change	no change

fluence calcium excretion, thyro-parathyroidectomy moderately increased it. In the case of thyro-parathyroidectomy, $NaHCO_3$ infusion did not further increase calciuria (Tables III, IV, V).

2. Citrate excretion was moderately decreased by acidification and significantly increased by alkalinisation (Fig. 3). Citraturia was further increased by metabolic alkalosis subsequent to parathyroidectomy (Tables III, IV, V).

Urine output was significantly increased by metabolic alkalosis and was not influenced by acidosis (Fig. 4).

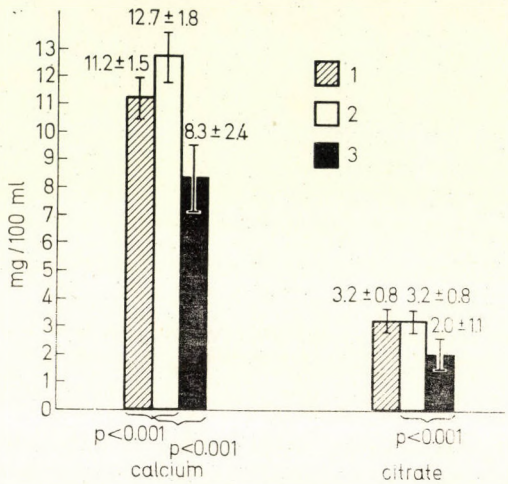

Fig. 1. Serum calcium and citrate levels 15 minutes after oxalate infusion as well as 3 to 4 days after parathyroidectomy. 1 Serum calcium and citrate levels 15 minutes after oxalate infusion; 2 serum calcium and citrate levels in normal dogs; 3 serum calcium and citrate levels following parathyroidectomy

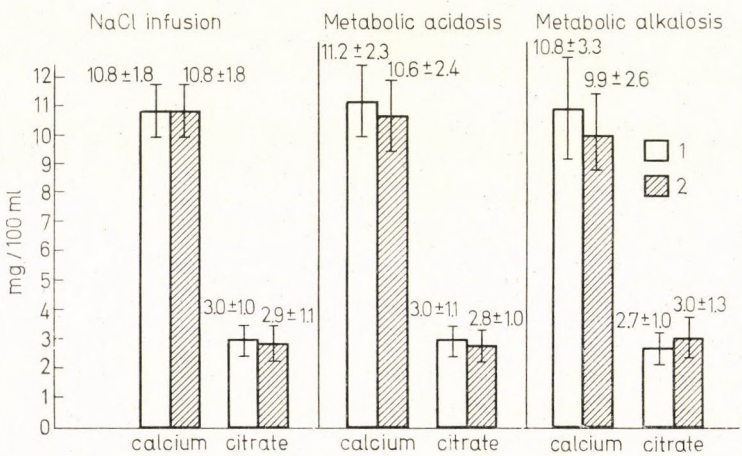

Fig. 2. Serum calcium and citrate levels before and after infusion of physiologic NaCl solution, metabolic acidosis, and metabolic alkalosis, respectively. 1 Serum calcium and citrate levels before infusion; 2 serum calcium and citrate levels after infusion

Table III

Effect of physiologic NaCl infusion on serum calcium and citrate levels and urinary excretion in the different groups

Group	Before Na oxalate infusion	at 0 min	at 60 min	at 120 min	at 180 min	Urinary excretion mg/kg/3 hrs	
						Ca	Ci
Se Ca		11.9±1.0	12.0±0.9	12.0±0.8	12.0±0.8		
I.a						0.85 ±0.48	0.83 ±0.49
Se Ci		2.8±0.7	2.9±0.7	2.8±0.7	2.7±0.5		
Se Ca	12.5±1.6	10.9±1.4	10.5±1.9	10.9±1.9	11.3±1.9		
I.b						0.80 ±0.54	0.60 ±0.45
Se Ci	3.4±0.8	3.4±0.8	3.2±0.6	3.4±0.6	3.3±1.0		
Se Ca		9.2±2.1	8.9±1.2	8.5±1.5	8.4±1.2		
I.c						1.14 ±0.36	0.93 ±0.44
Se Ci		2.8±1.4	2.8±1.6	2.7±1.5	2.8±1.7		

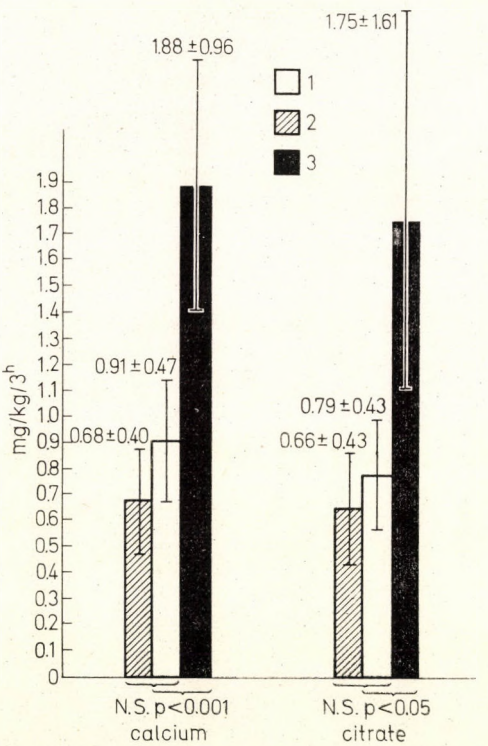

Fig. 3. Urinary calcium and citrate excretions in metabolic acidosis and metabolic alkalosis. 1 normal; 2 acidosis; 3 alkalosis

Table IV

Effect of 0.005 N HCl infusion on serum calcium and citrate levels and urinary excretion

Group	Before Na oxalate infusion	at 0 min	at 60 min	at 120 min	at 180 min	Urinary excretion mg/kg/3 hrs	
						Ca	Ci
Se Ca		12.5±1.9	12.1±1.8	11.7±1.6	11.5±1.8		
II.a						0.28±0.21	0.64±0.65
Se Ci		3.5±0.9	3.4±0.7	3.5±0.2	3.5±0.2		
Se Ca	13.2±0.9	11.6±1.5	11.3±1.9	11.6±1.5	11.7±1.4		
II.b						1.04±0.53	0.50±0.25
Se Ci	3.2±0.8	3.2±1.0	3.2±0.9	2.9±0.8	2.9±0.6		
Se Ca		8.5±2.4	7.9±2.2	7.2±2.4	7.1±2.4		
II.c						0.71±0.41	0.83±0.44
Se Ci		1.8±0.7	1.5±0.7	1.5±0.7	1.4±0.7		

Table V

Effect of 0.3 N $NaHCO_3$ infusion on serum calcium and citrate levels and urinary excretion

Group	Before Na oxalate infusion	at 0 min	at 60 min	at 120 min	at 180 min	Urinary excretion mg/kg/3 hrs	
						Ca	Ci
Se Ca		12.8±3.7	11.8±2.9	11.7±2.5	11.5±2.0		
III.a						2.25±1.09	1.24±1.07
Se Ci		3.3±0.9	3.2±0.7	3.6±1.3	3.2±1.6		
Se Ca	13.0±1.2	11.3±1.5	10.6±1.2	10.0±1.3	10.3±1.0		
III.b						1.96±1.06	2.04±2.04
Se Ci	3.0±0.8	3.0±0.6	3.0±0.8	3.0±0.8	3.3±1.1		
Se Ca		7.1±2.7	6.7±2.7	6.4±3.1	6.6±3.1		
III.c						1.20±0.79	1.97±1.75
Se Ci		1.5±1.0	1.4±0.9	2.0±0.7	2.2±0.8		

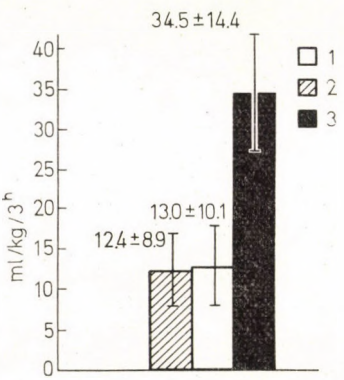

Fig. 4. Urine volume in metabolic acidosis and metabolic alkalosis. 1 Normal; 2 Metabolic acidosis; 3 Metabolic alkalosis

Discussion

1. Citrate excretion increased in metabolic alkalosis and decreased in acidosis. SIMPSON (1967) observed a significant decrease in citrate excretion following acidification. The decreased excretion observed by us following acidification was not significant statistically. Irrespective of the method by which alkalosis is induced, the changes in citrate excretion may be explained by a shift in cellular pH in the alkaline direction, releasing more HCO_3^- ions so that less citrate is oxidized in the mitochondria in the Krebs-Szentgyörgyi cycle. The citrate so accumulated in the tubular cell causes an increase in citraturia (SIMPSON 1967), either by reducing reabsorption, or by increasing tubular excretion (MORISSEY et al. 1963). Not only a shift in pH, but vitamin D may also reduce citrate oxidation in renal tissue (MAINTZER et al. 1961).

2. Calcium excretion increased in metabolic alkalosis and decreased in metabolic acidosis. The parallelism with citrate is obvious and suggests that the increased amounts of citrate excreted would take the calcium with them. Hypercalciuria in response to alkalinisation was observed also by WILLIAMSON and FREEMAN (1957). The cause of the increased excretion is the reduced resorption (WILLIAMSON and FREEMAN 1957) which may be correlated with the fact that calcium citrate is filtered across the glomerules, but masses of it cannot be reabsorbed from the tubules (BRODWALL and LAAKE 1967). A small proportion, however, may be reabsorbed in polar form from the proximal tubules (COHEN and PROUT 1964).

Normally, 50% of the excreted calcium is bound to citrate, this means that when citrate excretion increases, calcium excretion too, increases. This is of importance in two clinical conditions. One is nephrolithiasis, in which the formation of stones may be prevented by the administration of citrate if the stones are of a certain composition. The other is idiopathic hypercalciuria. In this condition, the cause of hypercitraturia is a reduction in the citrate utilization by tissues (BRODWALL and LAAKE 1967). This may load to a diminution of renal oxygen consumption (BRODWALL and LAAKE 1967), as 10% of the renal oxygen consumption is used for the oxidation of citrate. The hypercalciuria characteristic of the condition is a sequel of the hypercitraturia.

LEMANN et al. (1967) observed that following acidification with ammonium chloride, calcium excretion increased; the increase was ascribed to an alteration of tubular metabolism. This contradictory evidence suggests that calcium excretion may be modified depending on the use of NH_4Cl, HCl or acetazolamide for acidification. The different compounds act namely in different ways on the mitochondria. This shows that calcium excretion may change in different fashion depending on whether the acidosis is of the metabolic or the respiratory type (FREEMAN and JACOBSEN 1957).

Metabolic alkalosis induced subsequent to parathyroidectomy does not significantly enhance calciuria. This may be due to a calcium deficiency in the extracellular space. It may also be suggested that the absence of parathormone, causing by itself hypercalciuria, cannot further be enhanced by alkalinization. The fact that parathormone causes bicarbonate diuresis (DANOWSKI 1962) is another evidence pointing to a close interrelationship.

It is unlikely that other hormones, too, should be involved. As regards the two ions under discussion, thyrocalcitonin has no renal effect (COPP 1967), and in so short a time thyroxine deficiency may not play any important role.

3. Serum calcium and citrate levels. We have been unable to confirm the results published by FUJITA et al. (1965) concerning the influence of acidosis and alkalosis on the restitution of the serum calcium level following oxalate infusion. FREEMAN et al. (1957) found a faster rate of calcium disappearance from serum in response to the combined administrations of $NaHCO_3$ and calcium. In our experiments such an effect could be observed exclusively after oxalate infusion, i.e. with a decreased calcium level.

The present investigations, in harmony with earlier ones (MOSONYI et al. 1965), supply a new argument against the claim made by HARRISON (1956) that the citrate level would be regulated by the calcium level. Following oxalate infusion namely the serum calcium level decreased significantly without a similar change in the citrate level. On the other hand, parathyroidectomy and the parathormone deficiency so induced, significantly lowered the level of both ions.

An interesting clinical observation was made during the experiments. In 9 of the 18 animals blood appeared in the urine after the first hour of HCl infusion, while in the other 34 animals no haematuria was elicited by any of the infused compounds. We are unable to offer an explanation for this phenomenon.

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RELATIONSHIP BETWEEN WATER AND FOOD INTAKE IN THE RAT

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The rat consumes only about 80% of its daily total water requirement when allowed to drink only twice for 1 hour daily, both when kept on a dry diet or on food containing 50% water.

When kept on dry food, food intake and body weight were less in those animals which were offered water twice for one hour only, as compared with the ad libitum control group.

The absolute and relative values of 2×1 hour food intake were significantly higher in that group which was offered water only during these 2 hours, in contrast to the group with free water intake.

As an effect of the quantitative restriction of water intake, food intake decreased almost immediately and a stable lower level was reached within one week. Body weight decreased progressively until a new equilibrium had been established by the 3rd to 4th week. After terminating water restriction, the original level of water and food intake was rapidly restored after an overshoot to the original level, body weight increasing rapidly.

It has been generally accepted that food and water intakes are running parallel, so that alterations in one function are reflected by similar changes in the other (ADOLPH 1939; TEITELBAUM and STELLAR 1954). MORRISON and MAYER (1957a) observed parallel aphagia and adipsia following bilateral electrolytic lesions of the lateral hypothalamus in the rat, and — for 1 or 2 days (MORRISON and MAYER 1957b) — also following sham-operation, while MORGANE (1961) reported prolonged aphagia and adipsia after sham operations. In our earlier experiments (SCHMIDT et al. 1968) changes were found in food and fluid intake after both sham-operations and electrolytic lesions, in agreement with MORRISON and MAYER.

A strictly linear relationship between food and water intake was found during an experimental period of several years in the intact dog (CIZEK 1959). STROMINGER (1947) obtained similar results in the intact rat. According to the data of CIZEK and NOCENTI (1965) the consumed water/food rate rises with the age of the rat.

According to other results food and water intakes are, however, not strictly linked functions. For instance, GREER (1955) observed "pure water intake" after electrical stimulation of the hypothalamus in the rat. Similar results were found by ANDERSSON and MCCANN (1955) in the goat. In the rat, GROSSMAN and GROSSMAN (1963) observed variable changes of food and water

intake following electrical stimulation or lesion of different parts of the amigdaloid nuclei.

The question of priority between the mechanisms regulating food and fluid intake is also under debate. EPSTEIN and TEITELBAUM (1960) assumed that the lesioned animals do not eat because they do not drink.

The present experiments were designed to study the effect on water and food intake by limiting the time during which water was available to intact rats kept on dry and on wet (containing 50% water) diets. The effect of restriction of the amount of water available to the animal on food intake and body weight was also investigated.

Methods

Fortyeight male albino rats of the Institute's colony were used. The animals were housed in individual metabolism cages during pre- and postexperimental and experimental periods. Water was avaible from a measuring-tube furnished with a glass-sucker. Water and food intake was measured daily, body weight every 3rd day.

Composition of the wet diet (containing 50% water) was as described earlier (SCHMIDT and ANDIK 1969), and was prepared freshly every day. The dry diet in pellet form, made by the Hungarian Institute of Nutrition, had the following composition: wheat, 18%; barley, 18%; rye, 14%; oat, 14%; maize, 4%; hulled sunflower seed, 8%; dried lucerne flour, 5%; casein, 5%; fish flour, 5%; skimmed milk-powder, 4%; dried yeast, 4%; and adequate amounts of Ca, P, $MnSO_4$, $MgSO_4$, $FeSO_4$, $CuSO_4$, $ZnSO_4$, KI, NaCl; vitamins A, B_1, B_2, B_{12}, D_2, E, niacin, choline, methionine. Both diets had a nutritional value of 3.7 Cal/g dry weight. Evaporation from the wet diet was negligible.

Statistical evaluation was carried out by Student's *t*-test.

Results

In the first part of the experiment the animals were kept on the diet containing 50% water. In the pre-experimental period the animals were offered water ad libitum. During the experimental period of 26 weeks the animals were divided into two groups; group A was allowed to drink for 24 hours daily ad libitum, while group B had access to water for 1 hour in the morning and late in the afternoon. Total fluid intake (consisting of the water consumed in fluid form and of the water in the consumed food), water-free food intake and body weights are demonstrated in Fig. 1. It is obvious that the total fluid intake of group B fell markedly behind that of group A, the difference being about 20% ($p < 0.001$). There was, however, no significant difference in food intake and body weight between the groups.

In another experiment the animals were kept on dry food, so the only means of water intake was by drinking. The results are presented in Fig. 2. During the pre-experimental period both groups consumed the wet diet and were offered water ad libitum throughout the day. After restriction of free water consumption to one hour twice daily, water intake of group B was about 20% less than that of group A, but in contrast to the results of the 1st experiment, food intake of group B fell behind that of group A and the same was

true for body weight, the difference being statistically significant ($p < 0.01$ and $p < 0.01$, respectively).

Total water and food intake during a 10-week experimental period of the animals kept on wet or on dry diet, respectively, all allowed free access to water for 24 hours, are demonstrated in Table I. It is obvious, that the absolute

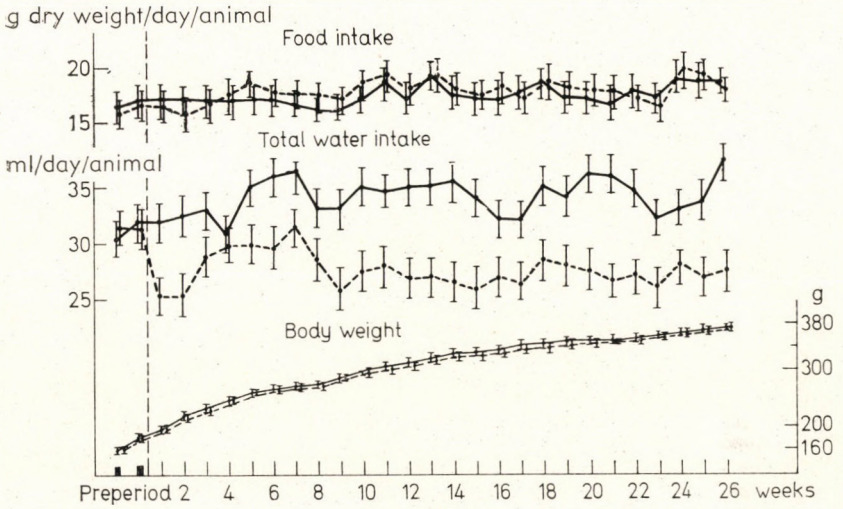

Fig. 1. Food intake ±SE, total water intake ±SE, and body weight ±SE of animals allowed to drink ad libitum (group A, n : 12, •—•) and of animals allowed to drink during one hour twice daily (group B, n : 12, •— —•), both groups kept on wet diet

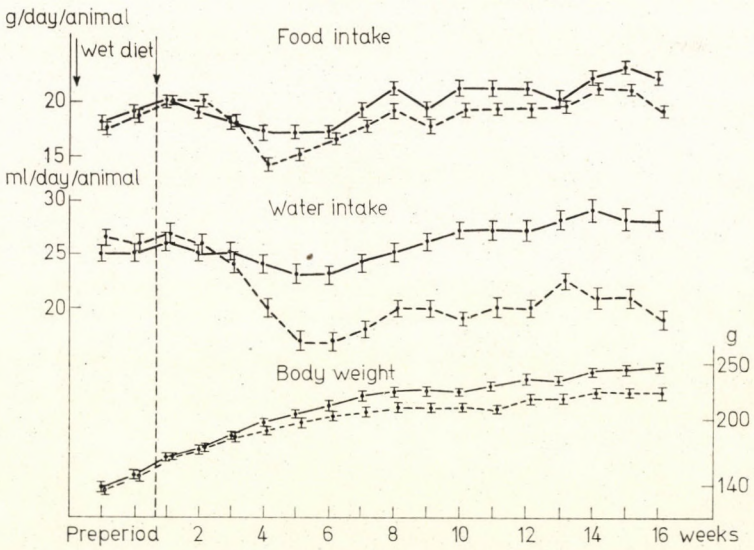

Fig. 2. Food intake ±SE, water intake ±SE, and body weight ±SE of animals allowed to drink ad libitum (group A, n : 12, •——•) and of animals allowed to drink during one hour twice daily (group B, n : 12, •— —•), both groups kept on dry diet

Acta Physiologica Academiae Scientiarum Hungaricae 38, 1970

Table I

Total water intake ±SE and food intake ±SE of animals kept on wet (n : 12) and on dry (n : 12) diets

		1	2	3	4	5	6	7	8	9	10	weeks
Total water intake ml/day/animal	on wet diet	32±**	33±**	34±**	31±**	35±**	36±**	33±**	32±*	32±*	34±*	
		0.76	0.84	0.88	0.86	0.90	0.79	0.80	0.92	0.96	0.88	
	on dry diet	24±	23±	23±	24±	25±	26±	27±	27±	27±	30±	
		0.68	0.73	0.50	0.55	0.72	0.84	1.09	1.09	1.03	0.84	
Food intake (g dry weight/day/animal)	on wet diet	17±	17±	17±	17±	17±	18±	18±	18±	18±	19±	
		0.72	0.82	0.80	0.76	0.78	0.79	0.82	0.83	0.74	0.75	
	on dry diet	16±	16±	17±	18±	19±	19±	20±	20±	20±	20±	
		0.67	0.62	0.86	0.73	0.79	0.62	0.68	0.88	0.84	0.78	

* $p < 0.01$
** $p < 0.001$

(degree of significance between corresponding weeks of total water intake)

amount of consumed water was significantly less with the animals kept on dry diet ($p < 0.001$), without any difference in food intake. This means that the physical condition of food affects total water intake, and demonstrates the absence of a strict correlation between water and food intake when both are supplied ad libitum.

Fig. 3 demonstrates daily food intake of groups A and B on the pellet diet for the 22 hours when there was no water available to group B, and for the 2 hours when group B was also allowed to drink. The absolute values are shown on the left side, while the percentual ones on the right. Both the absolute and percentual figures were higher in group B during the 2 hours of free water

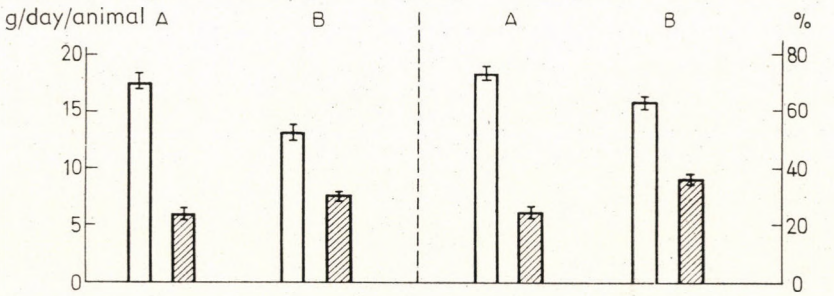

Fig. 3. Food intake ±SE of groups A and B, during 22 hours when no water was available to group B (□) and for the 2 hours when group B was allowed to drink (▨), in absolute values (g/day/animal) on the left side, and in percentual figures on the right side

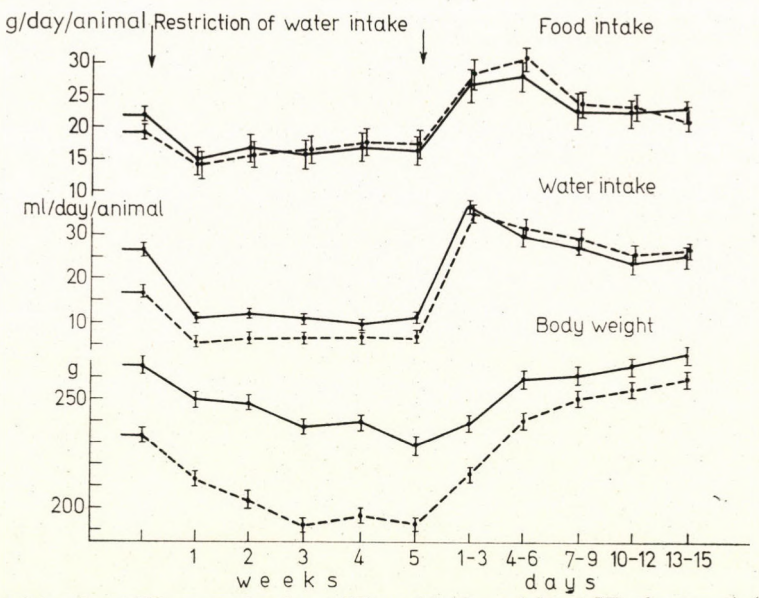

Fig. 4. Food intake ±SE, water intake ±SE, and body weight ±SE of group A (free water intake before restriction, n : 12, •—•) and B (allowed to drink during one hour twice daily before quantitative restriction, n : 12, •— —•) before, during and after 50% restriction of actual water intake; both groups were kept on a dry diet

intake, than in group A to which water was available all the time. The difference was significant, $p < 0.02$ and $p < 0.01$, respectively. The absolute level of 24-hour-food intake was higher in group A than in group B, which means that free water intake for the whole day ensured a higher food intake.

In the next series of experiments the effect on food intake and body weight of a 50% restriction of water intake was investigated. Fifty per cent of their average ad libitum daily water intake was offered to both groups A and B. The results are demonstrated in Fig. 4. Food intake significantly decreased in both groups, by 24% on the average in group A, and by 18% in group B. As a result of this, a marked decrease in body weight was observed, averaging 36 g and 40 g, respectively. Allowing free water to both groups, food and water intake increased promptly and markedly. In group B, in which water had been available for two hours only before the restriction to 50% of the actual intake, body weight, water and food intake were lower than in group A. After restoration of free water intake throughout the day a greater rise in all parameters was observed in group B; this resulted in a marked decrease of the difference in body weight between the two groups (from 37 g to 11 g by day 15). Body weight in group A reached the level observed before water restriction on the 15th day, while group B surpassed it already on the 7th day. This means that after terminating water restriction the regulative mechanisms normalise body weight in a few days by increasing water and food intake.

Discussion

Providing water for 1 hour twice daily does not ensure a water intake equal to that consumed when water is available ad libitum for 24 hours. However, in spite of an about 20% decrease in water intake, food intake and body weight were not affected when the food contained 50% water. On feeding a dry diet, both body weight and food intake decreased in response to a restriction of water intake to two hours daily. The cause of this apparent discrepancy might have been that total water (drinking + water in food) intake was sufficient to ensure an unchanged calorie intake in the first experiment, but not in the second, in which the food contained a minimum of water.

According to some authors, differences in the composition of diets other than the amount of water consumed, may also play a role (Asher and Hodes 1939; Bruce and Kennedy 1951; Radford 1959).

Lepkovsky et al. (1957) came to the conclusion that the amount of consumed food depends on how much water the organism can mobilize from the tissues and assume an osmotic regulation of food intake. According to Harper and Spivey (1958) the osmotic pressure of food is one of the decisive components in food intake. Recently, data have been published (Yin et al. 1970) which do not fit into the osmotic regulation of food intake.

In animals fed a wet and a dry diet, total water intake (both groups were allowed to drink ad libitum) differed significantly although calorie intake was the same in both groups. On restricting the time of free water intake (2 hours daily) of animals on the dry diet, water intake decreased by 20%, whereas food intake by 5%. Restriction of water intake to 50% of the amount consumed ad libitum, also decreased food intake but the relationship was not strict. All these indicate a close but not strict correlation in the regulation of food and water intake, and suggest that a number of factors exert an influence on their relationship.

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THERMOREGULATORY HEAT PRODUCTION AND THE REGULATION OF BODY TEMPERATURE IN THE NEW-BORN RABBIT

By

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Oxygen consumption and deep body temperature were recorded in new-born rabbits aged from a few hours to 18 days at ambient temperatures of 35, 30 and 20°C. Oxygen consumption remained unchanged in a thermoneutral environment throughout this period, and declined with age at lower temperatures. Deep body temperature increased with age at each ambient temperature. In response to exposure to an ambient temperature of 20 °C, deep body temperature varied within a wide range; some rabbits aged less than 12 hours maintained their colonic temperature within 0.5°C of that measured in the thermoneutral environment of 35 °C, while others responded with a fall in deep body temperature of 3 or 4°C. Similar results were obtained in a few days older animals and even some rabbits aged 10 to 18 days responded with a fall in body temperature approaching or exceeding 2 °C. No correlation could be established between body weight and the changes in body temperature, nor between body temperature in the thermoneutral temperature of 35 °C and the change in body temperature in response to transfer to an ambient temperature of 20 °C. A moderate correlation was found between oxygen consumption at an ambient temperature of 20 °C and the concomitant change in body temperature in rabbits aged up to 2 days, and 3 to 6 days, but not in the older ones. This correlation cannot be interpreted to indicate that oxygen consumption per se determines the response of body temperature to exposure to an ambient temperature of 20°C. The age at which animals responding with a marked fall in body temperature to a cool environment, began to maintain body temperature under the same conditions, varied from animal to animal. It is concluded that the differences in the responses are due to genetically determined functional differences in the central regulating mechanism.

In contrast to the rat, it was generally accepted that the new-born rabbit is able to increase heat production in response to cold exposure (ADAMSONS 1959; VÁRNAI 1961; DAWES and MESTYÁN 1963; BLATTEIS 1964; HULL 1964, 1965; DAWKINS and HULL 1964, 1965; HARDMAN et al. 1969a, b). Notwithstanding the increase in heat production, the new-born rabbit was found by all these investigators, with the exception of VÁRNAI (1961), to be unable to maintain body temperature even when exposed to a moderately cool environment, and when some observations of VÁRNAI (1961) were presented showing that new-born rabbits may be able to maintain their body temperature at an ambient temperature of 20°C, these were received with open incredulity (MOTT 1961; HAHN 1961). Certainly, differences in experimental setup also accounted for this discrepancy as pointed out by DONHOFFER (1961), since similar ambient temperatures do not necessarily mean equal thermal conditions and may represent great differences in actual heat loss. A detailed investigation

of the changes in body temperature in response to changes in the thermal environment under strictly standardized conditions led to the conclusion that, apart from technical details, genetic differences have to be considered.

Methods

The study comprised 159 experiments performed on unanaesthetized new-born rabbits of outbred mixed stock, aged from a few hours to 18 days. The rabbits remained in their mother's nest until they were transferred to the metabolic chamber (DONHOFFER et al. 1958) which was placed into a waterbath of 35°C. The measurements were started 30 to 60 min later and continued for 30 min, then the metabolic chamber was transferred to another waterbath of 30°C, and after another 30 min to a waterbath of 20°C. The essential feature of the metabolic chamber was that the animal was kept in a small wire cage, the bottom of which was covered with a few layers of thin blotting paper. The wire cage was separated from the wall of the brass metabolic chamber by a 1 cm layer of air, the feet of the wire cage being isolated from the brass wall by rubber. Thus heat loss by direct contact with the wall of the brass metabolic chamber was completely avoided. Oxygen consumption was measured by a modified Kipp-Noyons diaferometer with an air flow of 600 to 1900 ml/min, according to the size of the animal (DONHOFFER et al. 1958; VÁRNAI 1970a). Colonic temperature was measured 4.5 to 6 cm from the anus by a copper-constantan thermocouple enveloped in a flexible nylon sheath. Figures for oxygen consumption represent the means for the whole period of exposure to the relevant ambient temperature, whereas those for colonic temperature give the level reached by the end of exposure to that temperature. After the observation the animals were returned to the mother's nest and in many cases the observations were repeated a few days later on the same animal. Student's two tailed *t*-test was employed in the statistical evaluation of the differences between the means; in some instances Pearson's product-moment coefficient of correlation (*r*) was used and the significance of the coefficient of regression was also calculated (LACEY 1953). Body surface was calculated according to the formula $10 \sqrt[3]{kg^2}$.

Results

Oxygen consumption. Fig. 1 demonstrates the mean oxygen consumption of rabbits aged 0—2, 3—6, 7—9, and 10—18 days, respectively, at the three experimental ambient temperatures. At the neutral ambient temperature of 35°C, there was no significant difference between the different age-groups, whereas at the ambient temperatures of 30°C and 20°C a decline with age was observed. At 30°C, oxygen consumption of the two older age-groups was the same as the basic value observed at 35°C, indicating the broadening of the thermoneutral zone with age.

In some rabbits of the oldest age-group and in a few days older animals, however, not only a broadening of the thermoneutral zone was observed, but also a shift to lower ambient temperatures, since oxygen consumption was higher at 35°C than at 30°C (Figs 2 and 3).

At an ambient temperature of 30°C, the difference between the youngest and the two oldest groups was highly significant statistically ($p < 0.001$), the difference between the 3 to 6-day-old and the 10 to 18-day-old rabbits reached statistical significance ($p < 0.05$), while the difference between the second and third age-group failed to do so ($0.1 > p > 0.05$). At an ambient temperature

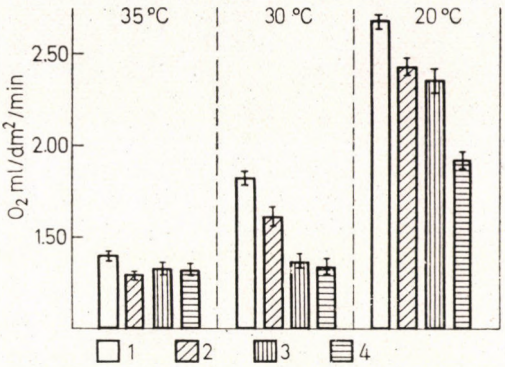

Fig. 1. Oxygen consumption of new-born rabbits (M ±S.E.) aged less than 2 days (1), 3 to 6 days (2), 7 to 9 days (3), and 10 to 18 days (4) at 35, 30, and 20°C ambient temperature. Number of observations at the respective ambient temperatures was in rabbits aged less than 2 days 54, 53, 55; in 3 to 6-day-old animals, 41, 45, 48; in 7 to 9-day-old rabbits, 18; and at the age of 10 to 18 days, 25, 26 and 22, respectively

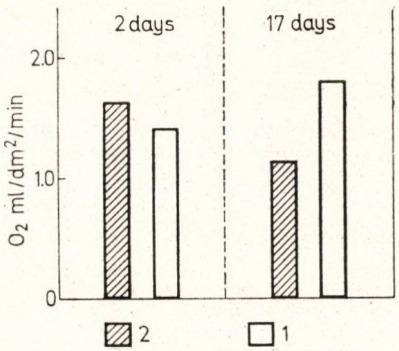

Fig. 2. Oxygen consumption of a rabbit at an ambient temperature of 35 (1) and 30°C (2) at 2 days and 17 days of age

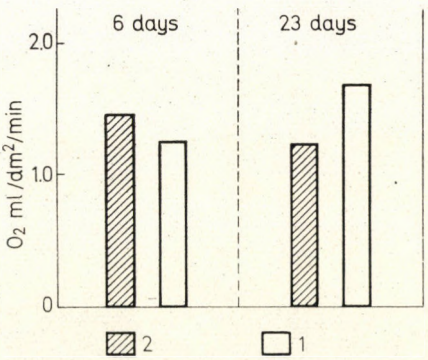

Fig. 3. Oxygen consumption of a rabbit at an ambient temperature of 35 (1) and 30°C (2) at 6 days and 23 days of age

of 20°C, the difference in oxygen consumption was significant between the youngest age-group and those aged 3 to 6 and 7 to 9 days ($p < 0.05$), and highly significant between the youngest and the oldest group ($p < 0.001$), and between the two oldest groups ($p < 0.01$).

To be able to compare our results conveniently with those obtained by others, but expressed per unit body weight instead of per unit body surface calculated as the 2/3rd power of body weight, Table I presents the figures cal-

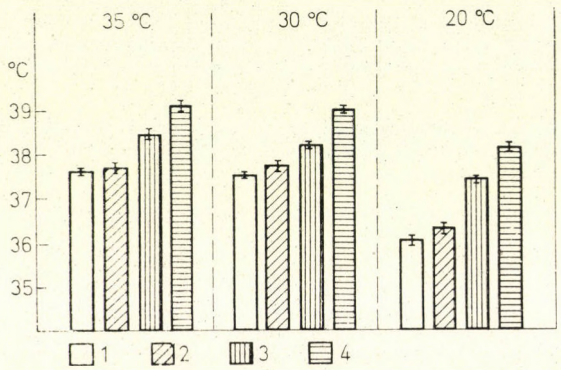

Fig. 4. Colonic temperature of new-born rabbits (M ±S.E.) aged less than 2 days (1), 3 to 6 days (2), 7 to 9 days (3), and 10 to 18 days (4), at 35, 30 and 20°C ambient temperature, Number of observations in the four age-groups was 69, 50, 18 and 22, respectively

Table I

Oxygen consumption of new-born rabbits of different ages, expressed as ml $O_2/dm^2 \cdot min$ and ml $O_2/kg \cdot min$ (M ±S.E.)

(In brackets, number of observations)

Age; days	Body weight g	Mean body surface dm^2	35°C	
			ml $O_2/dm^2 \cdot min$	ml $O_2/kg \cdot min$
0—2	79.6 ± 2.2	1.83 (54)	1.40 ± 0.05	32.4 ± 1.2
3—6	103.0 ± 6.1	2.20 (45)	1.29 ± 0.05	27.4 ± 1.0
7—9	174.0 ± 9.3	2.99 (18)	1.33 ± 0.07	24.2 ± 1.3
10—18	199.0 ± 13.0	3.63 (22)	1.32 ± 0.07	21.8 ± 1.2

Age; days	30°C		20°C	
	ml $O_2/dm^2 \cdot min$	ml $O_2/kg \cdot min$	ml $O_2/dm^2 \cdot min$	ml $O_2/kg \cdot min$
0—2	1.82 ± 0.06	42.1 ± 1.4	2.66 ± 0.08	61.5 ± 1.8
3—6	1.61 ± 0.10	34.3 ± 2.1	2.41 ± 0.08	51.2 ± 1.7
7—9	1.37 ± 0.08	24.9 ± 1.5	2.34 ± 0.12	42.6 ± 2.2
10—18	1.34 ± 0.08	22.1 ± 1.3	1.91 ± 0.09	31.6 ± 1.5

culated by both methods. The two ways of calculation yielded different results which, at an ambient temperature of 35°C, might lead to contradictory conclusions. On the basis of body surface, there was no change in heat production up to the age of 18 days, whereas on the basis of body weight, there was a highly significant reduction in oxygen consumption not only between the youngest and the oldest age-group ($t = 6.2$, $p < 0.001$), but also between the two youngest age-groups ($t = 3.2$, $p < 0.01$), between the youngest age-group and the rabbits aged 7 to 9 days ($t = 4.6$, $p < 0.001$), and between the animals aged 3 to 6 days and 10 to 18 days ($t = 3.6$, $p < 0.001$). At the ambient temperatures of 30°C and 20°C the differences were quantitative: in the youngest and the oldest age-group heat production, when calculated on the body surface basis, diminished by 36.8% and 28.3%, respectively, whereas on the body weight basis the corresponding figures were 47.3% and 48.6%.

Body temperature. Fig. 4 demonstrates, on the one hand, that body temperature increased with age at all three ambient temperatures, the increase having been confined largely to the two older groups and, on the other hand, that at an ambient temperature of 20°C, mean colonic temperature declined significantly in all four age-groups. The increase with age at 35°C was not significant between the two younger groups and highly significant between these and both older groups and also between the two oldest groups ($p < 0.001$). At 30°C, the difference between the two youngest age-groups was not significant statistically, whereas the difference between the youngest age-group and the two oldest age-groups, as well as between the two oldest groups ($p < 0.001$), and between the groups aged 3 to 6 and 7 to 9 days ($p < 0.01$) was highly significant.

At 20°C, the difference in mean temperature between the two younger age-groups was not significant statistically, whereas that between both younger and both older groups was highly significant ($p < 0.001$), and the difference between the two oldest groups also reached statistical significance ($p < 0.02$). Within the same age-group, body temperatures measured at ambient temperatures of 35 and 30°C were not significantly different statistically.

Fig. 4 shows correctly the general trend of the changes with age in the body temperature response to ambient temperature, but failed to disclose the wide variety of the response. Tables II and III demonstrate the differences in colonic temperature observed in the different age-groups at the ambient temperatures of 35°C and 20°C, and 30 and 20°C, respectively.

Table IV shows the distribution around the mean at an ambient temperature of 20° C. Whereas in the two older age-groups the mode lies within ± 0.5°C of the mean, as does the mode in all age-groups at ambient temperatures of 35°C and 30°C, at an ambient temperature of 20°C, however, the mode was shifted to the right in the two younger age-groups, which indicates, of course, that the mean had been influenced to a considerable extent by a group of

Table II

Changes in colonic temperature between ambient temperatures of 35°C and 20°C in new-born rabbits of different ages

Age, days	Decrease in colonic temperature				
	Less than 0.5 °C	0.5—1.5°C	1.5—2.5°C	2.5—3.5°C	More than 3.5°C
0—2	22 (33.8%)	14 (21.6%)	11 (16.9%)	5 (7.7%)	13 (20.0%)
3—6	11 (22.0%)	23 (46.0%)	7 (14.0%)	6 (12.0%)	3 (6.0%)
7—18	9 (22.5%)	19 (47.5%)	9 (22.5%)	3 (7.5%)	∅ —

Table III

Changes in colonic temperature between ambient temperatures of 30°C and 20°C in new-born rabbits of different ages

Age, days	Decrease in colonic temperature				
	Less than 0.5 °C	0.5—1.5°C	1.5—2.5°C	2.5—3.5°C	More than 3.5°C
0—2	21 (30.4%)	19 (27.6%)	15 (21.8%)	5 (7.2%)	9 (13.0%)
3—6	13 (26.0%)	18 (36.0%)	7 (14.0%)	9 (18.0%)	3 (6.0%)
7—18	17 (43.6%)	17 (43.6%)	5 (12.8%)	∅ —	∅ —

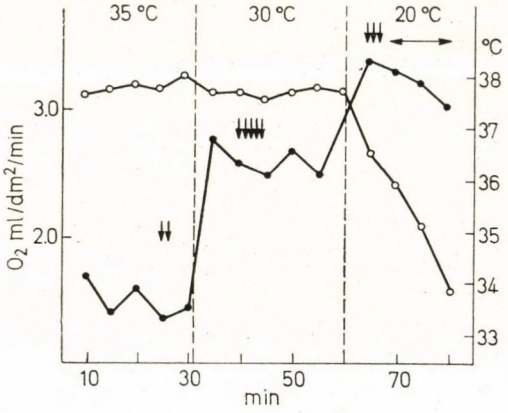

Fig. 5. New-born rabbit aged 12 hours; body weight 85 g. Oxygen consumption and colonic temperature at 35, 30 and 20°C ambient temperature. Arrows indicate motor activity, horizontal double-headed arrow indicates visible tremor (shivering)

rabbits with very low body temperatures, i.e. which when exposed to an ambient temperature of 20°C responded with a very marked fall of colonic temperature.

Individual experiments serve to demonstrate the variety of the response of colonic temperature to exposure to an ambient temperature of 20°C. Fig.

Table IV

Distribution of colonic temperatures around the mean in new-born rabbits of different ages at 20°C ambient temperature

Age, days	Mean body temperature S.E.	Deviation from mean °C						
		>−3.5	−3.5 to −2.5	−2.5 to −1.5	−1.5 to −0.5	± 0.5	+0.5 to +1.5	+1.5 to +2.5
0—2	36.04±0.21	5 (7.2%)	5 (7.2%)	3 (4.3%)	7 (10.2%)	14 (20%)	19 (27.6%)	16 (23.2%)
3—6	36.30±0.21	1 (2.0%)	1 (2.0%)	9 (18%)	5 (10.0%)	11 (22%)	17 (34.0%)	6 (12.0%)
7—9	37.53±0.14	—	—	1 (5.6%)	3 (16.7%)	10 (55.5%)	4 (22.2%)	—
10—18	38.10±0.16	—	—	1 (4.6%)	3 (13.0%)	14 (63.5%)	4 (18.2%)	—

5 and 6 show the records of two rabbits, both aged 12 hours. One of them responded to exposure to an ambient temperature of 20°C with a fall of more than 3.5°C in colonic temperature (Fig. 5), whereas the other rabbit maintained its body temperature under the same conditions (Fig 6). It should be noted that heat production was greater in the rabbit showing a marked fall in body temperature than in the animal which maintained its colonic temperature, and that the body weight of the former (85 g) also exceeded that of the latter (75 g).

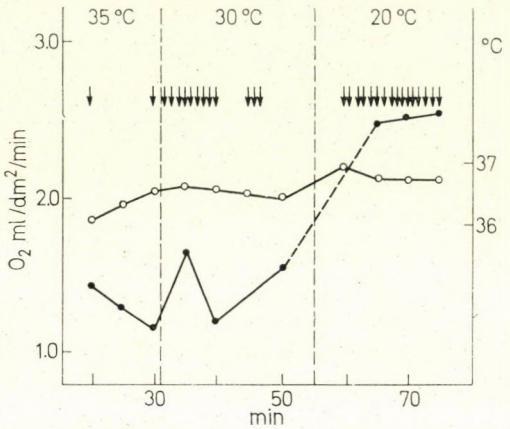

Fig. 6. New-born rabbit aged 12 hours; body weight 75 g. Oxygen consumption and colonic temperature at 35, 30 and 20°C ambient temperature. Arrows indicate motor activity

Animals maintaining their body temperature soon after birth or a few days later, retained their ability to do so. The age, however, at which colonic temperature was maintained at an ambient temperature of 20°C varied widely. Fig. 7 demonstrates observations made on the same animal on the first and the sixth day of life. On the first day, body temperature declined by about one centigrade; on the sixth day body temperature was maintained at a completely stable level, while heat production per unit surface area was less than at the age of one day. Fig. 8 shows the behaviour of another rabbit on the 3rd and the 7th day of life. On the 3rd day, colonic temperature fell by almost 2°C, on the 7th it was maintained. Fig. 9 records observations on a third rabbit at the age of 7 and 9 days. On the 7th day, colonic temperature fell by almost 2°C, on the 9th, it was maintained. The age at which body temperature was maintained at an ambient temperature of 20°C varied, therefore, within fairly wide limits, and even in the oldest age-group a number of animals showed a fall in colonic temperature greater than 1°C.

Table V shows the changes in colonic temperature in response to exposure to 20°C, regarding a change of more, or less, than 1°C as the arbitrary dividing line, a procedure of admittedly limited value. The fact that in the majority of

Table V

Change in colonic temperature of new-born rabbits transferred from an ambient temperature of 30 °C to one of 20 °C by the end of 30 min exposure

In brackets, number of observations

Age, days		Fall in colonic temperature		
		A More than 1.0°C	B Less than 1.0°C	C Less than 0.5°C
0—2	(69)	41 (59%)	28 (41%)	20 (71%)*
3—6	(50)	25 (50%)	25 (50%)	13 (52%)*
7—9	(18)	6 (34%)	12 (66%)	9 (75%)*
10—18	(22)	9 (41%)	13 (66%)	9 (69%)*

* In per cent of animals displaying a fall in body temperature of less than 1.0°C.

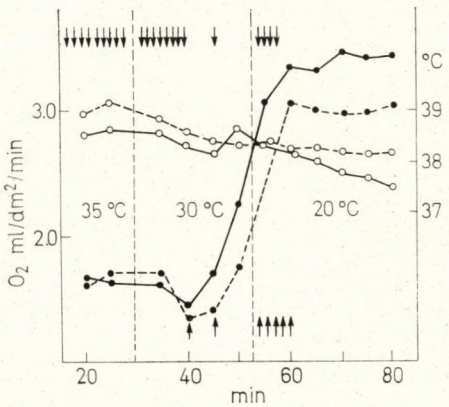

Fig. 7. Oxygen consumption and colonic temperature of a new-born rabbit at the age of 1 and 6 days at ambient temperatures of 35, 30, and 20°C. Oxygen consumption on day 1: ●——●, on day 6: ●------●; colonic temperature on day 1: ○——○, on day 6: ○---○. Arrows at the top refer to motor activity on day 1, arrows at the bottom, to that on day 6.

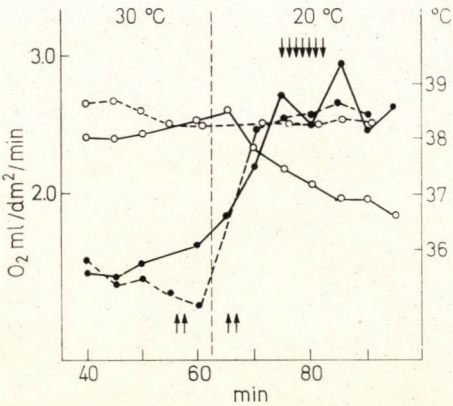

Fig. 8. Oxygen consumption and colonic temperature of a new-born rabbit at the age of 3 and 7 days at ambient temperatures of 30 and 20°C. Oxygen consumption on day 3: ●——●, on day 7: ●------●; colonic temperature on day 3: ○——○, on day 7: ○------○. Arrows at the top refer to motor activity on day 3, arrows at the bottom, to that on day 7

the rabbits showing a fall of less than 1.0°C in colonic temperature, the actual change was less than 0.5°C (Column C in Table V), appears, however, to be more meaningful, and suggests, as the skewed distribution in the two younger age-groups might also have suggested that one is dealing with a non-uniform population of rabbits.

Correlation of body weight and the change in colonic temperature in response to exposure to 20°C. It has been widely assumed that the unfavourable ratio

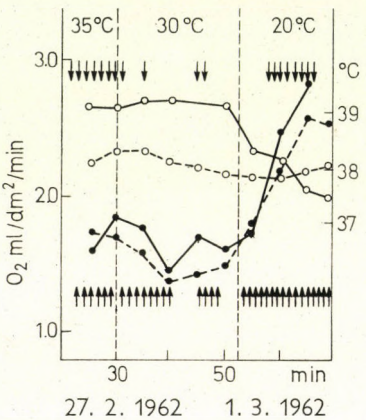

Fig. 9. Oxygen consumption and colonic temperature of a new-born rabbit at the age of 7 and 9 days at ambient temperatures of 35, 30 and 20°C. Oxygen consumption on day 7: ●——●, on day 9: ●------●; colonic temperature on day 7: ○——○, on day 9: ○------○. Arrows at the top refer to motor activity on day 7, arrows at the bottom, to that on day 9

Table VI

Body weight of rabbits responding to exposure to 20°C with a fall in colonic temperature of more than 1.0°C (I), and those maintaining colonic temperature within 1.0°C (II), and correlation between body weight and change in colonic temperature

In brackets, number of observations. (n.s. = not significant)

Age, days	Group	Body weight M±S.E.	Difference between group I and II		Coefficient of correlation r
			t	p	
0—2	I (42)	76± 3	1.65	>0.1	(69) 0.095 n.s.
	II (27)	83± 3			
3—6	I (25)	101± 8	0.28	>0.7	(50) 0.107 n.s.
	II (25)	104± 7			
7—9	I (6)	164±19	0.56	>0.5	(18) 0.037 n.s.
	II (12)	177±15			
10—18	I (9)	190±13	0.40	>0.6	(22) −0.195 n.s.
	II (13)	199±18			

Table VII

Oxygen consumption in ml/dm² · min at 20°C (M±S.E.), and the fall in colonic temperature in response to transfer from 35°C to 20°C ambient tmperature (Part A), and from 30 to 20°C (Part B)

In brackets, number of observations

Age, days	More than 1°C	Less than 1°C	t	p
		Part A		
0—2	2.52±0.12 (26)	2.85±0.09 (24)	2.20	<0.05
3—6	2.24±0.14 (22)	2.69±0.14 (20)	2.28	<0.05
7—9	2.37±0.19 (8)	2.53±0.21 (10)	0.07	>0.9
10—18	1.79±0.12 (12)	2.05±0.13 (10)	1.47	>0.1
		Part B		
0—2	2.53±0.14 (29)	2.77±0.08 (26)	1.48	>0.1
3—6	2.14±0.13 (19)	2.65±0.09 (25)	3.30	<0.01
7—9	2.20±0.30 (5)	2.42±0.16 (13)	0.65	>0.5
10—18	1.94—0.16 (9)	1.88±0.11 (13)	0.31	>0.7

of body mass and body surface is one of the most important factors in the thermal instability of the new-born of many species. The fact that some several-day-old rabbits respond to an ambient temperature of 20°C with a considerable fall in colonic temperature while, under the same conditions, other animals maintain their body temperature a few hours after birth, rend red it unlikely that body weight should have a dominant role in determining the response. Table VI compares body weights of rabbits responding to exposure to 20°C with a change in body temperature of less than 1.0°C with the body weight of those showing a fall greater than 1.0°C. In addition, the coefficient of correlation (r) between body weight and the change in body temperature was also calculated. Evidently, no correlation could be established between body weight and the change in body temperature in response to exposure to an ambient temperature of 20°C.

Correlation between oxygen consumption and the change in body temperature at an ambient temperature of 20°C. The possibility that oxygen consumption, i.e. heat production, might be the determining factor whether on exposure to an ambient temperature of 20°C the animal maintains its body temperature or responds with a considerable fall in colonic temperature, had also to be examined. Table VII analyses our data according to whether the difference in colonic temperature between the ambient temperatures of 35°C and 20°C

Table VIII

Fall in colonic temperature in response to transfer from an ambient temperature of 35 and 30°C, respectively, to one of 20°C, and the correlation with oxygen consumption

In brackets, number of observations

Age, days	Ambient temperature	Difference in colonic temperature M ± S.E.	Oxygen consumption at 20°C M ± S.E.	Coefficient of correlation (r)	Significance	Significance of coefficient of regression	
						t	p
0—2	35 and 20°C (50)	−1.30±0.21	2.66±0.08	0.177	n.s.	1.23	>0.2
	30 and 20°C (55)	−1.21±0.16	2.66±0.08	0.326	<0.02	2.52	<0.02
3—6	35 and 20°C (44)	−1.27±0.25	2.40±0.09	0.399	<0.01	2.74	<0.01
	30 and 20°C (42)	−1.15±0.16	2.45±0.08	0.413	<0.01	3.30	<0.01
7—9	35 and 20°C (18)	−0.84±0.16	2.36±0.15	0.045	n.s.	0.25	>0.9
	30 and 20°C (18)	−0.55±0.17	2.36±0.15	0.288	n.s.	1.16	>0.2
10—18	35 and 20°C (22)	−1.12±0.14	1.91±0.09	0.282	n.s.	1.33	>0.1
	30 and 20°C (22)	−0.83±0.12	1.91±0.09	0.237	n.s.	1.09	>0.2

Table IX

Correlation between colonic temperature at 35°C ambient temperature and fall in body temperature in response to exposure to 20°C ambient temperature

In brackets, number of observations

Age, days	Body temperature at 35°C (M±S.E.)	Change in body temperature at 20°C (M±S.E.)	Coefficient of correlation r	Significance of coefficient of regression	
				t	p
0—2 (68)	37.61±0.11	−1.59±0.21	−0.088 n.s.	0.715	>0.4
3—6 (49)	37.69±0.13	−1.37±0.17	−0.003 n.s.	0.024	>0.8
7—9 (18)	38.44±0.14	−0.84±0.15	−0.263 n.s.	1.004	>0.3
10—18 (22)	39.27±0.15	−1.17±0.24	−0.244 n.s.	0.980	>0.3

Table X

Correlation between colonic temperature at 30°C ambient temperature and fall in body temperature in response to exposure to 20°C ambient temperature

In brackets number of observations

Age, days	Body temperature at 30°C (M±S.E.)	Change in body temperature at 20°C (M±S.E.)	Coefficient of correlation r	Significance of coefficient of regression	
				t	p
0—2 (69)	37.50±0.12	−1.51±0.17	+0.211 >0.05	1.695	>0.05
3—6 (50)	37.67±0.11	−1.43±0.16	+0.288 <0.05	2.105	<0.05
7—9 (18)	38.18±0.12	−0.58±0.16	−0.216 n.s.	0.884	>0.4
10—18 (22)	38.97±0.12	−0.85±0.13	−0.157 n.s.	0.595	>0.5

(Part A), and between 30°C and 20°C (Part B) exceeded 1.0°C or was less than 1.0°C. In the youngest age-group, the difference reached statistical significance when body temperatures were compared at ambient temperatures of 35 and 20°C; in the group aged 3 to 6 days the difference was significant statistically also when changes in colonic temperature at ambient temperatures of 30 and 20 °C served as the basis of comparison. In the two older age-groups oxygen consumption was not significantly different in animals responding to an ambient temperature of 20 °C with a change in colonic temperature of less or more than 1°C. Calculation of the coefficients of correlation and regression (Table VIII) showed essentially the same; in the youngest age-group, however, no correlation could be established between oxygen consumption and the fall in colonic temperature between ambient temperatures of 35 and 20°C, whereas a correlation could be demonstrated between oxygen consumption and body temperature changes between 30 and 20°C.

The correlation between oxygen consumption, i.e. heat production, and the fall in colonic temperature in response to exposure to an environment of 20°C, appears, therefore, to be moderate even in the 3 to 6-day-old group, and cannot be interpreted to demonstrate that heat production in itself is the main determinant of the change in body temperature.

Correlation between deep body temperature in a thermoneutral environment and the subsequent fall in colonic temperature on exposure to an ambient temperature of 20°C. The possibility had to be considered that the fall in body temperature in response to exposure to an ambient temperature of 20 °C might be determined, at least to some extent, by the deep body temperature prevailing at thermoneutral or near-thermoneutral ambient temperatures. From individual experiments no definite conclusions could be drawn, since animals with similar deep body temperatures at 35°C, might respond to an ambient temperature of 20°C quite differently. The impression gained was nevertheless confirmed by statistical analysis, and Table IX and X show that no correlation could be demonstrated between the two variables considered excepting a loose one in one group in Table X.

Discussion

Comparing our results with those of others some of the differences can easily be explained by differences in the experimental procedure. For instance, ADAMSONS (1959) cooled smaller rabbits by removing external heat sources, and larger ones by placing, in addition, small ice-packs over the inguinal region, and HARDMAN et al. (1969a, b) immersed their rabbits in a beaker into the waterbath, so that a considerable and variable amount of heat must have

been lost by conduction. Our experimental conditions were most nearly approached by the setup described by DAWES and MESTYÁN (1963) and used by several other investigators. They placed the new-born rabbit into a Perspex chamber on a wire-grid raised a few mm from the floor to prevent contact with the wall of the chamber. This was, however, achieved only so far that it prevented the contact of the animal with the bottom of the chamber but not with its side-wall. Another factor which might account for some of the differences is the way in which colonic temperature was measured. The depth to which the thermocouple is introduced may influence the result obtained in response to cold exposure. HULL (1965), for instance, introduced the thermocouple to a depth of 2 to 3 cm, whereas in our experiments they were 4.5 to 6 cm from the anus.

Differences in experimental procedure could account, in part, also for the fact that in our experiments oxygen consumption at the neutral ambient temperature of 35°C was considerably higher in the youngest age-group ($32.4\pm\pm1.2$ ml/kg · min) than in similar experiments of DAWKINS and HULL (1964) in which oxygen consumption was 23.4 ± 0.46 ml/kg · min, whereas at an ambient temperature of 30°C, practically identical results were obtained ($42.1\pm\pm1.4$ and 42.6 ± 2.0, respectively). In a thermoneutral environment considerably higher values were observed by us also for older animals (see Table I) than those given, for instance, by HULL (1964), who found at an ambient temperature of 35°C an oxygen consumption of only 16.4 ± 0.6 ml/kg · min in rabbits aged 1 to 7 days, and HARDMAN et al. (1969b) who measured 18.7 ± 0.6ml/kg · ·min in animals aged 6 to 8 days. MOORE and KOMÁROMI's (personal communication 1969) figures were, however, close to ours.

As has been pointed out, relating oxygen consumption to body weight or body surface may lead to different conclusions. At the thermoneutral ambient temperature of 35°C, new-born rabbits showed a reduction in oxygen consumption when calculated per unit body weight in the experiments of HULL (1965) as well as in our observations, whereas on the body surface basis ($10\sqrt[3]{kg^2}$) no change in oxygen consumption occurred (Table I). Especially in a period of rapid growth with a wide range of body weight, calculations on the body surface basis appear to be physiologically more meaningful. The observation of HULL (1965) that the maximum rate of oxygen consumption as calculated for unit body weight, decreased with age, whereas calculated on the body surface basis it remained unchanged, points to the same conclusion.

Changes in oxygen consumption with age are remarkably different in the new-born rabbit and the new-born rat in a thermoneutral environment as well as in response to exposure to lower ambient temperatures. In the rabbit, oxygen consumption calculated on the body surface basis does not change with age in a thermoneutral environment and decreases with age at lower ambient temperatures (Fig. 1), whereas in the rat, oxygen consumption increased

during the first three weeks of life in a thermoneutral environment as well as at lower ambient temperatures (VÁRNAI 1970b; VÁRNAI and DONHOFFER 1970).

The most striking result of our experiments was that under the specified conditions some rabbits were able to maintain deep colonic temperature within 1.0 and even within 0.5°C when exposed to an ambient temperature of 20°C already on the first day of extrauterine life, while under similar conditions other rabbits, even at the age of 10 to 18 days, responded with a fall in colonic temperature exceeding 1.0 and even 2.0°C. The experimental procedure being strictly standardized, and none of the animals starved, the cause of this remarkable difference had to be sought evidently in differences in the rabbits. Such differences in the animals could explain also the incredulity with which experienced workers (MOTT 1961; HAHN 1961) received the results of VÁRNAI (1961) demonstrating that new-born rabbits could maintain their body temperature at an ambient temperature of 20°C.

Differences between rabbit populations might be indicated by differences in body weight at birth. DAWKINS and HULL's (1964) new-born rabbits ranged in body weight from 35 to 74 g, whereas our rabbits aged less than 12 hours ranged from 42 to 85 g with an average of 70 ± 5 g, and if one included those animals in which body weight was first measured during the second 12 hours of extrauterine life, the range was extended to 120 g, so that the birth-weight of several animals must have been well above 85 g, mean body weight being 74 ± 3 g ($n = 27$).

Differences in body weight cannot, however, account for the difference in the response to exposure to an ambient temperature of 20°C. The smallest rabbit which maintained its colonic temperature at 20°C within 0.5°C of that observed at an ambient temperature of 35°C, was aged 1 day and weighed 55 g, whereas another rabbit aged 12 days and weighing 165 g responded with a fall of 2.66°C, and a third rabbit aged 14 days and weighing 235 g, with a fall of 1.81°C. Convincing evidence that there is no correlation between body weight and the response of deep body temperature to exposure to an ambient temperature of 20°C has been presented in Table VI. The simple explanation that failure to maintain deep body temperature is due to the unfavourable body mass–body surface ratio has, therefore, to be discarded, at least in the case of the new-born rabbit.

The correlation between oxygen consumption and body temperature at an ambient temperature of 20°C, demonstrated for the two younger age-groups in Tables VII and VIII cannot be interpreted to show that oxygen consumption, i.e. heat production, was the only factor determining the maintenance or the fall of body temperature. Even in rabbits aged 3 to 6 days, the correlation was not very close, and in the older animals no correlation could be demonstrated between oxygen consumption and body temperature. Neither was oxygen consumption at its peak in the younger animals responding with a

marked fall in body temperature to exposure to an ambient temperature of 20°C. Higher oxygen consumptions were observed in the same animals after exposure to hypoxia at the same ambient temperature, body temperature having been lowered further during hypoxia.

The possibility that body temperature, whether high or low at an ambient temperature of 35 or 30°C, could be a factor determining the response of deep body temperature to exposure to an ambient temperature of 20°C, was unequivocally excluded (Table IX and X).

Considering all of the evidence, there remains no doubt that the differences in the response were due to functional differences in the central regulating mechanism. The fact that some animals maintain deep body temperature already on the first day of life, while others respond even between the age of 10 to 18 days with a fall exceeding 1.0°C to exposure to an ambient temperature of 20°C, suggests that the difference is a genetically determined one. The variability of age at which animals not maintaining their deep body temperature in response to exposure to 20°C, change over to maintain it under the same conditions, and that once this change had occurred, the animal will respond consequently so, points not only to the developmental nature of this process, but furnishes additional evidence for the genetic origin of the difference in the response. The skewed distribution of the changes in body temperature in the two younger age-groups at an ambient temperature of 20°C (Table IV) might also be interpreted to support this conclusion.

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EFFECT OF DENERVATION ON THE COMPOSITION OF PHOSPHOLIPIDS IN TONIC AND TETANIC MUSCLES

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Following unilateral denervation the amount of the choline phosphatides and the cephalins increases in denervated tonic and tetanic muscles, while it decreases in the contralateral innervated muscles. These inverse changes are more pronounced in tetanic than in tonic muscles.

The qualitative composition of phospholipids undergoes changes after denervation. The fatty aldehyde content of choline phosphatides and cephalins increases in both denervated and innervated muscles. This increase in both phospholipid fractions is smaller in denervated than in innervated muscles. Changes in the fatty aldehyde content of phospholipids are more pronounced in tetanic than in tonic muscles.

It is well known that denervation atrophy is associated with changes in the lipid composition of muscles (ARTOM 1941; HINES and THOMSON 1956). The fact, however, that the muscles are built up of tonic and tetanic muscles with different functions and metabolism, has not been taken into consideration in most of the papers on the subject. In a previous study (HEINER and DOMONKOS 1970) we have reported on differences in the qualitative and quantitative composition of lipids in the two kinds of muscle. It seemed therefore of some interest to examine the lipid composition of the muscles during denervation atrophy.

Methods

Adult rabbits kept on a normal diet were used. The muscles were inactivated by unilateral section of the sciatic nerve at the trochanter level. The semimembranosus muscle was used as a tetanic and the soleus muscle as a tonic one. Changes following denervation were studied at intervals of 2 to 6 weeks.

After removing the connective tissue, the muscles were homogenized in methanol in a glass homogenizer and extracted with chloroform-methanol (2 : 1 v/v) at room-temperature. Separation of phospholipids into two fractions — choline phosphatides (lecithin, sphingomyelin, lysolecithin) and cephalins (ethanolamine, serine, inositol phosphatides) — was performed in the same manner as in earlier studies (HEINER and DOMONKOS 1970).

Determination of the muscle cell phase was based upon the estimation of non-collagenous protein according to LILIENTHAL et al. (1950). After treating the homogenized muscle with 0.05 *N* NaOH, the eluted protein was determined according to HILLER et al. (1948).

Fatty acid ester was estimated by the method of STERN and SHAPIRO (1953) as modified by HARTREE and MANN (1959), fatty aldehyde by the method of WARNER and LANDS (1961), phosphorus according to FISKE and SUBBAROW (1925) after wet combustion with H_2SO_4.

Results

Choline phosphatides (Table I)

In the third week following denervation the phospholipid content increased in the denervated and decreased in the innervated muscle. This change continued in the fourth week, then the lipid-P content in the denervated muscle

Table I

Amount of lipid phosphorus, fatty acid ester and fatty aldehyde of choline phosphatides in tonic and tetanic muscle following denervation

Weeks after denervation		Tonic muscle					
		Lipid phosphorus		Fatty acid ester		Fatty aldehyde	
		μM/g muscle (wet weight)					
2	C	4.30±0.80*	(5)	7.55±1.20	(5)	0.30±0.09	(5)
	D	4.40±0.78	(5)	7.65±1.00	(5)	0.21±0.06	(5)
3	C	4.10±0.31	(6)	7.00±1.25	(6)	0.42±0.11	(6)
	D	4.95±0.52	(7)	8.65±1.72	(7)	0.18±0.07	(7)
4	C	3.95±0.40	(7)	6.70±1.00	(6)	0.60±0.12	(7)
	D	4.60±0.30	(7)	8.10±0.90	(6)	0.16±0.08	(7)
5	C	3.78±0.61	(5)	6.50±1.02	(5)	0.45±0.20	(5)
	D	4.30±0.41	(5)	7.43±1.36	(5)	0.13±0.06	(5)
6	C	4.05±0.57	(6)	6.80±0.66	(6)	0.42±0.16	(6)
	D	4.15±0.61	(6)	7.20±1.04	(6)	0.12±0.06	(6)

Weeks after denervation		Tetanic muscle					
		Lipid phosphorus		Fatty acid ester		Fatty aldehyde	
		μM/g muscle (wet weight)					
2	C	4.40±0.72	(5)	7.40±1.00	(5)	0.40±0.18	(5)
	D	4.75±0.33	(5)	8.10±0.63	(5)	0.45±0.15	(5)
3	C	4.10±0.52	(7)	6.45±0.73	(7)	0.80±0.43	(7)
	D	5.30±0.56	(6)	8.95±1.80	(6)	0.87±0.23	(6)
4	C	4.00±0.50	(7)	6.45±1.30	(6)	1.02±0.20	(7)
	D	5.75±0.40	(7)	9.55±1.50	(6)	1.23±0.30	(7)
5	C	3.90±0.43	(5)	6.10±0.47	(5)	0.92±0.32	(5)
	D	5.42±0.64	(5)	9.25±0.51	(5)	0.70±0.17	(5)
6	C	4.05±0.33	(6)	6.35±0.83	(6)	0.60±0.20	(6)
	D	4.90±0.67	(6)	8.80±1.03	(6)	0.45±0.16	(6)

C: contralateral innervated muscle; D: denervated muscle
* mean ± standard deviation. Number of experiments in brackets

Table II

Amount of lipid phosphorus, fatty acid ester and fatty aldehyde of cephalins in tonic and tetanic muscle following denervation

Weeks after denervation		Tonic muscle					
		Lipid phosphorus		Fatty acid ester		Fatty aldehyde	
		μM/g muscle (wet weight)					
2	C	3.45 ± 0.40*	(5)	5.60 ± 1.32	(5)	0.75 ± 0.29	(5)
	D	3.36 ± 0.24	(5)	5.47 ± 1.43	(5)	0.70 ± 0.15	(5)
3	C	2.95 ± 0.59	(6)	4.80 ± 1.68	(6)	0.85 ± 0.39	(6)
	D	3.75 ± 0.42	(7)	6.00 ± 2.00	(7)	0.82 ± 0.53	(7)
4	C	2.70 ± 0.22	(7)	4.20 ± 1.20	(6)	1.00 ± 0.20	(7)
	D	3.45 ± 0.40	(7)	5.40 ± 1.20	(6)	0.90 ± 0.30	(7)
5	C	3.08 ± 0.60	(5)	4.70 ± 0.95	(5)	1.30 ± 0.28	(5)
	D	3.40 ± 0.45	(5)	5.36 ± 1.11	(5)	1.17 ± 0.21	(5)
6	C	3.20 ± 0.56	(6)	4.78 ± 0.86	(6)	1.40 ± 0.25	(6)
	D	3.34 ± 0.51	(6)	5.20 ± 0.61	(6)	1.16 ± 0.26	(6)

Weeks after denervation		Tetanic muscle					
		Lipid phosphorus		Fatty acid ester		Fatty aldehyde	
		μM/g muscle (wet weight)					
2	C	2.25 ± 0.23	(5)	3.65 ± 0.92	(5)	0.60 ± 0.28	(5)
	D	2.45 ± 0.56	(5)	4.00 ± 0.82	(5)	0.72 ± 0.29	(5)
3	C	1.90 ± 0.47	(7)	3.00 ± 1.00	(7)	0.70 ± 0.34	(7)
	D	2.80 ± 0.40	(6)	4.50 ± 1.22	(6)	0.88 ± 0.44	(6)
4	C	1.80 ± 0.24	(7)	2.55 ± 0.74	(6)	0.80 ± 0.30	(7)
	D	3.30 ± 0.26	(7)	4.90 ± 0.80	(6)	1.25 ± 0.30	(7)
5	C	1.75 ± 0.28	(5)	2.55 ± 0.80	(5)	1.00 ± 0.31	(5)
	D	2.90 ± 0.44	(5)	4.35 ± 0.70	(5)	1.40 ± 0.40	(5)
6	C	1.90 ± 0.37	(6)	2.63 ± 0.80	(6)	1.20 ± 0.20	(6)
	D	2.58 ± 0.47	(6)	3.72 ± 1.37	(6)	1.50 ± 0.35	(6)

C: contralateral innervated muscle; D: denervated muscle
* mean ± standard deviation. Number of experiments in brackets

began to decrease. At the same time a slight and slow increase started in the innervated muscle. In the sixth week the lipid-P content was nearly the same in the denervated and the innervated muscle.

In the choline phosphatide fraction of the tetanic muscle the lipid-P content increased on the denervated side from the 2nd to the 4th week. Sub-

sequently a remarkable decrease was observed. In the innervated muscle the amount of lipid-P decreased during the first weeks and increased after the fourth to fifth weeks.

The fatty acid ester content of the choline phosphatides in both the denervated and the innervated muscles changed similarly to the lipid-P content.

The fatty aldehyde content of the choline phosphatides increased markedly in the innervated tonic muscle up to the fourth week, then a great decrease set in. At the same time no change could be observed in the denervated muscle. In contrast, in the tetanic muscle on both the innervated and the denervated sides the fatty aldehyde content showed a well expressed increase until the fourth week, to decrease in the subsequent period.

Cephalins (Table II)

In the lipid-P content of the cephalins of the denervated tonic muscle a slight increase occurred, while in the innervated tonic muscle a marked decrease was observed in the third week. The phospholipid content of the cephalin fraction of the denervated and innervated muscles was the same during the fifth and sixth weeks.

In the denervated tetanic muscle the lipid-P content of the cephalin fraction increased slowly up to the fourth week, then it decreased. In the innervated muscle until the fourth week following denervation a decrease and then a slow increase was noted. The difference in lipid-P content between innervated and denervated tetanic muscle persisted even during the sixth week.

Table III

Amount of non-collagenous protein in tonic and tetanic muscle following denervation

Weeks after denervation		mg protein/g muscle (wet weight)			
		Tonic muscle		Tetanic muscle	
2	C	155.0± 9.8*	(3)	194.3±12.0	(3)
	D	132.2±10.4	(3)	176.6±14.4	(3)
3	C	151.5±11.2	(4)	165.5±11.4	(4)
	D	114.5±12.4	(4)	131.5±16.2	(4)
4	C	146.0±10.0	(6)	167.0± 6.6	(6)
	D	98.1±18.2	(6)	124.1± 9.3	(6)
5	C	149.8± 9.8	(8)	154.7±12.1	(8)
	D	101.1±17.6	(8)	106.5±19.1	(7)
6	C	140.0±16.5	(9)	149.2±11.0	(9)
	D	102.1±14.1	(10)	82.5±17.4	(10)

C: contralateral innervated muscle
D: denervated muscle
* mean ± standard deviation. Number of experiments in brackets.

The fatty acid ester content of the cephalins showed changes similar to those of the lipid-P content.

The fatty aldehyde content of cephalins increased in both the innervated and the denervated tonic and tetanic muscles for 5—6 weeks after denervation. No decrease was recorded even after six weeks.

Non-collagenous protein content (see Table III) showed no change in the innervated tonic muscle, while in the innervated tetanic muscle a slight decrease was observed after the third week.

The non-collagenous protein content of the denervated tonic muscle decreased from the second week on; between the fourth and sixth weeks no further decrease was observed. In the denervated tetanic muscle the non-collagenous protein content decreased after the second-third week, and this decrease persisted even during the sixth week.

Discussion

The changes in the lipid content of the muscles after neurogenic damage have so far been illustrated by few and contradictory data. AUDOVA (1923) observed fat accumulation in the muscles after neurotomy in rabbits. ARTOM (1941) studying rats and cats 2—14 days after nerve dissection reported on an increase of the lipid-P and total lipid content of the denervated muscles; their protein content was not affected. FRIEDLANDER et al. (1941) observed an increase in the amount of phospholipids, while DREYFUS and SHAPIRA (1948) an increase in the lipid content of skeletal muscle after denervation. HUMOLLER et al. (1952) studied m. gastrocnemius after neurotomy in rats. In the first week after section of the nerve the phospholipids decreased by about 10%, on the 15th day the total lipid and phospholipid contents increased by 20—30%. SUTFIN et al. (1954) found in different rat muscles an increase of total lipid content starting from the 2nd—3rd week after denervation. Recently GRAFF et al. (1965) studied changes in the phospholipid content of the denervated rat gastrocnemius.

Evaluation of the above data is complicated by the fact that they had been obtained on different animals and at various times after denervation. Besides, the lipid content of the denervated muscle has always been compared with muscles of the opposite side and not with the lipid content of the muscles of normal control animals, leaving out of consideration the different biological functions and the different lipid composition of the skeletal muscles. In spite of the discrepancy, it may be stated that from the second—third week after denervation the lipid-P content of the muscles increases temporarily. The present findings have shown that in the values for the increase in the phospholipid content in the denervated muscles are included those for its decrease in the

innervated (control) muscles on the opposite side. The changes occurring in these various muscles are seen in Fig. 1. Under the effect of unilateral denervation, opposite changes could be observed in the denervated and the innervated muscles. In innervated muscles the amount of both choline phosphatides and cephalins decreased for a few weeks, then an increase took place. At the same time, in the denervated muscles the lipid-P content increased for a few weeks, then it decreased. Thus, the differences between the innervated and denervated sides could not be ascribed solely to the changes occurring in the denervated muscles.

A comparison of the changes in the two muscles shows that under the effect of neurogenic damage the amount of phospholipids in tonic muscles with static functions changes to a smaller extent than in tetanic muscles with locomotor functions. This was true for both choline phosphatides and cephalins. In tonic muscles 15—25%, in tetanic muscles 30—50% differences were observed between the innervated and denervated sides; they were the most expressed in tonic muscles in the third—fourth week, in tetanic muscles in the fourth—fifth week after denervation. In addition, in both muscles the changes were more expressed in the cephalins than in the choline phosphatides.

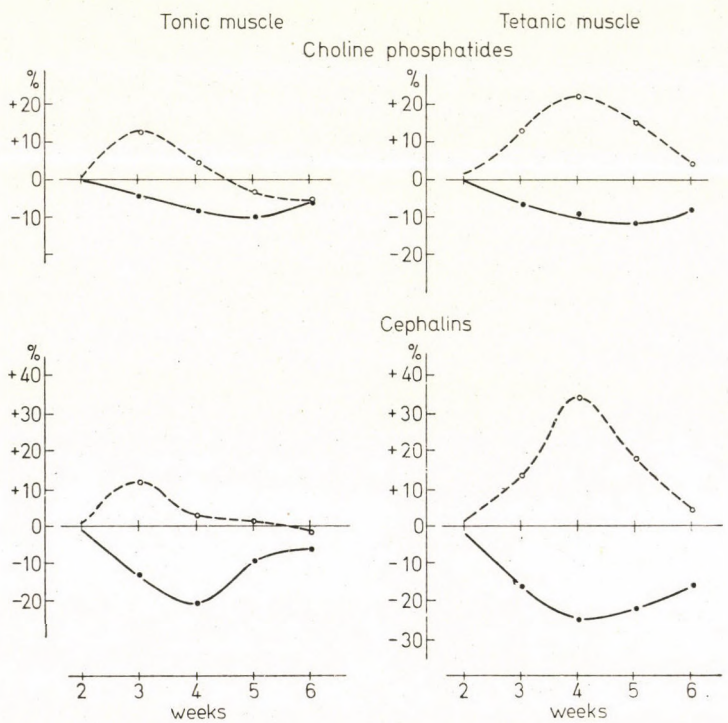

Fig. 1. Changes in lipid-phosphorus in tonic and tetanic muscles following denervation, per cent. ——— contralateral innervated muscles; – – – – – – denervated muscles. The values have been computed from the data of Tables I and II

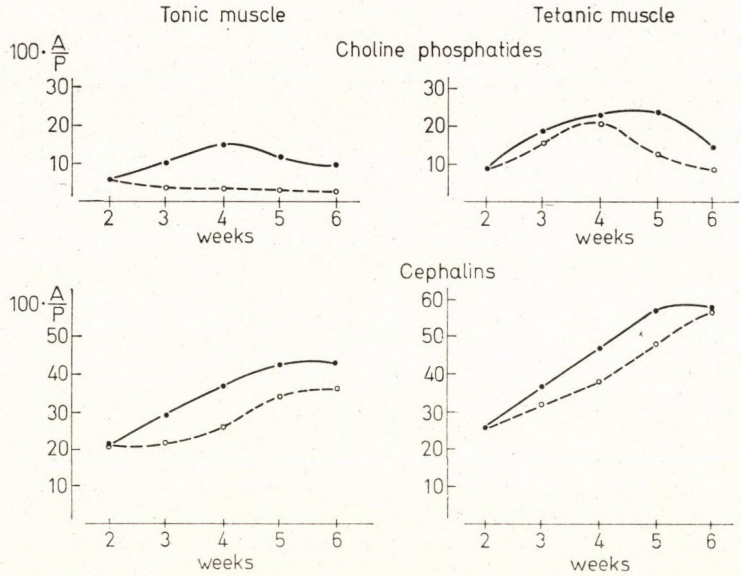

Fig. 2. Fatty aldehyde content of phosphatides in tonic and tetanic muscles following denervation, per cent. ——— contralateral innervated muscles; – – – – – – denervated muscles. The values have been computed from the data of Tables I and II

$$100 \cdot \frac{A}{P} : 100 \cdot \frac{\mu \text{ moles fatty aldehyde/g muscle (mean values)}}{\mu \text{ atoms lipid phosphorus/g muscle (mean values)}}$$

The changes in the muscles occurring after denervation are not limited to a decrease or increase in the amount of phospholipids. The A : P ratio as shown in Fig. 2 also indicates a qualitative change in the composition of the phospholipids. The change of the fatty aldehyde content does not go parallel with those of lipid-P. Disregarding the slight decrease in the choline phosphatides of the tonic muscles, the amount of fatty aldehydes in the phospholipids of both the denervated and the innervated muscles increases in the first weeks. This increase is more expressed and lasting in the cephalins than in the choline phosphatides. Also, the increase is always greater in the tetanic than in the tonic muscles. This is especially true for the cephalins of tetanic muscle, where in the sixth week after denervation on both the denervated and the innervated sides 28—30% of the phospholipids are present in the form of plasmalogen, in contrast to the initial 12—13%. The increase in the plasmalogen content of phospholipids suggests that under the effect of unilateral denervation the biosynthesis of vinyl ether bonds (plasmalogen) increases in both denervated and innervated muscles. The extent of the biosynthesis of plasmalogen is always less in the denervated muscles.

On the basis of the changes in the non-collagenous protein content it can be stated that different, sometimes even inverse changes in lipid-P and plasmalogen content are not connected with similar changes in the cell-phase content

The obtained results can be explained by changes occurring in the function of both the denervated and the innervated muscles. In denervated muscles the temporary increase of the lipid-P and fatty aldehyde content of phospholipids may be due to the pathological lipid metabolism increased by the fibrillation activity. However, the increase in plasmalogen concentration and the decrease in the amount of phospholipids in the innervated muscles may suggest that on the effect of unilateral denervation the function and consequently the lipid metabolism in the innervated muscles must have also suffered a transient change.

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ANALYSIS OF THE DEPOLARIZING EFFECT OF NICOTINE ON FROG'S SKELETAL MUSCLE

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The properties and mechanism of the slow depolarization induced by nicotine in the striated frog muscle may be outlined as follows.

1. The depolarizing effect of nicotine depends on the concentration (maximum between 5×10^{-2} and 2×10^{-1} mM), on the site of measurement (maximum in the end-plate area) and on seasonal conditions (the effect is more marked from October to March than in spring or summer).

2. The depolarizing effect is the most marked near the neuromuscular junction, may be inhibited by curarization and in the aneural part of the muscle even a thousandfold of the otherwise effective nicotine concentration causes no depolarization. Thus, the neuromuscular junction is apparently the site of the depolarizing effect.

3. Slow depolarization spreads exclusively in Ringer containing nicotine.

4. The cause of slow depolarization appears to be an increase in the Na permeability of the membrane and an about 4.5 fold increase of ^{24}Na influx, on the average.

5. Tetrodotoxin (TTX) at a concentration of 10^{-7} g/ml inhibits the spike propagation caused by nicotine in muscle fibre, but leaves the increase of ^{24}Na influx and slow depolarization unaffected.

6. At a concentration of 5×10^{-6} g/ml TTX diminishes both the depolarization and the increase of ^{24}Na influx induced by nicotine.

7. Nicotine increases ^{24}Na influx to the same extent at 0° C as at 20 to 23° C.

It is well-known that nicotine depolarizes neural structures. Yet, remarkably little is known about the potential changes and their mechanism occurring in muscle in response to nicotine. KUFFLER showed on the isolated frog nerve-muscle preparation that nicotine depolarizes the end-plate area and generates impulses capable of propagation, then slow potential changes develop. At first, only a slowly developing depolarization reaching 8 to 10 mV in 15 minutes was observed (KUFFLER 1943), then maintained large negative potentials of 20 to 25 mV, developing in 0.5 sec were also recorded (KUFFLER 1946). FLECKENSTEIN et al. observed marked depolarization of the frog rectus muscle in response to nicotine (FLECKENSTEIN et al. 1950). In his monograph published a few years later FLECKENSTEIN (1955) interpreted this phenomenon as one representing a direct depolarizing effect on muscle fibres, and independent of the effect of nicotine on the end-plate. In this respect he parallels nicotine with KCl. Although often quoted, this statement has not been corroborated decisively either by FLECKENSTEIN, or by others. THESLEFF fully confirmed KUFFLER's observations as regards spike generation, but described a *slowly ceasing* depolarization instead of a *slowly developing* one (THESLEFF

1955). Thus, despite the uniformity of observations concerning spike generation, few experiments have been carried out to study the slow depolarization and there is no agreement concerning its mechanism which is still unknown.

Methods

Freshly captured *Rana esculenta* frogs were used. The sartorius and the semitendinous muscles were prepared without curarization under microscope (magnification × 10 to 40) and the fibres were kept in Ringer's solution for 30 minutes; preparations showing intact fibres after that time were used in the experiments. Intactness of the preparations was checked at the end of the experiment, partly on grounds of the return of resting potential, partly by controlling the response to electrical stimulation. The individual differences between preparations from different frogs have made it necessary to use pairs of muscle where autocontrol was not feasible. The temperature of the incubating fluid ranged from 20 to 23° C, except when specified otherwise.

For measuring potentials Ling—Gerard type microelectrodes (LING and GERARD 1949) were used. Their tip potentials were below −5 mV, the resistances varied from 5 to 15 Mohm (ADRIAN 1956). Resting potential was measured by a Keithley 603 type electrometer. Action potentials were recorded by a DISA Universal Indicator with a DISA NCE amplifier. In experiments carried out on whole muscle the membrane potential values were determined from 5 to 10 impalements the ranges represent the mean deviation.

It was noted during the informative experiments that the different parts of the same fibre differed in the degree of depolarization. Presuming that in this relation the distance between the neuromuscular junction and the site of measurement might play a substantial role, in the experiments resting potential measurements were made consequently in the second one-fifth part from the pelvic end of the muscle.

The preparations were stretched to 4/3 of their slack length and were fixed in plexiglass chamber. In some experiments sartorius muscles were placed in separating cells, allowing to separate the pelvic end of the muscle which contains practically no end-plates (see PEZARD and MAY 1937) from the distal neural part (GESZTELYI and KOVÁCS, in preparation). Separation was effected by means of a thin rubber diaphragm, which does not block the spread of the action potential from one side to the other, but for instance K depolarization occurs only in that part of the muscle to which high K Ringer is applied. The fibre preparations made of semitendinous muscle were placed in a Perspex cell according to HODGKIN and HOROWICZ (1959), taking care to keep the preparation in Ringer's solution throughout. In the channel of the cell the rate of fluid flow was 1 ml/sec.

Na and K transport was studied in pairs of sartorius muscle dissected especially carefully, using ^{24}Na and ^{42}K. Activity was measured in a Gamma NK-108 type, energy-selective counter.

^{24}Na influx was determined by the method of KEYNES and SWAN (1959), except that incubation lasted 15 minutes in Ringer's solution of 0.125 mCi/ml activity.

In the tests for ^{24}Na outflow, the muscle was kept for 1 hour in Ringer's solution of 0.25 mCi/ml activity. To accelerate ^{24}Na uptake during the second 30 minutes of the incubation period, 1 Hz, 0.1 msec, suprathreshold amplitude stimulation was applied (on stronger stimulation the muscle was torn from the glass rod). Then the muscle was passaged in a series of test tubes containing inactive Ringer's solution moving it along slowly, to improve diffusion. The rate coefficients were plotted against time (KEYNES and SWAN 1959).

In the tests for ^{42}K output the muscles were kept for 8 hours in Ringer's solution of 0.025 mCi/ml activity at room temperature. Subsequently the muscle was washed for 20 seconds in inactive Ringer's solution then carried along a series of test tubes containing inactive Ringer's solution at 3, 5 and 10-minute intervals, as in the Na efflux tests.

To follow up ^{42}K uptake, the muscle was placed in Ringer's solution of 0.0025 mCi/ml activity, then taken out at 10-minute intervals and tested for activity.

Composition of Ringer's solution was KCl, 2.5 mM; NaCl, 115 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 2.15 mM; NaH_2PO_4, 0.85 mM; pH 7.0. Nicotine hydrogen tartarate (BDH) was dissolved in Ringer's solution. When more concentrated solutions were used, e.g. 5 to 10 mM nicotine, pH was adjusted to 7.0 with NaOH. The d-tubocurarine used was a preparation of Sigma Chemical Co., the tetrodotoxin was a three times recrystallized commercial Sankyo product. The pH 4 to 5 stock tetrodotoxin solution was kept about 0 °C and diluted when needed; every 15 days a fresh stock solution was prepared. $^{24}NaCl$ and ^{42}KCl were obtained from the Isotope Institute of the Hungarian Academy of Sciences.

Results

Seasonal variations, concentration and site dependence of nicotine depolarization

LANGLEY in 1908 observed seasonal variations in the mechanical action of nicotine; this suggested that similar variations may occur from season to season in the depolarizing effect of nicotine. In our first experiments we therefore compared the depolarization caused by 0.1 mM nicotine in frog sartorius muscle in different seasons. The resting potential of 5 to 10 fibres was measured after equilibration in normal Ringer for 30 to 60 minutes, then the normal Ringer was replaced by Ringer containing 0.1 mM nicotine. Fibrillation started immediately, lasting about 2 to 3 minutes. When it had ceased, the membrane potential was measured again, possibly at the same place (see Methods). As indicated in Fig. 1, from October to March 0.1 mM nicotine caused 28.7 $\pm$ 6.9 to 34.6 $\pm$ 2.1 mV depolarization. In April—May and September the depolarization values were 11.9 $\pm$ 2.02 to 22.1 $\pm$ 3 mV, respectively. In the three summer months temperature could not be kept around the desired 20 to 23 °C, therefore no such experiments were made.

In the next step the concentration dependence of nicotine depolarization was studied from early October to late March. As shown in Fig. 2, 1×10^{-3} to

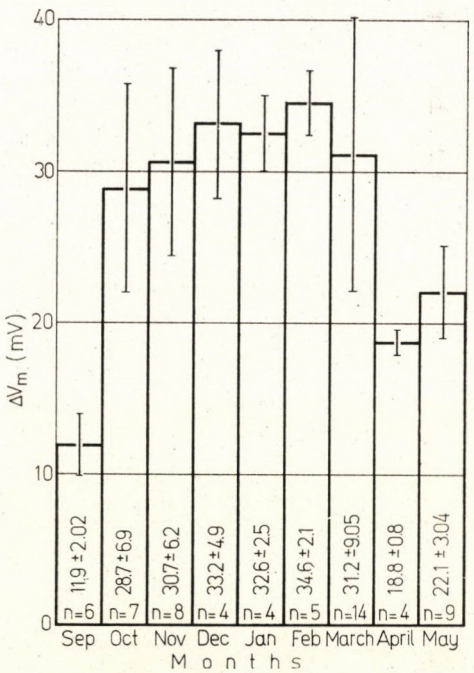

Fig. 1. Depolarization caused by 0.1 mM of nicotine in the period September to May. The height of the columns represents the mean of 10 resting potential measurements made in *n* number of sartorius muscles, the vertical lines indicate the mean deviation

5×10^{-3} mM concentrations of nicotine caused hardly any depolarization. On increasing the concentration, in the range of 5×10^{-2} to 2×10^{-1} maximum depolarization was attained. When the concentration had been increased to 10 mM, depolarization declined slightly. Thus, in subsequent experiments

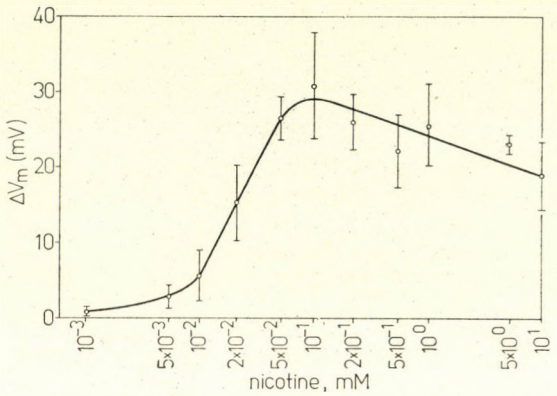

Fig. 2. Concentration dependence of nicotine depolarization in sartorius muscle. Semilogarithmic representation. The point of measurements represent means obtained in at least 5 sartorius muscle experiments. Vertical lines, mean deviation

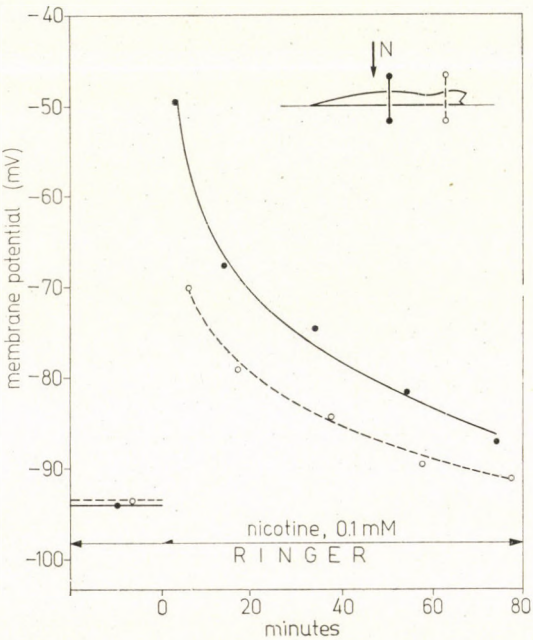

Fig. 3. Sartorius muscle. Changes in resting potential following nicotine treatment. ⁞ near the entry of nerve into muscle, ⁞ 3 to 4 mm from the pelvic end of muscle. N = site of nerve entry

0.1 mM nicotine was used. Since the time and site dependence of the nicotine effect was clearly observable in the preliminary tests, this was studied in the further experiments.

A typical experiment is presented in Fig. 3. The resting potential was measured at two sites of the sartorius muscle kept in normal Ringer's solution. One site was near the place where the nerve enters the muscle, the other was 3 to 4 mm from its pelvic end, i.e. in the aneural part.

As seen in Fig. 3, the two normal values agreed within the limits of error, while depolarization was more marked near the nerve than in the aneural part. The membrane potential measured near the end-plates returned less fast to the initial level, but there was nevertheless an obvious repolarization despite the presence of nicotine.

Nicotine depolarization vs. time

Fig. 4 shows the changes in potential in response to 0.1 mM nicotine of an isolated semitendinous fibre. Similarly conducted 8 experiments unequivocally indicated that two depolarization processes can be distinguished in time. At first, action potentials appear, followed by slow and lasting depolarization. The number of spikes varied over a wide range; it was remarkably high, from 10 to about 100 in the whole sartorius muscle, while less than 10, often only 1 or 2 in the isolated semitendinous fibre. The potential changes of a few mV following the first six spikes in Fig. 4 may be positive after-potentials, but they may also be artefacts, as every spike was followed by contraction. The further 3 spikes in Fig. 4 may also be artefacts because contraction had developed in the meantime. The maximum depolarization developing simul-

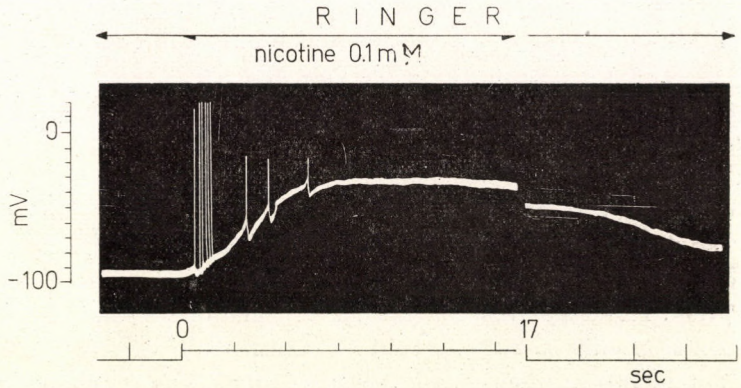

Fig. 4. Changes in potential in response to nicotine in a fibre isolated from semitendinous muscle. In the fibre placed into the Hodgkin—Horowicz measuring cell, the microelectrode was already impaled about 3 to 4 mm from the tendinous end when at time 0 the perfusion of Ringer's solution containing 0.1 mM nicotine was started. The second part of the curve shows the changes in repolarization after return to normal Ringer perfusion at 17 seconds

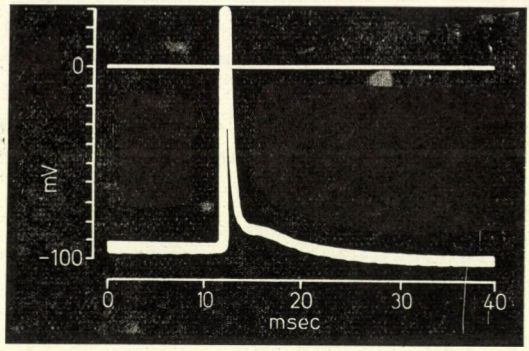

Fig. 5. One member of a spike series recorded on the aneural part of a sartorius muscle in normal Ringer's solution in the separating cell. The spike burst was generated by applying nicotine on the neural side

taneously with the disappearance of the spikes is reached in about 1 to 3 sec and depends on the concentration of nicotine, on seasonal differences, on the distance between the site of measurement and the neuromuscular junction (see Fig. 3).

Fig. 5 shows a typical nicotine spike recorded from the aneural part of a sartorius muscle in normal Ringer's solution in the Gesztelyi—Kovács separating chamber, after applying 0.1 mM nicotine to the neural side. In the neural part, nicotine generated a series of spikes. One of these is shown in Fig. 5. The resting potential was —93 mV, the overshoot 30 mV, with marked negative and positive after-potentials.

Site of action of nicotine

0.1 mM of nicotine always generated spikes and induced slow depolarization in the whole muscle or in the neural part of the sartorius muscle placed in the separating cell; on the other hand, even 10 mM of nicotine had no effect on the aneural part. The results confirmed the view that the site of nicotine action is the neuromuscular junction, in agreement with the classical experiments, concerning the mechanical effect of nicotine. Since, however, unequivocal data as regards depolarization were not available in the next step we studied whether nicotine depolarization could be blocked by curare.

As the results in Table I indicate, there was a correlation between the nicotine concentration used and the concentration of curare certainly blocking depolarization. The depolarization caused by 1 mM of nicotine was not inhibited by 0.01 mM of curare, but 0.05 mM blocked it.

To obtain information as to the spreading of slow depolarization, the neural part of the sartorius muscle in the separating cell was placed into Ringer's solution containing 0.1 mM of nicotine, while the aneural part remained in normal Ringer's solution. Depolarization of the usual size was measured

from the neural part, whereas this did not spread to the aneural part, in which the resting potential showed no change. When, however, 0.1 mM of nicotine was applied to the aneural part first, no depolarization resulted from that in agreement with our previous experiments and when at the neural part the normal Ringer's solution was substituted by one containing 0.1 mM of nicotine, depolarization of the neural part spread within seconds to the aneural part.

Evaluation of these results was difficult since spike bursts and slow depolarization may equally be propagated from the neural part and the above experiments did not reveal whether the lower resting potential values were due to the slow depolarization spreads to the aneural part, or nicotine delays the repolarization of the spikes spreading over the aneural part.

To clarify the problem, experiments were made with tetrodotoxin (TTX), which is known to block the propagation of action potentials (NARAHASHI et al. 1960). According to our observations TTX at a concentration of 10^{-7} g/ml blocked the propagation also of nicotine spikes in the frog muscle. Therefore, the previous experiments were so reproduced that prior to nicotine treatment the

Table I

Effect of nicotine on the membrane potential of curarized muscle

Membrane potential, mV					Date and protocol No
Ringer	Curare, 0.01 mM				
	Nicotine, mM				
	0.02	0.1	1	5	
−93.6	−93.8				May 5, 1969, — 28
−93.8	−93.6				May 5, 1969, — 28
−93.0	−93.2				May 5, 1969, — 28
−93.2	−93.2				May 5, 1969, — 28
−93.4	−93.1				Mean
−93.6		−93.0			April 24, 1969, — 28
−93.2		−90.4			April 24, 1969, — 28
−93.4		−90.4			April 24, 1969, — 28
−92.0		−93.0			April 29, 1969, — 28
−93.0		−89.4			April 29, 1969, — 28
−93.2		−91.0			April 29, 1969, — 28
−93.1		−91.2			Mean
−91.9			−70.2		March 6, 1969, — 12
−93.6			−85.6		April 24, 1969, — 28
−93.6			−82.8		April 24, 1969, — 28
−93.2			−84.4		April 24, 1969, — 28
−93.1			−80.7		Mean

Table I. (cont.)

Membrane potential, mV					Date and protocol No
Ringer	Curare, 0.05 mM				
	Nicotine, mM				
	0.02	0.1	1	5	
−92.8		−92.6			November 22, 1968, — 22
−92.8		−93.0			October 17, 1968, — 25
−96.0		−95.0			October 18, 1968, — 25
−95.6		−94.8			October 18, 1968, — 25
94.3		−93.8			Mean
−91.4			−90.0		March 6, 1968, — 12
−92.6			−92.2		April 17, 1968, — 22
−92.8			−91.4		April 17, 1968, — 22
−91.8			−91.0		April 17, 1968, — 22
−92.2			−91.8		April 17, 1968, — 22
−92.2			−91.3		Mean
−93.4				−89.6	March 14, 1968, — 12
−93.6				−93.0	April 25, 1969, — 28
−93.4				−89.2	April 25, 1969, — 28
−93.6				−89.2	April 25, 1969, — 28
−93.5				−90.2	Mean

After resting potential measurement of muscles equilibrated in Ringer's solution (first column) the muscles were transferred into Ringer's solution containing 0.01 mM and 0.05 mM curare. After 30 minutes incubation the muscles were transferred into Ringer's solution containing the same concentration of curare, as well as nicotine. The resting potential was measured after fibrillation had ceased. When fibrillation was prevented by curare, the resting potential was measured in the 3rd minute of nicotine action

neural part of the muscle was incubated in Ringer's solution containing 10^{-7} g/ml TTX. The results differed from the previous ones only in that nicotine produced neither spikes nor fibrillation. Slow depolarization developed as before, i.e. when there was nicotine at the aneural part, nicotine applied to the neural part depolarized the aneural part as well. Thus, in the muscle fibre the propagation of slow depolarization is bound to the presence of nicotine.

Mechanism of nicotine depolarization

The results presented in Table II indicate that if the frog sartorius was exposed to the influence of 0.1 mM of nicotine during the 15 minutes of incubation in ^{24}Na Ringer's solution, the mean ^{24}Na influx increased from 2.96 $pM/cm^2 \cdot sec$ to 13.71 $pM/cm^2 \cdot sec$. Further experiments involving other pairs of muscle revealed that the increase of nicotine concentration to as high as 5 mM failed further to enhance ^{24}Na influx.

The results of the previously described experiments showed that the effect of nicotine on skeletal muscle is reversible and repolarization begins even in the presence of nicotine. As there is reason to believe that an increase of ^{24}Na influx has a role in the development of depolarization, we have investigated the changes in ^{24}Na influx during nicotine treatment of different duration.

The results in Table III reveal that the sartorius muscles kept in Ringer's solution containing 0.1 mM of nicotine for 135 to 255 minutes showed invariably lower values for ^{24}Na influx than the other member of the muscle pair which had been left in Ringer's solution containing nicotine for 15 minutes only. Despite the small number of cases, we may venture to suggest on grounds of Table III that ^{24}Na influx in muscles incubated in nicotine for 255 minutes is not only lower than that of muscles incubated for 135 minutes, but the former is nearer to the normal values as well. (In the 45 experiments made so far, mean ^{24}Na

Table II

Increase of ^{24}Na influx in response to nicotine in frog sartorius muscle

^{24}Na influx, $pM/cm^2 \cdot sec$					Date and protocol No.
Ringer	Nicotine, mM				
	0.1	0.5	1	5	
2.05	14.70				February 20, 1969, — 25
2.30	11.00				February 20, 1969, — 25
3.00	14.70				February 20, 1969, — 25
4.92	14.27				February 20, 1969, — 25
3.34	14.79				October 9, 1969, — 28
3.29	13.56				October 9, 1969, — 28
1.82	13.00				June 12, 1969, — 28
2.96	13.71				Mean
3.70		14.70			March 20, 1968, — 12
7.40		10.00			March 20, 1968, — 12
5.55		12.35			Mean
5.08			15.50		January 10, 1968, — 12
5.10			10.20		January 10, 1968, — 12
5.09			12.85		Mean
5.20				13.70	January 10, 1968, — 12
3.08				13.40	January 10, 1968, — 12
4.14				13.55	Mean

One member of every muscle pair was incubated for 15 minutes in Ringer's solution of 0.125 mCi/ml activity, the other member was incubated in Ringer's solution of the same activity and containing nicotine

Table III

Effect of nicotine treatment of various duration on ^{24}Na influx in sartorius muscle

^{24}Na influx, pM/cm^2 · sec					
Ringer	Nicotine, 0.1 mM. Duration of incubation, minutes			Date and protocol No.	
	0+15	120+15	240+15		
2.05	14.70			February	20, 1969, — 25.
2.30	11.00			February	20, 1969, — 25
3.00	14.70			February	20, 1969, — 25
4.92	14.27			February	20, 1969, — 25
3.06	13.66			Mean	
3.81		6.85		March	20, 1968, — 12
4.96		9.25		March	20, 1968, — 12
4.38		8.05		Mean	
	12.32	7.20		April	11, 1968, — 22
	17.26	9.50		April	11, 1968, — 22
	17.04	8.11		April	11, 1968, — 22
	8.30	6.22		April	11, 1968, — 22
	14.23	7.75		Mean	
	14.44		4.47	May	8, 1968, — 22
	11.01		5.64	May	8, 1968, — 22
	12.72		5.05	Mean	

Prior to transfer for 15 minutes into Ringer's solution containing ^{24}Na and nicotine, the muscle had been incubated for 0, 120 or 240 minutes in inactive Ringer's solution containing 0.1 mM nicotine

influx in untreated sartorius muscles was 4.06 ± 1.45 pM/cm^2 · sec, closely tallying with the 4.2 pM/cm^2 · sec value obtained for the same muscle by KEYNES and SWAN.)

As pointed out beforehand, curare at adequate concentrations inhibited nicotine depolarization. There was reason to assume that under similar experimental conditions curare would block also the ^{24}Na influx enhancing effect of nicotine. As the results for 8 pairs of muscle presented in Table IV reveal, 0.05 mM curare completely blocked the ^{24}Na influx increasing action of 0.1 mM nicotine.

The data obtained seemed to justify the claim that nicotine depolarization may be due to an increase in Na permeability and Na influx. This hypothesis could be tested by means of TTX which does not affect the membrane potential, and the Na transport in the resting muscle or the development of

Table IV

Effect of nicotine on ^{24}Na influx in curarized sartorius muscle

^{24}Na influx, pM/cm^2 · sec		Date and protocol No.
Curare, 0.05 mM		
Ringer	Nicotine, 0.1 mM	
5.25	3.55	April 10, 1968, — 22
6.00	3.80	April 10, 1968, — 22
3.78	5.80	April 10, 1968, — 22
4.40	4.88	April 10, 1968, — 22
5.04	4.22	December 5, 1968, — 22
4.42	2.30	December 5, 1968, — 22
5.07	4.70	December 5, 1968, — 22
3.86	5.44	December 5, 1968, — 22
4.74	4.33	Mean

Prior to transfer into Ringer's solution containing ^{24}Na, nicotine and 0.05 mM curare for 15 minutes, the muscle had been incubated for 30 minutes in inactive Ringer's solution containing 0.05 mM curare. The other member of the muscle pair was transferred for 15 minutes into ^{24}Na Ringer's solution after 30 minutes of incubation in inactive Ringer's solution containing 0.05 mM curare

end-plate potential. On the other hand, even very low concentration block the propagation of the action potential and correspondingly the increase in Na influx during excitation (FURUKAWA et al. 1959; NARAHASHI et al. 1960; KAO 1966). The results in Table V show that at a concentration of 10^{-7} g/ml TTX exerted no influence either on the resting Na influx or on the increased Na influx in response to 0.1 mM nicotine. On the other hand, at a concentration of 5×10^{-6} g/ml, TTX lessened the increase of Na influx caused by nicotine. At the same time, in the presence of TTX at such a concentration, Na influx was increased by nicotine less markedly than in the controls.

If the increase in ^{24}Na influx in response to nicotine is closely correlated with depolarization, then TTX at 5×10^{-6} g/ml concentration must decrease depolarization as well. In fact, the results presented in Table VI show that the depolarization caused by nicotine was not influenced by 10^{-7} g/ml, but was reduced by 5×10^{-6} g/ml of TTX.

These findings showed clearly the important role of the increase of Na influx in the mechanism of nicotine depolarization. It was therefore deemed necessary to study Na efflux, too.

A typical experiment of this kind is presented in Fig. 6. As seen, in response to 0.1 mM of nicotine ^{24}Na efflux increased roughly twofold. The effect was, however, transitory and the rate coefficient was restored to the pre-nicotine level already during incubation with nicotine.

Table V

Increase of ^{24}Na influx in response to nicotine tetrodotoxin-treated sartorius muscle

^{24}Na influx, pM/cm^2 · sec						Date and protocol No.
Ringer			Nicotine, 0.1 mM			
Tetrodotoxin, g/ml						
0	10^{-7}	$5 \cdot 10^{-6}$	0	10^{-7}	$5 \cdot 10^{-6}$	
3.67	3.76					October 30, 1968, — 22
3.53	2.50					October 30, 1968, — 22
3.60	3.13					Mean
2.47		4.70				November 13, 1968, — 22
5.80		4.79				November 13, 1968, — 22
2.81		2.66				October 8, 1969, — 28
2.92		2.79				October 8, 1969, — 28
3.50		3.73				Mean
3.34			14.79			October 9, 1969, — 28
3.29			13.56			October 9, 1969, — 28
2.05			14.70			February 20, 1969, — 25
2.30			11.00			February 20, 1969, — 25
3.00			14.70			February 20, 1969, — 25
4.92			14.27			February 20, 1969, — 25
3.15			13.83			Mean
4.34					7.74	November 13, 1968, — 22
3.13					4.86	November 13, 1968, — 22
5.73					16.94	October 8, 1969, — 28
6.70					23.48	October 8, 1969, — 28
4.97					13.25	Mean
			8.46	9.91		October 30, 1968, — 22
			9.20	7.85		October 30, 1968, — 22
			11.51	11.32		October 23, 1969, — 32
			11.89	10.37		October 23, 1969, — 32
			10.75	10.27		October 23, 1969, — 32
			12.44	11.45		October 23, 1969, — 32
			10.70	10.19		Mean
			4.58		2.53	October 31, 1968, — 22
			5.78		3.37	October 31, 1968, — 22
			12.20		9.32	October 9, 1969, — 28
			14.20		10.67	October 9, 1969, — 28
			9.19		6.47	Mean

After preincubation for 30 minutes in Ringer's solution containing 10^{-7} to 5×10^{-6} g/ml TTX, the muscles were transferred into active Ringer's solution containing the same TTX concentration for 15 minutes. In the case of nicotine-treated muscles the active Ringer's solution contained 0.1 mM nicotine

Table VI

Effect of tetrodotoxin pretreatment on nicotine-induced depolarization

Membrane potential, mV							
Ringer	Nicotine, 0.1 mM Tetrodotoxin, g/ml			△ V (mV)			Date and protocol No.
	0	10^{-7}	$5 \cdot 10^{-6}$				
I	II	III	IV	I–II.	I–III.	I–IV.	
−92.8	−66.4	−66.2		26.4	26.6		November 3, 1969, — 28
−92.6	−70.0	−72.2		22.6	20.4		November 3, 1969, — 28
−92.2	−71.8	−67.0		20.4	25.2		November 11, 1969, — 28
−93.0	−53.2	−57.4		39.8	35.6		November 13, 1969, — 28
−93.6	−76.4	−76.4		17.2	17.2		November 13, 1969, — 28
				25.2	25.0		Mean
−92.6	−57.2		−76.2	35.4		16.4	October 30, 1969, — 28
−93.2	−66.2		−74.2	27.0		19.0	October 30, 1969, — 28
				31.2		17.7	Mean
−91.8		−61.6	−67.8		30.2	24.0	December 8, 1969, — 32

After measuring the resting potential in normal Ringer's solution (I) it was determined to what extent the given muscle was depolarized by 0.1 mM nicotine (II). Nicotine treatment lasted a few minutes only, because the measurement was made immediately after fibrillation had ceased, then the muscle was returned into normal Ringer's solution. After complete repolarization in normal Ringer's solution the muscle was incubated in the presence of TTX for 30 minutes, then it was tested again for nicotine depolarization (III and IV, respectively)

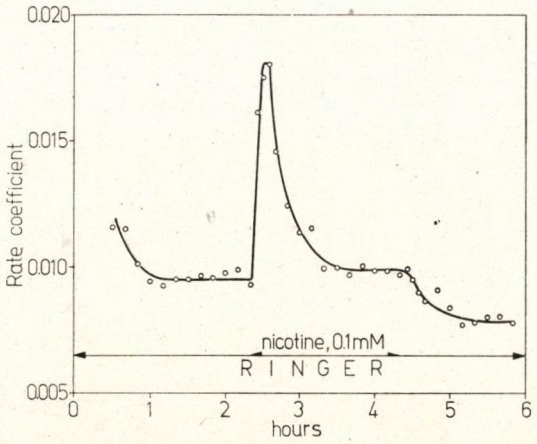

Fig. 6. Effect of 0.1 mM nicotine on ^{24}Na efflux in sartorius muscle

Table VII

Increase of ^{24}Na influx in response to nicotine at different temperatures

^{24}Na influx, pM/cm^2 · sec				Date and protocol No.
0°C		20–23 °C		
Ringer	Nicotine, 0.1 mM	Ringer	Nicotine, 0.1 mM	
4.17	14.10			January 22, 1969, — 25
1.34	11.70			January 22, 1969, — 25
3.05	12.91			January 22, 1969, — 25
1.31	12.80			January 22, 1969, — 25
2.46	12.87			Mean
0.51		1.34		April 16, 1969, — 28
0.73		2.99		April 16, 1969, — 28
2.16		6.14		December 18, 1968, — 25
0.61		2.65		December 18, 1968, — 25
1.00		3.28		Mean
	11.87		13.43	December 18, 1968, — 25
	11.00		15.00	January 23, 1969, — 25
	11.66		13.70	January 23, 1969, — 25
	14.20		17.18	January 23, 1969, — 25
	10.70		14.00	January 23, 1969, — 25
	11.74		14.46	Mean

Every muscle was preincubated for 1 hour at the specified °C before transfer to active Ringer's solution of the same temperature

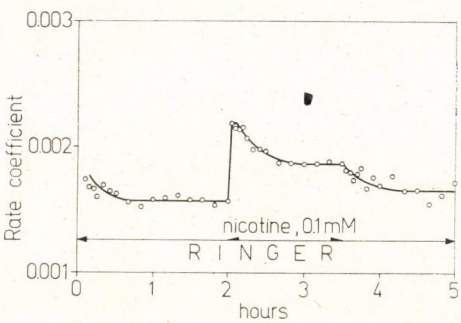

Fig. 7. Effect of 0.1 mM nicotine on ^{42}K efflux in sartorius muscle

The data in Table VII supply certain information as to the mechanism responsible for the Na influx-enhancing effect of nicotine. Na influx measured at 0 °C was about one third of that measured at 20 to 23 °C, while nicotine increased Na influx at 0 °C to the same extent as at 20 to 23 °C. The lower Na influx was apparently due to the circumstance that at 0 °C we measured merely the passive part of the resting Na influx. The fact that at 0 °C nicotine influences Na influx in the same way as at 20 to 23 °C suggests it is not the exchange diffusion on which nicotine acts; its effect consists in increasing the Na permeability of the membrane, significantly enhancing thereby passive influx.

The results presented in Fig. 7 indicate that 0.1 mM nicotine temporarily increased ^{42}K efflux in the muscle. This increase reached only 40% in 4 experiments. Under similar experimental conditions, however, nicotine produced no change in ^{42}K uptake.

Discussion

Attention must first of all be drawn on the slow depolarization caused by nicotine, the observations concerning the generation of spikes by nicotine being unequivocal.

In the aneural part of the sartorius muscle, even 10 mM of nicotine produced no depolarization, while in the neural part or in the whole muscle even 0.01 mM appreciably diminished the resting potential. Moreover, according to KUFFLER (1943) and our own data, curare at suitable concentrations inhibits the depolarization brought about by nicotine. It is suggested therefore that the site of action of nicotine is the neuromuscular junction also in the case of slow depolarization.

The fact that in response to nicotine ^{24}Na influx increased about 4.5-fold indicates the important role of Na permeability in nicotine depolarization. The correlation seemed to be even closer in the TTX experiments. TTX at 10^{-7} g/ml concentration left the slow depolarization unaffected and did not influence the increase of ^{24}Na influx caused by nicotine, either. In contrast, at a concentration of 5×10^{-6} g/ml, TTX diminished the rate of ^{24}Na influx as compared with the TTX untreated nicotine control; depolarization was also diminished.

Those outlined above suggest that the increase in ^{24}Na influx measured by us goes parallel with the slow depolarization. This does not mean that the nicotine spikes are not associated with an increase in Na influx. The findings indicate, however, that the relatively long lasting depolarization causes a much greater increase in Na influx than that caused by a spike burst lasting 2 to 3 minutes. As compared to the former, the latter apparently involves an uptake of a negligible amount of ^{24}Na and this explains the absence of demon-

strable differences in the nicotine enhanced Na influx between the series in which spikes were inhibited by TTX and those in which TTX was not used.

The view that slow depolarization and the increase of ^{24}Na influx caused by nicotine are correlated is apparently justified by the fact that curare at a concentration which inhibits depolarization prevents also the increase in ^{24}Na influx.

As it has been pointed out, ^{24}Na influx gradually decreased when the muscle was incubated with nicotine for 135 to 255 minutes. This explains why the muscle repolarizes despite the presence of nicotine.

There is a conspicuous difference between the increase in ^{24}Na influx observed by us and the results published by AHMAD and LEWIS (1962). While we recorded an average 4.5-fold increase, in the experiments of AHMAD and LEWIS the increase in the ^{24}Na content of the nicotine-treated muscle was less than 50%. The difference may be explained by the fact that we measured ^{24}Na influx by the method of KEYNES and SWAN (1959) and the muscle had been kept in ^{24}Na Ringer's solution for 15 minutes. AHMAD and LEWIS allowed the muscle to stay in the active solution for 90 minutes. As we have demonstrated, during that period influx is reduced substantially. It should be taken into consideration that part of the ^{24}Na taken up is also lost. As seen in Fig. 6, in response to nicotine there was a marked initial increase in Na efflux and this also may at any rate reduce the activity in the whole muscle.

Our results for K uptake also differ from those published by AHMAD and LEWIS (1962); the difference was probably due to the fact that we studied exclusively the influence on K uptake of 0.1 mM nicotine, while the concentration used by AHMAD and LEWIS was 1 mg/ml, about twentyfold of that used by us.

Our observations concerning the increase of K efflux caused by nicotine differ from the data published by HARDT and FLECKENSTEIN (1949). It seems likely that the relatively short-lived 40% increase in K efflux means an output of such minute amounts of K as may be measured by isotope methods, but not by microchemical procedures.

The mechanism by which nicotine enhances K efflux is unknown. The results of studies of Na transport suggest, however, that the increase in K efflux is not due directly to nicotine, but the depolarization resulting from the increase in Na permeability and Na influx is the factor directly responsible for the temporary increase in K efflux.

Finally, it seems worth-while to parallel the nicotine-generated spike with the slow depolarization. The results published by KUFFLER (1943, 1946) as well as DEL CASTILLO and KATZ (1957) make it most probable that the end-plate potential develops first in response to nicotine. When conditions are suitable and the end-plate potential is marked enough, action potentials will propagate along the muscle fibres. As to why the generation of further action

potentials ceases in a short time despite the presence of nicotine, THESLEFF (1955) supplied the most plausible explanation by demonstrating that during depolarization a neuromuscular block is brought about. This may inhibit the generation of further spikes. The great variability in the number of spikes found by us may be explained by that the neuromuscular junction is paralysed faster in isolated fibre because of more favourable conditions for diffusion. Moreover, an injury of the junction may have occurred and it should also be borne in mind that the isolated fibre preparations were made of semitendinous muscle. Finally, the number of spikes was usually higher in fresh preparations.

At any rate, if in response to nicotine an end-plate potential developed in the part of the fibre immersed in Ringer's solution containing nicotine, an electronic potential, too, will develop. Our experiments suggest that the cause of this is presumably an increase in the Na permeability of the membrane and an increase in Na influx. It is, however, remarkable that while the nicotine spike is propagated also in the part of fibre immersed in normal Ringer's solution, the electrotonic potential is propagated exclusively in the solution containing nicotine. It seems likely that two factors are required to produce these slow electrotonic potentials. One is that the nicotine must induce an end-plate potential at the junction, and the other, that the muscle membrane should be in Ringer's solution containing nicotine. In this way it seems certain that the mechanism of arisal of spikes and electrotonic potentials is identical only in that the induction of end-plate potential is a precondition to both but they differ in other aspects, for instance in propagation. This is indicated by the fact that 10^{-7} g/ml of TTX, which certainly blocks the nicotine spike propagation, leaves electrotonic depolarization absolutely unchanged.

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NEW METHOD FOR THE ELECTROPHYSIOLOGICAL ANALYSIS OF THE SEPARATED NEURAL AND ANEURAL PARTS OF FROG SARTORIUS MUSCLE

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A special chamber was designed for the mechanical separation of the pelvic part of the frog sartorius muscle, which practically does not contain end-plates, from the tibial part. Separation is attained by a suitable rubber membrane which by means of slight compression inhibits diffusion between the solutions bathing the two separated parts, without affecting the membrane potential of both parts of the muscle or interfering with the action potential propagation from one side to the other. On raising the concentration of extracellular potassium on one side, local depolarization occurred and could be maintained for considerable lengths of time without a depolarization on the other side.

The aneural part of the sartorius muscle separated by this method has been shown to contain no end-plates, since neither acetylcholine, nor nicotine, succinylcholine or carbachol were affecting the resting potential, and fibrillation indicating end-plate depolarization was not observed either.

It has been known since the observation of LANGLEY (1907) that the myoneural junctions of striated muscles are considerably more susceptible to certain chemical agents such as nicotine and acetylcholine than the extrajunctional areas. Several investigators have reported that in the frog sartorius muscle compounds with a selective action on the end-plates (nicotine, acetylcholine, carbachol, succinylcholine) give rise to depolarization not only in the end-plate but also in the extrajunctional membrane (FATT 1950; KATZ and MILEDI 1961; NASTUK and GISSEN 1965; VARGA et al. 1970). When studying this depolarization propagating to the extrajunctional membrane it is essential to have a preparation in which the electrophysiological properties of the muscle membrane not containing end-plates can be studied separately. Since the pelvic part of the frog sartorius muscle (about one-eighth to one-fifth of the full length) is practically free of end-plates (PEZARD and MAY 1937), it seemed especially suitable to study direct effects on the muscle membrane.

Methods

Recently captured frogs, *Rana esculenta*, were used. The sartorius muscles were dissected under a preparation microscope, kept in Ringer solution for 30 min and the intact preparations were transferred into the separating chamber.

The separating chamber (Fig. 1) consists of two parts made of plexiglass which are held together by screws. The windows of these two parts are separated by an about 0.10 to 0.15 mm thick rubber membrane made from surgical gloves. On the membrane an ellipsoid

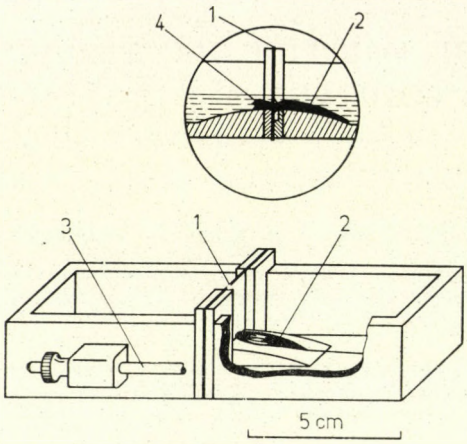

Fig. 1. Separating chamber (on the right, part of the front wall removed). Top right, cross-section of the central part of the chamber, with rubber membrane and muscle. 1. rubber membrane; 2. neural part of sartorius muscle; 3. fixing screw; 4. aneural part of sartorius muscle

opening was made, somewhat smaller in diameter than the muscle cross-section; thus, the rubber membrane by slight compression should inhibit the diffusion between the bathing solutions on the two sides. The sartorius muscle is pulled through the opening by means of a thread fixed to the tibial end, without damaging the preparation; thus the pelvic one-eighth is in one part of the chamber, and the larger tibial portion in the other part. The muscle is then stretched until its length is increased by one-third of the slack length. The two ends of the muscle are placed on pedestals at the level of the lower edge of the opening on the membrane. With the insertion of additional rubber membranes it is possible to separate the sartorius into more parts.

Electric potentials are recorded by Ling—Gerard microelectrodes. The tip-potential of the electrodes is lower than —5 mV, their resistance varying between 5 and 15 Mohm. The membrane potential was calculated from 5 to 10 measurements. The diagrams indicate the mean deviation.

The Ringer solution had the following composition: KCl, 2.5 mM; NaCl, 115 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 2.15 mM; NaH_2PO_4, 0.85. The 80 mM KCl solution contained, in addition to KCl, 37.5 mM NaCl, 1.8 mM $CaCl_2$, 2.15 mM Na_2HPO_4, and 0.85 mM NaH_2PO_4. The pH was adjusted to 7.0 in each experiment.

Results

When performing these experiments it was essential to establish whether the procedure had any deteriorating effect on the sartorius muscle, i.e. how long could the resting potential be maintained in the separating chamber. Fig. 2 shows that the membrane potential of the neural part of the muscle kept in Ringer solution, when measured at a point 15 mm from the rubber membrane, was stable for periods as long as 20 hours. On the aneural side, a Ringer containing 80 mM KCl was used which decreased the resting potential of —93 mV to —26 mV, which again remained remarkably stable, within a

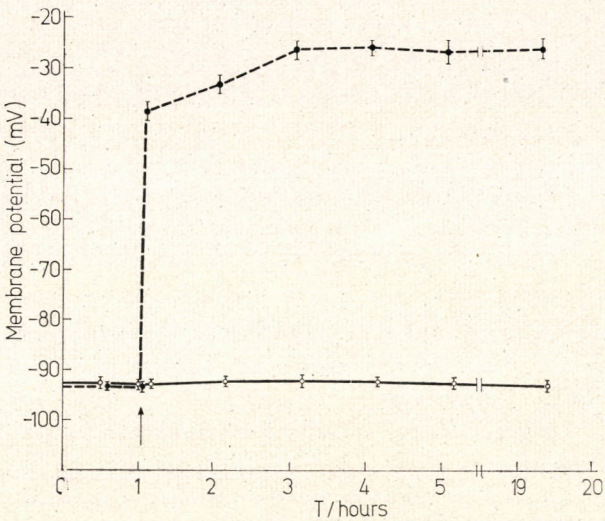

Fig. 2. Membrane potential values recorded on the neural and aneural parts of sartorius muscle. The neural part indicated by open circles and solid line was bathed in normal Ringer solution, while the aneural segment (solid circles, broken line) was, from the time indicated by arrow, incubated in Ringer solution containing 80 mM KCl

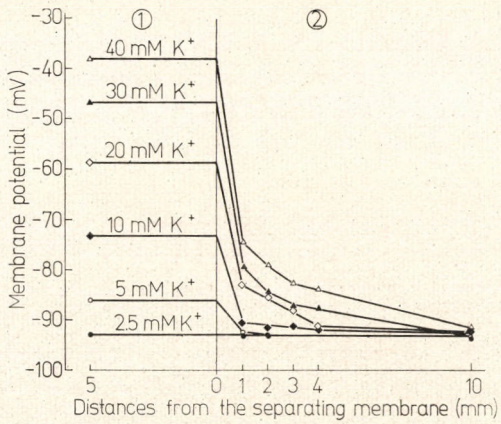

Fig. 3. Membrane potential values recorded from the two parts of the sartorius muscle separated in the middle by a rubber membrane, at various distances from the separating membrane. One part of the muscle (2) was incubated in Ringer solution, while the potassium content of the Ringer bathing the other side (1) was successively increased

certain range of scattering, over 20 hours. Thus, it could be shown that the rubber membrane did not damage the muscle fibres, the membrane potential of both the neural and aneural parts of the sartorius muscle remains for considerable lengths of time at the value determined by the ionic gradients.

Preliminary experiments with methylene blue have already indicated that there is practically no diffusion between the separated compartments

even after 10 to 12 hours. The experiment demonstrated in Fig. 2 shows that the compression exerted by the rubber membrane was sufficient to inhibit diffusion between the two parts of the chamber which could have affected the resting potential of either the neural or the aneural part of the sartorius muscle. The obtained data indicate that in the separating chamber there is no diffusion either between the rubber membrane and the surface of the muscle, or through the extracellular compartment of the muscle.

If one part of the sartorius muscle is depolarized by means of varying concentrations of KCl, the other part immersed in Ringer solution shows an electrotonic depolarization. Fig. 3 shows the magnitude of this electrotonic depolarization at various distances from the rubber membrane. Depolarization was greatest near the rubber membrane and gradually subsided at more distant points of the muscle. At a distance of 10 mm from the rubber membrane there was no depolarization even when the resting potential of the aneural side of the muscle was adjusted to −38 mV by the use of a Ringer solution containing 40 mM KCl. Thus, if there is a considerable difference between the resting potentials of the two parts of the sartorius muscle separated by the rubber membrane, the resting potential measured near the rubber membrane might be influenced electrotonically by the membrane potential of the other part. This circumstance has to be considered when using the separating chamber at membrane potential measurements.

In further parts of the experiments attempts were made to establish whether the compression exerted by the rubber membrane, while not affecting the resting potential, interfered with the integrity of the structures involved in the propagation of the action potential. It has been found that action potentials elicited by direct electrical stimulation of the muscle can be recorded without a loss in amplitude on the other side of the rubber membrane, moreover, the slope of the action potentials or of the afterpotentials remained unchanged. Fig. 4 shows the action potential recorded on the neural side and that from the same fibre on the aneural side, following stimulation on the neural side.

The separating chamber is suitable also for experiments using a sartorius nerve-muscle preparation. This means that the results of indirect stimulation can also be studied.

As it has been emphasized above, the pelvic one-eighth segment of the sartorius muscle may be regarded as free of end-plates. Thus, if the muscle is placed into the separating chamber in a manner leaving the pelvic end on the one side of the membrane, then the modifying effect of end-plate depolarization has not to be reckoned with. Experiments were also undertaken to establish whether the aneural part was in fact devoid of end-plates; such an experiment is shown in Fig. 5. Treatment of the aneural part of the muscle with 1 mM of nicotine, acetylcholine, carbachol or succinylcholine had no appreciable effect on the resting potential, and no fibrillation occurred

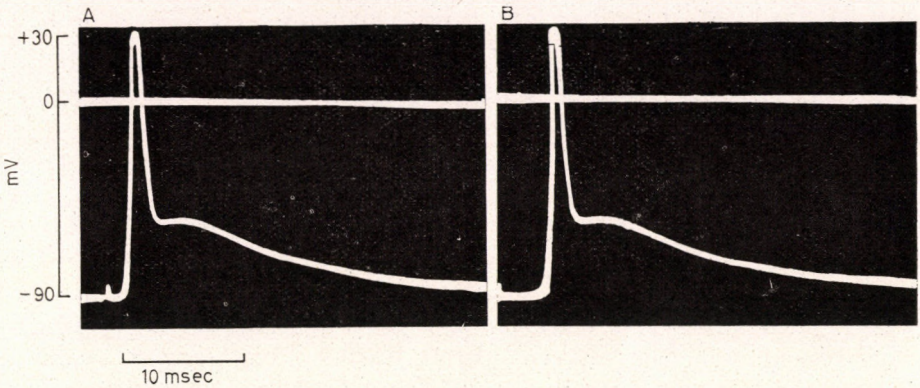

Fig. 4. Action potentials recorded from neural (A) and aneural (B) parts of separated sartorius muscle stimulated electrically on the neural side. Action potentials were recorded from the same fibre

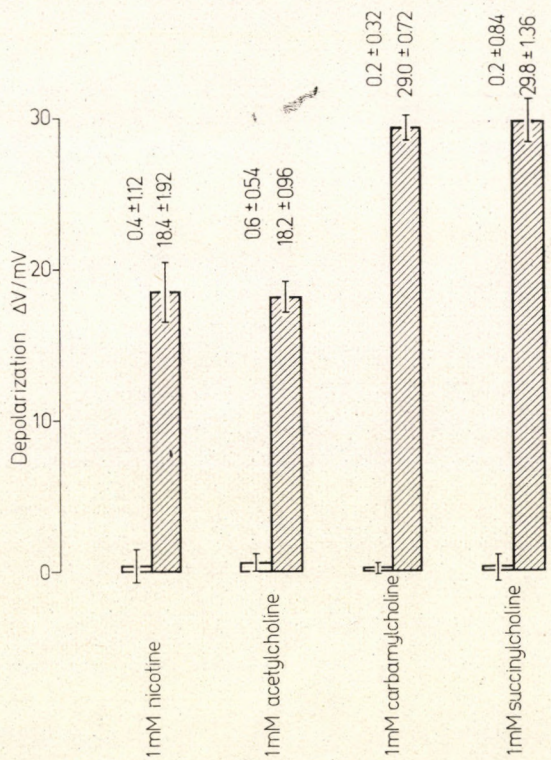

Fig. 5. Depolarizing effect of compounds on aneural (open blocks) and neural (hatched blocks) parts of sartorius muscle, within 5 min after application of the drug

on the affected part of the muscle. If these compounds were applied on the neural part of the sartorius muscle, there occurred a marked fibrillation and also depolarization, which is in accordance with the observations of others.

Discussion

Numerous attempts have been made to analyse the characteristics of the muscle membrane devoid of end-plates. MILEDI (1960) estimated the acetylcholine sensitivity of the end-plates and the extrajunctional membrane by means of iontophoresis. KATZ and MILEDI (1961) fixed the sartorius muscle vertically and immersed successively larger portions of the pelvic end into Ringer solution containing succinylcholine. By external electrodes it was possible to detect a potential difference between the pelvic and the tibial ends of the muscle, but only when the Ringer containing succinylcholine had already been bathing that part of the muscle which had end-plates, too.

The above methods are in many respects suitable for the analysis of the characteristics of the muscle membrane not containing end-plates. However, the simultaneous detection of changes in the resting potential of the two parts and the application of solutions of different composition require mechanical separation of the muscle into two parts, i.e. a neural and an aneural one. KAMIYAMA and MATSUDA (1966) designed a chamber divided into two parts in which the electrical properties of the papillary muscle of the heart could be studied. Unfortunately, the use of the stiff celluloid plate prevented its use in such experiments as outlined above.

The present results indicate that the resting potential of the muscle placed into the separating chamber constructed by us remains stable over considerable length of time, and the propagation of the action potential under the rubber membrane and on the extrajunctional areas is unaffected.

By the use of selective agents affecting only the neuromuscular junction (nicotine, acetylcholine, carbachol, succinylcholine) it was possible unequivocally to demonstrate that the pelvic segment of the muscle was in fact devoid of end-plates. The present method makes the use of end-plate blockers (dTC, cocaine) unnecessary when the effects of end-plate stimulation and changes in the potential of the muscle membrane are studied separately. These end-plate blocking agents might affect also the muscle membrane, although only at high concentrations (COOKE and GRINNEL 1964).

Finally, it should be emphasized that the separating chamber allows a thorough analysis of the action potentials elicited by direct and indirect stimulation. Action potentials evoked from the neural side or through the nerve propagate without appreciable change to the aneural side, where the deflections can be studied conveniently and interfered with while not affecting neuromuscular transmission or the primary action potential.

Acknowledgements

We should like to express our grateful thanks to Professor E. VARGA for his permanent encouragements and helpful support during our work.

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INSULIN UPTAKE BY ISOLATED RAT FAT CELLS

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Fat cells containing 35 μmoles triglyceride were isolated with collagenase according to RODBELL and incubated in the presence of 100 or 200 μU/ml insulin. Even in the case of a low fat cell concentration, a decrease of the amount of insulin in the medium was demonstrable by means of insulin immunoassay. The decrease was ascribed to an insulin uptake by the fat cells present in the medium.

In the disappearance of insulin an initial fast and a subsequent slower and continuous phase could be distinguished; the decrease was directly proportionate to the original insulin concentration.

In the experiments with 200 μU/ml insulin the disappearance of insulin and the glucose metabolism of fat cells suggested that insulin was bound to the cell membrane also non-specifically.

The qualitative and quantitative changes in the metabolism of insulin-dependent tissues in response to insulin are well known, but there is scarce evidence concerning the quantity of insulin bound to the cells of target organs, the nature of this combination and the subsequent fate of bound insulin.

Studying these problems, STADIE et al. (1949a, b, 1951) demonstrated in the rat diaphragm a rapid combination with insulin within 1 minute incubation, on the basis of an increase in glycogen production. The stimulation of glycogen production was not inhibited by repeated washings of the diaphragm following the short contact with insulin.

Many attempts have been made to study the uptake of labelled insulin by muscle and adipose tissues. However, the insulin was quickly deiodinated and degraded in the biological environment and the opinion began to prevail that labelled insulin was unsuitable for studying the phenomenon (CROFFORD 1968; BOUMAN et al. 1968).

The determination of immunoreactive insulin (IRI) introduced by YALOW and BERSON (1960) made it apparently possible to draw conclusions from the quantitative changes of insulin added to the incubated tissue medium as regards the extent and dynamics of insulin binding by tissues. ANTONIADES and GERSHOFF (1966) were the first to estimate the insulin disappearance, as based on the determination of the IRI content of the medium in experiments involving muscle and adipose tissue. They incubated rat epididymal adipose tissue with 400 μU/ml insulin for 90 minutes; by the end of incubation 200

to 250 μU/ml of insulin disappeared from the medium. BOUMAN et al. (1968) assayed the quantitative changes of 100 μU/ml insulin added to rat diaphragm and found that after 15 minutes of incubation only 40 to 50 per cent of the original amount of insulin was demonstrable. On prolonged incubation the insulin disappearance rate rapidly decreased, at 120 minutes there was still 10 per cent of the original amount of insulin in the medium.

A further advance was achieved in this respect by CROFFORD (1968), who studied isolated fat cells. He found that their insulin uptake occurred in two steps, a rapid combination taking place in the first minute, increasing linearly with the increase in the insulin concentration of the medium and amounting to about 10 per cent. In the subsequent phase of incubation the fat cells combined with insulin less rapidly but continuously. It is advantageous to use isolated cells instead of adipose tissue, because the hormones and drugs added to the incubated cell suspension may influence them without interference by disturbing factors.

Scarce evidence is available concerning the mode and rate of insulin inactivation in the cells of target organs. DIGIROLAMO et al. (1965) showed that the rat's adipose tissue contains an enzyme which splits insulin, thus comparable in function to the enzyme systems breaking down the pituitary peptide hormones.

Methods

Collagenase: Collagenase, *Cl. histolyticum* (Nutritional Biochemicals Corporation, Control No. 4447).

Insulin: Six times recrystallized pig insulin, 1 mg = 23.73 I.U. (Richter, Budapest).

Fat cells were isolated by the method of RODBELL (1964) from rat epididymal fat pad. Animals of the same breed, weighing 100 to 140 g were fed ad libitum.

After isolation, the cells were kept in suspension by means of a magnetic stirrer. To a plastic incubation vessel glucose-1-^{14}C ($7 \cdot 10^5$ DPM) of 0.2 μCi specific activity was added and its concentration was adjusted to 60 mg/100 ml (3.3 μmoles/ml). Then, using a semi-automatic micropipette, 100 μU/ml and 200 μU/ml of insulin, as well as 0.5 ml of cell suspension were added to the medium. To estimate the concentration of the cell suspension, 0.2 ml volumes were tested for glyceride-glycerol by the enzymatic method of EGGSTEIN and KREUTZ (1965). The results were related to the triglyceride content of cell suspension. The substances and the cell suspension were added to Krebs—Ringer-bicarbonate buffer containing 4 per cent bovine albumin and incubated in 2.0 ml volume. The plastic vessel was closed with a rubber stopper in which there were two glass tubes which could be closed by connecting them with a rubber tube. To collect CO_2, a 1.0 ml glass vessel was suspended on the inner end of the rubber stopper on a steel wire. Then the cell suspension was perfused for 10 minutes with a mixture of 95 per cent O_2 and 5 per cent CO_2.

Incubation in the Warburg apparatus was done at 37 °C with a shaking rate of 60/min for 5 to 120 minutes.

The experimental series were set up so that the suspension of fat cells was added to so many vessels that three of them were tested in parallel exclusively for IRI, and three others exclusively for CO_2. In the IRI assays the cell suspension was centrifuged immediately on completion of incubation for 1 minute at 3000 r.p.m. and the medium was sucked off from under the upper cellular layer by means of a pipette. To estimate CO_2, 0.5 ml of 7 *N* sulphuric acid was injected through a small-gauge needle into the closed incubation vessel and 0.3 ml of benzethonium into the suspended glass vessel. Then shaking in the Warburg apparatus was continued for another 30 minutes. On the next day the glass vessel containing

benzethonium was placed in 5.0 ml dioxane scintillator fluid, CO_2 activity was estimated in Packard Tri-Carb apparatus and the results were corrected taking into account the quench effect. The IRI determinations were made by the double antibody method of HALES and RANDLE (1963), except that, instead of microfiltration, centrifugation was applied for separating the insulin-antibody precipitate.

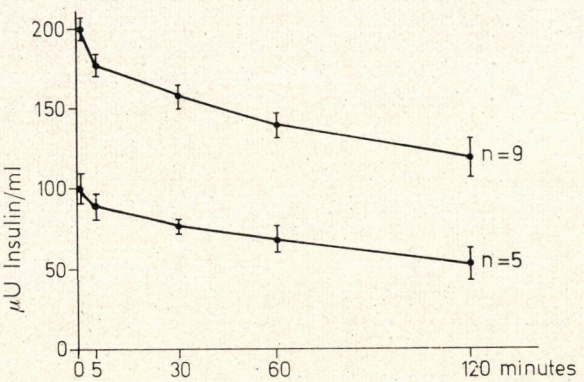
Results

IRI concentration in the medium

At 0, 5, 30, 60 and 120 minutes of incubation the cell-free medium was tested for IRI (Fig. 1). In the experiments with both 100 and 200 μU insulin two phases could be distinguished according to the shape of the insulin disappearance curves. During the first 5 minutes insulin disappeared faster, while later the disappearance rate was slower, but continuous. Comparison of the results obtained with 100 and 200 μU insulin showed that the curves ran an almost parallel course, thus, the decrease of insulin level in the medium of the incubated fat cells was proportionate to the initial insulin concentration.

CO_2 produced from glucose-1-^{14}C by fat cells

Glucose-1-^{14}C was used for two reasons. First, we wanted to prove that the metabolic activity of fat cells continues even after their isolation with collagenase, and secondly, to investigate whether there was a relationship



Minutes	0	5	30	60	120
200 μU/ml $\bar{x} \pm$ SD n = 9	200.0 ± 7.5	177.6 ± 7.0	158.8 ± 7.4	140.6 ± 8.3	120.6 ± 12.4
100 μU/ml $\bar{x} \pm$ SD n = 5	100.0 ± 9.4	89.0 ± 8.2	76.4 ± 4.3	68.6 ± 8.0	53.4 ± 9.6

Fig. 1. Decrease of insulin concentration in medium containing isolated rat fat cells (35 μmoles triglyceride) and 3.3 μmoles glucose during 2-hour incubation at 37 °C as determined from the IRI value at 0, 5, 30, 60 and 120 minutes. (Mean ± standard deviation). Four experiments in triplicate, one in duplicate

between the quantity of insulin disappearing from the medium and bound to fat cells and the effect by which glucose uptake is stimulated. In the presence of 100 μU/ml insulin CO_2 production by fat cells increased by 77 per cent as compared to the 113 per cent increase measured in the presence of 200 μU/ml insulin (Fig 2).

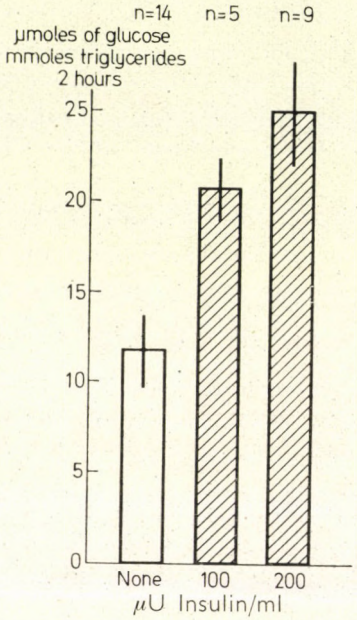


Fig. 2. Glucose uptake by isolated rat fat cells (35 μmoles triglyceride) calculated for mmole triglyceride from the amount of CO_2 produced from glucose-1-^{14}C (3.3 μmoles glucose/ml, specific activity 0.2 μCi/2 ml). Two-hour incubation at 37 °C. The columns represent the means, vertical lines denote standard deviation. Four experiments in triplicate, one in duplicate

The results showed that in the case of a double insulin concentration the decrease of insulin level in the medium was twice as great by the end of the 2-hour incubation period, while the increase of CO_2 produced by fat cells in the presence of 200 μU/ml insulin amounted to merely 36 per cent of the value measured in the presence of 100 μU/ml insulin.

Discussion

The linkage of insulin to effector tissue cells has been termed by STADIE et al. (1949a) a chemical combination, by ANTONIADES and GERSHOFF (1966) insulin uptake, by GARRAT et al. (1966) insulin association, by WOHLTMANN and NARAHARA (1966) insulin binding; CROFFORD (1968) called it insulin uptake and/or binding. The different terminology is due to the fact that the site at which insulin is bound to the cell membrane is still unidentified and

there is no unequivocal evidence concerning the kind of binding taking place. It is conceivable but has yet to be proved that not only the mode of action, but also the combination of insulin with the cell membrane depends on the dynamic condition of the adenyl cyclase enzyme system located in the cell membrane. GARRAT et al. (1966), as well as WOHLTMANN and NARAHARA (1966) offer, however, unquestionable proof that the cells are capable of binding insulin not only at the specific membrane receptor sites, but also outside them, in a non-specific fashion.

As regards the dynamics of combination with insulin, STADIE et al. (1949a, b), then CROFFORD (1968) demonstrated significant insulin binding during the first minute of incubation; this was attributed to an interaction of the hormone and the cell membrane. Our 5-minute values, too, reflect this phenomenon. In the present investigations indirect proof has been obtained showing that there had been both specific and non-specific insulin binding, as indicated by the glucose metabolism data. The insulin dose–response curves plotted from isolated fat cells ran a sigmoid course and the different authors claimed that the maximum stimulation of glucose metabolism would take place in the presence of 20 to 100 μU/ml insulin (RODBELL 1964; GLIEMANN, 1965; CROFFORD 1968). The present results also showed this phenomenon, inasmuch as the insulin disappearance rate in the presence of 200 μU/ml insulin was almost the double of that measured in the presence of 100 μU/ml insulin, while glucose metabolism rose only slightly. These data seem to prove that the insulin saturation of isolated fat cells takes place already at an insulin concentration of 100 μU/ml. At higher concentration non-specific binding also takes place. In man, a pathophysiological significance may be attributed to the upsetting of the balance between specific and non-specific combination ratios.

The present study served for developing a method by which isolated human fat cells, too, may be studied. By this method we hope to clarify whether there are alterations in the insulin-binding capacity of fat cells in obesity and in the various types of diabetes.

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EFFECT OF STRESS, DRUGS AND HYPOTHALAMIC EXTRACT ON ANTERIOR PITUITARY SOMATOTROPIN CONTENT IN THE RAT

By

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According to available tibia test data under the effect of vasopressin, epinephrin, insulin and some stressors or environmental factors the STH content of the rat adenohypophysis decreases or shows no change. A decrease was reported after intracarotid administration of crude hypothalamic extract or of purified hypothalamic factors.

In view of contradictions in the literature, the problem has been reinvestigated, estimating the pituitary STH content by means of polyacrylamide gel electrophoresis, a new specific and sensitive biochemical method.

Insulin, cold, blood loss, laparotomy, swimming, suckling stimulus, vasopressin and oxytocin failed to decrease the pituitary STH content in both male and female rats. Hypothalamic extract also failed to affect the STH content of the adenohypophysis.

According to earlier data obtained with the tibia test, acute and non-specific stressful stimuli significantly decrease the somatotropin (STH) content of the rat hypophysis (see for review: MARTINI and GANONG 1966; MCCANN et al. 1968; BRODISH 1968; SZENTÁGOTHAI et al. 1968).

Several authors (KRULICH and MCCANN 1966b; PECILE and MÜLLER 1966; MÜLLER et al. 1967a, 1968a; KATZ et al. 1967) have reported on a decrease also in the hypothalamic somatotropin releasing factor (STH-RF) after an acute stimulus. According to their opinion the effect of the factors investigated was brought about via the central nervous system. There are data showing a significant STH-RF activity in rat plasma after insulin-induced hypoglycaemia or on exposure to cold (KRULICH and MCCANN 1966b; MÜLLER et al. 1967a). On the other hand, radioimmune-assay determinations failed to show any change in pituitary and blood STH concentration after administration of different purified hypothalamic extracts (SCHALCH and REICHLIN 1968; RODGER et al. 1969; GARCIA and GESCHWIND 1968), making thus uncertain the value of the data for STH-RF.

In view of these contradictions it is difficult to take a stand on the extent and mechanism of the acute effect of different factors which influence the pituitary STH content and it was considered necessary to revise some earlier experiments and gather additional data. Accordingly, the effect of the following factors was studied.

1. Insulin
2. some stressors: a. cold
   b. blood loss
   c. laparotomy
   d. swimming
3. suckling stimulus
4. vasopressin and oxytocin
5. rat hypothalamic extract.

Methods

In the investigations, adult male and female and lactating R-Amsterdam-Hungary rats were used. At the end of the experiments each animal was decapitated under ether anaesthesia, the hypophysis removed immediately and the neurohypophysis separated from the adenohypophysis. The adenohypophysis was weighed with 0.2 mg accuracy on a torsion balance and homogenized in 0.02 *M* phosphate buffer pH 7.0. The samples were kept at 0 °C till electrophoretic separation.

Non-specific acute effect

1. Insulin. Male and female rats were injected subcutaneously with 2.0 or 4.0 I.U./kg crystalline insulin (Richter, Budapest) and decapitated 60 minutes later. In some cases the hypoglycaemic reaction was assessed by estimating the blood sugar after decapitation (HULTMAN 1959).

2. Stressors

a. Cold. The rats were placed in an environment of 0 °C and decapitated 60 minutes later.

b. Blood loss. From the male animals, 3—5 ml of blood was taken by cutting the tail; 180 minutes later the animals were decapitated. From the females, 2—3 ml of blood was taken in the same manner and they were decapitated 60 minutes later.

c. Laparotomy. The abdominal cavity of male and female animals was opened and closed under ether anaesthesia. Sixty minutes after surgery the animals were decapitated.

d. Swimming. Animals of both sexes were made to swim until exhaustion (30—45 minutes) and decapitated immediately thereafter.

3. Suckling stimulus. The litter (6 animals each) was removed from the mother for 12 hours on the 7th day of lactation. One group of the mother animals was decapitated without leaving them to lactate. In the other group, the litter was replaced and the mothers were killed 30—40 minutes thereafter.

4. a. Vasopressin. Male animals were injected into the carotid with 0.5—1.0 I.U. vasopressin (synthetic Vasopressin, Sandoz, Basel) and were decapitated 30 minutes later. The other group received 3.3 I.U. vasopressin intraperitoneally and was decapitated after 60—120 minutes.

b. Oxytocin. 2.5 I.U. oxytocin (synthetic Oxytocin, Richter, Budapest) was injected intraperitoneally into male animals. The animals were killed after 30, 60 or 120 minutes.

5. Hypothalamic extract.

Attempts were made to prepare the extract according to PECILE et al. (1965) and MEITES and FIEL (1965). Since in our hands these procedures were not reproducible, several modifications had to be made. The extracts were prepared from the stalk and the median eminentia of adult male and female rats which were dissected immediately after decapitation, homogenized in 0.1 *N* HCl at 0 °C and stored at —20 °C until processing.

Extraction and testing of the releasing activity of the extracts

a. 1.5 hypothalami/recipient animal. The acid extract was centrifuged at 3000 r.p.m. at 4 °C for 15 minutes and the pH of the clear supernatant was adjusted to 7.4 with *N* sodium carbonate. During neutralization a massive precipitation was observed. When the mixture was readjusted to pH 2.5 with HCl, the precipitate dissolved. The solution obtained in this

way was diluted with 0.9% saline so that 1.0 ml contained 1.5 hypothalami. One ml of this extract was injected into the left common carotid of male animals under ether anaesthesia. After 15 minutes the animals were decapitated. Untreated male animals served as controls. The hypophysis of these animals was removed after decapitation under deep ether anaesthesia.

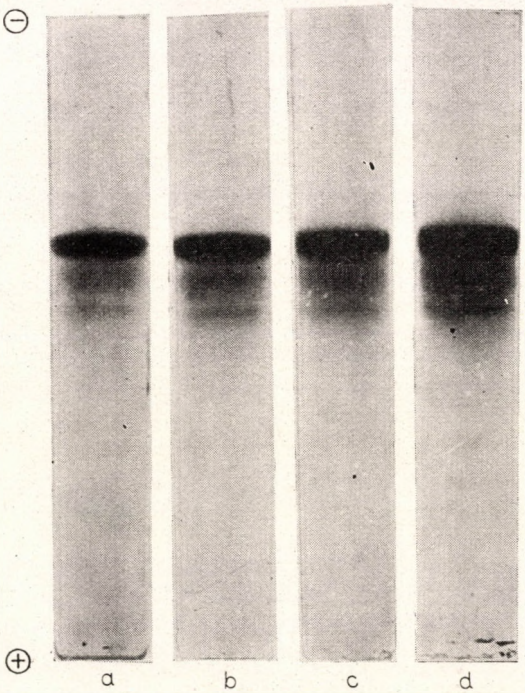

Fig. 1. Polyacrylamide gel electrophoretogram of different quantities of highly purified rat STH preparation. *a)* 50 μg; *b)* 100 μg; *c)* 150 μg; *d)* 200 μg sample (1.0 mg = 3.8 I.U.)

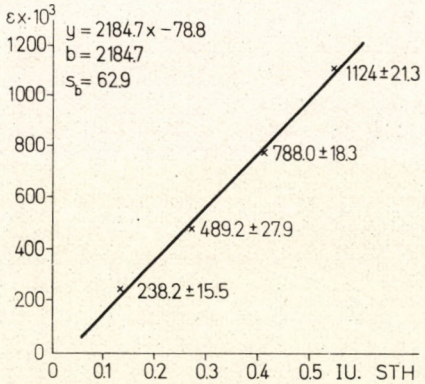

Fig. 2. Calibration curve for quantitative polyacrylamide gel electrophoresis of the STH content of rat pituitary. The figure demonstrates the correlation between the biological activity of the STH separated by electrophoresis and the optical density of the bound naphthalene black 10 B. v = equation for regression line; b = regression coefficient; s_b = deviation of regression coefficient

b. 2.5 hypothalami/recipient animal. The extract was prepared as described above with the difference that acid supernatant was adjusted to pH 6.0. In this way no precipitate had formed, but the water-clear supernatant became opalescent. Of this a suspension of 2.5 hypothalami/ml was prepared with 0.9% saline. The male animals were treated with this solution and decapitated 30 minutes later. The control group was treated intracarotidally with 1.0 ml of physiological saline pH 6.0.

c. 6.0 hypothalami/recipient rat. The supernatant of the acid extract was adjusted to pH 4 with *N* NaOH; at this pH range the solution was slightly opalescent. Dilution with saline was carried out in such a way that 1 ml contained 6.0 hypothalami. Then the procedure described under 5/b was performed with the exception that the control animals received saline of pH 4.

STH determination

STH content of the adenohypophysis was determined with polyacrylamide gel electrophoresis (NAGY et al. 1969, 1970). As a standard, a highly purified, high activity rat STH (3.8 I.U./mg) was used (Fig 1).

Different amounts were separated by electrophoresis, the naphthalene black 10 B (Reanal, Budapest, C.I. 20470) bound to the STH disk was eluted, the optical density was measured with a Beckman—Spinco M-150 ultramicrospectrocolorimeter at 610 mμ wavelength. and a calibration curve was plotted. On the basis of this curve was determined the biologial activity of the experimental STH disks.

Results

1. Pituitary STH content after insulin treatment (Table I). Sixty minutes after the administration of 4.0 I.U./kg of insulin, no significant increase could be observed in male rats (Group No. 5). Similar results were obtained in females with 2—4.0 I.U. regardless of the dose applied. The treated animals developed hypoglycaemia; 2.0 I.U./kg of insulin decreased the blood glucose from 108 to 64 mg per 100 ml.

2. STH content after different stressors (Table II). Swimming until exhaustion (30—45 minutes) increased STH content by 25% in the male animals but not in the females (Group No. 15). Sixty minutes exposure to cold slightly increased STH content in males but not in females (Groups Nos 7 and 12). Surgical stress produced a non-significant increase in both sexes (Groups Nos 9 and 14).

A blood loss of 3—5 ml in males resulted in a slight increase after 180 minutes (Group No. 8); in females the withdrawal of 2—3 ml of blood did not change significantly the adenohypophyseal STH content after 60 minutes (Group No. 13).

3. Suckling stimulus (Table III). Suckling is a physiological stimulus for lactating rats; it increased the STH content by 10% (Group No. 17) as compared with the mothers separated from their litter (Group No. 16). The increase was not significant statistically.

4. Vasopressin and oxytocin (Table IV). In male rats, the intracarotid injection of 0.5—1.0 I.U. vasopressin did not change the STH content (Group No. 19). Intraperitoneal injection of 3.3 I.U. caused no significant change

Table I

Effect of insulin on STH content of rat pituitary

Group No.	Sex	Treatment	No. of animals	Time till killing; minute	Weight of adenohypophysis, mg $\bar{x}\pm S\bar{x}$	Body weight, g $\bar{x}\pm S\bar{x}$	STH content, I.U./mg adenohypophysis $\bar{x}\pm S\bar{x}$	Statistical evaluation of the change in STH content
1.	♀	Control	29		6.3±0.2	141.6±2.3	0.0785±0.0025	
2.	♀	Insulin						
		2 I.U./kg	13	60	6.8±0.4	144.6±7.8	0.0859±0.0044	1—2 NS
3.	♀	Insulin						
		4 I.U./kg	5	60	6.0±0.3	141.4±4.7	0.0811±0.0069	1—3 NS
4.	♂	Control	20		5.5±0.2	184.1±9.4	0.1401±0.0049	
5.	♂	Insulin						
		4 I.U./kg	8	60	5.3±0.1	191.9±6.5	0.1539±0.0011	4—5 NS

$\bar{x}$ = mean
$S\bar{x}$ = standard error of the mean
NS = not significant

Table II

Effect of stressors on STH content of rat pituitary

Group No.	Sex	Treatment	No. of animals	Time till killing, minute	Weight of adenohypophysis, mg $\bar{x}\pm S\bar{x}$	Body weight, g $\bar{x}\pm S\bar{x}$	STH content, I.U./mg adenohypophysis $\bar{x}\pm S\bar{x}$	Statistical evaluation of the change in STH content
6.*	♂	Control	20		5.5±0.2	184.1±9.4	0.1401±0.0049	
7.	♂	Cold (0°C)	9	60	5.7±0.2	203.0±5.0	0.1640±0.0114	6—7 NS
8.	♂	Blod loss						
		(3—5 ml)	7	180	5.7±0.3	205.7±3.1	0.1531±0.0097	6—8 NS
9.	♂	Laparotomy	10	30—45	5.4±0.1	194.0±4.5	0.1465±0.0053	6—9 NS
10.	♂	Swimming	6		5.7±0.3	214.3±7.1	0.1803±0.0099	6—10 $p<0.001$
11.**	♀	Control	29	60	6.3±0.2	141.6±2.3	0.0785±0.0025	
12.	♀	Cold (0°C)	16	60	6.0±0.3	141.6±4.7	0.0798±0.0034	11—12 NS
13.	♀	Blood loss						
		(2—3 ml)	10	60	6.8±0.3	147.0±2.9	0.0937±0.0042	11—13 $p<0.01$
14.	♀	Laparotomy	6	30—45	5.3±0.6	137.2±4.4	0.0858±0.0074	11—14 NS
15.	♀	Swimming	8		5.1±0.4	129.3±3.8	0.0899±0.0052	11—15 NS

$\bar{x}$ = mean
$S\bar{x}$ = standard error of the mean
p = probability
NS = not significant

* = same as Table I, Group No. 4.
** = same as Table I, Group No. 1.

Table III

Pituitary STH content in lactating rats after suckling stimulus

Group No.	Treatment	No. of animals	Duration of separation, hour	Suckling stimulus, minute	Weight of AP, mg $\bar{x}\pm S\bar{x}$	Body weight, g $\bar{x}\pm S\bar{x}$	STH content, I.U./mg adenohypophysis $\bar{x}\pm S\bar{x}$	Statistical evaluation of the change in STH content	
16.	Without suckling stimulus	9	12	—	11.5±0.4	220.1±7.0	0.0520±0.0053		
17.	With suckling stimulus	10	12	30—40	10.7±0.9	211.1±10.7	0.0591±0.0035	16—17	NS

$\bar{x}$ = mean
$S\bar{x}$ = standard error of the mean
NS = not significant

Table IV

Effect of pharmacological doses of vasopressin and oxytocin on pituitary STH content in the rat

Group No.	Sex	Treatment	No. of animals	Time till killing, minute	Weight of adenohypophysis, mg $\bar{x}\pm S\bar{x}$	Body weight, g $\bar{x}\pm S\bar{x}$	STH content, I.U./mg adenohypophysis $\bar{x}\pm S\bar{x}$	Statistical evaluation of the change in STH content
18	♂	Control	20		5.5±0.2	184.1±9.4	0.1401±0.0049	
19	♂	Vasopressin 0.5—1.0 I.U./animal	15	30	5.5±0.3	178.0±8.4	0.1371±0.0050	18—19 NS
20	♂	Vasopressin 3.3 I.U./animal	9	30	5.1±0.3	169.0±5.8	0.1358±0.0076	18—20 NS
21	♂	Vasopressin 3.3 I.U./animal	8	60—120	5.9±0.2	201.0±6.8	0.1590±0.0063	18—21 $p = 0.02$
22	♂	Oxytocin 2.5 I.U./animal	9	30	5.9±0.2	199.5±9.6	0.1716±0.0114	18—22 $p<0.01$
23	♂	Oxytocin 2.5 I.U./animal	8	60—120	6.2—0.2	191.0±8.8	0.1483±0.0111	18—23 NS

$\bar{x}$ = mean
$S\bar{x}$ = standard error of the mean
p = probability
NS = not significant
* = same as Table I, Group No. 4.

Table V

Effect of hypothalamic extract given intracarotidally on pituitary STH content in male rats

Group No.	Treatment	No. of animals	Dose	Time till killing, minute	Weight of adenohypophysis, mg $\bar{x}\pm S\bar{x}$	Body weight, g $\bar{x}\pm S\bar{x}$	STH content, I.U./mg adenohypophysis $\bar{x}\pm S\bar{x}$	Statistical evaluation of the change in STH content	
24.	Control	7			8.2±0.5	287.7±7.6	0.1368±0.0042		
25.	Hypothal. extr. (pH = 2.5)	5	1,5 HP/* ml/rat	15	7.1±0.6	262.0±14.8	0.1511±0.0060	24—25	$p<0.05$
26.	Control pH = 6.5 (phys. NaCl)	7	1 ml/rat	30	5.2±0.5	179.5±9.9	0.1388±0.0095	26—27	NS
27.	Hypothal. extr. (pH = 6.5)	9	2.5 HP/** ml/rat	30	5.5±0.2	186.6±5.2	0.1494±0.0080		
28.	Control pH = 4.0 (phys. NaCl)	6	1 ml/rat	30	5.4±0.3	190.0±4.2	0.1099±0.0163	28—29	$p = 0.01$
29.	Hypothal. extr. (pH = 4)	8	6 HP/ml/*** rat	30	5.0±0.2	180.±3.1	0.1345±0.0059		

$\bar{x}$ = mean
$S\bar{x}$ = standard error of the mean
p = probability
NS = not significant

* = hypothalamic extract prepared according to 5/a
** = hypothalamic extract prepared according to 5/b
*** = hypothalamic extract prepared according to 5/c

after 30 minutes, while after 50—120 minutes a 15% increase was noted (Groups Nos 20 and 21).

After intraperitoneal administration of 2.5 I.U. oxytocin, a significant increase was observed after 30 minutes, which at 60 and 120 minutes showed a decreasing trend toward the control value (Groups Nos 22 and 23).

5. Hypothalamic extract (Table V). Using the tibia test, PECILE et al. (1965) observed a considerable decrease in pituitary STH content after the injection of crude hypothalamic extract. We could not support this result as our extract did not decrease the STH content but in two groups it increased it significantly.

The extraction procedure suggested by PECILE et al. (1965) and MEITES and FIEL (1965) failed to yield a usable preparation in our hands. On neutralization of the clear supernatant of the acid hypothalamic extract a precipitate had formed and if this solution was injected intracarotidally the animals died shortly thereafter. Similar was the effect of the non-centrifuged acid extract. However, the precipitate dissolved on acidification or on the addition of small amounts of $-HCO_3$ or NaOH to pH 6.0, and such solutions were successfully used for treatment. The controls received physiological saline of identical pH.

Discussion

There is convincing evidence that intact hypothalamo-hypophysial connections as well as the intactness of certain parts of the hypothalamus are needed for normal STH production (see for review: HARRIS and DONOVAN 1966; MARTINI and GANONG 1966; GUILLEMIN 1967; MCCANN et al. 1968; BRODISH 1968; SZENTÁGOTHAI et al. 1968; FROHMAN and BERNARDIS 1968). It is also generally accepted that the various prolonged endogenous and exogenous factors are influencing pituitary function beside a possible direct effect via the central nervous system. However, the extent and mechanism of action of non-specific acute stimuli on the pituitary STH content are questionable. One possible approach to the problem is an investigation of the STH content of the adenohypophysis.

In our studies, two to fourfold amounts of the generally used doses of insulin failed to decrease the STH contents in contrast to the results obtained with the tibia test (KRULICH and MCCANN 1966a, b; MÜLLER and PECILE 1966; MÜLLER et al. 1967b; 1968a; PECILE and MÜLLER 1966, KATZ et al. 1967). Our observation is in agreement with those of MÜLLER and DAL PRA (1968) made in mice. KATZ et al. (1967) reported that insulin treatment resulted in a significant decrease of the STH content in one out of four groups of rats, due probably to the stress-like effect of hypoglycaemia. Treatment with

α-2-D-desoxyglucose as well as with adrenaline led to a similar result (KRULICH and MCCANN 1966c; MÜLLER et al. 1967b, 1968a). Sixty minutes after administration of 2.0 I.U./kg of insulin the blood sugar showed a 40% decrease but such an alteration in carbohydrate metabolism did not affect the pituitary STH content. Our observations are in agreement with those of SCHALCH and REICHLIN (1968), GARCIA and GESCHWIND (1968) and DAUGHADAY et al. (1968) whose data were obtained with radioimmune-assay.

Exposure to cold is a classical stressor. Under its effect, KRULICH and MCCANN (1966a), MÜLLER et al. (1967a), SCHALLY et al. (1968a) observed a 50% decrease in pituitary STH content. In agreement with SCHALCH and REICHLIN (1968) and GARCIA and GESCHWIND (1968), no significant decrease was observed by us or by MÜLLER and DAL PRA (1968) in mice.

Data are divergent concerning the effect of other chemical, surgical and physical stressors. KRULICH and MCCANN (1966a) reported that a strong bell stimulus was followed by a significant increase after 30 minutes. Administration of formalin decreased the STH content only if it was combined with theophylline (MÜLLER et al. 1967b, 1969). According to others, formalin significantly increases the STH content (KRULICH and MCCANN 1966a). Fifteen minutes following splenectomy a decrease was shown by KRULICH and MCCANN (1966a), but after 30 and 60 minutes they found both increases and decreases. However, 60 minutes after laparotomy the STH content showed no change (MÜLLER et al. 1967a).

In agreement with SCHALCH and REICHLIN (1968) we found no decrease after physical exercise or trauma. Although swimming in males or a blood loss in females resulted in a significant increase, a change of such magnitude may have been due to errors in the determination, coincident with individual differences of the animals.

Unlike the factors described above, suckling is a physiological stimulus, which according to GROSVENOR et al. (1968) causes a fast decrease in pituitary STH content. SAR and MEITES (1969) observed a decrease after 180 minutes while our results showed a slight increase after 30 minutes suckling. According to results obtained with the tibia test, pituitary STH content is significantly decreased by many acute effects. To elucidate the mechanism of the phenomenon, many authors studied the effect of different drugs and neurohumoral agents but there was no agreement among their findings (MÜLLER et al. 1965, 1967b, 1968b; ISHIDA et al. 1965; KRULICH and MCCANN 1966c; ARIMURA et al. 1967; SAWANO et al. 1967).

In the present experiments, only the effect of pharmacological doses of vasopressin and oxytocin were studied. Concerning vasopressin, the conclusion can be drawn that the depleting effect on STH is dose-dependent (MÜLLER et al. 1965, 1967b, 1968b; ISHIDA et al. 1965; ARIMURA et al. 1967.) Eight to 100 mU given intraventricularly or intracarotidally were ineffective but

500 mU given intraperitoneally caused a 20% decrease. ARIMURA et al. (1967) reported that extract prepared from the posterior lobe of 8 rats, which contained approximately 800—1000 mU vasopressin, significantly decreased the STH content. Our investigations failed to support this observation.

After administration of a small dose of oxytocin, MÜLLER et al. (1965) as well as ISHIDA et al. (1965) observed no change in pituitary STH. Thirty minutes after giving a large dose we observed an increase, but after 60—120 minutes this was not significant any more. After the negative results obtained with vasopressin and oxytocin, the conclusion has been drawn that the two neurohumoral materials have no basic importance in the release of STH.

With the pituitary STH depletion method based on the tibia test as recommended by PECILE et al. (1965), a decreased STH-RF activity in the hypothalamus and the appearance of RF in peripheral blood was found after an acute stimulus (KRULICH and MCCANN 1966b; KATZ et al. 1967; PECILE and MÜLLER 1966). Although the importance of hypothalamic structures in STH production (secretion + release) has been verified, the data concerning STH-RF must be accepted with caution.

Probably because of difficulties in purification and extraction, STH-RF could not be isolated from the hypothalamus of any species in such a purified form which would allow the biological and biochemical characteristics to be described unambiguously. This might explain the differences in the opinion of DHARIWAL et al. (1965) and SCHALLY et al. (1968b) concerning the isoelectric point of the polypeptide isolated from the pig. The adequacy of the method of PECILE et al. (1965) for STH-RF determination based on the pituitary STH depletion test in vivo is disputable. SCHALCH and REICHLIN (1968) and GARCIA and GESCHWIND (1968) working with the more sensitive radioimmune-assay could not prove the STH depleting effect of the purified hypothalamic extract. Similar results were obtained with radioimmune-assay and tibia test by RODGER et al. (1969); the extract used by them did not increase the blood STH level, in contrast to the results of SAWANO et al. (1968). The data obtained with polyacrylamide gel electrophoresis are in agreement with those yielded by radioimmune-assay, in that injection of crude extract brought about no decrease. The hypothalamic STH inhibiting factor (KRULICH et al. 1968) might have had a role in the significant increase.

The STH-RF extraction method reported in the literature proved unsuccessful in our hands. The precipitate obtained in the course of neutralization probably contains tissue proteins with an isoelectric point identical with the pH obtained at neutralization. The non-neutralized acid extract could be used without difficulty as the first step of STH-RF isolation is an acid extraction (see for review: SCHALLY et al. 1968b).

Thus, the different non-specific and acute factors did not significantly influence the STH content of the rat adenohypophysis. Our polyacrylamide

gel electrophoretic results were in close agreement with those obtained by radioimmune assay (DAUGHADAY et al. 1968; SCHALCH and REICHLIN 1968; RODGER et al. 1969) and make the value of the biological determination questionable. According to our opinion, such data need revision in every respect. It is not likely that STH of the same mobility, biochemical characteristics and staining properties, isolated from the hypophysis of control and treated rats would induce different biological effects in the target organ (tibia) of the hypophysectomized test animal.

The results seem to support the hypothesis that, under normal resting conditions, basal STH secretion (synthesis + storage) is stable and that the pituitary contains a large quantity of "reserve" STH. Consequently, acute influences do not cause dramatic effects by an extensive STH release.

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Addendum

Since our manuscript was submitted STEINER et al. (Endocrinology 1970, 86: 1354—1360) have reported, that precipitate was formed by the neutralisation of the acidic extract of rat hypothalami and this was removed by centrifugation. This finding is in agreement with our experiences published in this paper.

The radioimmunological determinations are in accordance with the results of bioassays according to one of the latest papers of MÜLLER et al. (Proc. Soc. exp. Biol. N.Y. 1970, 135: 934—939). Using both methods they found circadian pattern of bioassayable and radioimmunoassayable growth hormone in the pituitary of female rats. The same paper reported that the intracarotid injection of hypothalamic extracts induced a depletion of bioassayable pituitary growth hormone. But MÜLLER et al. have not made radioimmunological determinations in these experimental groups.

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THE EFFECT OF SUCKLING, STRESS AND DRUGS ON PITUITARY PROLACTIN CONTENT IN THE RAT

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As revealed by polyacrylamide gel electrophoresis, a 30—40 minutes suckling stimulus does not result in a significant decrease in pituitary prolactin content in the rat. In non-lactating animals, different acute non-specific stressors such as cold, laparotomy, bleeding, swimming, insulin, oxytocin and vasopressin did not influence the pituitary prolactin content. The results do not exclude the possibility that the applied stimuli caused such a small prolactin release which was negligible as compared to the quantity found in the hypophysis and could not be detected by the method used. This small amount of prolactin might still cause significant changes in the target organ.

Decrease in pituitary prolactin content under the effect of suckling and the possible role in the phenomenon of the central nervous system had been reported by REECE and TURNER (1936a, b) and HOLST and TURNER (1939). These data have been confirmed by GROSVENOR and TURNER (1957, 1958a) and MOON and TURNER (1959). Then GROSVENOR (1965a, b), GROSVENOR et al. (1965, 1967a, b), GROSVENOR and MENA (1967) have shown that suckling stimulus and different aspecific factors after 8—10 hours separation caused a rapid and significant decrease in the pituitary prolactin content in lactating rats; SAR and MEITES (1969) could not confirm this rapid P release.

There are no data to show whether specific and non-specific acute stimuli could change the pituitary prolactin content in adult males and non-lactating females. Although a release of prolactin was observed following copulation (DESJARDINS et al. 1967), opinions differ concerning the effect of cervical stimulation carried out in oestrus (HERLYN et al. 1965; KUROSHIMA et al. 1966; SINHA and SCHMIDT 1969).

In view of the contradictions and the scarcity of data, it seemed interesting to investigate the acute effect of different factors such as suckling, stressors, hypoglycaemia, oxytocin and vasopressin on the pituitary prolactin content in non-lactating female and male rats.

Methods

Polyacrylamide gel electrophoresis (ORNSTEIN 1964 and DAVIS 1964) allows to determine prolactin and somatotrophin simultaneously from the same pituitary specimen (NAGY

et al. 1968, 1969; KURCZ et al. 1969; NAGY et al. 1970a). Prolactin determination was carried out in the same animals in which the pituitary STH content was investigated after different acute effects. For preparation of the hypophysis, see NAGY et al. (1970b).

A) Specific and non-specific effects

1. Suckling stimulus. On the 7th day of lactation the litters were removed from the mother for 12 hours, then replaced into the nest. After 30—40 minutes the mothers were decapitated in deep ether anaesthesia.

2. The effect of stressors was studied in adult non-lactating intact female and male rats.

a) Cold. The animals were exposed to 0 °C for 60 minutes and then decapitated.

b) Bleeding. Under anaesthesia the tail was cut and from the males 3—5 ml, from the females 2—3 ml of blood was taken. The males were decapitated 180, the females 60 minutes after bleeding.

c) Laparotomy. In male and female animals under ether anaesthesia the abdominal cavity was opened and then closed. 60 minutes following surgery the animals were decapitated.

d) Swimming. Female and male rats were made to swim till exhaustion (30—45 minutes) and decapitated.

3. Insulin. Hypoglycaemia was induced in male and female animals with 2.0 or 4.0 I.U./kg crystalline insulin (40 I.U./ml, Richter, Budapest) given subcutaneously. The animals were decapitated 60 minutes after insulin administration.

4. Oxytocin and vasopressin applied in pharmacological dose. The effect of these two drugs were investigated exclusively in male rats.

2.5 I.U. oxytocin (synthetic oxytocin, 5 I.U./ml, Richter, Budapest) was given intraperitoneally and the animals were killed after 30, 60 and 120 minutes.

Of vasopressin (synthetic vasopressin, 10 I.U./ml, Sandoz, Basel) 0.5—1.0 I.U./animal was given intracarotidally. The animals were decapitated 30 minutes later. Another group of animals was treated intraperitoneally with 3.3 I.U./animal. These animals were decapitated after 30, 60 and 120 minutes.

B) Prolactin determination.

Determination of pituitary prolactin content was carried out by our polyacrylamide gel electrophoretic micromethod (NAGY et al. 1968; KURCZ et al. 1969).

Results

1. Prolactin content of adenohypophysis 30—45 minutes after suckling (Table I, Groups 1, 2). On the 7th day of lactation, under the effect of suckling 12 hours after separation of the litter, pituitary prolactin content decreased by 10%. The decrease did not reach statistical significance.

2. Effect of acute stimuli on pituitary prolactin content (Table II). Exposure to 0 °C for 60 minutes caused a slight decrease in pituitary prolactin content in the females and an increase in the males. These changes were not significant statistically (Groups 3, 4, 8 and 9).

60 minutes after withdrawal of 2—3 ml of blood, pituitary prolactin content in the females increased by 21%. Blood loss in the males caused after 180 minutes a minimal increase (Groups 5 and 10).

Laparotomy 60 minutes after surgery caused no change in any of the animals (Groups 6 and 11) and swimming till exhaustion was also ineffective.

3. Effect of insulin (Table III). Hypoglycaemia was associated with no change in prolactin content after 60 minutes (Groups 13—17).

Table I

Pituitary prolactin content after 30—45 minute suckling stimulus in the rat

Group No.	Treatment	No. of animals	Weight of adenohypophysis (mg) $\bar{x} \pm S\bar{x}$	Body weight (g) $\bar{x} \pm S\bar{x}$	Prolactin content of adenohypophysis I. U /mg $\bar{x} \pm S\bar{x}$	Statistical evalutaion of the changes in prolactin content	
1	Lactating mothers without suckling stimulus	9	11.5 ± 0.4	220.1 ± 7.0	0.1138 ± 0.0097		
2	Lactating mothers after 30—45 minute suckling stimulus	10	10.7 ± 0.9	211.1 ± 10.7	0.1017 ± 0.0071	1—2	NS

$\bar{x}$ = average
$S\bar{x}$ = standard error of the mean
NS = not significant

Table II

Effect of different aspecific acute stimuli on pituitary prolactin content in the rat

Group No.	Sex	Treatment	No. of animals	Duration of stimulus (min)	Weight of adenohypophysis (mg) $\bar{x} \pm S\bar{x}$	Body weight (g) $\bar{x} \pm S\bar{x}$	Prolactin content of adenohypophysis I. U./mg $\bar{x} \pm S\bar{x}$	Statistical evaluation of the changes in prolactin content
3	♀	Control	29		6.3±0.2	141.6±2.3	0.0635±0.0035	
4	♀	Cold (0°C)	16	60	6.0±0.3	141.6±4.7	0.0551±0.0063	3—4 NS
5	♀	Bleeding (2—3 ml)	10	60	6.8±0.3	147.0±2.9	0.0769±0.0031	3—5 $p<0.05$
6	♀	Laparotomy	6	60	5.3±0.6	137.2±4.4	0.0672±0.0023	3—6 NS
7	♀	Swimming	8	30—45	5.1±0.4	129.3±3.8	0.0657±0.0068	3—7 NS
8	♂	Control	20		5.5±0.2	184.1±9.4	0.0280±0.0019	
9	♂	Cold (0°C)	9	60	5.7±0.2	203.0±5.0	0.0346±0.0009	8—9 NS
10	♂	Bleeding (2—3 ml)	7	180	5.7±0.3	205.7±3.1	0.0216±0.0025	8—10 NS
11	♂	Laparotomy	10	60	5.4±0.1	194.0±4.5	0.0228±0.0019	8—11 NS
12	♂	Swimming	6	30—45	5.7±0.3	214.3±7.1	0.0272±0.0030	8—12 NS

$\bar{x}$ = Average
$S\bar{x}$ = standard error of the mean
p = probability
NS = not significant

Table III

Effect of insulin on pituitary prolactin content in the rat

Group No.	Sex	Treatment	No. of animals	Time before killing (min)	Weight of adenohypophysis (mg) $\bar{x} \pm S\bar{x}$	Body weight (g) $\bar{x} \pm S\bar{x}$	Prolactin content of adenohypophysis I. U./mg $\bar{x} \pm S\bar{x}$	Statistical evaluation of changes in prolactin content
13	♀	Control	29		6.3±0.2	141.6±2.3	0.0635±0.0035	
14	♀	Insulin sc. 2.0 I. U./kg	13	60	6.8±0.4	144.6±7.8	0.0699±0.0044	13—14 NS
15	♀	Insulin sc. 4.0 I. U./kg	5	60	6.0±0.3	141.4±4.7	0.0632±0.0087	13—15 NS
16	♂	Control	20		5.5±0.2	184.1±9.4	0.0280±0.0019	
17	♂	Insulin sc. 4.0 I. U./kg	8	60	5.3±0.1	191.9±6.5	0.0293±0.0008	16—17 NS

$\bar{x}$ = average $S\bar{x}$ = standard error of the mean NS = not significant

Table IV

Effect of pharmacological doses of vasopressin and oxytocin on pituitary prolactin content in male rats

Group No.	Treatment	No. of animals	Time before killing (min)	Weight of adenohypophysis (mg) $\bar{x} \pm S\bar{x}$	Body weight (g) $\bar{x} \pm S\bar{x}$	Prolactin content of adenohypophysis I. U./mg $\bar{x} \pm S\bar{x}$	Statistical evaluation of changes in prolactin content
18	Control	29		5.5±0.2	184.1±9.4	0.0280±0.0019	
19	Vasopressin ic. 3.3 I. O/animal	15	30	5.5±0.3	178.0±8.4	0.0283±0.0012	18—19 NS
20	Vasopressin ip. 3.3 I. U/animal	9	30	5.1±0.3	169.0±5.8	0.0252±0.0010	18—20 NS
21	Vasopressin ip. 3.3 I. U/animal	8	60—120	5.9±0.2	201.0±6.8	0.0276±0.0014	18—21 NS
22	Oxytocin ip. 2.5 I. U/animal	9	30	5.9±0.2	199.5±9.5	0.0264±0.0013	18—22 NS
23	Oxytocin ip. 2.5 I. U/animal	8	60—120	6.2±0.2	191.0±8.8	0.0270±0.0014	18—23 NS

$\bar{x}$ = average $S\bar{x}$ = standard error of the mean NS = not significant

4. Oxytocin and vasopressin (Table IV). The effect was investigated in male rats only. Pituitary prolactin content did not change 30—120 minutes after intraperitoneal administration of 2.5 I.U. oxytocin (Groups 18—21) nor 30—120 minutes after intracarotidal or intraperitoneal administration of 0.5—3.3 I.U. vasopressin/rat (Groups 22, 23).

Discussion

According to the results a 30—40 minute suckling stimulus applied after removal of the litter from the mother animal for 12 hours caused no significant decrease in the pituitary prolactin content, in full agreement with the data of SAR and MEITES (1969) and REECE and TURNER (1936a, b) who observed a change only 180 minutes after the stimulus. All these findings contradict the observation (GROSVENOR and TURNER 1957, 1958a; MOON and TURNER 1959; GROSVENOR 1965a, b; GROSVENOR et al. 1965; GROSVENOR et al. 1967a, b; GROSVENOR and MENA 1967; MENA and GROSVENOR 1968) according to which a 30-minute suckling stimulus caused a 50—87% decrease in the prolactin content. GROSVENOR et al. (1967a) reported also that a two-minute stimulus was as effective as a 30-minute one and concluded that the stimulus reaching the central nervous system results in a maximum release. After such depletion the "releasing mechanism" reaches a refractory stage and the prolactin left behind in the hypophysis is not released. Thus the possibility arose that the adenohypophysis reacts on the basis of an "all or none" phenomenon to the suckling stimulus. All this is difficult to compare with the later findings of MENA and GROSVENOR (1968) according to which the pituitary prolactin content of the lactating rat and the extent of the decrease is significantly influenced by the number of the litter. This principle cannot be valid during the whole lactation period and GROSVENOR and TURNER (1957) have shown that on the 20th day of lactation the suckling stimulus cannot any more induce a decrease in the pituitary prolactin content.

Our results as well as those of SAR and MEITES (1969) failed to support the above hypothesis; it is not likely that the contradiction should have been due to differences in the rat strain used in these experiments.

Another principal contradiction is as follows. The decrease in prolactin was observed 8—12 hours after removal of the litter. Such a situation does not happen under physiological circumstances. The mother rat is in permanent contact with the young animals and keeps them warm. Twelve-hour-separation is a very long one considering the suckling rhythm of the rat and it is questionable whether such an artificially produced pituitary prolactin content should be considered a basal level and whether its changes allow for conclusions as to physiological rules.

All these refer to the recent data of CONVEY and REECE (1969), too. On 3—4th day of lactation, after 10-hour-separation from the litter, a 2-hour-suckling stimulus caused a 90% decrease in the pituitary prolactin. On the other hand, according to the data of GROSVENOR and TURNER (1957) restoration of this level to the pre-suckling one takes 10 hours. Such an experimental condition is wholly aphysiological just as a "2—3-hour-suckling stimulus" is also impossible. The litter separated from the mother and put back are suckling themselves full in 15—30 minutes then they hardly feed and the amount of milk they receive in the next two hours is negligible although they continue in holding the nipple and to influence the mother with different exteroceptive stimuli. The contact, especially in the first part of the lactation period, is practically permanent and a 2—30-minute suckling stimulus which causes a 50—90% decrease in prolactin content is hardly imaginable under such conditions.

SAR and MEITES (1969) could detect a significant decrease in the mother rat's pituitary prolactin content only if she had been together with her litter for at least 3 hours. Thus, during the lactation period the maintenance of prolactin release might depend on the repetition of suckling and on the continuous contact between mother and litter.

In our experiments, acute stressors, such as cold, bleeding, laparotomy, swimming, failed to influence the pituitary prolaction content in adult non-lactating female and male rats. It is difficult to compare our data with those found in the literature since to our knowledge similar investigations have been carried out only in lactating animals. An exception is the observation concerning the effect of cervical stimulation. In contradiction to HERLYN et al. (1965) and KUROSHIMA et al. (1966), SINHA and SCHMIDT (1969) could not find a decrease in the pituitary prolactin content after such stimulation. The effect of different kinds of stress on lactating rats was investigated by GROSVENOR (1965a, b), GROSVENOR et al. (1965, 1967b), GROSVENOR and MENA (1967). Seeing or hearing of the separated litter, beating, decapitation, laparotomy, bleeding, cervical dislocation significantly decreased, while milk ejection inhibiting acoustic, olfactory and optical stimuli did not change the pituitary prolactin content. We failed to observe such a change in non-lactating female and male rats. However, in the experiments of GROSVENOR et al. (1965, 1967b) the decrease occurred from an artificially high level, while in our control animals the pituitary prolactin level did not change. In this context it may arise that the mechanism of the prolactin release caused by stress might differ in the lactating and the non-lactating animal.

It is not clear whether the neurohypophyseal hormones can change the pituitary prolactin content. BENSON and FOLLEY (1957) inhibited mammary involution after weaning by means of oxytocin administration and concluded that oxytocin causes a release of prolactin and other galactopoietic as well

as lactotropic hormones. GROSVENOR and TURNER (1958b), MEITES et al. (1960a, b), MEITES and HOPKINS (1961) could not confirm this effect of oxytocin neither has it been supported by our present observations.

All the factors investigated by us have failed to cause a change in the prolactin content of the adenohypophysis or, at least, the released amount could not be detected with our method. The possibility cannot, however, be disregarded that such minute amounts of prolactin still exert a physiological effect at the periphery. It remains for the radioimmunological determination of the blood prolactin level to clarify whether the factors applied actually inhibited the pituitary release of small prolactin fragments which entering into the circulation may cause a significant increase in the blood prolactin concentration.

The cause of the contradictions found in the literature might be ascribed to the fact that prolactin determination by the pigeon crop test carries methodological errors and its evaluation depends greatly on subjective factors.

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Addendum

While our paper was in press GROSVENOR et al. (J. Endocrinol. 1970, 17; 339—346) reported that the reaccumulation of the diminished prolactin content of the rat anterior pituitary after suckling stimulus was accelerated in response to hypothalamic extracts or exogenous prolactin. The authors mention, that we have also found the decrease of the prolactin content of rat pituitary after suckling stimulus in starch-gel electrophoresis (KURCZ, M.: Studies on the neuro-humoral regulation of lactation. In: LISSÁK, K. (Ed.): Symposion of Reproduction, Akadémiai Kiadó, Budapest 1967, pp. 175—211; BARANYAI et al.: Clin. Chim. Acta 1966, 16; 449—451). However, we should like to emphasize that the above method was not a densitometric determination. Consequently results obtained are only semiquantitative and should be treated with due caution. The fall of prolactin content published in our earlier papers is mathematically not significant as shown by the quantitative results of polyacrylamide gel electrophoresis.

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EFFECT OF CORTICOSTEROIDS ON PASSIVE AVOIDANCE BEHAVIOUR OF RATS

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The effect of corticosteroids on passive avoidance behaviour of the rat has been investigated in two experimental situations based upon light-avoidance and thirst drive. Administration of a single dose of cortisone led to a suppression of both immediate and long term retention of passive avoidance elicited by a single shock trial in a light-avoidance situation. The effect of corticoids was a function of shock intensity eliciting the inhibition of inherent light-avoidance response. The higher the shock intensity, the more steroid was necessary to suppress passive avoidance.

Passive avoidance elicited by a single shock trial in a space discriminative conditioned situation motivated by thirst was also suppressed by a single dose of dexamethasone. Discrimination between the shocked and non-shocked side was, however, enhanced by dexamethasone.

A number of behavioural and electrophysiological experiments has demonstrated that the central nervous influence of corticosteroids is exerted through the enhancement of internal inhibitory processes of Pavlovian terminology (Endrőczi and Lissák 1962; Bohus and Endrőczi 1965; Bohus and Korányi 1969; Endrőczi 1969). It has also been shown that this effect of steroids is exerted directly on the forebrain structures (Bohus 1970) related to the elaboration of internal inhibitory influences (Lissák and Endrőczi 1967). However, some observations cast doubt on the view that an enhancement of internal inhibition is the sole mechanism of the central nervous action of corticosteroids. Thus, both behavioural (Bohus 1968, 1970; van Wimersma Greidanus and de Wied 1969) and electrophysiological (Slusher et al. 1966; Endrőczi 1969) observations suggest the involvement of the reticular activating system in the action of corticosteroids.

The present experiments were aimed at investigating the influence of corticosteroids on passive avoidance behaviour of rats in order to determine if any other mechanism than an enhancement of internal inhibition may be involved in the behavioural influence of corticosteroids.

Methods

Adult male albino rats of an inbred strain were used. They were fed on rat chow and allowed to drink ad libitum unless otherwise indicated.

Passive, one-trial shock avoidance behaviour of rats was studied in two different motivated situations. Inhibition of an inherent light-avoidance response was investigated in a conditioning apparatus which consisted of two plywood compartments connected by a 10×10 cm

opening in the centre of the partition at floor level. One compartment measured $35 \times 30 \times 48$ cm and was painted a light gray and lighted intensely by a 50 W bulb fixed on the lid of the chamber. The other compartment measured $30 \times 8 \times 48$ cm, it was painted black and the floor was made of metal rods which were connected to a shock scrambler.

Immediate passive avoidance was studied as follows. On day 1 the rats were placed in the lit compartment and were allowed to explore for 180 sec. Those rats which exhibited no substantial light-avoidance — i.e. did not enter the dark compartment within 10 sec — were excluded from further studies. On day 2 the rats were divided randomly into three groups and treated with 1.0 or 2.0 mg/100 g b.w. of cortisone acetate (Adreson, Organon, Oss) or with saline given subcutaneously 3 hours prior to the experiment. Then the rats were placed in the lit compartment. When they entered the dark chamber a shock of 0.5 mA, A.C. was applied through the grid floor. When the rat had escaped back into the lit compartment, a timer was activated and the time the animal took to reenter the dark chamber was recorded and served as the index of passive avoidance behaviour of each rat (retention score). If reenter had not occurred within 10 min the rat was removed from the apparatus and the retention score was recorded as 600 sec.

Long term inhibition of inherent light-avoidance behaviour by a single shock trial was studied as follows. The rats were randomly treated with 1.0, 2.0 or 4.0 mg/100 g b.w. of cortisone acetate or saline given subcutaneously. Three hours later the rats placed in the intensely lit compartment were allowed to explore for 180 sec. The time spent in the lit compartment was recorded. Those rats which showed no substantial light-avoidance, i.e. spent more than 60 sec in the lit chamber, were excluded from further experiments. Learning trial consisted of an avoidable intermittent A.C. shock at 0.5 or 1.0 mA intensity for 60 sec in the dark compartment. Immediately after the single learning trial the rats were removed from the apparatus. Retention of passive avoidance task was assessed 24 and 48 hours after the learning trial. The rats were placed in the lit compartment and the time spent in it was recorded. Passive avoidance retention was expressed by a retention score index for each rat. This was calculated from the time spent in the lit compartment during the retention test minus that during the exploration period.

Passive avoidance behaviour of rats was also studied after the suppression of a space-discriminative conditioned approach response for water reward. The experiments were performed in a rectangular box measuring $60 \times 50 \times 40$ cm. Water reinforcement was delivered through manually controlled water dish systems mounted 40 cm apart on one wall of the box. Electric shock could be applied through a metal plate mounted on the front of the water dish and the metal floor of the conditioning apparatus.

Male albino rats were deprived of water for 23 hrs and maintained on this schedule throughout the experiments. Conditional stimulus (CS) was the light of 6 W bulbs placed 5 cm above the water dishes. Ten conditioning trials were given daily. The CS was presented for 10 sec if no conditioned response had occurred. When the rats had begun to drink within 10 sec of CS presentation water was available for 5 sec. Then the dish was withdrawn and the CS was discontinued. The space-discriminative conditioning consisted of 6 trials to the left side and 4 trials to the right one in the sequence LLRLRRLRLL. Average intertrial interval was 60 sec.

The rats were trained to achieve the learning criterion of 60 correct responses during 6 consecutive days. The rats which had reached this criterion were randomly divided into two experimental and two control groups. The experimental animals were treated with dexamethasone phosphate (Oradexon, Organon, Oss) in a dose of 20 μg/rat while controls were injected subcutaneously with saline 3 hours before the next session. In this session the first CS on the left side was paired with an electric shock of 1.0 mA, A.C. Immediate and long term retention of passive avoidance task was then studied.

Studying the immediate retention of avoidance response one experimental and one control group was trained as follows: after the punishment of the first conditioned response at the left side the rats remained in the conditioning apparatus and discriminative trials were given continuously in the schedule described above. The rats were trained to reach a criterion of 10 correct conditioned drinking responses during 10 consecutive trials but to a maximum of 40 trials. On the next two days the rats were tested again with 10—10 trials. The number of conditioned drinking responses (CR's) scored during the test periods served as the index of passive avoidance behaviour of each rat. The less the number of CR's, the more effective the passive avoidance was.

When the long term retention of passive avoidance response was studied the rats of the other experimental and control groups were removed from the conditioning apparatus immediately after the single shock trial on the left side. Retention of passive avoidance response was tested 24 hours later, then daily for the next 6 consecutive days with 10 trials each.

The total number of CR's scored during the 70 retention trials served as the index of passive avoidance behaviour.

The influence of dexamethasone on the conditioned approach response, on water intake and open-field behaviour was also investigated. Male albino rats were trained to achieve the learning criterion of the conditioned discriminative drinking response as described above. Then a single injection of 20 μg/rat of dexamethasone was given subcutaneously 3 hours before the next session. The controls were treated with saline. The influence of the steroid was analysed during two consecutive sessions.

The effect of dexamethasone on water intake was determined on male albino rats kept in separate metabolic cages and deprived of water for 23 hrs. Water intake was measured for 6 consecutive days for 60 min daily. After the first 3 days the rats were matched into experimental and control groups according to their 3-day water intake. Then dexamethasone (20 μg/rat) or saline wall injected subcutaneously 3 hours before each of the next 3 daily sessions.

Open-field activity was estimated in a rectangular box measuring $60 \times 60 \times 50$ cm. The box wall was painted white and the floor was divided by black lines into 10×10 cm squares. A 150 W bulb was placed 100 cm above the floor. Test trials consisted of 3-min sessions daily for 3 consecutive days. Ambulatory (number of squares transversed), rearings (number of rearings), grooming (grooming movements during each 2.5 sec period) and defecation (number of boluses) scores were recorded. Male albino rats subjected to 23-hr water deprivation were divided randomly into two groups before the first session. Experimental animals were treated with dexamethasone (20 μg/rat) 3 hours daily before the test; to the controls, saline was given.

Statistical analysis of the data was carried out by means of variance analysis, Mann and Whitney's U-test or of the double-tail *t*-distribution test. A probability level of 0.05 or less was accepted as significant difference.

Results

The influence of cortisone on the immediate retention of a passive avoidance task in a light-avoidance situation is shown in Fig. 1. Passive avoidance appeared in the inhibition of a previously established somato-motor pattern — i.e. to reenter the dark compartment after a single shock in that chamber. Six of 10 saline-treated controls failed to enter the dark compartment during the 10 min test period; the mean latency was 424.8 $\pm$10.4 sec. Administration of cortisone acetate in a dose of 1.0 mg/100 g b.w. failed to affect the immediate retention of the passive avoidance task. Seven of 10 rats failed to enter the dark compartment and mean avoidance latency was 432.9 $\pm$27.0 sec. Administration of 2.0 mg/100 g b.w. of cortisone acetate, however, resulted in a significant ($p = 0.05$) decrease in avoidance latency; mean latency time was 156.6 $\pm$23.6 sec. Thus, 10 rats treated with this dose of cortisone reentered the dark compartment sooner than did either the rats treated with 1.0 mg of cortisone or the controls. Analysis of variance revealed a significant ($p = 0.05$) difference between rats treated with 1 or 2 mg of cortisone.

The effect of cortisone on long term retention of the passive avoidance task in a light-avoidance situation appeared to depend on both the shock intensity and the dose of steroid given prior to the single learning trial (Fig. 2). Significant interactions were found between the retention scores and shock intensities and between the doses of cortisone and the shock intensities. Observations in saline-treated rats revealed that the higher the shock intensity, the longer the time spent in the lit compartment, which results in high reten-

tion scores. Administration of cortisone in a dose of 1.0 or 2.0 mg/100 g b.w. resulted in a significant decrease of the retention score both 24 and 48 hours after the single learning trial when the shock intensity was 0.5 mA. This fact indicates that cortisone-treated rats spent less time in the lit compartment than the controls, thus their passive avoidance was suppressed. The differences between the rats treated with 1.0 mg cortisone and the controls were significant at the 0.01 (24-hour) and 0.005 (48-hour) levels. Controls and rats treated with 2.0 mg of cortisone differed at the 0.005 level (24th and 48th hour). A significant dose-response relationship was not observed.

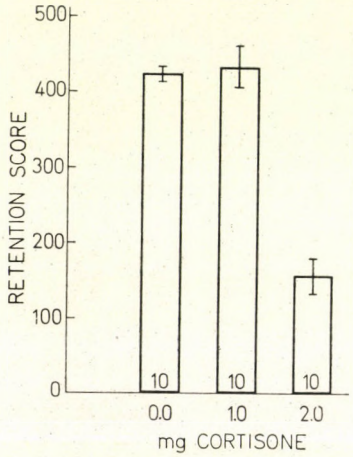

Fig. 1. Immediate retention of a passive avoidance task in a light-avoidance situation following the administration of a single dose of cortisone acetate

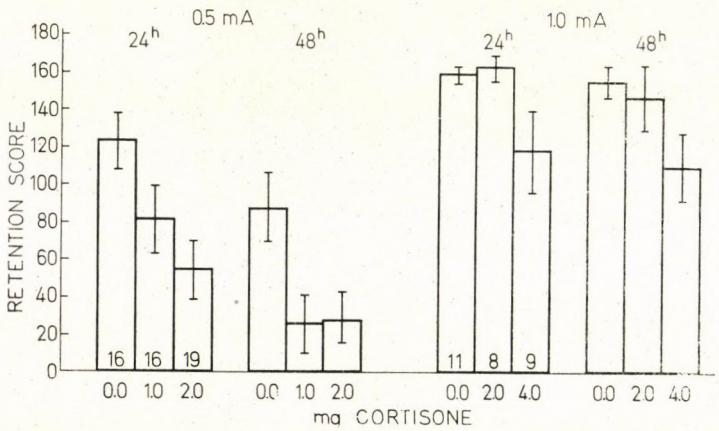

Fig. 2. Effect of cortisone acetate administered prior to the single learning trial on long term passive avoidance of the rat in a light-avoidance situation and its dependence on the shock intensity eliciting passive avoidance

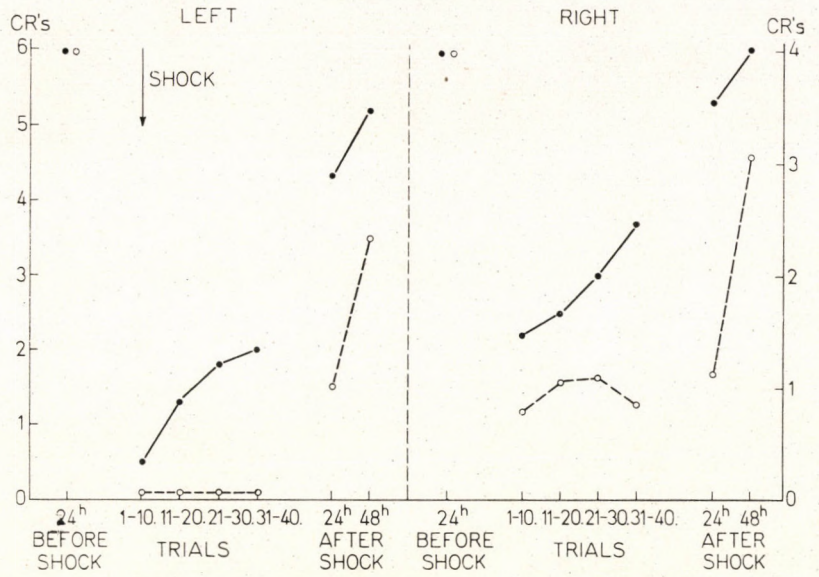

Fig. 3. Signal-controlled left-right discrimination behaviour of dexamethasone- (●—●) and saline-treated (○-----○) rats during immediate retention of passive avoidance elicited by a single shock trial on the left side

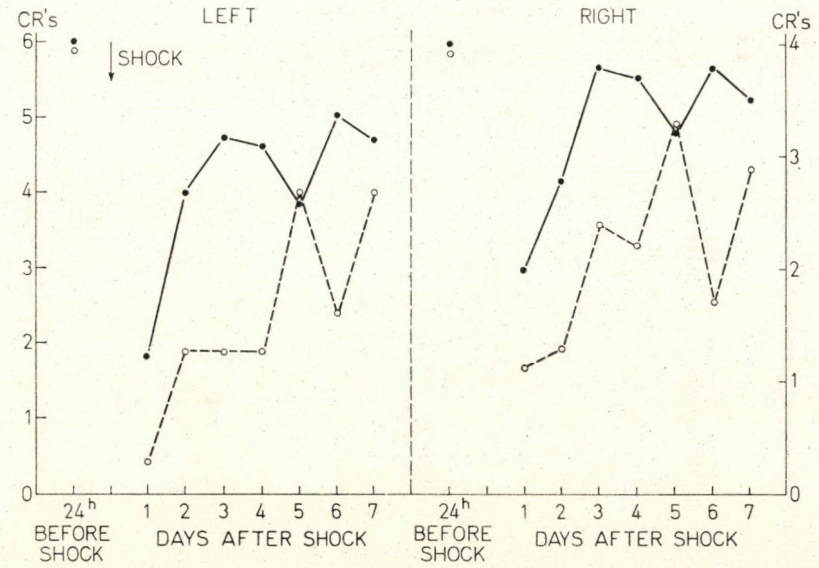

Fig. 4. Long term passive avoidance behaviour of rats in left-right discrimination situation following a single shock trial on the left side. Dexamethasone (●—●) or saline (○-----○) was given prior to the learning trial to water-deprived rats

Administration of 2.0 mg of cortisone prior to the learning trial failed to affect passive avoidance behaviour of rats while the shock intensity was 1.0 mA. A higher dose of steroid (4.0 mg/100 g b.w.) resulted in a decrease in the retention scores. The differences between cortisone-treated and control rats were significant both 24 ($p < 0.05$) and 48 hours ($p < 0.05$) after the learning trial. A similar difference could be found between rats treated with 2 and 4 mg of cortisone.

Immediate retention of a passive avoidance task in a space-discriminative drinking situation and the influence of a single dose of dexamethasone are shown in Fig. 3. Pairing the CS with an electric shock on the left water dish led to a complete suppression of the previously established conditioned behaviour towards the left side while the suppression of conditioned response towards the right, non-shocked side appeared partial during 40 trials immediately following the single shock trial in the controls. The rats exhibited substantial passive avoidance 24 and 48 hours after the learning trial towards the left side and a suppressed performance to the right one as well. Administration of dexamethasone 3 hours prior to the shock trial resulted in a less effective avoidance towards the shocked left side and a more effective response towards the right one as compared with the controls. The total number of conditioned drinking responses scored during 40 trials was significantly ($p < 0.05$; U-test) higher towards both sides in dexamethasone-treated rats than in the controls. Conditioned drinkig behaviour of dexamethasone-treated rats was nearly restored 24 hours after the shock trial. The number of CR's towards both sides was significantly higher in the treated rats. The probability level was 0.05 on the left side and 0.01 on the right one. 48 hours after the shock trial the differences were not significant.

The influence of dexamethasone administered prior to the single shock trial on the long term retention of passive avoidance in conditioned drinking situation is shown in Fig. 4. Marked suppression of the conditioned response performance towards the shocked side in space-discriminative situation was observed during the first and successive days after the learning trial in the controls. Conditioned behaviour then slowly improved but did not achieve the pre-shock level towards the left side during the 7-day test period. A significant suppression of conditioned behaviour towards the non-shocked right side was also observed. Meanwhile, the performance level was restored faster than on the left side. Significant passive avoidance towards the shocked side was observed in rats treated with a single dose of dexamethasone prior to the shock trial. However, the number of CR's scored during the first post-shock session was significantly higher than in the controls and the tendency to respond for signalled water reward was also higher during the next 6 sessions. The total number of CR's scored during the 7-day retention test was significantly ($p < 0.05$; U-test) higher in dexamethasone-treated rats than in

the controls. Conditioned response performance towards the non-shocked right side was also suppressed in dexamethasone-treated rats but this suppression was partial in comparison with the controls. The slope of the response curve was nearly the same in both groups but the total number of CR's scored during the retention trials was significantly ($p < 0.05$) higher in rats treated with dexamethasone.

Table I

Effect of a single dose of dexamethasone on conditioned discriminative drinking response in rats

Treatment	No. of rats	Conditioned responses					
		before treatment		during treatment		after treatment	
		Left	Right	Left	Right	Left	Right
Dexamethasone,	7	6.0±0.	4.0±0.	5.8±0.1	4.0±0.	6.0±0.	4.0±0.
Saline	7	6.0±0.	4.0±0.	6.0±0.	3.8±0.1	6.0±0.	4.0±0.

Table II

Effect of dexamethasone on 1-hr water intake of rats on a 23-hr water deprivation schedule

Treatment	No. of rats	Water intake			
		before treatment		during treatment	
		ml	ml/100 g b .w.	ml	ml/100 g b.w.
Dexamethasone	6	29.2±1.8*	25.6±1.1	27.6±2.5	24.1±1.8
Saline	6	31.6±1.3	26.9±1.0	30.1±1.3	25.7±1.6

* Mean ± S.E. of 3 days water intake

Table III

Effect of dexamethasone on open-field behaviour of water-deprived rats

Treatment	No. of rats	Ambulation	Rearing	Grooming	Defecation
Dexamethasone	14	153.5±18.5*	32.6±3.9	13.4±2.5	6.4±1.3
Saline	10	203.0±25.7	40.7±3.9	14.4±2.7	3.6±1.3

* Mean ± S.E.

Administration of a single dose of dexamethasone failed to influence the conditioned space-discriminative drinking behaviour of 7 rats as compared with 7 controls (Table I). Similarly, no change in 1-hour water intake was

observed following the administration of dexamethasone for 3 days to water-deprived rats (Table II).

Dexamethasone treatment resulted in a slight but not significant suppression of ambulatory activity in an open-field (Table III). Rearing, grooming and defecation scores of water-deprived rats were in the same order of magnitude in dexamethasone-treated rats and controls.

Discussion

The present findings suggest that corticosteroids are suppressing the passive avoidance of the rat based upon the inhibition of a previously fixed somato-motor pattern or of a genetically determined response. Suppression was apparent both in immediate and long term retention of passive avoidance responses. Thus, rats treated with dexamethasone exhibited a partial passive avoidance in a thirst-motivated, signal-controlled space-discriminative situation towards the shocked side in both immediate and long term retention tests. At the same time, discrimination between shocked and non-shocked sides was improved by a single dose of dexamethasone administered prior to the passive avoidance learning trial. Passive avoidance of rats treated with cortisone was also partial during immediate and long term retention of the inherent light-avoidance response inhibited by a single shock trial. The influence of steroid was a function of the dose of cortisone and of the intensity of shock. Passive avoidance elicited by a low shock intensity was significantly affected even by moderate doses of cortisone while the higher the intensity of shock, the more steroid was required to affect passive avoidance.

A profound influence of corticosteroids on conditioned avoidance behaviour has been demonstrated by this and other laboratories. Thus, a reduction of spontaneous intertrial activity in rats during active avoidance conditioning was observed due to the influence of corticosteroids whilst the acquisition of avoidance reponse was not affected (Bohus and Endrőczi 1965; Bohus and Lissák 1968). Furthermore, administration of various glucocorticosteroids enhanced the extinction of conditioned avoidance responses (de Wied 1967; Bohus and Lissák 1968). Since facilitation of extinction may result from an enhancement of internal inhibitory processes of Pavlovian terminology and the reduction of spontaneous intertrial activity may also be the function of enhanced discriminative internal inhibitory mechanisms (Endrőczi and Lissák 1962; Bohus and Endrőczi 1965), these and previous experiments in dogs and cats (Lissák and Endrőczi 1964) led to the hypothesis that the behavioural influence of corticosteroids manifests itself in an enhancement of internal inhibitory processes.

The present experiments seemed to indicate that the suppression of both the immediate and long term retention of passive avoidance was due to a

suppression of the fear-eliciting response inhibition. The facts which support this assumption are as follows. The failure of corticosteroids substantially to influence open-field activity, water intake of deprived rats and conditioned behaviour for reward indicates that neither exploratory activity nor the thirst-drive level and the previously fixed somato-motor patterns were primarily affected. Impairment of memorization, memory consolidation by corticosteroids may also be excluded since the immediate effect of shock trial was also suppressed. Evidence in favour of fear suppression by corticosteroids was presented in previous experiments. Spontaneous goal-directed activity may represent a drive level in signal-controlled situations (BRUSH 1962; ENDRŐCZI and LISSÁK 1962) and its reduction may be the function of discriminative internal inhibitory processes. Since corticosteroids are diminishing intertrial activity during one-way avoidance conditioning (BOHUS and ENDRŐCZI 1965; BOHUS and LISSÁK 1968) we have suggested that the mechanism of behavioural action of corticosteroids consists in a reduction of the fear level due to the enhancement of internal inhibitory processes (BOHUS and LISSÁK 1968; BOHUS and KORÁNYI 1969).

There are, however, observations suggesting that fear suppression by corticosteroids depends of several factors. Thus, in the present experiments the influence of cortisone on passive avoidance behaviour depended on the shock intensity eliciting fear. If the shock intensity was high, an aphysiologically high dose of steroid was necessary to affect passive avoidance retention. The fact that corticosteroid treatment failed to influence the acquisition of signalled avoidance responses (BOHUS and LISSÁK 1968; CONNER and LEVINE 1969) indicates that the repetition of reinforcement is also of importance in the fear-suppressing potency of corticosteroids. It was also shown that the inhibitory influence of fear conditioning prior to shuttle-box conditioning on avoidance acquisition was not affected by cortisone administered during fear or avoidance conditioning (BOHUS et al. 1971). Furthermore, cardiac acceleration which had developed during the signalled fear conditioning persisted throughout the extinction period in rats treated with cortisone while the extinction of cardiac acceleration due to non-signalled fear conditioning was not affected by the steroid (BOHUS et al. 1970). The above-mentioned data and the present findings may suggest that the suppression of fear by corticosteroids is highly dependent on the experimental situation whether fear-motivated response serves self-preservation like in active avoidance, or fear inhibits self-preservative response patterns like water intake after deprivation or light-avoidance as an inherent response. One cannot, however, exclude the possibility that the corticosteroids are influencing the relationship between concurrent drives — i.e. fear vs. thirst or fear vs. light-avoidance. The appearance of corticosteroid action then might be a function of the drive levels.

Electrophysiological and behavioural experiments suggest that the central nervous influence of corticosteroids manifests itself at the forebrain, mesencephalic and diencephalic levels (FELDMAN et al. 1961; FELDMAN and DAFNY 1966; FELDMAN and DAVIDSON 1966; SLUSHER et al. 1966; RUF and STEINER 1967; ENDRŐCZI et al. 1968; BOHUS 1968; VAN WIMERSMA GREIDANUS and DE WIED 1969; BOHUS 1970). Despite the fact that the mechanisms underlying the action of corticosteroids may be a facilitation of forebrain inhibitory influences upon the ascending activating system and a direct inhibitory influence on the reticular activating system (FELDMAN and DAFNY 1966; SLUSHER et al. 1966; ENDRŐCZI et al. 1968), the behavioural appearance of their action may be uniform in certain situations. Thus, it was observed that extinction of a one-way avoidance response is more rapid in rats with corticosteroid implants in both forebrain and diencephalic and mesencephalic reticular areas than in the controls (BOHUS 1968, 1970). Notwithstanding the fact that enhanced discrimination between shocked and non-shocked side in space-discriminative drinking conditioning during passive avoidance was found in dexamethasone-treated rats indicating an enhanced forebrain inhibitory function, it remains to be shown whether the corticosteroid-induced changes of passive avoidance behaviour are due to an action on the forebrain or on the reticular activating function.

It is generally known that stressful stimuli elicit an activation of the pituitary-adrenal axis. The observations discussed above indicated a profound influence of corticosteroids on the adaptive behavioural processes. Experimental evidence, however, suggests that ACTH may also affect animal behaviour through extra-adrenal mechanisms. Thus, it has been shown that ACTH facilitates active avoidance (BOHUS and ENDRŐCZI 1965; BOHUS et al. 1968) and passive avoidance learning in rats (LEVINE and JONES 1965) and mice (KORÁNYI et al. 1967), due presumably to their enhancing the fear level. Furthermore, ACTH and ACTH-like peptides delay the extinction of conditioned avoidance response (BOHUS and DE WIED 1966; DE WIED 1966; BOHUS et al. 1968). Since these effects of ACTH and that of corticosteroids act in an opposite way, hormonal conditioning of adaptive behavioural processes may assure a dynamic, situation-dependent control of animal behaviour.

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SUPERFICIAL AND DEEP CHEMONOCICEPTION: DIFFERENTIAL INHIBITION BY PRETREATMENT WITH CAPSAICIN

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Subcutaneous capsaicin pretreatment failed to block sensitivity to algogenic chemicals injected into deep tissues although it produced long-lasting insensitivity to irritants applied to the mucous membranes. In capsaicin-pretreated rats, sensitivity to intraarterial injections of acetylcholine and KCl was unchanged. The subplantar and intraarterial nociceptive threshold doses of capsaicin were elevated only by a factor of 10 to 100, and the inhibition was surmountable; the abdominal constriction response to intraperitoneal injection of acetic acid was not inhibited and that to intraperitoneal acetylcholine was even enhanced.

The nociceptive response to subplantar and intraarterial injections of capsaicin was blocked for at least 1 to 3 hours after intraperitoneal pretreatment with large doses of capsaicin (max. 200 mg/kg), but the sensitivity to injections of capsaicin was partially restored in 24 hours.

It is suggested that receptor sites on superficial nerve terminals may be inactivated by pretreatment with large doses of capsaicin without permanently impairing the response of some receptors mediating deep chemonociception.

There is general agreement that pain evoked from the surface of the body differs in quality from that originating in deeper structures. However BEECHER (1957) found no evidence of a difference in the mechanisms of superficial and deep pain, and LIM (1960) proposed that the same system of paravascular nerve endings transmits both cutaneous and visceral pain, with the difference that cutaneous nociception inevitably results in excitation of axons besides terminals while visceral pain is evoked by the consequence of noxious influences acting on the chemosensitive terminals.

In contrast, recent evidence strongly suggests that nerve fibres subserving chemonociception are different from the mechanonociceptors (JANCSÓ 1960; JANCSÓ et al. 1961, 1967; BURGESS and PERL 1967) and that there are various types of chemonociceptor mechanism (JANCSÓ et al. 1961; KEELE 1962; KANTOR et al. 1967; MAKARA et al. 1967).

JANCSÓ et al. (1961) have shown that in the rats and the guinea pig capsaicin pretreatment causes a long-lasting insensitivity to irritation by algogenic chemicals and suggested that the animals "become permanently insensitive to all kinds of chemical pain stimuli". However, this conclusion was based on experiments with irritants applied to the skin and mucous membranes, although it has been shown that both KCl applied to a lesioned skin area and capsaicin injected intraarterially evoked electrical activity in the

saphenous nerve of rats pretreated with capsaicin (PÓRSZÁSZ and JANCSÓ 1959). The present study had the aim of clarifying whether the same mechanism mediated superficial and deep chemonociception. Thus, the sensitivity to algesics applied by various routes in rats pretreated with large doses of capsaicin has been examined.

Methods

Animals. Rats of Wistar and CFE strains weighing 160 to 230 g were used. They were housed at 24 ± 1 °C and 65 ± 10% humidity and were given commercial rat food and tap water ad libitum. In all series the capsaicin pretreated and control rats were paired with regard of strain, sex and body weight.

Pretreatment with capsaicin. Systemic injections of capsaicin (a decylenic acid amide of vanillylamide, the pungent principle of red pepper), were used to inhibit chemonociception. The controls were injected with appropriate amounts of the vehicle simultaneously with the experimental groups.

All rats in group 1, 2, or 3 were injected subcutaneously with 4, 8, and 16 mg of capsaicin, respectively, at 8—12 hour intervals (JANCSÓ et al. 1961).

In group 1 the rats were tested by instilling 2% capsaicin solution into the eye one day after the last dose; if they responded with blepharospasm they were given an additional dose of 100 mg/kg of capsaicin. In group 2 the rats were also given a fourth dose of 200 mg/kg of capsaicin subcutaneously. In group 3 the rats were given intraperitoneally 10, 20, 40, 80, 120 and 200 mg/kg doses of capsaicin at intervals of 4 to 8 hours; the tolerance of these rats to capsaicin was considerably increased; normal rats usually died after the intraperitoneal injection of a 3 to 5 mg/kg dose.

Sensitivity to painful chemicals. The rats were placed singly in translucent plastic cages at least 30 minutes before the testing procedures began.

In subcutaneous tests, we injected 0.05 ml of sterile physiological saline or of capsaicin solution into the subplantar tissue of one hindpaw. We observed the rats for 240 seconds after the injection and measured the duration of lifting the hindpaw. When the test was repeated on the same day the second paw was injected at least 20 minutes after the first one.

Visceral chemonociception was studied by injecting intraperitoneally 10 ml/kg of 0.35% acetic acid in water or 16 mg/kg of acetylcholine bromide in 0.9% NaCl in rats of group 2. The rats were observed and the number of writhings was counted for 30 minutes.

In intraarterial tests we used the technique of DEFFENU et al. (1966). The rats were anaesthetized with ether and a fine polythene cannula was ligated into one carotid artery. At least 4 hours after recovery from the anaesthesia the drugs were injected in 0.5 ml/kg of saline and washed in with 0.15 ml of saline. In preliminary experiments there was no evidence of tachyphylaxis after injections repeated at 10 minute intervals. The threshold dose eliciting reproducible vocalization was established using a fixed sequence of drugs (capsaicin, acetylcholine and KCl) and increasing doses spaced by a factor of two. Injections were given at intervals of at least 10 minutes. When the tests were repeated the day after the cannulation, the thresholds usually differed by not more than a factor of two.

Drugs. Capsaicin of 98—99% purity (obtained from Industrija Paprike, Horgos, Yougoslavia) was dissolved in physiological saline at 2% concentration with 5% ethanol and 10% Tween 80 and was further diluted with saline. Acetylcholine bromide (Hoffmann—LaRoche), histamine dihydrochloride (Reanal), morphine sulphate and acetylsalicylic acid were also used.

Statistical methods. Intraarterial nociceptive thresholds were transformed to logarithms and the groups were compared with the sequential *t*-test (ARMITAGE 1960). Data concerning the subplantar test were analysed by analysis of variance and writhing responses by the Mann—Whitney method.

Results

Chemical instilled into the eye. When capsaicin solution was instilled into the eye and the duration of blepharospasm was measured in control rats, the log-concentration-response curve was linear and threshold concentration was

about 10^{-6} g/ml. Rats pretreated with capsaicin did not respond with blepharospasm even to $2 \cdot 10^{-2}$ g/ml capsaicin, 1% hydrochloric acid or 3% formaldehyde instilled into the eye.

Subplantar injection. In preliminary experiments capsaicin and other pain inducing substances were injected at different subcutaneous sites. Injections into the hindpaw produced the best response; the rats lifted the hindpaw injected with the algogen but only a few rats lifted for some seconds the

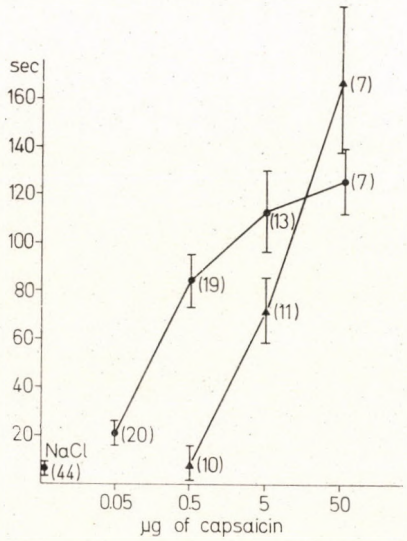

Fig. 1. Effect of capsaicin pretreatment on lifting the hindpaw induced by subplantar injection of capsaicin. Ordinate, duration in sec of hindpaw lifting; abscissa, logdose of capsaicin. Number of tests in brackets. Vertical bars give ±S.E. Rats pretreated with capsaicin ▲ or vehicle ●

paw injected with physiological saline. The response to 0.05 μg capsaicin was significantly longer ($p < 0.05$) than that to physiological saline (Fig. 1).

In group 1 we compared the approximate thresholds of capsaicin as the logdose-response curves of the control and capsaicin pretreated rats were not parallel. The threshold was between 0.005 and 0.05 μg in the controls and was elevated to about 0.5 μg in the capsaicin pretreated rats (Fig. 1).

In group 3 the rat's behaviour was normal but none of the ten rats responded even to 1 mg of capsaicin injected into the hindpaw two to three hours after the last intraperitoneal dose of the pretreatment. This meant a more than 20 000 fold increase in threshold. At the same time, there was a normal response to the subplantar injection of 0.25 mg of formaldehyde: the rats lifted the hindpaw for more than 240 seconds. The sensitivity to subplantar capsaicin returned after an interval of 22 hours; at this time even 5 μg of capsaicin produced a prolonged lifting of the hindpaw (61.3±11.7 seconds).

Intraperitoneal injection. According to CARROL and LIM (1958) the receptors for the writhing response appear to be in the gastrointestinal tract. In group 2 the sensitivity to visceral chemonociceptors was assessed by intraperitoneal injections of acetic acid and acetylcholine. Both substances produced significant responses in rats pretreated with capsaicin; when compared to the controls, the response to acetic acid was not inhibited and that to acetylcholine was enhanced (Table I).

Intraarterial injection. Behavioural responses to intraarterial algogens consisted of vocalization, torsion of the body to the side of the cannula, and running. The vocalization response was selected as an indicator of nociceptive activity (LIM 1968) since the other components of the reaction changed parallel with the vocalization. The response to threshold doses of capsaicin was significantly inhibited within 30 minutes after the intraperitoneal injection of morphine sulphate (10 mg/kg) or acetylsalicylic acid (500 mg/kg).

Table I

Writhing response after intraperitoneal injection of acetic acid and acetylcholine in rats pretreated with capsaicin (4, 8, 16 mg and 200 mg/kg, subcutaneously)

	Number of writhings in 30 minutes	
	control*	capsaicin pretreated*
0.35% acetic acid	21 (0—27)	12 (0—23)
16 mg/kg acetylcholine	3 (1—10)	14 (6—21)**

* Each group consisted of 12—13 rats. Figures are medians with the range in brackets
** Different from its control (Mann—Whitney test: $p < 0.05$)

Table II

*Nociceptive (vocalization) threshold doses of capsaicin, acetylcholine and KCl injected retrogradely into the carotid artery in rats pretreated with capsaicin (4, 8, and 16 mg subcutaneously)**

	Vocalization threshold dose (geometric means and 95% confidence limits)					
	Control rats			Capsaicin pretreated rats		
	No. of rats	threshold dose		No. of rats	threshold dose	
Capsaicin (μg/kg)	20	2.5	(1.3—4.6)	11	28	(16—49)**
Acetylcholine (mg/kg)	12	0.41	(0.22—0.76)	7	0.62	(0.22—1.97)***
KCl (mg/kg)	14	24	(12—36)	11	24	(17—35) ***

* 3 to 4 days after pretreatment
** control versus pretreated: $p < 0.05$
*** within the mean $\pm \sigma$ range of the controls ($p < 0.05$)

In rats of group 1, three to four days after subcutaneous pretreatment with capsaicin the threshold dose of capsaicin was about 10 times that found in the controls. In contrast, the intraarterial threshold doses of acetylcholine and KCl were the same in the control and the capsaicin pretreated animals (Table II). Fifty to 60 days after pretreatment, the intraarterial capsaicin threshold was still about 10 times higher than in the controls.

In group 3 we have attempted to block the response to intraarterial algogens by intensive intraperitoneal pretreatment. The carotid artery was cannulated the day after the last pretreatment dose. The rats responded with vocalization to 370 μg/kg (confidence limits, 725—100) of capsaicin; this threshold was about 100 times higher than that in the controls. Then the cannulated rats were given a further intraperitoneal injection of 200 mg/kg of capsaicin, which killed three of the ten rats. However, 1 to 3 hours later the surviving rats failed to vocalize even after intraarterial injection of 20 000 μg/kg capsaicin, while they responded with vocalization to pinching the tail with forceps. Four of them were tested one day later; their threshold then was about 300—400 μg/kg of capsaicin.

Discussion

The present experiments have confirmed that long-lasting insensitivity to algogenic chemicals applied to the skin and mucous membranes is induced by systemic pretreatment with capsaicin (JANCSÓ et al. 1961). The complete insensitivity of the superficial chemonociception contrasts sharply with the finding that the nervous elements subserving nociception in some subcutaneous, visceral and periarterial tissues remain chemically excitable. It follows that the conclusion drawn from experiments with superficial application of algogens cannot be generalized to all tissues and the rats pretreated with capsaicin are not permanently insensitive to all kinds of chemical painful stimuli.

There were quantitative and qualitative differences in the effect of capsaicin pretreatment on superficial and deep chemonociception. First, the superficial nociceptive threshold was elevated by more than $2 \cdot 10^4$, whereas the subplantar and intraarterial thresholds of capsaicin were elevated by a factor of 10 to 10^2 three days after pretreatment. Second, the skin and mucous membranes are permanently insensitive to *all* pain-causing chemicals (JANCSÓ et al. 1961) but the inhibition of the deep chemonociception was chemospecific: the intraarterial thresholds of acetylcholine and KCl were unchanged but that of capsaicin was elevated. Third, recovery of the superficial chemonociception takes several weeks (JANCSÓ 1960; JANCSÓ et al. 1961) whereas partial recovery of sensitivity to subplantar and intraarterial injections of capsaicin takes no more than 24 hours.

These differences may be explained by postulating at least two operationally different types of receptor sites in the rat. (i) The first type of receptor site may mediate chemonociceptive responses of the superficial nerve endings in the skin and the mucous membranes; irreversible and non-specific blockade of these receptors can be induced by capsaicin pretreatment. These characteristics are in accordance with the conclusions of JANCSÓ et al. (1961). This set of receptors may be differentiated by pharmacological blockade or desensitization. The receptors for pain production by K^+, serotonin and bradykinin are different from that activated by acetylcholine; and those activated by serotonin and bradykinin are not identical (JANCSÓ et al. 1961; KEELE 1962). (ii) A second type of receptor site on the nerves and terminals in subplantar, visceral and periarterial tissues is not permanently blocked by capsaicin pretreatment. These receptors mediate the nociceptive effects of capsaicin, acetylcholine and KCl. Their response to small amounts of capsaicin may be inhibited but the inhibition is surmountable. This difference in inhibition by capsaicin pretreatment might reflect some differences in the mechanism by which the neural action potential is initiated and/or inhibited at these receptor sites.

The suggestion that chemonociceptors at different anatomical loci have different functional characteristics may be supported by the facts (i) that acetylsalicylic acid did not readily block the nociceptive response to intradermal bradykinin (COLLIER and LEE 1963; LIM 1968) but it suppressed the response to the same drug injected intraperitoneally or intraarterially (GUZMAN et al. 1964; DICKERSON et al. 1965), and (ii) in man, small doses of bradykinin injected intraperitoneally or intraarterially produce intense pain whereas the same drug fails to evoke pain in 10^4 times higher doses when injected by the intramuscular and subcutaneous route (KANTOR et al. 1967). BARAZ et al. (1968) also postulated two different receptor sites for capsaicin in the vascular bed of the small intestine.

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Pathophysiologia

DIABETOGENICITY OF KETOHEPTOSES

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Eight ketoheptoses were tested in the rat. Blood glucose was determined by glucose oxidase method. D-mannoheptulose was verified as causing hyperglycaemia in the rat; D-glucoheptulose and L-galactoheptulose were also found to cause this effect. L-guloheptulose, D-idoheptulose, L-mannoheptulose, L-glucoheptulose and D-galactoheptulose did not significantly demonstrate such activity when tested at the 200 mg dose level.

In their studies with the rabbit, ROE and HUDSON (1936) concluded: "Mannoheptulose administration brought about a marked increase in fermentable sugar of the blood — when insulin was injected, fermentable sugar completely disappeared from the blood after mannoheptulose administration". SIMON and KRAICER (1957) first showed this fermentable sugar to be glucose. They then proceeded to identify other characteristics of the diabetic syndrome produced, including glucosuria, ketosis (SIMON et al. 1961), gluconeogenesis and the effect of adrenalectomy and hydrocortisone supplantation (SIMON et al. 1962). PAULSEN et al. (1967) have shown the effect of the diabetogen by following blood insulin levels directly. Sedoheptulose has been shown not to behave in this manner (SIMON et al. 1961).

In surveying the activity of other ketoheptoses in this regard, the simplest, most straight forward method was chosen — following the curve of blood glucose response to injections of relatively large doses of test material.

Methods

Male albino rats, weighing 180—220 g, were used. The animals were fasted 16 hr before injection. Each ketoheptose to be tested was injected s.c. into three animals at 200 mg/animal (2 ml of 10% soln.). The blood glucose was determined before injection, and then at 1/2, 1, 2, 3 1/2 and 5 hr after injection. Glucose oxidase method was employed. As controls, three animals were injected similarly with 2 ml normal saline and their blood glucose pattern followed in like manner.

D-mannoheptulose had m.p. 151°, D-glucoheptulose 171°, L-glucoheptulose 171°, L-galactoheptulose 101°; L-guloheptulose was prepared by *Acetobacter suboxydans* oxidation of sedoheptitol (m.p. 127—9°). D-idoheptulose was prepared by the method of JONES (1954) forming idoheptulosan, m.p. 169°. D-galactoheptulose and L-mannoheptulose were prepared by cyanohydrin reaction on D-galactose and L-mannose, followed by reduction and isomerization at 37° in saturated $Ca(OH)_2$. These failed to crystallize, however their identities and purities were verified by chromatographic homogeneity and comparison with their optical antipodes. All syrupy preparations were desiccated to constant weight before use.

Table I

		½ hr	1 hr	2 hr	3½ hr	5 hr
Saline Controls:						
number	1)	24	34	2	3	7
	2)	2	3	−15	−8	0
	3)	−24	10	−5	−5	0
D-Mannoheptulose:						
number	1)	33	58	88	60	48
	2)	30	82	82	10	−1
	3)	17	52	70	33	0
D-Glucoheptulose:						
number	1)	74	92	92	72	60
	2)	30	72	65	15	0
	3)	24	50	50	40	30
L-Galactoheptulose:						
number	1)	52	60	35	15	12
	2)	30	54	50	24	28
	3)	24	46	45	22	21
L-Mannoheptulose:						
number	1)	28	23	−5	5	10
	2)	21	28	10	8	12
	3)	16	26	−3	0	24
L-Glucoheptulose:						
number	1)	36	25	23	23	23
	2)	14	15	14	22	26
	3)	12	13	−5	0	5
D-Galactoheptulose:						
number	1)	27	24	−2	7	20
	2)	5	5	4	5	5
	3)	1	0	−25	−18	−5
L-Guloheptulose:						
number	1)	25	28	28	27	27
	2)	7	12	12	13	12
	3)	−8	−2	−24	−23	−23
D-Idoheptulose:						
number	1)	13	15	11	26	24
	2)	5	6	6	5	5
	3)	−24	−20	−26	−25	−23

Results

The Table I shows the data observed on treating the nine groups of three animals each. The values shown indicate the elevation in mg% glucose above the initial, fasting level of each animal. Negative values indicate drops below initial levels. In each group the first animal listed is that one demonstrating the most dramatic response, and does not reflect any chronological order. Controls indicate levels above which responses to ketoheptoses may be considered valid.

Discussion

While the number of animals thus far tested with each of the ketoheptoses is small, the work done to this pont indicates that only D-mannoheptulose, D-glucoheptulose and L-galactoheptulose may be said to exhibit diabetogenic activity in the rat. The others, if they do evoke any such response, must be relegated to a much lower potency.

Immediately one sees that the ketoheptoses include two, multimembered groups in regard to diabetogenicity, i.e. neither of the previously tested ketoheptoses, D-mannoheptulose or sedoheptulose, is unique in this respect. Further, these observations indicate that only one member of a D, L pair of ketoheptoses is active, and that this activity is not limited explicitly to either D or L conformers. This suggests that the actual shape of the ring is not a rigid steric requirement for the activity. However, the lower activity apparent in L-galactoheptulose does indicate a possible, partial dependence upon such a requirement.

In viewing the lower potency exhibited by L-galactoheptulose, however, there is an additional possible steric effect to be considered. The glucoheptuloses and mannoheptuloses are known not to mutarotate. The galactoheptuloses, as well as the taloheptuloses are known to mutarotate. Quantitative analyses of their anomeric equilibrium compositions are not definable. Similarly, it is not yet possible to assign any quantitative relative potency to each of the three ketoheptoses revealing diabetogenic activity. It remains a possibility nonetheless that the stereochemistry about the anomeric position may be of true importance. Testing of taloheptulose seems indicated in this connection.

Of importance issuing from such observations is the possibility that those ketoheptoses which undergo extensive heptulosan formation may not be subject to reliable testing by exogenous injection, as such heptulosan formation alters the anomeric structure. This group consists of the guloheptuloses, idoheptuloses and altroheptuloses, of the last of which the D conformer is the important mammalian metabolite, sedoheptulose.

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BESTIMMUNG DER GUANIDYLESSIGSÄUREKONZENTRATION IM BLUTSERUM UND LIQUOR CEREBROSPINALIS

Von

Katalin A. Szilágyi

AUS DEM BIOCHEMISCHEN FORSCHUNGSLABORATORIUM DER STAATLICHEN HEILANSTALT FÜR NERVEN- UND GEISTESKRANKHEITEN, BUDAPEST

(Eingegangen am 19. August 1970)

Eine zur Bestimmung von Guanidylessigsäure (GES) im Serum und Liquor geeignete, auf bekannten Trennprinzipien beruhende Methode wird beschrieben. Für eine Gruppe von Patienten mit verschiedenen neuropsychiatrischen Krankheitsbildern wurde in guter Übereinstimmung mit in der Literatur für normale Versuchspersonen angegebenen Werten ein Durchschnittswert von 1,8 μg GES/ml Serum ($\delta = 0{,}4$) erhalten. Für den Liquor-GES-Spiegel wurde ein Durchschnittswert von 0,23 μg/ml gefunden. Bei keiner der im untersuchten Krankengut vorkommenden Krankheiten konnten charakteristische Veränderungen der Serum- bzw. Liquor-GES-Konzentration beobachtet werden. An Hand dieser Feststellungen werden Ergebnisse früherer, sich mit der Konzentration bzw. Verteilung der gesamten monosubstituierten Guanidylverbindungen im Serum und Liquor befassenden Arbeiten präzisiert.

In früheren Arbeiten (Szilágyi und Pataky 1966a, b; Szilágyi 1967) haben wir monosubstituierte Guanidylverbindungen im Serum und Liquor von Patienten mit verschiedenen neuropsychiatrischen Krankheiten bestimmt und unter anderem festgestellt, daß im Serum von Epileptikern die Konzentration dieser Verbindungen signifikant erniedrigt ist (Mittelwert — als Arginin — 15,9 μg/ml gegenüber 20,0 μg/ml bei Kontrollen) und besonders bei Gehirnatrophien im Liquorsystem eine typische Konzentrationsverteilung in caudocranialer Richtung vorliegt.

Bei diesen Untersuchungen wurde die von Gilboa und Williams (1956) angegebene kolorimetrische, auf der Sakaguchi-Reaktion beruhende Methode zur Bestimmung von monosubstituierte Guanidylgruppen tragenden Verbindungen, und zwar ohne diese vorher aufzutrennen, angewandt. Zwar ist somit nur diese Verbindungsklasse als Ganzes erfaßt worden, aber es besteht kein Zweifel, daß die erhaltenen Werte im wesentlichen dem Arginin zuzuschreiben sind, denn die in der Literatur angegebenen, nach mehreren anderen Methoden und z.T. nach vorangegangener Trennung bestimmten Serum- und Liquorargininingehalte stimmen mit den von uns erhaltenen Konzentrationen an monosubstituierten Guanidylverbindungen gut überein. Andere die betreffende Farbreaktion gebende Verbindungen können somit nur in wesentlich geringerer Konzentration als Arginin vorliegen.

Von monosubstituierten Guanidylverbindungen wurden bisher im Serum außer Arginin nur die im Stoffwechsel als Zwischenprodukt bei der Kreatin-

synthese auftretende Guanidylessigsäure (GES) und — in sehr geringen und mit den zur Verfügung stehenden Methoden quantitativ nicht genügend zuverlässig zu erfassenden Mengen — Methylguanidin nachgewiesen. Nach VAN PILSUM und Mitarb. (1956) liegt die GES-Konzentration unter 3 μg/ml, die des Methylguanidine unter 0,2 μg/ml. Im Liquor wurden bisher außer Arginin noch keine anderen monosubstituierten Guanidylverbindungen nachgewiesen.

Bei unseren bisherigen Untersuchungen blieb die Frage offen, ob die beobachteten, oben erwähnten Unterschiede in der Konzentration der monosubstituierten Guanidylverbindungen in Seren bzw. Liquors nicht vielleicht gänzlich oder doch zu einem wesentlichen Teil auf ausgeprägte Veränderungen der Konzentration einer in wesentlich geringerer Menge als Arginin vorliegenden monosubstituierten Guanidylverbindung (wobei nur GES in Frage kommt) oder auf sich in Grenzen haltende Veränderungen des Argininingehaltes zurückzuführen sind. Um diese Frage entscheiden zu können, wurde in der vorliegenden Arbeit eine für die Trennung der Guanidylessigsäure von Arginin im Serum und Liquor geeignete Modifikation von im Prinzip bekannten Trennmethoden ausgearbeitet und eine Reihe derartiger Bestimmungen vorgenommen.

Methodik

Die auf der Sakaguchi-Reaktion basierende kolorimetrische Bestimmung von GES im Serum und Liquor setzt eine Abtrennung des Eiweißes und der anderen monosubstituierten Guanidylverbindungen voraus. GES ist wegen der benachbarten Stellung der Guanidyl- und Carboxylgruppen im Molekül wesentlich schwächer basisch als Arginin oder Methylguanidin. Diesem Umstand ist es zu verdanken, daß GES im Gegensatz zu den basischeren Verbindungen nicht von in der H-Form vorliegenden schwach sauren (Carboxylgruppen tragenden) oder in der Na-Form vorliegenden stark sauren (Sulfosäurengruppen tragenden) Kationenaustauschharzen sorbiert wird und somit eine Möglichkeit gegeben ist, GES relativ einfach und vollständig abzutrennen (GERBER und Mitarb. 1961; ROBIN und VAN THOAI 1967).

Da in einem ml Probeflüssigkeit größenordungsmäßig 1 μg der zu bestimmenden Substanz enthalten sein muß, um quantitativ gut auswertbare Extinktionen zu erhalten, darf folglich im Falle des Serums keine wesentliche Verdünnung der GES im Verlaufe der Abtrennung erfolgen. Im Falle des Liquors, wo eine wesentlich geringere GES-Konzentration zu erwarten ist, ist sogar eine Konzentrierung erforderlich.

Die bei der üblichen Abtrennung der Eiweiße nach Folin-Wu oder mit Trichloressigsäure eintretende Verdünnung der zu untersuchenden Körperflüssigkeiten und das Einbringen großer Mengen von Salzen (die Trichloressigsäure müßte neutralisiert werden, da die Trennung am Ionenaustauscher im neutralen Medium ausgeführt werden muß), die eine nachträgliche Konzentrierung durch Eindampfen unmöglich machen, schließen die Anwendung dieser Enteiweißungsmethoden aus. Dagegen hat sich die Enteiweißung durch Gelfiltration über Sephadex G25 oder G50 als zweckmäßig erwiesen. Als Elutionsmittel ist eine Salzlösung zu verwenden, um ein Ausfallen von Eiweißen auf dem Sephadex zu vermeiden, jedoch soll der Salzgehalt dieser Lösung möglichst gering sein, damit die Konzentrierung des durch die Austauschersäule durchgetretenen Eluates nicht erschwert wird. Diesem Kompromiß wird eine 0,1%ige Kochsalzlösung am besten gerecht.

Da bei diesem Trennverfahren der in den Körperflüssigkeiten enthaltende Harnstoff in der GES-Fraktion verbleibt und die nach den üblichen Methoden ausgeführte Sakaguchi-Farbreaktion etwas im Sinne einer Verringerung der Extinction beeinflußt, empfiehlt sich die Anwendung der von DUBNOFF und BORSOOK (1941) angegebenen Modifikation der Sakaguchi-Reaktion, bei der schon vor der Farbentwicklung ein großer Harnstoffüberschuß zugegeben und somit ein störender Effekt der aus den Körperflüssigkeiten stammenden Harnstoffmengen ausgeschaltet wird.

Im folgenden ist die genaue Vorschrift angegeben, nach der die im weiteren beschriebenen Untersuchungen ausgeführt wurden.

Die Abtrennung der Eiweiße erfolgt in einer mit Sephadex G50 gefüllten Säule (1 m Länge, 12 mm Durchmesser), die mit einer chloroformgesättigten, 0,1%igen NaCl-Lösung durchgespült wurde. Auf die Säule werden 3 ml Blutserum bzw. 5 ml Liquor aufgegeben und mit dieser NaCl-Lösung eluiert, wobei die Elutionsgeschwindigkeit etwa 15 ml/h beträgt. Die ersten 105 ml des Eluates, in denen das ganze Eiweiß enthalten ist, werden verworfen, die nächsten 60 ml aber über eine mit einem in der Na-Form vorliegenden stark sauren Kationenaustauscher gefüllte Säule (13 cm lang, 8 mm Durchmesser) gegeben, die danach mit 10 ml dest. Wasser ausgewaschen wird. Das gesamte Eluat wird im Falle des Blutserums auf 5 ml eingedampft, filtriert und zweimal je 2 ml des Filtrates für die photometrische Bestimmung verwendet. Im Falle des Liquors wird das Eluat auf 1 ml eingedampft, filtriert, das Filterpapier mit 1 ml dest. Wasser ausgewaschen und das gesamte Filtrat (2 ml) für die Farbreaktion verwendet, die folgendermaßen ausgeführt wird: Zu 2 ml des betreffenden, im Eisbad abgekühlten Filtrates werden 0,4 ml einer vor dem Gebrauch im Verhältnis 1 : 5 mit H_2O verdünnter, 0,2%iger alkoholischer α-Naphthol-Lösung, danach 0,4 ml einer 4%igen Harnstofflösung und schließlich 0,4 ml einer durch Auflösen von 0,3 ml Brom in 100 ml einer 5%igen NaOH-Lösung hergestellten Natriumhypobromit-Lösung zugegeben (alle Reagenzlösungen werden vor Gebrauch im Eisbad auf etwa 0°C abgekühlt). Nach 20 minutigem Stehen im Eisbad wird die Intensität der sich entwickelten orangeroten Färbung des Gemisches photometrisch bei 510 mμ bestimmt. Zu jeder Messung werden einige Blindversuche mit dest. Wasser ausgeführt. Die »recovery« der Methode, auf die später noch näher eingegangen wird, beträgt etwa 70%.

Für die Bestimmung der GES-Konzentration wurden Seren von 53 in stationärer Behandlung stehenden Patienten verwendet. Die Hauptdiagnosen waren (in Klammern die Anzahl der Fälle): Alcoholismus chr. (15), Epilepsie (14), Schizophrenie (5), Gehirnarteriosklerose (5), Taboparalyse (2), senile Demenz (2), Psychoneurose (2), Psychopathie (2), Depression (2), extrapyramidale Hyperkinese mit ungeklärter Ätiologie (1), M. Wilson (1), M. Pick (1), Contusio cer. (1).

Die Bestimmung der GES wurde an einem cysternal und 16 lumbal entnommenen Liquoren durchgeführt, die bei der pneumoencephalographischen Untersuchung von Patienten mit verschiedenen neuropsychiatrischen, zum Teil eine Hirnatrophie aufweisenden Krankheitsbildern anfielen. Die entnommenen Liquormengen waren von Fall zu Fall verschieden (bis 60 ml). In 2 Fällen wurde der Liquor in Fraktionen von je 10 ml in kalibrierten Röhrchen aufgefangen, um aus verschiedenen Höhen des Liquorsystems stammende Proben für die Untersuchung zur Verfügung zu haben.

Ergebnisse und Diskussion

Für die untersuchten 53 Seren wurde ein GES-Konzentrations-Mittelwert von 1,28 μg/ml mit einer Standardabweichung (σ) von 0,31 μg/ml erhalten. Diese Streuung ist aber nur zum kleineren Teil durch methodische Fehler bedingt, denn aus mit jeweils dem gleichen Serum ausgeführten Parallelbestimmungen (die stets dann durchgeführt wurden, wenn genügend Serum zur Verfügung stand) folgt, daß der experimentelle Fehler innerhalb von $\pm 12\%$ lag.

Es deutet auch nichts darauf hin, daß die Heterogenität des untersuchten Krankengutes mit der Streuung im Zusammenhang steht. Alkoholiker und Epileptiker in getrennten Gruppen zusammengefaßt wiesen praktisch den gleichen Mittelwert und die gleiche Standardabweichung wie die Gesamtgruppe auf. Für die anderen Krankheiten läßt zwar die jeweils zu geringe Zahl der Fälle keine statistische Auswertung zu, aber aus der Lage der einzelnen Werte innerhalb der Gesamtverteilung ist zu erkennen, daß bei keiner der in dem betreffenden Krankengut vorkommenden Krankheiten typische Verände-

rungen der Serum-GES-Konzentration nach kleineren oder größeren Werten hin auftreten bzw. zu erwarten sind.

Die Häufigkeitsverteilung der einzelnen Konzentrationswerte ist in Abb. 1 dargestellt. Aus dieser Abbildung ist — besonders wenn man die für eine solche Darstellung relativ geringe Zahl der Fälle berücksichtigt — zu ersehen, daß eine gut angenäherte Normalverteilung vorliegt. Es ist deshalb

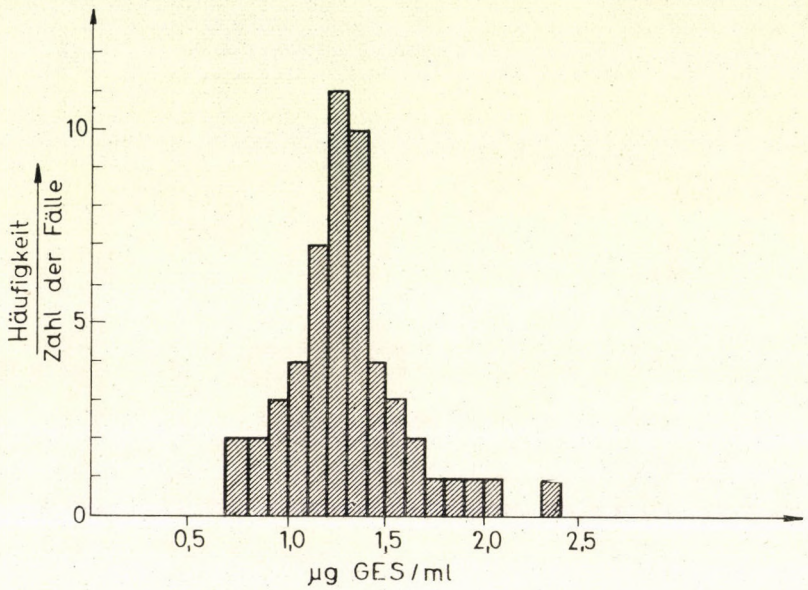

Abb. 1

wahrscheinlich, daß es sich hierbei entweder um die normale physiologische Verteilung handelt oder die Streuung auf verschiedene, nicht erfaßte oder zu erfassende, aber vom ätiologischen Standpunkt aus unwesentliche Faktoren (z. B. Diät, Muskelarbeit) zurückzuführen ist.

Wir haben ebenfalls untersucht, ob mit der Methode GES im Serum und Liquor vollständig oder nur teilweise erfaßt wird, also »recovery«-Bestimmungen durchgeführt. Zu diesem Zwecke wurden sowohl reine als auch Arginin im großen Überschuß enthaltende GES-Lösungen verschiedener Konzentration und mit bekannten GES-Mengen angereicherte Seren verwendet. Es wurde festgestellt, daß jeweils nur durchschnittlich 70% der angewandten bzw. zugesetzten GES-Mengen wiedergefunden werden, wobei die einzelnen recovery-Werte innerhalb der experimentellen Fehlergrenze der Methode zwischen 64 und 78% lagen. Es ist zu vermuten, daß der Verlust durch Adsorption eines Teiles der GES an den Säulen zustande kommt.

Unter Berücksichtigung der experimentell ermittelten recovery von 70% beträgt also der in dieser Arbeit festgestellte Mittelwert der GES-Konzentration in Blutseren 1,8 μg/ml.

SRIKANTIA und Mitarb. (1965) haben neben Kreatin, Kreatinin und Arginin auch GES im Serum von an Kwashiorkor und Marasmus leidenden Kindern untersucht. Die von diesen Autoren angegebenen Mittelwerte der GES-Serum-Konzentration betragen bei Marasmus 4,0 μg/ml und bei Kwashiorkor 2,9 μg/ml vor und 4.0 μg/ml nach der Behandlung. Da diese Krankheiten aber mit weitgehenden allgemeinen Störungen des Stoffwechsels einhergehen, ist ein Vergleich mit den Ergebnissen der vorliegenden Arbeit nicht angebracht. BERTOLINI und Mitarb. (1962) haben dagegen GES (neben Arginin, Kreatin und Kreatinin) im Serum and Urin von gesunden Versuchspersonen verschiedenen Alters nach der Methode von VAN PILSUM und Mitarb. (1956) bestimmt. Für den Serum-GES-Spiegel wurden für die dem Lebensalter nach in 10-Jahresgruppen zusammengefaßten 20- bis 60-jährigen Mittelwerte von 1,87 bis 2,06 μg/ml (σ = 0,4 bis 0,7), für die 60- bis 80-jährigen dagegen höhere Mittelwerte (3,0 und 2,75 μg/ml) gefunden.

Wir konnten bei über 60-jährigen Patienten keine Abweichung der Serum-GES-Konzentration nach höheren Werten hin feststellen, allerdings fielen auch nur 7 der von uns untersuchten 53 Personen in diese Altersgruppe.

Die von BERTOLINI und Mitarb. (1962) für gesunde 20- bis 60-jährige Versuchspersonen gefundenen Mittelwerte und Streuungen der Serum-GES-Konzentration stimmen gut mit den von uns für Patienten mit verschiedenen neuropsychiatrischen Krankheiten ermittelten Werten überein, was ebenfalls die obengetroffene Feststellung unterstützt, daß zwischen diesen Krankheiten und dem GES-Spiegel kein Zusammenhang besteht.

Die eingangs erwähnte, in einer früheren Arbeit (SZILÁGYI und PATAKY 1966b) beschriebene Beobachtung, daß bei Epileptikern die Konzentration der monosubstituierten Guanidylverbindungen im Serum signifikant erniedrigt ist, läßt sich nun an Hand der Befunde dieser Arbeit dahingehend präzisieren, daß es sich dabei um eine Erniedrigung des Arginin-Spiegels handelt, während der GES-Spiegel keine Abweichungen vom Normalwert zeigt.

Für die untersuchten Liquors wurde ein Mittelwert von 0,16 μg GES/ml (σ = 0,07) erhalten. Derartig geringe GES-Gehalte kommen aber den absoluten Fehlern der Methode schon ziemlich nahe, das heißt, daß die gemessenen Werte mit verhältnismäßig großen relativen Fehlern behaftet sind, deren Streubreite etwa der ermittelten Standardabweichung von annähernd 50% des Mittelwertes entspricht.

Berücksichtigt man, daß die recovery der Methode nur etwa 70% beträgt, so ergibt sich für den GES-Spiegel des Liquors ein Mittelwert von 0,23 μg/ml. In der Literatur liegen bisher noch keine Arbeiten über die Bestimmung bzw. den Nachweis von GES im Liquor vor.

Die Liquor-GES-Konzentration beträgt also nur etwa 10% der des Serums und ist — ebenfalls wie der Serum-GES-Spiegel — bei verschiedenen neuropsychiatrischen Erkrankungen innerhalb der allerdings ziemlich weiten Fehlergrenzen der Methode unverändert. Innerhalb dieser Fehlergrenzen liegen auch die Werte, die für den GES-Gehalt des untersuchten cysternalen Liquors und der aus verschiedenen Höhen des Liquorsystems stammenden Fraktionen erhalten wurden.

In einer früheren Arbeit (SZILÁGYI 1967) haben wir festgestellt, daß der Gehalt an monosubstituierten Guanidylverbindungen in den aus verschiedenen Höhen des Liquorsystems stammenden Fraktionen lediglich bei der Mehrzahl der Hirnatrophien gleich ist, im allgemeinen aber Unterschiede, nämlich in der Fraktion von 20 bis 30 ml und zum Teil auch in höheren Fraktionen um 1 bis 2 μg/ml höher liegende Werte als in den ersten 10 ml aufweist. Da die GES-Konzentration um eine Größenordnung niedriger liegt als diese Unterschiede und in allen Fällen nur innerhalb der experimentellen Fehlergrenzen variiert, ist somit als erwiesen anzusehen, daß diese typische Verteilung der monosubstituierten Guanidylverbindungen im Liquorraum nicht durch Veränderungen der GES-Konzentration, sondern lediglich durch die der Arginin-Konzentration bedingt sein kann.

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Pharmacologia

THE EFFECT OF CHLOROQUINE ON THE ANTIGEN-ANTIBODY REACTION

By

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Chloroquine has been shown to inhibit the formation of the antigen-antibody complex in vitro, and to enhance the dissociation of such complexes; both effects are dose-dependent.

In guinea pigs, treatment with daily 20 mg/kg of chloroquine over 4 weeks did not interfere with the anaphylactic reaction, while it inhibited the systemic and local manifestations elicited by the antigen-antibody complex.

It has been known for several years that chloroquine had a blocking effect on the antigen-antibody reaction (Haberland 1960; Domokos and Aszódi 1964c). Experiments have, however, been made only with cellular antigens, i.e. blood cells (Domokos and Aszódi 1964a; 1964b). The present study has been devoted to the effect of chloroquine on the reactions of ovalbumin and its antibody in vivo and in vitro.

Methods

The chloroquine preparation used in these experiments was 7-chloro-4-(4-diethylamino-1-methyl-butylamino)quinoline-diphosphate (Delagil, United Pharmaceutical Works, Budapest, Hungary).

In the first series of experiments the effect of different amounts of chloroquine has been studied on the quantitative precipitation of antiovalbumin immune sera in vitro at pH 7.2. Chloroquine was incubated with the immune serum at 37 °C for 30 min, then ovalbumin was added to the mixture. In another series of experiments anti-ovalbumin rabbit serum was incubated with the blood plasma of chloroquine-treated guinea pigs at 37 °C for 30 min, then the antigen was added. Finally, the immune serum was precipitated with ovalbumin, and the precipitate was incubated with chloroquine.

The antigen-antibody complex was dissolved by addition of four times the amount of antigen necessary to equivalence and subjected to gel-filtration on Sephadex G-200 column at pH 7.2, according to the method of Litt (1966), and the distribution of the components of the complex according to molecular weight was determined. The same complex was incubated with chloroquine at 37 °C for 30 min and the effect of chloroquine on the composition of the complex was studied.

Female guinea pigs weighing 250 to 350 g were used in the experiments in vivo; it was shown by McChesney et al. (1967) that the tissues of female guinea pigs take up more chloroquine than those of the males. The animals were given intraperitoneally 20 mg of chloroquine base per kg of body weight, every day over a period of 28 days. The weight of the animals was checked weekly, since chloroquine has been shown to increase body weight (Mc Chesney et al. 1965); we observed an average gain in weight of about 20% during the 4-week period.

A group of the guinea pigs was sensitized with 0.48 or 0.24 mg of antibody-N per kg body weight of rabbit serum raised against ovalbumin, by the intracardiac route. Passive

anaphylactic shock was elicited by the injection into the jugular vein of 4 mg ovalbumin 48 hours after sensitization.

In another group of guinea pigs, anaphylactic shock was elicited by an antigen-antibody complex containing 100 μg of antibody-N. The dissolved complex was injected into the external jugular vein, and the reaction was graded as follows

$\pm$ coughing, piloerection;
$+$ coughing, piloerection, defecation, micturition;
$++$ coughing, piloerection, defecation, micturition, scraping of nose and ear, dyspnoea;
$+++$ apnoea, falling to the side, convulsions.

The complex was injected also subcutaneously, under the depilated skin of the back, in untreated and chloroquine-treated female guinea pigs, eliciting thereby a cutaneous reaction. The response was evaluated after 24 hours, according to the criteria of COCHRANE and WEIGLE (1958):

$+$ slight oedema, erythema 15 mm in diameter;
$++$ slight oedema, erythema 20 to 30 mm in diameter;
$+++$ slight oedema, erythema 30 to 40 mm in diameter, surrounded by a 5 to 15 mm haemorrhagic halo.

The plasma chloroquine level of the guinea pigs was determined with trans-aconitic acid, by the method of STRUFE (1960).

Results

As shown in Fig. 1, binding of the antigen by antibody was inhibited by 3.12×10^{-3}M of chloroquine base, i.e. 5 mg in a total volume of 5 ml, the amount of precipitable antibody being substantially reduced in curve *b* as compared to *a*. Curves *c* and *d* demonstrate that the antibody content of the precipitate was decreasing with increasing concentrations of chloroquine.

The plasma of guinea pigs subjected to long-term chloroquine treatment contained 0.5 to 2.5 μg/ml of chloroquine base (Table II). At this concentration, chloroquine failed to block precipitation in vitro (Fig. 1, curves *e*, *f*).

When chloroquine was added to the formed antigen-antibody complex (Fig. 1, curves *g*, *h*), it not only blocked the formation of the precipitate, but though to a lesser extent also dissolved it.

The question arose whether the solution of the precipitate was due to a dissociation of the antigen-antibody complex. The gel-filtration diagram shows (Fig. 2) that the complex is made up to two components of a molecular weight higher than that of gamma-globulin, and of free ovalbumin. This composition of the complex is compatible with the finding of ISHIZAKA and CAMPBELL (1958) who obtained by electrophoresis two complexes containing antigen and antibody in different ratios, dissolved in a small excess amount of the antigen.

Curve 1 in Fig. 2 shows the original composition of the complex, while curve 2 demonstrates the effect of chloroquine on it. Both components of the complex diminished in amount while the amount of free ovalbumin successively increased. From this observation it may be concluded that chloroquine caused a dissociation of the antigen-antibody complex so that the solution of the

antigen-antibody precipitate on the addition of chloroquine must have been due to a dissociation of the complex.

In the experiments in vivo, the following results were obtained.

Fifteen guinea pigs had been subjected to long-term chloroquine treatment, then a passive anaphylactic shock was elicited. On the shock elicited

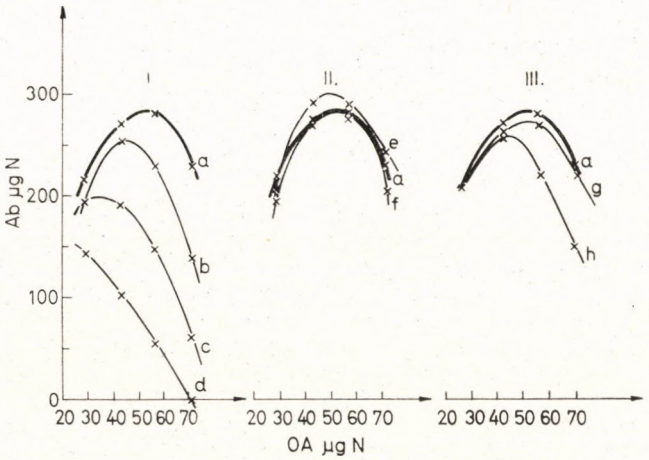

Fig. 1. Quantitative precipitation of anti-ovalbumin rabbit serum.
I. Immune serum treated with chloroquine in vitro. *a* Control serum; *b* 3.12×10^{-3} M chloroquine; *c* 6.25×10^{-3} M chloroquine; *d* 1.88×10^{-2} M chloroquine.
II. Antiserum incubated with guinea pig plasma (1.30 µg of chloroquine per ml). *a* Control serum; *e* 0.5 ml; *f* 1.0 ml.
III. Effect of chloroquine on the precipitate. *a* Control; *g* 3.12×10^{-3} M chloroquine; *h* 1.88×10^{-2} M chloroquine

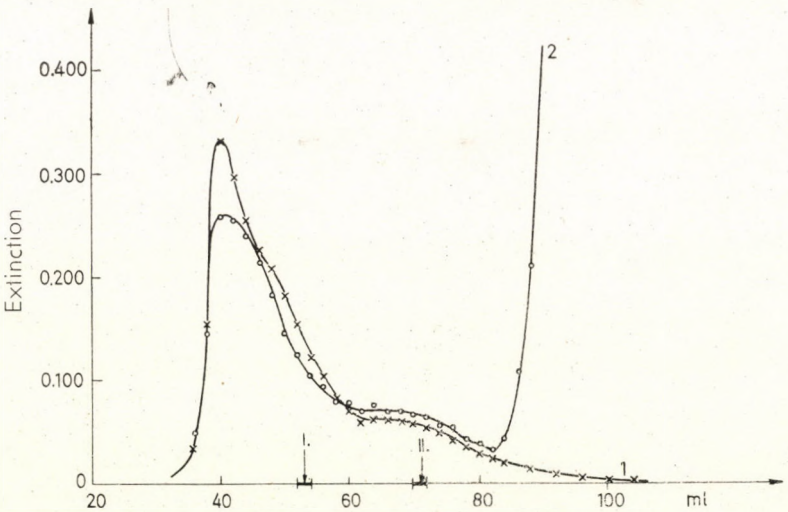

Fig. 2. Gel-filtration diagram of the antigen-antibody complex. 1. Without chloroquine. 2. After incubation with 1.88×10^{-2} M chloroquine. I. Separation site of gamma-globulin. II. Separation site of ovalbumin

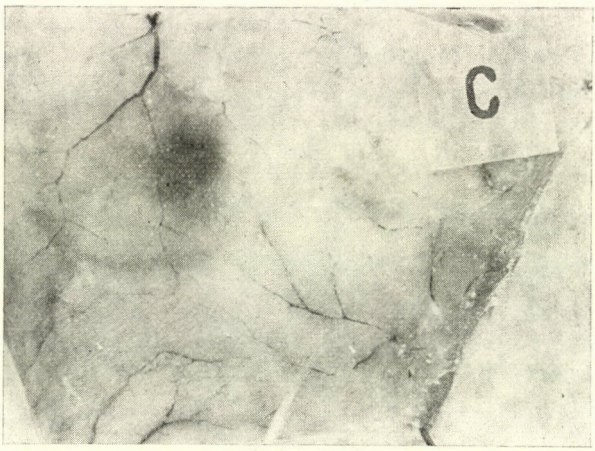

Fig. 3. Cutaneous reaction elicited by antigen-antibody complex on the back of guinea pig (control)

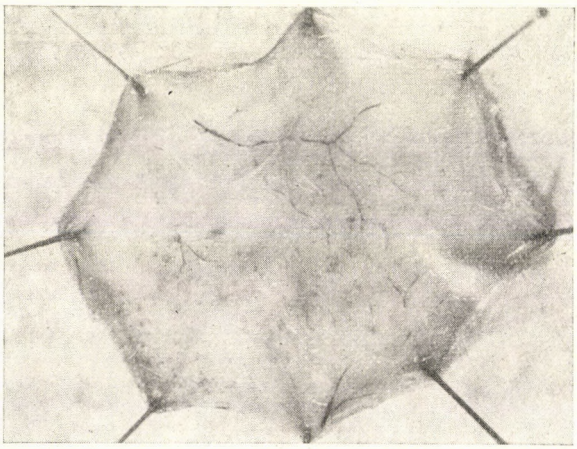

Fig. 4. Cutaneous reaction elicited by antigen-antibody complex on the back of guinea pig pretreated with chloroquine

in this way, chloroquine had no appreciable effect since the results were identical with those found in the untreated controls.

In another series of experiments an anaphylactic reaction was induced by the administration of the antigen-antibody complex. In the control guinea pigs, a severe anaphylactic response ensued within 3 hours (Table I), but none of the animals died. In 20 guinea pigs pretreated with chloroquine and given the same amount of the antigen-antibody complex the reaction which ensued within about the same time was considerably milder; some of the animals showed no reaction whatever.

Table I

Anaphylactic shock elicited with antigen-antibody complex

Control		Pretreatment with chloroquine	
No.	Grade of reaction	No.	Grade of reaction
1	+++	18	++
2	+++	19	ø
3	+++	20	ø
4	++	21	±
5	+++	22	±
6	++	23	++
7	+++	24	ø
8	++	25	+
9	++	26	ø
10	++	27	+
11	+++	28	ø
12	+++	29	+
13	+++	30	±
14	+++	31	ø
15	+++	32	+
16	+++	33	+
17	+++	34	ø

Table II

Cutaneous reaction elicited with antigen-antibody complex

Controls		Pretreated with chloroquine	
No.	Intensity of reaction	No.	Intensity of reaction
35	++	47	+
36	++	48	ø
37	++	49	ø
38	+	50	ø
39	+++	51	ø
40	++	52	ø
41	+++	53	ø
42	++	54	ø
43	+	55	ø
44	++	56	ø
45	++	57	ø
46	++	58	ø

No. 47, concentration of chloroquine base, 0.52 μg/ml plasma
Nos 50, 51, concentration of chloroquine base, 1.30 μg/ml plasma
Nos 55, 56, concentration of chloroquine base, 2.50 μg/ml plasma

In the next series of experiments, a cutaneous reaction was elicited in 12 control guinea pigs by injecting the antigen-antibody complex intradermally. Most of the animals exhibited mild oedema and erythema 20 to 30 mm in diameter. The reaction evaluated as ++ is shown in Fig. 3. Injection of a similar dose of the antigen-antibody complex into the skin of 12 guinea pigs pretreated with chloroquine was followed by a mild reaction in 1 case, whereas in the rest of the animals no response occurred. Thus, the reaction was inhibited by chloroquine pretreatment (Fig. 4). The results of these experiments are summarized in Table II.

Discussion

The present in vitro experiments have unequivocally demonstrated that chloroquine at sufficiently high concentrations inhibits the precipitation of the antigen-antibody complex. With increasing chloroquine concentrations, this blocking effect is enhanced. Chloroquine is potent first of all in inhibiting the antigen-antibody combination and, to a smaller extent, it is capable of dissociating the precipitate thus formed. The present experiments gave no indication that chloroquine would have been attached to either the antigen or the antibody. According to HABERLAND (1960), protein and chloroquine do not precipitate each other while in the case of cellular antigens chloroquine would be attached to the subcellular elements.

In view of the observation that chloroquine possesses inhibitory properties in the case of cellular as well as soluble antigens, and with complete as well as incomplete antigens, it might be assumed that chloroquine interferes with some basic mechanism of the antigen-antibody reaction. Further experiments are necessary to establish whether this inhibition is brought about by physico-chemical changes or by alterations in conformation.

Our experiments in vivo have shown that chloroquine pretreatment does not prevent the lethal systemic anaphylaxis elicited by antigen following sensitization with the antibody. On the other hand, chloroquine proved to be potent in reducing the moderate anaphylactic symptoms induced by the administration of the antigen-antibody complex; an even more pronounced blocking effect was observed on the cutaneous reaction elicited by the antigen-antibody complex.

The plasma concentration of chloroquine in the guinea pigs pretreated with the drug was lower than the concentration found to be effective in inhibiting the antigen-antibody reaction in vitro. However, with non-steroidal anti-phlogistics such as sodium salicylate (BROWN and MACKEY 1968), the in vitro reaction of ovalbumin with anti-ovalbumin is inhibited by concentrations 100 to 200 times higher than those found in the plasma of patients undergoing salicylate treatment. Similarly, epsilon-aminocaproic acid inhibits the forma-

tion of the antigen-antibody complex in vitro at much higher concentrations than in vivo (WOLD et al. 1967). Furthermore, it has been demonstrated by VARGA (1968) that the concentration of chloroquine in the lungs, skin and liver may attain 500 to 1000 times its concentration in plasma. Thus, it might be assumed that the relative concentrations of protein and chloroquine in the various organs are favourable when the drug is given in vivo.

In addition to its effect on the antigen-antibody reaction, chloroquine has other effects in vivo, e.g. antihistamine and antiserotonin properties, which might contribute to the observed differences between the in vivo and in vitro effects (OKEGAUA et al. 1966).

Finally, it has been suggested by ABRAHAM et al. (1968) that chloroquine has an effect on the lysosomes, first labilizing, then stabilizing, the lysosome membrane.

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Communicatio brevis

MORPHOLOGY AND FUNCTION OF INTERSCAPULAR BROWN ADIPOSE TISSUE IN THE NON-COLD-ADAPTED ADULT GUINEA PIG

By

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(Received June 25, 1970)

Brown adipose tissue is being gradually replaced by white fat soon after birth in most mammalian species when not reared in the cold (Smith and Horwitz 1969). In the new-born guinea pig, brown adipose tissue is replaced by white adipose tissue within 3 to 4 weeks after birth even when the animal is reared at an ambient temperature of 16 to 19 °C (Brück and Wünnenberg 1966a, b). Concomitantly with the replacement of brown adipose tissue by white adipose tissue, non-shivering thermogenesis is held to be replaced by shivering, and in response to cold the temperature of the interscapular fat was observed to fall in parallel with that of the colon, and to fail to rise above colonic temperature like in the new-born guinea pig (Brück and Wünnenberg 1965). In the adult non-cold-adapted guinea pig thermogenesis has been observed, however, to persist in the interscapular fat in response to cold exposure (Donhoffer and Szelényi 1965). Therefore, adult guinea pigs reared at a room temperature of 20 to 23 °C were exposed to cold and the temperature of the interscapular adipose tissue, of the underlying muscle and of the colon were measured with thermocouples, oxygen consumption being simultaneously recorded by a Noyons-Kipp diaferometer. The thermocouples were inserted into the interscapular adipose tissue and the underlying muscle under hexobarbital anaesthesia the evening before the experiment and so the observations were made on completely alert animals. After the experiment the interscapular adipose tissue was excised and serial sections were made.

Fig. 1 is a typical example of the histological picture seen in these animals. None of the serial sections contained cells resembling brown adipose tissue. Yet, as Fig. 2 demonstrates the same adipose tissue responded to cold exposure like typical brown adipose tissue. Colonic temperature continued to fall after oxygen consumption had increased, whereas the temperature of the interscapular adipose tissue had stabilized simultaneously with the increase

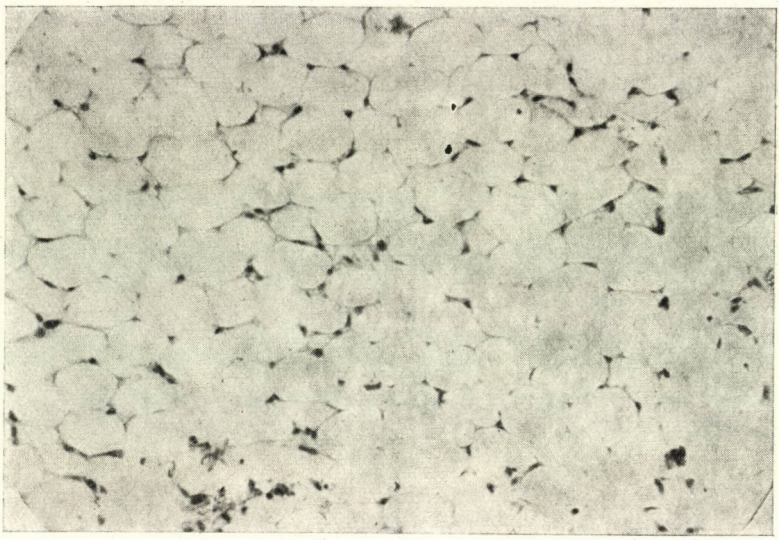

Fig. 1. One of the serial sections from the interscapular adipose tissue of a male guinea pig weighing 575 g reared at 20 to 23 °C

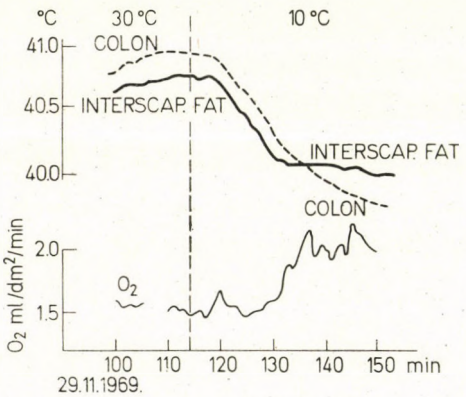

Fig. 2. The response of the same guinea pig to transfer from the thermoneutral temperature of 30 °C to an ambient temperature of 10 °C. The abscissa indicates the time elapsed since the measurements had been started

in heat production and was maintained well above colonic temperature. The temperature of the muscle underlying the interscapular adipose tissue was lower than, and fell parallel with, colonic temperature and, therefore, could have been by no means responsible for the stabilization of the temperature of the adipose tissue at a level well above both colonic and muscle temperature. It has, therefore, been concluded that the physiological thermogenetic function of brown adipose tissue is not dependent on the histological appearance.

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Recensiones

W. RÜDIGER (Ed.)

Lehrbuch der Physiologie

VEB Verlag Volk und Gesundheit, Berlin 1969. XVI + 614 pages, with 300 figures and 15 tables. Price: M 41.10

This Textbook of Physiology was written by G. KÜCHLER, M. LINDEMANN, W. RÜDIGER and E. SCHUBERT, all of the Institute of Physiology, Humboldt-University, Berlin, first of all for medical students, but it will meet the requirements of veterinary and biology students, too. The book has been written to suit the modern aspect in the education of physiology, placing in foreground the regulation of functional systems and the main chapters are the methods of research of functional systems such as the methods of research in physiology, energy exchange and thermoregulation, the system of respiration-heart-circulation, physiology of the kidney, physiology of the brain.

The reviewer is convinced that the book will fulfil its mission in the teaching of physiology.

K. LISSÁK

E. SCHUBERT

Praktikum der Physiologie

VEB Gustav-Fischer-Verlag, Jena 1969. 199 pages, with 35 figures. Price: M 10.70

This laboratory manual of physiology was written in collaboration by H. CHOINOWSKI, G. DYBOWSKY, K. ECKOLDT, Ch. PFEIFFER, D. RUFF, of the Institute of Physiology, Humboldt-University, Berlin.

In view of the limited possibilities in time and space, it is a difficult task in the teaching of physiology to follow the progress in the equipment of physiological and medical research. The manifold purpose of laboratory work in the teaching of physiology is first of all to illustrate the principles of physiology, to develop the ability of the student to make independent observations, and to teach him to make reasonable deductions from his own observations.

The book includes only the fundamental observations and experiments necessary to acquire some dexterity and a true understanding of the most fundamental principles of physiology, emphasizing the value of quantitative measurements instead of gathering qualitative data. The preliminary remarks will help the student in understanding his own observations and recall his knowledge from the lectures and textbooks. This laboratory manual will be a useful help in the teaching of physiology.

K. LISSÁK

G. A. KERKUT (Ed.)

Experiments in Physiology and Biochemistry

Volume 3. Academic Press, London and New York 1970. XVIII + 504 pages, with 184 figures. Price: 140s; US $ 7.00

The third volume of this very useful series contains fourteen contributions describing different methods of physiology and biochemistry as follows. 1. An investigation of the distribution and properties of some phosphagen phospho transferases, by D.C. WATTS and Barbara MORELAND. 2. Estimation of the glycine concentration in small tissue samples from the central

nervous system, by M. H. APRISON and R. P. SHANK. 3. A controlled humidity constant temperature cold box for dissection of small frozen tissue sections, by M. H. APRISON and C. J. HANCOCK. 4. Direct electrical currents in metabolising epithelial membranes, by O. WATHINGTON, T. C. SMITH and E. G. HUF. 5. Experiments on the transmembrane potential of chromatophores, by A. R. FREEMAN and M. FINGERMAN. 6. Electrical measurements on insect cuticle and integument, by P. O. SCHEIE. 7. Experiments on synaptic transmission using the frog sartorius nerve-muscle preparation, by Barbara J. EXCELL. 8. Experiments on amphibian respiratory and circulatory systems, by D. R. JONES. 9. Two experiments using the dog lung apparatus: study of deterioration of preparation and prolonged veno-venous perfusion, by J. L. RATLIFF. 10. Base ratio analysis of small samples of RNA, by J. P. MONJARDINO. 11. The push-pull cannula (PPC), by J. D. DUDAR and J. C. SZERB. 12. The spider heart, by R. G. SHERMAN and R. A. PAX. 13. Techniques for maintaining a stock of the blowthy *Pharmia regina*, by L. T. WIMER, R. H. LUMB and L. G. TATE. 14. The use of Sephadex in the separation, purification and characterisation of biological materials, by J. CURLING. The book is completed by an author and subject index, and with a cumulative Contents, which survey the first and second volumes of this series.

The third volume of the series follows the program of the first and second volumes to give sufficient practical detail to enable the reader to follow and carry out the experiments described.

This book will be useful for both the experimental research worker and the student in becoming acquainted with new techniques and new experimental animals. It is recommended to biologists, biochemists, physiologists and others interested in experimental research work.

K. LISSÁK

A. V. HILL

First and Last Experiments in Muscle Mechanics

Cambridge University Press, 1970. XV + 141 pages, with 68 figures. Price: 55s; US $ 9.50

This historically important book of a highly esteemed scientist presents a lifetime's work in muscle physiology, considering the force-velocity relation, the speed of fibre contractions, the development of the active state, the elastic energy in a contracting muscle, the tension–extension curve and the internal redistribution of length.

A. V. HILL's fundamental work in muscle physiology has its own decisive significance in physical education and athletics, too. A sports physiological delicacy is an unusual experiment on the force-velocity relation of human muscle by (and on) Charles H. BEST, at that time 29 year old. The book concludes with the inaugural address to the Congress of the International Union of Physiological Sciences in Tokyo, 1965, with many personal remembrances on the history of earlier International Physiological Congresses.

The book will be of interest not only to physiologists and biophysicists but also to sports physiologists.

K. LISSÁK

O. HUDLICKÁ (Ed.)

Circulation in Skeletal Muscle

Proceedings of an International Symposium held at Smolenice, Czechoslovakia, 1966. IX + 356 pages, with 182 figures. Pergamon Press, London, New York 1968

The Symposium on Circulation in Skeletal Muscle was sponsored by the Physiological Section of the Czechoslovak Medical Society and the Slovak Academy of Sciences in September, 1966. It was attended by fifty-five expert scientists working in this field from all over the world.

The papers presented reviewed the problems of Nervous regulation of circulation in skeletal muscle; Capillary circulation and transcapillary exchange; Interrelations between blood flow and metabolism; Exercise hyperaemia; Interrelations between blood flow and metabolism; Reactive hyperaemia; Autoregulation in skeletal muscle. Each paper was followed with a discussion and the Symposium ended with a general discussion.

All the papers contained the latest data of their authors, completed with the critical approach of the discussions. The book represents a comprehensive review of contemporary research in this field. It will be an important reference work for physiologists and for everyone in the medical sciences interested in recent developments concerning the circulation in skeletal muscle.

K. LISSÁK

H. VAN CAUWENBERGE and P. FRANCHIMONT

Assay of Protein and Polypeptide Hormones

International Series of Monographs in Pure and Applied Biology. Division: Modern Trends in Physiological Sciences. Volume 33. Pergamon Press, 1970. VI + 242 pages, with 52 figures. Price: 100s; $ 13.50

The book reviews in sixteen chapters the radioimmunological methods of protein and polypeptide hormone assay, which have opened a new possibility of experimental and clinical endocrinological investigations. The first two chapters review the general principles and the following chapters the particular hormones. Chapters 3 to 8 are discussing the assay of hypophyseal hormones: growth hormone, adrenocorticotropin, thyrotropin, gonadotropins, prolactin, vasopressin and oxytocin. Chapters 9 and 10 deal with the methods of insulin and glucagon assay and chapters 13 and 14 with the assay of hormones regulating the metabolism of phosphorus and calcium: parathormone, thyrocalcitonin. Chapters 15 and 16 review the assay of placental hormones: human chorionic gonadotropin and human placental lactogen.

After several years of experimental and clinical research in this field, it is timely to evaluate and view its current status. The book is a useful review of the current status of radioimmunological assay methods and will be indispensable for all research workers in endocrinology, physiology, biochemistry and those working in clinical laboratories.

K. LISSÁK

M. CEREIJIDO and C. A. ROTUNNO

Introduction to the Study of Biological Membranes

Gordon and Breach Science Publishers, New York, London, Paris 1970. X + 261 pages, with 112 figures. Price: £ 10 10s; US $ 25.20

The intention of the authors was to introduce a student or a non-specialist without previous acquaintance of membranes in the basic interdisciplinary knowledge and enable him to read and understand the original papers.

After a short historical introduction the book reviews the facts about the cell membrane which determine the rate of selective entry and exit of water and solutes into and out of the cell as well as the concentration levels. In connexion with membrane theory its essence and criticism, together, with one of its most interesting alternatives, Ling's association-induction hypothesis are discussed. Considering the present status of research, the authors conclude that the truth might be somewhere between these two extremes.

The bibliography with more than 700 references is far from being complete but still provides a good reading list to complete the reader's knowledge.

The book is a useful help for biophysicists, biochemists, physiologists and for graduate students in any branch of biology, unless the unusual high price of the book presents difficulties.

K. LISSÁK

N. LAKSHMINARAYANAIAH

Transport Phenomena in Membranes

Academic Press, New York and London 1969. XI + 517 pages, with 77 figures. Price: US $ 22.00

The book is a survey of the theoretical treatments of membrane phenomena and the experimental results obtained using a number of well-defined membrane systems, with the aim of enabling biologists with a limited mathematical background to cope with the material covered. The intention of the author was to facilitate the understanding between the physical and biological sciences in the aspects of transport phenomena in artificial and biological membranes.

The book is divided in nine chapters. Preparation of membranes; Basic principles; Membrane phenomena due to a gradient of chemical or electrochemical potential; Phenomena due to an applied electric field, to a pressure gradient, to gradient of temperature; Transport phenomena in nerve cell membrane; Properties of monolayers and bilayers are discussed, among others. Each chapter is completed with references and an appendix presents an annotated Bibliography i.e. short abstracts of papers published since the manuscript had been submitted. An author and subject index completes this excellent monograph, which will be useful to research workeres and students of chemical technology, biophysical chemistry, biophysics, physiology, pharmacology and molecular biology.

K. Lissák

F. Snell, J. Wolken, G. Iverson and J. Lani (Eds)

Physical Principles of Biological Membranes

Proceedings of the Coral Gables Conference on Physical Principles of Biological Membranes. Center for Theoretical Studies, University of Miami, December, 1968. Gordon and Breach Science Publishers, New York, London, Paris 1970. VII + 435 pages, with 174 figures. Price: £ 8; US $ 19.20

One of the problems of biology most in the foreground is the precise nature of the functional role of living membranes. The theoretical and experimental approach in the last decades has resulted in a great amount of new knowledge, but a complete description of biological membranes is still lacking. The Coral Gables Conference was attended by over 40 distinguished scientists representing a truly interdisciplinary group with prime interest in the structure and function of biological membranes.

The presented 19 papers were as follows. Dielectric properties of living membranes (K. S. Cole); Optical and electrophysiological evidence for conformational changes in membrane macromolecules during nerve excitation (I. Tasaki, W. Barry and L. Casnay); Theory of nerve excitation as a cooperative cation exchange in a two-dimensional lattice (G. Adam); A non-linear term in the ion transport across membranes (R. Schlägl); Ion distribution equilibria in bulk phase and the ion transport properties of bilayer membranes produced by neutral macrocyclic antibiotics (G. Szabó, G. Eisenman and S. M. Ciani); Possible mechanisms of ion transit (L. Onsager); Physical principles in monolayer and membrane permeation (M. Blank and J. S. Britten); Current rectification and action potentials across thin lipid membranes (D. R. Kalkware, D. L. Frasco and W. H. Brattain); Dispersion forces and stability of lipid bilayers (S. Ohki); Ion transport across lipid bilayer membranes (D. Länger); Role of water structure in various membrane systems (W. Drosthansen); Structural and functional properties of bacterial cell membranes (M. R. J. Salton); Structure of the mitochondrial crystal membrane (D. E. Green and G. Vander-Koi); Conformational basis of energy transductions in biological membranes (D. E. Green and R. A. Harris); Contrasting protein architectures of plasma and mitochondrial membranes (D. F. H. Wallach, A. S. Gordon, J. M. Graham and B. R. Fernbach); Cell and photoreceptor membranes (J. J. Walken); The indication of a light induced electrical field by pigments incorporated in chloroplast membranes (W. Junge, H. M. Emrich and H. T. Wit); Concept of the reactive site in biological transport (H. N. Christensen); Membrane-like properties in microsystems assembled from synthetic protein-like polymer (S. W. Fox, R. J. McCauley, P. O. B. Montgomery, T. Fukushima, K. Harada and C. R. Windsor). Each paper was followed by discussions.

The book is recommended to students and researchers of biophysics, biochemistry, neurophysiology, physics and chemistry.

K. Lissák

R. Porter (Ed.)

Breathing: Hering—Breuer Centenary Symposium

A Ciba Foundation Symposium. J. and A. Churchill, London 1970. XIV + 402 pages, with 116 figures. Price: 80s

This Ciba Foundation Symposium was organized in 1969 to commemorate the centenary of the publication of Hering and Breuer's work on Selfsteering of Respiration through the Vagus Nerve. The thirty participants of this multidisciplinary meeting discussed the recent work on the nervous control of breathing, particularly as mediated through afferent pathways

and through receptors in the thorax and the lung itself. The basic research workers in various branches of physiology discussed the relevance of their findings and techniques to the clinical problem of respiratory failure, defining the peripheral and central pathways involved in respiration and outlining the possibilities of future research.

A particularly valuable part of the book is the historical and biographical introductory note of Elisabeth ULLMANN about Ewald HERING and Joseph BREUER, and the translation of the two original papers written by HERING and BREUER in 1868.

The book is recommended to physiologists and clinicians interested in the contemporary problems of respiratory regulation.

K. LISSÁK

H. PILZ

Die Lipide des normalen und pathologischen Liquor cerebrospinalis

Schriftenreihe Neurologie, Neurology Series Band 4. Springer-Verlag, Berlin, Heidelberg, New York 1970. VIII + 123 pages, with 4 figures and 23 tables. Price: DM 48.-; US $ 13.20

The great progress in methodology has made it possible to study lipids in body-fluids and the introduction of new and simple separating techniques such as thin layer chromatography now allows their quantitative estimation in the CSF.

After a short introduction the author reviews the classification of lipids, then describes the analysis and the results of CSF lipid analysis, the fatty acids in CSF, the comparative analysis of lipids in brain, serum and CSF. Discussing and critically reviewing the relevant research work the book concludes with a complete bibliography on the subject.

The book will be of interest to biochemists, neurophysiologists and particularly to neuropathologists.

K. LISSÁK

W. LUDWIG

Das Rechts-Links-Problem im Tierreich und beim Menschen. Mit einem Anhang: Rechts-Links-Merkmale der Pflanzen

Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere. Springer-Verlag, Berlin, Heidelberg, New York 1970. Reprint. XI + 496 pages, with 143 figures. Price: DM 54.-; US $ 14.90

It is a foreseeing wise policy of the Publishers to reprint from the classical monograph series, the Territory of Physiology of Plants and Animals, this 27th volume, which was published in 1932. Since then the great progress in biology, the results in electronmicroscopy, the aspects of molecular biology, the discovery of the double helix by CRICK and WATSON, have thrown new brightness on the right-left-problem.

The book is divided in three parts. The first is an introductory, the second a special and the third a general part. The first part discusses the characteristics of the right-left-problem, the second part reviews the problem from the phylogenetic aspect, from the protozoa to the handedness of man and antropoids. The third part discusses the right-left-problem from the inorganic world to the mythic and metaphysical aspects.

This fundamental classical monograph of biology, nearly forty years after its first edition will offer much for every research worker in the large field of biological sciences.

K. LISSÁK

G. E. W. WOLSTENHOLME and JULIE KNIGHT (Eds)

Taste and Smell in Vertebrates

A Ciba Foundation Symposium. J. and A. Churchill, London, 1970. X + 402 pages with 152 figures. Price: 80s

This volume is the fifth of a series of symposia on sensory function in vertebrates organized by Professor O. E. LOWENSTEIN with the Ciba Foundation. The first of the series was on Colour Vision (1964), the second on Touch, Heat and Pain (1966), the third on Myotatic, Kinesthetic and Vestibular Mechanisms (1968), the fourth on Hearing Mechanisms (1969).

The eighteen papers presented on chemoception were as follows. The anatomy and ultrastructure of taste endings (R. G. MURRAY, A. MURRAY); Physiological and behavioural processes of the sense of taste (C. PFAFFMANN); Physiological properties of mammalian taste receptors (L. M. BEIDLER); Problems of taste specificity (H. T. ANDERSON); The influence of the circulation on taste receptors as shown by the summated chorda tympani nerve responses in the rat (G. HELLEKANT); Neural and perceptual responses to taste stimuli (G. BORG, H. DIAMONT, Y. ZOTTERMAN); Olfactory surface and central olfactory connections in some vertebrates (T. S. REESE, M. W. BRIGHTMAN); The olfactory neuron and the blood-brain barrier (A. J. D. DE LORENSO); Anatomy and ultrastructure of the olfactory bulb in fishes, amphibia, reptiles, birds and mammals (K. H. ANDRES); Experiments in olfaction (K. B. DOVING); Olfaction in mammals, two aspects: proliferation of cells in the olfactory epithelium and sensitivity to odours (D. G. MOULTON, G. CELEBI, R. P. FINK); Detection of hidden objects by dogs (E. H. ASHTON, J. T. EAYRS); Recent developments in the "penetration and puncturing" theory of odour (J. T. DAVIES); Computer correlation of molecular shape with odour: a model for structure-activity relationships (J. E. AMOORE); Odour similarity between structurally unrelated odorants (M. G. J. BEETS, E. T. THEIMER); Specific physicochemical mechanisms of olfactory stimulation (R. H. WRIGHT, R. E. BURGESS); Electrical signs of olfactory transducer action (D. OTTOSON); Higher olfactory centres (W. R. ADEY).

The book represents a comprehensive review of contemporary research into the physiology of taste and smell in vertebrates and will be an up-to-date reference work for the investigators of morphological and functional branches of biological sciences.

K. LISSÁK

K. N. LEIBOVIC (Ed.)

Information Processing in the Nervous System

Proceedings of a Symposium held at the State University of New York at Buffalo, October, 1968. Springer-Verlag, Berlin, Heidelberg, New York 1969. XVII + 375 pages, with 69 figures. Price: DM 79.20; US $ 19.80

The main intention of the Symposium was to correlate in some way the "neuronal machinery with psychophysiological phenomena". Prof. LEIBOVIC, the organizer of the symposium, calls attention to the urgency of achieving a common conceptual basis for neuroanatomy, neurophysiology and psychology, all of which refer to the same biological unity.

The volume contains six parts. I *Automata Theory*. 1. Automata Theory as an Abstract Boundary Condition for the Study of Information Processing in the Nervous System (M. A. ARBIT). 2. Hierarchial Organisation in Automata Theoretic Models of the Central Nervous System (R. ROSEN). 3. The Non-local Storage and Associative Retrieval of Spatio-Temporal Patterns (H. C. LONGUET—HIGGENS). Part II *Linguistics*. 4. On the Complexity of Language Processing by the Brain (P. S. PETER). 5. Context-Sensitive Coding in Speach Recognition, Articulation and Development (W. A. WICKELGREN). 6. The Speach Code and the Physiology of Language (I. G. METTINGLY, A. M. LIBERMAN). Part III *Psychophysiology*. 7. Repetition, Visual Persistence, Visual Noise and Information Processing (R. N. HABER). 8. Behavioral and Electrophysiological Studies of Visual Masking (P. H. SCHILLER). 9. Temporal Summation During Backward Visual Masking (R. M. BOYNTON). 10. Toward a Theory of Man: Precision of Essential Form in Living Communication (M. CLYNES). Part IV *Neurophysiology*. 11. Trigger Features, Adaptation and Economy of Impulses (H. B. BARLOW). 12. Information Processing in the Motorsensory Cortex (V. B. BROOKS). 13. The Dynamic Loop Hypothesis of Movement Control (J. C. ECCLES). Part V *Models and Theory*. 14. On Synaptic Transmission, Neural Signals and Psychophysiological Phenomena (K. N. LEIBOVIC, N. H. SABAH). 15. Of Digital Oscillators (W. S. MCCULLOCH). 16. The Reticular Formation Command and Control Systems (W. KILMER, W. S. MCCULLOCH). 17. "Autonomy" and "Dominance" in Neural Systems: Two Examples of Dynamic Properties Defined on Finite State Models (R. F. REISS). 18. Biological Information Processing Using the Concept of Interpenetrating Domains (O. H. SCHMITT). Each paper was followed with a stimulating discussion by the sixty-seven participants. Part VI *Final Session*, consisted of general comments, principally from session chairmen, and some discussion from the participants. The topics of concern were information theory, neural coding, reliability of signal transmission, and the general problems of communication between specialized fields and the process of education.

The book concludes with a glossary of terms, which has been compiled at the suggestion of Prof. ECCLES as an aid in communicating with people from different specialities.

The book will be an interesting and useful source of information first of all to anatomists, physiologists and psychologists interested in the problems of information processing in the nervous system.

K. LISSÁK

L. CHESTOK (Ed.)

Psychophysiological Mechanisms of Hypnosis

An International Symposium sponsored by the International Brain Research Organization and the Centre de Recherche, Institut de Psychiatrie. Springer-Verlag, Berlin Heidelberg, New York 1969. VIII + 207 pages, with 50 figures. Price: DM 48.-; US $ 13.20

The First International Symposium on the Psychophysiological Mechanisms of Hypnosis was held at Tourtour in the fall of 1967. The book contains the thirteen papers presented by twenty-seven eminent psychiatrists, psychologists and physiologists from various parts of the world. Unfortunately, the discussions have not been included, but the participants were encouraged to rewrite and make up-to-date their manuscripts and to include, when appropriate, some of the material that had emerged from the discussions, thus making available much of the content of the Symposium, explains in his foreword Martin T. ORNE.

The contributions cover all the recent important interdisciplinary aspects of hypnosis and present the theoretical and practical problems of physiology, psychology, psychiatry and psychoanalysis.

K. LISSÁK

K. DANZIGER (Ed.)

Readings in Child Socialization

Pergamon Press, Oxford 1970. X + 337 pages, with 11 figures and 41 tables. Price: hard cover 50/—; US $ 8.00; flexi cover 35/—; US $ 5.00

The book was published in The Commonwealth and International Library "Readings in Sociology" series. In the introduction the General Editor, Anthony H. RICHMOND pointed out that this is a collection on a specialized aspect of sociology and social psychology, which contains some selected readings on the state of knowledge and research in the field in question.

The Editor, K. DANZIGER, in his detailed introduction discusses the concept and the process of socialization, the family system, socialization and society, and, finally, the methodology.

The book contains thirteen papers; they will be of interest for psychologists, paediatricians, teachers and others interested in child sociology.

Stefanie LISSÁK

W. H. HOLLINSHEAD

Functional Anatomy of the Limbs and Back

W. B. Saunders Co. Ltd., London 1969. 420 pages, with 170 figures. Price: £ 5 6s.

The treatment of locomotor disorders is based on functional anatomy. A concise but appropriately profound work like HOLLINSHEAD's functional anatomy may arouse a keen interest from the clinicians dealing with the organs of locomotion. The author too emphasizes that he addressed his book first of all not to anatomists but to those who want to make use in their practice of functional experiences in combination with anatomy.

The title promises only a discussion of the limbs and back, but the book contains much more; its last chapter deals with the functional anatomy of the neck, the head, and the trunk. The first chapter is a review of knowledge on the tissues, the construction of the organism and offers an up-to-date anatomical terminology.

A book dealing with anatomy is not conceivable without figures. HOLLINSHEAD's work is abundantly illustrated. The reader, spoiled by the multicoloured, intaglio-printed figures of the big atlases of anatomy can hardly expect a reproduction of the different structures in black-and-white figures. However, this is the case, but the black-and-white-shaded figures are prominent with their remarkable exactness to details and they are hardly inferior to coloured reproductions. The figures describing the innervation of the individual muscle groups are particularly demonstrative and original.

It is a pity that this well-constructed book does not give a more detailed discussion of the function of the hands, of our subtlest and most precise organ of locomotion. The clinician too expects a comprehensive discussion of the hands, especially in view of the recent development of their surgery.

HOLLINSHEAD's functional anatomy will be useful to all specialists engaged in the treatment of the locomotor organs, especially for orthopaedic surgeons, traumatologists, psychotherapists, and last but not least to anatomists.

M. FORGON

W. DOERR

Spezielle pathologische Anatomie, I

Heidelberger Taschenbücher 69. Springer-Verlag, Berlin, Heidelberg, New York 1970. XVII + 403 pages. Price: DM 6.80, US $ 1.90

The present book is the first part of a textbook of special pathological anatomy intended for students. It treats the pathology of the following organ systems: Heart and vessels, haemopoietic organs, respiratory tract and uropoietic organ systems. Two more parts will follow. The material of the book represents the substance of the lectures by the author at the Pathological Institute, University of Heidelberg, and is considered the standard requirement for medical students; one must admit, it is a high standard.

The pathoanatomical and histological changes are described in the light of their pathogenetic relations against a broadly outlined background of pathophysiology and clinical pathology.

The treatment of the subject is dynamic and permeated with views and statements based on personal experience in practice and in research work. In this way the text is stimulative and able to lead the student to begin independent thinking about pathological problems. The information is up-to-date throughout the whole material; an aim certainly not easy to achieve in a one-author book, which offers much useful and new knowledge on many subjects also for the practitioner.

The chapters on the heart and vessels and the pathology of arteriosclerosis are remarkable in this respect, but the other organ systems are also well presented. Although no illustrations are included, the book is of great value but it would be welcomed if it could be supplemented with illustrations and developed into a complete book of pathology.

In view of the features mentioned, the following parts of the book are awaited with interest.

G. ROMHÁNYI

H. W. ALTMANN and F. BÜCHER (Eds)

Handbuch der allgemeinen Pathologie. Entwicklung, Wachstum, Regeneration, Hyperplasie, Cancerisierung

Vol. 6, Part 2. Springer-Verlag, Berlin, Heidelberg, New York 1969. XVI + 883 pages, with 314 figures. Price: DM 358.-; US $ 98.50

The vast field of present day knowledge concerning regeneration, growth and cancerization is treated in this comprehensive volume which contains five monographic sections.

The first section (pp. 1—128) by R. SCHINDLER gives a valuable review of the biochemical basis of regeneration, setting out the structures of DNA and RNA with their basic roles in protein synthesis, their intermediate pathways of synthesis and the key points of possible blocking by antimetabolites and cytostatics. The biochemistry of regeneration is treated

within a broad framework of cellular morphology and physiology. Radiation damage, its molecular effects on cell growth and mutation are reviewed as well as the regulation of cellular DNA, RNA and protein synthesis. Subsequently, the mechanism of biochemical regulation is discussed on the example of epithelial tissue regeneration in liver and kidney. The review gives a valuable general background of the biochemistry of regeneration at the molecular and cellular level in physiology and pathology.

The second section (pp. 129—243) by E. GRUNDMANN and H. J. SEIDEL is a review of liver regeneration following partial hepatectomy. This subject is of general interest and is now extensively studied since it represents an example of effective compensatory organ regeneration in the mammalian organism. Experimental studies of the question were mainly stimulated by the aim to obtain information on the causal factor(s) initiating and regulating the regenerative process. This section gives an excellent review of the relevant vast experimental material considering the exogenous and endogenous factors possibly related with the regulation of hepatic regeneration, great attention being paid to the question of humoral factor(s) initiating the regenerative nucleic and protein synthesis and the mitotic wave. The available evidence suggests that this might be an inhibitory factor (produced by the liver itself) initiating the regenerative proliferation of liver cells by a cellular feedback mechanism. Thus the problem seems intimately related with liver cell organisation and its further study may provide more knowledge on the basic phenomena of cell growth and its regulation.

The third section (pp. 245—375) by W. OEHLERT is a remarkable review of recent knowledge on the proliferation kinetics of the replacing tissues, obtained by using ^{3}H-thymidine autoradiography. This method has led in ten years to a wealth of information on the quantitative and qualitative aspects of cellular proliferation processes in physiology and pathology. The proliferation kinetics of some epithelial replacing tissues such as the stratified epithelium of skin, tongue, oesophagus, the epithelia of the gastrointestinal and the tracheobronchial tracts, are discussed in detail, based mainly on the experiments of the author and his team, which give a new insight into the biological changes of mitosis, the cell kinetics of physiological regeneration, and the proliferative process in cancerization.

The fourth section (pp. 374—495) by T. M. FLIEDNER and W. CALVO reviews the orthology and pathology of bone marrow regeneration. This is a field of general clinico-pathological interest with several unsolved problems, particularly with regard to cellular differentiation from the undifferentiated stem cell stores of the bone marrow. Investigations using ^{3}H-thymidine labelling in vivo and in vitro and especially the method of the complete labelling of bone marrow in vivo and by studying the capacity of the bone marrow stem cells of irradiated donors to repopulate bone marrow of lethally irradiated hosts led to completely new concepts on the qualitative and quantitative aspects of bone marrow cell regeneration and to the potential distinction of the different classes of the stem cell stores in bone marrow. The regeneration kinetics of each bone marrow cell line and the regenerative capacity of the bone marrow after different injuries with particular emphasis on radiation damage after whole and partial body irradiation are discussed. Valuable chapters are included on regenerative bone marrow hyperplasia in human pathology and on regenerative cell kinetics in human haemoblastoses.

All these data indicate how our knowledge concerning bone marrow cell kinetics has entered a new phase and that further development in this field may greatly influence our views on some basic problems of pathology.

Section V (pp. 496—766) by H. COTTIER et al. is a highly valuable review of the regeneration hyperplasia and oncogenesis of the lymphoreticular organs. This is probably the subject on which our knowledge has changed most radically in the last decade or so, mostly due to the rapid development in the field of immunology and immunohistology. The authors give a remarkable survey of this rapidly developing field of knowledge. Starting with the discussion of the phylogenesis and ontogenesis of the lymphoreticular tissues, chapters follow discussing the cell kinetics, the differentiation processes of cell lines and the basic problem of stem cells of lymphoreticular tissue. Differentiation, regeneration and proliferation in the lymphoreticular cell system are closely correlated with immunological activity (immunological competence, sensitization) during which complex functional interrelations exist between the representative organs of the system, the thymus, spleen, lymph nodes, bone marrow, and the cellular elements of the RES in different locations. These complex structural functional interrelations, the different cell populations, their life span, their migration into the tissues and recirculation are discussed in detail. The theoretical basis of the methods applicable in studies of such cell kinetics, too, is discussed critically.

Then chapters follow reviewing the mechanism of regulation of the lymphoreticular cell system in several pathological conditions in regeneration after ionizing radiation after the administration of antilymphocyte serum, lymphopenic corticoids, and cytostatics. Further chapters deal with the regeneration of the lymphoreticular cell system in immunologically

defective states, following RES blockade, with hyperplasia during antibody production in the course of antigenic stimulation, in the hypersensitivity states of the delayed type with homeo- and heterotransplantation, with transformation by phytohaemagglutinin or other mitogenic stimuli. A comprehensive chapter deals with cell kinetics in neoplastic processes of the lymphoreticular organs.

It is evident that this section will be of great value for a wide circle of readers. As a matter of fact this applies also to the subject of all the former sections of this comprehensive volume which therefore should be avaible not only in university libraries but also in those of pathological laboratories.

Each section has an extensive list of references and a subject and author index.

G. Romhányi

W. Doerr and G. Quadbeck

Allgemeine Pathologie

Heidelberger Taschenbücher 68. Springer-Verlag, Berlin, Heidelberg, New York 1970. XIII + 239 pages. Price: DM 5.80, US $ 1.60

This is a pocket book of general morphological pathology intended for medical students. General morphological pathology is becoming more and more interrelated with pathochemical data, so that an integrated form of teaching of the pathomorphological and chemical aspects of basic problems in general pathology seems to be a desirable approach.

A unique feature of this book is that the pathoanatomist and the pathochemist, both well experienced in their own field, have joined in writing a general pathology in such an integrated form. The authors have succeeded in blending pathomorphological aspects with the relevant pathochemical data. The book is well written; its text is not burdened with citations of authors or literary data and it shows much first-hand knowledge. There is a number of well-chosen and stimulative aphorisms as well as shortly inserted representative historical data. The material is treated under five headings, 1. General disturbances of blood circulation. 2. Morphological pathology of the metabolic disorders. 3. Inflammation. 4. Pathology of growth. 5. Basic characteristics of general aetiology.

Although written for the student and intended as a pocket book it can provide for the practitioner much useful information on issues of general pathology and stimulate to an integrative way of its teaching.

G. Romhányi

INDEX

PHYSIOLOGIA

Hantos Z., Lázár Z.: The Flow of Fluid through the Wall of Capillary Systems Studied by a Mathematical Model 265

Szilágyi G.: Effect of Acidification and Alkalinisation on Calcium and Citrate Metabolism in Normal and Parathyroidectomized Dogs 281

Schmidt P., Andik I.: Relationship between Water and Food Intake in the Rat 291

Várnai Ilona, Farkas Mária, Donhoffer Sz.: Thermoregulatory Heat Production and the Regulation of Body Temperature in the New-Born Rabbit 299

Heiner L., Domonkos J., Vargha M. jr.: Effect of Denervation on the Composition of Phospholipids in Tonic and Tetanic Muscles 317

Varga E., Kovács L., Gesztelyi I.: Analysis of the Depolarizing Effect of Nicotine on Frog's Skeletal Muscle 325

Gesztelyi I., Kovács L.: New Method for the Electrophysiological Analysis of the Separated Neural and Aneural Parts of Frog Sartorius Muscle 343

Balázsi I.: Insulin Uptake by Isolated Rat Fat Cells 351

Nagy I., Kurcz M., Halmy L., Mosonyi L., Baranyai P., Kiss Cs.: Effect of Stress, Drugs and Hypothalamic Extract on Anterior Pituitary Somatotropin Content in the Rat 357

Nagy I., Kurcz M., Kiss Cs., Baranyai P., Mosonyi L., Halmy L.: The Effect of Suckling, Stress and Drugs on Pituitary Prolactin Content in the Rat 371

Bohus B., Grubits J., Kovács G., Lissák K.: Effect of Corticosteroids on Passive Avoidance Behaviour of Rats 381

Makara G. B.: Superficial and Deep Chemonociception: Differential Inhibition by Pretreatment with Capsaicin 393

PATHOPHYSIOLOGIA

Nelkin W.: Diabetogenicity of Ketoheptoses 401

Szilágyi A. Katalin: Bestimmung der Guanidylessigsäurekonzentration im Blutserum und Liquor cerebrospinalis 405

PHARMACOLOGIA

Szilágyi T., Kávai Mária: The Effect of Chloroquine on the Antigen-Antibody Reaction 411

COMMUNICATIO BREVIS

Székely M., Kellermayer M., Sümegi Irene, Cholnoky Gabriella: Morphology and Function of Interscapular Brown Adipose Tissue in the Non-Cold-Adapted Adult Guinea Pig 419

RECENSIONES

Rüdiger W. (Ed.): Lehrbuch der Physiologie. VEB Verlag Volk und Gesundheit, Berlin 1969. (K. Lissák) 423

Schubert E.: Praktikum der Physiologie. VEB Gustav-Fischer Verlag, Jena 1969. (K. Lissák) 423
Kerkut G. A. (Ed.): Experiments in Physiology and Biochemistry. Vol. 3. Academic Press, London, New York 1970. (K. Lissák) 423
Hill A. V.: First and Last Experiments in Muscle Mechanics. Cambridge University Press, 1970. (K. Lissák) 424
Hudlická O. (Ed.): Circulation in Skeletal Muscle. Pergamon Press, London, New York 1968. (K. Lissák) 424
Van Cauwenberge H., Franchimont P.: Assay of Protein and Polypeptide Hormones. Vol. 33. Pergamon Press, London, New York 1970. (K. Lissák) 425
Cereijido M., Rotunno C. A.: Introduction to the Study of Biological Membranes. Gordon and Breach Science Publishers, New York, London, Paris 1970. (K. Lissák) 425
Lakshminarayanaiah N.: Transport Phenomena in Membranes. Academic Press, New York, London 1969. (K. Lissák) 425
Snell F., Wolken J., Iverson G., Lani J. (Eds): Physical Principles of Biological Membranes. Gordon and Breach Science Publishers, New York, London, Paris 1970. (K. Lissák) 426
Porter R. (Ed.): Breathing: Hering-Breuer Centenary Symposium. J. and A. Churchill, London 1970. (K. Lissák) 426
Pilz H.: Die Lipide des normalen und pathologischen Liquor cerebrospinalis. Springer-Verlag, Berlin, Heidelberg, New York 1970. (K. Lissák) 427
Ludwig W.: Das Rechts-Links-Problem im Tierreich und beim Menschen. Mit einem Anhang: Rechts-Links-Merkmale der Pflanzen. Springer Verlag, Berlin, Heidelberg, New York 1970. (K. Lissák) 427
Wolstenholme G. E. W., Knight Julie (Eds): Taste and Smell in Vertebrates. J. and A. Churchill, London 1970. (K. Lissák) 427
Leibovic K. N. (Ed.): Information Processing in the Nervous System. Springer-Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák) 428
Chestok L. (Ed.): Psychophysiological Mechanisms of Hypnosis. Springer-Verlag, Berlin, Heidelberg, New York 1969. (K. Lissák). 429
Danziger K. (Ed.): Readings in Child Socialization. Pergamon Press, Oxford 1970. (Stefanie Lissák) 429
Hollinshead W. H.: Functional Anatomy of the Limbs and Back. W. B. Saunders Co. Ltd., London 1969. (M. Forgon) 429
Doerr W.: Spezielle pathologische Anatomie I. Springer-Verlag, Berlin, Heidelberg, New York 1970. (G. Romhányi) 430
Altmann H. W., Bücher F. (Eds): Handbuch der allgemeinen Pathologie. Vol. 6, Part 2. Springer-Verlag, Berlin, Heidelberg, New York 1969. (G. Romhányi) 430
Doerr W., Quadbeck G.: Allgemeine Pathologie. Springer-Verlag, Berlin, Heidelberg, New York 1970. (G. Romhányi) 432

O. Szende and M. Nemessuri

THE PHYSIOLOGY OF VIOLIN PLAYING

With a foreword by Yehudi Menuhin and a preface by Paul Rolland

The authors, a violin teacher and a physiologist, survey the physiological and psychological problems arising in the learning and teaching of violin playing. The study is based on their unparallelled series of experiments and on their pedagogical and medical experience. The book uses the data of electro-myographical research concerned with the muscular functions involved in violin playing and treats the question of control through the nervous system while playing an instrument.

In English • 202 pages • 17×25 cm • Cloth

A joint edition — distributed in the socialist countries by KULTURA, in all other countries by COLLET'S HOLDINGS Ltd., Northants

RESULTS IN NEUROHISTOLOGY, NEUROPHYSIOLOGY, NEUROPHARMACOLOGY, NEUROPATHOLOGY

edited by K. Lissák

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РЕЗЮМЕ

ИССЛЕДОВАНИЕ ТРАНСМУРАЛЬНОГО ЖИДКОСТНОГО ТОКА МИКРОЦИРКУЛЯРНЫХ СИСТЕМ НА МАТЕМАТИЧЕСКОЙ МОДЕЛИ

З. ХАНТОШ и Ж. ЛАЗАР

Авторы исследовали условия течения жидкости на гидродинамической модели фиктивной капиллярной сети, прежде всего с точки зрения величины и интенсивности трансмурального тока. Главная цель модельного опыта состояла, кроме выявления его зависимости от ряда основных показателей (среднее капиллярное гидростатическое давление, онкотическое давление плазмы, отводящая способность лимфатических сосудов), в исследовании механизмов умеряющего снижения фильтрационного тока. Установлено, что движение трансмуральной жидкости и изменения онкотического давления протекающей плазмы взаимосвязаны. Эти влияния становятся более выраженными, если повышается способность стены капиллярной модели пропускать воду и растворенные в ней вещества. Исследование системы по наличию свойств математической модели производилось с помощью вычислительной машины.

ВЛИЯНИЕ АЦИДОЗА И АЛКАЛОЗА НА ОБМЕН КАЛЬЦИЯ И ЦИТРАТА У ТИРЕО-ПАРАТИРЕОИДЕКТОМИРОВАННЫХ СОБАК

Г. СИЛАДЬИ

1. Уровень цитрата в сыворотке крови регулируется паратиреоидным гормоном, а не колебаниями уровня кальция. Регуляция осуществляется в основном более через костную систему, а не через почки.

2. Метаболический ацидоз (введение 0.005 *N* HCl) и алкалоз (введение 0.3 *N* $NaHCO_3$) существенно не изменяют уровень цитрата и кальция в сыворотке.

3. Метаболический ацидоз снижает выведение кальция и цитрата с мочей, хотя эти изменения статистически не достоверны.

4. Метаболический алкалоз значимо повышает выведение кальция и цитрата с мочей, и увеличивает количество мочи. Эффект алкалоза на повышение выведения ионов связан с тем, что накопление HCO_3 в митохондриях снижает окисление цитрата.

5. Тирео-паратиреоидектомия препятствует влиянию метаболического алкалоза, приводящего к гиперкальцинурии. По мнению автора паратиреоидектомия снижает влияние метаболического алкалоза путем изменения энзиматической системы в клетках почечных канальцев (изменяя уровень двууглекислой соли внутри клеток) или путем потери резервного кальция.

СВЯЗЬ ПРИЕМА ПИЩИ И ВОДЫ У КРЫС

Т. ШМИДТ и И. АНДИК

Если крысам давать пищу или воду не ad libitum, а два раза в сутки по одному часу, то при этом крысы потребляют весь дневной рацион пищи, но потребление воды уменьшается до 80%-ов. Последнее не зависит от того, какую пищу они при этом получали, сухую или содержащую 50%-ов воды.

У крыс, которые питались сухой пищей и получали воду два раза по часу, вес тела и потребление пищи было меньше по сравнению с теми крысами, которые получали воду ad libitum.

Потребление пищи за два часа значительно больше как и по абсолютной, так и по процентной величине у тех крыс, которые получали воду лишь эти два часа, в отличие от тех крыс, которые имели свободный доступ к воде на протяжении 24-ех часов.

При ограничении приема воды на 50%-ов потребление пищи животными быстро уменьшается и через неделю устанавливается на более низком уровне. Вес тела снижается в течение 3—4 недель, а затем устанавливается на новом уровне. После окончания ограничения прием воды и пищи быстро достигает нормального уровня и вес тела тоже нормализуется.

ТЕРМОРЕГУЛЯЦИОННАЯ ТЕПЛОПРОДУКЦИЯ И РЕГУЛЯЦИЯ ТЕМПЕРАТУРЫ ТЕЛА У НОВОРОЖДЕННЫХ КРОЛИКОВ

И. ВАРНАИ, М. ФАРКАШ и С. ДОНХОФФЕР

У новорожденных кроликов, в возрасте от нескольких часов до 18 дней, измерялось потребление кислорода и температура ободочной кишки при окружающей температуре 35, 30 и 20°С. В термонейтральном окружении потребление кислорода не изменилось в исследованный период, но в более прохладном окружении теплопродукция по возрасту снижалась. Температура ободочной кишки при всех температурных режимах повышалась с возрастом. При температуре внешней среды 20°С, температура тела колебалась в широких пределах: ряд кроликов, не достигших 12-ти часового возраста сохранил температуру тела (с изменениями в 0.5°С) на том уровне, который был при нейтральной температуре, у других температура ободочной кишки снижалась на 3—4°С. Животные, несколькими днями старше, имели такие же сдвиги, и даже в 18-дневнем возрасте были кролики, у которых температура тела снижалась на 2°С. Не было корреляции между изменением температуры тела и весом тела, а также между температурой тела, измеренной при нейтральном окружении и изменениями температуры тела, наступившими при 20°С. Умеренная корреляция была найдена между потреблением кислорода при 20°С и наступившими изменениями температуры тела у крыс до 6-дневнего возраста. У более старших животных корреляция между потреблением кислорода и температурой ободочной кишки не обнаружена. Те животные, которые не сохраняли температуру своего тела, в прохладном окружении начинали поддерживать свою температуру в чрезвычайно различные возрастные периоды.

Авторы заключают, что причина различий в температурном гомеостазе заключается в генетически детерминированных функциональных различиях центральной регулирующей системы.

ВЛИЯНИЕ ДЕНЕРВАЦИИ НА СОСТАВ ФОСФОЛИПИДОВ В ТОНИЧЕСКИХ И ТЕТАНИЧЕСКИХ МЫШЦАХ

Л. ХЕЙНЕР, Й. ДОМОНКОШ и М. ВАРГА

После односторонней перерезки нерва количество холинофосфатидов и кефалинов в денервированных тонических и тетанических мышцах увеличивается, а в контрлатеральных иннервированных мышцах уменьшается. Эти изменения более выражены в тетанических мышцах, чем в тонических.

Качественный состав фосфолипидов после денервации также изменяется. В колинфосфатидах и кефалинах увеличивается содержание плазмалогена. Это увеличение в денервированных мышцах выражено меньше, чем в контрлатеральных иннервированных мышцах. Содержание плазмалогена в фосфолипидах больше изменяется после денервации в тетанических мышцах, чем в тонических.

АНАЛИЗ ДЕПОЛЯРИЗАЦИОННОГО ЭФФЕКТА НИКОТИНА НА СКЕЛЕТНУЮ МЫШЦУ ЛЯГУШКИ

Э. ВАРГА, Л. КОВАЧ и И. ГЕСТЕЛЬИ

Изучая особенности и механизм «медленной» деполяризации, формирующейся при действии никотина на поперечнополосатую мышцу лягушки, установлено следующее:

1. Деполяризационный эффект никотина зависит от его концентрации (между 5×10^{-2}—2×10^{-1} мМ максимален), от места измерения (максимален на краю пластинки) и от сезона (с октября до марта больше, чем весной или летом).

2. Деполяризационный эффект самый большой вблизи нервно-мышечного синапса; курарином можно тормозить этот эффект, а на аневральной части мышцы никотин, который обязательно деполяризирует, даже в 1000 раз большей концентрации не является эффектным. На основании этих фактов вероятно, что мишень для деполяризационного эффекта никотина — нервно-мышечный синапс.

Авторы предполагают, что введение капсаицина может инактивировать рецепторы на поверхностных нервных окончаниях без заметного нарушения деятельности хемоноцицепторов в более глубинных тканях. Наблюдаемая разница в тормозном влиянии капсаицина объясняется тем, что механизм возникновения нервного потенциала действия различен в поверхностных и глубоких хемоноцицепторах.

ДИАБЕТОГЕННЫЙ ЭФФЕКТ КЕТОХЕПТОЗОВ

В. НЕЛКИН

Влияние 8 кетохептозов исследовалось на крысах. Сахар крови определяли по глюкозо-оксидазе. Установлено, что d-маннохептулоза способна вызвать гипергликемию; d-глюкохептулоза и l-галактохептулоза имеют такой же эффект. l-гулохептулоза, d-идохептулоза, l-маннохептулоза, l-глюкохептулоза и d-галактохептулоза подобным влиянием не обладали, когда исследовались в дозе 200 мг.

ОПРЕДЕЛЕНИЕ ГУАНИДИНУКСУСНОЙ КИСЛОТЫ В СЫВОРОТКЕ И ЛИКВОРЕ

К. А. СИЛАДЬИ

Автор предлагает модификацию метода определения гуанидинуксусной кислоты (ГУК), основанную на известном принципе отделения, которая может быть использована при определении малых количеств ГУК в сыворотке и ликворе. Метод даёт возможность отделять ГУК от больших количеств аргинина, находящегося в тех же средах. Литературные данные о количестве ГУК в сыворотке больных с различными заболеваниями хорошо согласуются с данными, полученными автором при обследовании нейропсихиатрических больных: 1.08 γ/мл ($\sigma\pm$ 0.4). Среднее количество ГУК д ликворе 0.23 γ/мл. Характерные изменения концентрации ГУК у исследованных больных не отмечались ни в сыворотке, ни в ликворе. На основании этих данных, раннее полученные результаты об изменении концентрации дериватов монозамещенного гуанидина в сыворотке и ликворе могут быть уточнены, ввиду того, что они, вероятно, получены за счет сдвигов в содержании аргинина.

ДЕЙСТВИЕ CHLOROQUIN НА РЕАКЦИЮ АНТИГЕН-АНТИТЕЛА

Т. СИЛАДЬИ и М. КАВАИ

Chloroquin in vitro в соответствии с повышением его концентрации тормозит соединение антигена и антитела, и способствует диссоциации сформировавшегося комплекса антиген-антитело.

У морских свинок лечение chloroquin в дозе 20 мг/кг через 4 недели не влияет на систематическую анафилактическую реакцию, но тормозит местные анафилактические явления, вызванные комплексом антиген-антитело.

МОРФОЛОГИЯ И ФУНКЦИЯ ИНТЕРКАПСУЛЯРНОЙ БУРОЙ ЖИРОВОЙ ТКАНИ У МОРСКИХ СВИНОК, НЕ АДАПТИРОВАННЫХ К ХОЛОДУ

М. СЕКЕЙ, М. КЕЛЛЕРМАЙЕР, И. ШЮМЕГИ и Г. ЧОЛНОКИ

У взрослых морских свинок, содержащихся при температуре 20—23°С, интеркапсулярная бурая жировая ткань морфологически соответствует белому жиру, но сохраняет оригинальную функцию бурого жира. В прохладном окружении температура интеркапсулярного жирового тела стабилизируется параллельно с повышением потребления кислорода, и превышает температуру ободочной кишки и грудной мышцы.

3. Распространение медленной деполяризации наблюдается только в растворе Рингера, содержащем никотин.

4. Причиной медленной деполяризации является, вероятно, увеличение проницаемости мембраны для натрия и повышение входа ^{24}Na в среднем в 4,5 раз.

5. Тетродотоксин (TTX) в концентрации 10^{-7} г/мл тормозит появление никотиновых спайков в мышечном волокне, и не изменяет медленную деполяризацию и повышение ^{24}Na входа.

6. TTX в концентрации 5×10^{-6} г/мл одинаково уменьшает деполяризационный эффект никотина и его способность повышать скорость движения ^{24}Na.

7. При 0°C также как и при 20—23°C, никотин увеличивает вход ^{24}Na.

НОВЫЙ МЕТОД ЭЛЕКТРОФИЗИОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ ИЗОЛИРОВАННОЙ НЕВРАЛЬНОЙ И АНЕВРАЛЬНОЙ ЧАСТИ ПОРТНЯЖНОЙ МЫШЦЫ ЛЯГУШКИ

И. ГЕСТЕЛЬИ и Л. КОВАЧ

Авторы конструировали прибор, дающий возможность механически отделять тазовую часть (которая практически не содержит двигательных пластинок) портняжной мышцы от ее тибиальной части. Отделение происходит с помощью резиновой мембраны, которая путем мягкой компрессии препятствует диффузии из жидкостей, находящихся в двух частях сосуда. Мембранный потенциал обоих частей портняжной мышцы, а также распространение потенциал действия с одной стороны на другую не нарушается. Локальная деполяризация, вызванная увеличением внеклеточной концентрации калия, формируется и существует длительно только на той стороне, на которой применен раствор Рингера, содержащий высокую концентрацию калия.

Отделенная авторами аневральная часть мышцы, видимо, не имеет двигательных пластинок, т. к. под влиянием ацетилхолина, никотина, сукцинилхолина и карбамилхолина потенциал покоя не изменяется и не появляется фибрилляция, указывающая на возбуждение пластинки.

ПОГЛОЩЕНИЕ ИНСУЛИНА ИЗОЛИРОВАННЫМИ ЖИРОВЫМИ КЛЕТКАМИ КРЫС

И. БАЛАЖИ

При инкубации жировых клеток, изолированных с помощью коллагеназы по Rodbell и содержащих 35 μмол триглицерида, в присутствии 100 или 200 μед/мл инсулина, концентрация гормона в инкубационной среде, рассчитанная иммунореактивным методом, снижается. Это снижение происходит вследствие поглощения инсулина жировыми клетками.

При изучении скорости поглощения инсулина были определены две фазы: начальная быстрая и последующая более медленная, но более продолжительная фаза. Снижение инсулина в инкубационном растворе находится в соответствии с первоначальной концентрацией гормона.

В опытах, с содержанием в среде 200 μед/мл инсулина, на основании скорости его исчезновения и по метаболизму глюкозы в жировых клетках, автор допускает возможность неспецифического накопления инсулина на мембране клетки.

СОДЕРЖАНИЕ СОМАТОТРОФИНА В АДЕНОГИПОФИЗЕ КРЫС ПРИ СТРЕССОРНЫХ ВОЗДЕЙСТВИЯХ, ВВЕДЕНИИ НЕКОТОРЫХ ФАРМАКОЛОГИЧЕСКИХ ПРЕПАРАТОВ И ГИПОТАЛАМИЧЕСКОГО ЭКСТРАКТА

И. НАДЬ, М. КУРЦ, Л. ХАЛМИ, Л. МОШОНЬИ, П. БАРАНЬАИ и Ч. КИШШ

Согласно современным исследованиям, выполненным с помощью tibia теста, содержание STH в аденогипофизе крыс уменьшается или остается неизмененным после дачи вазопрессина, эпинефрина, инсулина, а также после некоторых стрессоров или изменения окружающих факторов. Из литературы известно также, что после интракаротидного введения гипоталамического экстракта или очищенных гипоталамических факторов наблюдается уменьшение гормона в аденогипофизе.

Ввиду того, что недостаточны и противоречны литературные данные, авторы считали необходимым повторить и дополнить выше упомянутые исследования. В своей работе они определяли содержание STH в гипоталамусе крыс с помощью чувствительного и специфичного биохимического метода, основанного на полиакриламид-гель электрофорезе.

По их результатам инсулин, различные стрессорные факторы (холод, кровопускание, лапаротомия, длительное плавание), сосательный стимул, вазопрессин и окситоцин не уменьшали содержания STH в гипофизе ни у самцов и ни у самок крыс. Гипоталамические экстракты также не изменяли содержания STH в аденогипофизе.

ДЕЙСТВИЕ СОСАТЕЛЬНОГО СТИМУЛА СТРЕССА И ФАРМАКОЛОГИЧЕСКИХ ВЕЩЕСТВ НА СОДЕРЖАНИЕ ПРОЛАКТИНА (Р) В ГИПОФИЗЕ КРЫС

И. НАДЬ, М. КУРЦ, Ч. КИШШ, П. БАРАНЬАИ, Л. МОШОНЬИ и Л. ХАЛМИ

Авторы, на основании результатов, полученных с помощью собственного полиакриламид-гель электрофоретического метода, установили, что 30—40-минутный сосательный стимул не оказывает значительного влияния на содержание пролактина в аденогипофизе крыс. У некормящих животных различные неспецифические стрессорные факторы (холод, лапаротомия, кровопускание, длительное плавание), а также инсулин, окситоцин и вазопрессин не оказывают быстрого эффекта на содержание Р в гипофизе. Результаты не исключают, однако, возможности того, что примененные стресс-факторы лишь незначительно изменяют концентрацию пролактина в гипофизе, и эти изменения трудно определить современными методами. При этом вполне возможно, что эти небольшие изменения в секреции Р, вызывают значительные изменения в органах мишенях.

ВЛИЯНИЕ КОРТИКОСТЕРОИДОВ НА ПАССИВНО-ОБОРОНИТЕЛЬНОЕ ПОВЕДЕНИЕ КРЫС

Б. БОХУШ, Й. ГРУБИЧ, Т. КОВАЧ и К. ЛИШШАК

Влияние кортикостероидов на пассивно-оборонительное поведение исследовалось в двух экспериментальных условиях, основанных на избегании света и лишении воды.

Однократное назначение кортизона снимало пассивно-оборонительную реакцию и сокращало время возврата крыс из освещенной камеры в темную, где они раньше получили однократный удар электрическим током.

В ситуации с лишением питья, когда на поилку однократно подавался электрический ток, дексаметазон также растормаживал пассивно-оборонительную реакцию. Дискриминация между сторонами покоя и шока дексаметазоном усиливалась.

ДИФФЕРЕНЦИРОВКА ТОРМОЖЕНИЯ ПОВЕРХНОСТНОЙ И ГЛУБОКОЙ РЕЦЕПЦИИ БОЛИ ВВЕДЕНИЕМ КАПСАИЦИНА

Г. Б. МАКАРА

Подкожное введение капсаицина не блокирует болевые эффекты, вызванные влиянием веществ, введенных в глубокие ткани, но вызывает длительную анестезию к веществам, раздражающим слизистую оболочку. У крыс, которые получали капсаицин:

1. чувствительность к ацетилхолину и KCl, вводимым внутриартериально, не изменена;
2. порог к субподошвенному и внутриартериальному введению капсаицина повышался в 10—100 раз; тормозной эффект может быть снят повышением дозы;
3. сокращение брюшных мышц, вызванное внутрибрюшинным введением ацетилхолина и уксусной кислоты, не заторможено.

Ноцицептивное влияние субподошвенного и внутриартериального укола капсаицина можно блокировать внутрибрюшинным введением капсаицина в повышающей дозе до 200 мг/кг через 1—3 часа после последнего укола. Через 24 часа чувствительность животных востанавливается.

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