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

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ACTA PHYSIOLOGICA

TOMUS XLIV

INDEX

PHYSIOLOGIA

Avar Z., Monos E., Kurucz M., Nagy I., Bukulya B.: Mechanism of Perinatal Reproductive Disorders Induced by Parafornical Hypothalamic Lesions in the Rat 45

Баклаваджян О., Бужаки Г., Андьян Л.: К электрофизиологическому анализу влияния атропина на реакцию самораздражения у кошек .....

Baló-Banga J. M., Antoni F., Altmann H.: Studies on Human Tonsillar Cell DNA. Preparation and Characterization of Nuclear and Non-Nuclear DNA from Isolated Tonsillar Cells 101

Batuev A. S.: Organization of Visual Cortex Primary Response 61

Csapo A. I., Fáy Erzsébet: Effect of Hypophysectomy on Ovariectomy-Induced Placental Hypertrophy 39

Farkas Mária, Donhoffer Sz.: Comparison and Correlational Analysis of the Effect of Hypoxia and Hypercapnia on Heat Production and Body Temperature in the Adult Guinea Pig 11

Kollai M., Fedina L., Kovách A. G. B.: Effect of Bleeding, Cooling and Asphyxia on the Activity of Vertebral and Cardiac Sympathetic Nerves 145

Munshi J. S. D., Dube S. C.: Oxygen Uptake Capacity of Gills in Relation to Body Size of the Air-Breathing Fish, *Anabas testudineus* (Bloch) 113

Nikolásev V., Resch B. A., Mészáros J., Szontágh F. E., Karády I.: Lipid Content of Human Embryonic Tissues 201

Puppi A., Kiss I.: Microelectrophysiological Analysis of the Correlation between the Redox State and the Type of Effect of Acetylcholine and/or 5-Hydroxytryptamine 133

Radev A. I. Effect of Simultaneous Oestriol and Progesterone Administration on Sodium and Potassium Excretion and on their Tissue Content in Female Rats 209

Rózsahegyi G., Telegdy G., Lissák K.: Diurnal Changes in Hypothalamic Serotonin Content and its Correlation with Adrenal Function in the Rat during the Oestrus Cycle ... 125

Salánki J., Vadász I.: Chemical Sensitivity at Different Temperatures of the Br-Type, Bimodal Pace-maker Neurone in the CNS of the Snail *Helix pomatia* L 51

Szalontai K., Rochlitz Sz., Molnár L.: Use of Radioactive Isotopes in the Examination of Cerebral Blood Flow 1

Varga B., Horváth Edit, Stark E.: Effects of ACTH and HCG on Ovarian Oestrogen and Progesterone Secretion in Dogs Pretreated with HCG 29

PATHOPHYSIOLOGIA

Bálint G. A., Kasirye E. M. B., Kakembo A. S. L.: Effect of Ricin Immunization on the Qualitative Composition of Plasma Proteins 77

Makkári Erzsébet, Kovách A. G. B., Nyáry I.: Acid-Base Balance and Regional CO_2 Content of the Brain in Hypovolaemic Shock 157

Maklári Erzsébet, Kovács A. G. B., Nyári I.: Effect on Central Nervous Tissue CO_2 Content of the Correction of Acidosis due to Haemorrhage 171

Spitzer A. Judy, Kovách A. G. B., Sándor P., Spitzer J. J., Storck R.: Adipose Tissue and Endotoxin Shock 183

PHARMACOLOGIA

Kenedi I., Losonci A.: Effect of Beta Adrenergic Blocking on the Arrhythmias Following Coronary Ligation in Rat 247

Phan D. V., Dóda Margit, Bite Ágnes, György L.: Antinociceptive Activity of Nicotine .. 85

Phan D. V., György L., Varga B., Szelényi Z.: Temperature Reducing Effect of Oxotrememorine in the Rat and the Mouse 259

Varga F., Fischer E., Szily Terézia: Inhibition of Microsomal Drug Metabolism by 2-{[2-(4-Pyridyl)-Methyl]-Thio} Benzimidazole (H—718) 227

Varga F., Hankovszky H. Olga, Hideg K., Fischer E., Szily Terézia: Inhibition of Hexobarbital Metabolism by Pyridyl Derivatives 235

RECENSIONES

Assali N. S. (Ed.): Pathophysiology of Gestation. Volumes I, II and III. Academic Press, New York 1972. (G. Telegdy) 99
Becker V.: Bauchspeicheldrüse (Inselapparat ausgenommen). Springer-Verlag, Berlin, Heidelberg, New York 1973. (G. Romhányi) 98
Béssis M., Weed R. I., Leblond P. F. (Eds): Red Cell Shape Physiology, Pathology, Ultrastructure. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 277
Costoff A.: Ultrastructure of Rat Adenohypophysis. Correlation with Function. Academic Press, New York, London 1973. (B. Mess) 96
De Mello W. C. (Ed.): Electrical Phenomena in the Heart. Academic Press, New York, London 1972. (K. Lissák) 195
Emmelin N., Zotterman X. (Eds): Oral Physiology. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. (K. Lissák) 96
Farner D. S., King R. (Eds): Avian Biology. Volumes I and II. Academic Press, New York, London 1971 and 1972. (K. Lissák) 95
Frisch K. v.: Erinnerungen eines Biologen. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 280
Gabelnick H. L., Litt M. (Eds): Rheology of Biological Systems. Charles C. Thomas Publisher Springfield, Illinois 1973. (K. Lissák) 95
Giese A. C. (Ed.): Photophysiology, Current Topics on Photobiology and Photochemistry. Vol. VII. Academic Press, New York, London 1972. (K. Lissák). 195
Hanke W.: Vergleichende Wirkstoffphysiologie der Tiere. VEB Gustav Fischer Verlag, Jena 1973. (K. Lissák) 277
Henley E. J., Williams R. A.: Graph Theory in Modern Engineering. Academic Press, New York, London 1973. (J. Czopf) 200
Hodge H. C., Sannard J. N., Hursh J. B. (Eds): Uranium, Plutonium, Transplutonic Elements. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 279
Iggo A. (Ed.): Somatosensory System. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 279
Jorpes J. E., Mutt V. P. (Eds): Secretin, Cholecystokinin, Pancreozymin and Gastrin. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 278
Jung R. (Ed.): Visual Centres in the Brain. Handbook of Sensory Physiology. Vol. VII/3. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 197
Krug M., Winter R. (Eds): Biochemical, Physiological and Pharmacological Aspects of Learning Processes. VEB Verlag Volk. und Gesundheit, Berlin 1972. (K. Lissák) 197
Langer H. (Ed.): Biochemistra and Physiology of Visual Pigments. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 196
Levi L. (Ed.): Stress and Distress in Response to Psychosocial Stimuli. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. (K. Lissák) 198
Levine Y. K.: X-Ray Diffraction Studies of Membrane. Vol. 3. Part. 4. Pergamon Press, Oxford 1973. (T. Lakatos) 97
Lüttge U. (Ed.): Microautoradiography and Electron Probe Analysis. Their Application to Plant Physiology. Springer-Verlag, Berlin, Heidelberg, New York 1972. (G. L. Farkas) 97
Rossmann M. G. (Ed.): The Molecular Replacement Method. Gordon and Breach Science Publishers, New York, London, Paris 1972. (A. Kálmán)..... 199
Salánki J. (Ed.): Neurobiology of Invertebrates. Mechanism of Rhythm Regulation. Akadémiai Kiadó, Budapest 1973. (A. Puppi) 199
Voss H. E., Oertel G. (Eds): Androgene I. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 278
Wehner R. (Ed.): Information Processing in the Visual Systems of Arthropodes. Springer-Verlag, Berlin, Heidelberg, New York 1972. (K. Lissák) 196

ABSTRACTS of the lectures delivered at the 38th Congress of the HUNGARIAN PHYSIOLOGICAL SOCIETY, Budapest, May 31—June 3, 1972 281

INDEX RERUM

Abortion 39
acetylcholine 51—59, 66, 92, 133—142, 225, 271, 272
4-acetylpyridine 233, 235
acid-base balance 157, 159, 161, 164
acidosis 157, 171—175
ACTH 29—36, 47
action potentials 148
actinomycin-D 35
adenohypophysis 47, 48
adenyl cyclase 34, 141
adipose tissue 183—192
adrenal blood flow 29, 35
— function 126
— gland 32, 34, 42, 47
adrenalectomy 32, 34, 40
adrenaline 66, 133, 134
adrenocortical system 167
albumin 79, 82, 186
aldosterone 209, 214
aminophenazone, N-demethylation of 232
amnion 47
amphetamine 272
amylnitrite 271, 272
anaesthesia, ether 227
analgesia 85
androgen 125
aniline, hydroxylation of 232
anoxia 191
antibody 77
antigen 77
aortic chemoreceptors 154
arecoline 85
arrhythmia 247, 248, 252, 255, 256
ascorbic acid 136, 137, 140
asphyxia 146, 151, 152, 154
asthma, bronchial 256
ATPase 141
atropine 87, 92, 260, 266, 272
— , its effect on evoked potentials 222
— , its effect on self-stimulation 217—223
auditory cortex 72
AV block 248, 249, 251

Barbital sleeping time 227, 229
baroreceptors 153
baroreflexes 145
behaviour, maternal 45, 47, 48
blood flow 185, 191
— — , adrenal 29, 35
— — , cerebral 1, 2, 5
— — , ovarian 29—36
— haemoglobin content 161
— lactate level 185
— lipides 104
— pressure 147, 153, 185, 270
— viscosity 180
blood-brain barrier 90
bleeding 146, 147, 171, 180, 185
body temperature 13—17, 24—27, 45, 47, 82, 260, 262
— weight 45, 46, 113—119
bradycardia 248, 249
brain stem 220
— tissue, buffer capacity of 166
— — , CO_2 level of 158, 162, 171, 178, 179
bromsulphophthalein 232
bronchial asthma 256
bronchitis, chronic 256
bronchospasm 256
Br-type neurone of *Helix pomatia* 52, 54, 58
buffer capacity of brain tissue 166
burst generation 51, 52

cAMP 34, 191
carbachol 260, 271—273
cardiac output 185
carotid chemoreceptors 153
castration 125, 127, 130
catecholamine 129, 166, 186
cerebral blood flow 1, 2, 5
chemoreceptor activity 153
— , aortic 154
— , carotid 153
chloral hydrate 271
chloralose 65—70, 74
chloroplast 101
chlorpromazine 66, 229, 271, 272
cholesterol 34, 201—204
— ester 201, 202
cholinergic receptors 138
cholinesterase 233

clearance, endogenous creatinine 213
CO_2 level of brain tissue 158, 162, 171, 178, 179
congenital abnormalities 205
colonic temperature 12—15, 20 25—27
cooling 146, 149, 150
coronary ligation 247—249, 252, 255
corpus luteum 34, 35, 39
cortex, auditory 72
— , frontal 166, 167, 171, 172, 179, 180
— , occipital 162, 179, 180
cortical response, primary 61, 62, 65—72
— — , — , its latency 67—70
corticosterone level, plasma 128—130
creatinine clearance, endogenous 213
cycloheximide 33, 35

Dehydroepiandrosterone 34
dendrites 74
denervation, sympathetic 192
depolarization 53 139, 141
dibenzyline 180
diglycerides 201—203, 206
diphenylamine 106
diuresis 213, 214
DMPP 90—92
DNA 101—110
dopamine 57—59, 125, 271
dopaminergic cells in the hypothalamus 125
drug metabolism, microsomal 227—235
D type cells of *Lymnea stagnalis* 134, 135

E. coli endotoxin 185, 187, 189, 191
EEF changes in shock 167
— hypersynchronization 222
embrionic tissues, lipid fractions in 201, 202
endoplasmic reticulum, liver 235
endotoxin shock 191
EPSP 57—59, 65, 66, 140
eserine 66
erythrocyte 106
ethanol 271, 272
ether anaesthesia 227
ethyl ether sleeping time 240
evoked potential 217, 221—225
extrasystole 248—252

Fat 183
fatty acid, free 185—192, 201, 206
fibrillation, ventricular 248—253
flavoprotein 189
flutter, ventricular 248—253
foetal disorders 45
— vitality 45, 47
— weight 41
food uptake 46
frontal cortex 166, 167, 171, 172, 179, 180
FSH 125, 126

GABA 65
gestrointestinal tract 229
globulin 77, 81—83
glomerular filtration rate 214
glucose 185—191
glutathione 232
— S-aryltransferase 233
glycerol 185 —190
glycogen 183
gonadotoropic hormones 29, 34—36, 125

H-718, 2-{[2-(4-pyridyl)-methyl]-thio}benzimidazole 227—240, 244
— , acute toxicity of 232
haematocrit 180
haemodilution 180
haemoglobin content, blood 161
haemorrhagic shock 157, 158, 167, 171, 180, 181, 191
HCG 29—35
heart rate 185
heat loss 260
— production 12—25, 260, 271
hexobarbital concentration, plasma and tissue 229, 230, 233, 240, 243
— metabolism 233, 239
— oxydation of 232, 242
— sleeping time 227—240
H type cells of *Lymnea stagnalis* 134
hydroquinone 137, 138
hypercapnia 12—27, 158, 164, 166, 179, 180
hyperlipaemia 204
hyperpolarization 55, 137, 139, 141
hypertension 270, 272
hyperthermia 260
hyperventillation 165
hypocholesterolaemia 205
hypophysectomy 40, 41
hypotension 171
hypothalamic lesion, parafornical 45, 47
— norepinephrine content 125
— serotonin content 126—130
— serotoninergic system 126
hypothalamus 125, 126, 145, 164—167, 171, 172, 178—180, 217, 218, 260, 272
— , dopaminergic cells in 125
hypothermia 260—262, 267, 271, 273
hypoxia 12—27, 153, 191

Immunization 77, 79, 83, 84
infarction 251, 255
IPSP 65, 66
ischaemia 255
isoniazide 233
isonicotinic acid hydrazide 235
isoprenaline 185
isoproterenol 247, 271

Kidney 201, 202, 206

Lactate level in blood 185
lactation 45
latency of primary cortical response 67—70
leucocytic infiltration 255
LH 34, 35
— release 126, 129
— releasing activity 125

lipid fractions in embrionic tissues 201, 202
— in blood 104
lipolysis 189—191
liver 201—203
— endoplasmic reticulum 235
LTH 45, 46, 48, 126
lung 201—203
lymphocytes 101, 102, 109, 110
lymphoid tissue 83

Maternal behaviour 45, 47, 48
mecamylamine 87, 90, 92
medial forebrain bundle 220
medulla oblongata 166, 178
membrane polarization 136,
— , postsynaptic 138
— potential 140
— proteins 142
metabolic changes during pregnancy 204
metabolism, drug 227—235
methylatropine 261, 266, 267, 271, 272
methylene blue 134, 135, 139, 141
α-methyl-p-thyrosine 125, 129
metyrapone 233, 235, 244
microsomal drug metabolism, hepatic 227—235
— procaine estherase 232, 233
milk excretion 48
mineralocorticoids 209, 213
mitochondria 101
monoglyceride 201, 202
motor reaction, contraversive 218
— — , ipsiversive 218
myocardial potassium content 256

NAD 233
NADPH 233
natriuresis 209
neostigmine 271
nephron 214
nerve, cardiac sympathetic 145—154
— , splanchnic 145
— , vertebral sympathetic 147—154
neurohypophysis 47
neuronal activity 55
neurone, Br-type 52, 54, 58
— , pyramidal 70
nicotine, its antinociceptice activity 85—92
nicotinic acid 135, 142
noradrenaline, norepinephrine 51, 57, 59, 186—192, 217—221, 271
— content, hypothalamic 125

Occipital cortex 162, 179, 180
oedema 30
oestradiol 35, 39, 40, 209
oestriol 209, 210, 213, 214
oestrogen 125, 129, 130
— secretion 29—36
oestrone 35, 127, 209
oestrus cycle 125—130
ontogenesis 70
osmolarity, plasma 213, 214
— , urine 213
ovarian blood flow 29—36
— weight 30, 47
ovariectomy 39—41
ovulation 125, 126
oxygen consumption 13—17, 24—26, 113—122, 256, 260—265, 271—273
— saturation 161, 164, 175
— uptake 120, 185
— — capacity 114, 121
oxotremorine 85, 88, 230, 233, 260—273

Palmitic acid 202
parafornical hypothalamic lesions 45, 47
paraoxon 233
parathion, acute toxicity of 231, 233
pentobarbital 64, 67, 70
pH changes 159, 174, 175
pehoxybenzamine 185
physostigmine 88, 92, 272
piloerection 154
pindolol 247, 249, 252—256
pituitary gland 42
placenta 39, 40, 47, 201—206
placental growth inhibitor (PGI) 40, 41
— hypertrophy 40, 41 42
plasma corticosterone level 128—130
— hexobarbital concentration 229, 230, 233, 240, 243
— osmolarity 213, 214
— potassium content 211
— potassium content 211
— protein level 77, 79
plasma-membrane 101, 102, 109
p-methyl-amino-phenosulphate 138, 139
pons 179
postsynaptic membrane 138
potassium content, myocardial 256
— — of renal structures 211, 214
— — , plasma 211
— excretion 209, 210
— reabsorbtion 214
practolol 247, 251, 252
pregnancy 39, 45
— , metabolic changes during 204
pregnanolone 34
procaine 272
— esterase, microsomal 232, 233
progesterone 39, 40, 127, 130, 209, 210, 213, 214
— secretion 29—35
propranolol 247, 248, 253, 255, 256
protein level, plasma 77, 79
puromycin 35
pyramidal neurones 70
pyrogenic effect of ricin 78, 82, 84

Redox state of the tissue 133, 141
— potential 133
reflex, righting 229
renal cortex 209
— medulla 209

renal papilla 209
— potassium content 211, 214
— sodium content 214
respiratory rate 178
— surface 113, 120
reticular formation 85
ricin 77, 83
— , its pyrogenic effect 78, 82, 84
RNA 101, 107

Salive secretion 271
scopolamine 87, 223
seizures, clonic 86
self-stimulation, effect of atropine on 217—223
serotonin 51, 55—59, 126, 133, 139—141
— content, hypothalamic 126—130
serotoninergic system, hypothalamic 126
shock, EEG changes in 167
— , endotoxin 191
— , haemorrhagic 157, 158, 167, 171, 180, 181, 191
sinus rhythm 248, 249, 251
skin temperature 260—270
— , vasodilatation in 260, 267, 271, 272
sodium content, renal 214
— excretion 209, 210
— reabsorbtion 209, 210, 213
spike activity 57, 72, 73
— generation 51, 52, 57, 136, 137
splanchnic nerve 145
staphylococci 108
stearic acid 202
steroidogeneses 34
STH 45, 48
streptococci 108
stress 48, 129
strychnine 65
sympathetic activity 146, 147, 153, 154
— denervation 192
— nerves, cardiac 145—154
symphatetic nerves, vertebral 147—154
sympathico-adrenal activity 185
synaptic potentials 57, 58

Tachycardia, ventricular 248, 249
tachyphylaxis 261, 272
thalamic afferents 62, 71
— nuclei 70
thalamo-cortical associative system 62
thalamus 162, 179, 180
temperature, body 13—17, 24—27, 45, 47, 82, 260, 262
— , colonic 12—15, 20, 25—27
— , core 262, 269, 272
— , skin 260—270
testis 34
theca interna 35
theophylline 192
thermoregulation 145
thermoregulatory mechanisms 11, 24, 27
thionine 135
tonsils 102, 108
toxicity of H-718, acute 232
— of parathion, acute 231, 233
tremor 86, 230, 233, 267
tremorine 88, 230, 259, 260, 269
triglyceride 186, 201, 202 206

Urethan 264—267, 272, 273
urine osmolarity 213

Vasoconstriction 272
vasodilatation in the skin 260, 267, 271, 272
vasopressin 271, 272
ventricular fibrillation 248—253
— flutter 248—253
— tachycardia 248, 249
visual projection zone 62
vitamin B_{12} 233

Water uptake 46

INDEX AUTORUM

A

Altmann H. vide *Baló-Banga J. M.* 101
Андьян Л. vide *Баклаваджян О.* 217
Antoni F. vide *Baló-Banga J. M.* 101
Avar Z., Monos E., Kurcz M., Nagy I., Bukulya B.: Mechanism of Perinatal Reproductive Disorders Induced by Parafornical Hypothalamic Lesions in the Rat 45

B

Баклаваджян О. Бужаки Г., Андян Л.: К электрофизиологическому анализу вмияния атлопина на реакцию самораздражения у кошек 217
Baló-Banga J. M., Antoni F., Altmann H.: Studies on Human Tonsillar Cell DNA. Preparation and Characterization of Nuclear and Non-Nuclear DNA from Isolated Tonsillar Cells 101
Batuev A. S.: Organization of Visual Cortex Primary Response 61
Bálint G. A., Kasirye E. M. B., Kakembo A. S. L.: Effect of Ricin Immunization of Plasma Proteins 77
Bite Á. vide *Phan D. V.* 85
Bukulya B. vide *Avar Z.* 45
Бужаки Г. vide *Баклаваджян О.* 217

C

Csapo A. I., Fáy Erzsébet: Effect of Hypophysectomy on Ovariectomy-Induced Placental Hypertrophy 39

D

Dóda M. vide *Phan D. V.* 85
Donhoffer Sz. vide *Farkas M.* 11
Dube S. C. vide *Munshi J. S. D.* 113

F

Farkas Mária, Donhoffer Sz.: Comparison and Correlational Analysis of the Effect of Hypoxia and Hypercapnia on Heat Production and Body Temperature in the Adult Guinea Pig 11
Fáy E. vide *Csapo A. I.* 39
Fedina L. vide *Kollai M.* 145
Fischer E. vide *Varga F.* 277, 235

G

György L. vide *Phan D. V.* 85, 259

H

Hankovszky H. O. vide *Varga F.* 235
Hideg K. vide *Varga F.* 235
Horváth E. vide *Varga B.* 29

K

Kakembo A. S. L. vide *Bálint G. A.* 77
Karády I. vide *Nikolásev V.* 201
Kasirye E. M. B. vide *Bálint G. A.* 77
Kenedi I., Losonci A.: Effect of Beta Adrenergic Blocking on the Arrhythmias Following Coronary Ligation in Rat 247
Kiss I. vide *Puppi A.* 133
Kollai M., Fedina L., Kovách A. G. B. Effect of Bleeding, Cooling and Asphyxia on the Activity of Vertebral and Cardiac Sympathetic Nerves 145
Kovách A. G. B. vide *Kollai M.* 145
— vide *Maklári E.* 157, 171
— vide *Spitzer A. J.* 183
Kurcz M. vide *Avar Z.* 45

L

Lissák K. vide *Rózsahegyi G.* 125
Losonci A. vide *Kenedi I.* 247

M

Maklári Erzsébet, Kovács A. G. B., Nyári I.: Acid-Base Balance and Regional CO_2 Content of the Brain in Hypovolaemic Schock 157

Maklári Erzsébet, Kovách A. G. B., Nyári I.: Effect of Central Nervous Tissue CO_2 Content on the Correction of Acidosis due to Haemorrhage 171

Mészáros J. vide *Nikolásev V.* 201

Molnár L. vide *Szalontai K.* 1

Monos E. vide *Avar Z.* 45

Munshi J. S. D., Dube S. C.: Oxygen Uptake Capacity of Gills in Relation to Body Size of the Air-Breathing Fish, *Anabas testudineus* (Bloch) 113

N

Nagy I. vide *Avar Z.* 45

Nikolásev V., Resch B. A., Mészáros J., Szontágh F. E., Karády I.: Lipid Content of Human Embryonic Tissues 201

Nyári I. vide *Maklári E.* 157, 171

P

Phan D. V., Dóda Margit, Bite Ágnes, György L.: Antinociceptive Activity of Nicotine 85

Phan D. V., György L., Varga B., Szelényi Z.: Temperature Reducing Effect of Oxotremorine in the Rat and the Mouse 259

Puppi A., Kiss I.: Microelectrophysiological Analysis of the Correlation between the Redox State and the Type of Effect of Acetylcholine and/or 5-Hydroxytryptamine 133

R

Radev A. I.: Effect of Simultaneous Oestriol and Progesterone Administration on Sodium and Potassium Excretion and on their Tissue Content in Female Rats 209

Resch B. A. vide *Nikolásev V.* 201

Rochlitz Sz. vide *Szalontai K.* 1

Rózsahegyi G., Telegdy G., Lissák K.: Diurnal Changes in Hypothalamic Serotonin Content and its Correlation with Adrenal Function in the Rat during the Oestrus Cycle 125

S

Salánki J., Vadász I.: Chemical Sensitivity at Different Temperatures of the Br-Type, Bimodal Pace-Maker Neurons in the CNS of the Snail *Helix pomatia* L 51

Spitzer A. Judy, Kovách A. G. B., Sándor P., Spitzer J. J., Storck R.: Adipose Tissue and Endotoxin Shock 183

Spitzer J. J. vide *Spitzer A. J.* 183

Stark E. vide *Varga B.* 29

Storck R. vide *Spitzer A. J.* 183

Szalontai K., Rochlitz Sz., Molnár L.: Use of Radioactive Isotopes in the Examination of Cerebral Blood Flow 1

Szelényi Z. vide *Phan D. V.* 259

Szily T. vide *Varga F.* 227, 235

Szontágh F. E. vide *Nikolásev V.* 201

T

Telegdy G. vide *Rózsahegyi G.* 125

V

Vadász I. vide *Salánki J.* 51

Varga B., Horváth Edit, Stark E.: Effects of ACTH and HCG on Ovarian Oestrogen and Progesterone Secretion in Dogs Pretreated with HCG 29

— vide *Phan D. V.* 259

Varga F., Fischer E., Szily Terézia: Inhibition of Microsomal Drug Metabolism by 2-{[2-(4-Pyridyl)-Methyl]-Thio} Benzimidazole (H-718) 227

Varga F.,Hankovszky H. Olga, Hideg K., Fischer E., Szily Terézia: Inhibition of Hexobarbital Metabolism by Pyridyl Derivatives 235

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Physiologia

USE OF RADIOACTIVE ISOTOPES IN THE EXAMINATION OF CEREBRAL BLOOD FLOW

By

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The radioactive clearance technique applied for measuring cerebral blood flow has been described. The procedures for the mathematical evaluation of clearance curves obtained in the course of investigations have also been dealt with. A proposal has been made for the application of a new mathematical method, by means of which evaluation of clearance-curves may provide data less ambiguous than those available previously.

1. The growth of interest in the physiology and pathology of cerebral blood flow is explained by the fact that disorders of cerebral blood supply are frequent causes of death (see DORNDORF and GÄNSHIRT 1972).

Since 1850, various methods have been applied to determine cerebral blood flow and metabolism (see MOLNÁR 1967). Work in this area received great impetus with the development of the nitrous oxide method by KETY and SCHMIDT (1945, 1948). In recent years, however, this technique has considerably been criticized (see HERRMANN 1972). KETY (1960) himself outlined its limitations. What the procedure mostly lacks is that it does not offer more than average data and for drawing reliable conclusions, a series of examinations have to be carried out. Another disadvantage of the procedure is that it gives information about the circulation of the brain as a whole. However, the majority of pathological processes (softening, haemorrhage, tumour, injuries) damage only circumscribed areas of the brain (see LASSEN and INGVAR 1972) and the technique does not permit the localization of circulatory changes which are of fundamental significance in clinical practice. Nor does it satisfy the needs of those who investigate theoretical principles since the procedure cannot record rapid circulatory changes (see BETZ 1972).

In order to eliminate these disadvantages, the application of radioactive isotopes has been suggested, mostly by Scandinavian researchers (LASSEN and INGVAR 1972; LASSEN and HØEDT-RASMUSSEN 1966; LASSEN et al. 1963; INGVAR and LASSEN 1972; HØEDT-RASMUSSEN et al. 1966). Two types of radioactive isotopes are employed: 1) non-diffusible substances, and 2) diffusible indicators, i.e. inert gases. The latter and widespread method

is considered suitable for measuring regional blood flow in the brain (LASSEN and INGVAR 1972; INGVAR and LASSEN 1972). According to these authors, the analysis of clearance curves for ^{85}Kr or ^{133}Xe injected into the internal carotid permits the quantitative determination of regional blood flow.

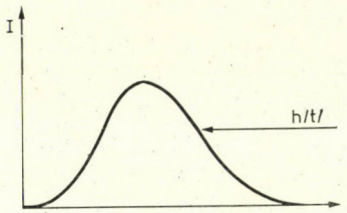

Fig. 1. Explanation in text

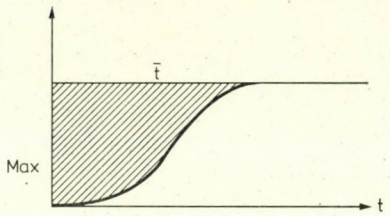

Fig. 2. Summation curve of Fig. 1.

The most widespread technique is the injection of 1.0 mCi/ml of ^{133}Xe in 3—5 ml physiological saline solution into the internal carotid within 1—2 seconds. Impulses, registered by scintillation counters, centred round the various regions of the brain, are recorded on tape. Changes of radioactivity are measured during ^{133}Xe wash out by plotting a clearance curve. Gamma activity of ^{133}Xe is registered by scalp detectors. Use of the beta emitting ^{85}Kr is restricted to animal experiments because its measurement requires craniotomy. The data obtained are recorded on tape, registered by ratemeters and evaluated graphically by the methods of MEIER and ZIERLER (1954), ZIERLER (1965) or LASSEN et al. (1963).

1.1. The method of MEIER and ZIERLER (1954).

Mean transit time ($\bar{t}$) of the radioactive substance representing blood flow is calculated as follows.

$$\bar{t} = \int_0^\infty th(t)\,dt$$

$$F = \frac{V}{t}$$

where $h(t)$ = density function,
$\bar{t}$ = mean transit time,
V = blood volume.

Graphic representation of the registered curve is seen in Fig. 1.

Its summation curve is $H(t) = \int_0^t h(t)dt$ (see Fig. 2)

(1.1) $$\bar{t} = \int_0^\infty th(t)dt = \int_0^\infty [1 - H(t)]\,dt$$

In a chosen case
$h(t) = \alpha e^{-\alpha t}$ (α is related to the biological half-time)

$$\bar{t} = \int_0^\infty t\alpha e^{-\alpha t}\, dt = \frac{1}{\alpha}$$

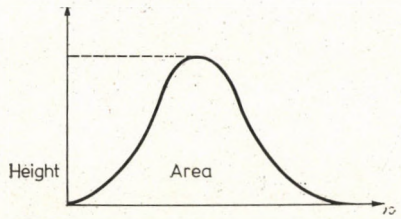

Fig. 3. Registered curve for calculation of t

$\bar{t}$ = the mean can also be calculated on the basis of

$$(1.1)\quad H(t) = \int_0^t h(t)dt = 1 - e^{-\alpha t}$$

$$\bar{t} = \int_0^\infty [1 - H(t)]\, dt = \int_0^\infty e^{-\alpha t} dt = \frac{1}{\alpha}.$$

Both methods of calculation yield the same result:

$$\bar{t} = \frac{1}{\alpha}$$

Technically, registration is carried out by means of a scaler and line-plotter or only a line-plotter.

1.2. According to ZIERLER (1965) the phenomenon cannot be described as a single exponential density function. If the results appear on a scaler, mean transit time is,

$$\bar{t} = \frac{A(rea)}{H(eight)} \text{ (Fig. 3)},$$

A = total number of decompositions,
H = initial maximum of the curve.

If the h(t) function appears only in the area recorded by the line-plotter, evaluation is carried out graphically by plotting the initial tangent (Fig. 4), or by measuring half-times if the ordinate of the line-plotter has a logarithmic

scale (Fig. 5). Half-time is the period during which the initial intensity of radiation decreases to half. (This biological half-time is not to be confused with the physical half-time of the isotope applied during measurement. In the case of ^{133}Xe the latter amounts to 5.5 days.)

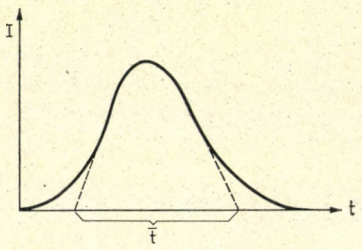

Fig. 4. Techniques of plotting the initial tangents

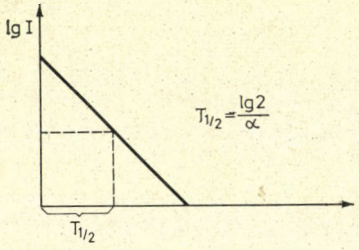

Fig. 5. Measurement of biological half-times

Blood flow is related to unit weight of the organ studied.

$$F = \frac{\lambda}{\bar{t}}$$

where λ is the solubility ratio between tissue and blood (defined by KETY as partition coefficient), and F is flow in ml/100 g tissue/min.

1.3. According to LASSEN et al. (1963), the majority of registrations does not give a straight on a logarithmic scale. It can, however, be divided into a fast and a slow component. The fast component represents the clearance curve of the grey, whereas the slow one that of the white matter.

$C(t) = I_g \exp(-k_g t) + I_w \exp(-k_w t)$,
I_g = initial intensity of radiation in grey matter,
I_w = initial intensity of radiation in white matter,
k_g and k_w = to be determined on the basis of the recorded diagram.
$f_g = \lambda_g\ k_g$ (the λ values are taken from a given chart).
$f_w = \lambda_w\ k_w$.
Relative weights:

$$W_g = \frac{\frac{I_g}{f_g}}{\frac{I_g}{f_g} + \frac{I_w}{f_w}} \qquad W_w = \frac{\frac{I_w}{f_w}}{\frac{I_g}{f_g} + \frac{I_w}{f_w}}.$$

It follows that

$$f = W_g f_g + W_w f_w, \quad \text{while } W_g + W_w = 1.$$

The resultant flow is the weighted sum of the individual components.

According to LASSEN et al. (1963) this method of curve analysis provides reliable information if the following conditions are met:

A) The curve is made up of two exponential components, theses being the clearance curves for the grey and white matter, respectively.

This is possible only if

B) the grey and white matters represent two homogeneous kinds of tissue, functioning next to each other and no diffusion of the radioactive substance occurs.

Fulfilment of these two conditions cannot be verified as:

a) The clearance curves of the grey and white matter cannot be regarded as monoexponential even if forced. On the basis of data obtained by other methods it can be verified unambiguously that in the various parts of the grey matter (cortex, striatum, thalamus) blood flow during a unit of time and calculated with respect to a mass unit is very diverse. The same is valid for the white matter (see REIVICH 1972).

During clearance, activity arising from the white and grey matter is recorded simultaneously by the detector and in the venous blood the parts coming from the grey and from the white matter cannot be separated. The veins contain mixed blood.

b) The assumption that inert gases remain in one single area of the same organ and do not diffuse into the other is inconceivable.

Division of the curve into two components seems to be arbitrary even if the conditions set up by LASSEN et al. (1963), HØEDT-RASMUSSEN et al. (1966) are fulfilled, for at least one more component has to be taken into consideration: the effect of radioactivity in venous blood.

Division of the clearance curves into two components, involves the danger of subjectivity, for the plotting of tangents depending on their placing yields various results.

One of the requirements in the evaluation of clearance curves is the continuity of flow during measurement. Significant practical difficulties may arise in biexponential evaluation, if e.g. the flow changes within one compartment. In these cases slope and shape modifications occur in the clearance curves. The slope of curves can be influenced by a change of flow just in one of the compartments, likewise if the activity originates from the other compartment.

When comparing the three methods, it must be taken into account that the measuring procedures applied do not provide curves of the same type. The choice of the appropriate method of calculation must depend on the technique of measurement.

The mentioned disadvantages and errors are eliminated by the use of the evaluation procedure to be described below.

2. Our considerations are based on PARSONS' work (1968), which represents one of the possible methods (GARDNER et al. 1959; MEDGYESSY 1961; PARSONS 1970).

To begin with, the recorded curve is made up of p components. These components are exponential density functions:

$$r(t) = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + \ldots + A_p e^{-a_p t}. \tag{2.1}$$

The values for this function are measured at given points of time: t_i $(i = 1, .. N)$.

Fulfilment of the relation

$$0 < a_1 < a_2 < \ldots < a_p \text{ can be assumed.} \tag{2.2}$$

Independent of the way of recording (scintillation counters or line-plotters), the fulfilment of

$$t_i = t_1 + (i-1)T \qquad i = 1, \ldots N$$

can be realized. If

$$x_j = e^{-a_j T} \tag{2.3}$$

and

$$B_j = A_j e^{-a_j t_1} \qquad j = 1, \ldots p$$

then

$$A_j e^{-a_j t_i} = B_j x_j^{i-1} \qquad i = 1, \ldots N$$
$$j = 1, \ldots p.$$

Then the solution of the original problem is equivalent to the determination of such $B_1, \ldots B_p, x_1, \ldots x_p$ constants with respect to which

$$r(t_i) = B_1 x_1^{i-1} + B_2 x_2^{i-1} + \ldots + B_p x_p^{i-1} \qquad i = 1, \ldots N. \tag{2.4}$$

It follows from our earlier assumption that

$$1 > x_1 > x_2 > \ldots > x_p > 0. \tag{2.5}$$

x_j values are determinable, namely as roots of the

$$x^p - c_1 x^{p-1} + c_2 x^{p-2} - \ldots + (-1)^p c_p = 0 \tag{2.6}$$

algebraic equation. The $c_1, \ldots c_p$ coefficients occurring in the equation are the solutions of the

$$r(t_{i+p-1})\, c_1 - r\,(t_{i+p-2})\, c_2 + \ldots + (-1)^{p+1} r(t_i) c_p = r(t_{i+p}) \tag{2.7}$$

$$i = 1, \ldots N-p$$

linear system of equations.

Then B_j values can be calculated from the (2.4) system of equations,

$$a_j = -\frac{\ln x_1}{T} \qquad j = 1, \ldots p$$

$$A_j = \frac{B_j}{x_j^{\frac{t_1}{T}}}.$$

During calculations, several difficulties appear. In the case of the (2.4) and (2.7) systems of equations, the equations can be greater in number than the unknown variables. Considering the fact that these contain values obtanied. by means of measurement, the existence of an exact solution cannot be expected. The systems of equations can be solved, however, by applying the method of least squares.

The solution of the (2.6) algebraic equation is not simple either, furthermore, if $p = 3$ or $p = 4$ it becomes extremely complicated and if $p \geq 5$ there is no exact formula to reach a solution. At such times, however, appropriate numerical methods can be used.

Determination of the value for p, i.e. the number of the components, is a difficult task.

The method recommended by PARSONS (1968) is extremely complicated. A much more practical solution can be found instead, if the analysis of curves is carried out — $p = 1, 2, \ldots N-p$, in turn. In the case of every p the

$$s_p = \sqrt{\frac{1}{N} \sum_{i=1}^{N} [r(t_i) - \bar{r}(t_i)]^2}$$

value can be calculated on the basis of (2.1).

If s_p is small enough as related to a given p value, or if s_p and s_{p+1} do not differ considerably, the presence of a p component can be stated. If in the case of some p value the requirements (2.2) and (2.5) are no longer met, r(t) can be considered to have a $p - 1$ component.

For the calculation of regional blood flow ($f = rCBF$) the $f = r\,(0) = A_1 + A_2 + \ldots + A_p$ formula is proposed. In the case of two components, this yields the same results as do the methods quoted, but its calculation does

not depend on the number of components. The generalization of relative weights, as described by LASSEN et al. (1963) in 1.3., is not regarded as soluble with respect to more components because the choice of λ values is arbitrary.

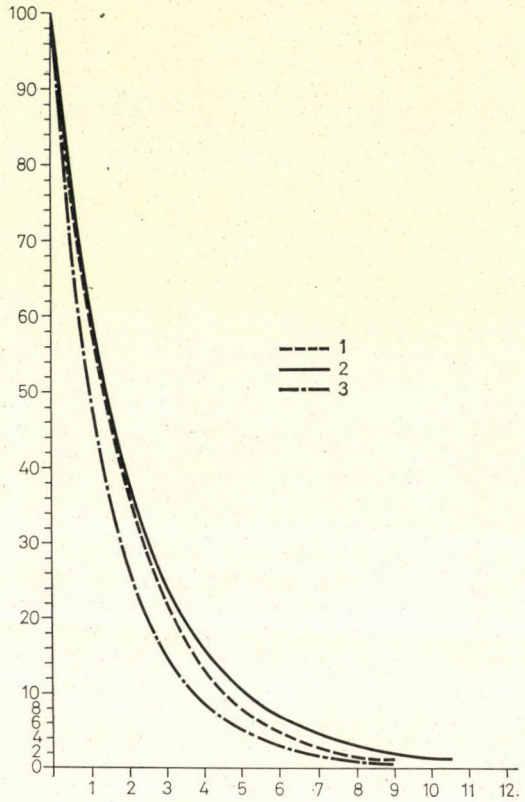

Fig. 6. Curves plotted on the basis of 1. $rt = 100\ e^{-0.5}$; 2. $rt = 22\ e^{-t} + 78\ e^{-.04t}$; 3. $rt = 30\ e^{-t} + 28\ e^{-0.4t} + 42\ e^{-0.7t}$

The suggested evaluation procedure has been used for the analysis of fictitious curves. It was possible to complete the analysis of a curve in a few seconds. This procedure was resorted to because instruments for measuring blood flow were not available. Curves were made using determined parameters and component number, and the applicability of the division method recommended here was controlled by analyses (Figs 6 and 7). The calculated values were in each case in complete agreement with the parameters contained in the computer program. The figures show well that the graphic evaluation of curves consisting of components varying in number is unreliable, especially if errors are allowed to remain in them. By means of the method outlined here, the

number of components can, however, be determined with complete certainty. The calculations were carried out using an ALGOL 60 program on an Odra-1204 type electronic computer at the Kossuth Lajos University of Debrecen.

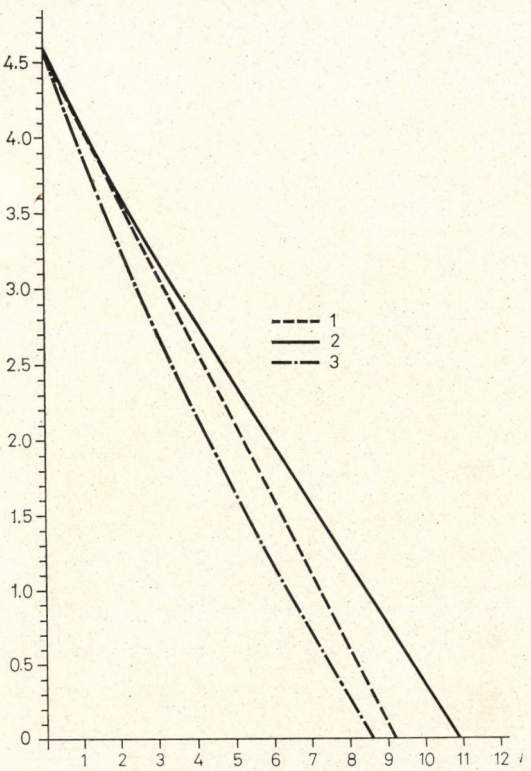

Fig. 7. The curves of Fig. 1 in a semilogarithmic co-ordinate system. (Curve 1 contains one, Curve 2 two, Curve 3 three components.)

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COMPARISON AND CORRELATIONAL ANALYSIS OF THE EFFECT OF HYPOXIA AND HYPERCAPNIA ON HEAT PRODUCTION AND BODY TEMPERATURE IN THE ADULT GUINEA PIG

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Oxygen consumption (VO_2) and colonic temperature (T_c) of adult guinea pigs of both sexes were recorded before, during, and after breathing 12% O_2, 8% O_2, and 6% CO_2 at ambient temperatures (T_a) of 20° and 10°C. It has been confirmed that heat producing and heat losing mechanisms participate in a different ratio in apparently similar thermoregulatory responses to hypoxia and to hypercapnia. A fairly close correlation was established between: a) the level of VO_2 before exposure and its subsequent change during hypoxia and hypercapnia, b) the change in VO_2 (ΔO_2) during exposure and the change subsequent to the restoration of air breathing, and c) the level of oxygen consumption (VO_2) during exposure and the change (ΔO_2) subsequent to the termination of exposure. No similar relationship could be established between the corresponding data on body temperature in the case of 12% O_2 and 8% O_2 at both T_a-s, and in the case of 6% CO_2 at T_a 20°C. At T_a 10°C, significant correlation was, however, demonstrated between: a) the level of body temperature (T_c) before exposure to hypercapnia and the change (ΔT_c) subsequent to the termination of exposure, b) the change in colonic temperature (ΔT_c) in response to hypercapnia and the change after termination of hypercapnia, and c) between T_c attained at termination of hypercapnia and the change (ΔT_c) subsequent to restoration of room air.

A relatively close correlation was revealed between the changes in oxygen consumption (ΔO_2) and body temperature (ΔT_c) during exposure to hypoxia and hypercapnia at both T_a-s, whereas no similar correlation could be demonstrated between the same parameters subsequent to the termination of exposure. A significant correlation was found between the changes in colonic temperature (ΔT_c) during hypoxia and the increase in heat production subsequent to the termination of hypoxia and, to a lesser degree, between T_c attained by the end of exposure and the subsequent increase in oxygen consumption. No correlation could be demonstrated at either T_a between the change in heat production in response to 8% O_2, on the one hand, and to 6% CO_2, on the other hand, whereas a close correlation was revealed between the corresponding changes in body temperature at both T_a-s. Various aspects of the results are discussed and the role of correlational analysis in studying highly complicated cybernetic systems is pointed out.

Homeostatic regulations of higher organisms, such as the thermoregulatory mechanism of homeotherms, are complex cybernetic systems which permit manifold responses to identical stimuli. Differences in the functioning of thermoregulatory mechanisms depend on genetic factors, age, adaptation to different environments, etc. Highly complex afferent and effector mechanisms are coupled with a complex central mechanism located in the hypothalamus, its function being modified by other central nervous systems responding to non-thermal stimuli. Considering the complexity of such systems, a strict correspondence between various parameters can hardly be expected, with

the exception of highly artificial conditions in which the working of a closed loop, isolated from all interference from stimuli arising elsewhere, could be studied provided such a complete isolation would be feasible.

The more complex a regulatory system, and the more its function is modified by other systems, closer or looser correlations between various parameters can be expected only. The results of an analysis of this kind of the thermoregulatory responses to changes in ambient temperature, to hypoxia and hypercapnia in the new-born rat and rabbit suggested that, by using correlational analysis as a tool, further insight might be gained into the cybernetics of thermoregulatory responses (VÁRNAI and DONHOFFER 1970; VÁRNAI et al. 1970a, b, 1971a, b; VÁRNAI 1973). A similar analysis was, therefore, undertaken of results obtained with adult guinea pigs exposed to 12% and 8% O_2, and to 6% CO_2, at ambient temperatures of 20° and 10°C.

Methods

Thirty-eight adult guinea pigs of both sexes, aged 107 to 399 days, weighing 525 to 850 g, reared in the Institute's colony, were used. Oxygen consumption and CO_2 production were determined by a modified Noyons-Kipp diaferometer. Colonic temperature was measured by copper-constantan thermocouples introduced to a depth of 10 cm. The whole experimental setup has been described earlier (DONHOFFER et al. 1957a, b, 1958; VÁRNAI et al. 1970a, b, c, 1971a, b; FARKAS and DONHOFFER 1973). After introducing the guinea pigs into the metabolic chamber, this was submerged into a water-bath providing an ambient temperature (T_a) of 30 °C. Measurements were started 30 to 45 min later at this temperature. 30 min thereafter the metabolic chamber was transferred into a water-bath of 20°C, at which temperature the effect of breathing air, 12% and 8% O_2 was observed for 30 minutes each, exposures to hypoxia being followed by 30 min periods of breathing air at the same T_a. Subsequently, 6% CO_2 in air was supplied for 30 min to the animal, followed by another 30 min of breathing room air. In the observations in which the effect of 12% O_2 was studied at T_a 10 °C, exposure to hypercapnia was omitted. At T_a 10°C, the response to 8% O_2 and to 6% CO_2 was recorded on separate days, with intervals ranging from 3 to 25 days. In these observations, oxygen consumption and colonic temperature were recorded at T_a 30°, 20°, and 10°C for periods of 30 min before exposure to hypoxia or hypercapnia. After termination of exposure the observations were continued at T_a 10°C for another 30 min.

Figures for oxygen consumption represent averages of readings taken every minute during the last 10—15 min of each 30-min period. Colonic temperatures represent values attained at the end of each period, the recording galvanometers were. however, read every minute throughout the experiment. Body surface was calculated according to the formula $10 \cdot kg^{0.67}$. Student's two tailed *t*-test and Pearson's product-moment correlation coefficient *r* was used.

Results

Table I summarizes the changes in heat production (ΔO_2) and in colonic temperature (ΔT_c) elicited by breathing 12% O_2, 8% O_2, and 6% CO_2 at ambient temperatures (T_a) of 20° and 10°C. The discrepancy between the changes in heat production and the concomitant changes in colonic temperature in response to hypoxia and hypercapnia, pointed out earlier (FARKAS 1973), has been confirmed. For instance, the reduction in heat production in response

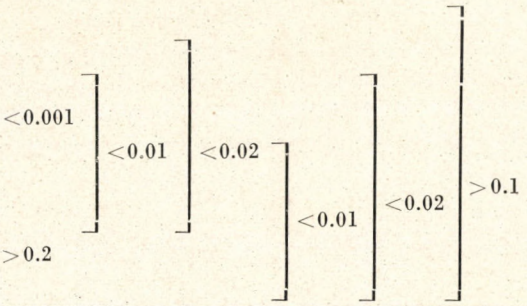
to exposure to 6% CO_2 was, at T_a 10°C, smaller, and the fall in body temperature, greater, than in response to breathing 12% O_2 at the same T_a. Although the differences in oxygen consumption and in body temperature both failed to reach statistical significance, they fit into the other results. Essentially, the same emerged from comparing the response to 8% O_2 at T_a 20°C and that to 6% CO_2 at T_a 10°C, or the response to 12% O_2 at 10°C and to 6% CO_2 at T_a 20°C. In both instances the reduction in heat production was significantly smaller in hypercapnia than in hypoxia, whereas no significant difference was found between the concomitant changes in colonic temperature.

Table I

Changes in oxygen consumption (Part A) and in colonic temperature (Part B) in response to hypoxia and hypercapnia at ambient temperatures (T_a) of 20 and 10°C

A

T_a	exposed to	n	ΔO_2, ml/dm² · min (M±S.E.)	p
20°	12% O_2	15	−0.02±0.02	<0.001
10°	12% O_2	10	−0.32±0.07	
20°	8% O_2	18	−0.38±0.05	<0.001
10°	8% O_2	10	−1.10±0.08	
20°	6% CO_2	12	−0.13±0.05	>0.2
10°	6% CO_2	13	−0.21±0.04	



B

			°C (M±S.E.)	
20°	12% O_2	15	−0.48±0.08	<0.05
10°	12% O_2	10	−0.88±0.15	
20°	8% O_2	22	−1.16±0.08	<0.001
10°	8% O_2	10	−2.18±0.10	
20°	6% CO_2	14	−0.94±0.07	0.1>p>0.05
10°	6% CO_2	13	−1.20±0.12	

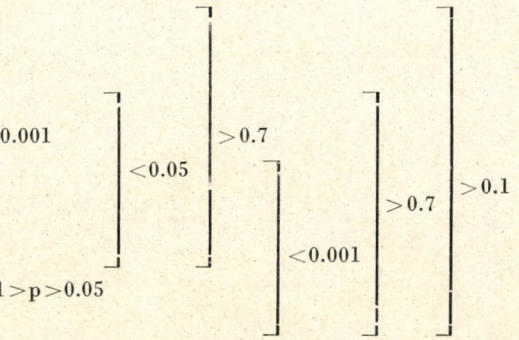


Table II contains corresponding data for the changes in heat production and in colonic temperature after termination of exposure to hypoxia and hypercapnia. Comparison of Tables I and II, i.e. of the changes in heat production and in colonic temperature during and after termination of the exposure to hypoxia and to hypercapnia revealed differences of some interest.

Table III shows that at T_a 10°C, body temperature increased highly significantly steeper after termination of exposure to 8% O_2 than after exposure to 6% CO_2. Despite this difference practically the same amount of heat had been produced during the post-exposure period. Evidently, a significantly

Table II

Changes in oxygen consumption (Part A) and in colonic temperature (Part B) after termination of hypoxia and hypercapnia at ambient temperatures (T_a) of 20 and 10°C

A

T_a		n	O_2, ml/dm² · min (M±S.E.)	p			
20°	after 12% O_2	15	+0.14±0.06	0.1>p>0.05			
10°	after 12% O_2	10	+0.33±0.09				
20°	after 8% O_2	18	+0.40±0.06	<0.001			
10°	after 8% O_2	10	+1.18±0.06		>0.3		>0.2
20°	after 6% CO_2	12	+0.32±0.06	0.1>p>0.05		<0.001	
10°	after 6% CO_2	13	+0.46±0.05				

B

			T_c, °C (M±S.E.)				
20°	after 12% O_2	15	+0.41±0.07	0.1>p>0.05			
10°	after 12% O_2	10	+0.69±0.14				
20°	after 8% O_2	22	+0.84±0.06	<0.02			
10°	after 8% O_2	10	+1.28±0.15		>0.3		>0.4
20°	after 6% CO_2	14	+0.71±0.13	>0.3		<0.001	
10°	after 6% CO_2	13	+0.55±0.11				

greater proportion of the heat produced had been retained after hypoxia than after hypercapnia. Essentially the same conclusion had to be drawn from Part B of Table III. The increase in body temperature subsequent to the termination of exposure to 12% O_2 at T_a 10°C and that after termination of

Table III

Colonic temperature (T_c) at termination of exposure to hypoxia and hypercapnia, and 30 min after restoration of air breathing; and the change in body temperature (ΔT_c) after restoration of air, and oxygen consumption (VO_2) in the post-exposure period (M ± S. E.). Part A: comparison of the effects of 8% O_2 and 6% CO_2 at T_a 10°C. Part B: comparison between the effects of 12% O_2 and 6% CO_2 at T_a 10°C. Part C: comparison of the effects of 8% O_2 and 6% CO_2 at T_a 20°C

n	exposure to	T_c at termination of exposure, °C	T_c 30 min after termination of exposure, °C	ΔT_c after exposure, °C	VO_2 after termination of exposure, ml/dm² · min
		A (T_a 10 °C)			
10	8% O_2	35.76±0.24	37.04±0.27	+1.28±0.15	2.44±0.09
13	6% CO_2	36.94±0.26	37.49±0.31	+0.55±0.11	2.47±0.08
8% O_2 vs. 6% CO_2	p	<0.01	>0.2	<0.001	>0.7
		B (T_a 10 °C)			
10	12% O_2	37.74±0.34	38.43±0.33	+0.69±0.14	2.08±0.08
14	6% CO_2	36.94±0.26	37.49±0.31	+0.55±0.11	2.47±0.08
12% O_2 vs. 6% CO_2	p	0.1>p>0.05	<0.05	>0.4	<0.01
		C (T_a 20 °C)			
22	8% O_2	37.61±0.16	38.45±0.18	+0.84±0.06	1.63±0.05
14	6% CO_2	37.49±0.28	38.20±0.33	+0.71±0.13	1.80±0.05
8% O_2 vs. 6% CO_2	p	>0.7	>0.5	>0.3	>0.1

hypercapnia were not different statistically, although the increase was somewhat greater after hypoxia than after hypercapnia. Oxygen consumption in the post-exposure period was, however, highly significantly greater after termination of hypercapnia than after termination of hypoxia. Again, it had to be concluded that a significantly greater proportion of the heat produced had been retained in the body after termination of hypoxia than after hypercapnia. Part C of Table III, comparing the effects of exposure to 8% O_2 and to 6% CO_2 at T_a 20°C, shows no differences of similar significance, the trend of the post-exposure levels of body temperature and of heat production was, however, consistent with the results obtained at T_a 10°C.

Table IV contains the correlational analysis between the level of heat production (VO_2) prior to exposure, and its subsequent change (ΔO_2) during hypoxia and hypercapnia (Part A), on the one hand, and that of the relationship between the level of colonic temperature (T_c) prior to exposure and the

subsequent changes (ΔT_c) during hypoxia and hypercapnia (Part B), on the other hand. A significant correlation was demonstrated between the level of heat production prior to exposure and the subsequent changes in response to 12% O_2, 8% O_2 and 6% CO_2 at T_a 20°C. At T_a 10°C, no correlation could

Table IV

The relationship between the level of heat production (VO_2) prior to exposure, and its changes (ΔO_2) during exposure to hypoxia and hypercapnia (Part A), and between the level of colonic temperature (T_c) prior to exposure and the changes (ΔT_c) during exposure to hypoxia and hypercapnia (Part B)

A

n	T_a	Variables X	Variables Y	$\bar{X}\pm$S.E. ml/dm²·min	$\bar{Y}\pm$S.E. ml/dm²·min	r	t	p
15	20°	VO_2	ΔO_2 12% O_2	1.62±0.05	—0.02±0.08	—0.696	4.602	<0.001
10	10°	VO_2	ΔO_2 12% O_2	2.07±0.08	—0.32±0.07	—0.318	0.944	>0.3
18	20°	VO_2	ΔO_2 8% O_2	1.67±0.05	—0.38±0.05	—0.590	2.922	<0.01
10	10°	VO_2	ΔO_2 8% O_2	2.36±0.12	—1.10±0.08	—0.505	1.654	>0.1
12	20°	VO_2	ΔO_2 6% CO_2	1.69±0.03	—0.13±0.05	—0.654	2.666	<0.05
13	10°	VO_2	ΔO_2 6% CO_2	2.20±0.08	—0.21±0.08	—0.514	1.986	0.1>p>0.05

B

			°C	°C			
15	20°	T_c	ΔT_c 12% O_2	38.46±0.13	—0.48±0.08	+0.014	0.050	>0.9
10	10°	T_c	ΔT_c 12% O_2	38.62±0.26	—0.88±0.15	+0.269	0.790	>0.4
22	20°	T_c	ΔT_c 8% O_2	38.77±0.13	—1.16±0.08	+0.155	0.703	>0.4
10	10°	T_c	ΔT_c 8% O_2	37.94±0.19	—2.18±0.10	—0.220	0.637	>0.5
14	20°	T_c	ΔT_c 6% CO_2	38.41±0.25	—0.92±0.07	+0.427	1.634	>0.1
13	10°	T_c	ΔT_c 6% CO_2	38.14±0.15	—1.20±0.12	+0.800	4.425	<0.01

be established between the corresponding parameters in response to breathing 12% O_2, and the coefficients of correlation fell short of statistical significance in the case of exposure to 8% O_2 and 6% CO_2. In the two latter instances, the relatively high coefficients ($r = -0.505$, and -0.514, respectively) suggest that the relationship is, nevertheless, very probably real.

No correlation whatsoever could be found between the pre-exposure level of body temperature (T_c) and its subsequent change (ΔT_c) during hypoxia. In contrast, a highly significant correlation was apparent between the pre-exposure level of body temperature and its change during hypercapnia at T_a 10°C, and the relatively high coefficient of correlation obtained at T_a 20°C ($r = +0.427$), although not significant statistically, suggests that the relationship might exist also at T_a 20°C as it does at T_a 10°C. It should be

noted that the coefficient r was negative between the level of oxygen consumption prior to exposure and its subsequent change during hypercapnia both at T_a 20°C and at T_a 10°C, whereas the coefficients between the pre-exposure level of body temperature and its subsequent changes in hypercapnia were positive at both ambient temperatures. This, of course, indicates that the greater was heat production before exposure, the greater the reduction in heat production tended to be during hypercapnia, whereas the higher was body temperature before exposure, the smaller tended to be its fall in response to the subsequent exposure to hypercapnia.

Table V presents data on the relationship between the changes in heat production (ΔO_2) in response to hypoxia and hypercapnia and the subsequent change after restoration of room air (Part A) and, in Part B, the relationship between the concomitant changes in body temperature (ΔT_c). Fairly high, statistically significant, coefficients of correlation were obtained between the changes in heat production during, and after termination of, exposure to 12% and to 8% O_2 at T_a 20°C. At T_a 10°C, however, the corresponding coefficients failed to reach statistical significance. The relatively high coefficients ($r = -0.596$, and -0.606, respectively) nevertheless suggest that the relationship is real at this T_a also. The correlation between the changes in heat production during, and after termination of, hypercapnia was statistically significant at T_a 10°C, whereas at T_a 20°C no correlation of the same parameters could be demonstrated.

In contrast, no significant correlation was observed between the corresponding changes in body temperature with the exception of exposure to hypercapnia at T_a 10°C. The positive sign of the coefficient indicates that the smaller the fall in body temperature in hypercapnia, the more it tended to increase after the termination of hypercapnia, whereas the negative coefficient between the changes in heat production indicates that the greater the reduction during hypercapnia, the greater tended to be the increase after restoration of air breathing.

Table VI contains an analysis of the relationship of the level of heat production (VO_2) during hypoxia and hypercapnia and the change in oxygen consumption (ΔO_2) subsequent to restoration of air breathing (Part A), on the one hand, and of the relationship of the level of body temperature (T_c) at termination of hypoxia and hypercapnia and the changes in colonic temperature (ΔT_c) subsequent to restoration of room air, on the other hand, (Part B).

Significant, or almost significant, negative coefficients were obtained between the level of heat production during, and its change after termination of, hypoxia at both ambient temperatures and with both 12% and 8% O_2, whereas no significant correlation could be demonstrated between the same parameters in hypercapnia. The relationship between the level of body temperature attained by the end of exposure and its change during the 30 min

Table V

The relationship between the changes in heat production (ΔO_2) during and after termination of hypoxia and hypercapnia (Part A) and between the concomitant changes in colonic temperature (ΔT_c; Part B)

A

n	T_a	Variables X	Variables Y	$\bar{X}\pm$S.E.	$\bar{Y}\pm$S.E.	r	t	p
				O_2, ml/dm²·min				
15	20°	ΔO_2 12% O_2	$-\Delta O_2$ after 12% O_2	−0.02±0.08	+0.14±0.06	−0.588	2.623	<0.05
10	10°	ΔO_2 12% O_2	$-\Delta O_2$ after 12% O_2	−0.32±0.07	+0.33±0.09	−0.596	2.099	0.1>p>0.05
18	20°	ΔO_2 8% O_2	$-\Delta O_2$ after 8% O_2	−0.38±0.05	+0.40±0.06	−0.562	2.718	<0.02
10	10°	ΔO_2 8% O_2	$-\Delta O_2$ after 8% O_2	−1.10±0.08	+1.18±0.06	−0.606	2.153	0.1>p>0.05
12	20°	ΔO_2 6% CO_2	$-\Delta O_2$ after 6% CO_2	−0.13±0.05	+0.32±0.06	−0.117	0.351	>0.7
13	10°	ΔO_2 6% CO_2	$-\Delta O_2$ after 6% CO_2	−0.21±0.08	+0.46±0.05	−0.697	3.228	<0.01

B

				°C				
15	20°	ΔT_c 12% O_2	$-\Delta T_c$ after 12% O_2	−0.48±0.08	+0.41±0.07	−0.081	0.317	>0.7
10	10°	ΔT_c 12% O_2	$-\Delta T_c$ after 12% O_2	−0.88±0.15	+0.69±0.14	−0.421	1.313	>0.2
22	20°	ΔT_c 8% O_2	$-\Delta T_c$ after 8% O_2	−1.16±0.08	+0.84±0.06	−0.264	1.224	>0.2
10	10°	ΔT_c 8% O_2	$-\Delta T_c$ after 8% O_2	−2.18±0.10	+1.28±0.15	−0.283	0.833	>0.4
14	20°	ΔT_c 6% CO_2	$-\Delta T_c$ after 6% CO_2	−0.94±0.07	+0.71±0.13	−0.406	1.541	>0.1
13	10°	ΔT_c 6% CO_2	$-\Delta T_c$ after 6% CO_2	−1.20±0.12	+0.55±0.11	+0.652	2.856	<0.02

Table VI

The relationship of the level of oxygen consumption (VO_2) during exposure to hypoxia and hypercapnia and its subsequent change (ΔO_2) after restoration of air breathing (Part A), and between the level of colonic temperature (T_C) at termination of exposure and its subsequent change (ΔT_C) after restoration of air breathing (Part B)

A

n	T_a	Variables X	Variables Y	$\bar{X} \pm$ S.E. ml/dm²·min	$\bar{Y} \pm$ S.E. ml/dm²·min	r	t	p
15	20°	VO_2 12% O_2	$-\Delta O_2$ after 12% O_2	1.59±0.05	+0.12±0.05	—0.692	3.455	<0.01
10	10°	VO_2 12% O_2	$-\Delta O_2$ after 12% O_2	1.75±0.09	+0.33±0.09	—0.585	2.039	0.1>p>0.05
18	20°	VO_2 8% O_2	$-\Delta O_2$ after 8% O_2	1.28±0.05	+0.40±0.06	—0.602	3.018	<0.01
10	10°	VO_2 8% O_2	$-\Delta O_2$ after 8% O_2	1.26±0.10	+1.18±0.06	—0.567	1.949	0.1>p>0.05
12	20°	VO_2 6% CO_2	$-\Delta O_2$ after 6% CO_2	1.49±0.04	+0.32±0.06	—0.335	1.232	>0.2
13	10°	VO_2 6% CO_2	$-\Delta O_2$ after 6% CO_2	1.99±0.08	+0.46±0.05	—0.392	1.413	>0.1

B

				°C	°C			
15	20°	T_C 12% O_2	$-\Delta T_C$ after 12% O_2	37.98±0.15	+0.41±0.07	+0.447	1.802	0.1>p>0.05
10	10°	T_C 12% O_2	$-\Delta T_C$ after 12% O_2	37.74±0.34	+0.69±0.14	—0.305	0.906	>0.3
22	20°	T_C 8% O_2	$-\Delta T_C$ after 8% O_2	37.61±0.16	+0.84±0.06	+0.076	0.340	>0.7
10	10°	T_C 8% O_2	$-\Delta T_C$ after 8% O_2	35.76±0.24	+1.28±0.15	—0.076	0.215	>0.8
14	20°	T_C 6% CO_2	$-\Delta T_C$ after 6% CO_2	37.49±0.28	+0.71±0.13	+0.234	0.834	>0.4
13	10°	T_C 6% CO_2	$-\Delta T_C$ after 6% CO_2	36.94±0.26	+0.55±0.11	+0.575	2.348	<0.05

subsequent to the termination of exposure, approached statistical significance in the case of exposure to 12% O_2 at T_a 20°C, and reached statistical significance in the case of exposure to hypercapnia at T_a 10°C. The positive sign of the coefficient r indicates that the higher body temperature was at the termination of exposure, the greater tended to be the increase after restoration of room air.

Table VII demonstrates the relationship between the changes in heat production during hypoxia and hypercapnia and the concomitant changes in body temperature (Part A), and the relationship between the corresponding parameters after termination of hypoxia and hypercapnia (Part B). The most striking result of this analysis was the contrast between the very close correlation of the change in heat production and the concomitant change in body temperature during hypercapnia ($r = +0.914$, and $+0.817$), on the one hand, and the lack of a significant correlation between the same parameters after termination of hypercapnia ($r = +0.273$, and -0.208), on the other hand. Essentially the same was observed at both T_a 20°C and T_a 10°C in the case of exposure to 8% O_2. The relatively high coefficients (T_a 20°C: $r = +0.424$; T_a 10°C: $r = +0.526$), although not significant statistically, appeared to indicate that the relationship was real. Similarly as after the termination of hypercapnia, no correlation could be demonstrated between the corresponding parameters after the termination of exposure to 8% O_2 ($r = +0.143$, and -0.014). No correlation could be demonstrated between the same parameters both during and after termination of exposure to 12% O_2 T_a 20 °C, whereas at T_a 10°C a relatively high coefficient ($r = +0.592$), approaching statistical significance, was obtained during exposure to 12% O_2.

Table VIII Part A summarizes the relationship of the level of body temperature (T_c) attained by the end of exposure and the changes in heat production (ΔO_2) subsequent to the termination of exposure; in Part B the changes in colonic temperature (ΔT_c) were taken as parameter instead of the level of colonic temperature (T_c). The relationship of the level of body temperature at the termination of exposure and the post-exposure change in heat production was statistically significant only in the case of exposure to 12% O_2 at T_a 10°C, and approached significance in the case of exposure to 8% O_2 at T_a 20°C. In contrast, the relationship between the changes in body temperature during exposure and the changes in heat production subsequent to the termination of exposure was statistically significant at both ambient temperatures in the case of exposure to either 12% or 8% O_2. The relatively high negative coefficient obtained in the case of hypercapnia at T_a 10°C suggests that a similar relationship might exist between both the end-exposure level of body temperature and the change in colonic temperature during exposure, on the one hand, and the change in heat production subsequent to the termination of exposure, on the other hand.

Table VII

The relationship between the changes in heat production (ΔO_2) and in colonic temperature (ΔT_c) in response to hypoxia and hypercapnia (Part A), and between the corresponding changes after restoration of room air (Part B)

n	T_a	Variables X	Variables Y	$\bar{X} \pm$ S.E. ml/dm²·min	$\bar{Y} \pm$ S.E. °C	r	t	p
			A					
15	20°	ΔO_2 12% O_2	$-\Delta T_c$ 12% O_2	-0.02 ± 0.08	-0.48 ± 0.08	$+0.261$	0.973	>0.3
10	10°	ΔO_2 12% O_2	$-\Delta T_c$ 12% O_2	-0.32 ± 0.07	-0.88 ± 0.15	$+0.592$	2.079	$0.1>p>0.05$
18	20°	ΔO_2 8% O_2	$-\Delta T_c$ 8% O_2	-0.38 ± 0.05	-1.15 ± 0.08	$+0.424$	1.872	$0.1>p>0.05$
10	10°	ΔO_2 8% O_2	$-\Delta T_c$ 8% O_2	-1.10 ± 0.08	-2.18 ± 0.10	$+0.526$	1.749	>0.1
12	20°	ΔO_2 6% CO_2	$-\Delta T_c$ 6% CO_2	-0.13 ± 0.05	-0.88 ± 0.08	$+0.914$	7.102	<0.001
13	10°	ΔO_2 6% CO_2	$-\Delta T_c$ 6% CO_2	-0.21 ± 0.08	-1.20 ± 0.12	$+0.817$	4.698	<0.001
			B					
15	20°	ΔO_2 after 12% O_2	$-\Delta T_c$ after 12% O_2	$+0.13 \pm 0.05$	$+0.41 \pm 0.07$	$+0.321$	1.223	>0.2
10	10°	ΔO_2 after 12% O_2	$-\Delta T_c$ after 12% O_2	$+0.33 \pm 0.09$	$+0.69 \pm 0.14$	$+0.364$	1.107	>0.3
18	20°	ΔO_2 after 8% O_2	$-\Delta T_c$ after 8% O_2	$+0.40 \pm 0.06$	$+0.85 \pm 0.07$	$+0.143$	0.576	>0.5
10	10°	ΔO_2 after 8% O_2	$-\Delta T_c$ after 8% O_2	$+1.18 \pm 0.06$	$+1.28 \pm 0.15$	-0.014	0.039	>0.9
12	20°	ΔO_2 after 6% CO_2	$-\Delta T_c$ after 6% CO_2	$+0.32 \pm 0.06$	$+0.72 \pm 0.14$	$+0.273$	0.896	>0.3
13	10°	ΔO_2 after 6% CO_2	$-\Delta T_c$ after 6% CO_2	$+0.46 \pm 0.05$	$+0.55 \pm 0.11$	-0.208	0.704	>0.4

Table VIII

Relationship between colonic temperature (T_c) at termination of hypoxia and hypercapnia and the changes in heat production (ΔO_2) after resumption of breathing room air (Part A), and between the change in body temperature (ΔT_c) during hypoxia and hypercapnia, and the change in heat production after restoration of air breathing (Part B)

n	T_a	Variables X	Variables Y	$\bar{X}\pm$S.E. °C	$\bar{Y}\pm$S.E. ml/dm²·min	r	t	p
			A					
15	20°	T_c 12% O_2	$-\Delta O_2$ after 12% O_2	37.98±0.15	+0.12±0.05	—0.118	0.647	>0.5
10	10°	T_c 12% O_2	$-\Delta O_2$ after 12% O_2	37.74±0.34	+0.33±0.09	—0.656	2.454	<0.05
18	20°	T_c 8% O_2	$-\Delta O_2$ after 8% O_2	37.64±0.16	+0.40±0.06	—0.457	2.457	0.1>p>0.05
10	10°	T_c 8% O_2	$-\Delta O_2$ after 8% O_2	35.76±0.24	+1.18±0.06	—0.091	0.259	>0.8
12	20°	T_c 6% CO_2	$-\Delta O_2$ after 6% CO_2	37.54±0.33	+0.32±0.06	—0.357	1.208	>0.2
13	10°	T_c 6% CO_2	$-\Delta O_2$ after 6% CO_2	36.94±0.26	+0.46±0.05	—0.472	1.776	>0.1
			B					
15	20°	ΔT_c 12% O_2	$-\Delta O_2$ after 12% O_2	—0.48±0.12	+0.12±0.05	—0.521	2.203	<0.05
10	10°	ΔT_c 12% O_2	$-\Delta O_2$ after 12% O_2	—0.88±0.15	+0.33±0.09	—0.714	2.882	<0.05
18	20°	ΔT_c 8% O_2	$-\Delta O_2$ after 8% O_2	—1.15±0.08	+0.40±0.06	—0.595	2.964	<0.01
10	10°	ΔT_c 8% O_2	$-\Delta O_2$ after 8% O_2	—2.18±0.10	+1.18±0.06	—0.663	2.566	<0.05
12	20°	ΔT_c 6% CO_2	$-\Delta O_2$ after 6% CO_2	—0.88±0.08	+0.32±0.06	—0.165	0.528	>0.6
13	10°	ΔT_c 6% CO_2	$-\Delta O_2$ after 6% CO_2	—1.20±0.12	+0.46±0.05	—0.420	1.535	>0.1

Table IX

The relationship between the change in heat production (ΔO_2) in response to 8% O_2 and to 6% CO_2 at T_a 20 °C and 10 °C (Part A), and between the concomitant changes in body temperature (ΔT_c; Part B)

A

n	T_a	Variables X	Variables Y	$\bar{X}\pm$S.E. ml/dm²·min	$\bar{Y}\pm$S.E. ml/dm²·min	r	t	p
8	20°	ΔO_2 8% O_2	ΔO_2 6% CO_2	−0.35±0.05	−0.14±0.05	−0.180	0.488	>0.6
10	10°	ΔO_2 8% O_2	ΔO_2 6% CO_2	−1.10±0.08	−0.12±0.05	+0.266	0.764	>0.4

B

				°C	°C			
12	20°	ΔT_c 8% O_2	ΔT_c 6% CO_2	−0.89±0.06	−0.93±0.08	+0.681	2.941	<0.02
10	10°	ΔT_c 8% O_2	ΔT_c 6% CO_2	−2.18±0.10	−1.05±0.11	+0.783	3.557	<0.01

Table X

The relationship between changes in body temperature in response to exposure to T_a 20° and T_a 10 °C (ΔT_c 30—20°C and ΔT_c 30—10 °C, respectively), on the one hand, and to exposure to 8% O_2 and 6% CO_2 at these T_a-s, on the other hand

n	Variables X	Variables Y	$\bar{X}\pm$S.E.	$\bar{Y}\pm$S.E.	r	t	p
21	ΔT_c 30—20°	ΔT_c 8% O_2 20°	−0.31±0.11	−1.12±0.07	+0.016	0.068	>0.9
14	ΔT_c 30—20°	ΔT_c 6% CO_2 20°	−0.56±0.24	−0.91±0.07	+0.227	0.806	>0.4
10	ΔT_c 30—10°	ΔT_c 8% O_2 10°	−1.38±0.26	−2.18±0.10	+0.343	1.031	>0.3
14	ΔT_c 30—10°	ΔT_c 6% CO_2 10°	−1.21±0.16	−1.19±0.11	+0.579	2.460	<0.05

In view of the differences between the effects of hypoxia and hypercapnia summarized in Tables I, II, and III, it was deemed interesting to study whether the responses to hypoxia and to hypercapnia were, nevertheless, correlated. Table IX is based on observations at T_a 20°C, in which the guinea pigs had been exposed to hypoxia and hypercapnia in the same experimental session, and on experiments performed at T_a 10°C, in which they were exposed to hypoxia and to hypercapnia on different days at intervals from 3 to 25 days. Part A of Table IX contains no indication of a relationship between the responses of heat production to hypoxia and to hypercapnia at either ambient temperature, whereas the data in Part B demonstrate a definite relationship between the changes in body temperature in response to exposure to 8% O_2 and to 6% CO_2 at both ambient temperatures.

Table X shows that a similar relationship could not be demonstrated between the changes in deep body temperature in response to changes in ambient temperature and those elicited by hypoxia at both T_a 20° and 10 °C, whereas at T_a 10°C a significant correlation was found between the fall in body temperature in response to cold, and its further decline during hypercapnia.

Discussion

Evaluating the results, it has to be recalled that whereas oxygen consumption is a straightforward representation of heat production, deep body temperature and its changes depend on the heat exchange between the animal and its environment, on the one hand, and on heat production, on the other hand. Knowing, however, the concomitant level or change in heat production, levels and changes in body temperature may serve as indicators of changes in physical thermoregulation.

The results summarized in Table I confirmed and extended the evidence which led to the conclusion that in the adult guinea pig (FARKAS 1973), as in the new-born rat and rabbit (VÁRNAI et al. 1970b, 1971b; VÁRNAI 1973) the actions of hypoxia and of hypercapnia on the thermoregulatory mechanisms are not identical, and that the ratio in which mechanisms of heat production and of heat loss participate in the responses differs significantly.

As has been pointed out earlier (FARKAS and DONHOFFER 1973), the fact that body temperature may fall markedly, whereas concomitant heat production may decline insignificantly, or not at all, or may even increase, does not permit to conclude that thermoregulatory heat production had not been affected, since even considerably smaller reductions in body temperature, e.g. in response to cold, would have elicited a very marked rise in heat production.

The most striking result of the correlational analyses was that whereas a fairly close relationship has been observed between

a) the level of heat production prior to exposure and its subsequent change during hypoxia and hypercapnia (Table IV, Part A);
b) the change in heat production during exposure and the change subsequent to restoration of air breathing (Table V, Part A); and
c) the level of heat production during exposure and the change subsequent to the restoration of air breathing (Table VI, Part A);

no similar relationship could be established between the corresponding data on body temperature, with the exception of hypercapnia at T_a 10°C.

At T_a 10°C a significant correlation could be established between

a) the level of colonic temperature before exposure, and the subsequent change during hypercapnia (Table IV, Part B);
b) the change in colonic temperature in response to hypercapnia, and the change subsequent to the termination of hypercapnia (Table V, Part B); and
c) the level of colonic temperature at the termination of hypercapnia, and the change subsequent to restoration of room air (Table VI, Part B).

In all of the three latter instances positive coefficients were obtained, indicating that, at T_a 10°C, a) the higher colonic temperature was before hypercapnia, the smaller tended to be the decrease in hypercapnia; b) the smaller the decrease in body temperature in hypercapnia, the more it tended to increase after the termination of hypercapnia; and c) the higher colonic temperature was at the termination of hypercapnia, the more it tended to increase after restoration of room air.

In interpreting these and similar results of correlational analyses, attention has to be paid to the fact that some of the parameters are closely related. For instance, the level of oxygen consumption during exposure and the change in oxygen consumption in response to exposure, as well as the corresponding parameters of body temperature are, evidently, not independent. Nevertheless, some differences emerged when using such closely related parameters (Tables V, VI and VIII).

The rather close relationship of the change in heat production and that in body temperature during exposure to hypoxia or hypercapnia (Table VII, Part A), might appear to confirm only what was to be expected. The absence of a similar relationship between the corresponding parameters subsequent to the termination of exposure (Table VII, Part B) indicates, however, that the relationship of changes in heat production and in body temperature may differ even under closely related conditions. Interestingly, the correlation of the changes in heat production and those in body temperature proved to be

much closer in hypercapnia (T_a 20°C: $r = +0.914$; T_a 10°C: $r = +0.817$) than in hypoxia. Despite the close correlation in hypercapnia, no correlation could be established between the corresponding parameters after the termination of hypercapnia.

Another point of interest emerged from the analysis presented in Table VIII. A significant correlation was revealed between the level of colonic temperature attained at the termination of exposure to hypoxia and the change in heat production subsequent to restoration of room air in the case of exposure to 12% O_2 at T_a 10°C, and statistical significance was approached in the case of exposure to 8% O_2 at T_a 20°C. Taking, however, as parameter the change during exposure instead of the level of colonic temperature attained at termination of exposure (Table VIII, Part B), the correlation proved to be significant in the case of exposure to 12% and 8% O_2 at T_a 10° as well as at T_a 20 °C, indicating that the change in body temperature during exposure to hypoxia serves as a more important determinant of the increase in heat production after restoration of room air than the level of body temperature at the termination of hypoxia.

Another aspect of these results deserves attention, although no satisfactory interpretation can be offered at present. Although Table VII showed a fairly close relationship between the changes in heat production during exposure and the concomitant changes in body temperature, and Table VIII demonstrated that the change in body temperature during exposure was an important determinant of the change in heat production subsequent to the restoration of air, no correlation was found after the termination of exposure between the changes in heat production and the concomitant changes in body temperature (Table VII, Part B).

Attention should be paid also to the important role played by the thermal environment in the determination of correlation between some of the parameters. In general, although not without exceptions, correlations tended to be more pronounced at T_a 10°C than at T_a 20°C, despite the fact that both temperatures provided an environment well below thermoneutrality. Correlation was more pronounced at T_a 10°C than at T_a 20°C, for instance, between the changes in oxygen consumption during, and after termination of hypercapnia: at T_a 20°C $r = -0.117$, $p > 0.7$; at T_a 10°C $r = -0.697$, $p < 0.001$ (Table V), between the level of body temperature at termination of hypercapnia and its change subsequent to restoration of room air: at T_a 20 °C $r = +0.234$, $p > 0.4$; at T_a 10°C $r = +0.575$, $p < 0.05$ (Table VI), between the level of body temperature at termination of exposure to 12% O_2 and the subsequent change in heat production: at T_a 20°C $r = -0.118$, $p > 0.5$; at T_a 10°C $r = -0.656$, $p < 0.05$ (Table VIII), and between the change in body temperature in response to transfer from thermoneutrality to the colder environment, and the change in colonic temperature subsequent to exposure

to hypercapnia: at T_a 20°C $r = +0.227$, $p > 0.4$; at T_a 10°C $r = +0.579$ $p < 0.05$ (Table X).

Considering the differences between the thermoregulatory responses to hypoxia and to hypercapnia, a correlation between these responses could hardly be expected. According to expectation, no correlation was found between the changes in heat production in response to hypoxia and to hypercapnia either at T_a 20°C or at T_a 10°C, whereas, rather unexpectedly, a definite correlation was established between the concomitant changes in colonic temperature (Table IX). The greater the fall in body temperature in hypoxia, the more it tended to decrease in hypercapnia.

The rather close correlation of the responses of body temperature to hypoxia, on the one hand, and to hypercapnia, on the other hand ($r = +0.681$ and $+0.783$), might suggest that an animal tends to respond with a decrease in body temperature of similar relative magnitude, independently of the nature of the causative stimulus. The observation presented in Table X demonstrates, however, that this is not the case, since no correlation of similar significance could be found between the changes in body temperature in response to transfer from a thermoneutral environment to a colder one, on the one hand, and its reduction in hypoxia, on the other hand.

Taken as a whole, the results are completely consistent with the conclusion arrived at earlier (FARKAS and DONHOFFER 1973) that in, as well as after the termination of, hypoxia and hypercapnia, differences in the participation of the two effector mechanisms of thermoregulation, i.e. of heat production and of heat loss, are the consequence of alterations in the function of higher integrative control mechanisms of temperature regulation.

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EFFECTS OF ACTH AND HCG ON OVARIAN OESTROGEN AND PROGESTERONE SECRETION IN DOGS PRETREATED WITH HCG

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The effects of ACTH and HCG on eostrogen (oestrone, 17β-oestradiol) and progesterone output in ovarian effluent blood were measured in dogs after HCG pretreatment for 5 days.

On the sixth day the infusion of 0.5 μg/kg/min of β_{1-24} ACTH for 10 and 60 minutes increased ovarian blood flow and oestrogen secretion. ACTH stimulated oestrogen secretion even after adrenalectomy, but there was no considerable change in progesterone secretion. Ovarian blood flow and oestrogen secretion normalized one hour after the termination of ACTH infusion.

A 10 minute infusion of 1.0 IU/kg/min of HCG was found to stimulate progesterone secretion, but ovarian blood flow and oesterogen secretion were increased only one hour after terminating the infusion, when progesterone secretion had returned to the control level.

ACTH administered immediately after 25.0 mg/kg of cycloheximide increased oestrogen secretion, while progesterone secretion decreased. After cycloheximide, HCG was ineffective.

It has been demonstrated recently that in dogs and cats ACTH increases blood flow not only in the adrenal, but also in the ovary (STARK et al. 1967; STARK and VARGA 1968). In the hamster, ovarian blood flow is significantly stimulated by ACTH while adrenal blood flow shows a slight or no change (VARGA et al. 1969).

In the anoestrous dog, ACTH infusion does not affect oestrogen secretion and only traces or no progesterone can be detected in the ovarian vein (HORVÁTH et al. 1969). HCG possesses some properties of the pituitary gonadotropic hormones (TELEGDY and HUSZÁR 1962; YOGO 1969) but in the anoestrous dog a single injection of HCG does not influence ovarian secretion only after some days pretreatment with HCG (TELEGDY and HUSZÁR 1962; ROMANOFF et al. 1962; NISHIZAWA and EIK-NES 1964).

The present experiments have been aimed at clarifying whether after HCG pretreatment ovarian oestrogen and progesterone secretion were increased by ACTH.

Methods

Thirty-nine female dogs weighing 10—15 kg each were treated with 100 IU/kg of HCG (Choriogonin, Richter) daily for 5 days. On the sixthy day the animals were anaesthetized with chloralose-urethane and a polyethylene catheter was inserted into the right ovarian vein. In some animals the adrenal glands had been removed before ovarian vein cannulation.

Then 0.5 μg/kg/min of synthetic β_{1-24} ACTH (Synacthen, Ciba) was infused into the femoral vein for 10 or 60 minutes and outflow from the vein was measured. Two hours after the termination of ACTH infusion, 1.0 IU/kg/min of HCG was infused for 10 minutes. In a group of animals ACTH and HCG infusion was given immediately after an intravenous injection of 25.0 mg/kg of cycloheximide (Sigma). Further details of the experimental arrangement are shown on the diagrams. Between two blood samplings the ovarian blood was led back into the systemic circulation. Blood pressure in the femoral artery was measured by a Statham transducer. To prevent a fall in blood pressure, blood from intact animals was infused during the experiment. Blood clotting was prevented by 3.0 mg/kg heparin (Richter). At the end of the experiment, the ovaries and adrenals were removed and weighed.

Oestrogen (oestrone, 17β-oestradiol) in the ovarian blood samples was determined by ITTRICH's method (1960) as modified by us (HORVÁTH et al. 1969). For the extraction of progesterone, TELEGDY and ENDRŐCZI's method (1961) was used which was followed by elution of progesterone separated by paper chromatography and measured fluorimetrically by the method of SHORT and LEVETT (1962).

Ovarian blood flow was expressed in ml/min and the rate of ovarian secretion in ng/min. For statistical evaluation, analysis of variance and the *t*-test were used.

Results

Effect of HCG pretreatment

HCG pretreatment increased the weight of the ovaries nearly two-fold. There was no difference in weight between the right and left ovary, showing that the cannulation did not cause a considerable blood stagnation or oedema in the right ovary.

HCG treatment left the weight of adrenals unaffected (Table I.).

Table I

Weight of ovaries and adrenals in dogs given 100 IU/kg HCG intramuscularly every day for 5 consecutive days and operated on day 6
(mean ± S. E.)

	Ovary, g			Adrenal, g		
	left	right		left	right	
Control	0.70 ±0.11	0.54 ±0.07	(6)*	0.83 ±0.10	0.90 ±0.15	(6)
HCG	1.35** ±0.17	1.26** ±0.13	(29)	0.89 ±0.08	0.88 ±0.08	(25)

* number of observations
** $p < 0.05$

Effect of ACTH infusion

During a 10-minute infusion of ACTH, ovarian blood flow and oestrogen secretion increased. One hour after the end of infusion blood flow and oestrogen secretion returned to the initial level. There was no change in progesterone secretion (Fig. 1).

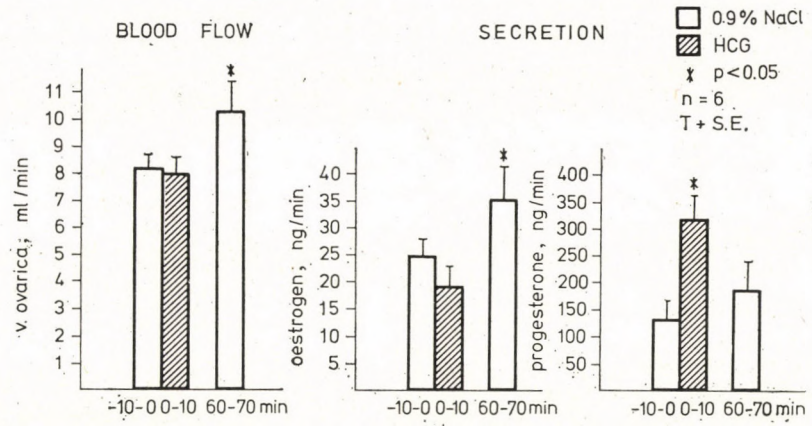

Fig. 1. Ovarian blood flow, oestrogen and progesterone secretion during a 10-minute infusion of ACTH and 60 minutes later

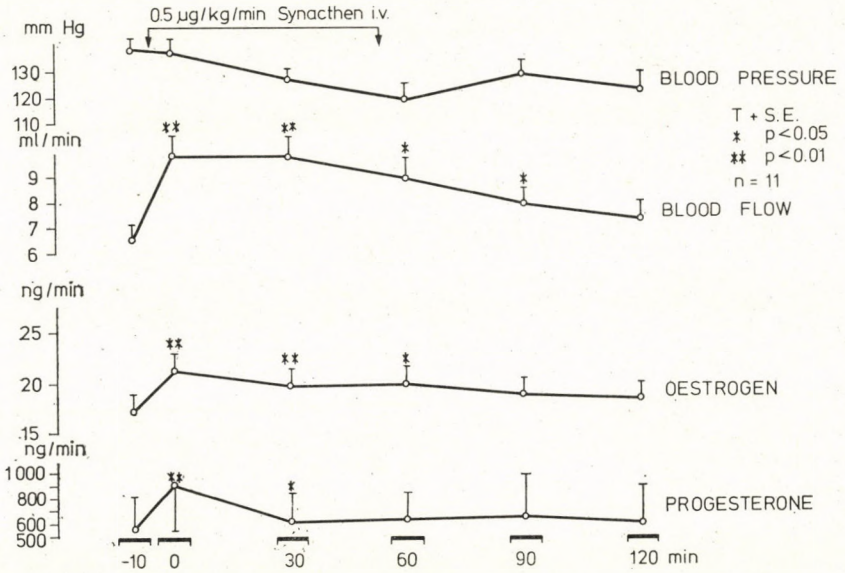

Fig. 2. Ovarian blood flow, oestrogen and progesterone secretion during and after a 60-minute infusion of ACTH

During a 60-minute infusion of ACTH, ovarian blood flow increased and remained high until 60 minutes after termination of the infusion. The one-hour ACTH infusion increased oestrogen secretion and kept it at a high level. Thirty minutes after the end of infusion, oestrogen secretion decreased to its initial value. Progesterone secretion was increased as well, but the increase was temporary and passed off just before the end of the infusion (Fig. 2).

To clarify the question whether ACTH influenced ovarian oestrogen secretion directly or through the adrenal gland, experiments were performed on adrenalectomized dogs. Two hours after adrenalectomy, ACTH was found to increase oestrogen secretion, while progesterone secretion was not affected (Fig. 3).

Effect of HCG infusion

During a 10-minute infusion of HCG there was no change in ovarian blood flow and oestrogen secretion, while progesterone secretion showed a considerable increase. One hour after the termination of HCG infusion, progesterone secretion returned to its initial value, while oestrogen secretion and ovarian blood flow increased (Fig. 4).

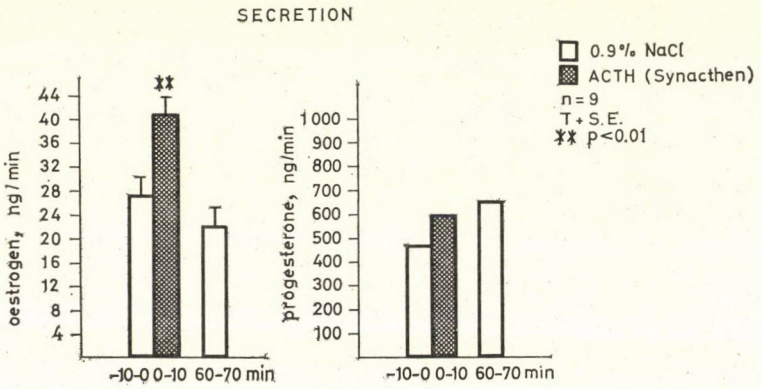

Fig. 3. Effect of ACTH on ovarian oestrogen and progesterone secretion after adrenalectomy

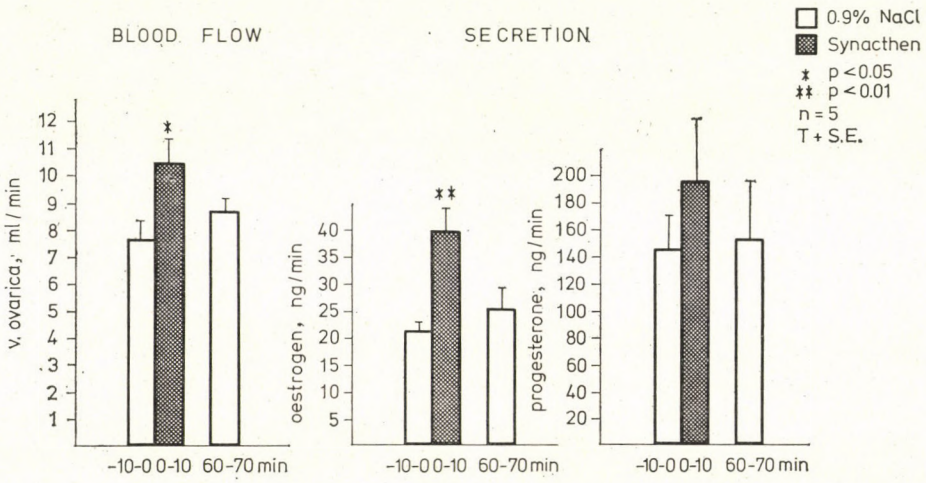

Fig. 4. Ovarian blood flow, oestrogen and progesterone secretion during a 10-minute infusion of HCG and 60 minutes later

Effect of ACTH and HCG infusion after cycloheximide administration

ACTH infusion increased ovarian oestrogen secretion in spite of cycloheximide administration. Progesterone secretion was decreased. Sixty minutes after the injection of cycloheximide, only traces of progesterone were detected in ovarian venous blood, when oestrogen secretion returned to its initial level.

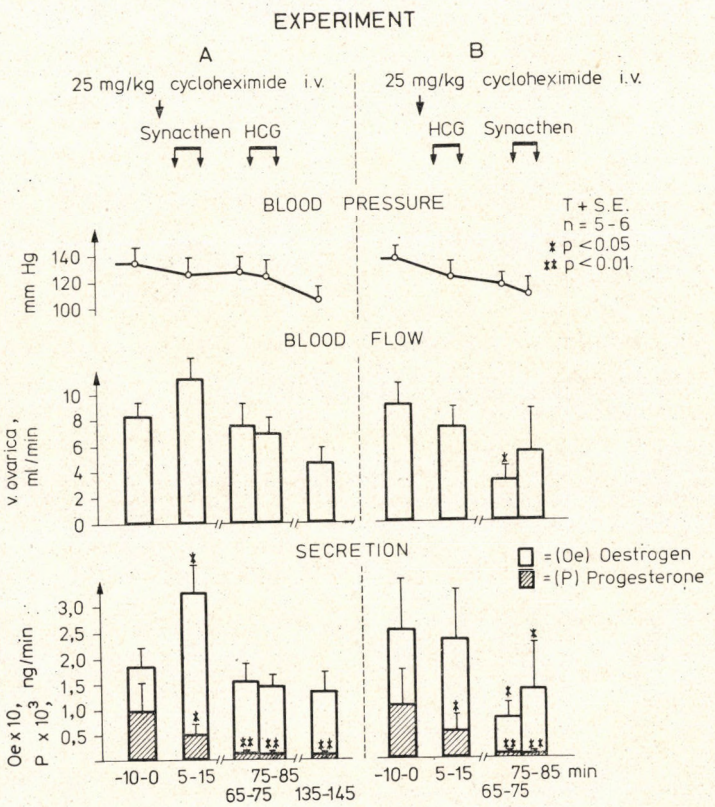

Fig. 5. Effect of ACTH and HCG on ovarian blood flow, oestrogen and progesterone secretion, following cycloheximide administration

Seventy minutes after the injection of cycloheximide, HCG was ineffective on ovarian blood flow as well as on oestrogen and progesterone secretion (Fig. 5A).

When HCG was administered immediately after cycloheximide treatment, progesterone secretion decreased to an unmeasurable level, but there was no change in blood flow and oestrogen secretion. Seventy minutes after cycloheximide injection, ACTH failed to affect ovarian blood flow or oestrogen and progesterone secretion (Fig. 5B).

Discussion

The aim of the above experiments was to establish whether the stimulating effect of ACTH on ovarian blood flow was connected with a change of ovarian secretion since steroidogeneses in the adrenal gland and the ovary coincide in many respects. LH in vitro increases the conversion of cholesterol into pregnanolone in the corpus luteum (HALL and KORITZ 1964). Cholesterol metabolism in the adrenal cortex is affected by ACTH in the same way (STONE and HECHTER 1954; KARABOYAS and KORITZ 1965). Cyclic AMP plays an important role in both the steroidogenic effect of LH (SAVARD et al. 1965) and in that of ACTH (HAYNES et al. 1960). It seems, however, that stimulation of adenyl cyclase activity in the target organs is hormone specific. Consequently, ACTH increases adenyl cyclase activity in the adrenal cortex of the rat (GRAHAME-SMITH et al. 1967), but it is not effective on the adenyl cyclase activity in the ovary of the rabbit in contrast to the stimulating effect of LH (DORRINGTON and KILPATRICK 1966).

In dogs treated with HCG for 5 days the higher level of progesterone in ovarian venous blood and the stimulating effect of HCG on progesterone secretion are attributed to luteinization (TELEGDY and HUSZÁR 1962; NISHIZAWA and EIK-NES 1964; STROTT et al. 1969). In these dogs on the sixth day, ACTH increases ovarian blood flow and oestrogen secretion. The effect of a single intravenous injection of ACTH was different from that of HCG; HCG immediately increased progesterone secretion and this was followed one hour later by a rise of oestrogen secretion. Since the effects of ACTH and HCG on ovarian blood flow and hormone secretion are different, ACTH presumably elicits its effect differently from gonadotrop hormones.

In the human, ACTH stimulates oestrogen secretion (SANDBERG et al. 1958; BROWN et al. 1959; BAIRD et al. 1969) and ketosteroid secretion (KECSKÉS et al. 1963) in the adrenal gland. It was supposed that the dehydroepiandrosterone (DHEA) produced in the human adrenal gland might be a mediator ovarian oestrogen production (BARLOW 1964). This opinion was not supported by the findings of AAKVAAG and FYLLING (1968), because in women the ovarian venous level of DHEA is higher than in peripheral blood. The DHEA level in the peripheral blood of dogs being lower than in man (OERTEL and EIK-NES 1959), the importance of this substance is questionable. On the other hand, testosterone production is considerably elevated in the dog testis perfused with adrenal venous blood (WASSERMANN and EIK-NES 1969), but ATCH leaves it unaffected (EIK-NES 1962).

ACTH increases ovarian blood flow even after adrenalectomy (STARK et al. 1967; STARK and VARGA 1968), and, as it has been shown in the present experiments, oestrogen secretion by the ovary in HCG pretreated animals as well. It seems that ACTH exerts its effect directly on the ovary, independently of the adrenal gland.

HCG immediately elevated progesterone secretion but HCG did not influence ovarian blood flow. Ovarian blood flow increased only one hour after HCG administration, and at the same time a considerable increase in oestrogen secretion could be noticed. This shows that the increase of ovarian blood flow and that of oestrogen secretion go parallel even after HCG stimulus. No close correlation could be found between the stimulating effect of ACTH on the ovarian blood flow and on the rate of progesterone secretion. In vitro, in the bovine corpus luteum (SAVARD et al. 1965) and in vivo, in pre-oestrous rats (ICHIKAWA et al. 1972) ACTH does not stimulate progesterone secretion.

In dogs, cycloheximide inhibited the effect of ACTH on corticoid secretion in 5 minutes while it took 30 to 60 minutes before it diminished the effect of ACTH on adrenal and ovarian blood flow (STARK and VARGA 1968). The synthesis of new proteins is important not only for the steroidogenic effect of ACTH but for the LH as well. In human corpus luteum slices, puromycin and actinomycin-D abolish the effect of LH on progesterone secretion (SAVARD et al. 1965). Under similar experimental conditions, cycloheximide inhibits the stimulating effect of LH on progestin secretion by the rabbit ovary (GORSKI and PADNOS 1966). The site of oestrogen secretion is the theca interna and the interstitium (SMITH and RYAN 1961), while practically all of the progesterone is produced in the corpus luteum (TELEGDY and HUSZÁR 1962; HAMMERSTEIN et al. 1964). These steroids differ from each other not only in the site of production but also in respect of the inhibition by cycloheximide of their steroidogenic action, as cycloheximide is immediately blocking progesterone secretion while it leaves oestrogen secretion unaffected. In mammalian ovarian tissue there are two biosynthetic pathways for oestrogen secretion, viz. (1) from pregnenalone to adrostenedion and oestrogen (RYAN and SMITH 1961a) and (2) conversion of progesterone into estrone and oestradiol (RYAN and SMITH 1961b). The first way seems to have an important role in the dog, as it is able to maintain a control oestrogen level in ovarian venous blood during the abolished progesterone secretion. ACTH increases ovarian blood flow (STARK and VARGA 1968) and oestrogen secretion after cycloheximide as well, but progesterone secretion decreases even if HCG is administered. The stimulating effect of ACTH on ovarian blood flow might be connected with an increase in ovarian oestrogen secretion.

On the other hand, ACTH increases ovarian blood flow in different periods of the ovarian cycle (STARK et al. 1967; VARGA et al 1969), while oestrogen secretion only after HCG pretreatment. An increase in oestrogen secretion might perhaps be achieved without HCG pretreatment, but the change then is too slight for our methods to show it. Presumably, a considerable effect of ACTH on ovarian oestrogen secretion could be brought about only in the luteal phase, in connection with effect of gonadotrophic hormones.

The fact that ACTH increases not only ovarian blood flow but under certain circumstances also ovarian oestrogen secretion, might suggest that in this way ACTH together with gonadotrophic hormones is able to influence female gonadal function under physiological and pathological conditions. Some authors have assumed that ACTH affected gonadal function through the corticoids (HAGINO et al. 1969; LIPTRAP 1970) or through the adrenal sex steroids (RESKO 1969; CHATTERJEE and HARPER 1970; FEDER et al. 1971). In contrast, our data show that ACTH has a direct effect on female gonadal function.

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EFFECT OF HYPOPHYSECTOMY ON OVARIECTOMY-INDUCED PLACENTAL HYPERTROPHY

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In 91 pregnant rats, the effect of hypophysectomy had been examined on the ovariectomy-induced placental and fetal hypertrophy. In the controls, hypophysectomy did not affect placental weight, but reduced fetal weight. In ovariectomized rats, hypophysectomy did not affect the hypertrophic response of the placentae, but prevented weight increase of the fetuses. It is concluded that the effect of ovariectomy on placental growth is not mediated by the pituitary, but that the pituitary controls fetal growth directly, by a mechanism independent of placental function.

As in humans (CSAPO et al. 1972, 1973), in rats the consequences of ovariectomy on pregnancy maintenance were determined by the gestational timing of surgery (CSAPO 1970; CSAPO and WIEST 1969; CSAPO and CSAPO 1973). When performed before the 16th day of pregnancy (the day of "sperm positivity" being designated day 0), ovariectomy in rats invariably provoked abortion, while a delay of 24 to 48 hours in surgery promoted pregnancy maintenance till term and the great majority of the litter survived.

These results suggested that the consequences of ovariectomy on pregnancy maintenance were determined by the degree of the "luteo-placental shift" in steroidogenesis at the time of surgery (CSAPO et al. 1972, 1973; CSAPO 1970; CSAPO and WIEST 1969; CSAPO and CSAPO 1973; YOSHIMI et al. 1969). Whenever functioning corpora lutea had been removed, progesterone (P) and estradiol 17β (E_2) levels decreased, regardless of gestational age and species. However, the degree of P and E_2-withdrawals provoked depended upon the species and the gestational timing of ovariectomy, since the relative contributions of the ovaries and placentae are different at various times of gestation in the different species.

These findings and the additional observations (CSAPO 1969; CSAPO and WIEST 1972) that pregnancy maintenance is controlled by the placenta even in the rabbit (a species in which luteal P-genesis is indispensable throughout pregnancy), because of the indispensability of placental luteotrophic

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support, fully exposed the significance of the placenta to pregnancy maintenance and fetal survival.

It was of considerable interest, therefore, that ovariectomy (at around the 12th day of pregnancy) provoked excessive placental hypertrophy in rats, provided that pregnancy was sustained through a prevention of ovariectomy-induced P and E_2 deficiencies, by treatment with P and E (CSAPO 1970; CSAPO and WIEST 1969; CSAPO and CSAPO 1973). Placental hypertrophy had been observed by several investigators (BUTTERSTEIN and LEATHEM 1970, 1972; CALLARD and LEATHEM 1971) who, however, explained its underlying mechanism differently. The finding was significant, therefore, that placental hypertrophy can be provoked in ovariectomized rats if their P and E_2 levels are sustained exogenously near physiological values (CSAPO and WIEST 1969, 1973). This finding substantiated the premise (CSAPO 1969, 1970; CSAPO and WIEST 1969, 1973; CSAPO and CSAPO 1973; CSAPO et al. 1972) that the growth potential of the placenta is greater than what is normally observed and that it is this growth "reserve" of the placenta which is released when an endogenous placental growth inhibitor (PGI) is removed by ovariectomy.

Since excessive placental hypertrophy only occurred in ovariectomized rats (excepting the intact "low" litter animals), it was concluded that PGI is of ovarian origin (CSAPO 1970; CSAPO and WIEST 1969, 1973; CSAPO and CSAPO 1973). It could be expected, therefore, that the replacement of this removed factor suppresses placental growth either directly or indirectly. If the effect of PGI is indirect, then the release of a hormone which stimulates placental growth must be suppressed in one of the endocrine glands other than the ovaries.

Adrenalectomy (BUTTERSTEIN and LEATHEM 1972) and hypophysectomy (CALLARD and LEATHEM 1971) did not affect the ovariectomy-induced placental hypertrophy in hormone-supported rats. These findings suggested that the effect of PGI on the placenta is a direct one. However, since only 6 animals were exposed to the combined procedures of ovariectomy and hypophysectomy, the effect of hormone treatment on the P and E_2 levels of the control and experimental animals had not been determined; fetal survival and fetal weight were low and unexplained; it was of interest to repeat these studies under improved experimental conditions (CSAPO and WIEST 1973; CSAPO and CSAPO 1973).

Methods

A total of 91 Holtzman bred, Sprague-Dawly rats were studied. Pregnancy was determined by "sperm positivity" and this day was designated day 0 of gestation. Throughout the experiments the animals were kept at a constant temperature, cyclically changing light and darkness and were fed a purine diet as described previously (CSAPO and WIEST 1973; CSAPO and CSAPO 1973).

Statistical significance: Placentae: between Groups 1 and 6, 7 or 8, $p < 0.001$. Fetuses: between Groups 1 and 4; 1 and 5; 1 and 6 + 7; or 8 and 6 + 7, $p < 0.001$. 1 + 3 and 4 + 5, $p < 0.001$

Table I

Effect on placental and fetal weight of hypophysectomy in intact, ovariectomized and hormone-treated pregnant rats

Groups	Surgery and treatment	Number of animals	Litter size	Fetal survival, %	Placental weight, g	Fetal weight, g
1	Sham only	21	10.3±0.3	100	0.59±0.01	5.6±0.1
2	Sham + P4E1	10	10.1±0.6	98	0.59±0.01	5.3±0.1
3	Sham + Hypox Op	6	7.2±1.3	97	0.59±0.04	5.5±0.2
4	Sham + Hypox	11	9.9±0.7	100	0.62±0.04	5.0±0.1
5	Sham + Hypox + P4E1	7	9.7±0.9	100	0.56±0.02	5.1±0.1
6	Ox + P4E1	11	9.8±0.6	94	0.89±0.03	6.5±0.1
7	Ox + Hypox Op + P4E1	13	10.7±0.4	97	0.89±0.04	6.1±0.1
8	Ox + Hypox + P4E1	12	9.8±0.7	95	0.99±0.04	5.5±0.2

Abbreviations: P4E1: 4 mg P + 1 μg E/day; Hypox Op: the operative procedure of hypophysectomy without removal of the pituitary gland; Hypox: hypophysectomy; Ox: ovariectomy

On the 13th day of gestation, all animals were subjected to a sham operation and the distribution of the conceptus sacs in the 2 uterine horns was recorded. Subsequently they were divided into 8 groups depending on further surgery and treatment (Table I).

Group 1, 21 animals were exposed to no further surgery other than sham and received no hormone treatment.

Group 2, 10 animals had no further surgery but received a daily dose of 4 mg progesterone (P4) and 1 μg estradiol dipropionate (E1) in oil, i.m., in 2 divided doses at 9 a.m. and 9 p.m. (labelled in Table I as P4E1).

Group 3, 6 animals were exposed to all the surgical procedures of hypophysectomy, except to the removal by suction of the pituitary gland itself. They received no hormone treatment.

Group 4, 11 animals were hypophysectomized (verified at autopsy by the complete absence of the pituitary gland) and received no hormone treatment.

Group 5, 7 animals were hypophysectomized and received P4E1 hormone treatment.

Group 6, 11 animals were ovariectomized and received P4E1 hormone treatment.

Group 7, 13 animals were subjected to hypophysectomy without removal of the pituitary gland. In addition, they were ovariectomized and received P4E1 hormone treatment.

Group 8, 12 animals were hypophysectomized (verified at autopsy by the complete absence of the pituitary gland). In addition, they were ovariectomized and received P4E1 hormone treatment.

Thus the effects on fetal and placental weights of each of the surgical procedures and hormone treatment had been separately examined in the experimental animals (Groups 2—8). The results obtained in the different groups were compared with one another and with the control values (Group 1). All studies began at 9—11 a.m. on the 13th day of pregnancy and were concluded by autopsy at 3 p.m. on day 21. Surgery was conducted in ether anesthesia and under strictly aseptic conditions. Extreme care was taken to avoid unnecessarily deep anesthesia and tissue damage. Ovariectomy was performed through laparotomy and hypophysectomy, using the transpharyngeal approach. Subsequently, the animals were observed daily at 9 a.m. and 9 p.m.

On day 21 all animals were hysterectomized in ether anesthesia and their uterine contents were removed and examined. The fetuses and placentas were separated from the fetal membranes and from one another and weighed individually. The placental implantation sites and the dead and resorbing fetuses were counted. In those animals which were exposed to the surgical steps of hypophysectomy, with or without the removal of the pituitary gland, the sella turcica was exposed and the presence, or absence, of the pituitary gland was determined. The data were placed on idividual study sheets, analyzed statistically and tabulated.

Results

As illustrated by Table I all surgical treatments were successful, as indicated by the high survival rate of the litter in all animals. As compared to the controls (Group 1), all the non-ovariectomized rats (Groups 1—5) had a normal placental weight regardless of the surgical procedure including hypophysectomy and hormone treatment. The differences in placental weight between non-ovariectomized (Groups 1—5) and ovariectomized (Groups 7—8) animals were statistically highly significant ($p < 0.001$), however.

These results provide definitive evidence that the ovariectomy-induced placental hypertrophy is independent of pituitary function, under such experimental conditions when fetal survival is normal and the P and E_2 levels are exogenously sustained at near physiological values.

However, hypophysectomy reduced fetal weight below the control value. The fetal weights in Groups 4 and 5 were significantly lower than in Group 1 ($p < 0.001$). Surgery alone, without the removal of the pituitary gland, had no effect on fetal weight, as demonstrated by the similarity of fetal weights in Groups 1 and 3, and the difference in fetal weights in Groups $1 + 3$ and $4 + 5$ ($p < 0.001$).

As in earlier studies (CSAPO and CSAPO 1973), ovariectomy increased fetal weights, as demonstrated by the difference in fetal weight between Groups 1 and $6 + 7$ ($p < 0.001$). Hypophysectomy (by reducing fetal weight) balanced this effect of ovariectomy, as illustrated by the similarity in fetal weight between Groups 1 and 8, or the difference between Groups $6 + 7$ and 8 ($p < 0.001$). Apparently, both the pituitary and the hypertrophied placentae promote the increase in fetal weight. Therefore, hypophysectomy cancels this effect of the hypertrophied placenta on fetal weight.

Discussion

The present study confirmed the earlier demonstrations (CSAPO 1969, 1970; CSAPO and WIEST 1969, 1973; CSAPO and CSAPO 1973) that ovariectomy in pregnant rats provokes placental hypertrophy even if by hormone substitution therapy the progesterone (P) and estradiol 17β (E_2) levels are sustained at near physiological values. It documented that this effect of ovariectomy on placental growth is not mediated through the pituitary, for hypophysectomy did not interfere with ovariectomy-induced compensatory process.

However, hypophysectomy (but not the surgical procedure alone without the removal of the pituitary gland) reduced fetal weight in the controls and it balanced the ovariectomy-induced weight increase of the fetuses.

These results suggest that the ovaries contain a placental growth inhibitor, the effect of which on placental growth is probably direct, since it is not mediated by the adrenals (BUTTERSTEIN and LEATHEM 1972) or the pitui-

tary (CALLARD and LEATHEM 1971). In contrast, the pituitary does affect fetal weight, for when it is removed fetal weight decreases, unless this deficiency syndrome is prevented by ovariectomy-induced placental hypertrophy which increases fetal weight beyond normal values.

Whether or not the presently exposed regulatory mechanisms, controlling placental and fetal growths, operate in the human is yet to be determined. Unfortunately, in those rare instances when the ovaries were removed in patients at advanced stages of gestation (when ovariectomy no longer terminates pregnancy; CSAPO et al. 1972, 1972) the placental weights were not measured after delivery. However, fetal weights had been reported occasionally. These measurements suggest that the effects of ovariectomy described in this report also occur in the human. If so, extensive clinical studies, focused upon the mechanism which controls placental and fetal growth, are mandatory.

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MECHANISM OF PERINATAL REPRODUCTIVE DISORDERS INDUCED BY PARAFORNICAL HYPOTHALAMIC LESIONS IN THE RAT

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Electrocoagulation of 50% of the parafornical area has been found to cause a considerable disturbance of maternal behaviour and foetal vitality. Food and water uptake of the damaged animals reached the initial level at the time of parturition. The decrease in body weight was not compensated due to foetal development. Body temperature was essentially normal. Maternal behaviour during delivery was normal, retrieving and nest-building activities appeared only in one third of the animals and nursing was also of a reduced degree. Adenohypophyseal LTH content was significantly higher than in the control group, but in STH content there was no difference between the two groups. The lower birth weights and live birth ratio pointed to intra-uterine foetal lesions.

It has been found earlier that electrocoagulation of the parafornical hypothalamic structures in the projection of the ventromedial nucleus leads to severe foetal disorders in the last third of pregnancy in the rat. None of the components of maternal behaviour appeared, the offspring died either in utero or on the first two days after birth. Disorders of motility, food uptake and lactation could not have played a decisive role in the perinatal lesion (AVAR and MONOS 1964, 1966, 1967, 1969a, b). Preliminary experiments indicated that even if the parafornical lesion was reduced in extent, maternal behaviour and foetal vitality may still be damaged. In a new series of experiments we have studied the effect of parafornical lesions of a smaller, roughly 50% extent on the following parameters.

I. In pregnant animals,
   - — changes in food and water uptake, body weight and temperature;
   - — maternal behaviour;
   - — LTH (prolactin) and STH content of the hypophysis;
   - — weight, and light and electron microscopic picture of the endocrine organs.

II. In the offspring,
   - — birth weight;
   - — ratio of live births;
   - — lactation readiness;
   - — light and electron microscopic picture of the endocrine organs.

Methods

Selection of the pregnant animals, determination of food and water uptake, measurement of body weight and temperature, operation and post-treatment were performed and maternal behaviour was tested as described earlier. Pituitary LTH and STH content was estimated by polyacrylamide gel electrophoresis (KURCZ et al. 1969; NAGY et al. 1970). Location of the hypothalamic lesion was checked histologically on serial sections. Seventeen rats were subjected to operation, 17 animals served as controls: of the latter, 8 were intact and 9 sham-operated. At evaluation, the two control groups were occasional pooled, as they displayed no differences in the investigated aspects. The animals were decapitated 24 hours following birth. Results were analyzed statistically by means of the *t*-test.

Results

Food uptake on the first two postoperative days of the damaged animals (Fig. 1/B) was significantly less than that of the controls ($p = 0.001$), but was again normalized by the time of parturition and higher than prior to operation. There was a similar change in water uptake. Body weight (Fig. 1A) decreased significantly between the operation and parturition as compared to the control

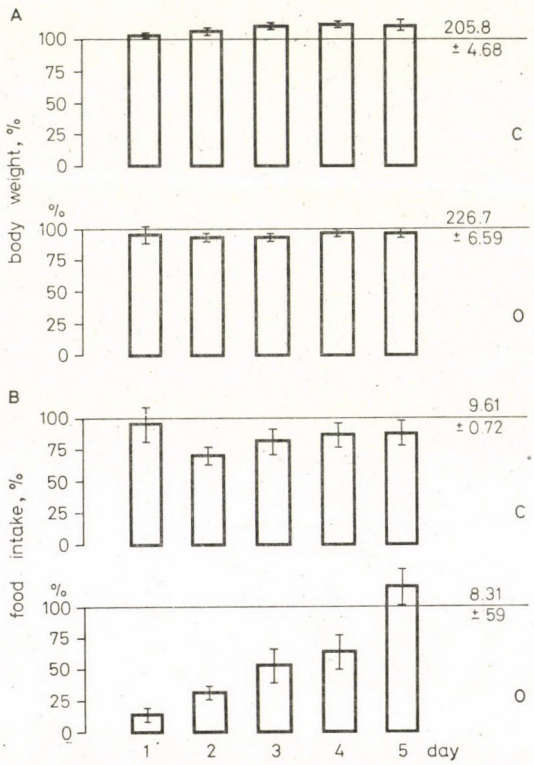

Fig. 1. A: Body weight in control (C) and operated (O) group between lesioning and parturition. B: Food intake of control (C) and operated (O) animals in the period between operation and parturition

group ($p = 0.001$). Temperature was not significantly affected by the hypothalamic lesion. Labour was normal in both groups, the offsprings were cleansed of the amnion and the placenta was eaten as usual. Only one third of the operated animals displayed retrieving and nest-building activities and nursing was also of a reduced degree. After birth there was no significant difference

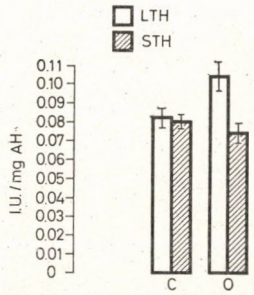

Fig. 2. LTH and STH content of adenohypophysis (AH) in control (C) and operated (O) group (mean ± S.D.)

between the groups in body and weight of the pituitary, thyroid, suprarenal gland and ovaries, neither was there a difference in weight between the two sides with respect to the adeno- and neurohypophysis and the paired endocrine organs. LTH content of the adenohypophysis (Fig. 2) was significantly higher in the damaged group ($p = 0.03$), while there was no change in STH content.

Next, the operated group was divided on the basis of the histological localization into a typical and a nontypical group. In the latter group (six animals) the lesions affected only a small part of the middle parafornical structures. When divided in this way in none of the cases was the increase in prolactin content significant, a fact in which the reduced number of animals must have played an essential role. In the operated group, the maternal suprarenal gland showed a histological change indicating an augmented, that of the neonatal suprarenal gland a reduced, ACTH action.

The lower birth weight, an average of 4.5 g compared to 5.6 g in the control group ($p = 0.004$) and the lower ratio of live births (95.4% vs. 99.3% in the control group, $p = 0.05$) indicated an intra-uterine lesion of the offsprings of damaged rats. In the majority of the new-born animals, milk could be detected in the stomach.

Discussion

The above data show that even if the parafornical lesion is reduced in extension, there will occur a significant damage to maternal behaviour and foetal vitality, though these will be less serious than in the case of such extensive

lesions as those investigated earlier. The body weight of the damaged mothers was less and did not reach the pre-operative level, owing to the lagging weight development or intra-uterine death of their foetuses. Behaviour during labour, an attitude linked to pituitary function was normal in the operated group.

No endocrine disturbance could be detected on the basis of the weight of the endocrine organs, neither did histological studies reveal anything beyond a stress effect. There was no LTH or STH deficiency in the adenohypophysis, and a higher amount of prolactin was found in the operated animals than in the controls. This couldn't have been the result of a complete blockade of release since milk excretion was noted in the operated animals and the milk found in the stomach of the new-borns indicated that despite deficient maternal behaviour some of the offspring were able to reach the mammae. It remains questionable whether there was a partial blocking of prolactin release in the damaged animals and, if so, whether this can be brought into connection with the defect in maternal behaviour. There are data according to which prolactin might have a role in inducing the mechanism of maternal behaviour (RIDDLE et al. 1942), this, however, is decisively a function of neural structures. This view is supported by tests performed on hypophysectomized rats and mice (COLLIP et al. 1933; LEBLOND and NELSON 1937; OBIAS 1957). The higher pituitary prolactin content of the lesioned mother might be related to a less intensive lactation stimulus exerted by new-borns of a vitality lower than that of the controls. We have not been able to judge quantitatively either lactation or the vitality of the new-borns, since we had only a single day after birth to make observations.

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CHEMICAL SENSITIVITY AT DIFFERENT TEMPERATURES OF THE BR-TYPE, BIMODAL PACE-MAKER NEURONE IN THE CNS OF THE SNAIL *HELIX POMATIA* L.

By

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The effect of ACh, 5-HT, NA and DA*, added into the bathing fluid was investigated on the Br-type neurone (RPal-cell) of the snail, *Helix pomatia* at 22°C, 7°C and above 30°C.

Acetylcholine caused an increase of activity at all the temperatures. The effect appeared instantaneously but lasted for a short time.

After serotonin application, synaptic potentials (EPSP-s) appeared at each temperature, evoking extra spikes. The characteristic bimodal activity pattern of the cell remained practically intact.

Noradrenaline blocked potential generation at each temperature.

Dopamine had a biphasic effect: it inhibited spike activity for 2—2.5 min, then action potential generation reappeared. In the later phase, depolarization and the presence of EPSP-s were characteristic.

It is supposed that ACh, NA and DA are directly influencing the permeability of the soma membrane, while the effect of 5-HT and the late effect of DA is the result of synaptic activation caused either by stimulation of the neurones connected synaptically to the Br-type cell, or by a direct effect on the synaptic region of the investigated cell.

One of the basic conditions for differentiated neuronal machinery is the heterogenous chemical sensitivity of neurones. The specifically determined response of soma and synapses to endogenous substances influencing the permeability of the membrane is of basic importance not only for impulse transmission but also in the determination of the excitability of spontaneously active neurones and probably in the generation of potentials in these cells. Starting from these considerations, a study of the chemical sensitivity and the effect of special chemical substances on neurones characterized with bimodal pace-maker activity seemed of some interest. In these latter cells different mechanisms may be responsible for spike generation and for burst generation. Such a type of neurone was described in the CNS of *Aplysia*(ARVANITAKI and CHALAZONITIS 1955), *Helix pomatia* (SAKHAROV and SALÁNKI 1969) and *Heliosoma* (KATER and KANEKO 1972). Examining the chemical sensitivity of this type of neurones, the excitatory effect of acetylcholine and serotonin (FRAZIER et al. 1967; KOROBTZOV and SAKHAROV 1971) and the inhibitory effect of noradrenaline (BOISSON and CHALAZONITIS 1972) were reported.

* Abbrevations: ACh: acetylcholine; 5-HT: serotonin; NA: noradrenaline; DA: dopamine.

In earlier investigations we found that the bimodal pace-maker, Br-type neurone of *Helix pomatia* receives synaptic inputs in some cases (SALÁNKI et al. 1972) and, further, that this neurone displays different types of activity at different temperatures, as its bimodality disappears both at low and at high temperatures (SALÁNKI et al. 1973). In the present investigations the chemical sensitivity of the Br-type cell has been studied at various temperatures where the activity pattern was characteristically different. The aim was to clarify (1) the type of reaction of this neurone to various bioactive substances, (2) eventual the temperature dependence of the reaction. The results were expected to allow conclusion concerning the mechanisms of spike and burst generation, and the specificities of the synaptic inputs.

Methods

Experiments were carried out on the Br-type cell of the snail *Helix pomatia* L. This giant neurone termed in earlier investigations as RPa1 neurone (SAKHAROV and SALÁNKI 1969) can be made visible together with its axon in the right parietal ganglion, by intracellular $CoCl_2$ staining (PITMAN et al. 1972; Fig. 1).

The isolated ganglionic ring was placed in a perfusion chamber of 3 ml. The temperature of the bathing fluid could be regulated between 5 and 40°C. Experiments were carried out at 5—7°C, and at 30—34°C. Recordings were made 10 min after the required temperature had been reached.

For recording membrane and action potentials, glass micropipettes filled with 2.5 mol KCl were used. The resistance of microelectrodes varied between 8—15 MOhm. For amplification and recording a negative capacitance, high input impedance amplifier (VÉRÓ 1971), an EMG 1552 type dual beam oscilloscope and film recorder were used.

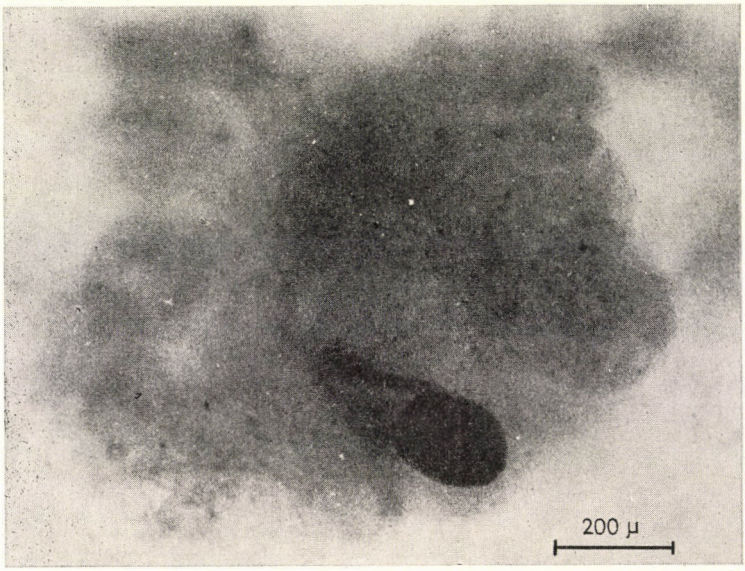

Fig. 1. Localization of the Br-type cell in the right parietal ganglion. Staining by intracellular $CoCl_2$ injection

The substances examined were dissolved in physiological saline and added to the bathing fluid. The substances applied were, acetylcholine chloride (ACh, Sigma), 10^{-4}—10^{-3} mol; 5-hydroxytryptamine (5-HT, Reanal), 10^{-5}—10^{-4} mol; noradrenaline bitartarate (NA; Sigma), 10^{-3} mol; dopamine (DA), 10^{-4} mol.

Composition of the physiological solution was NaCl, 3.0 g; KCl, 0.35 g; $MgCl_2 \cdot 6H_2O$, 2.4 g; $CaCl_2 \cdot 2H_2O$, 1.5 g; $NaHCO_3$, 0.2 g, per litre.

Results

The effect of ACh

a) At 22°C, when burst activity was characteristic, ACh caused in every case a slight depolarization followed by an increase in the frequency of activity. This occurred immediately after application, but after about 10 min the original activity was retorted. After ACh, the first burst was longer than the

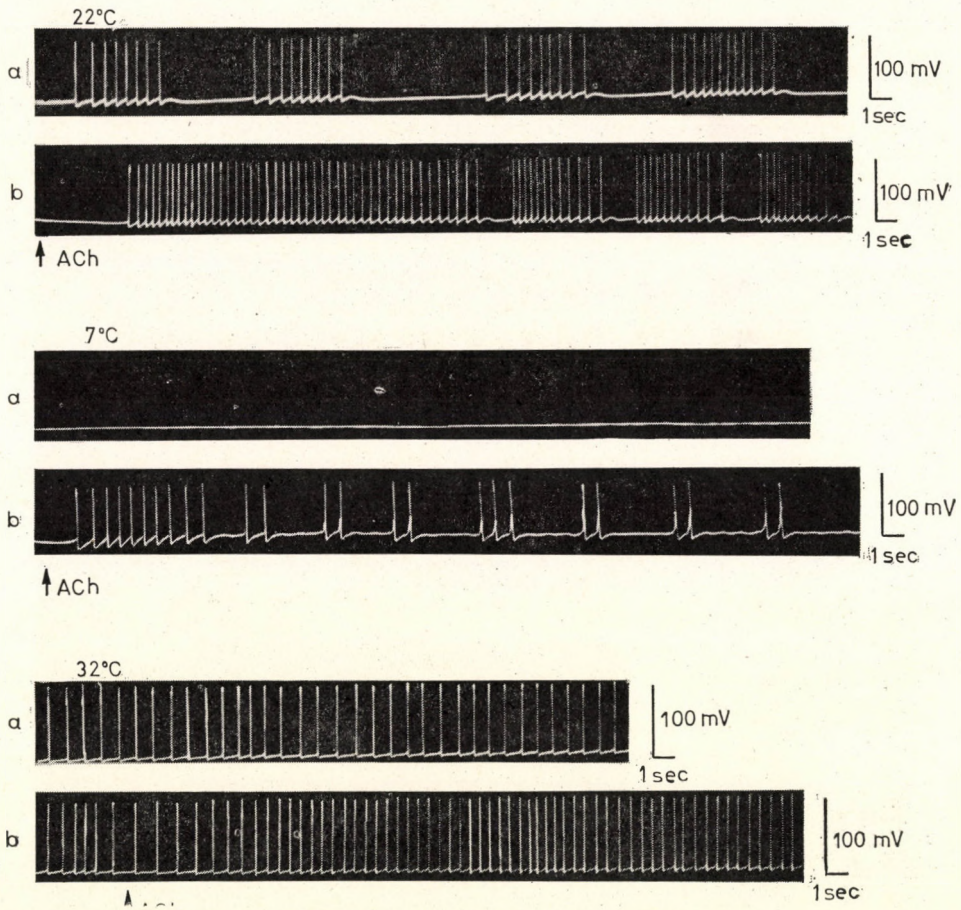

Fig. 2. Effect of ACh on spontaneous activity of the Br-type neuron at different temperatures; a: control; b: 10^{-4} mol ACh

control, but the number of spikes within the following bursts was similar to that in the control. In this way the duration of the bursts did not change significantly except in the case of the first one, while the duration of the interburst intervals decreased to about one third of the control. Impulse duration increased by about 20—25%, but no change was observed in spike amplitude (Fig. 2 upper).

b) At 7 °C activity of the Br-type cell was irregular. On decreasing the temperature, spike frequency gradually decreases and potential generation

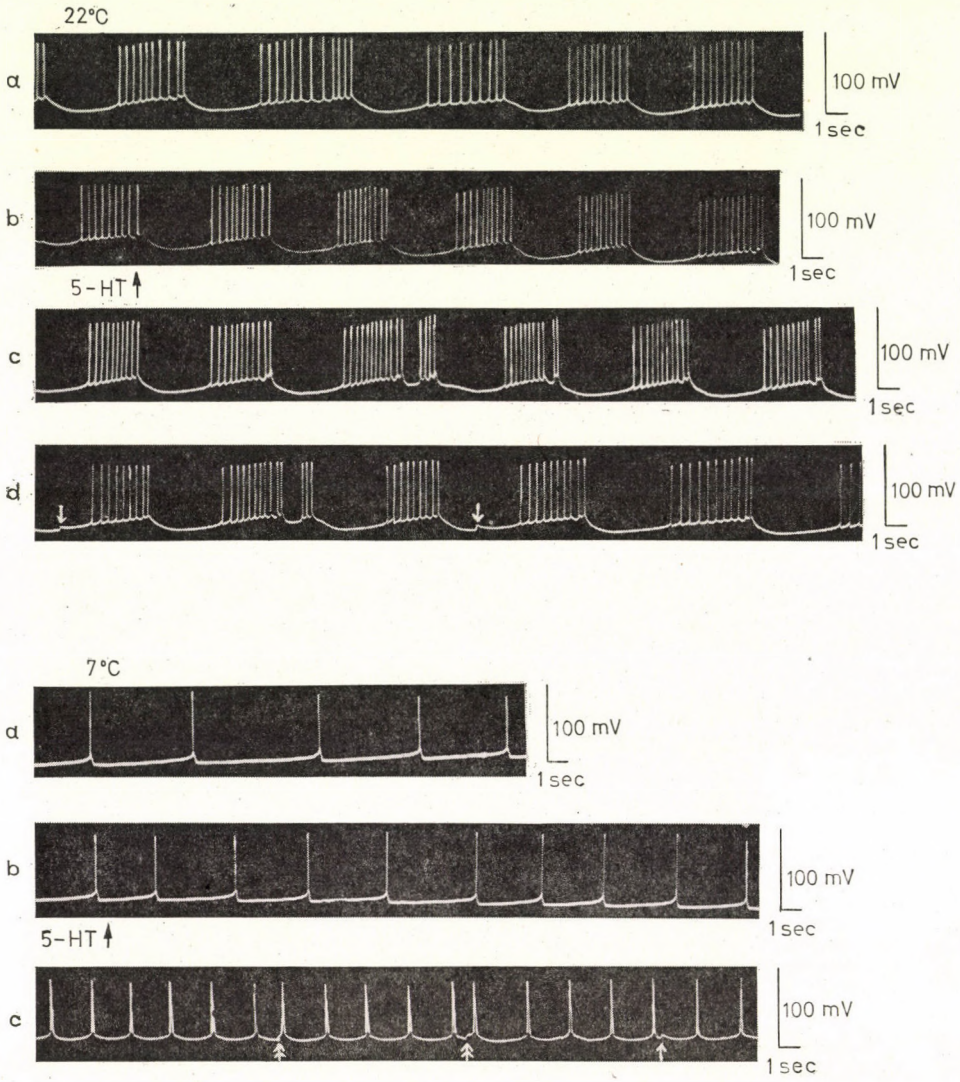

Fig. 3. Effect of 5-HT; a: control; b—d: 10^{-5} mol 5-HT; arrow: EPSP; doubled arrow: synaptically evoked spike

even stops. On ACh application, depolarization and an increase in spike frequency occurred. If the neurone had became silent, to ACh spiking reappeared. The activity type did not resemble the bursting pattern, although groups consisting of 2—3 spikes regularly occurred (Fig. 2 middle). The prolonged hyperpolarizing phase characteristic of the bursting pattern of this neurone was absent in the control and after ACh application. Spike duration increased by about 20% after ACh application, but the amplitude of the potentials remained unchanged. After ACh application some membrane oscillation occurred between groups of spikes.

c) Above 30°C, continuous, regular high frequency activity was recorded from the cell. On ACh application the activity became even more frequent, but its character did not change. Nor was a change detected in spike duration or spike amplitude (Fig. 2 lower).

The effect of 5-HT

a) At 22°C, 5-HT caused no immediate alteration in bursting activity. However, after 30—60 sec the regularity of bursts underwent a change in that at the beginning of afterburst hyperpolarization, a short train of spikes interrupted the interburst period. This interruption was followed by the hyperpolarizing phase characteristic of the Br-type neurone. At the same time, EPSP-s occurred in the interburst hyperpolarizing period, and the spikes appearing as a prolongation of the standard burst seemed to be elicited by EPSP-s (Fig. 3 upper). The other parameters of neuronal activity, the interspike interval, impulse duration, burst and interburst period, number of spikes within a burst and the amplitude of the spikes did not change significantly under the effect of 5-HT.

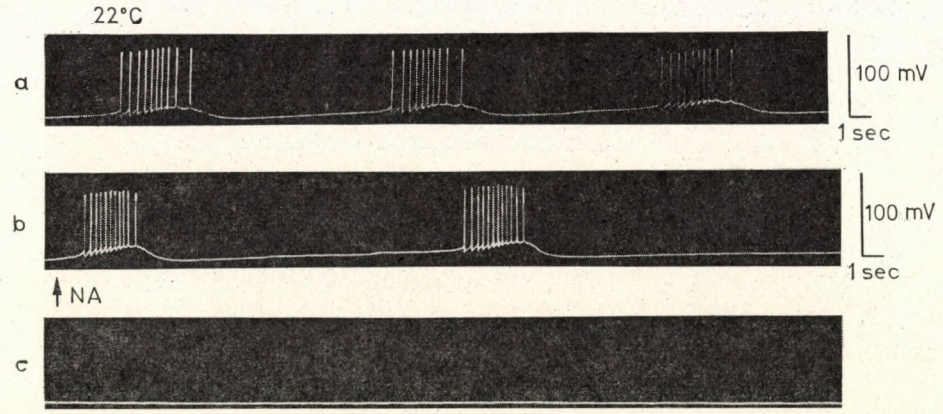

Fig. 4. Effect of NA at 22°C; a: control; b—c: 10^{-3} mol NA

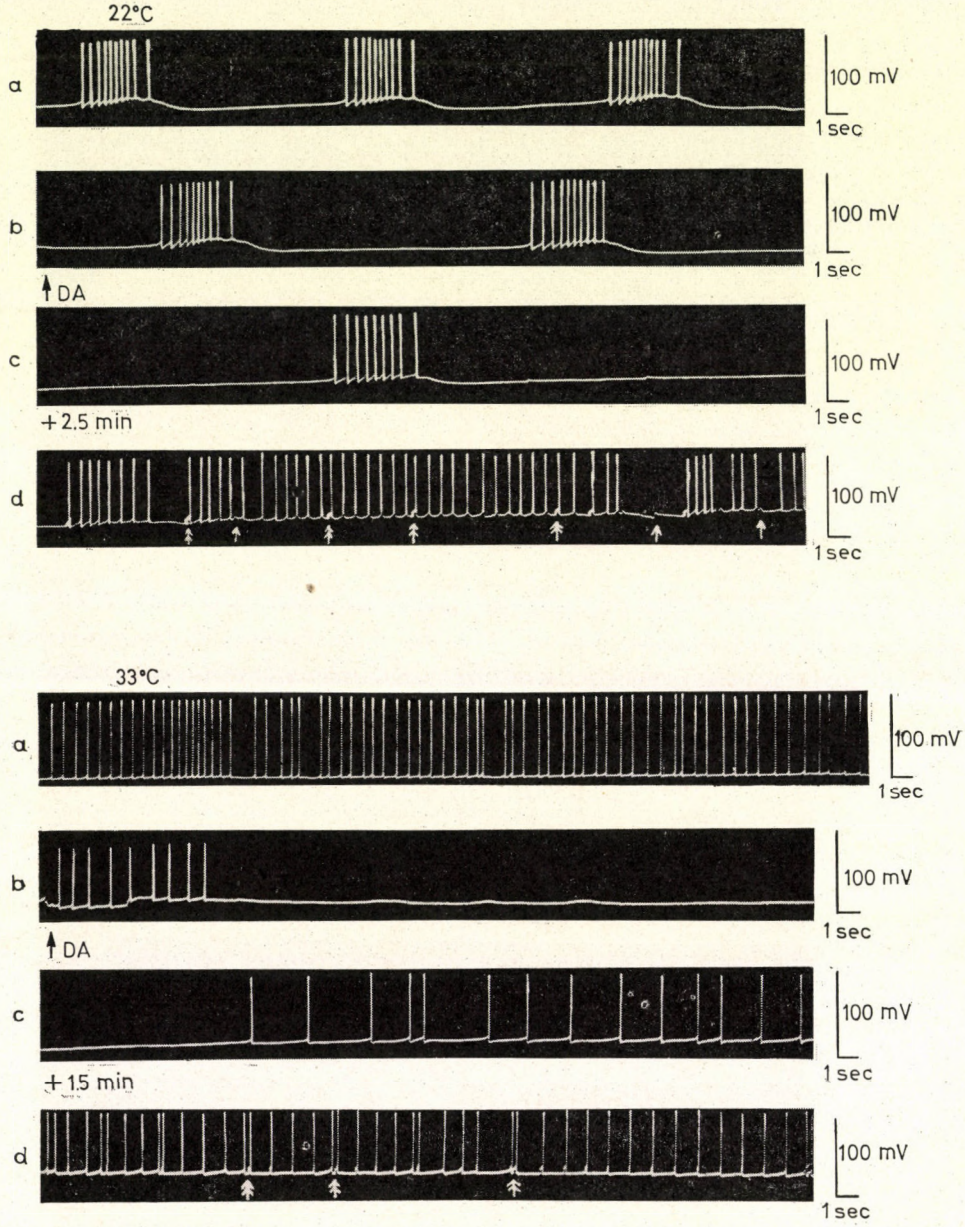

Fig. 5. Effect of DA; a: control; b—c: 10^{-3} mol DA; d: after 2.5 and 1.5 min; arrow: EPSP; doubled arrow: synaptically evoked spike

b) At 7°C, the control frequency of the activity increased by 100% and impulse duration was doubled. The comparatively regular activity pattern was sometimes interrupted by "arrhythmical" spikes evoked in every case by synaptic potentials (Fig. 3 lower).

c) Above 30°C, after 5-HT application control activity increased temporarily, the regular rhythm of activity became somewhat irregular and some of the spikes were evoked by EPSP-s while others were generated autoactively as before.

The effect of NA

NA had a similar effect at 22, 7 and above 30°C: immediately after application there was no change in the control activity, then in 30—60 sec the membrane potential suddenly increased by about 10 mV, and spike generation disappeared (Fig. 4).

The effect of DA

a) At 22°C, DA caused a progressive increase of the interburst intervals. This was followed by an elimination of spike activity. The silent period lasted 2—2.5 min, then action potentials reappeared but the typical bursting activity was masked by spikes evoked by EPSP-s. The spike series was sometimes interrupted by short silent periods corresponding to the interburst intervals characteristic of the Br-type activity. During the interburst periods, strong synaptic activity could be observed (Fig. 5a). The duration of spikes decreases by about 30% after DA application.

b) At 7°C, DA caused a complete elimination of spiking, and action potential generation failed to return.

c) Above 30°C, DA had a similar effect as at 22°C: continuous activity was abolished, then after about 1.5 min spikes were again present and later even an increase in the frequency was observed. Synaptic potentials were also recorded. Among the parameters of activity, only the duration of the spikes changed significantly: it decreased by about 30% (Fig. 5b).

Discussion

The effect of the investigated substances was characteristically different, due probably not only to chemical differences but also to their different sites of action. The results obtained at various temperatures allow some conclusions in this respect.

In case of ACh, the depolarizing effect and, as its consequence, the increase of spike activity was well visible at all the three temperatures. The increase

in the frequency of activity lasted for a short period, in agreement with the observations of KOROBTZOV and SAKHAROV (1971), but this stimulating effect started immediately after the application of ACh, referring to its direct neuronal effect. The shortening of the hyperpolarizing phase at 22°C, the membrane potential oscillation at 7°C, and the absence of synaptic potentials showed that the depolarization and the increase in frequency were caused probably by the effect of ACh on the soma membrane. Therefore this RPA1 neurone belongs among the D cells (TAUC and GERSCHENFELD 1960). The initial effect of ACh manifested itself also with an inhibition of the hyperpolarizing phase. The neurone was fast desensitized to ACh and it soon neutralized the ACh effect. This also shows that ACh is changing the permeability of the soma membrane being under the control of intraneuronal metabolic processes.

After serotonin application, the characteristic bursting pattern did not change at 22°C, and a comparatively fast effect manifesting itself in an increase in activity was observed only above 30%. However, in 30—60 sec synaptic potentials appeared at all the temperatures tested. At 22°C, the increased activity evoked by synaptic potentials (EPSP-s) formed groups of 2—4 spikes and they appeared at the beginning of the hyperpolarizing phase, while at 7°C 5-HT simply increased the frequency of activity. Both the delayed effect and the appearance of synaptic potentials and also the fact that the characteristic basic rhythm remained intact suggest that 5-HT at the concentrations applied does not influence the soma membrane of the Br-type neurone but influences cell activity synaptically, through other neurones. Nevertheless, the conclusion seems to be acceptable that the activity pattern of the Br-type cell can be influenced synaptically only to a slight degree.

The inhibitory effect of NA began with a hyperpolarization of the membrane, without IPSP-s, similarly to that described by BOISSON and CHALAZONITIS (1972) for the Br-cell of *Aplysia*. This suggests a direct soma effect, and probably NA blocks the mechanism responsible for the depolarizing phase of the slow oscillation. The elimination of spike activity is the result of hyperpolarization.

The initial effect of DA is identical with that of NA, and also the site of action seems to be the soma membrane. In a few minutes after application, however, DA evokes an increase of activity caused partly by a decrease of the membrane potential and partly by the appearance of EPSP-s. This secondary, stimulatory effect of DA may be the result of the synaptic effect of other neurones excited by DA. Considering the delayed depolarizing effect, DA might influence also the synaptic regions of the Br-type cell when causing the increased activity. This would mean that there are dopaminergic synapses on the Br-type neurone.

Our results thus show that ACh, NA and DA are exerting their effect on the soma membrane, while 5-HT influences the activity of the RPa1 neurone

only synaptically. In addition to 5-HT, DA also evokes EPSP-s, while ACh does not cause synaptic potentials on this cell. In the case of NA we did not record any effect beside hyperpolarization, although it cannot be excluded that together with its soma effect NA influences the synaptic membranes when causing strong inhibition. The unequivocal results obtained at various temperatures refer to the specificity of the drugs investigated, and shows that within the temperature range tested, the chemical sensitivity of the Br-type neuron of *Helix* is not temperature dependent.

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ORGANIZATION OF VISUAL CORTEX PRIMARY RESPONSE

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A detailed analysis of the visual primary response (PR) was performed by means of neurochemical analysis, local laminary and extracellular neuronal activity recordings. Unilateral effect of adrenergetics upon the PR positive phase only and its great sensitiveness to chloralose, atropine and eserine (judging by the speed of the maximum effect increase) was observed. Local PR analysis from different cortical layers has shown that the low level excitation was 1.6—2.9 msec late relative to the upper ones. PR components not reversing accross the cortex diameter were found under general chloralose anaesthesia. Inadequate impulse discharge correlation with the cortical layer PR was shown. The results obtained show the genesis of all the PR phases in the cortex upper layers and the chemical heterogenity of all the PR components. A supposition is made that the dipole component mechanism is essential within the limits of a single unit only and is not dominant in the visual PR form recorded from the cortical surface.

The origin of the primary cortical response (PR) phases presents one of the most intriguing and up-to-date problems in neurophysiological science. The majority of investigators proceeds from the conjecture that the surface PR consists of a negative and a positive phase, which are interrelated as cause and effect and a realization of the same afferent volley.

The hypothesis about the presence of physical dipoles in the cortex, which are responsible for the initial positive PR phase formation, has long been considered as the most trustworthy one and has been shared by many researchers (Adrian 1941; Eccles 1951; Roitbak 1955; Li et al. 1956; Bremer 1958; Chang 1959). However, some new experimental data gained in a number of laboratories (Durinyan and Polyakova 1967; Guselnikov and Supin 1968; Markel and Ádám 1969; Storozhuk 1970) are difficult to explain along the lines of the dipole relation theory.

It should be noted that the major PR studies have been carried out under deep barbiturate anaesthesia, whereas the application of other anaesthetics and the experiments on non-anaesthetized preparations have led to many contradictory facts. It is the type of anaesthetics which determines both the degree of the temporal dispersity of the afferent volleys and their spatial distribution in the cortical elements (Anokhin 1964, 1968).

Alongside with the classical positive-negative responses, the "—" responses are recorded under chloralose anaesthesia. The latter are topically

bound to certain cortical areas and differ from the "classical" PR by a number of properties. The responses of both types are supposed to be conducted to the cortex along independent afferent pathways (BATUEV and CHIVILEVA 1969). The initial negative deflection allows to suppose that there is an initial excitatory axon–dendrite volley (STERIADE 1964; GMIRYA-NOVI 1964; DEMBNOVETZKII 1967), which, in contrast to the dipole theory, implies the presence of contacts between the thalamic afferents and the surface cortical layer structures.

A peculiar group of responses, termed early negative reactions (ENR; KULLANDA 1964; BATUEV 1968) is revealed within the limits of the primary cortical projection by means of the same chloralose anaesthesia. Electrogenesis allows to distinguish pre- and postsynaptic ENRs and to connect their origin with the activation of the associative thalamo-cortical system which is facilitated by chloralose and has contacts in the surface cortical layers.

Correlation analysis, employed during the study of the positive and negative PR phase interrelation (BATUEV 1968), has revealed inversely proportional relations (the correlation coefficient with a negative sign) between negative and positive phases in the dynamics of the PR parameter changes to various intensities of adequate stimuli, but is has failed to reveal any directly proportional relations, as it should be the case according to the dipole theory of the PR phase origin.

As the PR is widely used in the investigations into the integrative and condition reflex elaborating mechanisms (ÁDÁM and KUKORELLI 1964), it seems justified to subject this electrocortical phenomenon to further detailed analysis. With this in mind we have studied the functional organization of the visual PRs by neurochemical analysis, focal layer-to-layer recording and extracellular recording of single neurone discharges (BATUEV and PIROGOV 1970a, b).

Methods and results

PR neurochemical analysis

The primary responses registered in the visual projection zone (field 17) under pentobarbital anaesthesia (60 mg/kg) are of the positive–negative wave type with a latency of 18—21 msec. The PR amplitude is relatively stable for 30 min after the dura mater has been opened and therefore the investigations of the drug effects have been carried out within this time limit. A true change in the PR phase amplitude (at the significance level of 0.01) has served as a criterion of the influence of the drug.

As seen in Fig. 1, the maximum effect was observed in 5 minutes after the administration of 25% sodium chloride solution, i.e. the positive phase

decreased by 69±3.0% and the negative phase by 68±0.0% of the initial value. The absence of changes in PR time parameters is typical. Although some authors hold the opinion that the effect of KCl is exerted mainly in the surface cortical layers (NEVERLEE and DE LAGET 1966; ROSENBLUTH and GARCIA RAMOS 1966), there are some data pointing to the vertical pattern

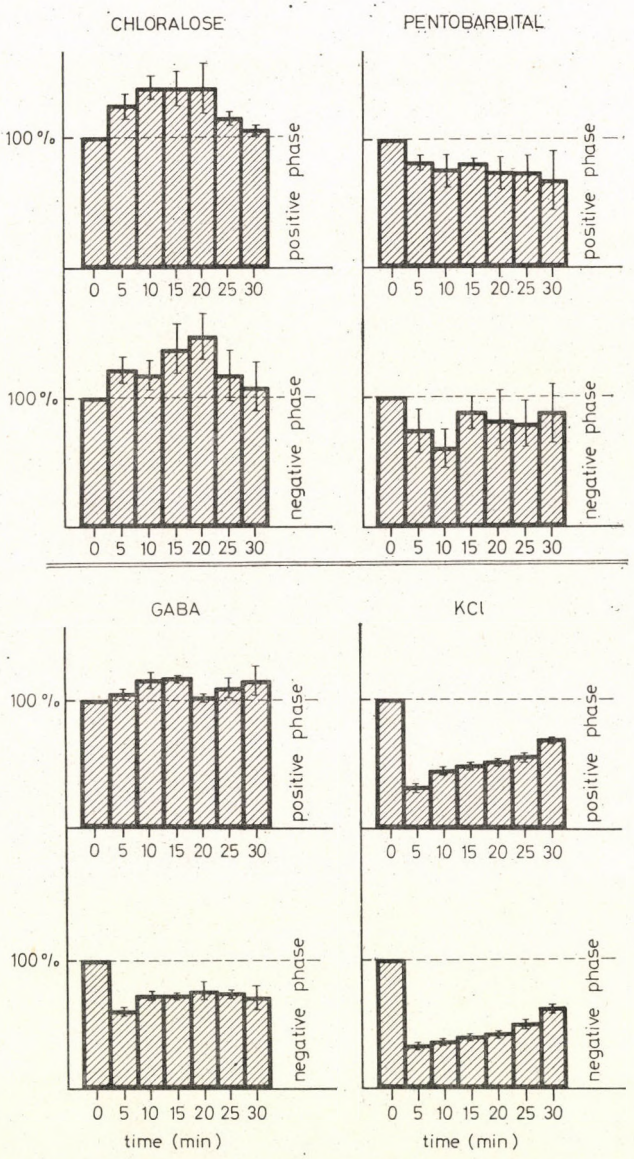

Fig. 1. Amplitude of PR phases on the application of drugs to the recording point. Ordinate: PR amplitude change per cent (normal = 100%). Abscissa: time in min. Every diagram presents the results of 8 experiments

of the slow negative potential distribution (MONAKOV et al. 1962). These observations testify to the cortical nature of both PR phases.

The application of 2% pentobarbital always resulted in a single sign effect and caused a PR phase reduction (Fig. 1). The positive phase decreased to 69±20% of its initial value in 30 minutes. The maximum decrease of the negative phase (to 69±20%) was observed at the 10th minute. Reliable latency changes have not been observed before the onset of the PR phases and before their maximum values.

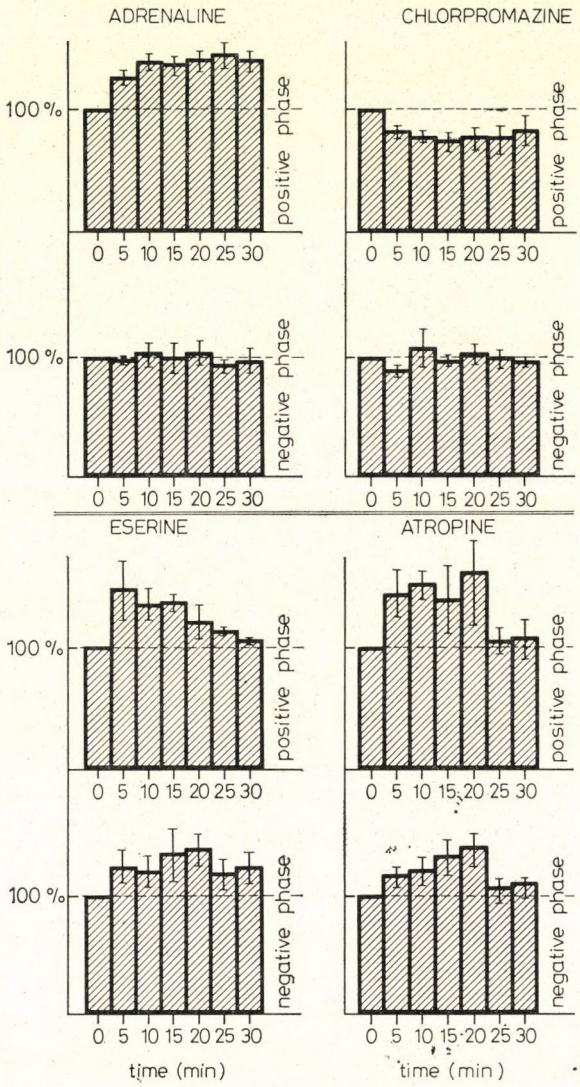

Fig. 1. (continuation)

The application of 2% chloralose solution brought about a different effect. The amplitudes of both the negative and positive components showed a mean increase of 37% and 49% correspondingly during the whole experimental series (Fig. 1). The maximum effect was observed at the 15th—20th minute of the drug influence. The amplitude correlation approached the initial value by the 30th minute. These facts bear testimony to the presence of some cortical elements which are activated by chloralose and are depressed by pentobarbital. It seemes likely that they participate in the genesis of both PR phases.

There was no single sign effect during the experiments with the application of 0.5% strychnine. An increase in both PR phases was observed during the majority of experiments. However, in some cases only the negative PR phase showed a progressive increase, whereas the positive PR phase decreased. Both effects might be considered the result of an intensification of the cell discharge. Alongside with this, strychnine facilitates EPSP while it reduces IPSP (ECCLES 1957; POLLEN and LUX 1966; STOROZHUK and PERFILIEVA 1967). The property of strychnine selectively to block the inhibitory synapses, i.e. to eliminate postsynaptic inhibition and simultaneously to reveal a weak background afferent impulsation in the form of convulsive discharges, has lead to the supposition that the elements of PR generation are different in their sensitivity to strychnine (TCHUPPINA 1966). Some of the above data may point to a predominance of hyperpolarizing PSP mechanisms in the positive PR phase and of depolarizing PSP mechanisms in the negative PR phase. However, this concept has not been fully confirmed as the strychnine effects are of a great variety.

Gamma aminobutryic acid (GABA) is considered an inhibitor of axon–dendrite excitatory synapses (PURPURA 1963), the inhibitory hypopolarizing system effect being stressed. Administration of a 10% GABA solution into the visual projection area revealed a PR phase change specific of this agent (Fig. 1). The negative phase amplitude showed a maximum decrease ($38\pm3.2\%$) at the 5th minute after the application. A reliable positive phase increase (by $17\pm7\%$) has been observed only at the 10th minute. Moreover, the changes affected also the negative phase latency period which increased by 20—25 msec, and consequently its peak time showed an increase of 30—40 msec. This is connected with an increase in the duration of the PR positive phase which directly passes over to the secondary negative element (ATA-MURADOVA 1969).

Thus the experimental results have supported ANOKHIN's theory (1968) of the heterochemical organization of the PR genesis systems. However, the correlation of the above drug effects makes us to favour the concept of the surface dendrite genesis of both PR components. The following observations may serve to support this opinion.

The problem of the presence of cortical synapses in the adrenergic or cholinergic mediator systems stays open for further detailed investigation. The participation of adrenergic contacts in the PR organization remains especially vague. A distinct PR reaction to the administration of 0.1% adrenaline solution was observed in our experiments (Fig. 1). It should be noted that the amplitude changes took place only during the positive phase which increased by $45 \pm 10\%$ while the negative phase amplitude remained practically unchanged. Application of adrenolytic (5% chlorpromazine solution) caused a reversed reaction (Fig. 1); in this case the positive phase amplitude decreased by $21 \pm 8\%$ and in some trials by 60%.

There is evidence in the literature indicating that a direct adrenaline influence on the central nervous system produces an inhibitory effect, whereas it has an excitatory effect when injected into the general blood stream. Such a high positive PR phase reactivity to adrenaline and chlorpromazine allows to suppose the surface location of the corresponding neuronal complexes. The positive phase increase under adrenaline influence might be regarded as a consequence of the change in the value and the correlation of EPSP and IPSP, generated in the given neuronal population by an afferent volley. It might be assumed that the hyperpolarizing synapses are activated by adrenaline influence and blocked by chlorpromazine which leads to the positive PR phase reduction.

Eserine, which blocks cholinesterase, thus preventing fast acetylcholine destruction, has been chosen for the investigation of the cholinoreceptive systems. Administration of 2% eserine resulted in an increase in both the positive and the negative phases; the former showed a mean increase of 46%, the latter one of 38% (Fig. 1). A PR amplitude rise was always recorded when a cholinolytic (2% atropine solution) was applied to the cortex. This is well in accordance with the findings of other authors (CHATFIELD and PURPURA 1954; KURPYAKOVA and SUPIN 1968; ABULADZE et al. 1970; and others). The positive and negative phases increased by $62 \pm 38\%$ and $37 \pm 9\%$, respectively (Fig. 1). This effect might be explained along the lines of the hypothesis which maintains that the block of cholinoreceptive synapses in the interneurone (the reverse inhibition block) appears under the influence of adrenaline which leads to an increase in the general synaptic activation in response to an afferent volley (CHATFIELD and PURPURA 1954; DUDAR and SZERB 1969). It might be assumed that the cholinoreactive systems have a wider representation in the cortex than the adrenoreactive ones, and are reponsible for the generation of both PR phases. The PR phase changes caused by chloralose, atropine and eserine are presented graphically in Fig. 2. A common feature of the curves is the development of the positive phase maximum effect within 5 to 15 minutes after the drug administration. The intervals for the negative phase are between 13 and 22 minutes. Thus, the positive phase is more sensitive to

these drugs and it is recorded, probably as well as the negative one, in the surface cortical layers which possess the highest chemical reactivity. It thus seems justified to suppose that both PR components represent physiological processes different in sign.

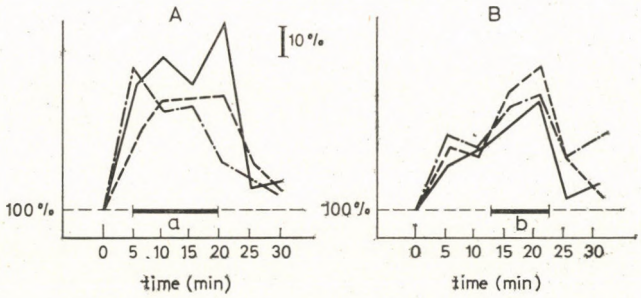

Fig. 2. Temporal regulation of PR positive and negative shifts on the application of different drugs to the cortex. A: dynamics of a positive phase; B: dynamics of a negative phase. Abscissa: time in min. Ordinate: amplitude changes in per cents; a, b: mean duration of maximum effect. Continuous line: atropine; broken line: chloralose; broken dotted line: eserine

PR laminar analysis

The above assumption is in accordance with the data gained in our experiments with PR recording from various cortical layers. The study was performed, in general, pentobarbital (75 mg/kg) and pure chloralose (75 mg/kg) anaesthesia.

As mentioned above, the PR latency before the onset of the first positive phase varies from 18 to 21 msec in trials under pentobarbital anaesthesia and from 24 to 26.4 msec before the positive phase reaches its maximum. As the electrode gradually penetrates deeper, the positive phase amplitude decreases, although its latency remains relatively stable. The so-called inversion of phases occurred at the level of 1300—1500 μ from the visual cortex surface, i.e. at this level the PR began with a negative deflection which was followed by a positive phase. As a rule, the electrode recorded a negative–positive complex at that position. There was either practically no PR or else it assumed the form of a monophasic slow deviation at 2000 μ. The dynamics and the parameters of the response phases corresponded to those described above when the electrode was pulled up from the deep cortical layers to the surface ones (Fig. 3).

The "inversion" of the PR phases was subjected to a detailed investigation. The same microelectrode was employed for the repetitive PR registration on the screen of a high speed beam oscillograph. The PRs were recorded from the surface and from that level at which the PRs had a contorted configuration, and the highest amplitude of the first negative phase. The calculations

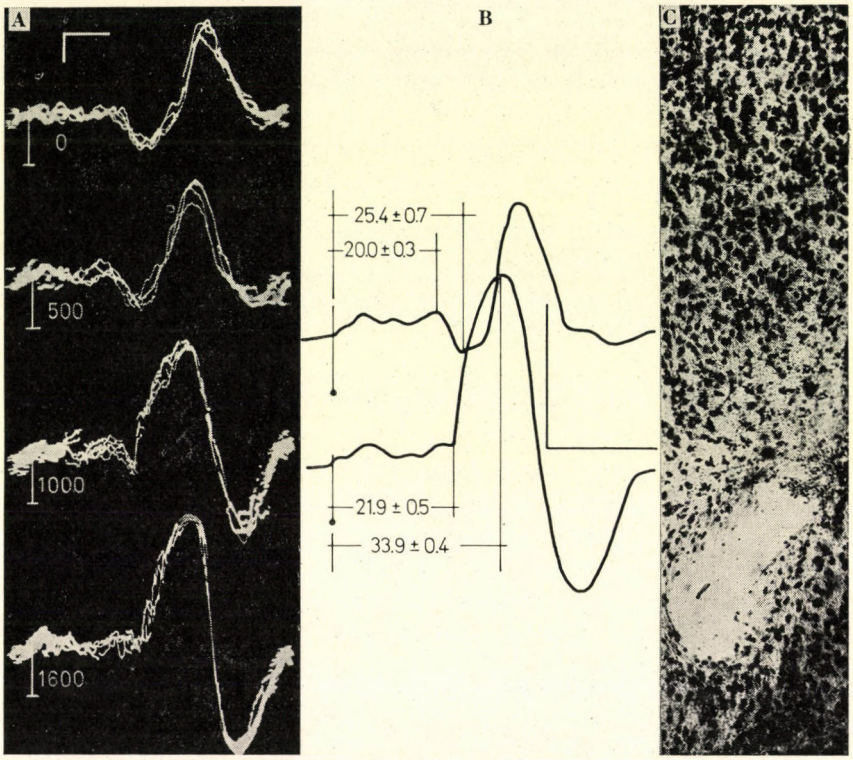

Fig. 3. PR focal recording of cat visual cortex under pentobarbital anaesthesia. A: PR at different levels of electrode penetration. Calibration: 10 msec, 10 μV. B: top — PR from cortical surface; bottom — at depth of 1600 μ. Figures: latencies before initial PR phase onset and before its maximum. The values for permissible intervals are given. Calibration: 20 msec, 50 μV. C: position of depth electrode in the cortical 5th layer

showed that the latency of the first depth negativity ranged from 18 to 24 msec but it reliably differed from that of the surface positivity in each individual trial. This difference, corresponding to 1.6—2.9 msec, was quite firm. It was calculated at the significance level of 0.01 and $\pi \gg 20$. The values for the Student–Fisher criterion exceeded the theoretical value by many times during the checking of the difference reliability of the surface positivity latencies and those of the depth negativity (Fig. 3).

The difference in latencies was calculated up to the maximum values of both phases in question. For example, the latency of the surface positive phase amounted to a mean of 25.6 msec and that of the depth negative phase to 28.1 msec during one of our trials. The difference of 2.5 msec turned out to be statistically real. This value was still higher and sometimes approached 10 msec during other trials.

Pure chloralose anaesthesia considerably widened the PR recording zone and caused the appearance of the late evoked response components.

PR latency ranged from 15.3 to 20.7 msec in field 17. Analysis of the PR layer-to-layer recording revealed some regularities of the phase "inversion" which differed from those observed under pentobarbital anaesthesia. The initial positive phase decreased when the electrode had penetrated deeper and it disappeared at 1500 μ (Fig. 4). In this case the first negative phase was recorded 1.5—3.4 msec later as compared to the surface positive one. This difference in latencies was statistically real as it exceeded, as a rule, its own error by more than three times at the chosen level of significance and value (Fig. 4). The initial negative phase amplitude was increasing toward 2000 μ and amounted to 540 mV during some trials but it was reduced on introducing the electrode lower.

It should be noted that no inversion was observed in the later components of the response. Thus the negative phase with a maximum latency of 73.8 msec at the cortical surface was still recorded at 2000 μ with a slight amplitude decrease (by 1.4 times) and an increase in its development time up to 93.0

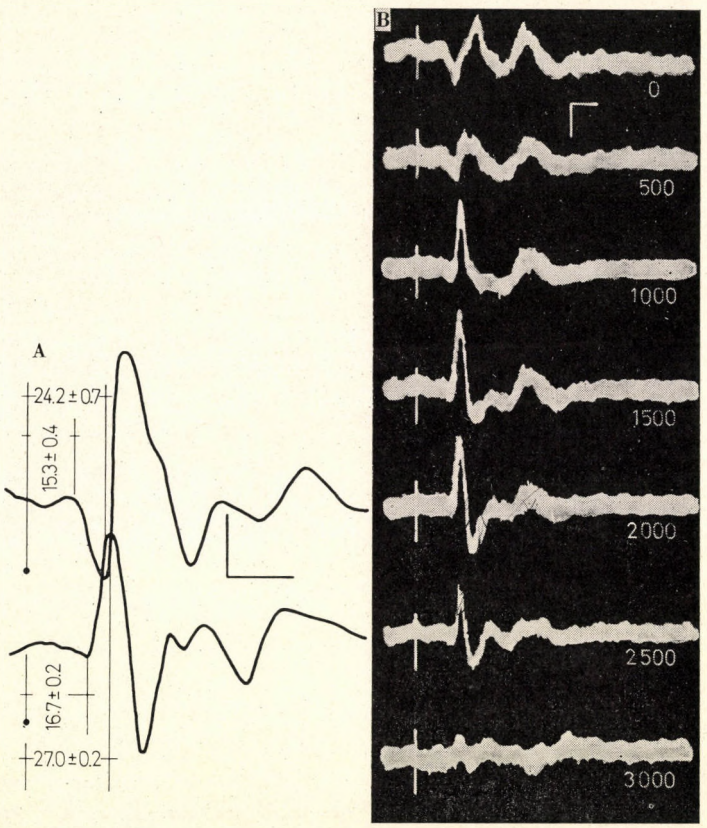

Fig. 4. PR focal recording on cat visual cortex under chloralose anaesthesia. A: top — surface recording; bottom — at depth of 1500 μ. Calibration: 20 msec, 50 μV. B: PR at different levels of electrode penetration

msec. The late wave complex, which was comparatively stable down to 2000 μ, was followed by a slow negative deflection below that level, i.e. in the white matter.

A number of indispensable conditions should be observed to prove the concept of the exceptionally dipolar origin of the positive PR phase. First, the surface positivity must be a mirror reflection of the depth negativity and their latencies must practically coincide. Second, the phenomenon of the PR phase inversion must have a universal nature during laminar recording, being determined by the neuronal geometry. Third, there must be no contacts between the thalamic afferents and the surface layer neurones.

The observations described above have shown that the depth negativity appears 1.6—2.5 msec later than does the surface positivity and there has been no mirror coincidence of them in any of the trials. KULLANDA (1968) and other authors (POLYANSKII 1965; HOLUBAR 1964; SUPIN 1969; SERKOV 1970) have described the same phenomenon in the somato-sensory PRs.

Bearing in mind the importance of the potential parameter characterizing the time necessary for the development of the maximum phase amplitude, the observations revealed a considerable difference in such values for the depth negativity and the surface positivity (2.4—10 msec).

The latency difference before the onset and before the peak of each phase ("phase time constant") is an indicator of the realization speed of the intracortical local potentials, the degree of synchronization and the rate of summation. It has been found that the frontal parts of the first surface and depth phase recordings have failed to coincide. Consequently, no pure inversion occurs, i.e. there is no passive inversion of the potential sign and, therefore, the above facts cannot satisfactorily be explained along the lines of the PR dipole theory.

Analysis of the PR dynamics under chloralose anaesthesia revealed still longer depth negativity delays (up to 3 msec), PR late components showing an inversion. It seems likely that chloralose creates other conditions than pentobarbital does for the realization of a disperse afferent volley across the cortex, and reveals a finer differentiation in the specific cortical projection areas.

There are facts proving the penetration of afferents into the 1st upper cortical layer at the early stages of ontogenesis (ATA-MURADOVA and CHERNISHEVSKAYA 1966). Their participation in PR component formation in adult animals is also known (ANOKHIN 1968). According to recent morphological studies, it is the third layer, located at the depth of 300 to 800 μ in the cat (VLADIMIROVA et al. 1968) that serves as a substrate, with which the fibres from the thalamic nuclei contact, and then the impulsation embraces the mass of the pyramidal neurones in the deep layers through a number of synaptic connections. SZENTÁGOTHAI (1967) does not exclude the possibility of

multiple contacts of one thalamic afferent with various pyramidal cell parts, and other authors (SCHEIBEL and SCHEIBEL 1966; GLOBUS and SCHEIBEL 1967; CREUTZFIELDT 1968) confirm the presence of synaptic connections with thalamopetal fibres in the 2nd cortical layer of the visual cortex. Therefore, the assumption that no thalamic afferent fibres have endings higher than in the 4th layer, cannot be considered fully acceptable in the light of these new findings.

Bearing in mind the independence of local and spreading excitations (GOLIKOV 1970), we have analyzed the correlation between the surface PRs and the individual neuronal discharge activity across the cortex under chloralose anaesthesia.

Analysis of PR extracellular recording

Background active neurones were observed under doses of 30—40 mg/kg of chloralose. As a rule, under large doses (60—70 mg/kg) the neurones responded with a spike only to a flash. As seen in Fig. 5, such neurones are found in deep layers, the number of active cells predominating at 1400—1500 μ. We have failed to locate any neurones responding to flash in the superficial visual cortical layers under our experimental conditions.

The post-stimulus histogram (Fig. 5) showed that the majority of neurones discharge during the occurrence of the surface positive PR phase, more precisely during its passing over to the negative phase. A negligible number of neurones is activated before the onset of the PR and during the second slow

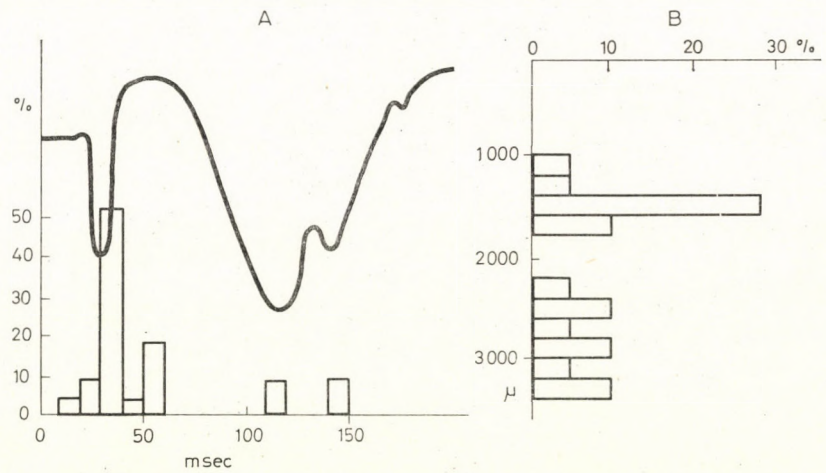

Fig. 5. A: correlation of neuronal activity post-stimulus histogram with the PR phases in the visual cortex (reconstruction). Abscissa: latency in msec. Ordinate: number of neurones in per cents. B: active neurone distribution in the cerebral cortex. Abscissa: number in per cents. Ordinate: depth in μ

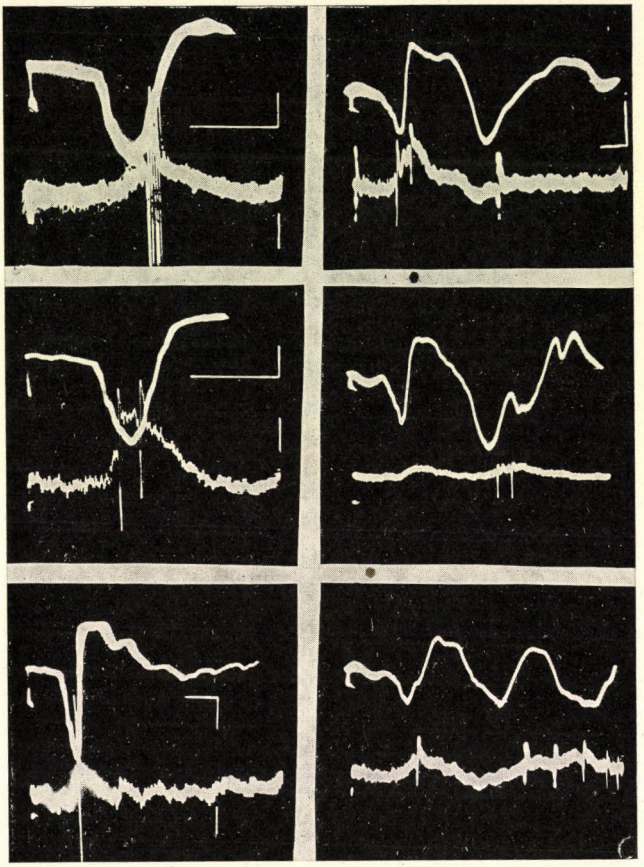

Fig. 6. Electrograms of the primary response and extracellular neuronal activity in response to a flash. Chloralose. Calibration against upper beam: 50 μV, 20 msec; against lower beam: 250 μV

positive phase. The prevalence of the neurones responding during the first positive phase was also confirmed by the high level of the depth focal potential, at the peak of which the spike discharges were usually recorded (Figs 6 and 7). This might be regarded as an evidence of the electrode's penetration into the zone with the highest density of active neurones. The focal potential corresponds to a considerably lower value or it is completely absent in cases when the spike latency coincides with the late PR phases (Fig. 6.) These data might point to a considerably even and diffuse distribution of such type of neurones along the cortex. Finally, there was singled out a group of cells whose discharges occurred both at the peak of the focal potential and with a longer latency, coinciding with the late PR phases but without a marked focal potential (Fig. 6). SERKOV (1970) obtained similar data for the auditory

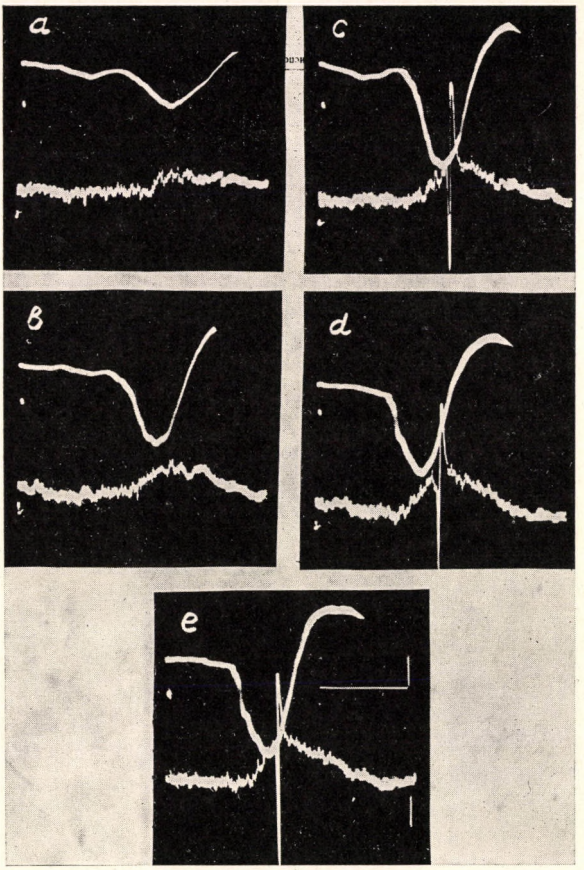

Fig. 7. Dependence on flash intensity of the total primary response and neuronal activity in depth of the cortex. From a up to e, flash intensity increases in conditioned device units

cortex neurones, and the significance of phase transition for spike activity modulation has been pointed out by DILL et al. (1968).

An analysis of the dynamics of the local surface PR and of the neuronal discharges under the conditions of a gradual stimulus intensification has shown that the threshold flash intensity evokes a more pronounced response at the cortical surface, whereas the deep focal potential has a considerably longer latency and a lower amplitude (Fig. 7). As the stimulus intensity increases, both local processes gain more power, their latencies become shorter and a high amplitude spike, whose latency progressively decreases, appears at the focal potential peak (Fig. 7). Even the maximum stimulus intensity brings about the focal potential with a steep frontal part considerably later than the surface positive PR phase and the spike appears when it reaches the highest degree of development.

Discussion

Under chloralose anaesthesia, local processes resulting from the first afferent volley arise first in the superficial cortical layers and then they occur in the depth at the cell soma and, probably, at the basal dendrites in case of an additional ascending volley (SHKOLNIK-YARROS 1963; SZENTÁGOTHAI 1969). The role of the surface local potentials, which probably appear at the apical dendrites (PURPURA 1963; ROITBAK 1965) may be limited to the electronic control of the somatic membrane excitatory level according to the physical dipole law (KULLANDA 1968).

If this assumption is true, the frontal part of the surface positive PR phase must be a reflection of the postsynaptic hyperpolarization processes in the apical dendrites, which may be of a thalamic genesis (CREUTZFIELDT and SAKMANN 1969) and cause an increase in cell soma excitability. The combination of the electronic influences, of the superficial dendrite cortical layers and the local soma dendrite processes in the depth leads to spike potential formation. Consequently, as it is really the case, the majority of active neuronal discharges must coincide with the onset of the positive PR phase. The late PR components seem to reflect a cortical neuronal integrative activity (KONDRATYEVA 1967) concerning the processing of the information contained in the heterogeneous and disperse afferent volley.

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EFFECT OF RICIN IMMUNIZATION ON THE QUALITATIVE COMPOSITION OF PLASMA PROTEINS

By

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The qualitative changes in plasma protein composition have been investigated in the course of immunization with ricin.

As a result of its hepatotoxic effect, ricin first caused a decrease in the albumin level, then with the progression of immunization the albumin level rose slowly, due probably to the gradual regeneration of the liver.

The gamma globulin fraction increased both in monkeys and in cats, and the increase corresponded to the appearance of a specific antibody, the "antiricin".

The beta fraction showed a significant decrease both in monkeys and in cats. This is assumed to represent a specific ricin effect closely associated with the disturbance in protein synthesis.

In the course of immunization, ricin loses its pyrogenic effect, in all probability as a result of an antigen–antibody interaction.

In ricin poisoning, the plasma protein level is known to diminish (THOMSON 1950). It has been shown earlier (BÁLINT et al. 1971; BÁLINT and HALÁSZ 1972; BÁLINT 1973) that the diminution was due to the hepatotoxic effect of ricin, and it is common knowledge that the bulk of the plasma proteins is synthetized in the liver (EVANS and EVANS 1968, etc.).

We have failed in finding data concerning the qualitative composition of plasma proteins beyond a reference claiming that ricin as an antigen gives rise to the production of antiricin, an antibody whose presence among the plasma proteins can be demonstrated (EHRLICH 1891).

In the present paper we shall discuss studies of the qualitative changes in plasma proteins during immunization with ricin.

Methods

African vervet monkeys *(Cercopithecus aethiops)* and cats were immunized with increasing doses of ricin and the qualitative and quantitative changes in plasma proteins were observed.

A total of 15 monkeys and 15 cats were treated as follows.

a) The control group consisted of 5 monkeys (3 males, 2 females) of 2800 to 4000 g body weight each and of 5 cats (2 males, 3 females) of 3000 to 3850 g body weight. These animals were treated subcutaneously with 0.8 ml of sterile, pyrogen-free physiological saline.

b) The monkeys (6 males, 4 females) of 2850 to 3900 g body weight each, and 10 cats (5 males, 5 females) of 2900 to 4000 g body weight each, were treated with ricin.

Ricin was administered in increasing doses dissolved in sterile pyrogen-free saline every 8th day as follows.

a) Monkeys; 20, 40, 80, 150, 300 and 1000 μg per kg of body weight, subcutaneously.

b) Cats; 15, 30,50, 80, 150, 300 and 1000 μg per kg body weight, intraperitoneally. (The DL_{50} of the applied ricin preparation was, depending on the species, between 35 and 40 μg per kg body weight).

Before each dose of ricin and eight days and one month after the last dose the quantitative and qualitative composition of the plasma proteins were determined electrophoretically.

Electrophoresis was performed on Oxoid-type cellulose acetate strips, in 5'-5-diethylbarbiturate buffer of 0.05 ionic strength at pH 8.6, 10 mA, for 2 hours. The strips were developed with Ponceau-S, and evaluated by means of the automatic extinction evaluator (Extinction of Schreiber type II of ZEISS, West Germany).

Body temperature of the animals was measured with a thermocouple introduced rectally at a depth of about 8 cm every day during the entire period of the experiment, at an ambient temperature of 26.0 $\pm$ 3.0°C.

Results

Results were evaluated statistically by means of Student's *t*-test; they are shown in Table I and in Figs 1 to 5.

The following conclusions have been drawn.

A) Monkey experiments

1. In full agreement with our earlier results (EPERJESSY et al. 1965; BÁLINT et al. 1966) it was found that ricin has a pyrogenic effect also in the African vervet monkey (Fig. 1). As regards the nature and duration of the pyretic reaction, no essential difference was observed from those found in the cat, rabbit, guinea-pig and rat.

Table I

Effect of ricin on plasma proteins during

Ricin dose	Albumin %		α_1 Globulin %		α_2 Globulin %	
i.p.	cats	monkeys	cats	monkeys	cats	monkeys
Control	34±2.00	28±2.00	1±0.00	1±0.00	15±3.00	7±1.00
15 μg/kg	32±2.00	—	3±0.50	—	21±2.50	—
20 μg/kg	—	32±4.00	—	2±0.25	—	8±0.75
30 μg/kg	37±1.50	—	2±0.50	—	10±0.25	—
40 μg/kg	—	33±2.75	—	1±0.00	—	8.5±1.00
50 μg/kg	36.5±1.75	—	2±0.50	—	20±3.00	—
80 μg/kg	39±4.00	27.5±5.00	2±0.25	1±0.00	18±1.25	9±1.00
150 μg/kg	39±1.50	28±4.25	2±0.25	3±1.00	16.5±1.75	11±1.25
300 μg/kg	45±0.50	29±2.50	2±0.00	2±0.00	15±0.50	10±1.00
1000 μg/kg	40±7.75	30±3.50	2±0.00	1±0.00	17±2.00	12±0.75
1 month later	43±3.00	32±2.50	3±0.25	2±0.50	15±0.75	14±1.25

2. In the course of immunization with ricin, in the qualitative composition of plasma proteins the changes illustrated in Fig. 2 were observed. These were as follows.

After a slight initial rise in the albumin level this decreased moderately and then returned to normal. None of these changes were significant statistically.

The initial slight rise in alpha globulin fraction was not significant statistically, in contrast to the slow rise of the alpha-2-globulin fraction which at the end of immunization and one month after the last dose showed a value significantly higher than the initial one ($p < 0.05$).

The beta globulin fraction after an initial decrease rose significantly but this rise was non-significant statistically ($p < 0.10$). The decrease following this rise which persisted till the end of the experiment was significant at two points of tissue, viz. after the 150 μg/kg dose $p = 0.05$) and one month after the last one ($p < 0.05$).

The gamma globulin level first decreased then rose steeply, but the changes were not significant statistically.

3. The quantitative changes observed in total protein and in the albumin globulin ratio were not significant either (Figs 4 and 5).

4. The control animals showed no deviations from the initial values.

B) Cat experiments

1. After a slight non-significant initial decrease the albumin level began to increase slowly and by the end of the experiment the increase had become significant statistically ($p < 0.05$).

immunization (cats and monkeys)

β Globulin %		γ Globulin %		Total protein g%		A/G Ratio (mean v.)	
cats	monkeys	cats	monkeys	cats	monkeys	cats	monkeys
33±8.00	31±2.00	17±3.00	33±1.50	4.23±1.07	6.04±0.23	1 : 1.95	1 : 2.54
28±9.00	—	18±5.00	—	2.91±0.45	—	1 : 2.09	—
—	28±5.00	—	30±1.00	—	7.06±0.59	—	1 : 2.17
25±3.00	—	25±1.50	—	7.28±0.62	—	1 : 1.70	—
—	29±6.25	—	28±4.75	—	6.69±0.97	—	1 : 2.17
16±2.75	—	25±4.00	—	8.06±0.39	—	1 : 1.70	—
14.5±1.25	39±5.00	27±4.25	24±3.00	8.10±0.73	6.42±1.06	1 : 1.56	1 : 2.13
16±0.25	21±5.50	26.5±2.75	37±3.25	7.82±0.9	6.84±1.34	1 : 1.56	1 : 2.22
13±1.00	28±4.00	25±0.50	31±2.75	8.00±1.25	8.24±1.37	1 : 1.23	1 : 2.35
19±8.25	26±4.00	22±1.25	31±3.00	7.18±1.24	6.05±1.52	1 : 1.50	1 : 2.33
18±3·00	22±3.25	21±·050	30±2.00	8.76±0.41	5.25±1.16	1 : 1.35	1 : 2.17

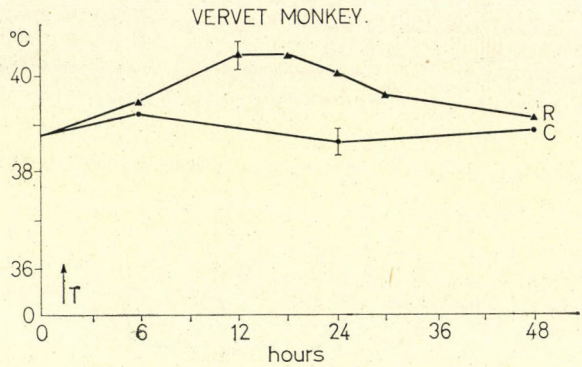

Fig. 1. Typical monkey fever curve: average results for 5 control and 5 treated animals. T: 20 μg per kg body weight of ricin subcutaneously; C: control; R: fever curve of ricin-treated animals

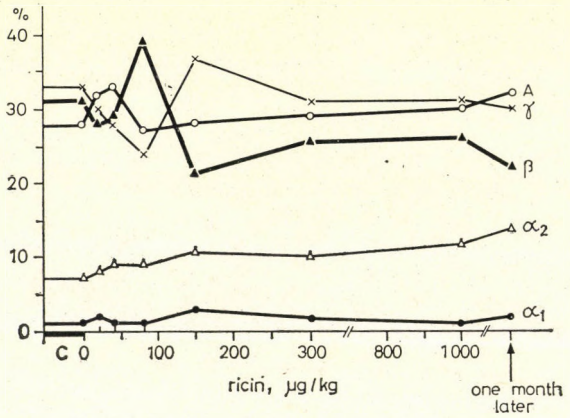

Fig. 2. Effect of ricin on plasma protein composition of monkeys (mean values). C: control values prior to immunization

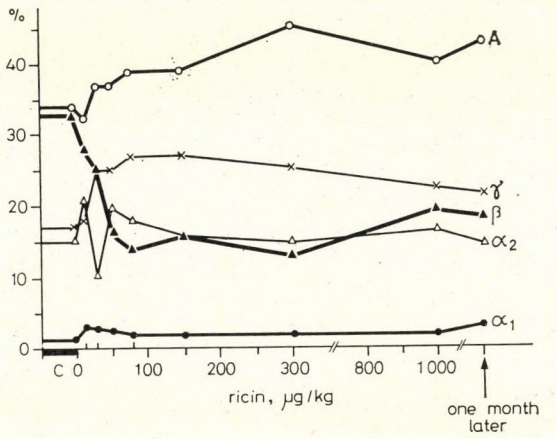

Fig. 3. Effect of ricin on plasma protein composition of cats (mean values). C: control value prior to immunization

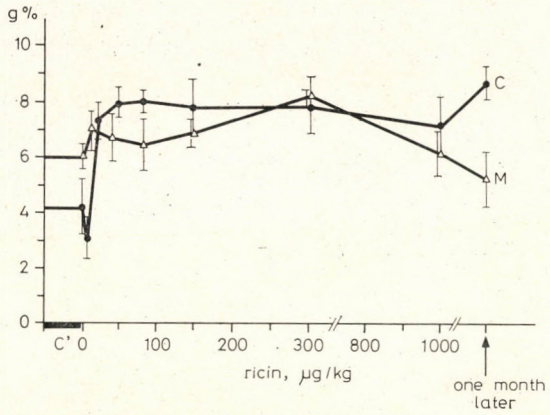

Fig. 4. Total plasma proteins during immunization with ricin (mean ±S.E.). C': control values prior to immunization; M: monkeys; C: cats

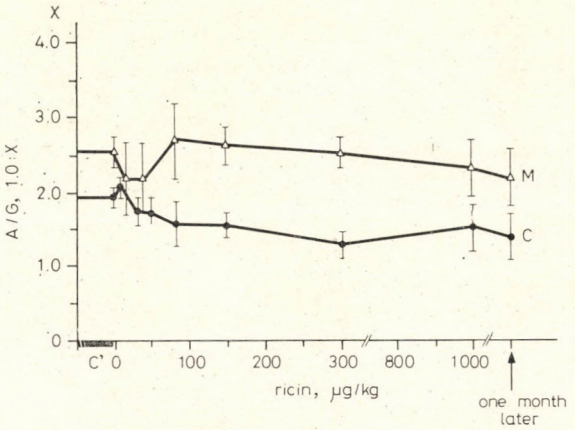

Fig. 5. Plasma albumin; globulin ratio during immunization with ricin. C': control values prior to immunization; M: monkeys; C: cats

As compared to the control value, the alpha-1-globulin fraction was significantly increased during the entire period of the experiment ($p = 0.05$ and $p < 0.05$).

The changes in the alpha-2-globulin fraction were not significant statistically ($p > 0.10$).

The beta globulin fraction decreased significantly during the whole period of immunization ($p = 0.05$ and $p < 0.02$).

In the gamma globulin fraction there was an immediate increase and after the 300 μg/kg dose the increase was significant ($p = 0.05$). Later the fraction decreased, but persisted at higher than the control level.

2. The quantity of total plasma proteins first decreased significantly and then underwent a steep rise; the difference versus the controls was significant ($p < 0.05$, see Fig. 4).

3. Changes in the albumin globulin ratio were not significant (Fig. 5).

4. The control animals showed no change whatever.

5. Ricin had a pyrogenic effect in these experiments, too. With the progress of immunization, the pyrogenic effect gradually diminished. The decrease was observed in the monkeys too, but was particularly marked in the cats (Fig. 6).

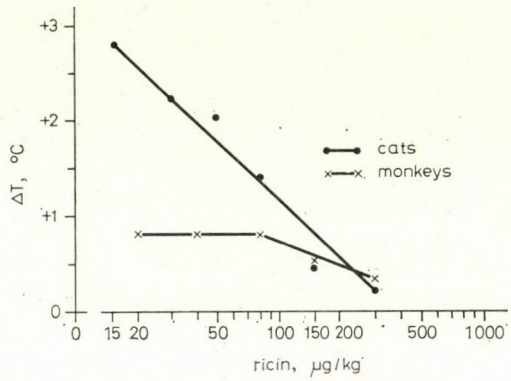

Fig. 6. Weakening of pyrogenic effect of ricin during immunization with ricin

After the first dose of ricin, the cats' body temperature rose by about 3 °C, while this increase was less than 1 °C in monkeys. After the fifth dose (300 μg per kg body weight) the pyrogenic effect practically disappeared.

Discussion

1. These experiments proved the pyrogenic effect of ricin on a further species, the monkey. This suggests the occurrence of a pyretic reaction in human ricin (castor oil seed) poisoning.

2. The slight initial decrease of the albumin level in cats might have been due to the hepatotoxic effect of ricin (BÁLINT 1973), since it is well-known that the liver alone is responsible for the production of albumin (MILLER and BALE 1954; EVANS and EVANS 1968, etc.) No explanation can be suggested for the fact that this initial decrease of the albumin level has not been observed in the monkeys. After regeneration of the liver (BÁLINT et al. 1971; BÁLINT 1974) albumin production is restored, a fact early demonstrated in the cat experiments.

3. The increase in the alpha-1-globulin fraction is not a specific reaction since it occurs in every pyretic reaction (BÁLINT 1968, etc.)

4. There was a close parallelity in the quantity of alpha-2-globulin and that of albumin in both monkeys and cats. Since the bulk of alpha- (and beta-) globulins is produced by the liver, the change might have been connected with the hepatotoxic effect of ricin. (In cats a decrease was followed by a slow increase; in monkeys a slow increase occurred, similar to the change in the albumin level.)

5. It is difficult to find an explanation for the changes observed in the beta globulin fraction. This decreased significantly in both species, so that in this case a specific ricin effect appears to be probable.

It has been shown (EVANS and EVANS 1968) that the major parts of the (alpha- and) beta globulins are produced in the liver, but it is also known (Miller et al. 1954) that these fractions, mainly the beta globulin, are formed also extrahepatically, for instance in the lymphoid tissues (KENNING and VAN DER SLIKKE 1950).

DIRHEIMER et al. (1966) have already pointed at the possibility of an interference by ricin with the protein synthesis in the liver. This was supported by our experiments (BÁLINT 1973), and unequivocally proven by JUNG-YAW LIN and his co-workers (JUNG-YAW LIN et al. 1971; JUNG-YAW LIN and TA-CHENG TUNG 1972).

The highly tocix effect of ricin on the lymphoid tissues has also been mentioned in the literature (HARANGHY 1934, 1966, etc.).

From the results of the above experiments, the following conclusions have been drawn.

In ricin poisoning both the hepatic and the extrahepatic sites of beta globulin production are damaged. It seems that in the liver ricin interferes mainly with the production of beta globulins. This impaired hepatic synthesis is not corrected by the extrahepatic production of beta globulin, since these production sites also become affected. For clarifying the details, further tests are needed.

6. The rise in gamma globulin level is related to the antigenic property of ricin (EHRLICH 1891). Antiricin production corresponds to a general immune reaction and the quantity of the immune substance formed increases the gamma globulin fraction.

7. The changes observed by us as in the quantity of total plasma protein and the albumin globulin ratio agree well with data in the literature (THOMSON 1950, etc.).

8. The decrease in the pyrogenic effect of ricin during immunization with the same substance is probably the result of a common immune reaction. The antiricin synthetized under the effect of ricin behaves as a complete antibody (EHRLICH 1897), and consequently the pyrogenic effect decreases propor-

tionally to the quantity of synthetized antiricin. Further investigations are needed to determine the period required for the return of the pyrogenic effect after the end of immunization. One month after immunization this was not yet detectable.

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Pharmacologia

ANTIN OCICEPTIVE ACTIVITY OF NICOTINE

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The antinociceptive activity of nicotine has been demonstrated in mice by means of the hot-plate technique. Repeated injections of nicotine given at intervals of 60 min produced and increase in the response. Prolongation of reaction time seemed to be independent of the neurotoxic effect of nicotine, since against the latter tolerance ensued rapidly as assessed by the rotating rod method. Mecamylamine did not prolong the reaction time but inhibited the antinociceptive activity of nicotine and so did 50 μg/kg of physostigmine given simultaneously, and 20 mg/kg of tremorine administered previously, while the said activity was increased by the simultaneous administration of small amounts of oxotremorine. The antinociceptive activity of nicotine in mice could be shown also by the acetic acid method.

Nicotine was found to have an antinociceptive activity also in the rat. In this animal, nicotine and DMPP given intraventricularly exerted an antinociceptive effect which was antagonized by mecamylamine given intraperitoneally.

The antinociceptive activity is most likely due to the stimulating effect of nicotine.

Cholinomimetic agents with a peripheral muscarinergic (M) stimulating effect such as oxotremorine and arecoline have been shown to possess analgetic properties (HERZ and YACOUB 1964; LÉVY and MICHEL-BER, 1967; METYS et al. 1969; LESLIE 1969; HOWES et al. 1969). According to OELSSNER and ANDREAS (1969) nicotine in doses as high as 320 μg/mouse does not interfere with the pain reaction elicited by electrical stimulation of the animal's tail; these authors suggested that only the M-cholinergic stimulants had antinociceptive properties. MATTILA et al. (1968) then succeeded in demonstrating the analgetic potency of nicotine in the mouse, rabbit and hamster. They presumed that the analgesia induced by nicotine was due to a blocking effect on the reticular formation rather than to a stimulating property but neglected to discuss their observation of a decrease in the analgetic activity of nicotine after treatment for one week and whether tolerance developed to the blocking effect.

In a preliminary study we have also observed the antinociceptive activity of nicotine. In the present work attempts were made to assess the specificity of this effect, and to clarify whether it was not a result of neurotoxicity, or of a central blockade or stimulation.

Methods

The experiments were performed on mice weighing 17 to 20 g and rats weighing 160 to 200 g. The animals were randomized, and the pain reaction was studied by the hot-plate method (WOLFE and MCDONALD 1944: PÓRSZÁSZ and HERR 1950), at a temperature of 52 and 56 °C, for mice and rats, respectively. In this test the licking response served as the specific response.

Acetic acid pain stimulation was also applied (KOSTER et al. 1959). At zero time we gave subcutaneously 5, 10 or 15 mg/kg of nicotine or saline and in the fifth minute 1% acetic acid in a quantity of 0.1 ml/10 g mouse intraperitoneally and the number of writhes in the subsequent 20 min period was counted at five-minute intervals. Every dosage was given to a group of 12 mice. In the rats, the effect of nicotine given intraperitoneally and intraventricularly by the method of VERSTER et al. (1971), was examined using the tail-flick test.

The neurotoxic property of nicotine was studied in mice with the rotarod technique (OELSSNER 1961): the experiments were performed on trained mice and the control animals were clinging for 3 min to the rod rotating at 7 r.p.m.

The doses indicated refer to mecamylamine HCl, nicotine hydrogen tartrate, oxotremorine oxalate, physostigmine salicylate, tremorine HBr and DMPP (1,1-dimethyl-4-phenylpiperazine) iodide.

Statistical comparisons were made by analysis of variance (Anova) and Student's *t*-test.

Results

Effect in mice

In agreement with MATTILA et al. (1968), the antinociceptive activity of nicotine subsides rapidly in the mouse. Ten to 15 min after an intraperitoneal dose of 7.5 to 10 mg/kg a marked effect on the hot plate could be demonstrated while 30 min after single doses of 5, 10, 15 or 20 mg/kg there was no measurable response. Remarkably, 10 mg/kg killed none of the 120 mice, but caused tremor in 82% and clonic seizures on 36% of the animals. Twenty mg/kg killed 9%, 7 of a total of 77; and caused tremor in 94% and clonic seizures in 88% of the animals. Thirty mg/kg killed 30%, caused tremor in 100% and clonic seizures in 90% of the mice. Tremor and seizures lasted 5—6 min after treatment.

If nicotine is given repeatedly a tolerance ("autoprotection") develops against its lethal and convulsive effects (HAZARD and SAVINI 1953). This was not the case with its antinociceptive activity which, as estimated 10 min after administration, was not diminished by the second and third dose given at intervals of 60 min (Table I).

Following pretreatment with nicotine for 4 days, the antinociceptive activity of a single dose given on the 5th day was markedly reduced as compared with results obtained in mice pretreated with solvent (Table II).

The neurotoxic effect of nicotine could be readily demonstrated: after an intraperitoneal dose of 10 mg/kg, but sometimes only after 15 or 20 mg/kg, the mice failed to cling to the rotarod for more than 5 to 30 sec. We also examined whether this neurotoxic activity of nicotine played a part in the prolongation of reaction time on the hot plate. Sixteen mice were given each 10 mg/kg of nicotine intraperitoneally at 0, 60 and 120 min, and they were put on

Table I

Antinociceptive activity assessed on the hot plate of three intraperitoneally administered 10 mg/kg doses of nicotine. Reaction time, means ± S.E. The agent was given at 0, 60 and 120 min and its effect was estimated 10 min later. There was a significant difference between the underlined values ($p < 0.05$)

Initial reaction time	n	Reaction time after dose		
		I	II	III
10.0±0.74	20	*33.7±4.2*	*45.7±4.1*	38.4±4.5
10.5±0.75	20	—	40.1±4.2	39.8±4.7
9.4±0.45	20	—	—	42.7±4.0

Table II

Effect of subacute nicotine pretreatment on the antinociceptive activity of nicotine. Each mouse was given 7 times 20 mg/kg of nicotine intraperitoneally, over a period of 4 days. Reaction time and its prolongation in seconds, mean ± S.E.

Treatment	n	Initial reaction time, 5th day	Increase of reaction time 10 min after 20 mg/kg of nicotine	
NaCl	16	8.7±0.9	38.4±4.9	$p<0.001$
20 mg/kg of nicotine	21	7.7±0.7	12.6±3.5	

the rotarod in the 10th, 20th and 50th min after every dose. The number of mice falling from the rod progressively diminished with subsequent doses, i.e. 12 of 16 after the first dose, 5 of 16 after the second, and 1 of 16 after the third dose. Thus, tolerance had developed to the neurotoxic effect of nicotine but not to its antinociceptive activity, making a causal relationship between the two effects highly improbably.

Relying on the suggestion of MATTILA et al. (1968) that the analgetic effect of nicotine was due to central blocking activity, we tested whether mecamylamine, this central nicotinergic blocking agent, possessed an analgetic property. As shown in Table III, mecamylamine in doses of 2, 5 and 10 mg/kg proved ineffective in the 30th min; in another series it had no appreciable antinociceptive activity 15 min after administration, and it was also ineffective when given in doses of 2×5 mg/kg. On the other hand, 5 mg/kg of mecamylamine prevented the antinociceptive activity of nicotine (Table IV), which was not affected by 5 to 10 mg/kg of atropine sulphate, while scopolamine in the same dosage caused prolongation of the reaction time to an extent not allowing the antinicotinic activity to be assessed. Smaller doses of scopolamine had no effect and even this high dosage did not prevent the antinociceptive activity of nicotine. The compound 2-dimethylamino-9-benzilyloxy-

Table III

Influence of mecamylamine on the reaction time of mice as measured by the hot-plate method. Reaction time and its prolongation in seconds, mean ± S. E. Each group contained 15 mice

Initial reaction time, 0 min	Dose of intraperitoneal mecamylamine, 30th min	Increase of reaction time, 60th min
9.5±1.2	2 mg/kg	2.3±1.28
11.9±1.4	5 mg/kg	1.9±1.1
13.2±0.9	10 mg/kg	−2.6±1.1

Table IV

Influence of mecamylamine on the antinociceptive activity of 10 mg/kg of nicotine, as measured on the hot plate. Reaction time and its prolongation in seconds, mean ± S.E. Each group consisted of 20 mice

Treatment subcutaneously at 0 min	Reaction time at 30 min	Treatment intraperitoneally at 90 min	Increase in reaction time at 100 min
NaCl	8.1±0.53	10 mg/kg of nicotine	18.9±3.1
Mecamylamine, 5 mg/kg	9.3±0.62	10 mg/kg of nicotine	6.4±1.74 ($p<0.01$)

bicyclo(3,3,1)nonane (N-1199; GYÖRGY et al. 1970b; BARTHELEMY et al. 1972) which has a more specific central anti-M activity, also proved ineffective in preventing the antinociceptive activity of nicotine.

MATTILA et al. (1968) found that physostigmine did not influence the antinociceptive activity of nicotine. In our experiments (Table V), however, 20 μg/kg of physostigmine did not affect, while 50 μg/kg inhibited, that activity. When applying doses of 100 μg/kg, the reaction time was so prolonged that the interference with antinociceptive activity could not be studied. This effect of large doses of physostigmine was observed earlier by PLEUVRY and TOBIAS (1971).

It has been shown in a previous paper (GYÖRGY et al. 1970a) that the lethal dose of nicotine was considerably reduced in the state of tolerance induced by repeated doses of oxotremorine, and a similar tolerance could be achieved by a single dose of tremorine (GYÖRGY et al. 1971). It should be noted that 18 hours after tremorine treatment, oxotremorine failed to exhibit any antinociceptive activity. In the present experiments, the antinociceptive activity of nicotine was considerably diminished 18 hours after tremorine treatment (Table VI). While tremorine pretreatment inhibited the antinociceptive activity of nicotine, small doses of oxotremorine, a metabolite of tremorine with a short lasting effect, did not do so and there was an additive effect (Table VII).

Table V

Influence of physostigmine on the antinociceptive activity of nicotine, measured on the hot plate. Reaction time and its prolongation in seconds, mean ± S.E. Two series of experiments

Initial reaction time, 0 min	n	Treatment, 30th min	Increase of reaction time, 40th min	
10.5±0.68	24	20 μg/kg physostigmine subcutaneously + NaCl	—0.1±0.21	
11.7±0.95	24	NaCl + 10 mg/kg nicotine intraperitoneally	25.3±4.30	
				$p > 0.4$
11.5±1.04	23	20 μg/kg physostigmine + 10 mg/kg nicotine	29.8±4.13	
11.6±0.81	28	50 μg/kg physostigmine + NaCl	2.7±1.5	
12.7±0.93	27	NaCl + 10 mg/kg nicotine	36.3±3.41	
				$p < 0.02$
11.9±0.76	26	50 μg/kg physostigmine + 10 mg/kg nicotine	23.3±3.96	

Table VI

Influence of tremorine pretreatment on the antinociceptive activity of nicotine, as determined on the hot plate. Reaction time and its prolongation in seconds, mean ± S.E.

Pretreatment (18 hours) intraperitoneally	n	Reaction time prior to nicotine	Increase of rection time 15 min after 10 mg/kg nicotine intraperitoneally	
NaCl	55	13.3±0.59	29.1±2.4	
				$p<0.001$
20 mg/kg of tremorine	58	12.4±0.51	11.6±1.9	

Table VII

Influence of small subcutaneous doses of oxotremorine on the antinociceptive activity of nicotine as determined on the hot plate. Reaction time and its prolongation in seconds, mean ± S.E. Oxotremorine — nicotine interaction: $p > 0.1$ (Anova)

Initial reaction time, 0 min	n	Treatment, 30th min	Treatment intraperitoneally, 45th min	Increase of reaction time, 55th min
9.1±1.5	14	20 μg/kg OT	NaCl	1.5±1.3
8.5±1.0	14	50 μg/kg OT	NaCl	4.3±2.4
6.9±0.9	14	100 μg/kg OT	NaCl	18.1±6.5
5.3±0.33	10	NaCl	10 mg/kg nicotine	20.7±5.9
6.2±0.81	10	20 μg/kg OT	10 mg/kg nicotine	23.9±5.8
5.7±0.63	10	50 μg/kg OT	10 mg/kg nicotine	38.0±6.8
6.1±0.65	10	100 μg/kg OT	10 mg/kg nicotine	48.9±3.5

OT: oxotremorine

The antinociceptive activity of nicotine was then tested on the pain reaction induced by acetic acid in mice. Figure 1 shows the inhibitory effect of 5, 10 and 15 mg/kg nicotine given subcutaneously.

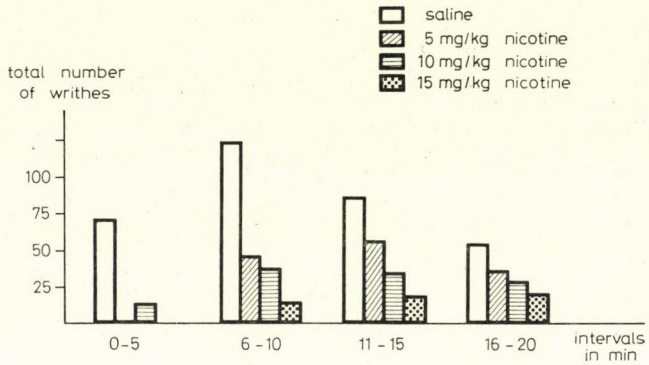

Fig. 1. Influence of nicotine on writhes induced by acetic acid in mice. Subcutaneous administration of saline or nicotine 5 min before intraperitoneal treatment with 1% acetic acid. Abscissa: intervals in minutes after acetic acid treatment; ordinate: total number of writhes in 12 mice in each group

Effect in rats

In several experiments we have succeeded in proving that nicotine has an antinociceptive activity on the hot plate also in the rat. In this species, doses as low as 5 mg/kg had a marked effect, and the response was prevented by mecamylamine similarly as in mice (Table VIII).

The antinociceptive activity of nicotine was examined also with the tail-flick test. There was a slight prolongation of the reaction time in 30 min after 5 and 7.5 mg/kg of nicotine given intraperitoneally.

150 μg per animal of nicotine given intraventricularly and tested in the 5th min exerted an antinociceptive activity; 2.5 mg/kg of mecamylamine given intraperitoneally and 30 min before nicotine, antagonized this effect. DMPP, a compound not passing across the blood–brain barrier, was given intraventricularly; 150 μg per animal had an antinociceptive activity in the 5th min and

Table VIII

Antinociceptive activity of nicotine in the rat, as assessed on the hot plate, and the antagonistic effect of mecamylamine. Reaction time and its prolongation in seconds, mean ± S.E. Each group consisted of 8 rats

Pretreatment subcutaneously, 0 min	Initial reaction time, 15th min	Treatment, 30th min	Increase of reaction time, 45th min
NaCl	6.8 ± 1.5	5 mg/kg nicotine intraperitoneally	24.9 ± 7.5
2 mg/kg mecamylamine	10.5 ± 3.4	5 mg/kg nicotine intraperitoneally	0.3 ± 2.9

the effect decreased till the 30th min. The antinociceptive activity of DMPP was antagonized by 2.5 mg/kg mecamylamine given intraperitoneally 30 min before DMPP (Table IX).

Table IX

Antinociceptive activity of nicotine and DMPP in the tail-flick test in rats and the effect of mecamylamine pretreatment Nicotine: 150 μg per animal given intraventricularly; DMPP: 150 μg per animal given intraventricularly; Mecamylamine: 2.5 mg/kg given intraperitoneally 30 min before administration of nicotine and DMPP. Reaction time and its prolongation in seconds, mean ± S.E.

Treatment	Number of animals	Reaction time				
		30 min before treatment	after mecamylamine	after treatment		
				5 min	10 min	30 min
Nicotine	8	2.2±0.1*		4.5±0.7*	2.7±0.7	2.0±0.7
Mecamylamine + nicotine	6	2.6±0.3	2.2±0.4	2.4±0.5	2.1±0.6	2.4±0.3
DMPP	10	2.4±0.04**		5.4±1.0**	3.9±0.5	3.5±0.4
Mecamylamine + DMPP	8	2.3±0.2	2.6±0.3	2.6±0.5	2.1±0.4	2.3±0.4

between * * $p < 0.01$
** ** $p < 0.005$
evaluated with one-sample *t*-test

Discussion

Our experiments confirmed the results of MATTILA et al. (1968) indicating that nicotine has an antinociceptive activity. The negative results of OELSSNER and ANDREAS (1969) are difficult to reconcile with these findings; most likely they measured the reaction time when 10 to 15 min after administration the peak activity had subsided.

The results obtained showed the antinociceptive activity of nicotine is not the result of the agent's neurotoxic activity: the antinociceptive effect of 3 subsequent injections of 10 mg/kg of nicotine given at intervals of 60 min was not diminished while the neurotoxic activity was abolished with the dosage schedule applied.

The increase in the response to nicotine after injections repeated every hour is apparently consistent with the view of MATTILA et al. (1968) attributing the antinociceptive effect of nicotine to a central block, as the depolarization block was usually augmented by successive treatments. However, the activity was reduced after pretreatment for 4 days in the present experiments and 2 weeks in the study of MATTILA et al. (1968), indicating that the antinociceptive effect was due rather to a stimulating effect exerted by nicotine on certain central nervous structures. It seems that a tolerance develops to the antinociceptive effect although it is not brought about as readily as the tolerance to the neurotoxic, the convulsive or the lethal effect of nicotine.

DMPP is the most specific ganglionic stimulant, practically without any blocking effect. This agent if given intraventricularly, exerts an antinociceptive effect and inhibits the tail-flick reflex in the rat. Mecamylamine, a drug with merely ganglionic blocking effects, has no antinociceptive activity.

If nicotine had exerted its antinociceptive activity by blocking N-cholinergic receptors of the CNS, a similar activity would have been observed with other central nicotinergic blocking agents. As shown in the present experiments, mecamylamine exerted such an activity neither in rats nor in mice.

Physostigmine inhibits the antinociceptive activity of nicotine, similarly as it blocks other stimulating effects such as the convulsive and lethal activities, as shown by our unpublished studies. This antagonism is readily explained by assuming that the antinociceptive effect of nicotine is the result of a stimulating effect, in other words a depolarization blockade is established which develops more rapidly in the presence of high acetylcholine levels. Interpretation of this phenomenon becomes more difficult if one presumes that the antinociceptive effect of nicotine is due to the blocking of certain synapses, it being known that the decamethonium-induced inhibition is not antagonized by the cholinesterase blockers in skeletal muscle.

Cholinergic stimulation, whether induced by muscarinergic (oxotremorine) or nicotinergic mechnisms, brings about an antinociceptive effect. The independence of the two cholinergic systems is clearly demonstrated by the fact that specific antagonists of oxotremorine (atropine, N-1199) fail to interfere with the antinociceptive activity of nicotine, while mecamylamine has no effect on the antinociceptive effect of oxotremorine. Oxotremorine is active only on the M-receptors, and there is reason to believe that specific blockade (tolerance, GYÖRGY et al. 1970a, 1971) established by repeated oxotremorine doses or a single tremorine dose has a similar site of action. The fact that the antinociceptive activity of both oxotremorine and nicotine is reduced in the state of tolerance points to the interrelationship of the two systems. It is possible that stimulus propagation in the N—M direction occurs also in the CNS, and the absence of an antinicotinic activity of the antimuscarine compounds might be due to a difficult blocking of the endogenous transmitter.

Acknowledgement

The authors are indebted to Mrs. A. HALMOS for skilful technical assistance.

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Recensiones

D. S. FARNER, R. KING (Eds)

Avian Biology

Volumes I and II. Academic Press, New York and London 1971 and 1972. Vol. I.: XIX + 586 pages, with 108 figures and 29 tables. Price: $ 34.00; Vol. II.: XXIII+612, pages, with 116 figures and 22 tables. Price: $ 32.00

These are the first two volumes of Avian Biology, a multivolume treatise of present knowledge of about 8600 living species of birds. International authorities of many biological disciplines present a comprehensive survey including ethology, ecology, population biology, evolution biology and physiological ecology. In addition, K. C. PARKES gives a short note on taxonomy.

Volume I is concerned primarily with systematics, population biology and ecology. With the preface of the Editors, Chapter 1 discusses Classification of Birds; Chapter 2: Origin and Evolution of Birds; Chapter 3: Systematics and Speciation in Birds; Chapter 4: Adaptive Radiation of Birds; Chapter 5: Patterns of Terrestrial Bird Communities; Chapter 6: Sea Bird Ecology and the Marine Environment; Chapter 7: Biology of Desert Birds; Chapter 8: Ecological Aspects of Periodic Reproduction; Chapter 9: Population Dynamics; Chapter 10: Ecological Aspects of Reproduction; Chapter 11: Ecological Aspects of Behaviour. The volume concludes with a comprehensive author subject and bird name index.

Volume II is concerned with the anatomy and comparative physiology of birds. Chapter 1 discusses The Integument of Birds; Chapter 2: Patterns of Molting; Chapter 3: Mechanism and Control of Molt; Chapter 4: The Blood Vascular System of Birds; Chapter 5: Respiratory Function in Birds; Chapter 6: Digestion and the Digestive System; Chapter 7: The Nutrition of Birds; Chapter 8: The Intermediary Metabolism of Birds; Chapter 9: Osmoregulation and Excretion in Birds. The volume ends with an author index, index to bird names and subject index.

The volumes of "Avian Biology" will be a reliable source of information for both research workers and teachers of avian biology and should find their place in the librairies of zoologists, ecologists, comparative physiologists and ornithologists.

K. LISSÁK

H. L. GABELNICK, M. LITT (Eds)

Rheology of Biological Systems

Charles C. Thomas Publisher, Springfield, Illinois 1973. XII + 319 pages, with 117 figures and 23 tables. Price $ 16.95

The volume presents the proceedings of an interdisciplinary symposium on biorheology held on November 30, 1971, in San Francisco, at the 64th Annual Meeting of the American Institute of Chemical Engineers. In this interdisciplinary symposium, 31 outstanding contributors to their fields, mostly Americans, and 7 discussants have participated. Biorheology, a new branch of biological sciences, means the study of deformation and flow of biological materials. The tremendous progress in industrial chemistry and engineering resulted in the extrapolation of results to biological systems in recent years. Biomedical sciences utilizing these results, contributed to the understanding some of the aspects of physiology, biophysics and medicine. In the two-part symposium, part I considers the rheological properties of blood, viz. 1. Rheology of the Red Cell and Capillary Blood Flow; 2. Rheological Correlation of Blood flow in the Macro- and Micro-Circulation; 3. Effect of Red Cell Deformability on Blood Viscos-

ity: 4. Viscosimetric Instrumentation for Studies of Red Cell Damage; 5. Criteria for Red Cell Haemolysis. Part II considers the rheological properties of other biomaterials, including: 1. Physico-Chemical Factors in Mucus Rheology; 2. Effect of Drugs on Rheology of Sputum and Saliva; 3. Novel Methods for Fluids; 4. Evaluation of Rheological Properties of Orthopaedic Implants; 5. Rheology of Synovial Fluid.

The book will be a useful information concerning biomedical sciences first of all in physiology, biophysics, and medicine.

K. Lissák

N. Emmelin, Y. Zotterman (Eds)

Oral Physiology

Proceedings of the International Symposium held in Wenner-Gren Center, Stockholm, August 1971. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. IX + 302 pages, with 215 figures and 12 tables. Price: £ 10.00

The book is the 20th volume of the Wenner-Gren Center International Symposium Series. The aim of this volume is to spread information about what is done in oral physiology, a basic knowledge for dental and medical surgeons and guide for clinical activity. The prominent international group of scientists discusses the physiology of salivary secretion, mastication and sensory functions of the organs in the oral cavity.

After the opening address by Y. Zotterman, the doyen of oral physiologists, twenty-eight papers were presented and discussed. The contents of the book reflects the wide field of this important branch of physiology: 1) control of salivary glands; 2) on the nerves of the parotid gland; 3) electrolyte transports involved in the formation of saliva; 4) neural mechanisms controlling the salivary secretion; 5) salivary secretion in disorders of the 7th and 9th cranial nerves; 6) on the variability of the parotid flow rate in healthy subjects on stimulation with citric acid; 7) responses of perfused main duct of rat submaxillary gland to pharmacological agents; 8) secretion of salivary glycoproteins; 9) neuro-effector sites in salivary glands; 10) electrolyte transport in the excurrent duct system of the submaxillary gland, I. Studies on the intact gland; 11) II. Microperfusion studies on the excretory ducts in vivo and in vitro; 12) circulation of the tongue; 13) nervous control of blood circulation in the dental pulp and the periodental tissues; 14) other techniques for monitoring of oral circulation; 15) effect of local anaesthetics on oral blood flow in human subjects; 16) adrenergic vasodilatation in the cat submandibular salivary gland; 17) recent advances in the physiology of mastication; 18) post-tetanic potentiation in masseter motoneurone; 19) the effect of repeated stimulation on the response from slowly adapting periodontal mechanoreceptors; 20) masticatory function in cases with denture, in cases with natural teeth, and the importance to digestion; 21) cross-adaptation between salts in the rat's chorda tympani response; 22) the morphological and functional development of foetal gustatory receptors; 23) the problem of sensation from teeth; 24) site of nerve fibres from the dental pulp; 25) the analysis and interpretation of phasic potentials from the tooth; 26) ultrastructural localization of cholinesterase activity in the dental pulp; 27) neural responses evoked by stimulation of dentin; 28) evidence that nerve impulses can be recorded from dentin.

The book presents important basic information for dentistry and oral surgery, and will greatly support teaching and research in oral physiology.

K. Lissák

A. Costoff

Ultrastructure of Rat Adenohypophysis. Correlation with Function

Academic Press, New York and London 1973. XIV + 220 pages. Price: US $ 14.50

This book is a modern and brief survey of our present knowledge about the histophysiology of the adenohypophysis. The work is divided into 12 chapters. After a general introduction and methodological survey, a special chapter is devoted to each cell type of

the anterior pituitary, as well as to the pars tuberalis and pars intermedia. The last two chapters deal with the hormonal activities and biochemical properties of isolated granule fractions of the different pituitary cells, and with the questions of synthesis, storage and release of anterior pituitary hormones. The documentation of the whole book consists of excellent electron micrographs of the different pituitary cells.

The book deals not only with the ultrastructure of the anterior pituitary in the normal intact rat but also after various treatments and changes in the animal's physiology. In addition to ultrastructural data, a method is given for the isolation of various populations of pituitary secretory granules that can be used in experimental endocrinological research.

A comprehensive work about the modern aspects of adenohypophysial structure and function was much needed in the field of experimental morphological research of the endocrine system. This book should therefore be of value not only to the student and the experimental investigator in endocrinology, anatomy, and pathology, but also to those in physiology, biochemistry, and related disciplines.

B. MESS

Y. K. LEVINE

X-ray Diffraction Studies of Membrane

Progress in Surface Science. Vol. 3. Part 4. Pergamon Press, Oxford 1973. Pages 279—352, with 17 figures and 2 tables. Price: £ 2.50

This book is part of a series of interdisciplinary studies in surface science. The Introduction summarizes the possibilities and limits of X-ray diffraction analysis. The method offers ample information about any periodic structure the distance between the elements of which is commensurate with the wavelength of the X-rays, but gives no indication e.g. that the lipid bilayer in the membrane is continuous. If the periods of structure are longer than 300 Å, their diffraction will hardly be observed.

The 2nd chapter presents the basic theory of diffraction and its interrelation with the Fourier transformation. Fourier analysis with the right corrections makes it possible to avoid any distortions of the reconstructed electron density profiles. The Fourier synthesis technique described in this chapter seems to present much more information than that achieved by the rather arbitrary "best fir" models. In the next chapters the structures of phospholipid–water mixtures, conformation of hydrocarbon chains in lipid bilayers, and bilayer parameters are summarized.

Electron density profiles of lamellar structures can be obtained by determining the phases of Bragg reflections and computing the corresponding Fourier synthesis. The protein organization on membrane surface can be determined in the same way.

In the last chapter of the book, a short appraisal is given concerning the square-wave models; in the author's opinion their usefulness has been overestimated.

The little book is a good introduction to the X-ray diffraction method and a clever guide in the up-to-date results obtained by X-ray diffraction studies of membranes. Seventy-seven references make the book complete.

T. LAKATOS

U. LÜTTGE (Ed.)

Microautoradiography and Electron Probe Analysis. Their Application to Plant Physiology

Springer-Verlag, Berlin, Heidelberg, New York 1972. III + 242 pages, with 78 figures. Price-DM 46.— ; US $ 14.60

Owing to a number of technical problems, the application of microautoradiography in plant sciences was retarded. Consequently, earlier works contained but a limited number of references concerning microautoradiographic work with plant material. The present volume fills this gap. It is a specialized treatise of the problem; it does not contain a description of the basic principles of autoradiography and the photographic process, problems well covered

elsewhere. After two introductory chapters on quantitative autoradiography, highly specialized sections, with special emphasis on the botanical aspects, are included. These (microautoradiography of highly insoluble organic compounds, autoradiography with the electron microscope, microautoradiography of water soluble inorganic ions and organic compounds) have been written by outstanding authorities having first hand experience in work with plant material. An important feature of the book is the critical attitude of the authors, who do not minimize the difficulties of the technique if applied to plant tissues or cells with high water content. In fact, they recommend other methods where they are more promising. Excellent illustrations and well-selected literary references add to the value of the text. The book, the first of its kind, will be indispensable in all botanical laboratories using isotope techniques.

G. L. FARKAS

V. BECKER

Bauchspeicheldrüse (Inselapparat ausgenommen)

Spezielle pathologische Anatomie. Band IV. Ed. by W. DOERR, G. SEIFERT, E. UEHLINGER. Springer-Verlag, Berlin, Heidelberg, New York 1973. X + 586 pages, with 296 figures. Price: DM 238.—; US $ 75.50

In this volume Professor V. BECKER, Director of the Pathological Institute of Erlangen-Nürnberg University presents a comprehensive treatise of the morphological, functional and clinical pathology of the pancreas, with a list of references on 64 pages. Included are embryonic development and developmental disorders, comparative anatomy of the pancreas with particular respect to the duct system and its relation to disposition to tryptic pancreatitis; the ultrastructural morphology, chemistry and physiology of pancreatic secretion, its functional variations such as hydrochylia and proteochylia and its regulative correlations; the pathological changes of the pancreas associated with extrapancreatic diseases, and those of pancreatic diseases as nosological entities.

Throughout the whole book special emphasis is laid on functional and clinico-pathological aspects. This results in a highly integrated presentation of the pathology of the organ as a whole, a good example for pathomorphology, worth-while to be followed.

There is a detailed chapter on the pathology of cystic fibrosis of the pancreas (dysporia bronchopancreatica) followed by chapters describing the functional disorders of pancreas secretion, the different forms of dyschylia and acute pancreatic oedema with reflux of pancreatic secretion from the duct system into the interstitium resulting from obstruction of the duct. This in itself is still no equivalent to tryptic necrotic pancreatities but may play a significant role as an initiating stage of pancreatities.

An excellent chapter (150 pages) deals with acute tryptic pancreatitis, presenting a detailed analysis of the complex biochemical mechanism of tryptic necrosis. A critical evaluation of the different pathogenic factors, possibly involved in this event is given, pointing out that duct obliteration, increased secretory stimulation, local activation of the enzymes and damage of cell metabolism are the basic pathogenic factors in the development of this fearful autodigestive necrotic inflammation of the pancreas.

The thoroughness in treatment of the subject and the depth in analysis of the problems in this chapter are remarkable. Chapters dicussing the pathology of chronic tryptic relapsing pancreatitis and the neoplastic diseases of the pancreas follow. A short closing chapter on conditions after total pancreatectomy and the problem of life without pancreas is attached.

The book contains a wealth of information not readily available elsewhere in such an integrated form and will be essential and of lasting value as a reference book not only for the pathologist but also for anatomists, physiologists and experimentators as well as for clinicians intersted in the many facets of pancreatic physiology and pathology. There is a good subject index. The volume maintains in every respect the high level of the preceding volumes of the series.

G. ROMHÁNYI

N. S. Assali (Ed.)

Pathophysiology of Gestation

Academic Press, New York 1972. Vol. 1. Maternal Disorders. XVII + 617 pages. Price: $ 29.00. Vol. 2. Fetal-Placental Disorders. XVI + 357 pages. Price: $ 25.00. Vol. 3. Fetal and Neonatal Disorders. XVI + 556 pages. Price: $ 29.50

The work is an interdisciplinary treatise giving an integrated account of the pathophysiology of gestation and of the rational management of the different disorders. Each volume is divided into chapters written by known experts. The authors of each chapter are giving a description of the physiological background of the subject, its pathophysiology, diagnosis and treatment. At the end of each chapter a bibliography is given.

Vol. 1 deals with the disorders of the maternal organism. The first two chapters are devoted to the pathophysiology of ovulation, gamete transport and implantation. Chapter 3 describes the disorders of uterine function during pregnancy, labour and puerperium. Chapter 4 deals with the disorders of the circulatory and respiratory system during pregnancy. In Chapter 5 the pathophysiology and pathology of water and electrolyte metabolism and renal function are discussed. Chapter 6 deals with hepatic disorders during normal and abnormal pregnancy. In Chapter 7 the disorders of lactation and mammary gland are summarized.

Vol. 2 is devoted to the foetal–placental disorders. Chapter I deals with disorders of placental transfer of gases, electrolytes, carbohydrates, amino acids, vitamins and water. Chapter 2 describes the pathophysiology of placental endocrine function. In Chapter 3 the disorders of amniotic function the use of amniotic fluid for the diagnosis of foetal disorders is described. Chapter 4 deals with the pathophysiology of foetal growth and development, genetic factors and inborn errors of metabolism associated with disorders of foetal development. Chapter 5 deals with teratologic impacts on of foetal development, environmental and maternal factors associated with teratologic abnormalities and their clinical manifestations.

Vol. 3 is devoted to the disorders of the foetal and neonatal organisms. Chapter 1 deals with the disorders of the cardiovascular system: pathophysiology of its mal adjustment to postnatal life, inborn cardiac malformations, etc. Chapter 2 describes selected disorders of respiration. Chapter 3 deals with the disorders of the endocrine system according to organs. In Chapter 4, a detailed description is given of the water, electrolyte and acid–base balance in physiological conditions and the disorders connected to the changes of the normal physiological parameters. Chapter 5 describes the disorders of the nervous system of genetic, environmental and iatrogenic origin. Chapter 5 deals with the disorders of the immunological mechanisms. Chapter 7 is devoted to the disorders of haemotopoiesis, separately analyzing the factors associated with the disorders of erythropoiesis, leukopoiesis and coagulation. The last chapter is devoted to the disorders of bilirubin metabolism.

The textbook is recommended to students, research workers and clinicians dealing with gestation.

G. Telegdy

INDEX

PHYSIOLOGIA

Szalontai K., Rochlitz Sz., Molnár L.: Use of Radioactive Isotopes in the Examination of Cerebral Blood Flow 1
Farkas Mária, Donhoffer Sz.: Comparison and Correlational Analysis of the Effect of Hypoxia and Hypercapnia on Heat Production and Body Temperature in the Adult Guinea Pig 11
Varga B., Horváth Edit, Stark E.: Effects of ACTH and HCG on Ovarian Oestrogen and Progesterone Secretion in Dogs Pretreated with HCG 29
Csapo A. I., Fáy Erzsébet: Effect of Hypophysectomy on Ovariectomy-Induced Placental Hypertrophy 39
Avar Z., Monos E., Kurcz M , Nagy I., Bukulya B.: Mechanism of Perinatal Reproductive Disorders Induced by Parafornical Hypothalamic Lesions in the Rat 45
Salánki J., Vadász I.: Chemical Sensitivity at Different Temperatures of the Br-Type, Bimodal Pace-maker Neurone in the CNS of the Snail *Helix pomatia* L. 51
Batuev A. S.: Organization of Visual Cortex Primary Response 61

PATHOPHYSIOLOGIA

Bálint G. A., Kasirye E. M. B., Kakembo A. S. L.: Effect of Ricin Immnization on the Qualitative Composition of Plasma Proteins 77

PHARMACOLOGIA

Phan D. V., Dóda Margit, Bite Ágnes, György L.: Antinociceptive Activity of Nicotine 85

RECENSIONES

Farner D. S., King R. (Eds): Avian Biology. Volumes I and II. Academic Press, New York and London 1971 and 1972. (K. Lissák) 95
Gabelnick H. L., Litt M. (Eds): Rheology of Biological Systems. Charles C. Thomas Publisher, Springfield, Illinois 1973. (K. Lissák) 95
Emmelin N., Zotterman Y. (Eds): Oral Physiology. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. (K. Lissák) 96
Costoff A.: Ultrastructure of Rat Adenohypophysis. Correlation with Function. Academic Press, New York, London 1973. (B. Mess) 96
Levine Y. K.: X-Ray Diffraction Studies of Membrane. Vol. 3. Part 4. Pergamon Press, Oxford 1973. (T. Lakatos) 97
Lüttge U. (Ed.): Microautoradiography and Electron Probe Analysis. Their Application to Plant Physiology. Springer Verlag, Berlin, Heidelberg, New York 1972. (G. L. Farkas) 97
Becker V.: Bauchspeicheldrüse (Inselapparat ausgenommen). Springer-Verlag, Berlin, Heidelberg, New York 1973. (G. Romhányi) 98
Assali N. S. (Ed.): Pathophysiology of Gestation. Volumes I, II and III. Academic Press, New York 1972. (G. Telegdy) 99

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РЕЗЮМЕ

ПРИМЕНЕНИЕ РАДИОАКТИВНЫХ ИЗОТОПОВ В ИЗУЧЕНИИ МОЗГОВОГО КРОВОТОКА

К. САЛОНТАИ, С. РОХЛИЦ и Л. МОЛНАР

Авторы описали радиоактивный «клиренс» метод для измерения мозгового кровотока. Они обсуждали распространенные математические методы для оценки кривых клиренса и предлагали использовать новый математический метод, с помощью которого оценка кривых клиренса более однозначна, чем с ранее использованными методами

СРАВНЕНИЕ И КОРРЕЛЯЦИОННЫЙ АНАЛИЗ ЭФФЕКТОВ ГИПОКСИИ И ГИПЕРКАПНИИ НА ТЕПЛОПРОДУКЦИЮ И ТЕМПЕРАТУРУ ТЕЛА У ВЗРОСЛЫХ МОРСКИХ СВИНОК

М. ФАРКАШ и С. ДОНХОФФЕР

Потребление кислорода (VO_2) и температура ободочной кишки (T_c) взрослых морских свинок обоих полов записывались перед, в течение и после вдыхания 12% O_2, 8% O_2 и 6% CO_2 при окружающей температуре (T_a) 20° и 10° С. Подтвердилось, что механизмы продукции и потери тепла участвуют в различной степени в терморегуляторных ответах при гипоксии и гиперкапнии. Давольно тесная корреляция наблюдалась между: а) уровнем VO_2 перед и его последующим изменением в течение гипоксии и гиперкапнии; б) изменением в VO_2 (ΔO_2) в течение гипоксии и его изменением после гипоксии; в) уровнем VO_2 в течение гипоксии и его изменением (ΔO_2) после гипоксии. Не было установлено подобной связи между эффектами вдыхания 12% O_2 или 8% O_2 при обоих T_a, и вдыхания 6% CO_2 при 20° С на температуру тела. При температуре 10° С, однако, значительная корреляция демонстрировалась между: а) температурой ободочной кишки (T_c) перед гиперкапнией и ее изменением (ΔT_c) в течение гиперкапнии; б) изменением температуры ободочной кишки (ΔT_c) при гиперкапнии и ее изменением после гиперкапнии; в) температурой ободочной кишки (T_c) при окончании гиперкапнии и ее изменением (ΔT_c) при вдыхании комнатного воздуха после гиперкапнии. Сравнительно тесное соответствие было обнаружено между изменениями в потреблении кислорода (ΔO_2) и температурой тела (ΔT_c) в течение гипоксии и гиперкапнии при обоих T_a, тогда как между этими же параметрами после гипоксии и гиперкапнии не могли демонстрировать подобных корреляций. Значительное соответствие было найдено между изменением температуры ободочной кишки (ΔT_c) в течение гипоксии и увеличением теплопродукции после гипоксии, и, в меньшей степени, между T_c при окончании гипоксии и последующим увеличением в потреблении кислорода. Не могли найти никакого соответствия при каждой температуре (T_a) между изменением теплопродукции в ответ на 8% O_2, с одной стороны, и 6% CO_2, с другой стороны, тогда как тесная связь была обнаружена между соответствующими изменениями в температуре тела при обоих T_a. Обсуждались различные аспекты результатов и роль корреляционного анализа в изучении сложных кибернетических систем.

ЭФФЕКТЫ АДРЕНОКОРТИКОТРОФНОГО ГОРМОНА (АКТГ) И ЧЕЛОВЕЧЕСКОГО ХОРИАЛЬНОГО ГОНАДОТРОПИНА (ЧХГ) НА СЕКРЕЦИЮ ЯИЧНИКАМИ ЭСТРОГЕНА И ПРОГЕСТЕРОНА У СОБАК, ПРЕДВАРИТЕЛЬНОГО ОБРАБОТАННЫХ ЧХГ

Б. ВАРГА, Е. ХОРВАТ и Е. ШТАРК

После предварительной пятидневной обработки собак с ЧХГ, измеряли количество эстрогена (эстрона, 17β-эстрадиола) и прогестерона в крови, вытекающей из яичников, при действии АКТГ и ЧХГ, введенных на шестой день.

B_{1-24} АКТГ, введенный 10- или 60-минутной инфузией, увеличивал как кровоток яичника, так и секрецию эстрогена. АКТГ стимулировал секрецию эстрогена даже после адреналэктомии, тогда как в секреции прогестерона не было значительного изменения. Кровоток яичника и секреция эстрогена нормализовывались через час после окончания инфузии АКТГ.

10-минутная инфузия 1,0 И. Е./кг/мин ЧХГ стимулировала секрецию прогестерона, а кровоток яичника и секреция эстрогена увеличивались только через час после окончания инфузии, когда секреция прогестерона возвращалась к контрольному уровню.

АКТГ, введенный непосредственно после 25,0 мг/кг циклогексимида, увеличивал секрецию эстрогена, тогда как секреция прогестерона уменьшалась. После циклогексимида ЧХГ был неэффективным.

ЭФФЕКТ ГИПОФИЗЭКТОМИИ НА ПЛАЦЕНТАРНУЮ ГИПЕРТРОФИЮ, ВЫЗВАННУЮ ОВАРИЭКТОМИЕЙ

А. И. ЧАПО и Е. ФАЙ

Исследовался эффект гипофизэктомии на плацентарную и зародышевую гипертрофию, вызванную овариэктомией у 91 беременной крысы. У контрольных крыс гипофизэктомия не оказывала влияния на вес плаценты, но уменьшала вес зародышей. У овариэктомированных крыс гипофизэктомия не оказывала влияния на гипертрофический ответ плаценты, но предотвращала увеличение веса зародышей. Было установлено, что эффект овариэктомии на гипертрофию плаценты не зависит от функции гипофиза, а рост зародышей частично контролируется гипофизом, через механизм, не зависящий от плацентарной функции.

МЕХАНИЗМ ПЕРИНАТАЛЬНЫХ РЕПРОДУКТИВНЫХ НАРУШЕНИЙ, ВЫЗВАННЫХ ПАРАФОРНИКАЛЬНЫМ ГИПОТАЛАМИЧЕСКИМ ПОВРЕЖДЕНИЕМ У КРЫС

З. АВАР, Е. МОНОШ, М. КУРЦ, И. НАДЬ и Б. БУКУЙА

Было найдено, что электрокоагуляция 50% парафорникального поля вызывала значительные нарушения материнского поведения и жизнеспособности зародышей. Потребление пищи и воды в начале значительно уменьшалось у нарушенных животных, а в предродовой период достигало уровня контрольных, но уменьшение веса тела не компенсировалось, отчасти, из-за понижения прибавления веса плодов и вследствие их смерти. Температура тела оставалась нормальной. Материнское поведение в течение родов было нормальным, а постройка гнезда и поисковая активность появлялись только у каждого третьего животного и кормление также уменьшалось. Аденогипофизарный LTH был значительно выше, чем у контрольной группы, но в STH не было различия между двумя группами. Уменьшение веса тела новорожденных и пропорции живорожденных по отношению к контрольным, указывают на внутриутробное нарушение развития плодов.

ХИМИЧЕСКАЯ ЧУВСТВИТЕЛЬНОСТЬ ПРИ РАЗЛИЧНЫХ ТЕМПЕРАТУРАХ БИМОДАЛЬНОГО ПЕЙСМЕКЕРНОГО НЕЙРОНА ТИПА Br В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ УЛИТКИ *Helix pomatia L.*

Й. ШАЛАНКИ и И. ВАДАС

Исследовались эффекты ацетилхолина (АХ), 5-гидрокситриптамина (5—ГТ), норадреналина (НА) и допамина (ДА), добавленных к раствору, где находился нейрон типа Br (RPa1-клетка) улитки *Helix Pomatia L.* при температурах 22° С, 7° С и выше 30° С.

АХ повышал активность нейрона при каждой температуре. Эффект его выявлялся мгновенно, но был не длительным.

При добавлении 5—ГТ синаптические потенциалы (ВПСП) появлялись при каждой температуре, вызывая отдельные разряды, тогда как характерная бимодальная картина активности клетки оставалась практически интактной.

НА блокировал генерацию потенциалов при всех исследованных температурах.

ДА имел бифазный эффект: вначале он тормозил спайковую активность, а через 2—2,5 мин генерация потенциалов действия снова появлялась. В последней фазе деполяризация и присутствие ВПСП были характерными.

Предполагается, что АХ, НА и ДА оказывают влияние прямо на проницаемость клеточной мембраны. Эффект 5—ГТ и поздний эффект ДА являются результатом синаптической активации, вызванной или стимуляцией нейронов, связанных синаптически с клеткой типа Br, или прямым эффектом вышеупомянутых веществ на синаптическую область данной клетки.

ОРГАНИЗАЦИЯ ПЕРВИЧНОГО ОТВЕТА ЗРИТЕЛЬНОЙ КОРЫ

А. С. БАТУЕВ

Предпринят детальный анализ зрительного первичного ответа (ПО] с помощью методов нейрохимического анализа, фокального послойного отведения и экстраклеточной регистрации активности нейронов. Выявлено одностороннее влияние адренэргетиков только на положительную фазу ПО и ее большая чувствительность (по скорости нарастания максимального эффекта аппликации) к хлоралозе, атропину, эзерину. Фокальный анализ ПО от различных слоев коры показывает запаздывание на 1,6—2,9 мсек возбуждения нижних слоев коры относительно верхних. Использование общего хлоралозового наркоза выявляет компоненты не реверсирующие по поперечнику коры. Показана нечеткая корреляция импульсных разрядов с отдельными фазами ПО, регистрируемого от повевхности коры. Полученные результаты показывают активный генез всех фаз ПО в поверхностных слоях коры, химическую гетерогенность его компонентов. Роль дипольных компонентов, по-видимому, существенная только в пределах единичного нейрона и не является доминирующей в конфигурации регистрируемых от поверхности коры зрительных ПО.

ЭФФЕКТ РИЦИНОВОЙ ИММУНИЗАЦИИ НА КАЧЕСТВЕННЫЙ СОСТАВ ПРОТЕИНОВ ПЛАЗМЫ

Г. А. БАЛИНТ, Е. М. Б. КАШИРИЕ и А. Ш. Л. КАКЕМБО

Исследовались качественные изменения в составе протеинов плазмы при иммунизации рицином.

Как результат своего гепатотоксического эффекта, вначале рицин вызывал уменьшение уровня альбумина, затем одновременно с прогрессивной иммунизацией, уровень альбумина медленно повышался, вероятно обусловливаясь градуальной регенерацией печени.

Фракция гамма-глобулина увеличивалась как у обезьян, так и у кошек, и это увеличение соответствовало появлению специфических антител, «антирицин».

Бета фракция показала уменьшение как у обезьян, так и у кошек. Прадполагается, что это специфический эффект рицина тесно связанный с нарушением в синтезе протеина.

При иммунизации рицин терял свой пирогенный эффект, по всей вероятности, в результате взаимодействия между антигеном и антителом.

АНТИНОЦИЦЕПТИВНАЯ АКТИВНОСТЬ НИКОТИНА

Д. В. ФАН, М. ДОДА, А. БИТЕ и Л. ДЬЁРДЬ

Антиноцицептивная активность никотина демонстрировалась на мышах с помощью техники «hot-plate». Повторные инъекции никотина, с интервалами 60 мин, вызывали повышение ответа. Продление времени реакции, определяемое методом «вращающегося стержня», казалось, не зависело от нейротоксического эффекта никотина, вследствие привыкания к нему. Мекамиламин не продлевал время реакции, но тормозил антиноцицептивную активность никотина. Такое же действие оказывали 50 мкг/кг физостигмина, введенного одновременно с никотином, и 20 мг/кг треморина, введенного предварительно, тогда как вышеупомянутая активность увеличивалась одновременным введением небольшого количества оксотреморина. Антиноцицептивная активность никотина у мышей была показана и методом «уксусной кислоты».

Было найдено, что никотин имеет антиноцицептивную активность также у крыс. У этих животных никотин и DMPP, введенные внутрь желудочков, оказывали антиноцицептивный эффект, который антагонизировался мекамиламином, введенным внутрибрюшинно. Антиноцицептивная активность вероятнее всего была обусловлена стимулирующим эффектом никотина.

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Physiologia

STUDIES ON HUMAN TONSILLAR CELL DNA. PREPARATION AND CHARACTERIZATION OF NUCLEAR AND NON-NUCLEAR DNA FROM ISOLATED TONSILLAR CELLS

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(Received June 27, 1973)

The preparation from human tonsillar cells of 570 μg of cytoplasmic DNA as compared to 800 mg of nuclear DNA, both determined by the diphenylamine reaction, is described.

The first step in the fractionation of cells was a treatment with the detergent Nonidet P-40. DNA extraction was carried out from the nuclear and the cytoplasmic fractions separately. The bulk (90%) of cytoplasmic DNA had a buoyant density in neutral CsCl gradients of 1.715 g/ml. Nuclear DNA consisted of two components, banding at 1.715 and 1.696 g/ml, respectively. Comparison of nuclear and cytoplasmic DNA on CsCl–ethidium-bromide gradients excluded the presence of covalently closed circular intact mitochondrial DNA in significant amounts. Alkaline sucrose velocity sedimentation revealed three main peaks in the nuclear DNA, while only two were found in the cytoplasmic material, positioned identically with two of three nuclear peaks. As our cytoplasmic DNA containing fraction consists as evidenced by fractionation on discontinuous sucrose gradients, of mitochondria and mostly of plasma membranes, the mitochondrial origin of cytoplasmic DNA remains a distinct possibility. The striking similarity with nuclear DNA, however, suggested that the DNA found in the cytoplasm of human tonsillar cells, represents a specific portion of nuclear DNA.

The discovery that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are distinct components of cytoplasmic organelles, in particular of mitochondria and chloroplasts, has prompted efforts to examine other cytoplasmic fractions for nucleic acid content (Nass 1969). Bell (1969) was the first in demonstrating "informational DNA" in the microsomes of embryonic cells of eucaryotes.

Lerner et al. (1971) and Hall et al. (1971) demonstrated the presence of DNA in a certain cytoplasmic fraction of cultured, synchronized lymphocytes and discussed it as "plasma membrane DNA". The paucity of evidence, however, supporting the plasma membrane bound nature of the DNA containing cytoplasmic fraction led to debates and stimulated further research in this field (Rogers et al. 1972).

Plasma membrane preparations from liver cells, tested for purity by biochemical criteria, were shown to contain very little DNA (BERMAN et al. 1969; TOUSTER et al. 1970). Preparations obtained from other eucaryotic cells, identified as membranes, by measuring marker enzymes are almost free of DNA (HENN et al. 1972).

The existence of plasma membrane associated DNA in lymphocytes, as contrasted with other cells, would be therefore of extreme interest not only from the point of view of genetic transmission in general, but also from that of the regulation of the immune functions.

Tonsils represent a readily available lymphoid organ (PIFFKÓ et al. 1970, 1972; MITROU et al. 1969). The aim of our experiments was to prepare DNA containing fractions from this natural source of human lymphocytes without culturing or stimulating tonsillar cells in vitro. We paid special attention to the homogeneity of the DNA containing nuclear and cytoplasmic fractions, which were prepared by procedures similar to the method of LERNER et al. (1971).

Methods

Preparation of nuclear and cytoplasmic DNA from human tonsils (Table I)

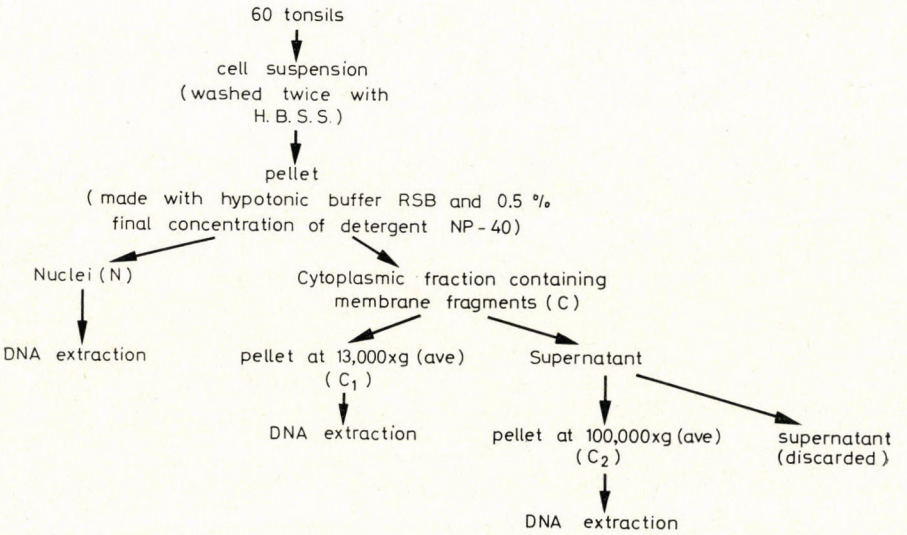
Sixty human tonsils, removed surgically from 4—7-year-old children (wet weight: 167 g), kept at 4°C for 2—10 hrs were cut into 1—3 cu. mm pieces and suspended in Hank's balanced salt solution (HBSS). The mixture was gently stirred with a magnetic stirrer at 0°C for 15 min and a cell suspension was prepared as described earlier (PIFFKÓ et al. 1970, 1972). The suspension was filtered through a double layer of gauze and centrifuged at 300 g* at 4°C for 10 min. The cell count was 1.64×10^8 per ml in a volume of 650 ml. The cells were washed twice with cold HBSS and resuspended in 300 ml of the same solution. After centrifugation at 600 g for 20 min, the pellet obtained was resuspended in 250 ml RSB buffer (0.01 M Tris-HCl, pH 7.4; 0.01 M NaCl; 0.0015 M $MgCl_2$) and lysed by the addition of 27 ml 5% NP-40** at 0°C for 5 min. Nuclei were separated by centrifuging the lysate at 2000 g for 5 min and washed again with cold RSB buffer (pellet N). The supernatants were carefully decanted, pooled and centrifuged at 13,000 g, 0°C for 60 min (pellet C_1). Next the supernatant (340 ml) of the latter centrifugation was spun again at 100,000 g for 60 min in a Beckman ultracentrifuge, Model $L_2$65B (pellet C_2). The supernatant of this fast centrifugation was discarded. DNA was extracted from the pellets N, C_1 and C_2 by the method of HIRT (1967). After 1% sodiumdodecylsulphate (SDS) treatment under gentle stirring at room temperature for 15 min precipitation by 1.0 M final concentration NaCl was followed and samples were kept at 0°C overnight. The precipitate was then removed by centrifugation at 17,000 g for 60 min in a Model J21 Beckman centrifuge and the supernatants were recovered. DNA was precipitated from the supernatants by adding two volumes absolute ethanol according to ZAMENHOF (1958). By a rotating glass stick, DNA could be removed from material N in form of fibres and filaments and was dissolved in 150 ml of buffer STE (1.0×10^{-1} M NaCl; 1.0×10^{-3} M EDTA; 5.0×10^{-2} M Tris-HCl, pH 7.4). C_1 and C_2 did not show any filamentous DNA, and a white pellet was obtained after centrifuging at 3500 g for 25 min. Pellet C_1 was dissolved in 50 ml, C_2 in 70 ml of STE buffer and HIRT's extraction procedure (1967) was repeated with the ethanol-precipitated material. Table II demonstrates the chemical composition of fractions N and C_1 after the second SDS deproteinization (N dissolved completely in 450 ml, C_1 in 10 ml of SE buffer (1.0×10^{-1} M NaCl; 1.0×10^{-3} M EDTA). Fraction C_2 contained no measurable DNA and was not analyzed further.

* All given g values represent averages.
** Nonidet P-40.

As seen from the above description the heavy nuclear DNA components, forming the pellet after precipitation by 1.0 M NaCl, were removed by centrifugation at 17,000 g and were not subjected to further analysis.

Table I

Fractionation of surgically removed tonsils
(preparations were carried out at 0—4°C)



Neutral caesium chloride (CsCl) and CsCl–ethidium-bromide (Eth-Br) isopycnic gradient centrifugation

In experiments analysing the isopycnic sedimentation of DNAs, prepared from fractions N and C_1, highly purified CsCl was used (Riedel de Haen Ag., Seelze-Hannover, West Germany, batch no 14718). 3 ml gradients were used. 1.326 ml of CsCl stock solution (p_{20} = = 1.80 g/ml) formed the bottom layer and an appropriate volume of "light" solution (p_{20} = = 1.50 g/ml), carrying the DNA sample for analysis, was overlayered. 100 μg of DNA, diluted on the basis of estimations by BURTON's diphenylamine reaction (1956) were analyzed in each tube. In parallel runs, 50 μg of Eth-Br (B grade, Calbiochem, Luzern, Switzerland) was mixed with the DNA containing "light" CsCl solution to provide fluorescence label. Preformed gradients were run at 20°C, 33,000—44,700 r.p.m., 15 hrs, rotor type SW 50·1, in a Beckman preparative ultracentrifuge, Model $L_2$65B. Two drop fractions from the bottom were collected and analyzed for refractive indices and after dilution by 0.5 ml of distilled water, for absorbancy at 260 nm in microcuvettes. For spectral readings, a spectrophotometer Model SP 500, Unicam, was used.

Alkaline sucrose rate zonal (velocity) sedimentation

100 μg of DNA, diluted from standard solutions N and C_1 (for composition, see Table II) were made 1.0 N by adding 4.0 N NaOH and overlayered upon 10.5 ml 5—20% alkaline sucrose gradients (CABELA et al. 1972). Centrifugation was carried out at 0°C, 65,000 r.p.m., for 180 min in an angle rotor No 65 Beckman preparative ultracentrifuge, Model $L_2$65B. Eight drop fractions collected from the bottom were analyzed for refractive indices and after diluting tenfold by distilled water, for absorbancy at 260 nm in 1 cm Q cuvettes.

Results

Chemical composition of fractions N and C_1

As DNA could not be detected in fraction C_2 either by BURTON's reaction (1956) or by means of CsCl isopycnic gradient centrifugation (see below) only fractions N and C_1 were studied.

Table II shows that the differences between N and C_1 in DNA/protein ratios were far more striking than those in DNA/lipid ratios. It has to be noted that BRAGDON's procedure (1951), described originally for determination of blood lipides, might provide somewhat higher values for lipides than any more detailed determination of these compounds.

Table II

Chemical composition of DNA containing standard solutions obtained from nuclear and C_1 cytoplasmic fractions

Fraction	Components in μg/ml			Ratios	
	DNA	protein	lipid	DNA/prot.	DNA/lipid
N	279.00	100.00	500.00	2.79	0.558
C_1	71.25	230.00	350.00	0.31	0.203

The total amount of DNA isolated by HIRT's procedure (1967) — from supernatants, after removing the 1.0 M NaCl precipitate —, was for N, 800 mg; for C_1, 570 μg. A double-stranded chicken erythrocyte DNA hydrolyzate (Reanal, Hungary) was used as standard. DNA was determined by the diphenylamine reaction (BURTON 1956). Thus, $C_1/N \times 100 = 0.071\%$. Protein was estimated by the method of LOWRY et al. (1951), using bovine albumin as standard. Lipides were estimated after repeated extraction by chloroform : methanol 25 : 1, by BRAGDON's procedure (1951). The extent to which nucleic acids interfered with protein estimations has not been calculated.

Analysis of fractions N and C_1 by isopycnic neutral CsCl and CsCl–Eth–Br sedimentation

Different banding patterns of fractions N and C_1 are demonstrated in Fig. 1A and B. The presence of two peaks is indicated in Fig. 1A for N at the densities 1.696 and 1.715 g/ml, respectively. The bulk of cytoplasmic DNA had its buoyant density at 1.715 g/ml. Two small shoulders are distinguishable in C_1, at the densities of 1.696 and 1.740 g/ml, but none of them is representing more than 2—5% of DNA, recovered from the peak at 1.715 g/ml (Fig. 1B). They cannot be visualized in Fig. 2, for the fluorescent band of C_1 seems to be homogeneous.

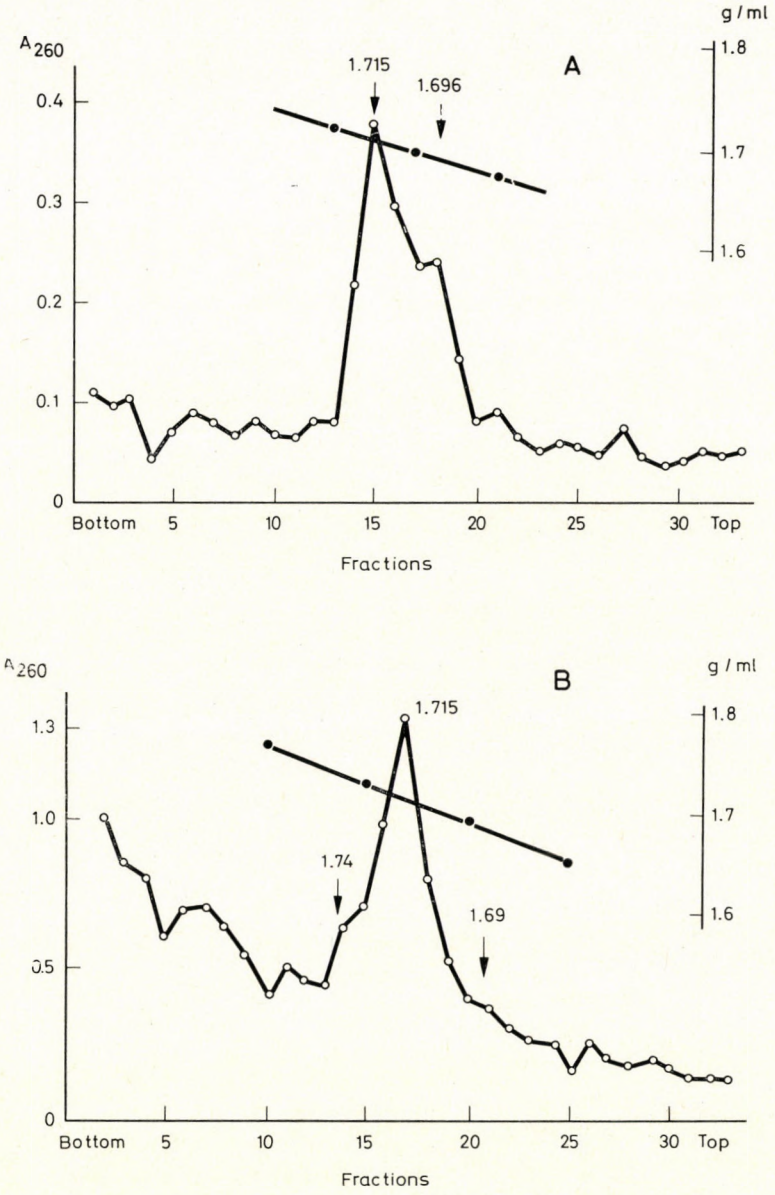


Fig. 1. CsCl equilibrium gradient sedimentation of isolated DNA containing fractions. 100 μg DNA from N and C_1 (see Table I) as determined by BURTON's reaction (1956), dissolved in 1.674 ml of SE buffer (0.1 M NaCl; 0.001 M sodium EDTA, pH 7.0) was mixed with solid CsCl to form the "light" solution (p = 1.50 g/ml; n_{t20} = 1.3840). This was overlayered on 1.326 ml "dense" CsCl stock solution (p = 1.80 g/ml; n_{t20} = 1.3960) to form a 3 ml preformed gradient. Gradients were covered with 2 ml of paraffin oil and centrifuged 44,700 r.p.m. at 20°C for 15 hrs as described in "Methods". ●——●: density; ○——○: A_{260}. A: fraction N (a total of 1.7 O.D. units recovered from the gradient); B: fraction C_1 (a total of 2.87 O.D. units recovered from the peak at 1.715 g/ml)

BURTON's estimation (1956), using as standard chicken erythrocyte double stranded DNA hydrolyzate yielded 1.7 to 2 times less DNA in C_1 than the real amount on the basis of UV absorbancy measurements at 260 nm. In contrast, nuclear DNA showed good correlation between diphenylamine reaction and UV absorbancy; 2 Optical Density units (O.D.) corresponded to about 100 μgs. As the legend to Fig. 1A and B points out the recovery of UV absorbing material from the peak fractions was 1.7 times higher in C_1 then in

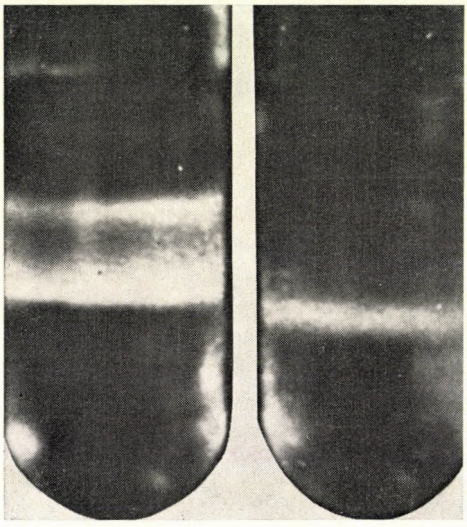


Fig. 2. Fluorescence label of different DNAs extracted from tonsillar cells. Preformed gradients were prepared as described in "Methods" and Fig. 1. 50 μg of ethidium-bromide were mixed with the "light" component of the preformed CsCl gradient. After centrifugation at 44,700 r.p.m., at 20°C for 15 hrs the tubes were irradiated with an UV lamp (Minuvis, Desaga, Heidelberg; West Germany); standard wavelength 366 nm, and photographed using an UV filter at a distance of 500 mm. Magnification, about $\times 4$

N, although we loaded the gradients with the same amounts of DNA according to diphenylamine estimations. Measurements of molecular extinction E(P) were not carried out considering the narrow reproducibility of such experiments even in the same sample.

Fig. 2 indicates the presence of two separated fluorescent bands in N and only one in C_1. (For technical details see "Methods".) We could not detect any DNA specimen banding at an intermediate position or binding less dye. In preliminary experiments we have demonstrated that if the g value was lowered by centrifuging gradients at 33,000 r.p.m., while other conditions remained unaltered, one single peak was obtained in N.

Analysis of fractions N and C_1 by rate zonal alkaline sucrose gradient sedimentation

Alkaline sucrose "velocity" sedimentation revealed the existence of three different peaks in N at the densities 1.10—1.08 and 1.055 and 1.037 g/ml, respectively, while only two were found with C_1 (Fig. 3A and B). Furthermore, in the bottom fractions of both the nuclear and cytoplasmic solutions, additional peaks of rapidly sedimenting materials are evident. This material has passed through the gradient. It has, however, to be noted that the peak at the "dense region" of the gradients (density $\geqq 1.13$ g/ml) did not form any compact pellet.

The use of alkaline rate zonal gradients for N and especially for C_1 had the purpose conclusively to eliminate the possibility of RNA contamination, considering the high susceptibility of RNA to alkali degradation.

It was reported that angle rotors provide a ten times higher resolving capacity than swinging bucket rotors (Flamm et al. 1966b). This allowed a better separation of the complementary strands of alkaline-denaturated linear DNA molecules and linear DNA from covalently closed circular-duplex DNA (Borst 1972). It should remembered that covalently closed circular DNA structures, present in all animal mitochondria, do not permit separation under mild alkaline conditions.

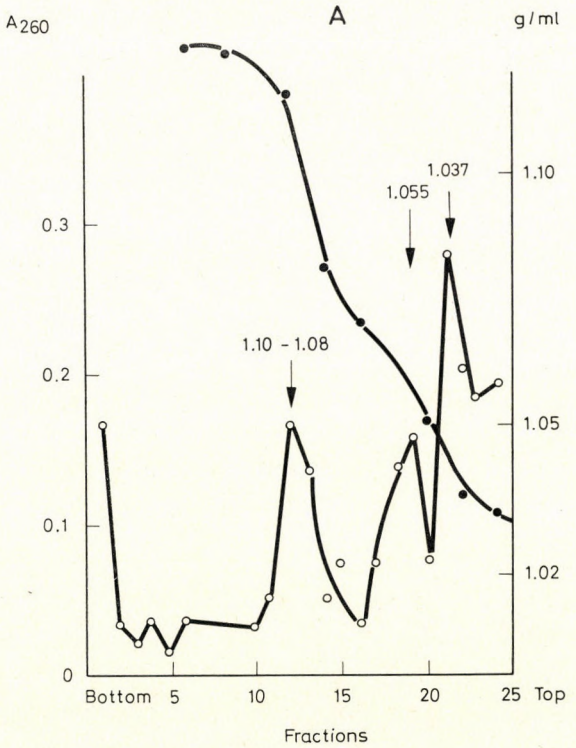


Fig. 3A.

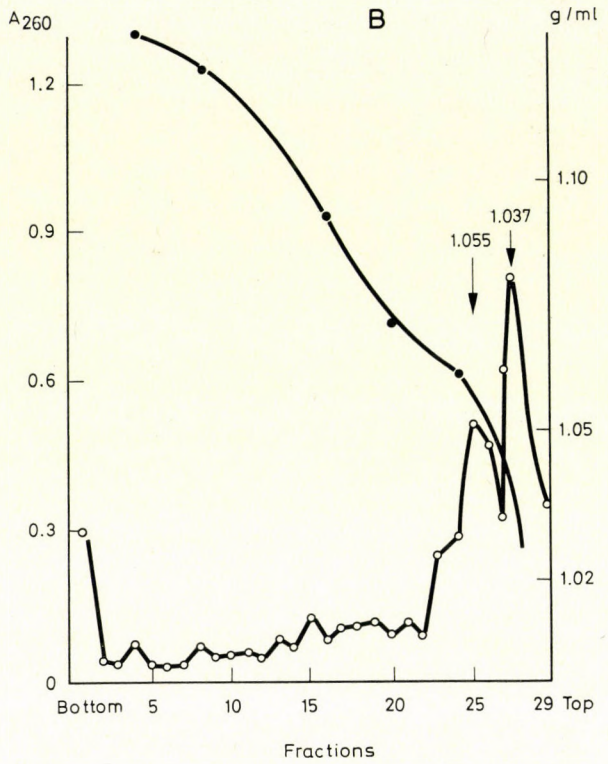


Fig. 3. Analysis of DNA containing specimens by rate zonal alkaline sucrose velocity sedimentation. 1 to 2 O.D. units of fractions N (A) and C_1 (B), in form of the standard solution (see Table II) dissolved in SE buffer (0.1 M NaCl; 0.001 M sodium EDTA) were made to 1.0 N by the addition of 4.0 N NaOH and overlayered on 11 cm linear, preformed 5—20% alkaline sucrose gradients (pH 11.8). The sucrose stock solutions contained: NaCl, 40.9 g/l; NaOH, 12.0 g/l; sodium EDTA, 0.37 g/l; Tris (solid), 1.21 g/l. Centrifugation was carried out in a Beckman preparative ultracentrifuge Model $L_2$65B, at 65,000 r.p.m., at 0°C for 180 min. 8 drop fractions of 0.35 ml each were collected from the bottom. ●———●: density; ○———○: A_{260}

Discussion

Recently, tonsils have been reported to consist of a heterogeneous population of cell with a 6% incidence of rosette-forming lymphoid elements (Zucker-Franklin and Berney 1972).

Their location in the oral cavity raised the question of contaminating microorganisms. Washed cell-suspensions of 10^8 tonsillar cells cultured on nutrient plates at 37°C for 24 hrs yielded not more than 10^3—10^4 bacteria per ml cell-suspension, mostly monocultures of streptococci or staphylococci.

DNA containing nuclear and cytoplasmic fractions were prepared from isolated tonsillar cells including slight modifications such as ethanol precip-

itation of extracted DNA after deproteinization, as described earlier (LERNER et al. 1971; HALL et al. 1971). The applied nonionic detergent NP-40 was recommended for the disruption of lymphocytes (BORUN et al. 1967) by acting primarily on plasma-membranes which they can solubilize, while the nuclear membranes are left intact. The isolated fractions seemed, however, inhomogeneous if tested by further fractionation on discontinuous neutral sucrose gradients (BERMAN et al. 1969; TOUSTER et al. 1970; HENN et al. 1972). The presence of mitochondria was evident in fractions N and C_1, only their proportion is different. Most mitochondria were found in the nuclear fraction but some could have contaminated fraction C_1. Thus there is good reason to expect that the cytoplasmic DNA is inhomogeneous, contaminated by mitochondrial DNA, or even identical with mitochondrial DNA.

The latter may exist in two forms (NASS 1969). Predominating is the covalently closed, circular "component I". This is greatly resistant to denaturation and strand separation by heat and alkali. With the use of CsCl–Eth–Br gradients it is possible to separate "component I" from the linear DNA of the nucleus and from the open or loosely twisted linear mitochondrial DNA ("component II"; i.e. denatured and nicked DNA). "Component I" binds less dye and bands therefore at higher densities than linear DNA (RADLOFF et al. 1967). Alkaline sucrose velocity sedimentation also separates linear and circular DNA forms from each other. Up to now, no single-stranded mitochondrial DNA has been observed (BORST 1972).

Figs 1 and 2 show no evidence of the presence of circular DNA in our CsCl–Eth–Br gradients. Thus, any contamination by mitochondrial DNA must be limited to DNA molecules in "component II" form. This might happen if the majority of circular DNA molecules were converted by denaturation to linear DNA. This, however, does not seem likely.

The applied method of DNA extraction (HIRT 1967) selectively extracts the lower sedimenting components, while 80—90% of the fast sedimenting cellular DNA is precipitated. We have applied gentle stirring in the course of DNA extraction during SDS treatment, allowing an about 70—80% yield of all cellular DNA in supernatants of fraction N, in a form of identically shaped pieces like C_1 DNA.

Figure 1A and B show that the buoyant density of cytoplasmic DNA, 1.715 g/ml, was exactly the same as that of the "denser" band of nuclear DNA. The band separated at 1.696 g/ml in N (see also Fig. 2) indicates the position of satellite DNA (FLAMM et al. 1966a, b) which amounts to about 10% of all nuclear DNA and is specific only for nuclei or whole cells. A random contamination with nuclear fragments in the cytoplasm must have led to the appearance of the satellite band in C_1-DNA.

Alkaline sucrose velocity sedimentation revealed a striking similarity of C_1 and N. As seen in Fig. 3A and B, the peaks at 1.055 and 1.037 g/ml are

identical. Material sedimenting at "dense regions" of the gradients is also demonstrable in both fractions. It should be noted that 1.055 g/ml represents under our conditions 16 S sedimentation, a figure in good agreement with earlier data on DNA secreted by lymphocytes (ROGERS et al. 1972). We therefore assume that the alkaline sucrose sedimentation of fractions N and C_1 has separated the bulk of linear molecules from any contaminating DNA that sedimented significantly faster. The portion of fast sedimenting DNA did not exceed 17% of the total DNA contents (Fig. 3A and B). This could be the mitochondrial DNA contaminating fractions N and C_1 to about the same extent. The peaks appearing in Fig. 3A might therefore represent the complementary strands of alkali-denatured nuclear DNA. The peaks at 1.10—1.08 and 1.055 g/ml could indicate the position of complementary strands. The peak at 1.037 g/ml may be the position of smaller DNA fragments.

The presented data do not eliminate conclusively the possibility that the isolated cytoplasmic DNA consists of degraded mitochondrial DNA. On the other hand, on the basis of experiments in which we determined the melting profiles of nuclear and cytoplasmic DNA (BALÓ-BANGA and ANTONI 1973), we should suggest that the cytoplasm of tonsillar cells may contain a single-stranded fraction of nuclear DNA. This would also explain the striking difference between the diphenylamine-reaction determined amount of DNA and UV absorbing material. If the used standard is prepared from double-stranded DNA, irrationally low values are obtained with single-stranded DNA, considering that only deoxyribose bound to puriness will give a colour reaction. This fits well with the prediction based on the similar buoyant density to nuclear DNA (i.e. similar GC contents), that C_1 must have less purines and more pyrimidines, especially cytosine. This latter fact might correct also the DNA/protein ratio for C_1 presented in Table II.

The plasma-membrane association of this unique DNA species and detailed characterization of nuclear and non-nuclear DNA are open to further research.

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OXYGEN UPTAKE CAPACITY OF GILLS IN RELATION TO BODY SIZE OF THE AIR-BREATHING FISH, *Anabas testudineus* (Bloch)

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Oxygen consumption through the gills in relation to body weight was estimated in the air-breathing fish, *Anabas testudineus* under two experimental conditions, viz., (i) when air-breathing was allowed and (ii) when it was prevented. The lower value of the slope (b = 0.67) of the line for oxygen consumption to body weight (when air-breathing was allowed) in comparison to water-breathing teleosts (b = 0.85) was related to the air-breathing habit of the fish and the increase in the relative proportion of air-breathing surface with the increase in body weight of the fish. A further lowering of the slope (b = 0.5259) on preventing air-breathing has been related to the decrease in the efficiency of the respiratory membrane of gills under stressed conditions. The observed capacity of smaller fishes to survive for longer periods depending upon gill-breathing slope than those of large fishes has been related to the decrease in the oxygen uptake capacity of gills as fishes grow in size. The higher rate of oxygen consumption per unit body weight in small fishes than in larger ones has been related to their higher metabolic rate and the larger surface area of gills per unit body weight available for gaseous exchange.

In recent years, several investigations have dealt with the function of teleostean gills and the respiratory surface has been studied in relation to body weight in several species of teleosts (PRICE 1931; GRAY 1954; HUGHES 1966, 1970, 1972; MUIR and HUGHES 1969) to understand the nature of growth of the gill-sieve. Similarly, the rate of oxygen consumption as a function of body weight has been studied in many teleost species (SMITH 1935; SCHOLANDER et al. 1953; JOB 1955; WINBERG 1956; PARVATHESWARARAO 1960; SAUNDERS 1963; PALOHEIMO and DICKIE 1965; BRETT 1965). These results indicated that the relationship of oxygen consumption to weight bears the same relationship as gill area to body weight. Thus, it has been suggested that some relationship might exist between the size of the respiratory surface and the rate of oxygen consumption among fishes (WINBERG 1956). As most of these growth studies were restricted to water-breathing fishes (having only gills for gaseous exchange), nothing is known about this relationship in air-breathing fishes, where gaseous exchange takes place both from air and water through the accessory respiratory organs and gills. Recently, HUGHES et al. (1973, 1974) studied the size of the respiratory surface of gills and air-breathing organs in relation to body weight in *Anabas testudineus* and *Saccobranchus (= Heteropneustes) fossilis*. In the present study an analysis has

been made of the oxygen uptake capacity of the gills in relation to body weight of the air-breathing climbing perch, *Anabas testudineus*, when it is free to breath both air and water and when air-breathing has been prevented. These results will be discussed as related to body weight, the extent of gill area and the asphyxiation of *Anabas* when not allowed to breath air.

Methods

Living specimens of different sizes of *Anabas testudineus* (Bloch) were collected from Purnea and nearby places in Bihar, India and maintained in large glass aquaria, with continuous flow of water. The fishes were fed on goat liver pieces on alternate days during a minimum acclimation period of one month in the laboratory.

The rate of oxygen consumption through the gills was measured in *Anabas* of different body weights under two experimental conditions, viz. (1) when access to air was allowed and (2) when it was prevented. Two types of self-designed spirometers were used. In the first series of experiments, a rectangular acroplex box of approximately three litre capacity, having a small air chamber at the top (Fig. 1A) was used; here the fish was free to breath

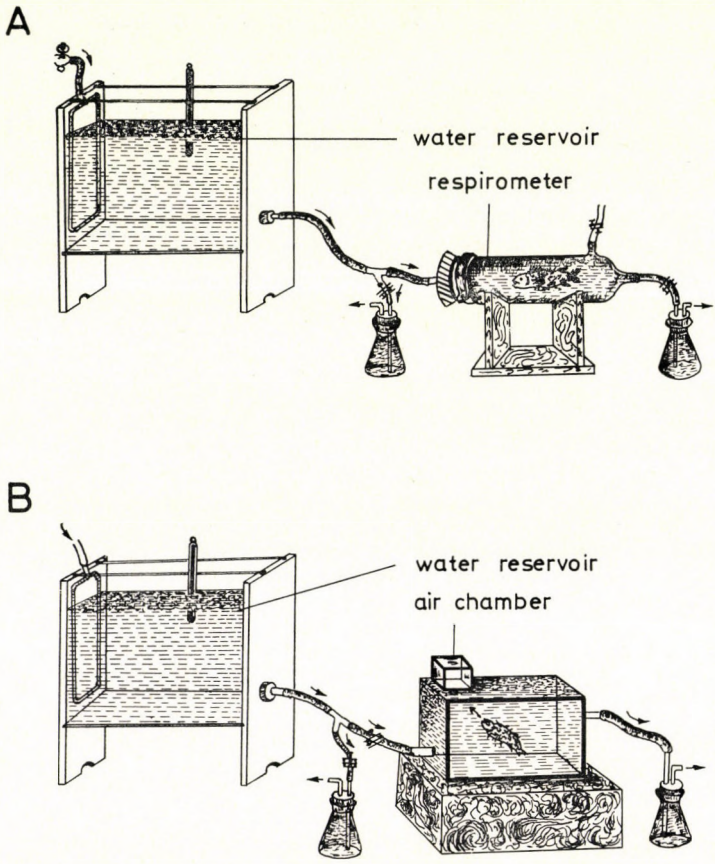

Fig. 1. Experimental setup for measuring oxygen consumption through gills with (A) and without (B) air-breathing

air in addition to gill-breathing. The air chamber at the top of the spirometer was constructed to minimize gaseous exchange at the air-water interface. The fish becomes adapted to this spirometer in a few hours and then goes freely up to the air-chamber to inhale air. For the second series of experiments, a big cylindrical glass spirometer was constructed with arrangements for continuous water flow through it and the removal of enclosed air (Fig. 1B).

The fish was introduced in the spirometer which was connected to a large constant level water tank to maintain the flow of water under constant hydrostatic pressure. The water entered the spirometer at one side and the rate of its flow per unit time was measured as it left the other side. The flow of water through the spirometer was adjusted according to the size of the fish, so that its metabolism did not reduce the oxygen content of the ambient water to below 80% of the air saturation level. The fish was acclimated to the spirometer at least overnight before the observations has begun. Two bottles were connected at the two ends of the spirometer to collect the water supplied and the ambient water.

Concentration of the dissolved oxygen in the samples collected was estimated by Winkler's volumetric method (WELCH 1948). The difference in the oxygen levels between the ambient water and that supplied to the spirometer, together with the rate of water flow and the weight of the fish were used to calculate the rate of oxygen consumption per unit body weight of the fish. Subsequent readings were taken at one hour intervals. Opercular frequencies were counted for a period of one minute at intervals of one hour and the amplitude of their movements was estimated by visual observation. The observations were recorded at $27.5 \pm 1°C$. Regression analysis using logarithmic transformations was made in order to find out the relationship of body weight with oxygen consumption through the gills of *Anabas*.

Results

Anabas testudineus is a habitual air-breathing fish and has well developed organs for respiration (MUNSHI 1968). When kept in water it comes to the surface at irregular intervals to inhale air in addition to normal gill-breathing.

The interval between the air-breaths vary from two minutes to fifteen minutes depending on the concentration of oxygen dissolved in the water. In water containing 4.2 to 5.6 ml/lit. dissolved oxygen and with free access to air, *Anabas* shows 30—45 opercular movements per minute. When access to air is prevented, the fish becomes restless in search of air, but the restlessness slowly decreases and the fish settles down at the bottom of the spirometer. However, the amplitude and frequency of opercular movements increase to 60—65 per minute.

Mean oxygen consumption per unit time of different weight groups of fishes under the two experimental conditions are summarized in Table I. Results indicated a marked effect of body size on the rate of oxygen consumption. When oxygen consumption has been plotted against body weight on log/log co-ordinates and the scores were fitted by the least square regression method, it gave a straight line. Thus the relationship between the rate of oxygen consumption and the body weight obeyed the equation,

$$X = aW^b$$

$$\text{or } \log X = \log a + b \log W, \tag{1}$$

where X = rate of oxygen consumption (ml/hr)
a = rate of oxygen consumption for 1 g fish (ml/hr)
b = regression coefficient (slope)
W = body weight (g).

I. Oxygen consumption from water when the fish has free access to air

When *Anabas* was kept in the spirometer with free access to air, its oxygen consumption from water ranged from 0.71 to 2.83 ml/hr with an increase in the body weight from 8.0 to 68.0 g. The log/log plot of the rate of

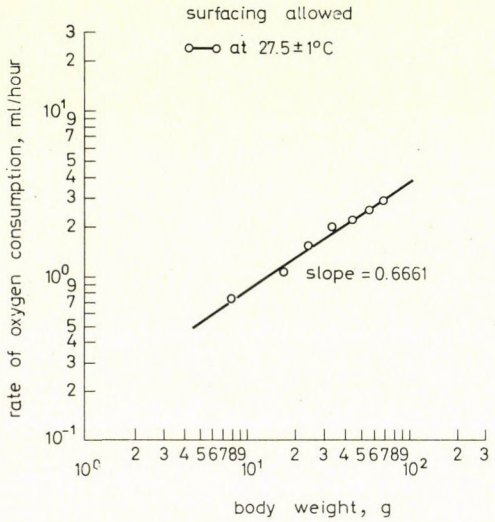

Fig. 2. Log/log plot of oxygen consumption rate (ml/hr) and body weight when air-breathing was allowed

oxygen consumption to weight gave a straight line with a slope of 0.6661 (Fig. 2) and the estimated value of oxygen consumption for one gram fish was found to be 0.1764 ml/hr (Table I). Thus, equation (1) after substitution becomes

$$\log X = \log 0.1764 + 0.6661 \cdot \log W . \qquad (2)$$

Equation (2) indicates that with a unit increase in the body weight of *Anabas*, the rate of oxygen consumption will increase by a fractional power of 0.6661 and thus oxygen consumption per g body weight remains higher in smaller fishes (88.99 ml/kg/hr) than in large ones (41.74 ml/kg/hr; Table I; Fig. 3). There was a good correlation between body weight and oxygen consumption rate, the correlation coefficient being 0.9947 (Table II).

Table I

Rate of oxygen consumption via gills of Anabas testudineus *different in size under two experimental conditions*

Mean oxygen consumption rate with access to air			Mean oxygen consumption rate with no access to air		
body weight (g)	$\dot{V}O_2$ (ml/hr)	$\dot{V}O_2$ (ml/kg/hr)	body weight (g)	$\dot{V}O_2$ (ml/hr)	$\dot{V}O_2$ (ml/kg/hr)
8.0	0.71	88.99	8.0	1.48	185.34
17.0	1.08	63.47	17.0	2.89	169.94
24.0	1.51	62.82	29.0	3.52	119.25
34.0	2.01	59.12	33.0	3.02	91.38
44.0	2.20	40.96	42.0	3.82	90.93
55.0	2.53	45.92	54.0	4.02	74.45
68.0	2.83	41.74	65.0	5.26	80.93

Table II

Regression and correlation coefficients to show the relation of O_2 consumption via gills and body weight

Experimental condition	Regression coefficient	Correlation coefficient	Intercept a
In flowing water with access to air	0.6661	0.9947	0.1764
In flowing water with no access to air	0.5259	0.9569	0.5453

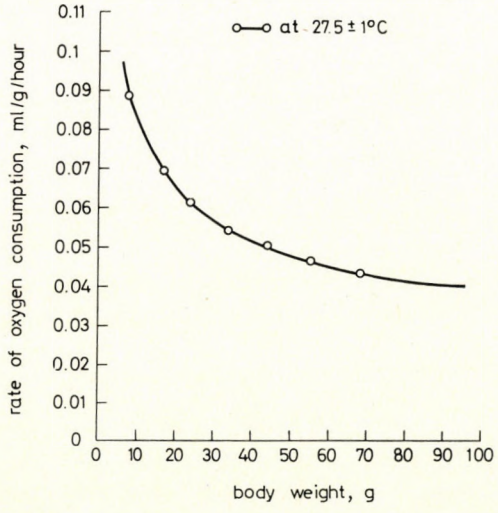

Fig. 3. Oxygen consumption (ml/g/hr) and body weight plot, when fish had free access to air

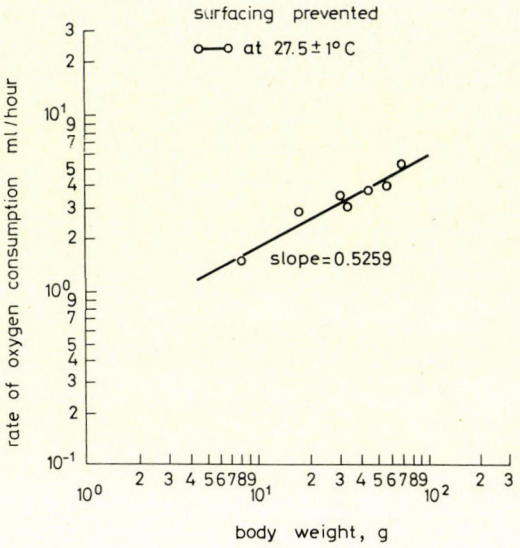

Fig. 4. Log/log plot of oxygen consumption (ml/hr) and body weight when air-breathing was prevented

II. Oxygen consumption from water with surfacing prevented

Under such conditions, the fish could obtain oxygen only from water. The marked increase in the ventilatory movements of the fish resulted in an increased oxygen consumption. Mean oxygen consumption under this particular experimental condition ranged from 1.48 to 5.26 ml/hr for the different weight groups of *Anabas* (Table I). This was much more than the oxygen consumed by *Anabas* of practically the same body weights under the first experimental conditions. The log/log plot of the rate of oxygen consumption against body weight gave a straight line on fitting the scores by the least square regression method (Fig. 4). The slope of the line was 0.5259, much less than the value obtained when air-breathing was allowed (Table II). The estimated value of a, i.e. of the rate of oxygen consumption for one g fish was 0.5453 ml/hr. Thus, after substitution, the equation is

$$\log X = \log 0.5453 + 0.5259 \cdot \log W \qquad (3)$$

indicating that the rate of oxygen consumption of *Anabas* under such conditions increases by a fractional power of 0.5259 with every unit increase in body weight. Thus, the rate of increase in oxygen consumption from water alone with the increase in the size of *Anabas* was comparatively less when air-breathing was not allowed than when the fish was breathing both air and water. However, the rate of oxygen consumption per unit body weight re-

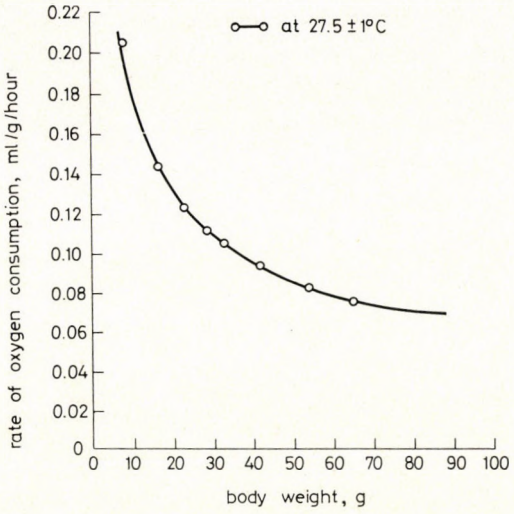

Fig. 5. Oxygen consumption per unit weight against body weight when air-breathing was prevented

mained high (80.93 to 185.34 ml/kg/hr) when air-breathing was prevented than when it was allowed (Table I). Oxygen consumption per unit body weight was higher in small fishes than in large ones (Fig. 5).

The correlation coefficient ($r = 0.9569$) again indicated that the rate of oxygen consumption was closely related to the weight of the fish.

Discussion

The rate of oxygen consumption through the gills of *Anabas* gives a straight line when the logarithm of the rate is plotted against the logarithm of body weight. Thus, the relationship of the metabolic rate to body weight in this air-breathing fish was similar to that found in other water-breathing teleosts. The slope of the logarithmic line for standard metabolism against body weight varied slightly among different species of teleosts. JOB (1955) found 0.85 for *Salvelinus fontinalis;* WINBERG (1956) obtained 0.81 as an average value for a number of species; and PALOHEIMO and DICKIE (1965) suggested 0.80 to be the value characteristic for most species of teleosts. In comparison to these values, the obtained value of the slope (0.67) for oxygen consumption through the gills to body weight (log/log) was much less when the fish was allowed to breath both air and water than under normal conditions. This was obviously due to the air-breathing habit of the fish which supplements the total oxygen consumption of the species. Measurements of the surface of gills and of the accessory respiratory organs of *Anabas* (HUGHES

et al. 1973) indicated that the rate of increase in the area of accessory respiratory organs (b = 0.713) was higher than that for the gills (b = 0.615) during their growth. Therefore, it is expected that in large fishes the proportion of oxygen uptake through the air-breathing organs will dominate over that of the gills. Secondly, the thickness of the blood–water barrier in the secondary gill lamellae increases in large fishes (b = 0.2272; DUBE and MUNSHI 1973a). Such an increase in the thickness of the barrier in secondary gill lamellae of tench has been suggested to decrease their diffusing capacity as the body size increases (HUGHES, 1972). It is, therefore, suggested that in larger fishes there will be an increasing dependence on the air-breathing organs for extracting oxygen from the environment which results in a lowering of the slope of oxygen consumption through the gills to the body weight line.

When *Anabas* is not allowed to breath air, its ventilatory movements increase in both frequency and amplitude which seems to be a homeostatic attempt (HUGHES, 1964) to compensate the functioning of the accessory respiratory organs in meeting the total oxygen demand of the fish. Under normal conditions of water and air-breathing, the rate of oxygen consumption via the gills of *Anabas* ranges from 41.74 to 88.99 ml/kg/hr, which increases to 80.93 to 185.34 ml/kg/hr when air-breathing is prevented (Table I). This increase is up to a certain extent due to the increased activity of the fish. Such an increase in the rate of oxygen consumption associated with an increase in the ventilatory movements was observed also by SAUNDERS (1962) in carps, white suckers and brown bullheads and by HUGHES and SINGH (1970) in *Anabas*, which they related to the increase in the effectiveness of gaseous exchange through the gills due to the increased volume of water irrigating the gills per unit time.

The slope of log oxygen consumption to log body weight line was 0.5259 when air-breathing was not allowed. This was less than the value of 0.6661 found when air-breathing was allowed. This reduction in the slope of the logarithmic line indicates that under stressed conditions the capacity of the gills to take up oxygen decreases as the fish grow in size. As a result, the rate of oxygen consumption did not increase satisfactorily with the increase in body weight and the logarithmic line was depressed towards the upper end of body size (Fig. 4).

The respiratory surface area of the gills (HUGHES et al. 1974), when expressed as the area across which each ml of oxygen is transferred per minute (surfacing prevented), the ratio of the surface area to oxygen consumed per unit time was less in small fishes, and increased with the increase in body weight (Table III). This means that more gill surface is required for the uptake of each ml of oxygen per unit time in large fishes, and perhaps the respiratory membrane of the gills of small fishes is more efficient in oxygen uptake than that of large ones.

The drowning experiments conducted on *Anabas* by BOAKE (1865—66), DOBSON (1874), DAS (1927) and GHOSH (1934) indicated that fishes belonging to this species become asphyxiated within 12 minutes to 3 hours and 30 minutes, when not allowed to breath air. HORA (1935), on the other hand, observed that while some of the specimens of *Anabas* were asphyxiated within 15 minutes, others survived indefinitely under similar conditions. Thus, his

Table III

Respiratory area for unit volume of oxygen consumed (ml O_2/min) Anabas *of different sizes (computed by extrapolation of regression lines for gill area and oxygen consumption rate against body weight)*

Body weight (g)	Gill area/ml oxygen consumed/min (mm^2)
10.0	382.0
20.0	398.6
30.0	416.8
40.0	426.3
50.0	433.9
60.0	437.6
70.0	445.6
80.0	451.8
90.0	456.7
100.0	460.7

observations were in agreement with the results of BOAKE (1865—66) in that the period of drowning varies with different individuals. The cause of this intraspecific variation in asphyxiation time during drowning experiments was not clear in the above mentioned studies. In the present study it has been observed that the asphyxiation time for *Anabas*, when not allowed to breath air, varies with the size of the fish, and as the body size increases the asphyxiation time decreases. This again supports the previously discussed view that the capacity of gills to take up oxygen decreases as the fish grows in size and, as a result, gill-breathing by itself can sustain a larger fish for a shorter period than a small one. This was further supported by the morphometric determinations of the diffusing capacity of gills of *Anabas* (DUBE and MUNSHI 1973b).

HUGHES and SINGH (1970) found oxygen consumption (from water) of *Anabas* at 25 $\pm$ 1°C to vary from 45.44 to 61.88 ml/kg/hr, when the fish was allowed to breath both air and water, and from 70.7 to 85.0 ml/kg/hr when air-breathing was prevented. When these values have been compared with

the present observations (Table I), the latter seem to be slightly higher, which was related to the higher temperature (27.5 ± 1°C) of the ambient water during the present observations.

Oxygen consumption per unit body weight was higher in smaller fishes but as their weight increases, the rate of oxygen consumption decreases sharply in the beginning up to about fifty g body weight and thereafter very slightly (Figs 3 and 5). In water-breathing fishes this phenomenon has been related to the higher metabolic rate of smaller fishes (FRY 1957). It may also be related to the larger surface area of gills per unit body weight of *Anabas* (HUGHES et al. 1973) available for gaseous exchange in smaller fishes than the large ones.

HUGHES and SINGH (1970) reported that specimens of *Anabas* weighing about 29 to 51 g consume about 53.6% of their total oxygen requirement through the air-breathing organs. DUBE (1972) found that the air-breathing surface in fishes of this size comprises about 42 to 43% of the total respiratory area. Thus it is suggested that larger fishes having a larger respiratory surface will naturally depend much more on air than on gill breathing because of the higher diffusing capacity of air-breathing organs than of gills (HUGHES et al. 1973). It will be interesting to investigate the relative rates of oxygen consumption through gills and air-breathing organs in different weight groups of *Anabas*.

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DIURNAL CHANGES IN HYPOTHALAMIC SEROTONIN CONTENT AND ITS CORRELATION WITH ADRENAL FUNCTION IN THE RAT DURING THE OESTRUS CYCLE

By

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Hypothalamic serotonin content and plasma corticosterone level have been measured daily at 8 a.m., 4 p.m., and 11 p.m., during different phases of the oestrus cycle in rats.

The hypothalamic serotonin level showed a diurnal variation with a peak at 8 a.m. and the lowest level at 11 p.m. The morning level during prooestrus and oestrus was significantly lower than in metoestrus and dioestrus. The decrease in the hypothalamic serotonin level observed at 11 p.m. was significant in each phase of the cycle but it was the most marked during prooestrus.

The plasma corticosterone level showed an opposite trend. It was low in the morning and high at 11 p.m. in each phase of the oestrus cycle except during oestrus when there was no significant difference between the morning and the late evening value. The morning values in prooestrus and oestrus were twice as high as those in the metoestrus and dioestrus phases.

Ovariectomy did not abolish the daily serotonin rhythm in the hypothalamus, but the morning value was significantly lower than in the prooestrus, metoestrus and dioestrus phases. Oestrone and progesterone treatment increased the morning hypothalamic serotonin level of castrated female rats.

In the last years increasing number of data pointed to the participation of monoamines in the release of gonadotropic hormones.

Histochemical studies showed that dopaminergic cells in the hypothalamus undergo marked changes during the oestrus cycle (FUXE and HÖKFELT 1966). Castration was followed by a reduced dopamine fluorescence in the hypothalamus, which was reversed on the administration of oestrogen or androgen (LICHTENSTEIGER 1969).

Dopamine in vitro increases the FSH releasing activity of the hypothalamus (KAMBERI et al. 1970b), and, if injected into the 3rd ventricle, it increases the LH releasing activity of pituitary stalk plasma (KAMBERI et al. 1969, 1970a). Norepinephrine and epinephrine given intracerebrally facilitate ovulation in barbiturate-blocked animals (RUBINSTEIN and SAWYER 1970). The hypothalamic norepinephrine content changes with the cycle; it is highest during prooestrus (STEFANO and DONOSO 1967). Drugs inhibiting catecholamine synthesis, such as α-methyl-p-tyrosine are inhibiting ovulation (BROWN 1967; KORDON and GLOWINSKI 1969).

Serotonin injected intraventricularly decreases luteinizing hormone secretion (SCHNEIDER and MCCANN 1970; KAMBERI et al. 1970a) while on systemic administration or when injected into the hypothalamus, it inhibits the gonadotropin-induced ovulation in immature animals (O'STEEN 1964, 1965; KORDON et al. 1968).

Monoamine oxydase inhibitors are also inhibiting ovulation (KORDON and GLOWINSKI 1972; LABSETWAR 1972). Injection of serotonin into the 3rd ventricle stimulates prolactin secretion but blocks FSH and LH release (KAMBERI et al. 1971; SCHNEIDER and MCCANN 1970).

In previous experiment it has been shown that the hypothalamic serotoninergic system exerts an inhibitory influence on the hypothalamo-pituitary-adrenal system (VERMES and TELEGDY 1972; VERMES et al. 1972; TELEGDY and VERMES 1973).

In the present study, the correlation between the diurnal rhythm of the hypothalamic serotonin content and adrenal function during different stages of the oestrus cycle and the role of sexual steroids in the regulation of hypothalamic serotonin content has been studied in rats.

Methods

Female adult CFY rats weighing 150—200 g were used. The animals were kept under a standard diet and light schedule, with 12 hours light and 12 hours dark periods. The light period started at 6 a.m. The oestrous cycle was controlled by vaginal smears at least two weeks prior to sacrifice and only regular 4-day-cycle-animals were used. The oestrus cycle was divided into four stages: prooestrus, oestrus, metoestrus and dioestrus. In each stage the animals were killed by decapitation at 8 a.m., 4 p.m., and 11 p.m.

In order to study the effect of castration on the hypothalamic serotonin content, the animals were overiectomized three weeks prior to testing. The hypothalamic serotonin content was measured at 8 a.m., 4 p.m., and 11 p.m.

To test the action of oestrogen and progesterone, the castrated animals were treated with 20 μg/animal of oestrone-acetate (Hogival, Richter, Budapest) and with 1 mg/animal of progesterone (Glanducorpin, Richter, Budapest) for six days. The animals were sacrificed 24 hours after the last injection.

Hypothalamic serotonin content was estimated by the method of SNYDER et al. (1967), the plasma corticosteron according to PURVES and SIRETT (1965).

Statistical analysis was done by Student's t-test.

Results

The hypothalamic serotonin content showed a remarkable fluctuation during the oestrus cycle (Table I, Fig. 1). It was significantly higher in the early morning hours than in the evening in each stage. However, in prooestrus and oestrus the morning values were significantly lower than in metoestrus and dioestrus. The most pronounced diurnal changes occurred during prooestrus, when in the hypothalamus the evening serotonin level was lowest.

Table I

Diurnal changes in hypothalamic serotonin content (μg/g) during oestrus cycle and after castration

Cycle	8 a.m.[a]	4 p.m.[b]	11 p.m.[c]	Significance
1. Prooestrus	1.90 ± 0.04* (21)**	2.13 ± 0.07 (22)	0.65 ± 0.04 (29)	1a vs. 3a $p < 0.01$ 1a vs. 1c $p < 0.001$ 1a vs. 4a $p < 0.05$
2. Oestrus	1.86 ± 0.08 (17)	1.00 ± 0.05 (15)	1.39 ± 0.10 (15)	2a vs. 2c $p < 0.001$ 2a vs. 3a $p < 0.01$ 2a vs. 4a $p < 0.05$
3. Metoestrus	2.17 ± 0.08 (17)	1.36 ± 0.06 (15)	1.22 ± 0.14 (15)	3a vs. 3c $p < 0.001$
4. Dioestrus	2.05 ± 0.05 (27)	1.02 ± 0.09 (15)	1.11 ± 0.07 (31)	4a vs. 4c $p < 0.001$
5. Castrated	1.73 ± 0.04 (22)	1.42 ± 0.03 (16)	1.30 ± 0.05 (16)	5a vs. 5c $p < 0.001$ 5a vs. 1a $p < 0.01$ 5a vs. 3a $p < 0.001$ 5a vs. 4a $p < 0.001$

* Mean ± S.E.
** In brackets, number of rats

Following castration the hypothalamic serotonin content was significant ly lower in the morning than in any other stage of the oestrus cycle except oestrus (Table I). Oestrone and progesterone treatment significantly increased

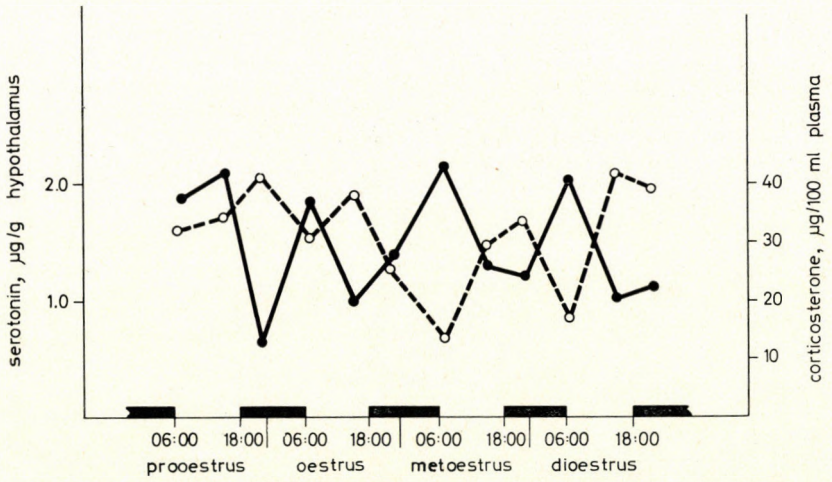

Fig. 1. Diurnal changes in hypothalamic serotonin content and plasma corticosterone during oestrus cycle. Solid line, serotonin; dotted line, corticosterone

Table II

Diurnal changes in plasme corticosterone content (μg/100 ml) during oestrus cycle and after castration

Cycle	8 a m.[a]	4 p.m.[b]	11 p.m.[c]	Significance
1. Prooestrus	30.2 ± 2.8* (41)**	33.8 ± 2.8 (22)	41.3 ± 2.6 (29)	1a vs. 1c $0 < 0.001$ 1a vs. 3a $p < 0.01$ 1a vs. 4a $p < 0.01$
2. Oestrus	32.6 ± 3.8 (34)	38.2 ± 3.2 (15)	25.6 ± 3.4 (15)	2a vs. 2c $p < 0.01$ 2a vs. 3a $p < 0.01$ 2a vs. 4a $p < 0.001$
3. Metoestrus	13.4 ± 1.7 (17)	29.3 ± 3.9 (15)	33.8 ± 2.6 (15)	3a vs. 3c $p < 0.001$
4. Dioestrus	16.5 ± 4.6 (27)	42.5 ± 4.3 (15)	38.0 ± 2.7 (31)	4a vs. 4c $p < 0.001$
5. Castrated	10.4 ± 1.0 (22)	28.0 ± 1.9 (16)	23.3 ± 1.4 (16)	5a vs. 5c $p < 0.001$

* Mean ± S.E.
** In brackets, number of rats

the morning serotonin level in the hypothalamus as compared to the castrated controls ($p < 0.05$ and $p < 0.01$, respectively; Fig. 2).

The plasma corticosterone level showed an opposite trend (Fig. 1). The morning level was higher than the evening level (11 p.m.) in each stage of the cycle (Table II). In prooestrus and oestrus, the plasma corticosterone level was twice that observed during metoestrus and dioestrus.

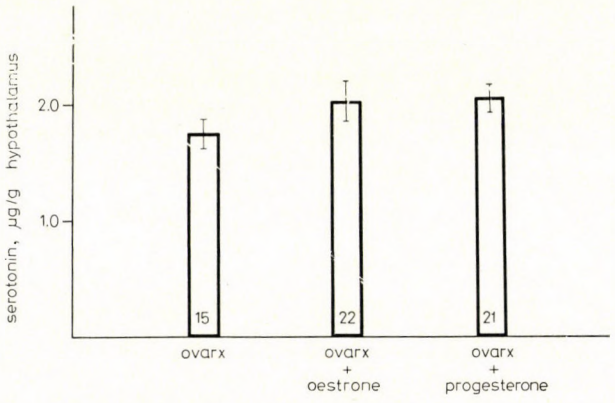

Fig. 2. Effect of oestrone and progesterone treatment on the hypothalamic serotonin content in castrated female rats. Number in the bars represents the number of animals used, the height of the bar the mean values and the standard error

Discussion

The presented data show a diurnal fluctuation of the hypothalamic serotonin level in female rats, with a high value in the morning and a low one in the evening. Similar results have been reported for the whole mouse (ALBRECHT et al. 1956) and the rat brain (DIXIT and BUCKLEY 1967; SCHEVING et al. 1968; ASANO 1971; OKADA 1971) for the telencephalon, diencephalon, mesencephalon and rhombencephalon (BOBILLIER and MOURET 1971), for different cortical (QUAY 1965) and subcortical areas (QUAY 1968) and for the hypothalamus (HÉRY et al. 1972). There are, however, authors who found no differences (SCAPAGNINI and PREZIOSI 1972).

The evening drop of the hypothalamic serotonin content was most significant during prooestrus, the time when LH is released from the hypophysis (FEDER et al. 1969; BROWN-GRANT 1972). This is in agreement with the inhibition of LH secretion by serotonin (KAMBERI et al. 1970a). Our finding would indicate that in the "critical period" of prooestrus the serotonin is released from the hypothalamus and this way the hypothalamus is deliberated from the inhibition of serotonin which is associated with an increase in LH secretion. It seems that the event of ovulation is controlled by a reciprocal innervation in which catecholamines and serotonin are involved. Catecholamines are stimulating LH secretion (KAMBERI et al. 1970a, b; RUBINSTEIN and SAWYER 1970) while serotonin is inhibiting it (SCHNEIDER and MCCANN 1970; KAMBERI et al. 1970a). During the critical period in prooestrus, catecholamines are increased (STEFANO and DONOSO 1967) while serotonin according to our data is decreased in the hypothalamus. The proper balance of these two factors would result in ovulation. Ovulation is blocked if there is a lack of catecholamines after the inhibition of catecholamine synthesis by α-methyl-p-tyrosine (BROWN 1967; KORDON and GLOWINSKI 1969) if serotonin content is increased by the local application of monoamine oxidase inhibitors (KORDON et al. 1968; KORDON 1969). Drugs which increase the level of serotonin in the hypothalamus are blocking LH release and consequent ovulation.

The diurnal fluctuation of serotonin is characteristic phenomenon. It has been shown previously (VERMES and TELEGDY 1972) that serotonin is inhibiting the stress mechanism on the hypothalamic level. The reciprocal correlation with the hypothalamic serotonin level and plasma corticosterone would also indicate that a low hypothalamic serotonin level is associated with a high plasma corticosterone level.

The fluctuation of plasma corticosterone during the oestrus cycle has been well documented (CRITCHLOW et al. 1963; HIROSHIGE 1973). Oestrogen in physiological amounts is stimulating the pituitary-adrenal axis (KITAY 1963) while in large doses it inhibits it (TELEGDY et al. 1964, 1967). Oestrogen may act also at the hypothalamic level (TELEGDY et al. 1962).

The morning plasma corticosterone level is highest and the hypothalamic serotonin level is lowest during prooestrus and oestrus. This would indicate that the ovarian hormones may modify the hypothalamic serotonin level and in return change the reactivity of the hypothalamo–pituitary–adrenal axis. This is further supported by the fact that the hypothalamic serotonin content decreases after castration and increases following oestrogen and progesterone treatment. Similar results have been obtained in mice by GREENGRASS and TONGE (1972).

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MICROELECTROPHYSIOLOGICAL ANALYSIS OF THE CORRELATION BETWEEN THE REDOX STATE AND THE TYPE OF EFFECT OF ACETYLCHOLINE AND/OR 5-HYDROXYTRYPTAMINE

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Experiments were carried out on giant neurones of *Lymnea stagnalis* and it was established that, (1) the depolarizing effect of acetylcholine on D type cells was enhanced by oxidizing agents (methylene blue, thionine) and at the same time, H type cells became D type ones after the application of the said agents; (2) redox catalysts of the reducing type such as ascorbic acid, hydroquinone, p-methyl-aminophenolsulphate, caused an expressed inhibition of ACh depolarization on D type cells. In some cases, the D type character of cells was converted into H type one. Experiments with nicotinic acid gave variable results. ACh depolarization was inhibited in some cases and augmented in others presumably owing to the bimodal antagonistic site of effect of nicotinic acid.

The depolarizing effect of 5-TH was inhibited by both the oxidizing and the reducing agents, although less markedly than in the case of ACh.

On the basis of the results it seems that the actual redox state of cells plays a decisive role in the definition of the D or H type of mediator effect.

The sites of the redox effect may be explained at the levels of a) changes in the activity of transport enzymes; b) changes in the molecular conformation and charges of plasmamembrane; and c) quantum biological changes of some charge transfer processes between the receptor and the mediator.

In previous studies a close correlation was shown to exist between the redox state of the tissues and the type of the effect of acetylcholine (PUPPI et al. 1971a), adrenaline (PUPPI et al. 1971b) and 5-hydroxytryptamine (PUPPI et al. 1973).

It has been observed that a shift towards the oxidizing direction results in a strengthening or manifestation of the cholinergic depolarizing and the adrenergic hyperpolarizing tendencies, while the tryptaminergic effects do not change following such a drift in the E_0'. At the same time, following a shift in the reducing direction, cholinergic hyperpolarizing and adrenergic and tryptaminergic depolarizing effects occur.

Considering that a similar correlation can be observed between the original redox potential of various tissues and the characteristic type of mediator effect on these tissues, it was concluded that the different types of effects of mediators on different tissues were due to differences in the E_0' of the latter.

To demonstrate the common character of the aforementioned phenomenon, experiments were done to elucidate whether the correlations referred to above are true for bioelectrical parameters of the some of neuronal cells.

The experiments were carried out on giant neurones of *Lymnea stagnalis*, for its well identifiable H and/or D type cells, offering the possibility that depolarizing and/or hyperpolarizing type ACh and/or 5-HT effects can be examined on the same ganglion (ZEIMAL and VULFIUS 1968; KISS and SALÁNKI 1971).

Methods

Examinations were conducted on the abdominal and right parietal ganglia of CNS of *Lymnea stagnalis*. The isolated ganglion ring was placed in a chamber containing 3 ml saline (JULLIEN and RIPPLINGER 1948). The thick connective tissue was removed from the dorsal surface of the ganglia, thus the cells were visible.

Membrane and action potentials were recorded by means of glass microelectrodes filled with 2.5 M KCl. The electrode resistance ranged between 5—10 Mohm. The electrodes were connected with an amplifier with FET input and negative capacitance (VÉRÓ 1971).

In the course of the experiments, the electric signs were recorded on tape and the desired parts were later photographed by means of an EMG indicator and a DISA photorecorder. In experiments where the membrane potentials were shifted artificial a measuring bridge was connected before the amplifier (KISS et al. 1972). The chemicals used were given near to the ganglia into the chamber by pipettes. After the reaction had subsided, the chamber was washed until the starting activity level had been restored.

The following chemicals were used: Acetylcholine hydrochloride (Fluka) 30 μg/ml; 5-hydroxytryptamine creatinine sulphate (Fluka) 30 μg/ml; methylene blue (Reanal) 30 μg/ml; thionine (Fluka) 30 μg/ml; nicotinic acid (KGYT) 30 μg/ml; p-methyl-amino-phenol-sulphate (Reanal) 30 μg/ml; ascorbic acid (Reanal) 30 μg/ml; hydroquinone (Reanal) 30 μg/ml

Results

I. Acetylcholine (ACh)

1. Oxidizing agents

a) Methylene blue. This electron acceptor agent increased the depolarizing effect of ACh on D type cells (Fig. 1) while on H cells it suspended the hyperpolarizing ACh effect or in some cases transformed it into a depolarizing type

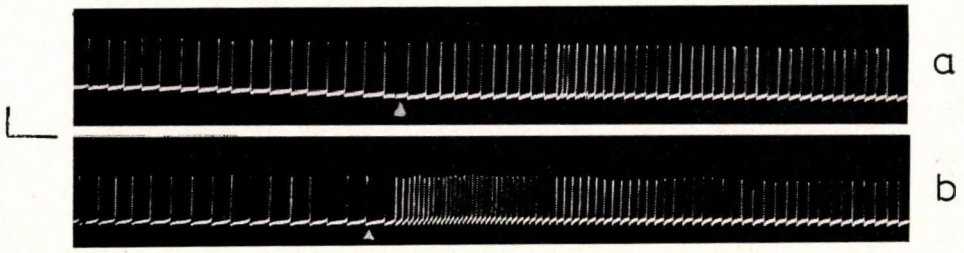

Fig. 1. Influence of methylene blue on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh action on the same cell after washing and 3 minutes methylene blue pretreatment. Δ: Administration of ACh. Calibration: 50 mV, 0.33 sec

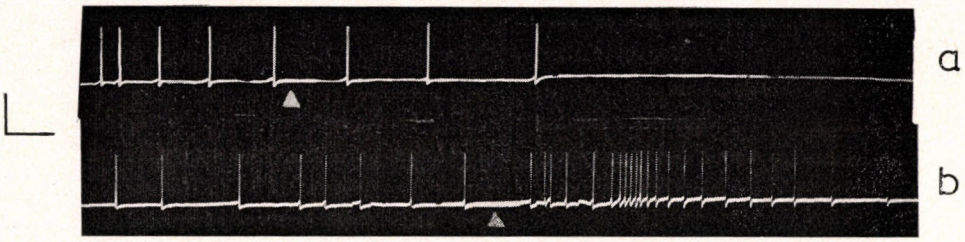

Fig. 2. Influence of methylene blue on the hyperpolarizing effect of ACh (H cell). a: Control ACh action; b: ACh action on the same cell after washing and 3 minutes methylene blue pretreatment. ▲: Administration of ACh. Calibration: 50 mV, 0.33 sec

one (Fig. 2). This feature was characteristic in 90% of the experiments. In the rest, methylene blue was without effect but a methylene blue inhibiting effect was never observed.

In the D cells the Δ cps% values of control ACh actions were equal to +67%, while on the same cells this Δ cps% value increased to +102 ($p < 0.002$) following methylene blue treatment. (Δ cps% = per cent difference between basis frequency and the frequency after ACh treatment).

b) Thionine. After pretreatment with this typical electron acceptor, the type of the ACh effect changed similarly as in the case of methylene blue. On D type cells the Δ cps% values for control ACh oscillograms were equal to +87, but after thionine pretreatment this value rose to +114 ($p < 0.01$). In 88% of our experiments, thionine increased the depolarizing action of ACh, while in 12% this effect was absent. A typical thionine effect is demonstrated in Fig. 3.

c) Nicotinic acid. The effect of this compound was not so definite as that of methylene blue and thionine. In 40% it enhanced, while in 50% it inhibited the depolarization, in 10% it had no effect. In the experiments where nicotinic acid increased ACh depolarization, the control Δ cps% values were elevated from +29 to +155 ($p < 0.001$) while in the experiments in which nicotinic

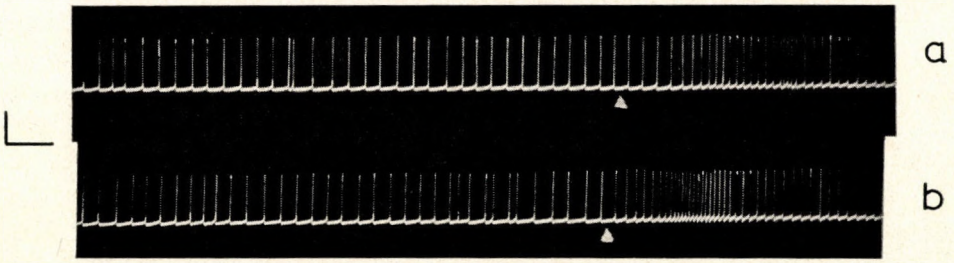

Fig. 3. Influence of thionine on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh action on the same cell after washing and 3 minutes thionine pretreatment. ▲: Administration of ACh. Calibration: 50 mV, 0.33 sec

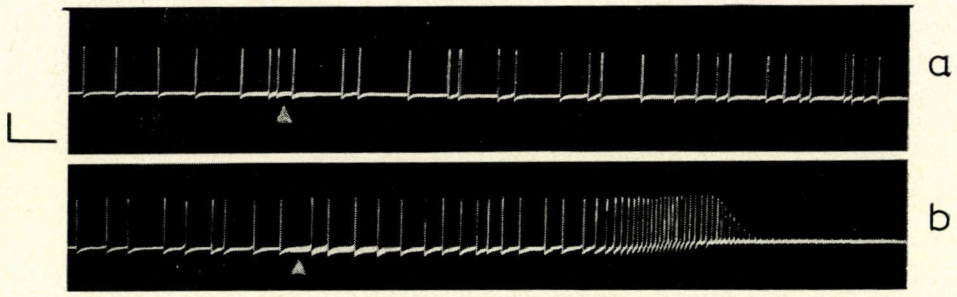

Fig. 4. Increasing effect of nicotinic acid on the depolarizing action of ACh (D cell). a: Contro ACh action; b: ACh effect on the same cell after washing and 3 minutes nicotinic acid pretreatment. *Δ*: Administration of ACh. Calibration: 50 mV, 0.33 sec

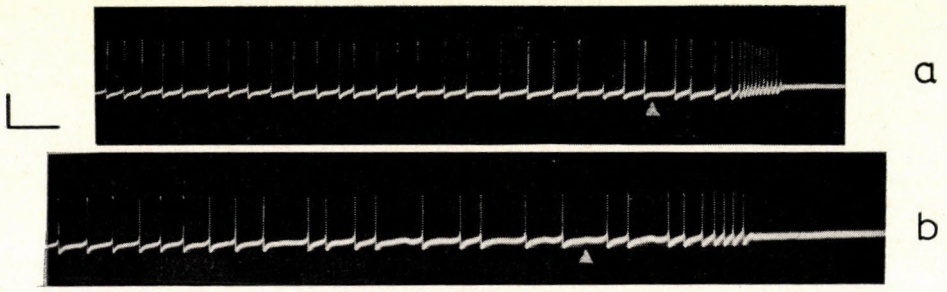

Fig. 5. Inhibitory effect of nicotinic acid on the depolarizing action of ACh (D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes nicotinic acid pretreatment. *Δ*: Administration of ACh. Calibration: 50 mV, 0.33 sec

acid was inhibitory, these values changed from +201 to +42 ($p < 0.001$). The enhancing type is demonstrated in Fig. 4, the inhibiting one in Fig. 5; in both cases, significant changes occurred in spike frequency as well as in membrane polarization.

2. Reducing agents

a) Ascorbic acid. This characteristic electron donor definitely inhibited the depolarizing and frequency enhancing effect of ACh; sometimes even a complete elimination of spike generation occurred. The corresponding *Δ* cps% values were, control ACh = +21; ascorbic acid + ACh = −38 ($p < 0.001$).

Fig. 6 shows that the original spike generating depolarizing action of ACh was eliminated by previous treatment with ascorbic acid. If such a cell with stopped spike generation is depolarized exogenously (Fig. 6c), spike generation could still be elicited, but after return to the starting membrane polarization level the cell is again silent. At the same time, if the return was

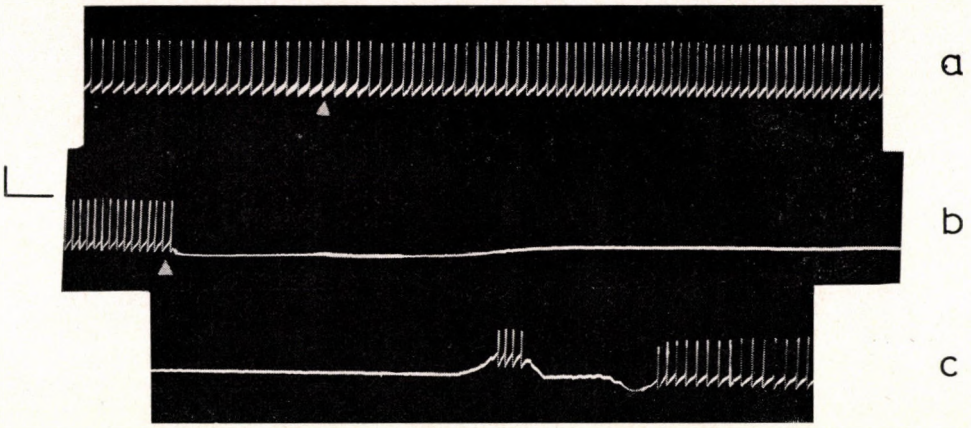

Fig. 6. Influence of ascorbic acid on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes ascorbic acid pretreatment; c: artificially evoked depolarization and hyperpolarization during the effect of ascorbic acid + ACh. ⊿: Administration of ACh. Calibration: 50 mV, 0.33 sec

preceded by exogenous hyperpolarization, spike generation started spontaneously.

Considering that the ascorbic acid diminished the pH of the medium by 0.3—0.3 units, experiments were performed to elucidate whether the effect was due to a shift of the pH or an increase of the electron donor character of the medium. In these experiments the pH of the medium was decreased by 0.3 units by acetic acid, which has no redox capacity. As it is seen in Fig. 7, changes could not be observed either in the qualitative character of the depolarizing wave evoked by ACh, or in spike frequency (control Δ cps% = +32; after ACh + acetic acid treatment = +37 [$p < 0.10$]).

b) Hydroquinone. Similarly to ascorbic acid, this reducing agent also inhibited the depolarizing effect of ACh. This type of action was significant in

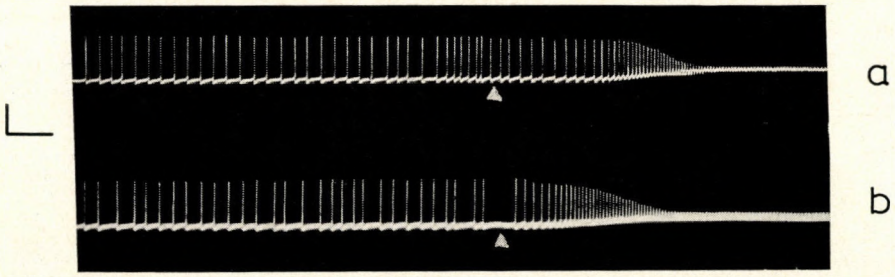

Fig. 7. Influence of acetic acid on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes acetic acid pretreatment. ⊿: Administration of ACh. Calibration: 50 mV, 0.33 sec

83% of the experiments, failed to appear in 7% and depolarization was enhanced in 10%. Thus, the increased reductive character of the medium caused by hydroquinone decreased the ACh effect less than it was done by ascorbic acid. The fact that following hydroquinone administration negative Δ cps% values were never obtained, also corroborates the said assumption. The mean Δ cps% values were, ACh control $= +51$; hydroquinone $+$ ACh $= +14$ ($p < 0.02$).

The two types of hydroquinone effect are demonstrated in Figs 8 and 9. As regards the bioelectric activity of the cell shown in Fig. 8, spike frequency, as the measure of membrane depolarization, changed significantly after hydroquinone treatment. At the same time, in the case of the cell demonstrated in Fig. 9, the decrease of membrane depolarization seems to dominate over the diminution of spike frequency. In the former case there is a pacemaker, and in the second a synaptically driven cell, therefore the cause of the difference must be in that the postsynaptic membrane and/or the cholinergic receptors of the soma of the pacemaker cell react in a different way to the reduction of the redox state of the biophase.

c) p-Methyl-amino-phenolsulphate (MAP). This potent reducing agent exerted an inhibitory effect on the depolarizing action of ACh in 92% but in 8% of the cases this effect was not significant. We have not succeeded in dem-

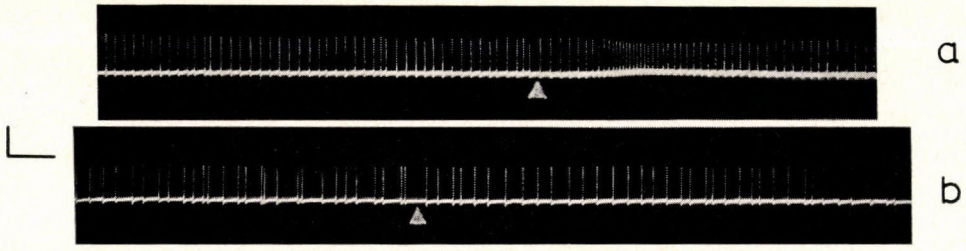

Fig. 8. Influence of hydroquinone on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes hydroquinone pretreatment. Δ: Administration of ACh. Calibration: 50 mV, 0.33 sec

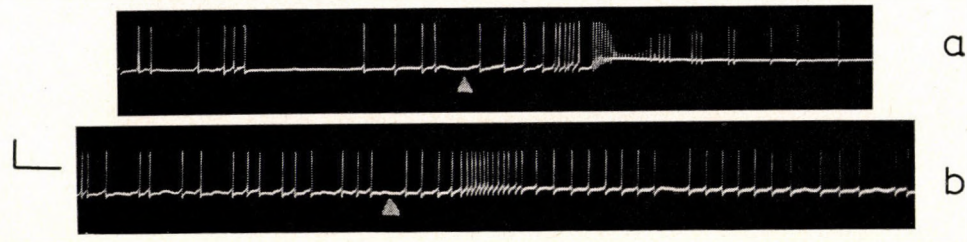

Fig. 9. Influence of hydroquinone on the depolarizing effect of ACh (D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes hydroquinone pretreatment. Δ: Administration of ACh. Calibration: 50 mV, 0.33 sec

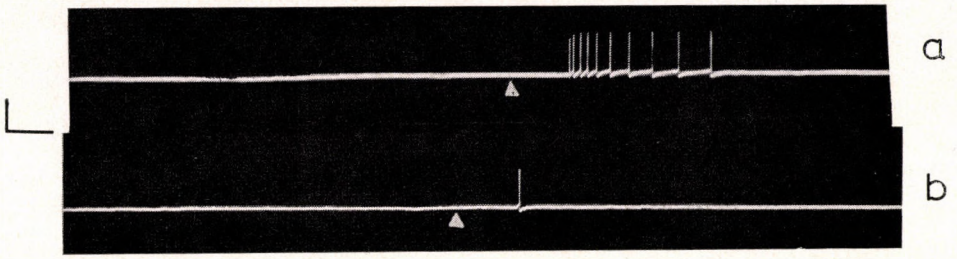

Fig. 10. Influence of metol on the depolarizing effect of ACh (silent D cell). a: Control ACh action; b: ACh effect on the same cell after washing and 3 minutes metol pretreatment. Δ: Administration of the ACh. Calibration: 50 mV, 0.33 sec

onstrating an enhancing action in any of the cases. The Δ cps% values were, ACh control $= +20$; ACh + MAP $= -30$ ($p < 0.002$).

In Fig. 10 the activity of a silent neurone is demonstrated before and after the administration of p-methyl-amino-phenolsulphate.

II. 5-hydroxytryptamine (5-HT)

In our experiments, a shift into the oxidative and/or the reducing side of the redox state of the biophase did not influence the depolarizing action of serotonin so clearly than after the application of ACh. (Serotonin is a reducing agent in itself!)

a) Methylene blue. An augmentation of the electron acceptor capacity resulted in an inhibition of the depolarizing action evoked by 5-HT in 70% of the experiments, while in 30% the methylene blue had no effect. The inhibition was not so considerable that the depolarization should have transformed to a hyperpolarization. The Δ cps% values were, serotonin control $= +26$; serotonin + methylene blue $= +3$ ($p < 0.01$).

In Fig. 11, activation of a silent neurone by serotonin and a partial inhibition of this activation by methylene blue is demonstrated.

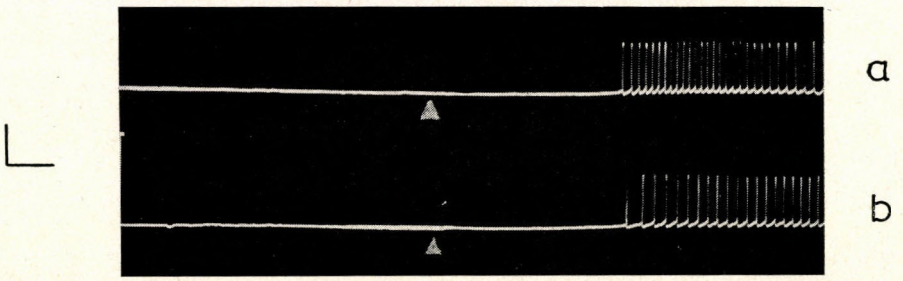

Fig. 11. Influence of methylene blue on the depolarizing effect of 5-HT (silent D cell). a: Control ACh action; b: 5-HT effect on the same cell after washing and 3 minutes methylene blue pretreatment. Δ: Administration of 5-HT. Calibration: 50 mV, 0.33 sec

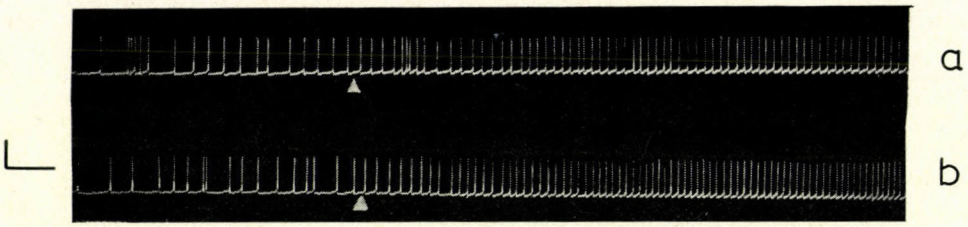

Fig. 12. Increasing effect of ascorbic acid on the depolarizing action of 5-HT (D cell). a: Control 5-HT action; b: 5-HT effect on the same cell after washing and 3 minutes ascorbic acid pretreatment. ⊿: Administration of 5-HT. Calibration: 50 mV, 0.33 sec

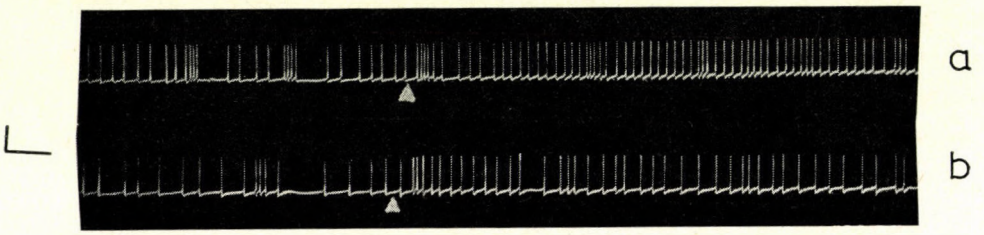

Fig. 13. Inhibiting effect of ascorbic acid on the depolarizing action of 5-HT (D cell). a: Control 5-HT action; b: 5-HT effect on the same cell after washing and 3 minutes ascorbic acid pretreatment. ⊿: 5-HT administration. Calibration: 50 mV, 0.33 sec

b) Ascorbic acid. Augmentation by this agent of the electron donor capacity did not change the original serotonin action. Ascorbic acid caused in 60% of the cases an inhibition of the depolarization evoked by serotonin. In 20% it had no effect and in another 20% it increased the action of serotonin (Fig. 12).

Fig. 13 demonstrates an inhibition evoked by ascorbic acid. Ascorbic acid diminished not only mean spike frequency, but also inhibited the spontaneous frequency of the trains evoked by the EPSP-s, perhaps by inhibiting the effect of the endogenous transmitter (ACh?).

In some experiments we have examined the changes of the membrane potential after methylene blue and ascorbic acid administration. It was found that after methylene blue treatment (shift towards the oxidative side) the membrane potential gradually diminished by -3.2 ± 0.7 mV, but this level returned to normal after 4—5 minutes. At the same time a shift towards the reducing side of the biophase caused by ascorbic acid resulted in an increase in the membrane potential by $+11 \pm 3.5$ mV. This increment failed to return to normal in 4—5 minutes. Since the ACh was administered less than 4—5 minutes after the redox catalysator pretreatment these changed membrane potentials have to be taken into consideration when discussing the role of the E_{ACh} phenomenon.

Discussion

The validity of the redox phenomenon for the activities evoked by ACh could be proved also with respect to the bioelectric parameters of the soma of the nerve cells. At the same time, in the case of activities evoked by 5-HT after both a reducing and/or oxidizing shift in the redox state, they resulted in a decrease of the depolarizing tendency in contradiction to our earlier findings (PUPPI et al. 1973) that in various structures (isolated mussel heart, *Lumbricus* muscles, *Anodonta* muscles) the depolarization evoked by 5-HT was augmented by ascorbic acid while methylene blue had no effect.

In the mentioned organs there is an ACh–5-HT antagonism while on *Lymnea* neurones there is mostly an ACh–5-HT synergism, the only explanation of these observations is to assume that the mechanisms manifesting with depolarization or hyperpolarization is similar on these cells, and so the redox changes resulted in a similar chemo-pharmacodynamic effect.

In general, the redox state of the biophase plays an important role in the definition of the depolarizing and/or hyperpolarizing type of effect, both with acetylcholine and with serotonin. The definition of the site of action presents more of a problem; changes in the value of the electron donor/acceptor quotient may result in actions at the following sites.

1) Changes in the activity of transport enzymes. ACh is well-known to inhibit adenyl cyclase (MÓZSIK 1969; MURAD et al. 1962) and following changes in the redox state of the milieu the activity of $(Na^+ + K^+)$ transport ATPase is also considerably affected (HEGYVÁRI 1966; DIKSTEIN 1971) so the influence on the activity of the transport processes has to be taken into consideration.

2) Conformational changes in the membranes. Differences in the charges and the hydrated ion radius of the chloride, potassium and sodium ions may play a role in the phenomenon because the diameter and the charge of the ionic channels depends on the secundary (URRY 1972) and/or tertiary (DEL CASTILLO et. al. 1972) structure of the proteins, which are influenced by electron donor/acceptor quotient of the biophase.

3) An influence on the interrelations between the mediator and the receptor.

The connection between the cationic head of the ACh and the carboxyl group of the anionic site of the ACh receptor are deeply influenced by the level of electron delocalizability (Dr^N). More exactly, the higher the value of (Dr^N), the stronger the inhibitory effect of the ACh on the isolated frog heart (INOUYE et al. 1963). Oxidizing agents as potent electrophilic substances, may influence the charge transfer reactions by masking the anionic site of the receptor and so the type of action of the mediator. As to the esteric receptor site, SHEFTER and MAUTNER (1969) showed that the depolarizing activity of ACh was in an inverse proportion to the ability of the side chain heteroatoms to form hydrogen

bridges. Methylene blue, as a hydrogen acceptor, may diminish the possibility of hydrogen bridge formation, and at the same time ascorbic acid, as a potent hydrogen donor, may promote this type of interaction.

Here the question arises whether the redox phenomenon manifests itself with changes in the E_{ACh}? E_{ACh} is equal to that equilibrium potential of the ion, through the fluxes of which the effect of ACh is realized (CHIARANDINI and GERSCHENFELD 1967). The problem becomes more complicated if we consider that we do not know which is the ion in question. Some data in the literature refer to the fact that on a definite part of giant neurones of invertebrates, the ACh actions correlate with Cl^- fluxes through membranes, but in another group of neurones the enhancement depends on the influx of sodium ions (CHIARANDINI and GERSCHENFELD 1967). The existence of similar mechanisms is supported by the E_{ACh} measurements performed on the central nervous system of *Lymnea stagnalis* (KISS and SALÁNKI 1971). The changes in the membrane potentials evoked by oxidizing and/or reducing agents refer to an influence on the E_{ACh} value.

The possibility, too, has to be taken into consideration, that if a definite ion mechanism is bound to a definite molecular conformation of the membrane proteins, the conformational change followed by a redox action favours the fluxes of Na^+ ions instead of Cl^- fluxes, and conversely, so that the E_{ACh} value will be determined by the equilibrium potential of the other ion.

Since all the sites of action mentioned above seem to be plausible, the enzyme activity, the changes in the conformation of membrane proteins, and also the level of the charge transfer processes may from part of a complex regulatory system.

It has been mentioned earlier that the effect of nicotinic acid was not significant statistically. Since, however, the mean nicotinic acid effect is made up of characteristic enhancing and inhibitory actions, in other words the effect is the resultant of two antagonistic actions, one may assume that the nicotinic acid effect shows individual deviations depending on whether the agent exerts predominantly an oxidizing action on the redox receptor or, as a molecule possessing a N^+ group, forms a complex with the carboxyl group of the glutaminic acid of the ACh receptor. In the former case, like the other oxidizing agents, it increases the depolarizing action of ACh, while in the second case it causes a nicotinergic inhibition.

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EFFECT OF BLEEDING, COOLING AND ASPHYXIA ON THE ACTIVITY OF VERTEBRAL AND CARDIAC SYMPATHETIC NERVES

By

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The effect of different factors influencing sympathetic efferent activity has been studied in order to find differences in regional sympathetic outflow. The experiments were performed on anaesthetized cats; integrated vertebral nerve activity (VNA), cardiac nerve activity (CNA), systemic blood pressure and respiration were recorded. During gradual, step-wise bleeding the increase of VNA exceeded that of CNA in all phases of bleeding. As a result of cooling the surrounding air from 30 to 15°C, both VNA and CNA displayed a slight increase, on further cooling to 10°C a steep rise of the activity level occurred in VNA. In the course of 1 min asphyxia VNA increased steadily and considerably while the increase in CNA began later and was slighter than that of VNA. The results revealed that the cardiac and vertebral sympathetic nerves give quantitatively different responses to the stimuli applied.

The sympathetic efferent system had for long been regarded as a uniform system: the spontaneous activities recorded on the cervical, thoracic and abdominal sympathetic efferent nerves showed similar cardiac and respiratory rhythm and the different stimulations evoked more or less uniform responses (ADRIAN et al. 1932; BRONK et al. 1936; ALEXANDER 1946, WEIDINGER et al. 1961).

GREEN and HEFFRON (1966) were the first to note differences in the activity of vertebral and cardiac sympathetic nerves; a comparison of the activities revealed that bursts occasionally occurred in the former when none were seen in the latter and vice versa. WALTHER et al. (1970) and IRIKI et al. (1971) cooled the spinal cord and the hypothalamus of rabbits and found that the sympathetic activity supplying the skin of the ear increased, while the activity recorded in the splanchnic nerve decreased. RIEDEL et al. (1972) found similar responses to natural thermal stimuli, i.e. heating and cooling of the rabbit's skin. In rabbits the ear is a special organ which takes part in thermoregulation, in cats the above-mentioned authors could rarely evoke similar sympathetic reactions. HAGBARTH et al. (1972) and DELIUS et al. (1972a, b, c) found qualitative differences in the activity of sympathetic fibres supplying the muscles and skin in human subjects. Stimuli like Valsava's manoeuvre and tilting of the body which evoke baroreflexes influenced only the activity which supplies the muscles, while activity running to the skin remained unchanged. During cooling and heating of the body, a reverse phenomenon was

observed. These results raise the question of a regional specificity in sympathetic outflow.

In the present experiments, by means of quantitative measurements and comparison of sympathetic activities supplying the forelimb and the heart, we have attempted to differentiate between the reactions during bleeding, cooling and asphyxia.

Methods

The experiments were performed on 13 cats of both sexes, weighing 2.0—3.5 kg. After ethyl chloride induction, anaesthesia was maintained with a mixture of 50 mg/kg chloralose and 200 mg/kg urethan injected intravenously. For muscular paralysis, 4 mg/kg of gallamine was administered and, if necessary, the dose was repeated. The trachea was cannulated and artifical ventilation was performed by means of a Starling pump. Blood pressure was measured in the left femoral artery through a polyethylene catheter. For drug injections the left femoral vein was used. Body temperature of the animals was kept at 38—39°C by external heating. The sympathetic nerves were prepared on the left side.

After preparation, the animals were fixed in the upright position in a frame and head holder. It is to be noted that the blood pressure values given below refer to heart level; in consequence of the upright position, blood pressure in the head was 5 mm Hg less. The exposed tissues were protected by warm paraffin oil.

Discharges of the vertebral and cardiac nerves were recorded with fine bipolar stainless steel electrodes and were fed into differential amplifiers with a frequency range of 8 cps—2 K cps. The amplified discharges were recorded a) directly on an oscilloscope, b) after full-wave rectification and integration on a compensograph. The time integral was used as basis for the analysis of variation in full nerve activity. When the integral reached a certain value the integrating capacitor was automatically short-circuited thus forming sawtoothlike impulses. There was a direct and quantitative correlation between the integrated activity and the frequency of these impulses. The sawtooth frequency was computed and recorded with a rate-meter and compensograph. When evaluating the changes in the activities, deflection of the direct writer was measured in mm. The integrator was not calibrated in μVsec as during evaluation the relative changes were always considered. We dismissed the idea to determine activity in μVsec, standardized by the number of fibres located in the nerve, because the activity recorded by this technique is greatly dependent on such factors as the position of the nerve on the electrodes or the thickness of the connective tissue sheath that remained on the nerve. The nerve–electrode position was kept unchanged throughout the recording period. On the diagrams, the starting level of activity measured in mm deflection of the direct writer was taken as 100% and the activity changes were expressed in relative percentage.

The effect of bleeding was examined in 8 animals. For bleeding, the abdominal aorta under the origin of the renal arteries was cannulated by a metal T cannula so that the blood supply of the hind limb remained undisturbed. Intravenous heparin was used as anticoagulant. The T cannula was then connected to a reservoir in which a buffer system kept the pressure at the desired level. By means of this system, blood pressure was kept with a fluctuation of 1—2 mm Hg around the set level. Bleeding was produced by a gradual reduction of the pressure in the reservoir. Each bleeding steps lasted 5—10 min. The animals were bled in 5—6 steps to 40—50% of the initial blood pressure level, then the whole withdrawn amount of blood was retransfused.

The effect of cooling of ambient air was investigated in 5 animals; they were placed in the upright position together with the frame into an insulated box. Cooled air circulated in the box and reduced the temperature in 15 min from 30°C to 10°C. During the first min, temperature fell from 30°C to 25°C, with a further decrease of 1°C per min. Rewarming was performed by opening the side of the box: the cold air was driwen out in some sec. The effect of asphyxia was investigated in 8 animals. Asphyxia was induced by switching off the respirator for 1 min. In consequence of muscle paralysis, spontaneous respiration ceased.

Results

1. Effect of bleeding. On gradual, step-wise bleeding, sympathetic efferent activity running to the two investigated regions increased in different degrees. The increase of vertebral nerve activity (VNA) exceeded the increase of cardiac nerve activity (CNA) in all phases of bleeding. The initial respiratory and cardiac rhythm disappeared in both nerves. Fig. 1 illustrates a typical experiment. In the first step, when the animals were bled from a pressure of 140 to one of 125 mm Hg, an increase took place in the activities running to both regions; at 85 and 50 mm Hg blood pressure levels VNA displayed a further increase while CNA did not change considerably. At 40 mm Hg the

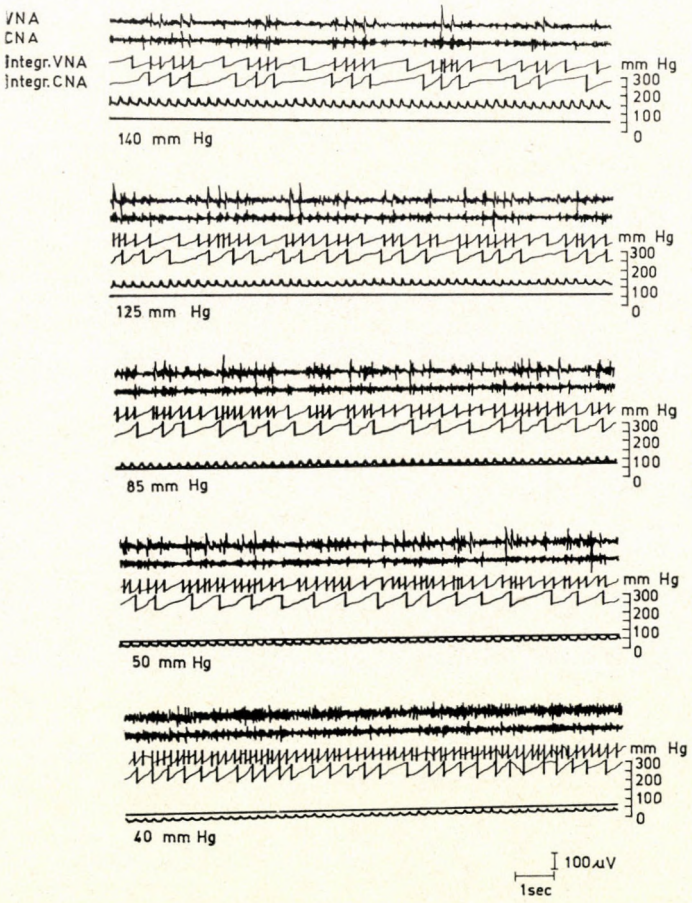

Fig. 1. Effect of bleeding on sympathetic efferent activity running to the forelimb (VNA: vertebral nerve activity) and to the heart (CNA: cardiac nerve activity). From top downwards: VNA, CNA, integrated VNA and CNA, arterial blood pressure. Blood pressure levels set by the buffer system: 140, 125, 85, 50 and 40 mm Hg

activity took a different appearance, mainly in VNA: the amplitude of action potentials decreased and their frequency increased as did also the total amount of activity. After each pressure fall, VNA rose steeply in 30 sec, and after another 30 sec it settled at a somewhat lower level; during evaluation, this level was always considered and the records of Fig. 1 were taken in this state. With CNA no such phenomena were observed.

In Fig. 2 activity changes are graphically plotted in the percentage of the initial activity using the data of 8 experiments. In all experiments the starting level of activity in both nerves was about 3—5 "short-circuit impulses" per sec at the output of the integrator. During the bleeding period, the increase

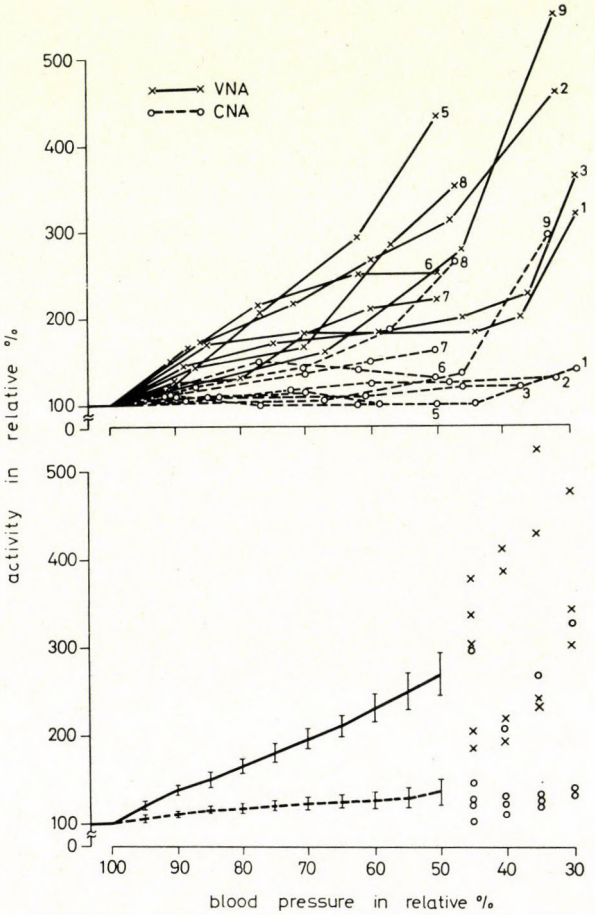

Fig. 2. Effect of bleeding on integrated VNA and CNA. Ordinate: integrated activity in percentage of control value. Abscissa: blood pressure levels in percentage of control value set by the buffer system. Control blood pressure values range between 100—140 mm Hg. Upper diagram: plots of 8 experiments; lower diagram: mean values and standard error of means calculated on the basis of 8 experiments

in VNA surpassed that of CNA at all blood pressure levels, both in the individual experiments and in the calculated mean values. At 50% of the initial pressure level, VNA reached 270%, and under 50% a further characteristically steep rise occurred. A fall of blood pressure induced a much smaller increase in CNA. At 50% of the initial pressure level, activity was only 135% and under 50% a steep activity increase occurred in a single case only.

2. Effect of cooling. Since the skin of the forelimb is supplied by the vertebral nerve we examined its activity as compared to CNA on cooling ambient air. It is well-known that anaesthetized animals start shivering at 10—15°C ambient temperature. Here the question arises as to the changes in sympathetic efferent activity when ambient temperature hits the threshold of shivering. Thus, ambient air was cooled to serve as a cold stimulus. In the first min, the temperature fell from 30°C to 25°C, then it dropped 1°C per min. Rectal temperature decreased by 1—1.5°C during the 15-min cooling period. In Fig. 3, initial ambient air temperature was 30°C. On cooling to 15°C, the

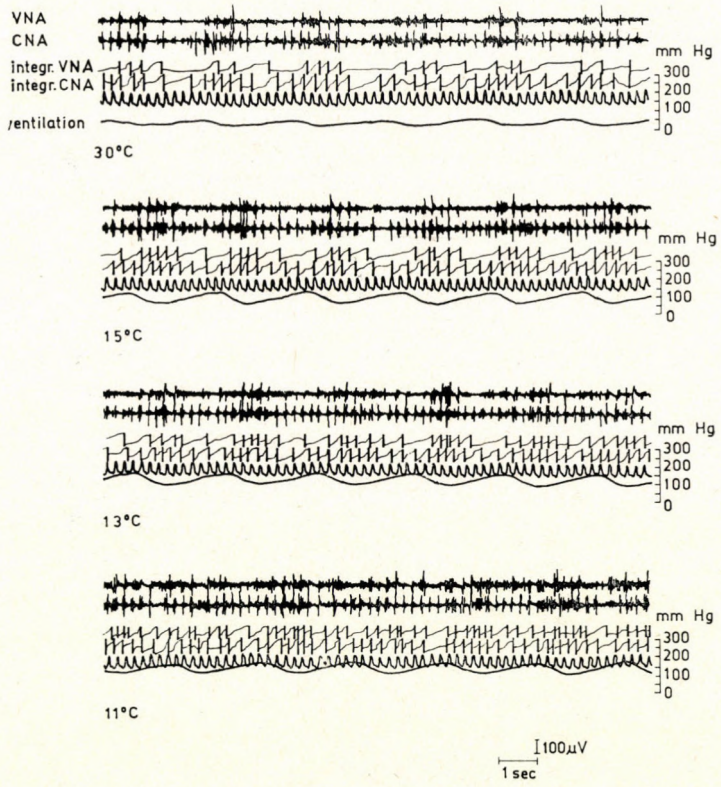

Fig. 3. Effect of cooling on VNA and CNA. From top downwards: VNA, CNA, integrated VNA and CNA, arterial blood pressure, ventilation. Temperature levels from top downwards: 30, 15, 13, 11°C

activity of both nerves was found to increase slightly. A significant change occurred only on further cooling. At 11°C the increase was much higher in VNA than in CNA. The pattern of VNA changed: most of the activity increase on the vertebral nerve was composed of fast potentials, the number of high amplitude slower waves did not change significantly. Owing to the activity increase, respiratory group formation disappeared. The characteristics of CNA did not change during cooling, its quantitative increase was smaller than that of VNA. During cooling, blood pressure displayed no significant change.

The activity changes are graphically plotted against ambient air temperature in Fig. 4. VNA increased slowly but steadily during cooling from 30°C to 15°C; at 15°C the activity was 125%. On further cooling a steep rise of VNA occurred and at 10°C it reached 178%. The increase in CNA on cooling

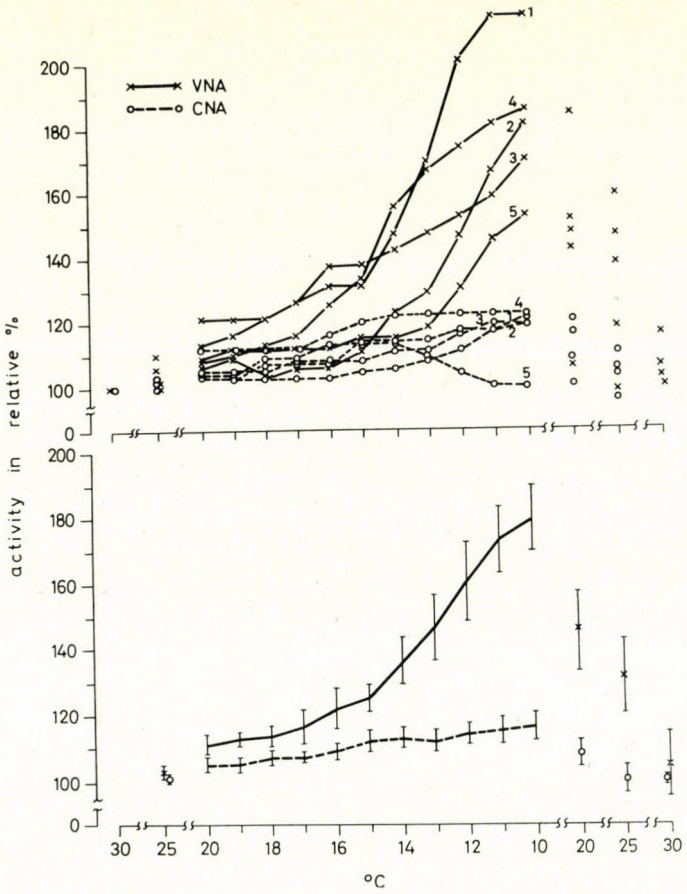

Fig. 4. Effect of cooling on integrated VNA and CNA. Ordinate: integrated activity in percentage of control values. Abscissa: temperature (°C). Upper diagram: data of 5 experiments; lower diagram: mean values and standard error of means calculated on the basis of 5 experiments

was much less; from 30°C to 10°C it was steady, at 10°C it amounted to 117%. Rewarming of ambient air to 30°C resulted in a decrease of activity and in a return to the precooling level.

3. Effect of asphyxia. Asphyxia is widely used for inducing an increase activity of the sympathetic structures. Fig. 5 shows the influence of asphyxia on VNA and CNA. A few sec after artificial ventilation had been switched off, VNA began to increase, while CNA remained uninfluenced. In the course of prolonged asphyxia, VNA steadily increased while the increase in CNA was considerably delayed. CNA showed an increase only after 50 sec, but this increase was lagging behind the activity level of the vertebral nerve.

Fig. 6 shows the effect of asphyxia in an arrangement similar to those given in Figs 2 and 4. The quantitatively different behaviour of the vertebral and cardiac nerves is conspicuous in both figures. VNA increased steadily

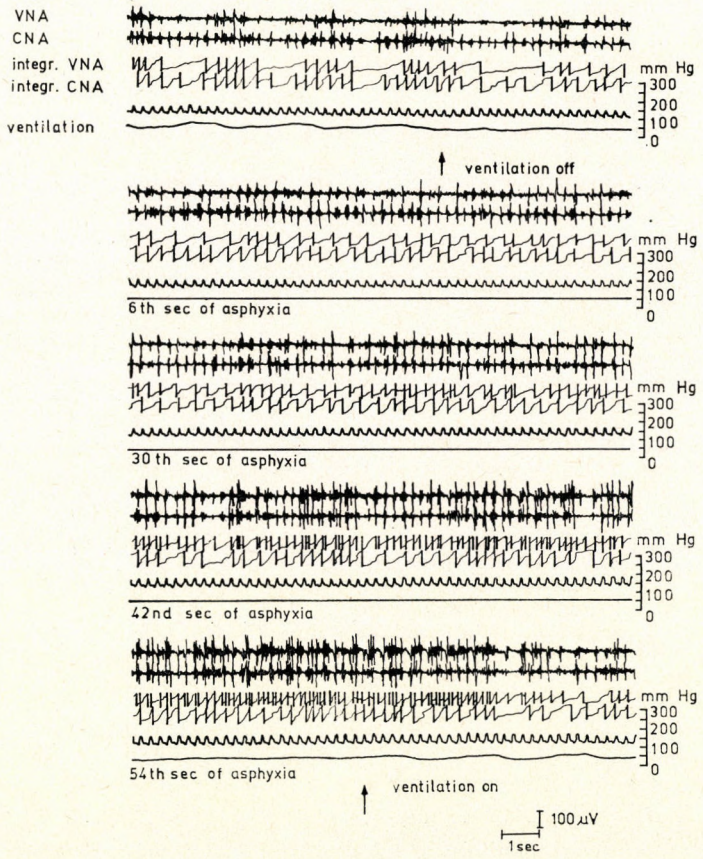

Fig. 5. Effect of asphyxia on VNA and CNA. From top downwards: VNA, CNA integrated VNA and CNA, arterial blood pressure, ventilation

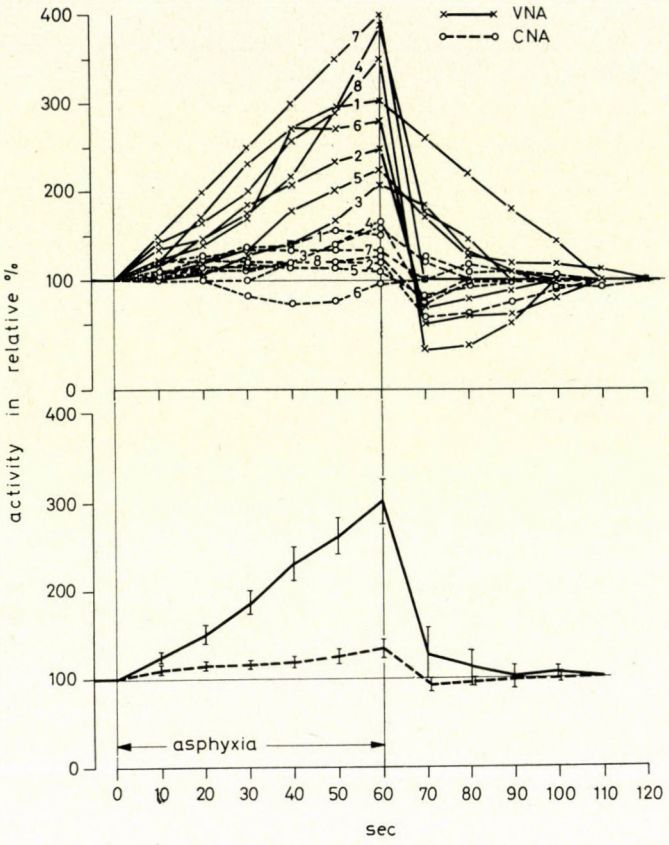

Fig. 6. Effect of asphyxia on integrated VNA and CNA. Ordinate: integrated activity in percentage of control value. Abscissa: time in sec. Upper diagram: data of 8 experiments, lower diagram: mean values and standard error of means calculated on the basis of 8 experiments

during asphyxia and reached 300% in the 60th sec. CNA displayed a much smaller increase; it rose to only 133% in the 60th sec of asphyxia. Blood pressure increased by 125% on the average.

Discussion

Beside the selective sensitivity of vascular smooth muscle and the role of local vasoactive metabolites, the specific sympathetic control of various organs might be the underlying mechanism for the development of differentiated cardiovascular reactions. However, such specific sympathetic control could not be revealed by comparing the activities of cervical, thoracic and

abdominal sympathetic efferent nerves (KOIZUMI et al. 1971). The contradiction between circulatory and electrophysiological results tends to disappear with recently applied methods. One way is to choose natural stimuli which have a selective influence on the sympathetic activity innervating certain regions. Thermal stimuli may selectively influence the sympathetic activity supplying the cutaneous vascular bed (WALTHER et al. 1970, 1971; RIEDEL et al. 1972). The other way is to investigate sympathetic fibres of different functions by the microelectrode technique. By means of this method HAGBARTH et al. (1972), DELIUS et al. (1972a, b, c) found qualitative differences in activity between the sympathetic fibres supplying the skin and muscle. The method applied in our experiments determines sympathetic activity differences by a quantitative measurement of full nerve action potentials. This method has the advantage of surveying the whole sympathetic outflow of a certain region. It has, however, the disadvantage that it is hard to decide whether in the case of increasing activity the same neurons fire more intensely or new ones are recruited.

In cats subjected to blood loss the activity of the vertebral nerve, which supplies the forelimb, increases more markedly than the activity of the cardiac nerve which innervates the heart. The vertebral nerve carries sympathetic control to the cutaneous and muscular vascular beds and to the pilomotor and sudomotor apparatuses. DELIUS et al. (1972c) found that the sympathetic activity supplying the skin was not influenced by baroreceptors. We presume that during slight decreases in blood pressure the enhanced VNA consists mainly of vasoconstrictor impulses affecting the muscular bed as a result of baroreceptor activity withdrawal. In a moderate hypotonic state the difference between vertebral and cardiac nerve activities is due to various baroreceptor influences on the centres regulating the two regions. In another series of experiments we found that occlusion of the common carotid arteries evoked a stronger and more prolonged increase in VNA than in CNA (FEDINA et al. in preparation). At pressure levels low enough to ignore the influence of baroreceptor activity (40—50 mm Hg) the further increase in sympathetic nerve activity can be ascribed partly to elevated chemoreceptor activity and partly to the low O_2 tension in brain tissue due to the reduced flow. During severe haemorrhage, a steep rise occurs in VNA while such a marked increase in CNA was detected in a single case only. This finding may be explained by a difference either in the sensitivity to O_2 lack or in the chemoreceptor afferentation of central structures controlling VNA and CNA. In the case of CNA, the central and peripheral effects of hypoxia may work against each other resulting in a less marked increase in CNA: the activity increase due to CNS hypoxia might partly be counteracted by the stimulation of carotid chemoreceptors which exert a diminishing effect upon CNA (DOWNING and SIEGEL 1963; ALANIS et al. 1968; STERN and RAPAPORT 1967; DOWNING 1972). The role of

aortic chemoreceptors does not, however, fit into this theory as their stimulation enhances CNA (STERN and RAPAPORT 1967).

NINOMYA et al. (1971) made comparative investigations of the sympathetic activities supplying the heart, kidneys and spleen. During haemorrhage they found the greatest increase in splenic sympathetic activity. This results in a contraction of the spleen which in turn leads to a redistribution of cardiac output to the vital organs. In our experiments, the extreme rise of VNA could produce a similar shift in cardiac output.

RIEDEL et al. (1972) found that on direct cooling of the body surface sympathetic activity running to the skin of the rabbit's ear increased, while the activity recorded on the splanchnic nerve decreased. In our experiments, cooling of the ambient air resulted in an increase in VNA, but at the same time CNA showed no significant change. As the muscle vascular bed is not involved in thermoregulation, we suppose that among the fibres of the vertebral nerve those which control the vessels of the skin and piloerection were responsible for the increase in VNA. This increased activity was accompanied by a slight increase in blood pressure. Our results together with those of RIEDEL et al. (1972) allow to assume that the increased VNA may have been compensated by the decreased activity of some other nerve (e.g. splanchnic nerve), and in this way the blood pressure remains unchanged.

When gradually cooling the ambient air, 15°C is a critical point for VNA. Below 15°C, VNA increases at a much higher rate than it did before and its characteristics are also markedly changed. As shivering is known to start around 15°C we suppose that below this temperature a different regulation related to shivering is released. Another question to be clarified is whether it is the thermoreceptors of the skin or the 1—1.5°C lower core temperature which induce the increase in activities. In view of the fact that on reheating the ambient air all the activities return to their initial level even though core temperature remains low, we may be certain that the thermoreceptors localized in skin are responsible for the increased sympathetic activity.

According to the literature, asphyxia elicits a general activation of the sympathetic efferent system. Our experiments have corroborated these findings and in addition they revealed that the increase in activity in different regions varied in extent. During the whole period of asphyxia the increase of VNA exceeded the increase of CNA. In this respect the effect of asphyxia and that of severe blood loss with an impaired O_2 supply, our results showed a good agreement.

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ACID-BASE BALANCE AND REGIONAL CO_2 CONTENT OF THE BRAIN IN HYPOVOLAEMIC SHOCK*

By

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The acid–base parameters of arterial, right atrial and sagittal blood as well as the tissue CO_2 content of various brain regions were studied in conditions of hypovolaemia in anaesthetized dogs. Within 30 min after bleeding to a 55 mm Hg mean arterial pressure, in arterial blood BE and pCO_2 decreased while the pH was unaffected. In right atrial blood, with the decrease of BE, a rise in pCO_2 took place and the pH was in the acidotic range. In the first 30 min of bleeding I, in sagittal blood no essential change was detectable. At 35 mm Hg mean arterial pressure, severe acidosis was found in all three blood samples. A—V O_2 and pCO_2 differences increased significantly in the first 30 min both in mixed venous and in sagittal sinus blood. Tissue CO_2 content in the various brain regions showed significant differences. Thirty min after the first bleeding, tissue CO_2 content in the hypothalamus rose from 14.5 ± 1.5 mM/kg to 31.7 ± 3.7 mM/kg and following the second bleeding it reached 38.5 ± 6.1 mM/kg. The frontal cortex displayed a similar but slighter elevation. CO_2 content in the pons increased only at the end of the second phase of bleeding, while the occipital cortex, thalamus and medulla oblongata did not show essential changes.

After the correction of acidosis by bicarbonate infusion hypercapnia occurred in the hypothalamus, while in the other brain regions normal or only slightly different values were obtained.

Numerous papers have discussed acid–base disturbances developing in haemorrhagic shock. Opinions vary as to the correlation between the degree of acidosis measured in arterial blood and the severity of shock (LILLEHEI et al. 1964; MEHMEL et al. 1966; CHICK et al. 1968). Similarly, data are not uniform concerning the therapeutic effect of the correction of acidosis with buffer solutions (DARBY and WATTS 1964; SCHOEPPNER 1965; CORAN et al. 1971; IRVING 1971), while HALMÁGYI et al. (1970) found a correlation to exist between venous pCO_2 and the prognosis of shock.

Previous experiments have revealed changes in central nervous function, blood flow and oxygen metabolism in dogs with haemorrhagic shock (KOVÁCH 1970). In order to study the development of this damage, we investigated the acid–base balance in arterial and sagittal blood samples along with the CO_2

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content of brain tissue in haemorrhagic shock. In irreversible haemorrhagic shock, at the end of the hypovolaemic period and in the normovolaemic stage following retransfusion, pCO_2 in sagittal sinus blood rose significantly with the simultaneous occurrence of a considerable hypercapnia in certain brain regions (MAKLÁRI and KOVÁCH 1968).

In view of the fact that the changes which render the process of shock irreversible develop progressively during bleeding, we were interested to know the time when the rise in tissue CO_2 content occurs and furthermore the time correlation of these changes to the acid–base parameters of the blood. In the present experiments the acidosis due to haemorrhage was corrected by infusing a buffer solution in the last third of blood loss, and the effect of the intervention has been studied upon the CO_2 level of brain tissues.

Methods

The experiments were carried out on 36 mongrel dogs of both sexes under 100 mg/kg α-D(+)-glucochloralose (Merck, Darmstadt) anaesthesia. Blood coagulation was inhibited by the administration of 5 mg/kg heparin (Richter, Budapest). Irreversible shock was brought about using a modification of WIGGER's method (1950). A pressurized reservoir system was employed and arterial blood pressure of the animals was stabilized at 50—60 mm Hg (bleeding I) for 90 min, then at 30—40 mm Hg (bleeding II) for 90 min. The procedure invariably resulted in irreversible shock in the dog (KOVÁCH 1970).

The acid–base values in the blood samples and the tissue CO_2 content of various brain regions were determined prior to bleeding, then in the 30th and 90th min of the bleeding I and bleeding II periods. Blood samples were obtained from the carotid artery and the sagittal sinus as described previously (MAKLÁRI and KOVÁCH 1968). Mixed venous blood was obtained via a catheter inserted through the external jugular vein in the right atrium. The pH was determined with a Beckman 160 type gas analyser using PNP standard buffer (Metrohm

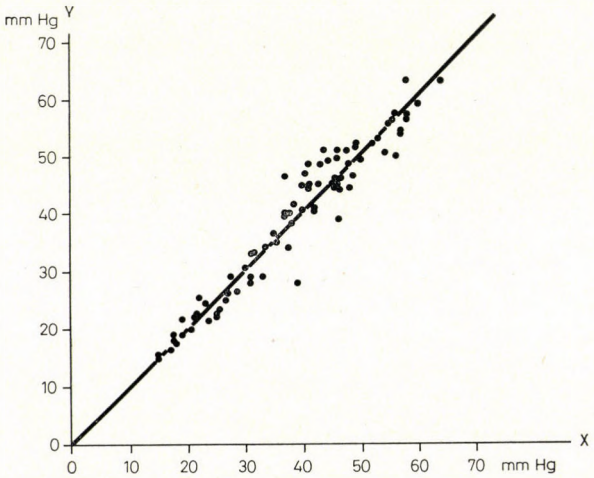

Fig. 1. Correlation between directly measured pCO_2 values and those derived from nomogram. Ordinate (Y): values measured directly with Severinghaus type electrode. Abscissa (X): values derived from Siggaard-Andersen nomogram on the basis of pH and total CO_2. The solid line represents the 45 degree axis

A.G., Herisau). Total CO_2 and standard bicarbonate were estimated by the method of PETERS and VAN SLYKE (1932). pCO_2 actual bicarbonate and base excess (BE) were determined from SIGGARD-ANDERSEN's nomogram (1963). In some experiments, pCO_2 was measured directly with a Severinghaus type electrode connected to the Beckman 160 gas analyser. pCO_2 derived from the nomogram and the directly measured CO_2 tensions were compared in 79 parallel determinations and consistent results were obtained (Fig. 1). The equation of regression was $y = 0.9913x + 0.9244$ ($r = 0.9963$, $p < 0.005$). Oxygen saturation was estimated by means of an Elema-Schönander oxymeter and Hb by the cyan methaemoglobin method (BAKOS 1963). Tissue CO_2 content was determined in the frontal and occipital cortex, thalamus, hypothalamus, pons and medulla oblongata with the modified method of ANREP et al. (1936) as described previously (MAKLÁRI and KOVÁCH 1968). For measuring tissue CO_2, 6 dogs each were decapitated at the points of time mentioned above.

In 6 animals infusion of 0.3 M sodium bicarbonate was started in the 40th min of the second phase of bleeding, to correct the acidosis during the remaining period of hypotension. Blood bicarbonate determinations were made at the 30th min of bleeding II and at 15 min intervals thereafter; adjustment of the bicarbonate infusion rate was made accordingly (ZIMMERMANN 1965). The CO_2 content of the brain tissue of these animals was determined in the 90th min of bleeding II. Blood samples were taken simultaneously from the treated and control dogs.

During the experiments, arterial blood pressure and respiratory rate were registered continuously and the amount of drained blood was measured.

Evaluation of results was done by Student's *t*-test.

Results

Acid–base balance

Table I shows the data yielded by analysis of acid–base values in carotid artery, right atrium and sagittal sinus blood.

In arterial blood, both bleedings were followed by rapid changes in acid–base values. Actual bicarbonate and pCO_2 dropped by about 25% in the first 30 min, BE fell from —6 mEq/l to —11 mEq/l. The values remained at these levels in the later part of the first period. The pH changed little, the difference was not significant even at the end of bleeding I ($p > 0.05$).

After the second haemorrhage, simultaneously with the repeated drop in actual bicarbonate and BE, pCO_2 diminished, pH fall considerably ($p < < 0.0005$) and an incompensated acidosis developed.

In right atrial mixed venous blood, BE showed a decrease identical with that of arterial blood, but pCO_2 began to rise and until at the end of the 30th min of bleeding I, it became significantly higher than prior to haemorrhage ($p < 0.05$). This was well reflected in the pH changes, as pH values dropped significantly during the first stage of bleeding ($p < 0.05$). During bleeding II, acidosis was further aggravated in consequence of the repeated decrease of the bicarbonate level and of the rise in pCO_2.

In sagittal sinus blood prior to haemorrhage, BE was the same as in mixed venous blood, while pCO_2 was somewhat elevated and pH slightly decreased. In sagittal blood the first phase of bleeding did not produce such rapid changes as in the mixed venous blood. BE and pH did not diminish much in the first 30 min; lower values were observed only at the end of the period. pCO_2 was practically unchanged. In the first 30 min of the second bleeding period, the drop in BE and pH in sagittal sinus blood was similar to

Table I

Results of blood sample analyses in hypovolaemia

Blood sample site		time	pH	pCO_2 (mm Hg)	Actual bicarbonate (mEq/l)	BE (mEq/l)	O_2 saturation (per cent)	O_2 content (vol. per cent)
Carotid artery	untreated	before bleeding	7.32 ± 0.01	38 ± 1	18.4 ± 0.5	− 6 ± 1	88 ± 1	19.3 ± 0.5
		bleeding 30′	7.31 ± 0.02	28 ± 2^{++}	13.4 ± 0.5^{++}	−11 ± 1^{++}	88 ± 2	17.5 ± 0.9^{+}
		90′	7.26 ± 0.02	28 ± 2^{++}	13.6 ± 1.1^{++}	−14 ± 1^{++}	88 ± 2	17.7 ± 1.0^{+}
		bleeding II 30′	7.01 ± 0.04^{++}	23 ± 2^{++}	7.5 ± 0.5^{++}	−21 ± 1^{++}	83 ± 4	16.1 ± 1.1^{++}
		90′	7.08 ± 0.06^{++}	23 ± 4^{++}	6.7 ± 0.9^{++}	−23 ± 2^{++}	89 ± 2	18.9 ± 1.0
	bicarbonate-treated	bleeding II 30′	7.03 ± 0.05^{++}	26 ± 4^{++}	7.5 ± 0.8^{++}	−22 ± 1^{++}	81 ± 5	15.0 ± 2.0^{++}
		90′	7.32 ± 0.04□□	36 ± 6□	17.8 ± 1.9□□	− 2 ± 3□□	91 ± 5	11.0 ± 1.0^{+}□□
Right atrium	untreated	before bleeding	7.30 ± 0.01	47 ± 2	20.4 ± 0.5	− 5 ± 1	61 ± 2	13.1 ± 0.6
		bleeding I 30′	7.19 ± 0.03^{+}	54 ± 4^{+}	18.5 ± 0.4^{++}	−10 ± 1^{++}	41 ± 3^{++}	8.0 ± 0.7^{++}
		90′	7.13 ± 0.02^{++}	56 ± 3^{+}	17.6 ± 0.7^{++}	−12 ± 1^{++}	36 ± 3^{++}	6.9 ± 0.7^{++}
		bleeding II 30′	7.00 ± 0.04^{++}	60 ± 6^{+}	13.3 ± 0.8^{++}	−20 ± 2^{++}	32 ± 3^{++}	5.8 ± 0.6^{++}
		90′	6.98 ± 0.04^{++}	62 ± 4^{++}	13.4 ± 1.2^{++}	−20 ± 2^{++}	31 ± 2^{++}	6.5 ± 0.5^{++}
	bicarbonate-treated	bleeding II 30′	6.95 ± 0.06^{++}	64 ± 10^{++}	12.8 ± 1.3^{++}	−22 ± 2^{++}	35 ± 5^{++}	6.0 ± 1.0^{++}
		90′	7.15 ± 0.04□□	67 ± 6^{++}	20.9 ± 1.3□	− 6 ± 2□□	35 ± 4^{++}	5.0 ± 1.0^{++}
Sagittal sinus	untreated	before bleeding	7.24 ± 0.01	56 ± 2	22.2 ± 0.5	− 5 ± 1	47 ± 3	10.5 ± 0.6
		bleeding I 30′	7.20 ± 0.02	53 ± 2	19.2 ± 0.6^{++}	− 7 ± 1	35 ± 2^{++}	7.2 ± 0.7^{++}
		90′	7.16 ± 0.02^{++}	56 ± 3	18.3 ± 0.5^{++}	−10 ± 1^{++}	34 ± 2^{++}	6.7 ± 0.8^{++}
		bleeding II 30′	7.00 ± 0.04^{++}	54 ± 4	12.6 ± 0.7^{++}	−18 ± 2^{++}	26 ± 3^{++}	5.2 ± 0.6^{++}
		90′	6.92 ± 0.06^{++}	62 ± 5	12.0 ± 1.0^{++}	−23 ± 3^{++}	35 ± 3^{++}	7.8 ± 0.8^{+}
	bicarbonate-treated	bleeding II 30′	6.94 ± 0.05^{++}	58 ± 7	11.9 ± 1.1^{++}	−20 ± 2^{++}	25 ± 4^{++}	5.0 ± 1.0^{++}
		90′	7.24 ± 0.05□□	49 ± 5□	19.1 ± 1.6^{++}□□	− 2 ± 3□□	37 ± 4	5.0 ± 0.4^{++}

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values
□ $p < 0.05$; □□ $p < 0.01$ as compared with simultaneous control values

that found in arterial and mixed venous blood. pCO_2 rose at the end of the haemorrhage but the difference was not significant statistically ($p > 0.05$).

In keeping with the above described changes, the V–A pCO_2 difference rose steadily in the course of the experiment (Fig. 2); it became prominent as early as the 30th min of bleeding I, both in mixed venous–arterial ($p < 0.01$) and sagittal–arterial blood ($p < 0.025$).

V–A actual bicarbonate difference in the right atrial–arterial blood also increased gradually during the hypotensive period, while, the sagittal–arterial bicarbonate difference increased markedly only by the end of the haemorrhage ($p < 0.01$).

Respiration is an important factor influencing acid–base balance. After the first stage of bleeding its rate increased (Table II) and by the end of the first phase it exceeded the initial value by 100%. In the second period of haemorrhage, hyperventilation increased further.

Table II

Blood pressure, respiratory rate, amount of blood loss and Hb during haemorrhage

Time	Mean arterial pressure (mm Hg)	Respiratory rate (N/min)	Drained blood (ml/kg b.w.)	Haemoglobin (g/100 ml)
Before bleeding	129 ± 3	11 ± 1		16.0 ± 0.4
Bleeding I 30′	58 ± 1	$17 \pm 1^{++}$	27 ± 2	$14.6 \pm 0.7^{++}$
90′	54 ± 1	$21 \pm 1^{++}$	30 ± 3	$14.6 \pm 0.7^{++}$
Bleeding II 30′	35 ± 1	$23 \pm 2^{++}$	34 ± 3	$14.1 \pm 0.8^{++}$
90′	36 ± 1	$25 \pm 2^{++}$	27 ± 3	15.7 ± 0.7
Before bicarbonate infusion Bleeding II 30′	35 ± 1	$26 \pm 4^{++}$	44 ± 4	$13.8 \pm 1.4^{++}$
After bicarbonate infusion Bleeding II 90′	34 ± 1	$28 \pm 3^{++}$	40 ± 6	$10.6 \pm 1.2^{++}$

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values

The blood Hb content diminished in the first third of the hypovolaemic period then, in agreement with data in the literature (BAUBKUS and KIRCHHEIM 1966; HOLLENBERG et al. 1970) it rose in the last hour. O_2 saturation of arterial blood was unaffected by bleeding, but the O_2 content changed due to the change in Hb content (Table I). On the other hand, O_2 saturation of right atrial and sagittal blood were reduced after bleeding, and the fall in O_2 content was even more pronounced. These low values did not change in spite of the haemoconcentration, even at the end of bleeding II.

The A–V O_2 difference increased in the first 30 min of haemorrhage in both mixed venous and sagittal blood (Fig. 2) and remained at an elevated level during the hypotensive period.

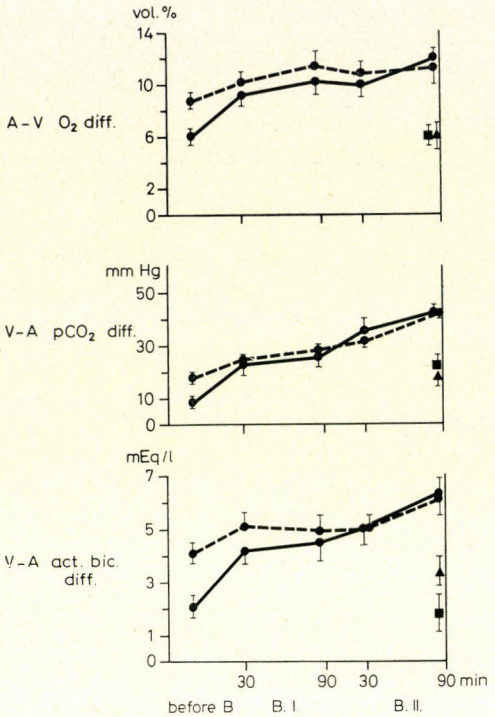

Fig. 2. A–V O_2 difference, pCO_2 and actual bicarbonate during haemorrhage. Continuous line: mixed venous–arterial difference. Broken line: sagittal sinus–arterial difference. ■: Mixed venous–arterial difference after bicarbonate infusion; ▲: sagittal sinus–arterial difference after bicarbonate infusion. B: bleeding

CO_2 content of brain tissues

In the 6 brain regions studied, a significant difference in tissue CO_2 content was observed (Table III). The most pronounced change was seen in the hypothalamus, where CO_2 rose from 14.5 ± 1.6 mM/kg to 31.7 ± 3.7 mM/kg in the first 30 min of haemorrhage, to remain significantly higher than the initial level throughout the hypovolaemic period. At the end of bleeding I, there was a tendency toward recovery.

In the frontal cortex there was a similar though smaller change in the CO_2 content. In the pons the CO_2 content was significantly elevated only at the end of bleeding II. In the medulla, the CO_2 level diminished in the first phase of bleeding, while in the second phase it increased transiently, and at the end of haemorrhage it again approached the normal level.

In the occipital cortex and the thalamus there was no significant change during hypovolaemia.

Table III

Tissue CO_2 content of brain regions during haemorrhage (mM/kg wet weight)

Time		Frontal cortex	Occipital cortex	Thalamus	Hypothalamus	Pons	Medulla oblongata
Before bleeding		18.2 ± 1.0	18.0 ± 1.0	16.8 ± 1.3	14.5 ± 1.6	15.6 ± 1.6	13.4 ± 1.5
Bleeding I	30′	$25.7 \pm 3.4^{++}$	19.3 ± 3.4	18.9 ± 1.8	$31.7 \pm 3.7^{++}$	16.8 ± 1.7	$8.0 \pm 1.4^{++}$
	90′	$12.4 \pm 1.4^{++}$	14.5 ± 2.3	18.1 ± 2.9	$19.9 \pm 2.5^{+}$	15.7 ± 2.7	11.5 ± 2.4
Bleeding II	30′	24.1 ± 5.0	19.7 ± 3.5	20.5 ± 3.4	$38.5 \pm 6.1^{++}$	19.8 ± 5.6	$20.1 \pm 3.9^{+}$
	90′	$28.5 \pm 3.9^{++}$	21.1 ± 2.6	17.6 ± 2.4	$39.9 \pm 4.2^{++}$	$22.5 \pm 3.0^{++}$	16.2 ± 1.4
Bicarbonate treated							
Bleeding II	90′	$17.3 \pm 2.9\square$	$12.8 \pm 1.7\dot{\square}^{+}$	$25.4 \pm 3.8\dot{\square}^{+}$	$35.1 \pm 3.9^{++}$	$11.8 \pm 3.2\square$	13.7 ± 2.3

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values
$\square$ $p < 0.05$; $\square\square$ $p < 0.01$ as compared with simultaneous control values

Effect of the correction of acidosis

In this group of animals the blood acid–base values were identical with those for the control group before the bicarbonate infusion had been started. Therefore a separate registration of these data did not seem necessary, and only the values measured during bleeding II, i.e. prior to infusion (30th min) and at the end of the infusion (90th min) have been listed in Table I.

In these treated animals at the end of the hypovolaemic period not even a compensated acidosis could be demonstrated, BE and pH were identical with prebleeding values.

In arterial and sagittal blood, pCO_2 was within normal limits but as it remained elevated in the mixed venous blood therefore the pH was lower, though the difference was not significant ($p > 0.05$) as compared to the prebleeding state.

V–A pCO_2 difference returned to normal, while the actual bicarbonate difference dropped below the initial value (Fig. 2).

Respiratory rate was not affected by the intervention (Table II). Values before and after the infusion did not differ from the parallel controls ($p > 0.15$). As a consequence of the infusion, the blood Hb content diminished. At the end of haemorrhage, O_2 saturation was the same as in the control group; only the O_2 content of arterial blood was found to be lower than the controls. The decrease in A–V O_2 difference was caused by the decrease of O_2 content in arterial blood as a consequence of haemodilution.

In this group the amount of drained blood was 50 ml/kg on the average at the end of the second phase of bleeding. The loss was substituted by the infusion (10 ml/kg on the average) as shown in Table II where these corrected data are shown in order to facilitate comparison with the untreated dogs. There was no significant difference between the two groups ($p > 0.15$).

Correction of the acidosis by bicarbonate infusions was found to influence the CO_2 content of brain tissues in a different degree in the different cerebral regions. In the hypothalamus, a considerable hypercapnia was invariably found at the end of the second stage of bleeding. In the thalamus the CO_2 content rose significantly, while values for the cerebral cortex and medulla oblongata remained considerably lower in the bicarbonate treated group at the end of the haemorrhagic period.

Discussion

In our experiments, after the first bleeding, respiratory rate increased, arterial bicarbonate and pCO_2 diminished; but the pH did not considerably deviate from the prebleeding value even at the end of the first haemorrhagic period. A considerable fall in pH took place after the second stage of bleeding,

together with a further drop in the bicarbonate level. This was in agreement with the results of BAUBKUS and KIRCHHEIM (1966), according to whom the reduction in bicarbonate is compensated by hyperventilation in the first phase of bleeding and a shift in pH occurs only after a prolonged hypovolaemic condition, at the time when retransfusion is started.

However, study of the venous blood samples revealed that the compensated condition observed in arterial blood in the first period of bleeding was only apparent. In mixed venous blood, the drop in pH was obvious in the first 30 min. The different behaviour of the acid–base components in arterial

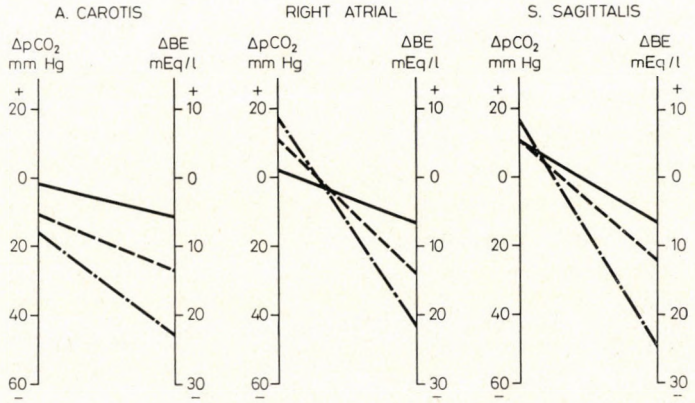

Fig. 3. Diagrammatic representation of ΔBE and ΔpCO_2 changes in carotid, right atrial and sagittal sinus blood, according to KINTNER. Continuous line: before bleeding. Broken line: 90 min, bleeding I. Dotted line: 90 min, bleeding II

and venous blood is well illustrated by KINTNER's scheme (1967) demonstrating the plus or minus deviation of the basic (ΔBE) and acid (ΔpCO_2) factors from the normal values. The divergence of the straight line connecting the two points from the horizontal line indicates the extent of the pH shift; and the deviation of ΔBE and ΔpCO_2 from O point indicates the role of metabolic and respiratory factors in the pH changes (Fig. 3).

The considerable drop of ΔBE in arterial blood refers to the decisive role played by the metabolic components in the development of acidosis. The decrease in ΔpCO_2 is at first nearly the same as the fall in ΔBE, while in the second phase of bleeding it becomes relatively smaller and acidosis develops.

In mixed venous and sagittal sinus blood the ΔBE decreased nearly identically, but the ΔpCO_2 increased, suggesting that both components had an active role in the pH shift.

The increase V–A pCO_2 difference indicated an increase in tissue CO_2 content shortly after the first stage of bleeding. This may have been the consequence of an elevated tissue metabolism. HALMÁGYI et al. (1970) analyzing

pulmonary artery blood have also observed the rise in pCO_2 during hypotension. In their view, the high venous pCO_2 was the result of an increased metabolism in the cells, elicited by the elevated cathecholamine level. The composition of mixed venous blood is obviously influenced by changes of the entire organism. From the results obtained only general conclusions can be drawn. On the other hand, analysis of sagittal blood yielded information solely of the changes occurring in the central nervous system. Metabolism being relatively high in brain tissue, the brain A–V O_2 and pCO_2 differences were more pronounced prior to bleeding than in mixed venous blood.

In sagittal sinus blood, the change following the first stage of bleeding did not reach the extent of those found in right atrial blood. It would appear that due to a circulatory autoregulation the compensating mechanisms are more effective in brain tissue (GOTTSTEIN 1965; DROST et al. 1971). Numerous data in the literature support the view of the relatively wide buffer capacity of brain tissue (SIESJÖ and PONTÉN 1966; WEYNE et al. 1968). The investigations of SIESJÖ and ZWETNOW (1970) into the tissue metabolism of the whole brain indicated considerable changes in the bicarbonate content of the tissues a mean arterial pressure of less than 50 mm Hg. Increase in sagittal sinus–arterial pCO_2 difference in the first period of bleeding indicated, however, the involvement of brain tissues in this early stage of shock. Brain A–V O_2 difference also increased in this period. After the second bleeding the differences between the mixed venous and sagittal sinus blood disappear, the acid–base parameters show identical values. SIESJÖ and ZWETNOW (1970) found in rat experiments that the CSF–arterial pCO_2 difference increased parallel with bleeding. On the contrary, according to their calculations, intracellular pH in the brain tissue remained unchanged. To explain this contradiction they assumed that in certain brain regions there may occur alterations which are not revealed by studies concerned with the whole brain. In our previous experiments carried out in dogs, a blood less was followed by essential differences in the tissue CO_2 content of various brain regions (MAKLÁRI and KOVÁCH 1968). The present results have corroborated these observations in that in the thalamus and occipital cortex the CO_2 content remained unchanged throughout the entire hypotensive period. At the same time in the hypothalamus and frontal cortex the CO_2 level rose in the first 30 min of bleeding. These brain regions displayed the most considerable changes during the experiments. In the pons and medulla oblongata, the differences observed were slight. The metabolic changes due to haemorrhage did not have an effect uniform either in extent or in time upon the studied brain regions. The local tissue hypercapnia found in certain brain regions explains the increase observed in the sagittal sinus–arterial pCO_2 difference.

In the elevation of the tissue CO_2 content, the reduction of blood flow by the blood loss may play a role. According to studies on cerebral circulation in

haemorrhagic shock, regional variations occur within the brain; the most conspicuous drop was recorded in the hypothalamus and frontal cortex (KOVÁCH et al. 1973). Numerous experimental data seem to indicate that these haemodynamic variations are not sufficient to explain the tissue changes (HALMÁGYI et al. 1970; KOVÁCH et al. 1965; KOVÁCH et al. 1959; HALMÁGYI et al. 1971; BYNUM et al. 1971). The tissue CO_2 content seems to have undergone changes independent of the acidosis involving the entire organism, because, according to our findings, correction of acidosis in the last third of the hypovolaemic period did not influence identically the CO_2 level in the different regions of the brain. For this, intracellular biochemical changes must be held primarily responsible. NANA et al. (1965) studying the changes in gamma-aminobutyric acid concentration of brain tissues in shock, also noted differences in the various brain regions and found the highest values just in the hypothalamus and frontal cortex. The metabolic changes, as well as the change in CO_2 content itself may affect the function of the involved areas. KRNJEVIC et al. (1965) studying the irritability of the cerebral cortex, observed that even slight variations in pCO_2 lead to changes in cell activity. DOWNING et al. (1963) showed that cerebral hypercapnia enhanced sympathetic activity. The role of the hypothalamus in the activation of the adrenocortical system is well-known. KOVÁCH and FONYÓ (1960) found a correlation between the development of irreversible shock and the EEG changes and considered the latter to be the most sensitive indicator of the shock condition. FINK et al. (1970) have corroborated this observation.

Our experiments seem to indicate that within the central nervous system the hypothalamus and frontal cortex are the sites most affected in haemorrhagic shock. Pathological changes, which presumably play a role in the evolution of the process, occur even at the beginning of the hypovolaemic period.

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EFFECT ON CENTRAL NERVOUS TISSUE CO_2 CONTENT OF THE CORRECTION OF ACIDOSIS DUE TO HAEMORRHAGE*

By

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The effect of bicarbonate infusion upon the acid–base balance and CO_2 content of brain tissue was studied in haemorrhagic shock in dogs. When physiological saline infusion is given in haemorrhage a similar but more moderate acidosis develops as compared to untreated dogs. Among the brain regions studied only the hypothalamus displayed a considerable rise in CO_2 at the end of the bleeding. Two hours after retransfusion, beside a marked hypercapnia of the hypothalamus, a significant increase in CO_2 level was observed in the frontal and occipital cortex.

During haemorrhage, the development of acidosis can be prevented by appropriate bicarbonate infusion. At the end of the bleeding no tissue hypercapnia was found in any of the brain regions, and 2 hours after retransfusion a slight CO_2 accumulation could be demonstrated only in the hypothalamus. Physiological saline infusion seems to reduce by an alteration of blood viscosity, the pathological changes arising in the cells.

In view of the favourable effect of bicarbonate infusion, acidosis is assumed to represent an aggravating factor in the deterioration of cell metabolism.

The relationship between the acidosis which develops in the course of hypovolaemia and the irreversibility of shock is not fully clarified. SCHMIDT and SCHMIER (1968) presume that local acidaemia may be an important factor in the deterioration of cardiac function in shock. SIEGEL and DOWNING (1970) demonstrated that the decrease in the O_2 tension of arterial blood with a normal pH reduces only minimally, while in acidosis considerably, the contractility of the myocardium. Thus, in haemorrhagic shock acidosis has a potentiating effect upon the deterioration of cardiac function.

In previous experiments we have studied the CO_2 content of various central nervous areas and have come to the conclusion that among the brain regions studied, the hypothalamus and the frontal cortex showed the most significant change in CO_2 content (MAKLÁRI and KOVÁCH 1968). Tissue hypercapnia in these brain regions developed shortly after bleeding, at a time when in arterial blood a decrease in bicarbonate level was found while the pH was still normal. After prolonged hypotension, when the already existing arterial acidosis was corrected with sodium-bicarbonate infusion, the high CO_2 con-

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centration of the hypothalamus did not decrease while in the frontal cortex and in the pons a normal CO_2 level was measured (MAKLÁRI et al. in preparation).

The aim of the present experiments was to find an answer as to the changes in the CO_2 content of brain tissue if the development of acidosis was prevented throughout the bleeding period. For this reason, blood pH and bicarbonate content were kept at a normal level in the hypotensive stage by appropriate sodium-bicarbonate infusion; the CO_2 content of various brain regions was studied at the end of the bleeding period and 2 hours after retransfusion. The same parameters were also examined in control dogs which received a similar volume of physiological saline infused during the entire bleeding period.

Methods

The experiments were carried out on 43 dogs of both sexes under 100 mg/kg α-D(+)-glucochloralose (Merck, Darmstadt) anaesthesia. For the prevention of blood clotting, 5 mg/kg heparin (Gedeon Richter, Budapest) was administered before starting the experiment. Haeminorrhagic shock was brought about by the modified method of WIGGERS (1950). By collecting the drained blood into a reservoir, blood pressure of the animals was maintained between 50—60 mm Hg in the first 90 min period, and between 30—40 mm Hg in the second period, then the shed blood was retransfused.

Blood sampling was done from the carotid artery, sagittal sinus, and right atrium before bleeding, then in the 30th and 90th min of bleeding I, in the 30th and 90th min of bleeding II, and finally 60 and 120 min after retransfusion. A Beckman 160-type gas analyser was used to measure blood pH and pCO_2. Actual bicarbonate and base excess (BE) were estimated from the SIGGAARD-ANDERSEN nomogram (1963). O_2 saturation was determined by means of an Elema-Schönander oxymeter and Hb was estimated with the cyan-methaemoglobin method (BAKOS 1963). During bleeding, standard bicarbonate determination was performed at 15 min intervals using the method of VAN SLYKE (PETERS and VAN SLYKE 1932).

Tissue CO_2 content of the brain tissues was determined in dogs decapitated at the termination of haemorrhage or 120 min after retransfusion as done in our previous experiments with the modified method of ANREP (MAKLÁRI and KOVÁCH 1968).

Thirty-one dogs received an infusion of 0.3 M bicarbonate dissolved in physiological saline throughout the bleeding period, by carefully controlling the drop count in harmony with the standard bicarbonate values according to the scheme mM base = mM BE $\times$ 0.3 $\times$ body weight. Twelve dogs, serving as controls, received the same amount of physiological saline infusion as did the treated groups, at identical intervals. Blood pressure, respiration and the amount of blood loss were registered. From the latter value we deducted at every reading the amount of the already administered infusion and added the volume of blood samples. These corrected values are given in Table I in order to render our data comparable with those of untreated dogs of previous experiments. On retransfusion, the blood in the reservoir was replaced with donor blood in an amount used for the tests.

Student's *t*-test was applied for statistical evaluation of the results.

Results

Acid–base balance

Table I comprises the acid–base values for the physiological saline-treated control group. The tendency of the parameters studied during bleeding and after retransfusion agreed essentially with the changes observed in previous experiments on untreated dogs (MAKLÁRI and KOVÁCH 1968).

Table I

Acid–base values for dogs treated with physiological saline infusion

Blood sample site	time	pH	pCO_2 (mm Hg)	Actual bicarbonate (mM/l)	BE (mEq/l)	O_2 saturation (per cent)	O_2 content (vol per cent)	pO_2 (mm Hg)
Carotid artery	before bleeding	7.37 ± 0.02	32 ± 2	17.8 ± 0.7	-6 ± 1	89 ± 2	20.3 ± 0.8	82 ± 4
	bleeding I 30′	7.36 ± 0.02	$24 \pm 2^{++}$	$13.2 \pm 1.0^{++}$	$-11 \pm 2^{++}$	90 ± 2	$17.8 \pm 1.4^{+}$	$91 \pm 3^{++}$
	90′	$7.33 \pm 0.02^{+}$	$25 \pm 2^{++}$	$12.6 \pm 0.8^{++}$	$-12 \pm 1^{++}$	90 ± 1	$19.2 \pm 0.8^{+}$	$93 \pm 4^{++}$
	bleeding II 30′	$7.28 \pm 0.02^{++}$	$24 \pm 2^{++}$	$11.2 \pm 0.8^{++}$	$-14 \pm 1^{++}$	90 ± 1	$18.6 \pm 0.8^{++}$	$95 \pm 3^{++}$
	90′	$7.24 \pm 0.03^{++}$	$23 \pm 2^{++}$	$9.8 \pm 0.5^{++}$	$-17 \pm 1^{++}$	90 ± 1	$18.4 \pm 0.9^{++}$	$95 \pm 3^{++}$
	after retransfusion							
	60′	$7.27 \pm 0.02^{++}$	$27 \pm 2^{+}$	$13.0 \pm 1.1^{++}$	$-13 \pm 2^{++}$	88 ± 1	20.7 ± 1.2	82 ± 9
	120′	$7.27 \pm 0.02^{++}$	$29 \pm 3^{+}$	$12.7 \pm 1.1^{++}$	$-14 \pm 1^{++}$	88 ± 2	20.5 ± 1.2	84 ± 2
Right atrium	before bleeding	7.32 ± 0.01	42 ± 2	19.9 ± 0.6	-5 ± 1	52 ± 4	11.8 ± 1.1	39 ± 3
	bleeding I 30′	$7.22 \pm 0.02^{++}$	$47 \pm 3^{++}$	$18.1 \pm 1.0^{+}$	$-8 \pm 1^{+}$	$25 \pm 3^{++}$	$5.2 \pm 0.5^{++}$	$26 \pm 4^{++}$
	90′	$7.19 \pm 0.02^{++}$	$47 \pm 2^{++}$	$17.0 \pm 0.7^{++}$	$-11 \pm 1^{++}$	$31 \pm 5^{++}$	$6.7 \pm 1.0^{++}$	$25 \pm 3^{++}$
	bleeding II 30′	$7.15 \pm 0.02^{++}$	$50 \pm 3^{++}$	$16.1 \pm 1.0^{++}$	$-13 \pm 1^{++}$	$25 \pm 3^{++}$	$4.8 \pm 0.6^{++}$	$26 \pm 4^{++}$
	90′	$7.09 \pm 0.03^{++}$	$53 \pm 3^{++}$	$15.3 \pm 1.2^{++}$	$-15 \pm 2^{++}$	$24 \pm 3^{++}$	$4.7 \pm 0.4^{++}$	$30 \pm 4^{+}$
	after retransfusion							
	60′	$7.20 \pm 0.01^{++}$	45 ± 3	$16.5 \pm 0.9^{++}$	$-11 \pm 1^{++}$	$39 \pm 4^{+}$	$9.2 \pm 1.0^{+}$	40 ± 8
	120′	$7.20 \pm 0.02^{++}$	44 ± 2	$16.2 \pm 0.8^{++}$	$-12 \pm 1^{++}$	$42 \pm 4^{+}$	$9.8 \pm 1.1^{+}$	33 ± 9
Sagittal sinus	before bleeding	7.28 ± 0.01	52 ± 2	22.5 ± 0.5	-4 ± 1	37 ± 5	8.5 ± 1.2	29 ± 2
	bleeding I 30′	7.22 ± 0.03	$47 \pm 2^{+}$	$18.3 \pm 1.3^{++}$	-9 ± 2	$21 \pm 3^{++}$	$3.9 \pm 0.5^{++}$	25 ± 2
	90′	$7.20 \pm 0.01^{++}$	49 ± 2	$17.9 \pm 0.8^{++}$	$-10 \pm 1^{++}$	$26 \pm 2^{++}$	$5.4 \pm 0.5^{++}$	25 ± 2
	bleeding II 30′	$7.16 \pm 0.02^{++}$	51 ± 2	$16.6 \pm 0.7^{++}$	$-12 \pm 1^{++}$	$23 \pm 2^{++}$	$4.8 \pm 0.5^{++}$	25 ± 2
	90′	$7.12 \pm 0.02^{++}$	49 ± 2	$15.2 \pm 0.6^{++}$	$-14 \pm 1^{++}$	$24 \pm 2^{++}$	$4.9 \pm 0.4^{++}$	27 ± 2
	after retransfusion							
	60′	$7.17 \pm 0.02^{++}$	53 ± 3	$17.5 \pm 0.8^{++}$	$-11 \pm 2^{++}$	$27 \pm 2^{+}$	$6.4 \pm 0.5^{+}$	28 ± 6
	120′	$7.14 \pm 0.03^{++}$	57 ± 5	$17.6 \pm 0.8^{++}$	$-12 \pm 1^{++}$	$26 \pm 2^{+}$	$6.2 \pm 0.6^{+}$	26 ± 3

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values

The first haemorrhage was immediately followed by a decrease in the bicarbonate level and BE. During this period pH in arterial blood did not diminish considerably, but in the right atrial and sagittal blood the pH was significantly lower even at the end of the first 30 min. The A–V pCO_2 difference increased in the first 30 min in both the mixed venous and sagittal blood (Fig. 1).

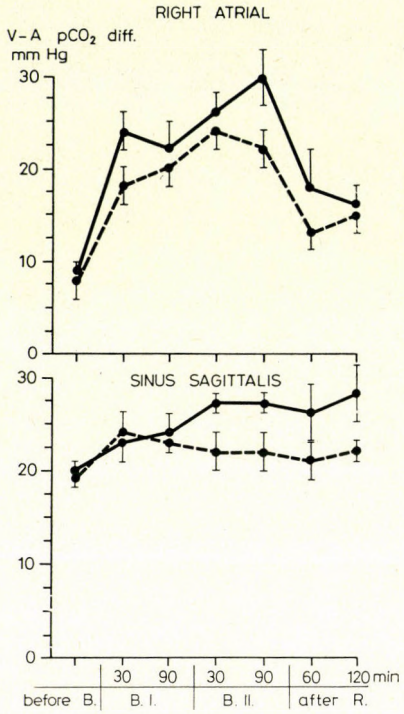

Fig. 1. V–A pCO_2 difference during haemorrhagic shock in physiological saline-treated and in bicarbonate-treated dogs. Continuous line: physiological saline treatment. Broken line: bicarbonate treatment. B: bleeding; R: retransfusion

The second bleeding period was followed by a further increase of acidosis with a simultaneous further drop of the bicarbonate level and pH. This fall, however, was not so apparent in this group as it was in the untreated dogs. The pH values at the end of the second bleeding period as compared to the corresponding data of untreated dogs were as follows (with the previous values in parenthesis), in arterial blood, 7.24 (7.08) (MAKLÁRI et al. in preparation); in the sagittal sinus, 7.12 (6.92); in mixed venous blood, 7.09 (6.98). pCO_2 also rose sometwhat in both mixed venous and sagittal blood; this was reflected also in the more moderate increase in the V–A pCO_2 difference.

The same was true for the acid–base values found 60 min after retransfusion. Acidosis was less grave in the physiological saline-treated group than

in untreated dogs. No difference was demonstrable 2 hours after retransfusion, BE, pCO_2 and pH values agree fully in the untreated and physiological saline-treated animals.

O_2 saturation of arterial blood did not change during bleeding. It diminished considerably, however, in all types of venous blood. There was a significant increase in A–V O_2 difference (Fig. 3). O_2 content of the mixed

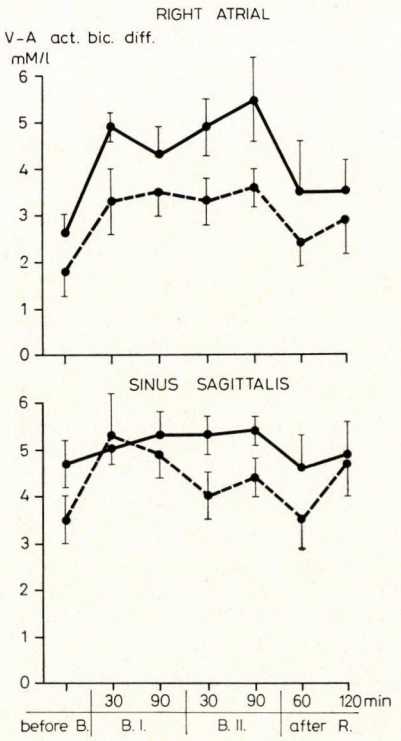

Fig. 2. V–A actual bicarbonate difference during haemorrhagic shock in physiological saline treated and in bicarbonate-treated dogs. Continuous line: physiological saline treatment Broken line: bicarbonate treatment. B: bleeding; R: retransfusion

venous blood was higher after retransfusion, the reduction in A–V O_2 was similar to that found in the untreated animals. Retransfusion did not produce changes in the O_2 content of the sagittal sinus, thus, the A–V O_2 difference remained invariably high.

Blood values for the animals receiving bicarbonate infusion are summarized in Table II. Development of acidosis was prevented by appropriate infusion throughout the bleeding period. BE and actual bicarbonate did not decrease either in the arterial or the venous blood samples. In arterial blood a slight rise in pH took place, but in venous blood the pH remained unchanged,

no acidosis could be demonstrated. One and 2 hours after retransfusion the results obtained were identical with the pre-experimental ones. In mixed venous blood, the rise in pCO_2 could be detected also in this experimental group, consequently the V–A pCO_2 difference also increased during bicarbonate infusion, but at the end of the haemorrhage it did not reach such a high value as in control shock (Fig. 1). After retransfusion, the reduction in pCO_2 difference was the same as in the animals receiving physiological saline.

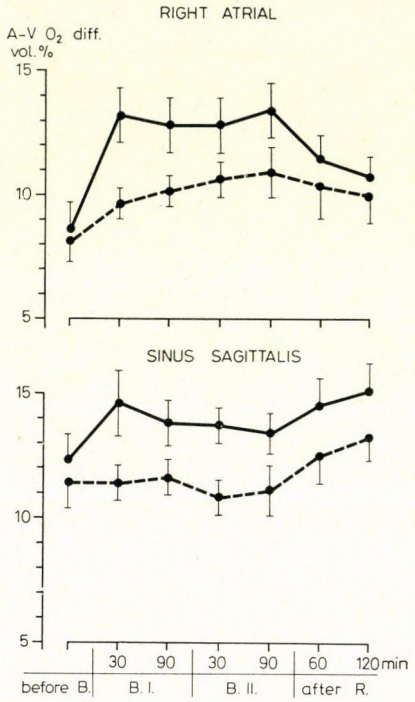

Fig. 3. A–V oxygen difference during haemorrhagic shock in physiological saline-treated and in bicarbonate-treated dogs. Continuous line: physiological saline treatment. Broken line: bicarbonate treatment. B: bleeding; R: retransfusion

During haemorrhage, pCO_2 does not change much in the sagittal sinus. V–A pCO_2 difference increased somewhat in the first phase of bleeding, but in the second phase no further rise took place. Even retransfusion failed to affect the values.

The mixed V–A actual bicarbonate difference did not increase to such an extent in the bicarbonate-treated group as in the physiolgical saline-treated shock group, and after retransfusion the difference tended to diminish gradually (Fig. 2). The sagittal sinus–arterial actual bicarbonate difference changed during the first bleeding period similarly as in the physiological saline-treated group, and diminished during the second bleeding period to produce

Table II

Acid–base values for dogs treated with bicarbonate infusion

Blood sample site	time	pH	pCO_2 (mm Hg)	Actual bicarbonate (mM/l)	BE (mEq/l)	O_2 saturation (per cent)	O_2 content (vol per cent)	pO_2 (mm Hg)
Carotid artery	before bleeding	7.33 ± 0.02	37 ± 2□	18.7 ± 0.6	−5 ± 1	85 ± 2	19.0 ± 0.8	86 ± 3
	bleeding I 30′	7.41 ± 0.03++	31 ± 3□	18.9 ± 1.2□□	−4 ± 1□□	91 ± 1++	17.5 ± 0.7+	86 ± 4
	90′	7.39 ± 0.02+	28 ± 2++	16.9 ± 0.8+	−6 ± 1□□	89 ± 2++	17.3 ± 0.9+	92 ± 4
	bleeding II 30′	7.42 ± 0.02++	30 ± 3+	18.4 ± 1.2□□	−5 ± 2□□	90 ± 2++	16.8 ± 1.2+	86 ± 4
	90′	7.41 ± 0.02++	29 ± 2+□+	17.9 ± 0.8□□	−5 ± 2□□	91 ± 2++	17.0 ± 1.1+	89 ± 4□
	after retransfusion 60′	7.36 ± 0.02□□	32 ± 3+	17.2 ± 0.9+□	−7 ± 1+□	93 ± 1+□	19.9 ± 0.8	89 ± 5
	120′	7.37 ± 0.02□□	30 ± 3+	16.3 ± 1.5+	−7 ± 2+□	93 ± 1+	19.9 ± 0.9	85 ± 4
Right atrium	before bleeding	7.27 ± 0.02	44 ± 2	19.5 ± 0.5	−5 ± 4	49 ± 4	10.8 ± 1.1	43 ± 4
	bleeding I 30′	7.30 ± 0.02□	50 ± 3++	22.0 ± 0.7++	−3 ± 1++	40 ± 3++	7.6 ± 0.8+□+	30 ± 3++
	90′	7.25 ± 0.03□	49 ± 3+	20.3 ± 0.7++	−6 ± 1□□	39 ± 3++	7.3 ± 0.7+□+	29 ± 3++
	bleeding II 30′	7.26 ± 0.02□□	53 ± 3++	21.6 ± 1.0□□	−5 ± 1□□	36 ± 2++	6.3 ± 0.6+□+	24 ± 2++
	90′	7.23 ± 0.02□□	52 ± 4++	20.5 ± 1.5□□	−6 ± 2□□	36 ± 2++	6.2 ± 0.5+□+	25 ± 2++
	after retransfusion 60′	7.27 ± 0.02□□	45 ± 3	19.2 ± 0.9□	−7 ± 1□	46 ± 4+	9.6 ± 1.0	35 ± 2+
	120′	7.29 ± 0.02□□	45 ± 3	19.7 ± 1.0□	−5 ± 1□	43 ± 4+	9.6 ± 1.1	35 ± 3+
Sagittal sinus	before bleeding	7.24 ± 0.02+□+	54 ± 2	21.8 ± 0.6	−4 ± 1	34 ± 3	7.3 ± 1.0	27 ± 3
	bleeding I 30′	7.30 ± 0.02+□	55 ± 3	24.1 ± 0.6+□	−1 ± 1□□	30 ± 2□	5.6 ± 0.6+	22 ± 2
	90′	7.28 ± 0.02 □	50 ± 3	21.7 ± 0.9□□	−4 ± 1□□	30 ± 2	5.6 ± 0.5+	22 ± 2
	bleeding II 30′	7.29 ± 0.02□□	51 ± 4	22.4 ± 1.3□□	−3 ± 2□□	35 ± 2□□	5.9 ± 0.5	23 ± 2
	90′	7.27 ± 0.02□□	49 ± 4+	21.5 ± 1.4□□	−5 ± 2□□	35 ± 2□□	5.9 ± 0.4	22 ± 2
	after retransfusion 60′	7.23 ± 0.02□	54 ± 3	20.7 ± 1.0□	−6 ± 1□	33 ± 3□	7.4 ± 0.9	28 ± 5
	120′	7.25 ± 0.02□□	54 ± 3	21.6 ± 1.1□	−5 ± 2□□	31 ± 3	6.7 ± 0.4	26 ± 4

+ $p < 0.05$; ++ $p < 0.01$ as compared with prebleeding values
□ $p < 0.05$; □□ $p < 0.01$ as compared with physiological saline treated values

a strongly significant difference. Two hours after retransfusion there was no difference in the two experimental groups.

An essential difference in O_2 content could be demonstrated in favour of the bicarbonate-treated group. In these dogs the O_2 content of mixed venous blood did not fall during haemorrhage to such an extent as in the physiological saline-treated animals and changed even less in the sagittal sinus. This is well illustrated in Fig. 3 which shows the difference in A–V O_2 content . The arterial–mixed venous O_2 difference during haemorrhage increased only slightly in the physiological saline-treated group and the arterial–sagittal sinus O_2 difference remained practically unchanged throughout the experiment. The difference between the two groups was significant statistically both during bleeding and after retransfusion.

Evaluating the data characteristic of the general condition (Table III), the respiratory rate was accelerated in the same degree both in the physiological saline-treated and in the bicarbonate-treated dogs during bleeding, and retransfusion did not result in a difference either. The same amount of blood was drained in both experimental groups.

Table III

Blood pressure, respiratory rate, reinfusion of lost blood and Hb level in physiological saline-treated and in bicarbonate treated dogs

Group	Time	Mean arterial pressure	Respiratory rate/min	Corrected blood loss	Amount of infusion administered	Haemoglobin
Physiological saline treated	before bleeding	115 ± 4	21 ± 3			16.3 ± 0.5
	bleeding I 30′	$61 \pm 2^{++}$	$27 \pm 3^{++}$	27 ± 3	4 ± 0.4	$15.3 \pm 0.7^{++}$
	90′	$58 \pm 2^{++}$	$31 \pm 4^{++}$	19 ± 4	8 ± 0.6	$15.6 \pm 0.5^{++}$
	bleeding II 30′	$38 \pm 2^{++}$	$30 \pm 3^{++}$	20 ± 4	14 ± 0.8	$15.1 \pm 0.6^{++}$
	90′	$36 \pm 2^{++}$	$33 \pm 4^{++}$	15 ± 4	20 ± 0.7	$15.0 \pm 0.6^{++}$
	after retransf. 60′	$55 \pm 6^{++}$	$32 \pm 6^{+}$			17.1 ± 0.9
	120′	$60 \pm 6^{++}$	$32 \pm 6^{+}$			17.1 ± 0.9
Bicarbonate treated	before bleeding	106 ± 5	$12 \pm 2^{\square\square}$			16.3 ± 0.5
	bleeding I 30′	$59 \pm 1^{++}$	$17 \pm 2^{++}_{\square\square}$	22 ± 2	4 ± 0.4	$14.2 \pm 0.4^{++}$
	90′	$55 \pm 1^{++}$	$24 \pm 3^{++}$	19 ± 2	9 ± 0.6	$13.9 \pm 0.5^{+}_{\square}$
	bleeding II 30′	$36 \pm 1^{++}$	$25 \pm 3^{++}$	22 ± 3	14 ± 0.8	$13.3 \pm 0.7^{+}_{\square}$
	90′	$35 \pm 1^{++}$	$24 \pm 3^{++}$	18 ± 3	20 ± 1.0	$13.3 \pm 0.7^{+}_{\square}$
	after retransf. 60′	$69 \pm 5^{+}_{\square}$	$28 \pm 6^{++}$			15.5 ± 0.6
	120′	$72 \pm 5^{++}$	$24 \pm 2^{++}$			15.7 ± 0.8

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values
$\square$ $p < 0.05$; $\square\square$ $p < 0.01$ as compared with physiological saline treated values

Changes in the CO_2 content of brain tissues

In the physiological saline-treated control group a higher CO_2 content was found in the hypothalamus and medulla oblongata at the end of the haemorrhage (Table IV). In the hypothalamus, the increase in CO_2 level was

not so considerable as in the previous, untreated experimental group (39.9 mM/kg). Also, an increase in CO_2, as found in the untreated group, failed to occur during the infusion of physiological saline both in the frontal cortex and in the pons.

Table IV

CO_2 content of brain tissues (mM/kg wet weight) in physiological saline treated and bicarbonate treated dogs

Group	Time	Frontal cortex	Occipital cortex	Thalamus
Physiological saline treated	bleeding II 90′	17.4 ± 1.9	16.0 ± 2.5	15.9 ± 1.5
	after retransfusion	$21.9 \pm 2.2^{+}$	$24.5 \pm 3.8^{+}$	22.9 ± 4.5
Bicarbonate treated	bleeding II 90′	14.9 ± 1.9	16.6 ± 1.9	14.7 ± 2.2
	after retransfusion	18.2 ± 3.2	14.4 ± 2.7	15.2 ± 1.8

Group	Time	Hypothalamus	Pons	Medulla oblongata
Physiological saline treated	bleeding II 90′	$25.1 \pm 2.3^{++}$	17.5 ± 3.0	$21.0 \pm 2.2^{++}$
	after retransfusion	$42.8 \pm 3.4^{++}$	16.8 ± 1.7	18.2 ± 4.2
Bicarbonate treated	bleeding II 90′	$18.4 \pm 2.2\square$	12.1 ± 2.1	$12.8 \pm 2.7\square$
	after retransfusion	$21.3 \pm 2.7^{+}\square\square$	16.4 ± 3.1	11.8 ± 2.8

$^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$ as compared with prebleeding values
$\square$ $p < 0.05$; $\square\square$ $p < 0.01$ as compared with physiological saline treated value

Two hours after retransfusion, a marked hypercapnia, similar as in the untreated group, was found in the hypothalamus. Of the other brain regions studied, the frontal and occipital cortex displayed a moderate rise in CO_2, while in contrast to the untreated animals, in the thalamus and medulla oblongata there was no hypercapnia.

In the brain tissues of the bicarbonate infusion-treated dogs, at the end of the bleeding, a normal CO_2 content was found in every case.

Two hours after retransfusion, tissue CO_2 was significantly higher only in the hypothalamus, while in the other brain regions there was no change as compared to the pre-experimental data.

Discussion

Analysis of the acid–base values of blood samples obtained from animals treated with physiological saline infusion indicated that the infusion reduced the extent of acidosis due to haemorrhage in the later stages of hypotension. The differences were still observable one hour after retransfusion, but two

hours later they already corresponded to the acid–base values of untreated dogs. Less marked were also the differences in the CO_2 content of brain tissues in the physiological saline-treated animals than in the untreated ones. Of the brain areas examined the hypothalamus displayed the most extensive alterations and at the end of the haemorrhage a moderate rise in CO_2 occurred and two hours after retransfusion a marked hypercapnia was found. The picture was more favourable in the saline group in the other brain regiones examined. In the frontal and occipital cortex, as well as in the thalamus, the CO_2 content was elevated at two hours after retransfusion but it failed to approach the very high values found in the untreated group. Even then the pons and the medulla oblongata showed a normal CO_2 level.

It is well-known that in haemorrhagic shock in the later period of bleeding, parallel with the retransfusion of blood the plasma protein content increases and so do the haematocrit and blood viscosity (ROTHE and SELKURT 1964; SHOEMAKER 1965; LUTZ 1971). This enhances cell aggregation in the blood and contributes to the reduction of cardiac output and to the deterioration of circulation. Blood viscosity has an important role also in cerebral circulation (GOTTSTEIN 1965). Deterioration of local blood flow of tissues may have a role in the pathological alterations of cell metabolism. In view of these findings, the question arises that in the treatment of shock, beside volume substitution, haemodilution may also be an important factor in the amelioration of circulation and tissue microcirculation (EHRLY 1971; MESSMER and BRENDEL 1971; BAVE et al. 1967). HOLLENBERG et al. (1970) attributed an importance to haemodilution in the mechanism of action of dibenzyline pretreatment. The inhibitory effect of physiological saline infusion on the development of hypovolaemic acidosis might be explained in this way.

Our observation that in the brain regions the tissue CO_2 content did not change so much as in untreated dogs may indicate a slighter derangement of tissue metabolism.

The present results thus supported the earlier observations that it is in the hypothalamus that haemorrhage causes the earliest and most extensive changes.

When maintaining the blood bicarbonate and pH values at a normal level by bicarbonate infusion throughout the entire bleeding period, we did not find CO_2 accumulation in any brain region at the end of the 3-hour hypotonic period. Two hours after retransfusion, a slight rise in CO_2 could be demonstrated only in the hypothalamus. Correction of arterial acidosis reduced this or at least delayed the development of those metabolic changes in the brain cells which manifest themselves with an increase in tissue CO_2 content. Thus, an aggravating role must be attributed to acidosis in the cerebral alterations elicited by bleeding. Data in the literature are not uniform concerning the addition of bicarbonate infusion to the preventive or therapeutic

measures in haemorrhagic shock (SCHWEIZER and HOWLAND 1964; NORTHFIELD et al., 1971; IRVING 1971; JONES 1971). Our experimental results, however, strongly support the favourable effect of bicarbonate infusion.

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ADIPOSE TISSUE AND ENDOTOXIN SHOCK[1]

By

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Adipose tissue metabolism was studied in the isolated subcutaneous (SC) and omental (O) regions in female mongrel dogs before and after *E. coli* endotoxin administration. Of the haemodynamic parameters monitored, MABP, CO and BF decreased significantly, while HR remained unchanged after endotoxin injection. Arterial concentration of FFA remained unchanged, GLY and L increased progressively, and GLU decreased only >60 min after endotoxin administration. GLY output and GLU uptake by SC adipose tissue decreased significantly >60 min after endotoxin injection.

Both basal and NE stimulated lipolysis by isolated fat cells were studied in the presence and absence of GLU. Adipocytes of O adipose tissue consistently released more FFA and GLY upon NE stimulation, than adipocytes from SC adipose tissue, both before and after endotoxin administration. M adipocytes behaved like those of O origin in 3 out of 4 instances under control conditions, and in each instance after endotoxin. Endotoxin shock increased the NE induced lipolytic response in SC, O and M fat cells, as illustrated by increased FFA and GLY release.

SC and O fat cells showed greater NE stimulated FFA release in the absence than in the presence of GLU, indicating decreased reesterification under these conditions. Endotoxin tended to reduce this difference. The NE dose–response curve was not shifted after endotoxin administration. However, the lipolytic response was increased by all (including maximum) doses of NE applied.

Adipose tissue holds by far the largest reserve of metabolic energy in the body. On the average, a normal man may store about 10,000 g of fat (MORSE and SOELDNER 1965), but only 500 g of glycogen (DAVSON and EGGLETON 1962). Thus the lipids in adipose tissue represent a nearly inexhaustible supply of energy. In life processes the demand for energy takes precedence over all other requirements. It is therefore of crucial importance to gain detailed knowledge of the regulation of energy supply in face of an inimical internal environment. With these considerations in mind we have studied the metabolic changes occurring in the subcutaneous and omental adipose tissue regions following endotoxin administration in dogs.

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Abbreviations used: MABP: mean arterial blood pressure; CO: cardiac output; HR: heart rate; BF: blood flow; NE: norepinephrine; FFA: free fatty acids; GLY: glycerol; L: lactate; GLU: glucose; SC: subcutaneous; O: omental; M: mesenteric; TG: triglyceride; cAMP: cyclic 3',5'-adenosine monophosphate.

Methods

Female mongrel dogs weighing 16 to 23 kg were used after an 18-hour fast. They were anaesthetized with sodium pentobarbital given intravenously initially in a dose of 30 mg/kg and throughout the experiment at a constant infusion rate of 4 mg/kg/hr. Following splenectomy, each animal received 700 U.S.P. units per kg body weight of heparin. Blood pressure was measured in the left common carotid artery, systemic arterial blood samples were taken from the left femoral artery. Cardiac output was determined by the dye dilution technique (KINSMAN et al. 1929).

The subcutaneous fat pad in the suprapubic region was completely isolated from the abdominal muscles. It remained in connection with the dog's circulation and nervous system only by one supplying artery, one vein and a sympathetic nerve (ROSELL 1966). The vein was cannulated and the outflowing blood collected in order to determine the subcutaneous tissue blood flow. Venous blood was continuously returned to the animal by transfusion through the right femoral or saphenous vein. Tissue samples for isolated fat cell studies were taken from the contralateral subcutaneous fat pad.

The omental adipose tissue was prepared according to BALLARD's technique (BALLARD and ROSELL 1969). The mesenteric adipose tissue was prepared in the following manner. An approximately 25 cm long segment of the small intestine, 30—40 cm below the duodenum, was isolated with a number of fine ligatures from the mesenteric adipose tissue. This segment was removed while the two lateral parts of the bowel with intact circulation were connected to each other by end-to-end anastomoses. The main mesenteric adipose tissue vein was then cannulated.

Blood and adipose tissue were sampled before, < 60 minutes after, and >60 minutes after the injection of *Escherichia coli* endotoxin (Difco) in a dose that corresponded to approximately LD_{90}. Each blood sample for FFA, glucose (GLU), glycerol (GLY) and lactate (L) analysis was anticoagulated in vitro with heparin and kept in an ice bath. Blood GLY and L concentrations were determined enzymatically (WIELAND 1963; HOHORST 1963). Plasma FFA was measured by the Dole—Meinertz method (DOLE and MEINERTZ 1960), plasma GLU by autoanalyzer. Fat cells were obtained by enzymatic digestion of subcutaneous and omental adipose tissue, following the procedure of RODBELL (1964) with minor modifications. The cut pieces of adipose tissue were blotted and placed in 30 ml plastic bottles. Each bottle contained 3 ml of 4% albumin (Fraction V, Pentex Co., Kankakee, Ill.) in Krebs–Ringer bicarbonate buffer, pH 7.4, prepared fresh each time, and 1 mg of crude collagenase per ml (Clostridium histolyticum, from Worthington). At the end of the 1 hr digestion period in a metabolic shaker at 37°C, undigested pieces (if any) were removed with a forceps, and the isolated cells were washed 3 times with Krebs–Ringer bicarbonate buffer. The washed cells were incubated in plastic capped culture tubes, in Krebs–Ringer bicarbonate buffer containing 4% bovine serum albumin in the presence of GLU (5.5 μmoles/ml), and norepinephrine (NE, 0.2 μg/ml). The gas phase for incubation was 95% O_2 — 5% CO_2. Incubation was carried out in a metabolic shaker at 37°C for 2 hours. At the end of the incubation period aliquots of the medium were taken for subsequent FFA and GLY analysis, the latter by an enzymatic fluorometric micromethod (LAURELL and TIBBLING 1966).

Release of FFA and GLY was expressed in units per triglyceride (TG) content of the cells (FAIN and REED 1970), calculated on the basis of total fatty acid content after saponification. Protein was also measured in aliquots of washed, suspended cells (LOWRY et al. 1951).

Fat cell size distribution was estimated by measuring the diameter of 100 washed cells using a light microscope equipped with a calibrated ocular. From the mean diameter and standard deviation, mean volume was calculated on the assumption that the cells were spherical and using the equation:

$$\text{Volume} = \frac{\pi}{6}(3\sigma^2 + \bar{x}^2)\bar{x},$$

where σ^2 is the variance of the diameter, and $\bar{x}$ the mean diameter (HIRSCH and GALLIAN 1968).

Results and discussion

Table I shows the haemodynamic changes after *E. coli* endotoxin injection. Mean arterial blood pressure (MABP), cardiac output (CO) and blood flow (BF) decreased significantly, heart rate (HR) remained unchanged. The percentage decrease in CO was quite similar to decrements of BF at the two adipose tissue sites.

Table I

Haemodynamic changes following endotoxic shock (n = 13)
(mean ± S.E.)

	Control	After endotoxin	
		< 60 min	> 60 min
MABP (mm Hg)	126 ± 7	67 ± 7 (−47%)	36 ± 3 (−71%)
HR (per min)	154 ± 8	146 ± 12	141 ± 15
Cardiac output (ml/min)	1592 ± 141	754 ± 104 (−53%)	531 ± 79 (−67%)
Blood flow (ml/min/100 g)			
in subcutaneous adipose tissue	3.74 ± 0.66	1.98 ± 0.76 (−47%)	1.49 ± 0.56 (−60%)
in omental adipose tissue	3.27 ± 0.33	1.73 ± 0.40 (−47%)	1.27 ± 0.35 (−61%)

In earlier studies (KOVÁCH et al. 1970) it was demonstrated that during haemorrhage blood flow in subcutaneous adipose tissue was severely diminished due to the pronounced rise in peripheral resistance, and as a consequence, tissue metabolism was impaired as indicated by the marked decrease in oxygen uptake. Blood flow diminution was much less pronounced in phenoxybenzamine-treated animals during haemorrhage. Infusion of isoprenaline counteracted the pronounced suppression of blood flow in subcutaneous adipose tissue during bleeding to 55 mm Hg (KOVÁCH et al. 1971).

Mean values for the arterial and venous levels of the metabolites studied are presented in Table II. FFA levels remained unchanged throughout the experiments, GLY levels increased significantly 60 min following endotoxin injection. L levels rose progressively and significantly at both sampling times, GLU levels remained unchanged at <60 min, and decreased at >60 min after endotoxin administration.

In earlier experiments the arterial plasma FFA and release rate of FFA from the subcutaneous adipose tissue did not increase during bleeding, despite a presumably high sympathico-adrenal activity (KOVÁCH et al. 1970), with significantly elevated blood GLY levels (ROSELL et al. 1973).

The observed elevated blood lactate level is an accepted hallmark in the various forms of shock. Lactate may also serve as a feedback regulator of FFA mobilization (WEIL et al. 1965), bringing about an inverse relationship between blood lactate and plasma FFA levels (MILLER et al. 1964). The progressively increasing plasma GLY levels after endotoxin administration were con-

Table II

Arterial and venous levels of various metabolites before and after endotoxin administration
(mean ± S.E.)

		Control	After endotoxin < 60 min	After endotoxin > 60 min
FFA				
	A	0.608 ± .039 (14)	0.587 ± .042 (15)	0.687 ± .093 (12)
	V	0.708 ± .082 (13)	0.644 ± .083 (13)	0.839 ± .122 (12)
	O	0.646 ± .077 (12)	0.556 ± .062 (12)	0.684 ± .087 (10)
Glycerol				
	A	0.063 ± .010 (13)	0.087 ± .014 (13)	0.195 ± .065 (11)
	V	0.164 ± .031 (12)	0.261 ± .071 (11)	0.314 ± .089 (11)
	O	0.117 ± .017 (11)	0.225 ± .068 (13)	0.425 ± .129 (12)
Lactate				
	A	1.105 ± .196 (14)	2.874 ± .421 (15)	3.365 ± .455 (11)
	V	1.194 ± .163 (13)	2.270 ± .286 (13)	3.224 ± .403 (11)
	O	1.213 ± .183 (12)	2.167 ± .237 (13)	2.731 ± .603 (9)
Glucose				
	A	7.795 ± .831 (14)	8.582 ± .961 (15)	5.838 ± 1.344 (13)
	V	6.523 ± .730 (13)	5.675 ± .725 (13)	4.938 ± 1.375 (10)
	O	6.566 ± .828 (12)	5.952 ± 1.034 (12)	4.517 ± 1.123 (10)

All concentrations are expressed in μmoles/ml. Numbers in parentheses refer to the number of experiments
A: blood from femoral artery
V: venous blood coming from subcutaneous adipose tissue
O: venous blood coming from omental adipose tissue

current with high circulating catecholamine levels. This finding corroborates the report by BALLARD and ROSELL (1971) that the outflow of GLY from canine O adipose tissue was increased during sympathetic nerve stimulation or NE infusion.

Enzymatic breakdown of adipose tissue TG is the source of blood GLY and FFA. The simultaneous occurrence of increased GLY and unchanged FFA levels may be ascribed to the fact that GLY can diffuse freely from the fat cell and become distributed in the body's water compartment, while FFA has to be transported bound to albumin. The significantly reduced blood flow after endotoxin administration imposes serious limitations on the availability of albumin and possibly other protein fractions for the transport of FFA.

Table III presents information on the effect of endotoxin on glycrol output and glucose uptake by the two adipose tissue sites studied in vivo. The subcutaneous site released significantly less glycerol ($p < 0.025$) and took

Table III

Effect of endotoxin on glycerol output and glucose uptake by adipose tissue (mean ± S.E.)

	Control	
	SC	O
Glycerol output	0.383 ± 0.096 (n = 11)	0.198 ± 0.051 (n = 12)
Glucose uptake	6.46 ± 1.91 (n = 13)	5.58 ± 1.36 (n = 12)

	< 60 min after endotoxin	
	SC	O
Glycerol output	0.199 ± 0.074 (n = 11)	0.140 ± 0.039 (n = 13)
Glucose uptake	4.16 ± 0.91 (n = 13)	5.53 ± 1.44 (n = 12)

	After endotoxin > 60 min	
	SC	O
Glycerol output	0.159* ± 0.067 (n = 11)	0.124 ± 0.029 (n = 10)
Glucose uptake	2.36** ± 1.07 (n = 10)	3.16 ± 1.21 (n = 10)

All values are expressed in μmoles/min/100 g tissue
SC: subcutaneous adipose tissue
O: omental adipose tissue
* $p < .025$
** $p < .05$

up significantly less glucose ($p < 0.05$) at > 60 min after endotoxin administration, than under control conditions.

The isolated fat cell preparation was used for in vitro metabolic investigations. Relative homogeneity of the cell population as well as unimpaired access of hormones to the cells are the two greatest advantages of this preparation.

From Table IV it can be observed that the NE stimulated release of FFA and GLY was higher in O and M, than in SC fat cells both under control conditions and after endotoxin administration. Following endotoxin administration, the NE evoked lipolytic response was increased at all three sites studied.

Under control conditions the NE stimulated release of FFA was significantly higher ($p < 0.05$) by O cells than by SC cells. In 3 out of 4 experiments performed with M cells, they also released more FFA than did SC fat cells.

Table IV

NE stimulated FFA and glycerol release by dog fat cells of subcutaneous (SC), omental (O), and mesenteric (M) adipose tissue, μmoles/mmole triglyceride/2 hr (mean ± S.E.)

	SC	O	M
	before endotoxin		
FFA	7.17 ± 1.83 (n = 11)	14.40 ± 2.64 (n = 11)	32.71 ± 14.20 (n = 4)
Glycerol	4.96 ± 0.69 (n = 11)	7.57 ± 1.52 (n = 11)	13.58 ± 2.89 (n = 4)
	after endotoxin		
FFA	11.76 ± 2.20 (n = 13)	28.40 ± 4.34 (n = 13)	53.78 ± 23.50 (n = 4)
Glycerol	5.77 ± 1.02 (n = 13)	11.68 ± 1.53 (n = 13)	18.24 ± 4.00 (n = 4)

In refers to the number of experiments. Each experimental value represents the average of duplicate incubations

GLY release under the same experimental conditions was significantly higher by M cells ($p < 0.05$) than by SC cells. After endotoxin administration, the NE evoked release of both FFA and GLY was significantly higher by O ($p < 0.01$) and M adipocytes ($p < 0.05$ for FFA and $p < 0.01$ for GLY) than by SC fat cells. The differences in lipolytic activity did not reflect differences in cell diameter, cell volume, or protein content, as can be seen from Table V.

Table V

Size, volume and protein content of canine fat cells (mean ± S.E.)

	Size (μ)	Volume (picoliters)	Protein content (mg/mm TG)
SC	64.73 ± 2.03 (14)	161.98 ± 15.02 (14)	5.67 ± 1.66 (13)
O	66.02 ± 2.29 (14)	176.53 ± 17.26 (14)	5.26 ± 1.28 (13)

Numbers in parentheses refer to the number of experiments
SC: subcutaneous adipose tissue
O: omental adipose tissue

Although properly prepared fat cells always responded in the same fashion qualitatively, they showed a considerable quantitative variability. Therefore, a truer measure of relative rates of FFA and GLY release by O and

Table VI

Comparative rates of NE stimulated FFA and glycerol release by omental (O) and subcutaneous (SC) fat cells before and after endotoxin administration

Ratio of O : SC activity			
before		after	
FFA	glycerol	FFA	glycerol
2.75 ± 0.69 (n = 11)	1.87 ± 0.49 (n = 11)	2.97 ± 0.71 (n = 13)	2.54 ± 0.62 (n = 13)

In parentheses, number of experiments

SC fat cells can be obtained by comparing the two lipolytic activities within the same animal and expressing them as ratios. These data are shown in Table VI. The same holds true for a comparison of NE stimulated lipolysis after and before endotoxin administration (Table VII).

Table VII

Effect of endotoxin administration on NE stimulated lipolysis by isolated canine fat cells

Adipose tissue site	Ratio of after endotoxin: before endotoxin activity	
	FFA	glycerol
Subcutaneous	2.29 ± 0.56 (n = 12)	1.48 ± 0.38 (n = 12)
Omental	2.60 ± 0.42 (n = 13)	2.16 ± 0.39 (n = 13)
Mesenteric	1.65 ± 0.12 (n = 4)	1.43 ± 0.22 (n = 4)

In parentheses, number of experiments

It has been reported previously that the response to epinephrine and NE varies with adipose tissue location in the rat (WERTHEIMER et al. 1960) and in the human (CARLSON and HALLBERG 1968). Regional differences exist in enzyme patterns and flavoprotein content in canine adipose tissue (SPITZER 1967).

The effect of endotoxin on NE stimulated lipolysis by isolated fat cells was also studied in the absence of GLU. It is known that reesterification cannot proceed in the absence of GLU, since glycerophosphate made available during the glycolytic breakdown of GLU is not present, and white adipose tissue — lacking appreciable amounts of glycerokinase — cannot reutilize free GLY. Indeed, in the absence of GLU there is increased FFA release by adipocytes from all 3 locations under investigation as seen in Table VIII. There is

a somewhat increased GLY release also under these conditions, although not as consistent as FFA release. Out of 11 experiments with SC cells incubated in the absence of GLU, GLY release increased in 6, decreased in 2, and remained essentially unchanged in 3. Out of 12 experiments with O cells under the same conditions, GLY release rose in 9, decreased in 2, and was practically unchanged in 1. Examination of the ratio of FFA/GLY output by fat cells of SC and O adipose tissue (Table IX) suggests more reesterification (lower ratio) at the SC than at the O site before endotoxin administration. After endotoxin, the ratios suggest nearly complete lipolysis with very little reesterification at both regions.

Table VIII

Effect of glucose in the incubation medium upon NE stimulated lipolytic activity

Ratio of FFA and glycerol release in the absence of glucose: presence of glucose

subcutaneous		omental		mesenteric	
FFA	glycerol	FFA	glycerol	FFA	glycerol
before endotoxin					
1.99 ± 0.27 (n = 11)	1.31 ± 0.21 (n = 11)	1.79 ± 0.34 (n = 12)	1.45 ± 0.19 (n = 12)	1.64 ± 0.26 (n = 4)	1.31 ± 0.12 (n = 4)
after endotoxin					
1.55 ± 0.21 (n = 12)	1.47 ± 0.33 (n = 12)	1.40 ± 0.31 (n = 13)	1.33 ± 0.28 (n = 13)	1.32 ± 0.31 (n = 4)	1.05 ± 0.21 (n = 4)

In parentheses, number of experiments

Table IX

Ratio of FFA : glycerol output by isolated fat cells of subcutaneous (SC) and omental (O) adipose tissue

Subcutaneous		Omental	
(+glucose)	(−glucose)	(+glucose)	(−glucose)
before endotoxin			
1.55 ± 0.18 (n = 11)	1.99 ± 0.32 (n = 12)	2.18 ± 0.28 (n = 12)	2.45 ± 0.19 (n = 12)
after endotoxin			
2.56 ± 0.36 (n = 13)	2.67 ± 0.26 (n = 13)	2.57 ± 0.24 (n = 13)	2.56 ± 0.17 (n = 13)

In parentheses, number of experiments

We have also investigated the effect of various concentrations of NE upon the lipolytic response of isolated fat cells. These NE dose–response studies revealed that the dose customarily used for stimulation, 0.2 μg/ml, was far from saturating in our system. Furthermore, at each concentration of NE, including saturation levels, the lipolytic response was greater after endotoxin administration than before. Fig. 1 illustrates this point, showing the results of one of six experiments performed for this purpose.

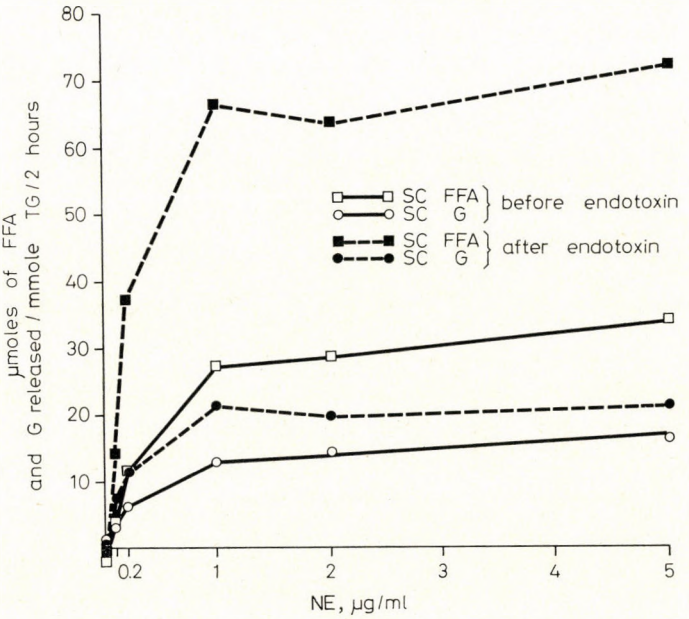

Fig. 1. NE dose–response curves. Subcutaneous adipose tissue

Cyclic 3',5'-adenosine monophosphate (cAMP) has repeatedly been implicated in the activation of hormone-sensitive lipolytic activity of adipose tissue (Butcher et al. 1968). It is reasonable to assume that the accentuated lipolytic response to NE brought about by endotoxin is mediated — in part — by changes in cAMP metabolism. Since in endotoxin shock regional blood flow in adipose tissue is severely reduced, tissue hypoxia in some way may be a contributing factor in rendering the cells more responsive to lipolysis. In this context it is of interest that anoxia results in striking increase in the levels of cAMP in mouse brain in situ (Goldberg et al. 1970). Previously reported instances of increased metabolic activity in anoxia include an augmented uptake of GLU by the rat diaphragm, which is independent of the increased permeability of muscle cells (Randle and Smith 1958) and a greater than normal GLU uptake and L production by diaphragm of the rat subjected to haemorrhagic shock (Drucker and DeKiewiet 1964).

Hypersensitivity of adipose tissue to the lipolytic action of NE has been observed in patients with idiopathic orthostatic hypotension (ENGELMAN et al. 1964). The NE hypersensitivity was caused presumably by sympathetic denervation of the adipose tissue. Fat cells of the denervated interscapular fat pad of guinea pigs exhibited an increased maximum lipolytic response to NE in comparison with cells of the intact innervated fat pad (FORN et al. 1970). The development of hypersensitivity to NE in adipose tissue of hyperthyroid rats has been described (KRISHNA et al. 1968). Adipose tissue of rats pretreated with agents blocking FFA mobilization (e.g. 5-carboxy-3-methyl pyrazole, or nicotinic acid) was more sensitive than normal tissue to the lipolytic action of NE or theophylline (BIZZI et al. 1969). Although the sympathetic neurohumoral system is known to have an important influence on the vascular and metabolic regulation of adipose tissue, the mechanisms which bring about hypersensitivity to NE under such diverse conditions are still incompletely understood.

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A. C. GIESE (Ed.)

Photophysiology, Current Topics in Photobiology and Photochemistry

Volume VII. Academic Press, New York and London 1972. XVII + 353 pages, with 78 figures and 22 tables. Price: US $ 22.50

In our era, man's interference with his environment is coming more and more into prominence. The biological equilibrium of life on earth is endangered by the interference with the photic environment. So the significance of new findings in fundamental research on the problems of photochemistry and photophysiology obtains a practical importance.

The present volume of the Photophysiology series has been written by 15 eminent representants of various aspects of photophysiology. The ten chapters of the volume are: 1. Electron Resonance Studies of Photosynthetic Systems; 2. Photochrome, a Photochromic Sensor; 3. Photosynthetic Nitrogen Fixation by Blue-Green Algae; 4. The Role of Light in Nitrate Metabolism in Higher Plants; 5. Mode of Photosensitizing Action of Furocoumarins; 6. Photosensitivity in Porphyria; 7. Ultraviolet Action and Photoreactive Action in Algae; 8. Chromosome Aberrations Induced by Ultraviolet Radiation; 9. Photochemistry of Nucleic Acids and their Constituents; 10. Enzymatic and Non Enzymatic Bioluminescence.

Research workers in plant chemistry, biochemistry and physiology will find this volume a useful source of information on the current results of research work in their field.

K. LISSÁK

W. C. DE MELLO (Ed.)

Electrical Phenomena in the Heart

Academic Press, New York and London 1972. XV + 415 pages, with 130 figures and 12 tables. Price: US $ 26.—

This book was published in the Clinical Engineering Series, edited by C. A. CACERES. The 16 contributors of the volume are discussing a wide range of subjects, from the ultrastructure of heart cells and the molecular organization of the heart cell membrane to the mechanisms of sealing and excitation–contraction coupling.

The thirteen chapters of the book reflect the abundant content. Chapter 1 discusses Electrical Properties of Embryonic Heart Cells by N. SPERELAKIS; Chapter 2: Correlation between the Ultrastructure and Function of Intercellular Contacts by W. K. BERGER; Chapter 3: Membrane Lipids and Cardiac Electrogenesis by C. DE MELLO; Chapter 4: Excitation Process in Heart Cells by J. DUDEL; Chapter 5: Depolarization Process of Heart Cells by D. NOBLE and R. W. TSIEN; Chapter 6: Active Ion Transport in Heart Muscle by G. HAAS; Chapter 7: Electrophysiology of the Sinoatrial Node by T. C. WEST; Chapter 8: Membrane Capacitance of Heart Cells by H. A. FONARD; Chapter 9: Cardiac Innervation and Synaptic Transmission in the Heart by M. ANDERSON and J. DEL CASTILLO; Chapter 10: Atrioventricular Transmission by C. MENDEZ and G. K. MOE; Chapter 11: Comparative Aspects of Electrogenesis in Myocardial Cells by F. V. MCCANN; Chapter 12: The Healing-Over Process in Cardiac and Other Muscle Fibers by W. C. DE MELLO; Chapter 13: Calcium Movements and Excitation–Contraction Coupling in Cardiac Cells by J. B. BASSINGTHWAIGHTE and H. REUTER. Each chapter gives abundant references, and the book is completed with an author and subject index.

This book marks an important milestone in the physiology and biophysics of the heart, and will be a useful reference work for scientists interested in cardiac physiology.

K. LISSÁK

H. LANGER (Ed.)

Biochemistry and Physiology of Visual Pigments

Springer-Verlag, Berlin, Heidelberg, New York 1973. XIII + 366 pages, with 202 figures. Price: DM 31.80; US $ 11.80

The volume contains the Proceedings of a Symposium held at the Institut für Tierphysiologie, Ruhr-Universität, Bochum, W. Germany, August 27—30, 1972. The 52 participants of this symposium dealing with various aspects of the visual process discussed the function of visual cells of both vertebrates and invertebrates, offering a good orientation in the recent progress of biochemistry and physiology of visual pigments. The papers presented at the symposium were divided into six main topics: 1. Pigment Structure and Chemical Properties; 2. Photolysis and Intermediates of the Pigments; 3. Regeneration of the Pigments; 4. Excitation and Adaptation of Photoreceptor Cells; 5. Ionic Aspects of Excitation and Regeneration; 6. Enzymology and Molecular Architecture of the Light Sensitive Membrane.

A great advantage of the volume is the registration of the lively and productive discussions which followed each paper, and finally the general discussion moderated by S. L. BONTING, in which an attempt was made at integrating the various new findings, and to reconcile certain points of disputable details.

The book presents the up-to-dat state of the biochemistry and physiology of visual pigments, and will be a useful source of information first of all for scientists working in this field, and also for teachers and postgraduate students of biochemistry and physiology.

K. LISSÁK

R. WEHNER (Ed.)

Information Processing in the Visual Systems of Arthropodes

Symposium held at the Department of Zoology, University of Zurich, March 6—9, 1972. Springer-Verlag, Berlin, Heidelberg, New York 1972. XI + 334 pages, with 263 figures. Price: DM 36.—; US $ 11.50

This symposium discussed the anatomical, neurophysiological and behavioural knowledge on the compound eye and the visual pathway of arthropods. In the morphological as well as the physiological aspect, the visual system of arthropodes provides a model suitable for the study of information processing in neuronal networks and so means a great help in studies of vertebrate neurophysiology.

The papers presented by the 77 participants of the symposium dealt with specialized topics in optics, electrophysiology and neuroanatomy, and the discussions made it clear that the visual system of arthropodes really have much to contribute to neurophysiology. The 9 main chapters of the volume are: 1. Anatomy of the Visual System; 2. Optics of the Compound Eye; 3. Biochemistry of Visual Pigments; 4. Intensity-Dependent Reactions; 5. Wavelength-Dependent Reactions; 6. Pattern Recognition; 7. Visual Control of Orientation Patterns; 8. Storage of Visual Information; 9. Methods of Quantifying Behavioural Data.

For investigators of sensory systems, the proceedings of the symposium will offer valuable help in interpreting on vertebrates the uptake, transmission and processing of optical data.

K. LISSÁK

R. JUNG (Ed.)

Visual Centres in the Brain

Handbook of Sensory Physiology. Volume VII/3. Central Processing of Visual Information. Part B. Springer-Verlag, Berlin, Heidelberg, New York 1973. VIII + 738 pages, with 216 figures. Price: DM 248.—; US $ 70.—

The volume VII of the new Handbook of Sensory Physiology presents a survey of recent knowledge of the physiology of vision. Volume VII consists of four parts: Part 1. Photochemistry of Vision; Part 2. Physiology of Photoreceptor Organs; Part 3. Central Processing of Visual Information; Part 4. Visual Psychophysics. Volume VII, Part 3 consists of Parts A and B, which deal with central visual function. Part A deals with the integration of the functions of vision and their mechanisms in the brain. Part B deals with the function and the structure of the visual centres in the brain. This Part B is the volume at issue; its 16 chapters on the "Morphology and Function of Visual Centres in the Brain" deal with the visual cortex and other higher visual centres in the temporal lobe. The 16 chapters are:

13. Anatomy and Synaptology of the Optic Tectum by G. SZÉKELY; 14. The Role of the Superior Colliculus and Pretectum in Vision and Visually Guided Behavior by J. M. SPRAGUE, G. BERLUCCHI and G. RIZZOLETTI; 15. Neurophysiology of the Accessory Optic System by E. MORG; 16. The Parietal Eye (Pineal and Parietal Organs) of Lower Vertebrates by E. DODT; 17. Neuronal and Synaptic Architecture of the Lateral Geniculate Nucleus by J. SZENTÁGOTHAI; 18. Neuronal Mechanisms of the Lateral Geniculate Body by H. J. FREUND; 19. Projection of Optic Pathways to the Visual Cortex by D. WHITTERIDGE; 20. Synaptology of the Visual Cortex by J. SZENTÁGOTHAI; 21. Neuronal Physiology of the Visual Cortex by B. BROOKS and R. JUNG; 22. Recording from Single Cells in the Human Visual Cortex by E. MARG; 23. Visual Functions of Inferotemporal Cortex by C. G. GROSS; 24. Ablations of Visual Areas in the Central Nervous System by R. W. DOTY; 25. Forebrain Commissures and Vision by R. W. DOTY and N. NEGRÃO; 26. Sensory Effects of Electrical Stimulation of the Visual and Paravisual Cortex in Man by G. S. BRINDLEY; 27. Electrophysiology and Topographical Distribution of Visual Evoked Potentials in Animals by O. D. CREUTZFELDT and U. KUHNT; 28. Visually Evoked Potentials and Visual Perception in Man by D. M. MACKAY and D. A. JEFFREYS. The volume ends with a comprehensive Author and Subject Index.

The editorial and aestheric quality of this volume, which is as excellent as the other volumes of the Handbook of Sensory Physiology, is a credit to the old traditions of the Springer-Verlag. This volume and the others of this series ought to be found in every library. They will remain for long an indispensable source of information in neuroanatomy, neurophysiology and neurobiology.

K. LISSÁK

M. KRUG, R. WINTER (Eds)

Biochemical, Physiological and Pharmacological Aspects of Learning Processes

Volume 10 of the series "Ergebnisse der experimentellen Medizin". VEB Verlag Volk und Gesundheit, Berlin 1972. 280 pages, with P 154 figures and 10 tables. Price: M 31.40

The volume presents the Proceedings of the III. Neurobiological Symposion organized by Prof. H. MATTHIES in Magdeburg, 5—7 May, 1971.

The 32 prominent participants of different disciplines presented 26 papers to offer a better understanding of the brain. The 26 papers are as follows. 1. G. D. SMIRNOV: Functional and Structural Aspects of Plasticity; 2. E. GLASSMAN and J. E. WILSON: Macromolecules and the Nervous System; 3. H. MATTHIES: The Intracellular Regulation of the Interneuronal Connectivity, the Molecular Foundation of Learning Processes; 4. T. OTT: The Significance of Hippocampus for the Extinction Delay after Application of Uridinemonophosphate; 5. B. LÖSSNER, S. SCHMIDT and N. POPOV: The Incorporation of L-leucine-1-^{14}C into the Brain Protein of Different Regions of the Rat Brain during Learning; 6. W. PHOLE: Microautoradiographic Investigation of the Nucleic Acid Metabolism in the Brain of Rats under Training Conditions; 7. H. LIEBMANN and CHR. RAUCA: Changes in the Cholinergic System

during a Learning Experiment in the Rat; 8. J. SCHMIDT, E. KAMMERER and H. RÜTHRICK: The Change of Neuronal Activity in the Neocortex by Conditioning; 9. H. BAUMANN, K. HECHT and J. LÄUTHER: Changes of the Neuroelectrical Functional State Depending on Conditioned Reflectory Learning Experiments; 10. M. CUÉNOD, R. E MEIER, J MIHAILOVIC and M. PERISIC: Interhemispheric Transfer of Visual Information in the Pigeon; 11. K. HECHT and K. TREPTOV: The Unity of Plasticity and Stereotype Action as Working Algorithms of the CNS in the Reproduction of Stored Information; 12. K. TREPTOW and K. HECHT: Mechanisms of Conditioning and Adaptation in Individual Clock Systems; 13. R. Y. ILYUTCHENOK: Involvement of Cholinergic Structures of the Limbic and Reticular Systems in the Mechanisms of Memory: Pharmacological Aspects; 14. F. KLINGBERG: Stage of Vigilance, General State of Behavioural Activity and Learning; 15. Z. VOTAVA and M. VOJTECHOVSKY: The Effect of Cholinergic Drugs on Storage and Information; 16. H. ZIPPEL and G. F. DOMAGK: Versuche zur Informationsverarbeitung und zur Übertragung erworbener Informationen beim Goldfisch *(Crassius aureatus)*; 17. B. CSILLIK: Function-Dependent Structural Alterations in the Neuromuscular Junction; 18. R. HASCHKE, W. HASCHKE, K. H. SCHMEIDUCH and P. ULLSPERGER: A Modulating Action of the Hippocampal Formation on Photic Evoked Potentials as a Model of Information Processing; 19. W. HARTMANN, H. KLUGE, W. ZAHLTEN and V. WIECZOREK: The Influence of Chlorpromazine on the Brain 5'-Nucleotidase in vivo and in vitro; 20. K. KANIG and W. OESTERLE: The Influence of Encephalotropic Substances on the Nucleic Acid Metabolism of the Brain; 21. ST. NIEMIERKO: Biochemical Markers of Axoplasmatic Flow; 22. W. OELSZNER: Pharmacological Models of Central Cholinergic Functions; 23. S. S. OJA: On the Measurement of Metabolic Rates of Brain Proteins; 24. A. H. STAIB, K. ANDREAS und SCHERBER: Die Wirkung von Glyzin und Gaba auf den Cholinerg ausgelösten Tremor und die Azetylcholinfreisetzung in die Hirnventrikel der Ratte; 25. S. THEIL, D. GELBRICH and F. KLINGBERG: Mathematical Modelling of Neuronal Networks; 26. P. TEICHGRÄBER: Influence of Visual Stimulation on the Incorporation of Leucine into Proteins of the Optic Pathway of the Rat.

The contents show the really interdisciplinary aspect of the symposium, which resulted in live discussions of the presented new facts in brain research. The volume will be interesting for students of all fields of neurobiology.

K. LISSÁK

L. LEVI (Ed.)

Stress and Distress in Response to Psychosocial Stimuli

Laboratory and Real-Life Studies on Sympatho-Adrenomedullary and Related Reactions. International Series of Monographs in Experimental Psychology. Vol. 17. Ed. by H. J. EYSENCK. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. VI + 166 pages, with 44 figures and 24 tables. Price: £ 3.50

The volume is introduced by Hans SELYE. The literature concerning the emergency function of the sympatho-adrenal system has greatly increased since the time of Walter B. CANNON. Out of this immense field this small volume deals only with the psychosocial aspects of stress in the human and reflects the four-year work of the editor and his group.

The important and interesting results are summarized in eight chapters: 1. Introduction: Psychosocial Stimuli, Psychophysiological Reactions, and Disease; 2. Methodological Considerations in Psychoendocrine Research; 3. Sympatho-Adrenomedullary Responses to "Pleasant" and "Unpleasant" Psychosocial Stimuli; 4. Sympatho-Adrenomedullary Activity, Diuresis and Emotional Reactions during Visual Sexual Stimulation in Females and Males; 5. Stressor-Induced Changes in Plasma Lipides and Urinary Excretion of Catecholamines, and their Modification by Nicotinic Acid; 6. Conditions of Work and Sympatho-Adrenomedullary Activity; Experimental Manipulations in a Real Life Setting; 7. Psychological and Physiological Reactions to and Psychomotor Performance during Prolonged and Complex Stressor Exposure; 8. General Discussion. The volume ends with summaries in English, German, French and Russian, and with a Subject Index.

The volume should find his place in every medical library, and in particular on the desk of investigators and physicians interested in the problem of psychophysiological stress.

K. LISSÁK

J. SALÁNKI (Ed.)

Neurobiology of Invertebrates

Mechanism of Rhythm Regulation. Proceedings of a Symposium held at Tihany, 2—5 August 1971. Akadémiai Kiadó, Budapest 1973. 494 pages

Organization of this Symposium was based on the concept that rhythmicity, being one of the basic properties of life processes has to be investigated also in invertebrates. Studies on the regulatory mechanisms of basic processes in lower phyla often resulted in discoveries important from a general biological and practical aspect.

In the preface by J. SALÁNKI it is emphasized that "The endogenous rhythms and the mechanisms of their basic regulatory processes were selected as the basic subject material".

After the opening address given by K. LISSÁK we can read some introductory remarks by J. SALÁNKI who emphasized that "When watching the behaviour of an animal or observing the living processes either of an organ or on the cellular level, one of the most striking phenomena is their repeated character, their rhythmicity".

The volume contains 33 papers and discussions on recent research in the field of rhythm regulation. The subjects cover primarily rhythmic phenomena, independent of external factors but based on structural and functional characteristics of cells, organs or the whole organism, i.e. endogenous rhythms.

The book is divided into four main parts. The first contains papers dealing with rhythms on the cellular level, analysing mainly the mechanisms of rhythmic firing in neurones. The second and third parts deal with rhythms appearing at the organ level. Here the papers are focussed on the rhythmic functioning of the heart. The fourth part contains results on behavioural rhythms and their neuronal regulation in the whole organism.

The material is particularly interesting from a comparative physiological point of view, as a number of different species — Vermes, Gastropoda, Pelecypoda, Crustacae and Insecta — were investigated by specialists of 14 countries attending the Symposium.

In the concluding remarks C. L. PROSSER emphasized that "The gap between molecular and organismic biology is real, and this conference helped to bridge this gap".

A. PUPPI

M. G. ROSSMANN (Ed.)

The Molecular Replacement Method

A Collection of Papers on the Use of Non-Crystallographic Symmetry. International Science Review Series 13. Gordon and Breach Science Publishers, New York, London, Paris 1972. VIII + 267 pages. Price: £ 6.25

This book is more than a simple collection of a few dozen well-selected papers which deal with the theoretical and technical problems of structure determination of large and macromolecules of biological importance. It is a review of the promising solutions of the phase problem in protein crystallography.

It was shown by the 1960's that the isomorphous replacement method applied with success first by M. PERUTZ (1953) in solving the haemoglobin structure was promising only if at least two proper substitutions of one amino acid residue in the asymmetric unit of a protein crystal with two related heavy-atom-containing organic groups could be performed. Generally, these chemical procedures were time-consuming or simply impossible. Success was never certain when only one heavy atom derivative could be obtained. In order to avoid the large number of impasses, invention of some new solution was imperative. The new idea was coming from M. G. ROSSMANN (1960) who suggested that the phase problem could presumably be solved by using the phenomenon of identical molecular sub-units, observed first by CRICK and WATSON (1956) and CASPAR (1956), and proved by HARRISON (1959) by crystallographic evidence, the di- (or poly-) morphism and the non-crystallographic symmetries of the protein crystals. This fundamental idea led to the mathematical equations of the molecular replacement method developed by distinguished crystallographers, as e.g. D. M. BLOW, R. A. CROWTHER, P. MAIN, M. G. ROSSMANN, P. TOLLIN and others. Their works published during the last ten years (mainly in Acta Cryst. J. molec. Biol. and the J. Amer. chemical Soc.) have now been edited in a separate volume which is introduced by M. G. ROSSMANN

giving a summary of the so-called three-stage "Rocket" method. Any identical molecular sub-unit can be related to another one by the simple formula $x' = (C)x + d$, where (C) is the rotation matrix determined in the first stage, and d the translation vector determined in the second stage. In the third stage the molecular replacement equations are to be set up on the basis of the previous steps. Examples of the successful application of the methods are also given. The use of these methods needs a good knowledge of the theory of X-ray crystallography together with proper mathematics.

Thus, this book can be recommended to crystallographers, biochemists and biophysicists who seek to enter the realm of structural molecular biology.

A. KÁLMÁN

E. J. HENLEY, R. A. WILLIAMS

Graph Theory in Modern Engineering

Computer Aided Design, Control, Optimization, Reliability Analysis. Mathematics in Science and Engineering, Vol. 98. Academic Press, New York, London 1973. XVI + 303 pages. Price: US $ 16.—

This book covers the theory and applications of directed and nondirected graphs with emphasis on the combinatorial approach to flow-graph analysis. It treats signal flow graphs, their construction, manipulation, and application to the solution of algebraic and differential equations, develops a method for the systematic analysis of large flow graphs, and extends the graph theory to encompass practical engineering problems in computer aided design, control, and optimization.

The versatility and power of the signal-flow-graph techniques are demonstrated by applying them to static and dynamic engineering problems in control, heat exchanger design, distillation column design, and flow-sheet and network analysis.

A new linear programming method based on flow-graph manipulations is constructed and tested.

Nondirected graphs, their matrix representation and application to optimization and system analysis are discussed.

The book includes a generalized treatment of branch and bound, tree search methods.

The authors give appendixes on matrix theory, linearization, Boolean and Modulo 2 algebra, linear programming, the Coates graph, and Fibonacci research methods. A two hundred-page appendix of computer programs is available from the authors.

This book will be of value to chemical and electrical engineers, applied mathematicians, computer scientists, optimization and control specialists.

J. CZOPF

X ACTA ENDOCRINOLOGICA CONGRESS

The tenth Acta Endocrinologica Congress will be held in Amsterdam at the Free University, from August 56-29, 1975.

The program includes nine symposia on current important topics within the scope of endocrinology. The congress should be of interest for all scientists in the field of clinical and experimental endocrinology.
For further information and registration write to the Secretary-General, Dr. P. G. Smelik, Dept. of Pharmacology,
Free University, Medical Faculty, Amsterdam - 1011,
The Netherlands.

"*Erratum*": B. VARGA, Edit HORVÁTH and E. STARK: Effects of ACTH and HCG on ovarian oestrogen and progesterone secretion in dogs pretreated with HCG.

Acta phys. Acad. sci. hung. **44**, (1) 29—37 (1973). In the cited paper legend to **Fig. 1.** is accordance with Fig. 4., legend to Fig. 4. in accordance with Fig. 1.

INDEX

PHYSIOLOGIA

Baló-Banga J. M., Antoni F., Altmann H.: Studies on Human Tonsillar Cell DNA. Preparation and Characterization of Nuclear and Non-Nuclear DNA from Isolated Tonsillar Cells 101
Munshi J. S. D., Dube S. C.: Oxygen Uptake Capacity of Gills in Relation to Body Size of the Air-Breathing Fish, *Anabas testudineus* (Bloch) 113
Rózsahegyi G., Telegdy G., Lissák K.: Diurnal Changes in Hypothalamic Serotonin Content and its Correlation with Adrenal Function in the Rat during the Oestrus Cycle 125
Puppi A., Kiss I.: Microelectrophysiological Analysis of the Correlation between the Redox State and the Type of Effect of Acetylcholine and/or 5-Hydroxytryptamine 133
Kollai M., Fedina L., Kovách A. G. B.: Effect of Bleeding, Cooling and Asphyxia on the Activity of Vertebral and Cardiac Sympathetic Nerves 145

PATHOPHYSIOLOGIA

Maklári Erzsébet, Kovách A. G. B., Nyáry I.: Acid–Base Balance and Regional CO_2 Content of the Brain in Hypovolaemic Shock 157
Maklári Erzsébet, Kovách A. G. B., Nyáry I.: Effect on Central Nervous Tissue CO_2 Content of the Correction of Acidosis due to Haemorrhage 171
Spitzer A. Judy, Kovách A. G. B., Sándor P., Spitzer J. J., Storck R.: Adipose Tissue and Endotoxin Shock 183

RECENSIONES

Giese A. C. (Ed.): Photophysiology, Current Topics in Photobiology and Photochemistry. Vol. VII. Academic Press, New York, London 1972. (K. Lissák) 195
De Mello W. C. (Ed.): Electrical Phenomena in the Heart. Academic Press, New York, London 1972. (K. Lissák) 195
Langer H. (Ed.): Biochemistry and Physiology of Visual Pigments. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 196
Wehner R. (Ed.): Information Processing in the Visual Systems of Arthropodes. Springer-Verlag, Berlin, Heidelberg, New York 1972. (K. Lissák) 196
Jung R. (Ed.): Visual Centres in the Brain. Handbook of Sensory Physiology. Vol. VII/3. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 197
Krug M., Winter R. (Eds): Biochemical, Physiological and Pharmacological Aspects of Learning Processes. VEB Verlag Volk und Gesundheit, Berlin 1972. (K. Lissák) 197
Levi L. (Ed.): Stress and Distress in Response to Psychosocial Stimuli. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1972. (K. Lissák) 198
Salánki J. (Ed.): Neurobiology of Invertebrates. Mechanism of Rhythm Regulation. Akadémiai Kiadó, Budapest 1973. (A. Puppi) 199
Rossmann M. G. (Ed.): The Molecular Replacement Method. Gordon and Breach Science Publishers, New York, London, Paris 1972. (A. Kálmán) 199
Henley E. J., Williams R. A.: Graph Theory in Modern Engineering. Academic Press, New York, London 1973. (J. Czopf) 200

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ACTA PHYSIOLOGICA

ТОМ 44— ВЫП. 2

РЕЗЮМЕ

ИЗУЧЕНИЕ ДНК ТОНЗИЛЛЯРНОЙ КЛЕТКИ ЧЕЛОВЕКА. ПРЕПАРАЦИЯ И ХАРАКТЕРИЗАЦИЯ ЯДЕРНЫХ И НЕ ЯДЕРНЫХ ДНК ИЗ ИЗОЛИРОВАННЫХ ТОНЗИЛЛЯРНЫХ КЛЕТОК

Й. М. БАЛО-БАНГА, Ф. АНТОНИ и Х. АЛТМАНН

Описывалась препарация 570 мкг цитоплазматической ДНК и 800 мкг ядерной ДНК из тонзиллярных клеток человека (оба определялись дифениламиновой реакцией). Первым этапом в фракционировании клеток была обработка детергентом Nonidet P-40. Экстракция ДНК из ядерных и цитоплазматических фракций проводилась отдельно. У 90% цитоплазматической ДНК плавучая плотность в нейтральных градиентах CsCl была 1,715 г/мл. Ядерная ДНК состояла из двух компонентов с плотностью 1,715 г/мл и 1,696 г/мл соответственно. Сравнение ядерной и цитоплазматической ДНК при градиентах CsCl-этидиум-бромида исключило наличие значительного количества ковалентно связанных циркулярных интактных митохондриальных ДНК. Щелочное сахарозное скорость-осаждение показало три главных пика в ядерной ДНК, тогда как в цитоплазматическом материале было найдено два пика, идентичных с двумя из трех ядерных пиков. Так как фракция, содержащая цитоплазматическую ДНК, состоит из митохондрий и, главным образом, из плазматических мембран (как было доказано фракционированием в прерывистых сахарозных градиентах), можно предположить вероятность митохондриального происхождения цитоплазматической ДНК. Однако, его удивительное сходство с ядерной ДНК указывало, что ДНК, найденная в цитоплазме тонзиллярных клеток человека, представляет собой специфическую часть ядерной ДНК.

СПОСОБНОСТЬ ПРИЕМА КИСЛОРОДА ЖАБРАМИ ПО ОТНОШЕНИЮ К РАЗМЕРУ ТЕЛА У РЫБ *Anabas testudineus (Bloch)*, ВДЫХАЮЩИХ ВОЗДУХ

Й. Ш. ДАТТА МУНШИ и Ш. Ц. ДУБЕ

Потребление кислорода через жабры по отношению к размеру тела у рыб ***Anabas testudineus***, вдыхающих воздух, исследовалось при двух экспериментальных условиях: 1) при вдыхании воздуха, и 2) когда вдыхание прерывалось. Более низкая величина наклона ($b = 0{,}67$) кривой для потребления кислорода относительно к весу тела (при первом условии) по сравнению с телеостами, вдыхающими воду ($b = 0{,}85$), была связана со свойством рыб вдыхать воздух и со сравнительным увеличением поверхности, вдыхающей воздух, параллельно с повышением веса тела рыбы. Дальнейшее понижение наклона ($b = 0{,}5259$) при втором условии было связано с уменьшением эффективности респираторной мембраны жабр под стрессовыми условиями. Наблюдаемая способность маленьких рыб выживать более длительный период (зависящий от дыхания только жабрами), чем способность больших рыб, была связана с уменьшением способности приема кислорода жабрами, которое происходило параллельно с ростом размера рыб. Более высокая пропорция потребления кислорода по отношению к весу тела у маленьких рыб, чем у больших, была связана с их более высокой метаболической пропорцией и с большей газообменной поверхностью жабр по отношению к весу тела.

ДНЕВНЫЕ ИЗМЕНЕНИЯ В СОДЕРЖАНИИ СЕРОТОНИНА В ГИПОТАЛАМУСЕ И ЕГО КОРРЕЛЯЦИЯ С НАДПОЧЕЧНИКОВОЙ ФУНКЦИЕЙ У КРЫС В ТЕЧЕНИЕ ЭСТРАЛЬНОГО ЦИКЛА

Г. РОЖАХЕДЬИ, Г. ТЕЛЕГДИ и К. ЛИШШАК

Содержание серотонина в гипоталамусе и уровень кортикостерона в плазме измерялись в 8 часов утра, в 16 и 23 часа вечера, в течение различных фаз эстрального цикла крыс.

Уровень гипоталамического серотонина показал дневное изменение с пиком в 8 часов утра, и самым низким уровнем в 23 часа вечера. Утренний уровень в течение проэструса и эструса был значительно ниже, чем при метэструсе и диэструсе. Уменьшение уровня гипоталамического серотонина, наблюдаемое в 23 часа, было значительным при каждой фазе цикла, но наиболее выраженным было в течение проэструса.

Изменение уровня сывороточного кортикостерона показало противоположную тенденцию. Оно было низким утром и высоким в 23 часа при каждой фазе эстрального цикла, исключая эструс, когда не было значительного различия между утренней и вечерней величинами. Утренние величины были в два раза выше в течение проэструса и эструса, чем в течение метэструса и диэструса.

Овариэктомия не уничтожала дневной ритм серотонина в гипоталамусе, но утренние величины были значительно ниже, чем при проэструсе, метэструсе и диэструсе. Введение эстрона и прогестерона повышало утренний уровень серотонина в гипоталамусе у кастрированных крыс-самок.

МИКРОФИЗИОЛОГИЧЕСКИЙ АНАЛИЗ КОРРЕЛЯЦИИ МЕЖДУ ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНЫМ СОСТОЯНИЕМ И ТИПОМ ЭФФЕКТА АЦЕТИЛХОЛИНА И/ИЛИ 5-ГИДРОКСИТРИПТАМИНА

А. ПУРРИ и И. КИШШ

Эксперименты были выполнены на гигантских нейронах ***Lymnea stagnalis***. Было установлено, что деполяризующий эффект ацетилхолина на клетки типа D был увеличен окислительными агентами (метиленсиний, тионин), и в то же время клетки типа H становились клетками типа D после применения вышеуказанных агентов. Окислительно-восстановительные катализаторы, восстанавливающего типа, такие, как аскорбиновая кислота, гидрохинон и р-метил-аминофенолсульфат, оказывали выраженное торможение на ацетилхолиновую деполяризацию на клетках типа D. В некоторых случаях характер типа D клеток превращался в тип H. Эксперименты с никотиновой кислотой дали различные результаты; ацетилхолиновая деполяризация в некоторых случаях тормозилась, а в других увеличивалась. Это, по-видимому, обуславливалось бимодальным антагонистическим местом эффекта никотиновой кислоты.

Деполяризующий эффект 5-гидрокситриптамина тормозился как окислительными, так и восстановительными агентами. Это торможение было менее выраженным, чем в случае ацетилхолина.

На основании результатов можно предположить, что действительное окислительно-восстановительное состояние клеток играет решающую роль в определении характера типа D или H эффекта медиаторов.

Места окислительно-восстановительных эффектов можно искать на трех уровнях. На уровне а) изменений активности энзимов, играющих роль в транспортных процессах ионов; б) изменений в молекулярной конфигурации плазматических мембран; в) квантобиологических изменений в некоторых процессах переноса между рецептором и медиатором.

ЭФФЕКТ ВЫПУСКА КРОВИ, ОХЛАЖДЕНИЯ И АСФИКСИИ НА АКТИВНОСТЬ ВЕРТЕБРАЛЬНЫХ И СЕРДЕЧНЫХ СИМПАТИЧЕСКИХ НЕРВОВ

М. КОЛЛАИ, Л. ФЕДИНА и А. Г. Б. КОВАЧ

Эффекты некоторых факторов, влияющих на симпатическую эфферентную активность, изучали для того, чтобы найти различия в региональной симпатической отдаче. Эксперименты были выполнены на анестезированных кошках. Записывались интегри-

рованная активность вертебральных нервов (АВН), активность сердечных нервов (АСН), системное кровяное давление и дыхание. В течение градуального, ступенчатого выпуска крови увеличение АВН превышало АСН во всех фазах. При понижения температуры окружающего воздуха от 30° до 15°С АВН и АСН показывали слабое увеличение; при дальнейшем охлаждении до 10°С наблюдалось крутое повышение уровня в АВН. В течение 1-минутной асфиксии АВН увеличивалась устойчиво и значительно, тогда как увеличение АСН начиналось позднее и было слабее, чем АВН. Результаты обнаружили, что сердечные и вертебральные симпатические нервы дают количественно разные ответы на примененный стимул.

КИСЛОТНО-ЩЕЛОЧНОЕ РАВНОВЕСИЕ И СОДЕРЖАНИЕ CO_2 В МОЗГОВОЙ ТКАНИ ПРИ ГИПОВОЛЕМИЧЕСКОМ ШОКЕ

Е. МАКЛАРИ, А. Г. Б. КОВАЧ и И. НЯРИ

У анестезированных собак при условиях гиповолемии исследовались килотно-щелочные параметры артериальной, правой предсердной и саггитальной крови, а также содержание CO_2 в тканях различных областей мозга. В течение 30 мин после выпуска крови, когда среднее артериальное давление устанавливалось на уровне 55 мм рт. ст., BE (избыток основания) и pCO_2 в артериальной крови уменьшались, тогда как pH не менялся. В правой предсердной крови с уменьшением BE имело место повышение pCO_2, а pH был в кислотном ряду. В первые 30 мин после первого выпуска крови не было отмечено существенных изменений в саггитальной крови. При среднем кровяном давлении 35 мм рт. ст. обнаружили сильный ацидоз во всех трех пробах крови. Различия между артерио-венозными O_2 и pCO_2 значительно увеличивались в первые 30 мин как в смешанной венозной крови, так и в крови саггитального синуса. Тканевое содержание CO_2 в различных мозговых областях показало значительные различия. В течение 30 мин после первого выпуска крови тканевое содержание CO_2 в гипоталамусе повышалось от $14{,}5 \pm 1{,}5$ мМ/кг до $31{,}7 \pm 37$ мМ/кг и после второго выпуска достигало $38{,}5 \pm 6{,}1$ мМ/кг. Во фронтальной коре повышение было подобное, но слабое. Содержание CO_2 в мосту повышалось только к концу второй фазы выпуска крови, тогда как в окципитальной коре, таламусе и продолговатом мозге не было существенных изменений.

После коррекции ацидоза введением бикарбоната, в гипоталамусе наблюдалась гиперкапния, тогда как в других областях мозга наблюдались нормальные или различающиеся только незначительно от нормы величины.

ЭФФЕКТ КОРРЕКЦИИ АЦИДОЗА, ВЫЗВАННОГО ГЕМОРРАГИЕЙ НА СОДЕРЖАНИЕ CO_2 В ЦЕНТРАЛЬНЫХ НЕРВНЫХ ТКАНЯХ

Е. МАКЛАРИ, А. Г. Б. КОВАЧ и И. НЯРИ

Изучался эффект введения бикарбоната на кислотно-щелочное равновесие и содержание CO_2 в мозговой ткани при геморрагическом шоке у собак. При введении физиологического раствора в геморрагическом состоянии можно было наблюдать подобный, но более умеренный ацидоз по сравнению с необработанными собаками. Только в гипоталамусе, из исследованных областей, наблюдалось значительное повышение CO_2 в конце выпуска крови. Через 2 часа после ретрансфузии, помимо выраженной гиперкапнии гипоталамуса, уровень CO_2 значительно повышался и во фронтальной и окципитальной коре.

В течение геморрагии развитие ацидоза можно было предотвратить введением бикарбоната. К концу выпуска крови ни в одном из областей мозга не была обнаружена тканевая гиперкапния. Через 2 часа после ретрансфузии только в гипоталамусе можно было наблюдать слабое накопление CO_1. Предполагалось, что физиологический раствор уменьшал патологические изменения, возникающие в клетках, путем изменения вязкости крови.

Ввиду благоприятного эффекта введения бикарбоната, авторы считают, что ацидоз может быть представлен как усугубляющий фактор в ухудшении метаболизма клеток.

ЖИРОВАЯ ТКАНЬ И ШОК, ВЫЗВАННЫЙ ЭНДОТОКСИНОМ

Й. А. ШПИТЗЕР, А. Г. Б. КОВАЧ, П. ШАНДОР, Й. Й. ШПИТЗЕР и Р. ШТОРК

Исследовался метаболизм жировой ткани в изолированных подкожных и сальниковых областях у нечистокровных самок собак, перед и после обработки эндотоксином *E. coli*. Среднее артериальное давление, минутный объем и кровоток в жировой ткани значительно уммньшались из изученных гемодинамических параметров, тогда как частота сердца оставалась неизмененной после введения эндотоксина. После введения эндотоксина концентрация свободных жировых кислот в артериальной крови не изменялась, концентрации глицерина и лактата прогрессивно повышались, а концентрация глюкозы понижалась, но только после 60 мин. Введение эндотоксина вызывало значительное уменьшение отдачи глицерина и приема глюкозы через 60 мин.

Исследовались основной и стимулированный норэпинефрином липолиз изолированных жировых клеток при наличии или отсутствии глюкозы. Как перед, так и после введения эндотоксина адипоциты сальниковой жировой ткани последовательно освобождали больше свободных жировых кислот и глицерина при стимуляции норэпинефрином, чем адипоциты подкожной жировой ткани. Адипоциты жировой ткани брыжейки вели себя так, как сальниковые подоциты, в 3 из 4 случаев при контрольных условиях, и в каждом случае после введения эндотоксина. При эндотоксиновом шоке повышался липолиз, вызванный норэпинефрином, как в подкожных, так и в сальниковых и брыжеечных жировых клетках. Это было показано повышением освобождения свободной жировой кислоты и глицерина.

Подкожные и сальниковые жировые клетки, стимулированные норэпинефрином, показали большее освобождение свободной жировой кислоты при отсутствии глюкозы, чем при ее наличии. Этот факт указывал на понижение реэстрификации при описанных условиях. Эндотоксин уменьшал это различие. Кривая дозы-действия норэпинефрина не изменялась после введения эндотоксина. Однако, липолитическое действие увеличивалось после каждой примененной дозы порэпинефрина (включая и максимальную дозу).

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Physiologia

LIPID CONTENT OF HUMAN EMBRYONIC TISSUES

By

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The lipid content of brain, liver, heart, lung and kidney of 12-week-old human embryos and of the placenta obtained at induced abortion was tested. The quantity of cholesterol, cholesterol ester, free fatty acid, monoglyceride, 1,2-diglyceride, 1,3-diglyceride and triglyceride in the tissues was determined and the fatty acid composition of the lipid fractions studied, to furnish data to the lacking standard values for early pregnancy.

The lipid content and the ratio of the lipid fraction in embryonic tissues have scarcely been investigated. There are some data for the rat, lamb, rabbit and guinea pig (Barcroft and Popják 1945; Van Duyne and Havel 1959; Popják 1954; Okuda and Tohoku 1960), and for the brain of the human foetus (Schnek et al. 1971) but none for the tissues of the human embryo. We have therefore studied the brain, liver, heart, lung, kidney and the placenta of 12-week-old embryos for their cholesterol, cholesterol ester, free fatty acid, triglyceride, 1,2-diglyceride, 1,3-diglyceride and monoglyceride content and the fatty acid composition of the lipid fractions.

Methods

The investigated tissues of embryos obtained at induced abortion in the 12th week of pregnancy were studied. Gestational age was taken as the period between the first day of the last menstruation and the day of abortion by means of curette and was determined also by bimanual palpation and from the weight and position of the embryo. The uterine contents were placed on a gauze sheet stretched in a metal frame, so that the embryo and its organs (liver, kidney, lung, heart, brain) the decidua or the placenta could easily be identified and rapidly separated. The organs were weighed, frozen in liquid air and stored at —30°C until processing. For each determination the corresponding tissues of 8 to 10 embryos were used.

Lipids were extracted and purified by the method of Folch et al. (1951). Neutral lipids were separated in one dimension on Silicagel G layer. The thin layer was previously activated at 100°C for 1 hour and as solvent petrol ether : diethylether : acetic acid : methanol 90 : 5 : 2 : 2 was used. Phospholipoids stayed at the starting point, while neutral lipids ran in the following order: monoglyceride, 1,2-diglyceride, 1,3-diglyceride, cholesterol, free fatty acid, triglyceride and cholesterol ester.

Quantitative estimation of the lipid fractions was done according to Kremer et al. (1969), extinction measured at 350 nm against cholesterol (Reanal) tripalmitate (BDH) and

palmitic acid as the standards. After methylation the lipids separated on the thin layer were subjected to gas chromatography for the separation of fatty acids. These were methyl-esterified on Silicagel with 5% methanol in hydrochloric acid. Separation was performed in a Jeol 810 type gas chromatograph, using a 2 m long column which contained 10% of 1,4-butanediol succinate on a Chromosorb GL support.

The temperature of the column was 175°C, that of the injecting block 210°C, and of the flame ionization detector 240°C. Nitrogen was used as carrier gas. The chromatograms were calibrated by means of the methyl fatty acids FAME K-101 and FAME K-108.

Results

Table I contains the concentration values for monoglyceride, 1,2-diglyceride, 1,3-diglyceride, triglyceride, cholesterol, cholesterol ester and free fatty acid in the embryonic heart, kidney, brain, liver, lung and in the placenta. For wet weight, the embryonic heart contained the highest and the placenta the lowest amount of lipid; considerable amounts were present in the kidneys and brain. Of the neutral lipid components, the heart and the kidneys contained the highest proportions of triglyceride. Monoglyceride content was highest in the brain; 1,2-diglyceride content in the heart, brain and in the kidneys; while 1,3-diglyceride in the kidneys. Free fatty acids and cholesterol occurred in the highest percentage in the heart, while the liver and the heart contained most of the cholesterol ester.

The fatty acids of the various lipid components were separated by gas chromatography. Tables II to VI show the amounts of fatty acids in the various lipid fractions.

Table II contains values for the fatty acids in the monoglycerides of the investigated embryonic tissues. Large amounts of palmitic acid ($C_{16:0}$), and in the placenta a considerable amount of stearic acid ($C_{18:0}$) was found. In the liver almost identical quantities of $C_{16:0}$, $C_{16:1}$, $C_{16:2}$, $C_{18:0}$, $C_{18:2\omega6}$ and

Table I

Mean neutral lipid content of organs of the 12-week-old human embryos
(μg/g wet weight $\pm$ S.D.)
(n = 10)

	Heart	Kidney	Brain	Liver	Lung	Placenta
Monoglyceride	655± 45	724± 65	859± 94	190± 25	538±42	157±22
1,2-Diglyceride	1223±109	1076± 98	1334±145	719± 84	567±39	274±32
1,3-Diglyceride	309± 25	492± 39	315± 43	194± 31	264±38	156±19
Cholesterol	660± 54	283± 39	408± 52	389± 49	233±29	287±31
Free fatty acid	539± 48	250± 41	199± 28	294± 37	246±32	130±18
Triglyceride	1577±164	1629±172	968±107	806±104	570±48	330±42
Cholesterol ester	665± 59	396± 42	419± 37	693± 52	406±51	296±31

$C_{18:3\omega3}$; in the lung, $C_{16:2}$, $C_{16:0}$; in the brain, $C_{14:0}$, $C_{16:0}$, $C_{16:2}$ and $C_{18:0}$; in the kidney $C_{16:0}$; in the placenta $C_{16:0}$, $C_{18:0}$; in the heart $C_{14:1}$, $C_{16:0}$ and $C_{16:1}$; were present.

In Table III the results for 1,2- and 1,3-diglycerides are given. In the diglycerides, $C_{16:0}$ and $C_{18:0}$ were present in largest quantities, while the other fatty acids at lower concentrations.

The fatty acids in the triglycerides and cholesterol esters of the various organs are presented in Table V and VI, respectively, the ratio of free fatty acids is shown in Table IV. Of the free fatty acids $C_{16:0}$ was present in the largest quantity in the investigated organs. Of the triglycerides, $C_{18:1}$ occurred in considerable quantities in the heart, the liver and the placenta; $C_{16:0}$, $C_{16:1}$ and $C_{18:3\omega3}$ in the lung; $C_{16:0}$ and $C_{18:1}$ in the brain and kidney; while of the cholesterol esters $C_{16:3}$ was found in significant amounts in the heart; $C_{18:1}$ in the liver, the lungs and the placenta; $C_{16:1}$ in the brain; $C_{16:0}$ and $C_{16:2}$ in the kidney.

Table II

Percentage distribution (± S.D.; n = 10) of fatty acids in the monoglycerides in the organs of the 12-week-old human embryo

Fatty acids	Heart	Liver	Lung	Brain	Kidney	Placenta
$C_{14:0}$	5.13±0.7	8.49±0.7	1.0 ±0.05	15.8 ±0.8	5.2±0.6	—
$C_{14:1}$	16.8 ±1.1				11.8±0.9	
$C_{15:0}$	3.05±0.2	1.78±0.9	11.21±0.9	1.05±0.07	5.9±1.0	2.2±0.1
$C_{15:1}$			0.5 ±0.04		12.6±1.1	2.0±0.2
$C_{16:0}$	23.11±1.7	14.6 ±0.6	15.62±1.1	24.62±0.5	17.7±0.9	27.6±0.9
$C_{16:1}$	20.26±2.1	11.3 ±1.1	3.82±0.2	2.34±0.1	2.3±0.4	6.0±0.3
$C_{16:2}$	4.79±0.5	11.05±0.9	19.28±0.9	21.9 ±0.8	13.3±0.8	7.7±0.2
$C_{16:3}$	6.84±0.7	3.55±0.2	1.17±0.1	2.1 ±0.1	4.5±0.3	5.3±0.1
$C_{17:0}$						5.8±0.4
$C_{18:0}$	6.15±0.5	11.2 ±0.6	14.1 ±0.4	14.8 ±0.4	3.7±0.2	38.5±2.4
$C_{18:1}$	5.47±0.6	0.56±0.3	0.32*	4.7 ±0.2	2.6±0.1	
$C_{18:2\omega6}$		10.41±1.2	9.57±0.7	7.7 ±0.3		
$C_{18:3\omega6}$	9.58±0.8	2.53±0.4	6.38±0.4		4.1±0.2	
$C_{18:3\omega3}$		11.3 ±0.8	9.81±0.7	5.6 ±0.4		0.54±0.2
$C_{20:1}$		7.3 ±0.6	0.32*		3.8±0.4	
$C_{20:2\omega6}$				2.11±0.1	3.4±0.5	2.7±0.1
$C_{20:3\omega6}$		1.13±0.2	8.38±0.6			2.4±0.2
$C_{20:4\omega6}$		3.26±0.2	3.27±0.2			
$C_{22:4\omega3}$		1.49±0.1				
$C_{22:5\omega3}$			0.42*			

* S.D. was not calculated for distributions lower than 0.5%

Table III

Percentage distribution (± S.D.) of fatty acids in the diglycerides of the organs of the 12-week-old human embryo

Fatty acids	Heart (n = 8)	Liver (n = 8)	Lung (n = 8)	Brain (n = 9)	Kidney (n = 9)	Placenta (n = 9)
$C_{14:0}$	0.6±0.1	5.9±0.3	0.5±0.08	5.5±0.2	8.4±0.2	5.7±0.2
$C_{14:1}$	1.9±0.1	8.8±0.2	2.7±0.1	7.9±0.3	3.9±0.1	5.8±0.4
$C_{15:0}$	0.8±0.2	1.2±0.008	0.9±0.1	3.7±0.1		4.6±0.2
$C_{15:1}$	1.6±0.09	5.5±0.4	3.6±0.2	3.9±0.1		3.4±0.1
$C_{16:0}$	36.5±1.4	32.6±2.1	34.5±1.5	32.2±2.1	18.8±0.9	20.4±1.2
$C_{16:1}$	4.9±0.2	5.2±0.7	5.4±0.2	5.9±0.2		4.9±0.3
$C_{16:2}$	5.3±0.3	2.7±0.1	2.4±0.1		16.3±0.7	2.7±0.4
$C_{16:3}$	4.2±0.2	6.2±0.2	3.6±0.3	4.8±0.1	2.9±0.1	7.8±0.2
$C_{18:0}$	21.4±2.1	8.8±0.2	18.9±1.0	25.1±1.0	12.6±0.5	11.2±0.3
$C_{18:1}$	2.5±4.1	2.5±0.1	16.4±0.7	7.6±0.4	5.3±0.6	4.6±0.1
$C_{18:2\omega6}$	1.5±0.1		1.1±0.07		9.9±0.4	
$C_{18:3\omega6}$	2.5±0.3				1.2±0.1	
$C_{18:3\omega3}$	2.7±0.1	3.1±0.1	2.8±0.09	5.9±0.3	7.3±0.2	6.2±0.2
$C_{18:4\omega3}$			0.5±0.06		0.9±0.2	0.4*
$C_{20:1}$	3.2±0.1	2.9±0.3	1.9±0.09		7.9±0.3	2.8±0.1
$C_{20:2\omega6}$		1.8±0.2	1.0±0.02			1.2±0.1
$C_{20:3\omega6}$	2.1±0.2	5.8±0.2			0.3*	
$C_{20:4\omega6}$	4.5±0.1	3.7±0.3	0.4*		3.8±0.1	2.5±0.3
$C_{22:4\omega6}$					0.9±0.05	
$C_{22:4\omega3}$	3.2±0.2	7.9±0.2			0.6±0.02	
$C_{22:5\omega3}$						6.8±0.5

* For ratios lower than 0.5% S.D. was not calculated

Discussion

A typical occurrence in the course of the metabolic changes during pregnancy is an about 50% increase in the lipid content of maternal blood (Peters and Man 1951; Russ et al. 1954; Studnitz 1955; Alvarez et al. 1959). This increase involves practically all the lipid fractions (Friedberg 1969).

The hyperlipaemia during normal pregnancy is, however, characteristic for the mother only, the lipid content of foetal blood remains low (Popják 1954). The placenta is impermeable to lipids (Popják 1953; Sternberg 1962), and, according to rat and rabbit experiments, it is only partly permeable for cholesterol and free fatty acids (Goldwater and Stetten 1947; Popják and Beeckmans 1950; Biezenski et al. 1971), thus the embryo must provide its

Table IV

Percentage distribution (± deviation) of free fatty acids in the organs of the 12-week-old human embryos

Fatty acids	Heart (n = 8)	Liver (n = 8)	Lung (n = 8)	Brain (n = 8)	Kidney (n = 10)	Placenta (n = 10)
$C_{14:0}$	2.7±0.1	2.2±0.09	2.8±0.3	2.8±0.4	3.6±0.2	7.6 ±1.1
$C_{14:1}$	4.6±0.2	1.5±0.08	1.7±0.4	1.1±0.4	2.5±0.1	
$C_{15:0}$	1.7±0.1	0.7±0.07	1.0±0.2	0.4*	0.9±0.2	0.9 ±0.2
$C_{15:1}$	7.7±0.2	0.9±0.05	2.7±0.1	0.3*	0.2*	1.5 ±0.2
$C_{16:0}$	20.5±1.2	38.8±1.8	34.1±2.5	52.4±3.8	20.4±1.9	21.6 ±1.8
$C_{16:1}$	14.2±1.3	7.3±0.8	5.9±0.8		4.8±0.7	3.1 ±0.7
$C_{16:2}$	1.2±0.05	0.5±0.1	1.4±0.3	2.7±0.4	5.2±0.4	2.6 ±0.5
$C_{16:3}$	9.9±0.5	0.1*	4.2±0.7	0.9±0.3	2.5±0.2	
$C_{18:0}$	12.1±0.7	11.4±0.9	19.5±1.7	34.6±4.1	19.5±1.2	17.6 ±0.9
$C_{18:1}$	6.9±0.2	29.3±2.1	17.3±2.8		9.8±0.8	6.2 ±0.2
$C_{18:2\omega6}$	10.6±0.6	0.8±0.5	0.6±0.3		4.2±0.5	0.5 ±0.2
$C_{18:3\omega6}$		0.4*			3.2±0.4	1.2 ±0.3
$C_{18:3\omega3}$		0.2*	0.2*	2.8±0.3	2.1±0.3	2.1 ±0.1
$C_{18:4\omega3}$	8.0±0.3				0.5±0.1	2.4 ±0.4
$C_{20:1}$			0.2*			1.6 ±0.2
$C_{20:2\omega6}$	4.2±0.2				4.2±0.3	0.2*
$C_{20:3\omega6}$		3.9±0.7	0.2*	2.2±0.4		
$C_{20:4\omega6}$	1.4±0.1	0.8±0.2			6.8±0.4	18.8 ±2.1
$C_{20:4\omega3}$					4.5±0.5	1.6 ±0.3
$C_{20:5\omega3}$	2.9±0.2					1.03±0.3
$C_{22:4\omega6}$	0.6±0.1				2.4±0.3	0.5±0.2
$C_{22:4\omega3}$			2.9±0.5		3.2±0.4	
$C_{22:5\omega3}$					0.6±0.2	
$C_{22:6\omega3}$						6.8 ±0.3

* For ratios lower than 0.5% S.D. was not calculated

necessary quantity of lipids. Synthesis in the embryo takes probably place starting from low molecular weight components (e.g. from acetate) which easily permeate through the placenta (KRANTZ and KUBLI 1967). During later development a considerable accumulation of lipids takes place (VAN DUYNE and HAVEL 1959).

It has been considered necessary to investigate the standard values for the lipoid content of embryonic organs, since disorders in lipoid metabolism have a teratogenic effect (TUCHMANN-DUPLESSIS and MERCIER-PAROT 1964). Hypocholesterolaemia as well as the compounds inducing hypercholesterolaemia lead to congenital abnormalities (TUCHMANN-DUPLESSIS and MERCIER-PAROT 1964).

Table V

Percentage distribution of fatty acids in thetriglycerides of the organs of the 12-week-old human embryo (± S.D. n = 10)

Fatty acids	Heart	Liver	Lung	Brain	Kidney	Placenta
$C_{14:0}$	0.6 ±0.1	1.9±0.2	1.9±0.3	2.6±0.3	3.2±0.4	1.4±0.4
$C_{14:1}$	7.7 ±0.3	2.5±0.1	0.4*	6.4±0.8	4.8±0.3	0.7±0.3
$C_{15:0}$	0.3*	0.1*	0.4*	0.3*	0.7±0.2	0.2*
$C_{15:1}$	8.7 ±0.2	3.4±0.4	4.9±0.2	8.2±0.7	2.3±0.3	0.9±0.4
$C_{16:0}$	7.26±0.3	12.3±1.7	12.3±0.8	24.5±2.1	36.9±2.8	10.2±1.5
$C_{16:1}$	5.41±0.4	4.4±0.5	18.6±0.9	9.5±0.8	6.3±0.7	2.9±0.5
$C_{16:2}$	0.4*	0.4*		0.4*	2.0±0.4	0.4*
$C_{16:3}$	18.5 ±1.1	4.2±0.2	2.2±0.1	9.5±0.9	3.0±0.5	1.5±0.7
$C_{18:0}$	3.3 ±0.1	3.3±0.1	5.4±0.6	6.0±0.3	7.8±0.4	20.2±1.8
$C_{18:1}$	23.5 ±1.9	58.6±4.9	4.2±0.5	15.8±2.1	18.3±1.7	33.5±2.9
$C_{18:2\omega6}$	2.5 ±0.2	1.4±0.4	5.4±0.7	0.4*	1.2±0.3	9.3±1.0
$C_{18:3\omega6}$	2.4 ±0.3	0.9±0.3			0.6±0.4	
$C_{18:3\omega3}$	9.4 ±0.7		12.7±1.7	6.9±0.9		1.4±0.4
$C_{18:4\omega3}$					1.2±0.5	
$C_{20:1}$	5.5 ±0.8	0.8±0.4	3.6±0.5	3.7±0.7	1.1±0.2	1.1±0.5
$C_{20:2\omega6}$				0.2*		
$C_{20:3\omega6}$						2.7±0.3
$C_{20:4\omega6}$	3.9 ±0.4	0.2*	9.7±0.8	0.3*	4.4±0.7	12.4±0.9
$C_{20:4\omega3}$			2.7±0.1		2.5±0.4	0.7±0.4
$C_{20:5\omega3}$	1.7 ±0.2	0.2*	11.7±2.4	0.7±0.1		
$C_{22:4\omega6}$	0.8 ±0.1		0.4*	3.7±0.7		
$C_{22:4\omega3}$		5.2±0.7	3.5±0.4			
$C_{22:5\omega3}$						0.3*
$C_{22:6\omega3}$	0.2*	0.2*			1.7±0.5	0.3*

* For ratios lower than 0.5% S.D. was not calculated

The foetal heart contains the highest quantity of lipid per wet weight and the placenta contains the lowest quantity, but the brain and the kidneys have also a considerable lipid content. Free fatty acid concentration is highest in the heart, the triglyceride content in the kidneys and in the heart. The kidney heart and brain contain in addition high quantities of diglycerides. The high diglyceride and free fatty acid content of certain embryonic organs can probably be explained with the high energy requirements of these tissues. A comparison of the ratio of fatty acid components in the lipid fractions shows that mostly the saturated fatty acids occur in larger quantities, but the ratio of fatty acids varies in the different fractions and organs.

Table VI

Percentage distribution (± S.D.; n = 9) of fatty acids in the cholesterol esters of the organs of the 12-week-old human embryo

Fatty acids	Heart	Liver	Lung	Brain	Kidney	Placenta
$C_{14:0}$	2.1±0.1	0.3*	3.8±0.2	8.5±0.7	8.3±0.5	3.4±0.2
$C_{14:1}$	8.3±0.7	0.2*	7.7±0.8		3.9±0.6	1.5±0.3
$C_{15:0}$	1.9±0.4	0.4*	1.8±0.2	4.9±0.8		4.6±0.5
$C_{15:1}$	8.3±0.9	0.6±0.1	6.9±0.5	8.5±0.5		
$C_{16:0}$	4.8±0.6	10.7±0.9	20.0±1.5	18.4±1.2	18.8±1.5	19.1±1.3
$C_{16:1}$	10.7±1.8	13.6±2.2	9.7±0.5	23.2±1.9		11.5±0.9
$C_{16:2}$	4.5±0.2		3.9±0.6		16.3±1.2	1.6±0.2
$C_{16:3}$	17.8±1.2	1.2±0.4	4.5±0.2	7.4±1.0	0.8±0.1	4.6±0.5
$C_{18:0}$		1.5±0.3	3.4±0.4	13.4±0.9	12.7±1.6	6.0±0.2
$C_{18:1}$		63.2±5.7	25.0±2.1	7.9±0.6	5.3±0.7	21.7±1.5
$C_{18:2\omega6}$		7.8±0.5	3.9±0.5		9.9±0.5	4.5±0.6
$C_{18:3\omega6}$	7.5±0.8			8.0±0.5	1.2±0.4	4.5±0.3
$C_{18:3\omega3}$	9.3±0.5		5.5±0.4		7.2±0.8	
$C_{18:4\omega3}$	5.7±0.2				0.8±0.2	1.0±0.2
$C_{20:1}$					7.9±0.5	4.7±0.3
$C_{20:2\omega6}$	2.6±0.3					
$C_{20:3\omega6}$	5.1±0.7				0.3*	5.9±0.6
$C_{20:4\omega6}$					3.8±0.4	
$C_{20:5\omega3}$	1.5±0.5				0.6±0.2	1.1±0.3
$C_{22:4\omega6}$					0.4*	1.9±0.3
$C_{22:4\omega3}$					1.2±0.9	
$C_{22:5\omega3}$		0.6±0.1				2.8±0.4

* For ratios lower than 0.5% S.D. was not calculated

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EFFECT OF SIMULTANEOUS OESTRIOL AND PROGESTERONE ADMINISTRATION ON SODIUM AND POTASSIUM EXCRETION AND ON THEIR RENAL TISSUE CONTENT IN FEMALE RATS

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Intact female rats received by subcutaneous injections 2.0 μg of oestriol and 0.002 g of progesterone daily for 10 days. During the whole period urinary output, the urinary levels of sodium, potassium and other osmotically active substances and endogenous creatinine concentration in the last urine portion were measured daily. At the end of the experimental period we determined the sodium, potassium and water content in the renal cortex, medulla and papilla and the clearance of sodium, potassium, osmotically active substances and endogenous creatinine. The simultaneous administration of the two hormones increased glomerular filtration rate and sodium and potassium reabsorption. The renal cortical and medullary sodium and potassium content increased, but that of the papillary area remained unchanged.

Oestriol has been shown to increase sodium reabsorption, most likely through the medium of mineralocorticoid hormones whose production is enhanced by oestriol (KATZ and KAPPAS 1967). It has also been found that in renal tissue the oestriol level is higher than that of oestradiol and oestrone (BROWN et al. 1964; BALIKIAN et al. 1968), and its amount increases during the lutein phase of the ovarian cycle (BARLOW and LOGAN 1966).

The studies of LANDAU et al. (1955), LANDAU and LUGIBIHL (1961), have demonstrated a natriuretic effect during the first few days of progesterone administration, due to its ability to eliminate aldosterone at the distal tubular level on the principle of concurrent antagonism. However, after prolonged administration of progesterone which is known to be the precursor of aldosterone (AYRES et al. 1960) the amount of aldosterone is increased to such an extent as to suppress the natriuretic effect and to enhance sodium excretion (PECHORSKAYA 1965).

We have studied the effect of the simultaneous administration of oestriol and progesterone on sodium and potassium excretion and the changes induced by the hormones in the electrolyte level in the renal cortex, medulla and papilla. In the available literature we found no report of similar investigations. This study is part of an investigation into the effect of ovarian hormones on renal function.

Methods

The experiments were carried out on 45 intact female Wistar rats, 2.5—3.5 months of age, weighing from 100 to 170 g. They were divided in two groups: a) control, 30 rats; and b) experimental, 15 rats. The animals were kept on a constant liberal food and fluid intake. Mean potassium content of the diet was 0.95 mEq and its sodium content, 2.32 mEq, daily. The room temperature was 20° ± 4°C. After several days of adjustment to the experimental conditions, each control animal received by subcutaneous injection 0.2 ml of sunflower oil for 10 days; experimental animals received instead 2.0 μg of oestriol and 0.002 g of progesterone each dissolved in 0.2 ml of sunflower oil. The hormone doses were within the physiological range (ZARROW et al. 1964). Thirteen control and 8 experimental animals' urine was collected from 18.30 p.m. to 6.30 a.m. on the 1st, 4th, 7th and 10th days. We measured diuresis, sodium and potassium excretion, osmolarity, and in the last sample, the concentration of endogenous creatinine. Sodium and potassium were estimated by flame photometry with internal lithium standard; endogenous creatinine was examined by VARLEY's modification (1966), osmolarity with an osmometer.

Twenty-four hours after the last injection the animals were decapitated, their blood was collected and the kidneys were removed rapidly. Parts of either kidney were treated by the method of HAWKINS and NIXON (1958) and served for determining potassium, sodium and water levels in the papilla and medulla, in the cortical zone lying opposite the papilla and in the whole kidney (Tables I and II).

Statistical analysis was made with Student's *t*-test. Oestriol was supplied by Organon Co., The Netherlands, progesterone by Schering Co., GFR.

Results

In control animals natriuresis was decreasing on the first four days, probably due to adjustment; thereafter it stayed at a fairly constant level (Fig. 1). Sodium excretion in hormone-treated rats was unaffected during the first few days, but later dropped from 246.907 mEq/100 g/10 hrs on the fourth day down to 143.642 mEq/100 g/10 hrs ($p < 0.025$) on the seventh day (Fig. 1) and remained at this level until the end of observation. At the end of the observation period natriuresis in rats receiving oestriol and progesterone was practically the same as in the control animals, in spite of the higher diuresis and filtration in the latter (Table I). In the experimental animals the percentage of reabsorbed sodium increased ($p < 0.001$, Table I).

The hormonal combination preserved corticomedullary concentration gradient for sodium–sodium per 100 g of dry tissue and 100 g of tissue water (cortex–medulla $p < 0.01$, $p < 0.001$; medulla–papilla $p > 0.10$) and against this background leads to an increase in the sodium content of the cortex ($p < 0.01$) and medulla ($p < 0.001$) with no detectable changes in whole kidney and papillar sodium levels (Table II).

Simultaneous administration of oestriol and progesterone increased potassium reabsorption (Fig. 1). At the end of the experimental period, the reabsorbed part of filtered potassium was increased ($p < 0.001$, Table I), while potassium excretion was maintained at a level similar to that in the control group, regardless of the higher filtration ($p < 0.01$) and greater urinary output (Table I).

Table I

Renal function in control and experimental rats receiving oestriol and progesterone simultaneously

No.	Parameters	Control group[1] ($\bar{x} \pm S_{\bar{x}}$)[4]	Experimental group[2] ($\bar{x} \pm S_{\bar{x}}$)	p[3]
1.	C_K, ml/100 g/10 h[5]	5.88 ± 0.29	5.33 ± 0.55	> 0.10
2.	$T_K/F_K \cdot 100$[6]	93.51 ± 0.29	97.89 ± 0.44	< 0.001
3.	$U_K/F_K \cdot 100$[7]	6.48 ± 0.29	2.10 ± 0.52	< 0.001
4.	P_K, mEq/l[8]	8.82 ± 0.26	10.49 ± 0.64	< 0.025
5.	U_K, μEq/100 g/10 h[9]	52.81 ± 2.63	54.90 ± 4.81	> 0.10
6.	C_{Na}, ml/100 g/10 h[10]	1.21 ± 0.11	1.29 ± 0.17	> 0.10
7.	$T_{Na}/F_{Na} \cdot 100$[11]	98.66 ± 0.11	99.52 ± 0.07	< 0.001
8.	$U_{Na}/F_{Na} \cdot 100$[12]	1.33 ± 0.11	0.47 ± 0.07	< 0.001
9.	P_{Na}, mEq/l[13]	131.73 ± 3.64	131.93 ± 2.67	> 0.10
10.	U_{Na}, μEq/100 g/10 h[14]	163.76 ± 16.73	169.92 ± 21.47	> 0.10
11.	C_{cr}, ml/100 g/10 h[15]	94.13 ± 5.29	306.26 ± 47.37	< 0.01
12.	P_{cr}, mg%[16]	0.82 ± 0.03	0.25 ± 0.03	< 0.001
13.	U_{cr}, mg%[17]	120.00 ± 5.34	71.25 ± 7.12	< 0.001
14.	$T^c_{H_2O} = C_{csm} - U_v$[18]	5.17 ± 0.27	4.31 ± 0.41	> 0.05
15.	U_v, ml/100 g/10 h[19]	0.65 ± 0.05	1.04 ± 0.16	> 0.05
16.	C_{osm}, ml/100 g/10 h[20]	5.83 ± 0.30	5.35 ± 0.44	> 0.10
17.	P_{osm}, mosm/l[21]	251.07 ± 2.42	259.75 ± 2.14	< 0.025
18.	U_{osm}, mosm/l[22]	2293.76 ± 107.70	1516.37 ± 199.29	< 0.002

[1] No. of cases, n = 13; [2] n = 8; [3] on comparison of the two groups; [4] $\bar{x} \pm S_{\bar{x}}$: arithmetical mean ± standard error; [5] C_K: potassium clearance, ml; [6] $T_K/F_K \cdot 100$: amount of reabsorbed potassium in per cents of filtered amount; [7] $U_K/F_K \cdot 100$: amount of excreted potassium in per cents of filtered amount; [8] P_K: plasma potassium, mEq/l; [9] U_K: urinary excretion of potassium, μEq; Nos [10], [11], [12], [13] and [14] correspond to [5], [6], [7], [8], [9], but refer to sodium; [15] C_{cr}: endogenous creatinine clearance, ml; [16] P_{cr}: plasma endogenous creatinine, mg per 100 ml; [17] U_{cr}: urinary endogenous creatinine, mg per 100 ml; [18] $T^c_{H_2O}$: amount of reabsorbed osmotically free water, ml; [19] U_v: urinary volume, ml; [20] C_{osm}: osmotic clearance, ml; [21] P_{osm}: milliosmols of osmotically active substances per litre of plasma; [22] U_{osm}: milliosmols of osmotically active substances per litre of urine; Nos [5], [9], [10], [14], [15], [19] and [20] have been estimated per 100 g of body weight for 10 hours

The hormones appeared to increase the potassium content of all renal structures; only in the papillar area failed this increase to search statistical significance (Table II). A potassium corticomedullary concentration gradient appeared to exist when calculating potassium per 100 g of dry tissue and 100 g of tissue water (cortex–medulla $p < 0.05$, $p < 0.001$; medulla–papilla $p > 0.10$).

Plasma potassium content was increased in the experimental animals but not in the control animals ($p < 0.025$, Table I).

Table II

Potassium, sodium and water in renal tissue of female rats receiving oestriol and progesterone simultaneously and in the control group

Tissue types	No. of cases (n)	K, mEq/100 g dry tissue ($\bar{x} \pm S_{\bar{x}}$)[1]	K, mEq/100 g tissue water ($\bar{x} \pm S_{\bar{x}}$)	Na, mEq/100 g dry tissue ($\bar{x} \pm S_{\bar{x}}$)	Na, mEq/100 g tissue water ($\bar{x} \pm S_{\bar{x}}$)	H_2O, g/100 g dry tissue ($\bar{x} \pm S_{\bar{x}}$)	H_2O, % in fresh tissue ($\bar{x} \pm S_{\bar{x}}$)
Whole kidney							
a) controls	10	23.49 ± 0.67	5.30 ± 0.24	21.42 ± 0.33	4.83 ± 0.13	446.53 ± 10.58	81.77 ± 0.40
b) oestriol + progesterone	5	26.55 ± 0.58	5.21 ± 0.33	19.11 ± 2.96	3.64 ± 0.48	527.79 ± 39.52	83.67 ± 0.40
		$p < 0.025$[2]	$p > 0.10$	$p > 0.10$	$p > 0.10$	$p < 0.025$	$p < 0.05$
Examined cortical area							
a) controls	20	22.59 ± 0.47	5.72 ± 0.13	29.95 ± 0.90	7.57 ± 0.22	395.62 ± 4.24	79.79 ± 0.17
b) oestriol + progesterone	10	26.09 ± 0.56	6.36 ± 0.22	34.65 ± 0.79	8.43 ± 0.36	412.55 ± 9.48	80.33 ± 0.33
		$p < 0.001$	$p < 0.025$	$p < 0.01$	$p < 0.05$	$p > 0.05$	$p > 0.05$
Medulla							
a) controls	20	35.73 ± 1.40	5.65 ± 0.18	62.17 ± 2.30	9.84 ± 0.36	634.39 ± 14.07	86.28 ± 0.26
b) oestriol + progesterone	9	47.21 ± 2.28	7.54 ± 0.52	105.74 ± 4.82	16.73 ± 0.95	630.10 ± 20.03	86.21 ± 0.38
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.10$	$p > 0.10$
Papilla							
a) controls	12	40.86 ± 1.08	5.92 ± 0.30	94.49 ± 7.21	14.92 ± 1.08	672.48 ± 45.15	86.64 ± 0.64
b) oestriol + progesterone	9	48.17 ± 3.66	6.54 ± 0.60	102.64 ± 8.93	13.99 ± 1.41	788.25 ± 41.97	87.43 ± 1.91
		$p > 0.05$	$p > 0.10$	$p > 0.10$	$p > 0.10$	$p > 0.10$	$p > 0.10$

[1] arithmetical mean $\pm$ standard error; [2] significance level on comparing a) with b)

Endogenous creatinine clearance (HARVEY and MALVIN 1965) was elevated by the two hormones ($p < 0.01$, Table I).

Diuresis was greater in the treated animals than in the control group (Fig. 1, Table I). Urine osmolarity in the experimental group was lower than in the control group (Fig. 1). Plasma osmolarity was increased by hormonal treatment ($p < 0.025$, Table I).

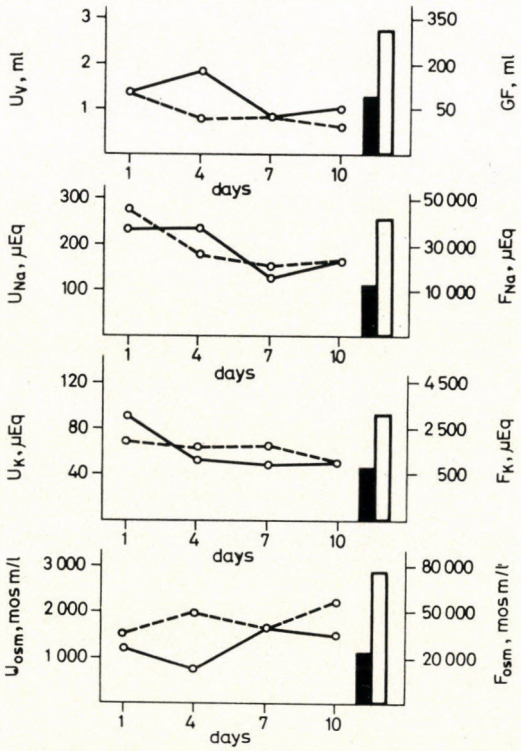

Fig. 1. Characteristics of diuresis, glomerular filtration, excretion and filtration of sodium, potassium and osmotically active substances. ■, ---, control; □, ——, oestriol + progesterone simultaneously. U_V urinary volume, ml; GF: glomerular filtration, ml; U_{Na}: amount of excreted sodium; F_{Na}: amount of filtered sodium; U_K: amount of excreted potassium; F_K: amount of filtered potassium; U_{osm}: osmotically active substances in urine; F_{osm}: filtered osmotically active substances. Each value without U_{osm} has been calculated per 100 g of body weight for 10 hours

Oestriol and progesterone produced no changes in the water content of the renal structures studied (Table II).

Discussion

The results showed that the combination oestriol and progesterone enhanced sodium reabsorption, obviously as it stimulated mineralocorticoid production (LANDAU et al. 1955; LANDAU and LUGIBIHL 1961; KATZ and KAPPAS 1967). The two hormones must have induced such a rise in the aldosterone level as to suppress the natriuretic effect of progesterone (PECHORSKAYA 1965) and so to enhance the sodium-retaining effect of oestriol.

Our observations on the increasing amount of sodium and potassium in the corticopapillary direction agree with those of WIRZ et al. (1951) who demonstrated that cortical osmolarity was lower than medullary osmolarity. The fact that tissue water (sodium and potassium referred to 100 g of tissue water) failed to inhibit the renal tissue sodium and potassium corticomedullary concentration gradient showed that both sodium and potassium were involved in the formation of an effective corticomedullary concentration gradient. Together with the increase of the intracellular potassium (MOORE et al. 1963), its extracellular fraction also increased as a result of the enhanced reabsorption. We are not able to give a satisfactory explanation of this increased potassium reabsorption observed in our study. However, divergences from the well-known aldosterone effect manifested by increased sodium reabsorption and potassium excretion have been described in the literature. For example, AUGUST et al. (1958) observed increased sodium and potassium excretion in spite of the administration of high doses of aldosterone. FINN and WELT (1963) demonstrated that aldosterone may increase sodium reabsorption without affecting potassium excretion. The higher plasma potassium level may be associated with the enhanced reabsorption of this electrolyte, with the ability of progesterone to activate protein metabolism resulting in potassium release (LANDAU and LUGIBIHL 1961; KERPEL-FRONIUS 1964) and with the ability of oestrogen hormones to extract potassium from the body cells *via* glucocorticoids whose production they stimulate (MULROW 1967).

The increased sodium content of the examined parts of the cortex and medulla (Table II) seemed to be due to the enhanced sodium reabsorption (Table I, Fig. 1) induced by aldosterone whose production increased under the effect of progesterone and oestriol (LANDAU et al. 1955; LANDAU and LUGIBIHL 1961; KATZ and KAPPAS 1967).

The different sodium content in the whole kidneys end in the examined part of the cortex may have been due to the uneven distribution of sodium in the renal cortex, owing to the functional heterogeneity of the nephrons (JAMISON 1970). With its large mass (THURAU 1964) the cortex is the part determining the individual fractions in the whole kidney.

The increased glomerular filtration rate is believed to be due to an enlargement of the extracellular compartment (MULROW 1967; GUYTON 1971) secondary to the increased sodium reabsorption (Table I).

Diuresis varies in conformity mainly with sodium ion dynamics (Fig. 1). Urine osmolarity is reduced parallel with the increased diuresis. The lower urine osmolarity in the experimental group, as compared to the control group, may have been associated with the enhanced sodium and potassium reabsorption (Fig. 1). The same mechanism seemed to be responsible for the increase in plasma osmolarity (Table I).

The greater water content in the medulla than in the cortex ($p < 0.001$) which has been observed by other authors as well (LEVITIN et al. 1962) may have been due to the higher medullary sodium and potassium content (Table II) with a secondary osmotic retention of a major amount of water.

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К ЭЛЕКТРОФИЗИОЛОГИЧЕСКОМУ АНАЛИЗУ ВЛИЯНИЯ АТРОПИНА НА РЕАКЦИЮ САМОРАЗДРАЖЕНИЯ У КОШЕК

О. БАКЛАВАДЖЯН, Г. БУЖАКИ и Л. АНДЬЯН
ИНСТИТУТ ФИЗИОЛОГИИ МЕДИЦИНСКОГО УНИВЕРСИТЕТА, ПЕЧ

(Статья поступила в редакцию 5 июня 1973 г.)

Опыты проведены на кошках с хронически вживленными электродами в области заднего гипоталамуса. Реакция самораздражения вырабатывалась на электрическое раздражение мозга биполярными электродами, локализованными в задне-латеральной области гипоталамуса. Соматосенсорные вызванные потенциалы гипоталамуса регистрировались биполярно как электродами «самораздражения», так и соседней парой электродов. Регистрация ЭЭГ осуществлялась эпидурально при помощи шариковых серебряных электродов. Установлено, что атропин, введенный внутримышечно, наряду с двигательными расстройствами вызывает и уменьшение частоты или повышение порога реакции самораздражения. При изучении влияния атропина на соматосенсорные вызванные потенциалы гипоталамуса, усредненные на анализаторе НТА—512, установлено, что атропин вызывает увеличение амплитуды сенсорных вызванных потенциалов как под электродами «самораздражения», так и в других зонах гипоталамуса.

Данные о роли М-холинергических структур задне-латерального гипоталамуса в формировании эмоциональных реакций весьма разноречивы. По данным ВАЛЬДМАНА и КОЗЛОВСКОЙ (1969), АЛЛИКМЕТСА (1972), ИЛЬЮЧЕНКА (1972), БУРОВА и КУРОЧКИНА (1972) и др., ряд эмоциональных реакций, вызываемых раздражением гипоталамуса, блокируются антихолинергическими веществами. Вероятно описанная ВИКЛЕРОМ (1952) и БРЭДЛИ (1958) фармакологическая диссоциация между поведением и биоэлектрической активностью касается только поведенческих проявлений, связанных с локомоторной активностью, которые сравнительно устойчивы к антихолинергическим веществам, в частности к атропину. Очевидно интеграция более сложных форм мотивационного проявления связана с холинергическими структурами гипоталамуса.

В данной работе изучено возможное участие М-холинергических структур гипоталамуса в осуществлении реакции самораздражения. Действие атропина на феномен самораздражения сопоставлено с действием норадреналина. С целью выяснения механизма действия нейротропных средств на эмоционально-мотивационные реакции, изучено также влияние атропина на сомато-сенсорные вызванные потенциалы (ВП) в участке самораздражения.

Методика

Опыты проведены на семи кошках, из них три с хронически вживленными электродами в области заднего гипоталамуса. Реакцию самораздражения вырабатывали в видоизмененной камере Скиннера. Животное приучалось нажатием лапкой на педаль замыкать

электрическую цепь стимулятора и запускать пачку стимулов частотой 100 гц длительностью 0,1 мсек в течение 0,25 сек. Счет частоты самораздражения проводился 15-и секундными интервалами. Определялась частота самораздражения при различных интенсивностях раздражающего стимула. Для раздражения и регистрации биопотенциалов гипоталамуса использовались биполярные игольчатые электроды, изолированные на всем протяжении кроме кончика острия с диаметром 50—80 микрон, расстояние между электродами — 1 мм. Стереотаксическая ориентация электродов осуществлялась по координатам атласа ДЖАСПЕРА и АЖМОН-МАРСАНА (1954). Обычно вживлялись восемь электродов, по четыре в каждом полушарии, ориентированных в медио-латеральном направлении. Регистрация ЭЭГ осуществлялась на восьмиканальном энцефалографе фирмы Медикор. ВП регистрировали биполярно как электродами, которые использовались для самораздражения, так и соседней парой электродов. 20—40 ВП усреднялись на анализаторе НТА—512 и результирующая кривая усредненного ВП записывалась на бумаге при помощи самописца, связанного с аналоговым выходом анализатора. При изучении влияния атропина и норадреналина на реакцию самораздражения препараты вводили внутримышечно. В исследованиях с регистрацией ВП после завершения опытов с самораздражением те же животные под легким наркозом подвергались трахеотомии и после вскрытия седалищного нерва и прохождения действия эфира обездвиживались флакседилом и переводились на искусственное дыхание. Влияние атропина на соматосенсорные ВП гипоталамуса изучено и на четырех кошках, у которых предварительно не вырабатывалась реакция самораздражения. После завершения опытов локализация электродов проверялась гистологически.

Результаты

При выработке реакции самораздражения на 8—10-й день после операции животные брались в видоизмененной камере Скиннера и проверялся характер двигательной реакции при стимуляции латеральной и медиальной областей гипоталамуса обоих полушарий при последовательном подключении к стимулятору четырех пар вживленных электродов. При выборе электродов для самораздражения исходили из данных ГРАШТЬЯНА и соавт. (1968) показавших, что у кошек реакция самораздражения вырабатывается легко, если раздражение гипоталамуса вызывает контраверсивное моторное последействие, контраверсивный «рибаунд» (отдача), независимо от направления движения первичного эффекта раздражения — ипсиверсивное или контраверсивное по отношению к раздражаемой стороне. Выбрав пару раздражающих электродов на основании вышеуказанных критерий, в течение двух-трех дней приучали животных нажатием лапкой на педаль вызывать «самораздражение». На этом этапе проверялось еще раз поведение животного к педали при последовательном попарном сочетании различных электродов. Реакция самораздражения считалась выработанной, если животное спонтанно начинало нажимать на педаль, по крайней мере в течение 15 минут. На Рис. 1 показана видоизмененная камера Скиннера и реакция самораздражения одной из подопытных кошек. При последующем гистологическом контроле оказалось, что у 3-х кошек электроды« самораздражения» локализовались в области гипоталамуса соответствующей координатам атласа ДЖАСПЕРА и АЖМОН-МАРСАНА: F 9—10,5, L 2,5—3,3 и V 4—5, т. е. в области медиального пучка переднего мозга.

На втором этапе экспериментов проверялась зависимость частоты самораздражения от интенсивности раздражающего стимула. После получения

стабильной фоновой частоты самораздражения приступали к изучению влияния атропина и норадреналина на феномен «самораздражения». Было установлено, что введение атропина в дозе 0,75—1,0 мг/кг внутримышечно вызывает облегчение общей моторной активности с рядом двигательных расстройств, характерных для «делириозного» синдрома, возникающего у человека при введении атропина: двигательное возбуждение, расстройство ориента-

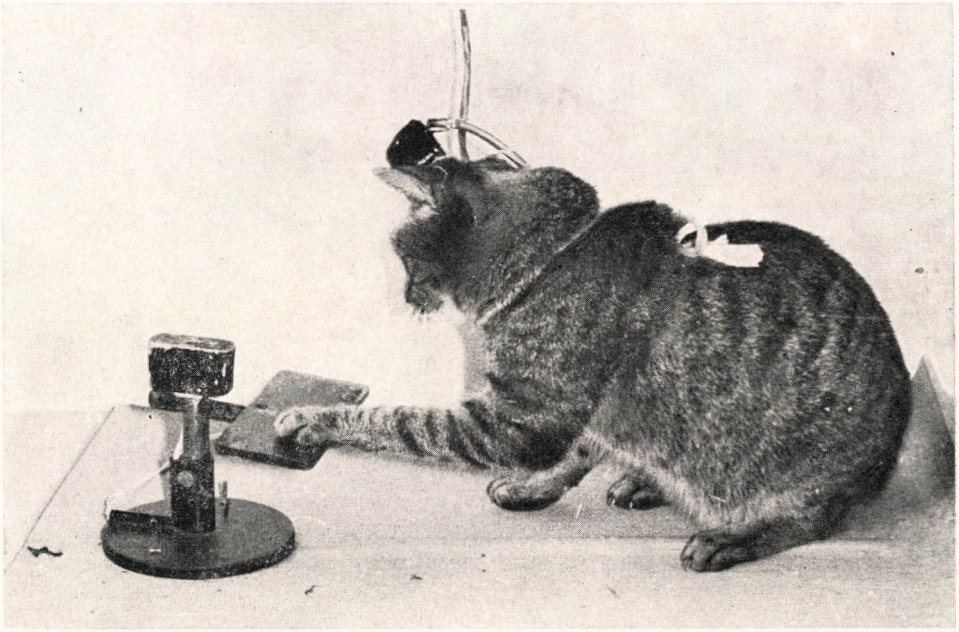

Рис. 1. Кошка с хронически вживленными электродами во время сеанса «самораздражения»

ции, животные натыкаются на препятствия и т. д. Как показано на Рис. 2 частота самораздражения уменьшается после введения атропина. Так, у кошки Б3, если частота самораздражения составляла в среднем 14 в 15 секунд, на 40-ой минуте после введения атропина снизилась до 3 в 15 секунд. Из графика, представленного на Рис. 2, видно, что усиление раздражения гипоталамуса не восстанавливает реакцию самораздражения до исходного уровня. У кошки Б2 эффект той же дозы атропина выражался только в повышении порога реакции самораздражения. При повышении интенсивности раздражающих стимулов частота самостимуляции соответствовала фоновой частоте самораздражения (Рис. 4С). У данной кошки атропин в указанной дозе не вызывал и столь выраженных нарушений общей двигательной активности, как у остальных двух кошек. Следовательно имеется некоторое индивидуальное различие в характере влияния атропина на реакцию самораздра-

жения, однако, общим для всех животных являлось повышение порогов «самораздражения».

Введение норадреналина в дозе 0,5 мг/кг внутримышечно по нашим предварительным данным не вызывало заметных изменений поведенческих реакций животного и как показано на графике Рис. 2 не менялась заметным образом и частота самораздражения. Возможно отсутствие четкого эффекта

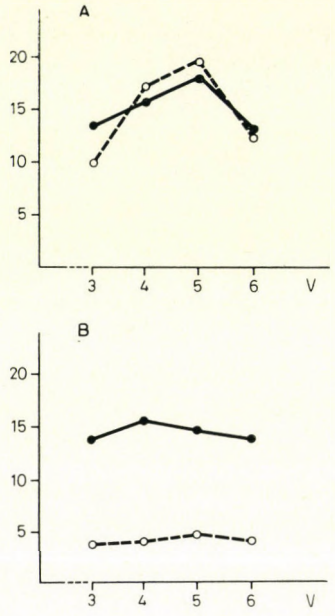

Рис. 2. Влияние норадреналина и атропина на реакцию самораздражения. На оси абсцисс — сила раздражающих стимулов в вольтах. На оси ординат — число нажатий на педаль за 15 секунд. Каждая кривая представляет усредненные данные трех опытов (30-и 15 секундных периодов) у кошки Б3. Черные кружки — до введения, светлые — после введения препаратов. A: эффект внутримышечного введения норадреналина в дозе 0,5 мг/кг. B: эффект атропина в дозе 1 мг/кг внутримышечно

норадреналина связано с плохой проницаемостью гемато-энцефалического барьера для катехоламинов. По данным ОЛДСА и ИТО (1971), ВАЙЗА и ШТЕЙН (1969), АРБУТНОТА и соавт. (1971) и др., реакция самораздражения связана с активностью норадренергических структур ствола мозга и гипоталамуса. Гистохимическими исследованиями ГЕЛЛЕРА и соавт. (1966) показано, что субстрат самораздражения, медиальный пучок переднего мозга (МИЛХАУЗ 1969) представляет главный диэнцефальный путь восходящих норадренергических волокон. Однако, по данным ряда авторов (ВАХИНГ и АЛЛИКМЕТС 1970; ФРАНКЕНХАУЗЕР 1971; БАКЛАВАДЖЯН и ДАРБИНЯН 1971, и др.) внутригипоталамическая инъекция норадреналина не вызывает выраженных изменений эмоционального поведения

животных. Данные о действии норадреналина на поведение животных при внутригипоталамическом и внутрижелудочковом введении препарата довольно противоречивы. Подробный обзор этих данных приводится в монографии ИЛЬЮЧЕНКА (1972). Очевидно этот вопрос нуждается в дальнейшем изучении, такие исследования проводятся нами в настоящее время.

Итак, по нашим данным психотропный эффект атропина проявляется и в отношении феномена «самораздражения». Каков механизм деприми-рующего эффекта атропина на реакцию самораздражения? Связан ли эффект атропина с блокадой афферентного звена или эфферентных механизмов центральной интеграции эмоционально-мотивационных реакции? Для выяснения этого вопроса в серии эксперментов изучено влияние атропина на сомато-сенсорные ВП, зарегистрированные в задне-латеральной области гипоталамуса теми же электродами, которые использовались для самораздражения. Влияние внутримышечного введения атропина на сомато-сенсорные ВП изучено в семи опытах. В четырех из них ВП гипоталамуса на раздражение седалищного нерва регистрировались у кошек, которые брались на острый эксперимент без предварительного изучения реакции самораздражения. Остальные три опыта проведены на трех хронических кошках после завершения опытов с самораздражением. Опыты проводились на ненаркотизированных кошках, обездвиженных флакседилом. О функциональном состоянии центральной нервной системы до введения атропина судили по реакции десинхронизации на ЭЭГ при высокочастотном раздражении центрального конца перерезанного седалищного нерва. Сомато-сенсорные ВП гипоталамуса, вызванные одиночным раздражением седалищного нерва, регистрировались до и в разные сроки после введения атропина. Контролем эффекта атропина

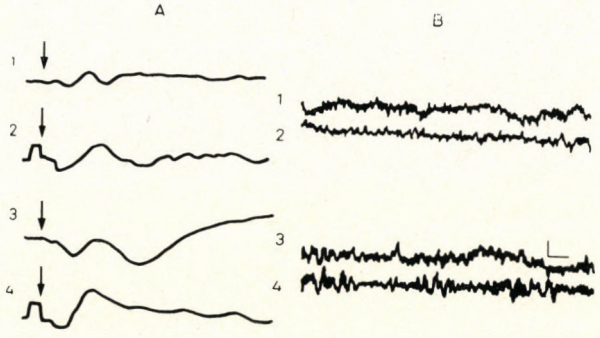

Рис. 3. Влияние атропина на вызванные потенциалы (ВП) и на ЭЭГ гипоталамуса. 1,3: Биполярное отведение от задне-медиального гипоталамуса ипсилатерально раздражаемому седалищному нерву до (1) и после (3) внутривенного введения 0,05% раствора атропина в дозе 1,3 мл/кг. 2,4: Биполярное отведение от задне-латерального гипоталамуса (электроды «самораздражения»), контралатерально раздражаемому нерву до (2) и на 26 минуте после (4) атропина. А: усредненный ВП 20-и ответов гипоталамуса. В: ЭЭГ отводимые теми же электродами до (1,2) и после (3,4) атропина. Острый опыт от 17 мая 1972 г. после завершения серии хронических опытов по самораздражению. Кошка Б3. На осциллограммах А2, 4 калидровка: 20 мкв, 10 мсек. На кривых В калибровка 100 мкв, 1 сек

на центральную нервную систему являлось изменение ЭЭГ, выражающееся в появлении типичной атропиновой медленной гиперсинхронной активности в ЭЭГ. Как показано на Рис. 3А и 4А, введение атропина вызывает увеличение амплитуды ВП гипоталамуса. «Облегчение» наблюдается как под электродами «самораздражения» (Рис. 3 и 4А 2,4), так и в других зонах гипоталамуса (Рис. 3А 1,3 и Рис. 4А 1,3). Результаты являются достоверными, так как при помощи анализатора НТА—512 регистрировались усредненные ответы 20—40 ВП, что сводило к минимуму флюктуацию фона. Здесь следует отметить, что учитывая большую вариабельность параметров ВП гипоталамуса у ненаркотозированных животных (БАКЛАВАДЖЯН и АСТВАЦАТРЯН 1973)

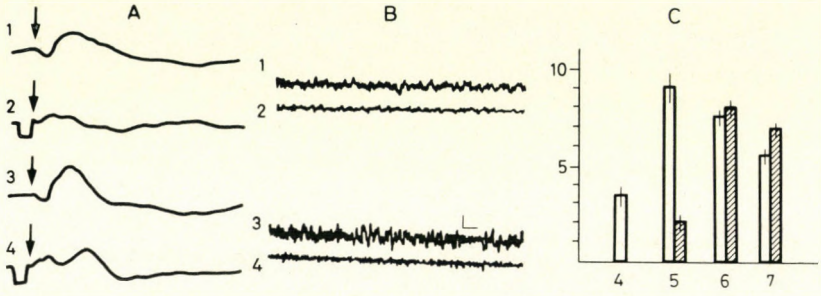

Рис. 4. Влияние атропина на ВП (А), на ЭЭГ (В) и на реакцию самораздражения (С). Кошка Б2. А, В: острый опыт от 18 мая 1972 г. после завершения серии опытов с самораздражением. А: усредненный ВП 20-и ответов гипоталамуса; В: ЭЭГ отводимая теми же электродами до (1,2) и после (3,4) атропина. 1,3: Биполярное отведение от задне-латерального гипоталамуса ипсилатерально раздражаемому седалищному нерву до (1) и на 30-й минуте после (3) введения 0,05% атропина в дозе 1 мл/кг внутривенно. 2,4: Биполярное отведение от задне-латерального гипоталамуса (электроды «самораздражения»), контралатерально раздражаемому седалищному нерву до (2) и после (4) атропина. Калибровки: как на рис. 3. С: усредненные данные трех опытов с самораздражением у кошки Б2. На оси абсцисс — сила раздражающих стимулов в вольтах. На оси ординат — число нажатий на педаль за 15 секунд (среднее 30-и 15 секундных периодов). Светлые столбики — до введения атропина; заштрихованные — на 40-й минуте после введения 0,05% раствора атропина в дозе 2 мл/кг внутримышечно. Вертикальные метки на столбиках — квадратическая ошибка

применение метода усреднения десятков ответов является желательным и пожалуй, необходимым при изучении влияния фармакологических веществ на сенсорные ВП гипоталамуса. Увеличение амплитуды ВП гипоталамуса при действии атропина наблюдалось и у кошек, которые брались на эксперимент и без предварительной выработки реакции самораздражения.

Обсуждение

Депримирующее влияние атропина на реакцию самораздражения свидетельствует о возможном участии М-холинореактивных структур гипоталамуса в осуществлении реакции самораздражения. Связан ли подавляющий эффект атропина с нарушением координации двигательных актов,

гиперреактивности животных или с психотропным и галлюциногенным эффектом препарата? В ряде работ показано изменение высшей нервной деятельности под влиянием атропина. При этом установлено избирательное влияние атропина на пищевые условные рефлексы, которые угнетаются меньшими дозами атропина, чем оборонительные (ВАНЕЦЕК и ВОТАВА 1956; ГРИГОРЯН 1961 и др.). Возможно положительные эмоциональные реакции более чувствительны к антихолинергическим веществам, чем отрицательные. Такое допущение согласуется с представлениями БОВАРДА (1962), который считает, что система, связанная с положительными эмоциями, осуществляет парасимпатические функции, отрицательная же система, при раздражении которой вызывается страх, гнев, агрессия — симпатическую функцию. По данным автора, положительная система локализована в переднем гипоталамусе, отрицательная — в заднем. Эти данные Боварда не согласуются с данными о локализации электродов «самораздражения» в наших экспериментах в задне-латеральной области гипоталамуса, при электрическом раздражении которой, как известно, вызываются выраженные симпатические реакции.

По данным ряда авторов, одна из причин нарушения сложных форм поведения при введении антихолинергических веществ состоит в ослаблении внутреннего торможения в результате блокады возвратного и ретикулярного торможения, имеющих холинергическую природу (ИЛЬЮЧЕНОК и соавт. 1970, и др.). При этом неизбежно меняется и функциональное состояние корково-гипоталамической системы интеграции. Так или иначе, изменение реакции самораздражения, вызываемое антихолинергическими веществами, связано, без сомнения, с блокадой центральных холинорецепторов. Имеются данные, что скополамин снижает у кроликов на 86% реакцию самораздражения переднего и заднего гипоталамуса и облегчает реакции избегания при стимуляции тех же точек (ГОРОЯН и КАЛЮЖНЫЙ 1969). По данным КРАУЗ и ЛАПИНОЙ (1972), в осуществлении положительных реакций, наблюдаемых при самостимуляции у собак, принимает участие холинергическая система, так как при угнетении М-холинореактивных структур метамизилом реакция исчезает полностью и появляются отрицательные эффекты. В исследованиях СТАРКА и БОЙДА (1963) установлено, что у собак внутривенное введение атропина в дозе 0,2 мг/кг не влияет заметным образом на феномен самораздражения, вызванный стимуляцией мамиллярной области гипоталамуса.

Наши исследования показывают, что атропин увеличивает амплитуду сомато-сенсорных ВП в области самораздражения гипоталамуса. Вероятно изменение реакции самораздражения связано с уровнем активности афферентных систем гипоталамуса. Увеличение амплитуды ВП гипоталамуса при действии атропина отражает, по всей вероятности, состояние гиперполяризации сенсорных нейронов гипоталамуса в результате уменьшения притока

афферентных импульсов и снятия фоновой деполяризации этих нейронов (дефасцилитация). О том, что уровень возбудимости заднего гипоталамуса отражается в амплитуде сенсорных ВП гипоталамуса, свидетельствуют данные одного опыта, в котором ВП на электрокожное раздражение задней лапки регистрировались электродами «самораздражения» заднего гипотала-

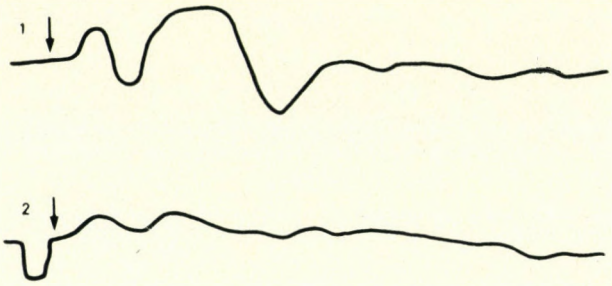

Рис. 5. Влияние реакции самораздражения на соматосенсорные ВП гипоталамуса. Кошка Б2. 1: ВП задне-латерального гипоталамуса на электрокожное раздражение контралатеральной задней конечности до самораздражения. 2: То же сразу после сеанса самораздражения. Усредненный ВП 20-и ответов гипоталамуса. Калибровка как на Рис. 3

муса до и сразу же после 20-минутного самораздражения. На рис. 5 показано, что сразу после сеанса самораздражения наблюдается заметное уменьшение амплитуды ВП в той же точке гипоталамуса. Учитывая данные ряда авторов о повышении возбудимости гипоталамуса при реакции самораздражения (САБО 1973 и др.), нужно полагать, что наблюдаемое в нашем опыте уменьшение амплитуды ВП гипоталамуса аналогично феномену маскировки сенсорных ВП коры во время реакции десинхронизации (БРЕМЕР 1958), результатом окклюзивного взаимодействия «неспецифических» и «специфических» импульсов, конвергирующих на одни и те же нейроны гипоталамуса. В свете этой концепции «подавление» ВП центральной нервной системы при возбуждении активирующей системы мозга есть результат не истинного торможения, а интерференции гетерогенных стимулов. Если редукция ВП гипоталамуса отражает состояние повышенной возбудимости этой области, то «облегчение» их вероятно соответствует состоянию торможения холинергических структур гипоталамуса. Об этом свидетельствуют и наши данные о повышении порога реакции самораздражения под влиянием мускаринового антихолинергического препарата. Эффект атропина на сенсорные ВП гипоталамуса аналогичен эффекту атропина на первичные ответы коры. И в этом случае наблюдается рост амплитуды первичных положительно-отрицательных корковых ответов на раздражение сенсорных афферентов (ЧАТФИЛЬД и ПУРПУРА 1954; БАКЛАВАДЖЯН 1964 и др.). В исследованиях последних лет при использовании метода микроионтофоретической аппликации фармакологических препаратов в область отдельных нейронов гипоталамуса и регистрации его импульсной активности установлена чувствительность

нейронов заднего гипоталамуса не только к норадреналину, но и к ацетилхолину (САЛМОЙРАГИ и СТЕФАНИС 1965 и др.). ШУТЕ и соавт. (1966) считают, что у кошек и у крыс по всему гипоталамусу проходит холинергический путь, по гистохимическим данным авторов ход холинергических волокон сходен с ходом моноаминергических.

Вопрос об изменении реакции самораздражения в результате изменения уровня активности афферентных систем гипоталамуса под влиянием психотропных фармакологических веществ нуждается в дальнейшем изучении с применением методики внутригипоталамической микроинъекции препаратов симпато- и парасимпатотропного действия при параллельной регистрации реакции самораздражения и сенсорных ВП гипоталамуса.

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Pharmacologia

INHIBITION OF MICROSOMAL DRUG METABOLISM BY 2-{[2-(4-PYRIDYL)-METHYL]-THIO}-BENZIMIDAZOLE (H-718)

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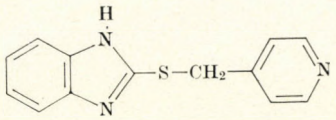
The intraperitoneal LD_{50} value of H-718 amounted to 314 mg/kg in rats. At dose levels from 10 to 15 mg/kg intraperitoneally, H-718 caused a 4—5-fold prolongation in hexobarbital sleeping time and increased the half-life of plasma hexobarbital concentration. The compound prevented the tremorine-induced tremor but failed to inhibit oxotremorine tremor. It prolonged the survival of rats treated with lethal doses of parathion, but did not affect the toxicity of paraoxon. The hepatic microsomal oxidation of hexobarbital, hydroxylation of aniline and N-demethylation of aminophenazone were markedly inhibited by H-718 at a concentration of 10^{-4} M, but procaine esterase activity was depressed only moderately. Conjugation of bromsulphophthalein with glutathione was not affected even by an intraperitoneal dose of 50 mg/kg. The data suggest that H-718 inhibits the microsomal NADPH and cytochrome P_{450}-dependent drug metabolizing enzyme system of the liver.

Preliminary pharmacological screening of 2-(thioalkylpyridyl)-[1,3]-diazole derivatives synthetized by HANKOVSZKY and HIDEG (1969) indicated that several of these compounds considerably prolonged hexobarbital sleeping time in the rat but failed to prolong the anaesthesia induced by ether or barbital. The duration of action of hexobarbital is determined by the rate of its biotransformation (BRODIE 1956), while barbital (GOLDSCHMIDT and WEHR 1957) and ether (VAN DYKE et al. 1964) are metabolized to a small extent only. It was therefore assumed that the prolongation of hexobarbital sleep by these drugs was a result of the inhibition of the hepatic microsomal drug metabolizing enzyme system. This assumption was supported also by the finding that hexobarbital sleeping time was prolonged by doses so low which did not show any other significant pharmacological effect.

Of the investigated compounds, H-718 was the most potent inhibitor of microsomal drug biotransformation. The present paper reports on studies of that compound.



Methods

Male Wistar rats of 140 to 180 g b.w. and albino mice of both sexes (20 to 30 g b.w.) were used. H-718 was dissolved in distilled water or in 0.9% NaCl solution and warmed to 30°C prior to administration.

In vivo methods

Determination of hexobarbital and barbital sleeping time. Both mice and rats were injected intraperitoneally with 75 mg/kg of hexobarbital (Evipan®), and the time from loss to return of the righting reflex was recorded. H-718 was given intraperitoneally 30 minutes prior to the injection of hexobarbital. Sleeping time being prolonged by hypothermia, the animals were kept under a bulb to prevent their body temperature from falling below 37°C. The effect of H-718 on barbital (250 mg/kg i.p.) sleep was tested as described above. H-718 was given intraperitoneally 30 minutes prior to the administration of barbital.

Determination of plasma and tissue hexobarbital concentrations. Rats were injected intraperitoneally with 75 mg/kg hexobarbital and on return of the righting reflex they were bled to death through the carotid artery. Plasma and tissue hexobarbital concentrations were determined by the method of BRODIE et al. (1953).

The antitremor activity of H-718 was determined in mice by the method of AHMED and TAYLOR (1959). H-718 was given one half hour prior to the injection of the tremor-inducing agent. Following the intraperitoneal administration of tremorine (25 mg/kg) or oxotremorine (0.2 mg/kg) the intensity of tremor was recorded three times for 2 minutes each, at 10, 15 and 20 minutes. The tremor index was expressed as the ratio of the length of the light track recorded after administration of the tremor-inducing agent, and the length of the light track in 0.9 NaCl control experiments performed on the preceding day.

Effect of H-718 on parathion and paraoxon toxicity. Eighty rats were divided into ten groups. The first group received 4 mg/kg parathion and each consecutive group a dose 50% higher than the preceding one. One half hour before the administration of parathion, 50 mg/kg of H-718 was given and this dose was repeated twelve hours later. In the next days treatment with 50 mg/kg of H-718 twice daily was continued until the animals died. Paraoxon was administered in doses of 2, 3, 4.5, 6.7 and 10 mg/kg to groups consisting of ten rats each. H-718 was given as described above.

Determination of bromsulphophthalein–glutathione conjugate. Rats were anaesthetized intraperitoneally with uretan (1.2 g/kg) and their common bile duct cannulated with PE-50 tubing. Bromsulphophthalein (50 mg/kg) was given into the femoral vein and the bile was collected for one hour. Five to ten μl of bile were applied on Whatman's No. 1 filter paper, and the chromatograms were developed in an ascending system consisting of n-butanol, acetic acid and water (4 : 2 : 1, v/v). The paper was exposed to ammonia vapour, the spots of free and conjugated bromsulphophthalein were cut out and the dye was eluted with 0.5 N NaOH. Optical density was measured at 580 mμ. H-718 was given 30 minutes prior to the administration of bromsulphophthalein.

Acute toxicity of H-718. A 3-day toxicity test was performed in rats and mice after intraperitoneal and oral administration. The LD_{50} values were estimated according to LITCHFIELD and WILCOXON (1949).

In vitro methods

Microsomal metabolism of hexobarbital, aniline and aminophenazone. The livers of three or four rats were forced through a tissue press to remove fibrous tissue. The liver pulp was homogenized in a Potter–Elvehjem homogenizer with two volumes of cold 1.15% KCl solution, containing 0.03 M tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 9000 $\times$ g at a temperature of 4°C for 20 minutes. The supernatant was decanted and centrifuged again at 100,000 $\times$ g for 60 minutes. The microsomal fraction was rehomogenized with 1.15% KCl solution and stored at —20°C till use, but not longer than 24 hours. The protein concentration of the microsomal fraction was determined by the method of LOWRY et al. (1951). Bovine albumin (Cohn fraction V, Koch-Light) was used for standard. Enzyme assay: to the microsomal fraction corresponding to 0.6 g liver were added 500 μMol of tris-HCl buffer, pH 7.4, 1 μMol of NADP (Boehringer), 25 μMol of nicotinamide (Reanal), 25 μMol of $MgCl_2$, 50 μMol of glucose-6-phosphate (Reanal), 0.5 units of glucose-6-phosphate dehydrogenase (Boehringer) and 1.15% KCl solution in a final volume of 4 ml. The amount of hexobarbital, aniline or aminophenazone in the reaction mixture was 5 μMol. The reaction mixture was incubated

in the presence of oxygen at 37°C in a shaking incubator for 45 minutes. H-718 was added two minutes prior to the pipetting of substrates to the medium. At the end of incubation, hexobarbital (BRODIE et al. 1953), p-aminophenol (KATO and GILLETTE 1965) and 4-amino-antipyrine (La DU et al. 1955) were determined in reaction mixtures. The mean values for six control experiments were,

hexobarbital, 3.13 ± 0.27 nMol/min · mg protein;
aniline, 0.73 ± 0.12;
aminophenazone, 0.62 ± 0.08.

Procaine esterase activity of the microsomal fraction was measured by the method of HEINRICH and KLINGER (1968). The control value was 1.55 ± 0.17 nMol/min · mg protein in the average of six experiments.

Statistical analysis. Student's *t*-test was used for statistical analysis.

Results

Prolongation by H-718 of hexobarbital and barbital sleeping time. Hexobarbital sleeping time was prolonged by H-718 in proportion to the dose in both mice and rats (Table I). H-718 was absorbed well from the gastrointestinal tract and at a dose level of 50 mg/kg the prolongation of hexobarbital sleep was marked even 8 to 10 hours following the administration (Table II). When the same dose was administered immediately after the righting reflex had returned, the rats did not fall asleep again, only the sedation lasted longer than in the control animals. In this respect, H-718 was different from chlorpromazine. When 20 mg/kg of chlorpromazine was given after restoration of the righting reflex, the animals fell asleep (21 ± 4 min, $n = 10$).

Following an intraperitoneal dose of 250 mg/kg of barbital, sleep lasted for 162 ± 24 min in the average of ten rats. 100 mg/kf og H-718 did not prolong this sleeping time (141 ± 35 min, $n = 10$, $p > 0.05$).

Plasma and tissue hexobarbital concentration in rats pretreated with H-718. Twelve rats were injected with 75 mg/kg of hexobarbital and another twelve

Table I

Effect of H-718 on hexobarbital sleeping time in mice and rats

Dose, mg/kg i.p.	Sleeping time, min	
	mice	rats
—	21 ± 4*	15 ± 3
6	41 ± 7	31 ± 6
12	57 ± 10	56 ± 9
25	102 ± 17	138 ± 25
50	145 ± 22	196 ± 34

* Mean ± S.D. of ten to fifteen animals
Dose of hexobarbital: 75 mg/kg intraperitoneally for both mice and rats

Table II

Hexobarbital sleeping time at various intervals after intraperitoneal or oral administration of H-718

Time in hours	H-718 (50 mg/kg)	
	intraperitoneal, min	oral, min
1	202 ± 32* (15)	—
2	215 ± 41 (10)	152 ± 34 (15)
4	128 ± 28 (12)	135 ± 37 (12)
6	83 ± 19 (10)	122 ± 22 (6)
8	54 ± 13 (6)	78 ± 14 (10)
10	33 ± 10 (6)	
12	17 ± 7 (6)	31 ± 8 (6)

* Mean ± S.D. Number of rats in brackets
Sleeping time of control rats: 16.4 ± 3.5 min (n = 44)

Table III

Tissue and plasma hexobarbital concentrations in control rats and in rats pretreated with 50 mg/kg of H-718

Tissue	Hexobarbital concentration, μg/g	
	controls	H-718
Brain	33.4 ± 4.8*	35.3 ± 3.7
Liver	35.1 ± 4.2	38.1 ± 5.2
Kidney	26.4 ± 3.9	24.3 ± 4.1
Fat	85.1 ± 15	76.4 ± 12
Plasma	28.3 ± 5.1	30.9 ± 4.2

* Mean ± S.D. of twelve rats. H-718 was given 30 minutes prior to the injection of 75 mg/kg of hexobarbital. Tissue and plasma hexobarbital concentrations were measured after return of righting reflex. Control animals slept for 17 ± 3 min, animals pretreated with H-718 slept for 126 ± 12 min

with 50 mg/kg of H-718 half an hour prior to the administration of hexobarbital. On return of the righting reflex they were bled to death and the plasma and tissue hexobarbital concentration was measured. There was no significant difference between the plasma and tissue hexobarbital levels of the two groups, in spite of the fact that the H-718-treated rats slept 7.4 times longer than the controls (Table III). This indicated that the half-life of plasma hexobarbital concentration was longer in H-718-treated than in control rats. Measurement of plasma hexobarbital levels verified this assumption (Fig. 1).

Prevention by H-718 of tremorine tremor. Tremorine-induced tremor was markedly inhibited by H-718, while oxotremorine tremor was not affected

significantly (Table IV). It was therefore assumed that H-718 itself did not possess any antitremor activity, it only inhibited the biotransformation of tremorine to oxotremorine.

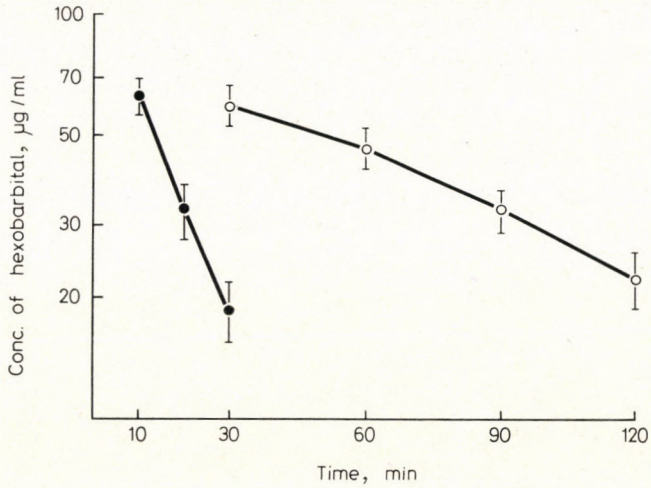


Fig. 1. Decrease of plasma hexobarbital concentration in control rats (●—●) and in rats pretreated with 15 mg/kg of H-718 (○—○) intraperitoneally. Each point represents the mean ± S.D. of twelve animals. Dose of hexobarbital 75 m/kg intravenously

Effect of H-718 on the acute toxicity of parathion. The oral LD_{50} of parathion amounted to 8.6 mg/kg. Most deaths occurred within 3 hours following the administration of parathion. Although the LD_{50} of parathion was hardly increased by H-718, survival of the rats was significantly prolonged (Table V). Against paraoxon, H-718 failed to show a protective effect.

Table IV

Intensity of tremor induced by tremorine or oxotremorine in mice after H-718 pretreatment

	Dose, mg/kg i.p.	Tremor index	
		tremorine	oxotremorine
Controls	—	2.65 ± 0.24*	2.81 ± 0.32
H-718	10	2.11 ± 0.26	2.50 ± 0.23
	30	1.23 ± 0.15	2.96 ± 0.42
	100	0.92 ± 0.08	2.48 ± 0.35

* Mean ± S.D. of ten mice. H-718 was given 30 minutes prior to the intraperitoneal administration of tremorine (25 mg/kg) or oxotremorine (0.2 mg/kg)

Inhibition of microsomal drug biotransformation. H-718 inhibited the oxidation of hexobarbital, the hydroxylation of aniline and the N-demethylation of aminophenazone (Table VI). Microsomal procaine esterase activity was depressed only moderately by H-718, even at a concentration of 10^{-3} Mol/litre. A preliminary investigation into the distribution of H-718 in rat tissues showed that 10^{-3} Mol/kg liver was the maximum concentration of H-718 achieved by the administration of doses which were already toxic (250—300 mg/kg intraperitoneally).

Conjugation of bromsulphophthalein with glutathione. In the bile of control rats, conjugated bromsulphophthalein amounted to $75 \pm 7\%$ ($n = 5$) of the total amount administered, while in the bile of H-718-treated (50 mg/kg) rats, to $73 \pm 6\%$ ($n = 5$).

Acute toxicity of H-718. The LD_{50} for the rat was 314 mg/kg (confidence limits, 266—370) by the intraperitoneal route and 940 mg/kg (confidence limits, 617—1416) by the oral route. Mean survival time was 26 hours, whatever the route of administration. Death was preceded by sedation, but no sleep. LD_{50} values for the mouse were, 420 mg/kg (confidence limits, 323—546) by the intraperitoneal route, and over 800 mg/kg by the oral route.

Table V

Effect of H-718 on parathion and paraoxon toxicity in rats

	LD_{50} (mg/kg)					
	Controls 24 hrs	Pretreated with H-718				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Parathion	8.6	49	27	17	13	13
Paraoxon	5.6	5.4	5.4	—	—	—

For details, see Methods

Table VI

Inhibition by H-718 of the metabolism of various drugs in rat liver microsomes

H-718, Mol/litre	Inhibition in per cents			
	Hexobarbital oxidation	Aniline hydroxylation	Aminopyrine *N*-demethylation	Procaine hydrolysis
1×10^{-3}	—	80	—	25
3×10^{-4}	82	75	68	13
1×10^{-4}	77	51	57	—
3×10^{-5}	48	45	41	—
1×10^{-5}	22	15	28	—
3×10^{-6}	20	—	—	—

For details, see Methods

Discussion

H-718 in itself had no hypnotic effect, but it increased the duration of action of hexobarbital. This effect may be brought about by one of two distinct mechanisms: by increasing the sensitivity to hexobarbital of the central nervous system, or by decreasing the rate of hexobarbital metabolism. H-718 did not potentiate the effect of subhypnotic plasma hexobarbital concentrations nor did it prolong barbital sleep. However, the half-life of plasma hexobarbital concentration was increased by H-718, and the microsomal oxidation of hexobarbital in vitro was also inhibited.

Tremorine-tremor is caused by oxotremorine, a metabolite of the parent compound (Cho et al. 1965). Parathion is also a "pro-drug" which is biotransformed by desulphuration into paraoxon, a more active inhibitor of cholinesterase (Davison 1955). H-718 inhibited the effect of these two "pro-drugs", but did not antagonize the effect of oxotremorine or the toxicity of paraoxon. The oxidation of hexobarbital, hydroxylation of aniline, N-demethylation of aminophenazone, oxidation of tremorine and desulphuration of parathion to paraoxon are catalyzed by hepatic microsomal NADPH- and cytochrome P_{450}-dependent drug metabolizing enzyme system. All these reactions were inhibited by H-718. Microsomal procaine esterase activity and cytoplasmic glutathione S-aryltransferase (Combes and Stakelum 1961) which are not cytochrome P_{450}-dependent reactions, were not affected by H-718.

H-718 contains a benzimidazole moiety. Benzimidazole cannot be regarded as a xenobiotic, since it is present in many biological substances, for instance in vitamin B_{12}. In experiments performed on beaf-spleen slices (Alivisatos et al. 1962) and on wheat-germ extract (Kapoor and Waygood 1965) benzimidazole could be substituted for the nicotinamide moiety of NAD in the reaction catalyzed by NAD nucleosidase. It seemed therefore justified to assume that H-718 inhibited the generation of NADPH. Still, our preliminary experiments failed to support this hypothesis. In addition, it was shown that the benzimidazole in H-718 could be replaced by other ring systems without any significant loss of the inhibitory effect on microsomal drug metabolism. However, substitution of the pyridine ring with a benzene ring abolished the inhibitory effect (unpublished observations). It was shown that 4-acetylpyridine (Coper et al. 1968), isoniazide (Coper et al. 1968), metyrapone (Netter and Kajuschke 1967) and some other pyridine derivatives inhibited microsomal drug metabolism by binding to cytochrome P_{450}. H-718 seems to be a more potent inhibitor of microsomal drug metabolism than the other pyridine derivatives. Though in rats SKF-525-A is 1.6 times more effective in prolonging hexobarbital sleeping time, it is about twice as toxic as H-718.

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INHIBITION OF HEXOBARBITAL METABOLISM BY PYRIDYL DERIVATIVES

By

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The prolonging effect of 34 pyridyl derivatives on hexobarbital sleeping time was compared in rats. It was found that the compounds increased the duration of hexobarbital action by inhibiting the microsomal oxidation of the drug. The carrier of the effect is the pyridine ring. N-methylation of the pyridine ring abolished the inhibitory action. The 4-pyridyl derivatives proved always more effective than the 3-pyridyl analogues and these than the 2-pyridyl analogues. The haptophore molecular moiety is, by itself, ineffective, and may widely differ in chemical structure. The role of the haptophore group is to amplify 10—100-fold the slight inhibitory action of pyridine.

Several compounds containing pyridine ring are known to exert an inhibitory effect on drug metabolism. For instance, 4-acetylpyridine (COPER et al. 1968), isonicotinic acid hydrazide (COPER et al. 1968) or metyrapone (NETTER and KAJUSCHKE 1967). To these belongs the compound H-718 (2-{[2-(4-pyridyl)-methyl]-thio}benzimidazole) described in a previous paper (VARGA et al. 1973). This compound has been shown considerably to prolong the duration of action of certain drugs by inhibiting their metabolism and to retard development of the action of "pro-drug" type compounds. H-718 proved much more effective than the compounds mentioned above. Therefore, we have investigated a series of further compounds in order to elucidate the relationship between chemical structure and pharmacological action.

The most simple, most reliable and most often used method for the estimation of the in vivo activity of the NADPH-dependent drug metabolizing enzyme system of the liver endoplasmic reticulum is the hexobarbital sleeping time test. The decisive part in the elimination of hexobarbital is played by biotransformation; correspondingly, the duration of hexobarbital action depends mainly on the activity of the drug metabolizing enzyme system. However, when evaluating the inhibition of hexobarbital oxidation in vivo other pharmacological effects of the inhibitor have also to be taken into account, thus the central depressant action, muscle relaxant effect, etc. The alteration of hexobarbital sleeping time can be regarded as an indicator of the activity of the drug metabolizing enzymes only in the case when the prolongation of sleeping time is exclusively due to the decreased activity of the enzymes. According to a preliminary screening, the majority of the pyridyl

derivatives to be described in this paper failed to show any significant muscle relaxant, sedative and ethyl ether anaesthesia prolonging effect even when applied in amounts corresponding to half their LD_{50} value.

Methods

General. Male albino rats of our own breed weighing 100—120 g were used in the experiments. The compounds bearing the code-letter H were synthetized by HANKOVSZKY and HIDEG (1966, 1969). Some of the derivatives were investigated by VÁCZI et al. (1968) for antiviral effect. The letter H in the tables and the text always refers to 4-pyridyl derivatives the 3- and 2-pyridyl analogues of which bear no special number. Some compounds were commercial preparations of analytical grade. Nearly all of the drugs investigated were easily soluble in distilled water or in 0.9% sodium chloride. All the drugs were administered by the intraperitoneal route.

Determination of hexobarbital sleeping time. The rats were injected with 75 mg/kg of hexobarbital (Evipan®) intraperitoneally, and the time from loss to return of the righting reflex was recorded. The drugs to be investigated were given 30 minutes prior to the injection of hexobarbital. Since the sleeping time is prolonged by hypothermia, the animals were kept under a bulb to prevent their body temperature from falling below 37°C. The drugs were administered in increasing doses to at least 5 animals each, and the PD_{300} values were determined. PD_{300} = estimated dose of a compound that prolongs sleeping time to fourfold of the control value. A minimum of 3 doses the effect of which amounted to between 100% and 500% were used for the calculation.

Determination of ethyl ether sleeping time. Two rats were placed in a 4.8 l wide-necked bottle standing in a water bath of 40°C, 5 ml ether was pipetted into it and it was stoppered immediately. Five minutes later the animals were taken out, and the time till return of the righting reflex was measured. The anaesthesia prolonging effect was compared with the average sleeping time of 8—12 control rats tested on the same way. The hexobarbital anaesthesia prolonging effect was estimated in the same way. Mean sleeping time of the control groups was 212 $\pm$ 42 sec (n = 56). Under the given experimental conditions, 5 mg/kg of chlorpromazine administered 30 min prior to ether anaesthesia caused a 82%, 10 mg/kg a 206%, and 20 mg/kg a 450% prolongation of the sleeping time (10 rats in each group).

Hexobarbital concentration in plasma was measured according to BRODIE et al. (1953); the animals received 75 mg/kg of hexobarbital into the tail vein.

Estimation of the microsomal oxidation of hexobarbital. Preparation of microsomal fraction from rat liver and determination of hexobarbital oxidation were performed according to the method described previously (VARGA et al. 1973). The inhibitory effect of a given compound was calculated from 4 parallel measurements for each concentration and expressed in per cents of the control value.

Determination of LD_{50}. The compounds were administered by the intraperitoneal route, each dose to at least 6 animals. The rats were observed for three days. The LD_{50} value was computed according to the method of LITCHFIELD and WILCOXON (1949). Confidential limits (95%) were calculated except for cases when only two doses fell between the 16% and 84% limits, or when only approximative values were needed.

Statistical analysis. Significance was calculated according to the *t* method. The tables and the text give mean values $\pm$ S.D.

Results

Relationship between chemical structure and prolongation of hexobarbital sleeping time

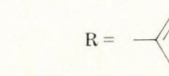
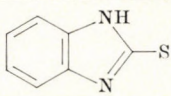
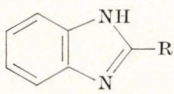
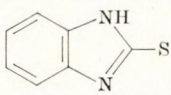
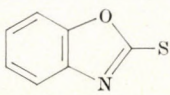
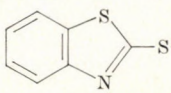
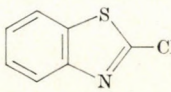
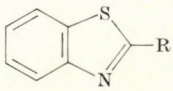
In our previous investigations (VARGA et al. 1973) H-718 was shown considerably to prolong hexobarbital sleeping time. Substitution of the benzimidazole ring in H-718 by benzoxazole (H-717) or benzthiazole (H-716)

did not affect the hexobarbital anaesthesia prolonging effect (Table I). Thus, these three ring systems may be considered bioisosteric as far as the prolonging effect on hexobarbital sleeping time is concerned. Lengthening (H-1256) or omission (H-227) of the alkyl chain connecting the benzimidazole and pyridine ring decreases, though does not abolish, the activity of the compound.

The presence of the pyridine ring is necessary for the action, as proved by the data of Table II. Even very high doses of benzimidazole or methylmercaptobenzimidazole fail, by themselves, to prolong hexobarbital sleeping time. Also ineffective are H-775 and H-941. However, the 4-pyridyl analogues of these two compounds (H-792 and H-950) are markedly prolonging hexo-

Table I

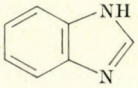
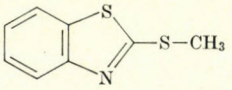
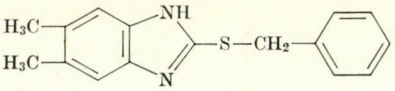
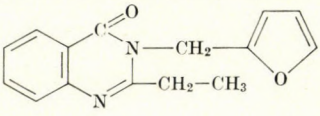
PD_{300} and toxicity of H-718 and some related compounds in the rat

Compound	R = 	PD_{300}, mg/kg i. p.	LD_{50} (mg/kg) (95% c. l.) i. p.
H-718		10.5	314 (266—370)
H-227		44	230
H-1256		15.2	154 (133-177)
H-717		10	293 (244-351)
H-716		9.3	364 (298-444)
H-827		9.1	
H-223		38	280 (215-364)

The compounds were administered 30 minutes prior to hexobarbital (75 mg/kg i.p.). Duration of action of hexobarbital in control rats: 17 $\pm$ 4 min (n = 82). PD_{300} = estimated dose of a compound that prolongs hexobarbital sleeping time to fourfold of the control value

Table II

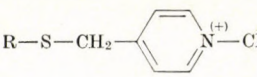
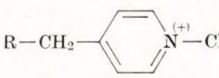
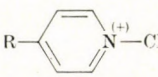
Prolongation of hexobarbital sleeping time (HST) in rats after pretreatment with benzimidazole, 2-methyl-mercaptobenzthiazole, H-775 and H-941, and the toxicity of the compounds

Compound	Structure	Dose, mg/kg i.p.	HST prolongation vs. controls, per cent	LD_{50}, mg/kg i.p.
		300	18	>700
		300	−25	>600
H−775		75	128	275
H−941		50	−15	180

The compounds were administered 30 minutes prior to hexobarbital (75 mg/kg i.p.). Sleeping time of control rats: 18 ± 3 min (n = 12)

Table III

Effect of N-methyl-pyridine analogues of H-716, H-827 and H-223 on hexobarbital sleeping time (HST) in the rat

Compound	R =	Dose, mg/kg i.p.	HST prolongation vs. controls, per cent	LD_{50}, mg/kg i.p.
H−716−Q		40	16	120
H−827−Q		10	−18	35
H−223−Q		30	25	80

The compounds were administered 30 minutes prior to hexobarbital (75 mg/kg i.p.). Sleeping time of control rats: 14 ± 3 min (n = 24)

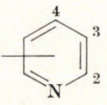
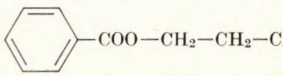
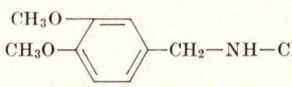
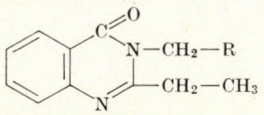
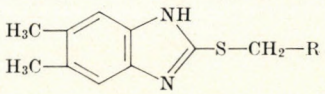
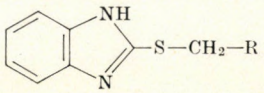
barbital sleep (Table IV), due to the inhibition of the metabolic transformation of hexobarbital (Table VII).

Quaternization of the nitrogen atom of the pyridine ring abolishes the hexobarbital anaesthesia-prolonging effect; no such action can be demonstrated even in subtoxic doses (Table III).

The position of the pyridine ring can also modify the strength of the action. All the seven compounds shown in Table VII prolonged hexobarbital

Table IV

Prolongation of hexobarbital sleeping time (HST) in rats after pretreatment with some 2-, 3- and 4-substituted pyridine derivatives

Compound	R = 	Position of pyridine bond	Dose, mg/kg i. p.	HST prolongation vs. controls, per cent	LD_{50}, mg/kg i. p.
-	$Cl—CH_2—R$	2	200	77	500
		-	–	-	–
		4	200	186	500
-	$OH—CH_2—CH_2—CH_2—R$	2	200	−26	900
		3	200	128	940
		4	200	155	920
H − 552		2	150	66	430
		3	150	138	425
		4	150	165	475
H − 595		2	100	42	235
		3	100	147	270
		4	100	283	250
H − 950		2	50	33	325
		3	50	174	285
		4	50	320	310
H − 792		2	30	85	
		-	–		
		4	30	550	
H − 718		2	25	58	380
		3	25	240	345
		4	25	721	314

The compounds were administered 30 minutes prior to hexobarbital (75 mg/kg i.p.). Sleeping time of control rats: 15.8 ± 3.1 min (n = 68). For determination of HST and LD_{50}, see Methods

sleeping time significantly longer ($p < 0.05$) than their 2-pyridyl analogues. The activity of the 3-pyridyl analogues was between the two former ones. An intraperitoneal dose of 25 mg/kg of the 2-pyridyl analogue of H-718 prolonged sleeping time by 58%. The PD_{300} value of this compound is 165 mg/kg, while that of the 4-pyridyl congener (H-718) 10.5 mg/kg. With the majority of the compounds shown in Table IV, the dose effect curves of the 2-pyridyl and the 4-pyridyl derivatives were not parallel, these of the latter running steeper. The PD_{300} of some 2-pyridyl derivatives could not be determined. For instance, the 2-pyridyl analogue of H-595 prolonged sleeping time by 230% even in a dose of 250 mg/kg. Great differences in efficiency were revealed on comparing the seven groups of compounds. For instance, the PD_{300} of 4-picolylchloride was more than 300 mg/kg while that of H-716, 9.3 mg/kg.

It is obvious from the data presented that the presence of the pyridine ring is necessary for the prolonging effect on hexobarbital sleeping time. Pyridine by itself prolongs hexobarbital sleeping time, its PD_{300} is more than 350 mg/kg. Its exact PD_{300} value could not be determined since the substance exert a marked central depressant effect in doses from 200 to 500 mg/kg. Moreover, pyridine pretreatment caused anaesthesia even in animals given a subhypnotic dose of hexobarbital (30 mg/kg intraperitoneally). The sleeping time was 43 ± 6 min after 200 mg/kg ($n = 5$), and 77 ± 9 min after 400 mg/kg ($n = 5$). The efficiency of the compound is determined by the moiety of the molecule attached to the pyridine ring. As seen from Tables I, IV and V, the structure of the carrier molecular part may be widely different, without any common structural–chemical resemblance.

In the case of the most active compounds, the sleep-prolonging effect was evident after doses as low as 1/60—1/30 of the LD_{50}. In this dose range the compounds exerted no other pharmacological effect.

Prolongation of ethyl ether sleeping time

When compared with the controls only two compounds out of the substances shown in Tables I, IV and V prolonged ethyl ether sleeping time. These were H-729 (85% prolongation after 50 mg/kg), and H-1484 (175% prolongation after 50 mg/kg). Lower doses (25 mg/kg) did not potentiate ethyl ether anaesthesia; on the other hand, they prolonged hexobarbital sleeping time.

The duration of ethyl ether anaesthesia was prolonged by 93% after 200 mg/kg of pyridine and by 217% after 400 mg/kg of it.

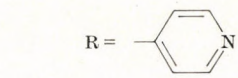
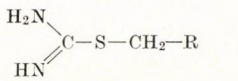
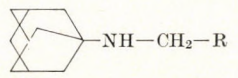
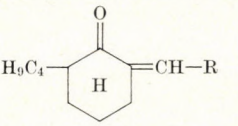
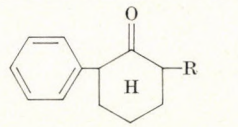
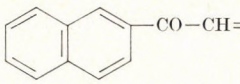
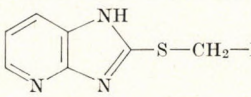
Changes in the plasma hexobarbital level

In Fig. 1, the plasma hexobarbital concentrations of control and treated animals are plotted against time. Of the compounds seen in Fig. 1, it was the

preparation H-716 which most markedly increased the plasma half-life of hexobarbital, while H-1212 was the least effective. The order of efficiency showed a good agreement with the prolonging action on hexobarbital sleeping time (cf. Tables I, IV and V).

Table V

PD_{300} of some 4-substituted pyridine derivatives

Compound	R = 	PD_{300}, mg/kg i.p.	LD_{50} (mg/kg) (95% c.l.) i.p.
H-1212		135	165
H-1239		46	145 (120-174)
H-1484		15.5	96 (74-125)
H-1482		28	203 (155-264)
H-1461		19	224 (174-280)
H-756		16	

The compounds were administered 30 minutes prior to hexobarbital (75 mg/kg i.p.). Duration of action of hexobarbital in control rats: 16.5 ± 3 min (n = 52). PD_{300} = estimated dose of a compound that prolongs hexobarbital sleeping time to fourfold of the control value

As seen in Table IV, H-718 prolonged sleeping time longer than did the 3-pyridyl or 2-pyridyl analogue of the compound. This difference was also manifest in the effect on the plasma hexobarbital level (Table VI).

Pyridine, in an intraperitoneal dose of 400 mg/kg, also retarded the decrease of the plasma hexobarbital level. Thirty minutes after the intra-

venous administration of 75 mg/kg of hexobarbital, its plasma level was 23 ± 5 μg/ml in control animals ($n = 7$) and 41 ± 5 μg/ml in pyridine-treated rats ($n = 10$). The difference was significant statistically ($p < 0.05$).

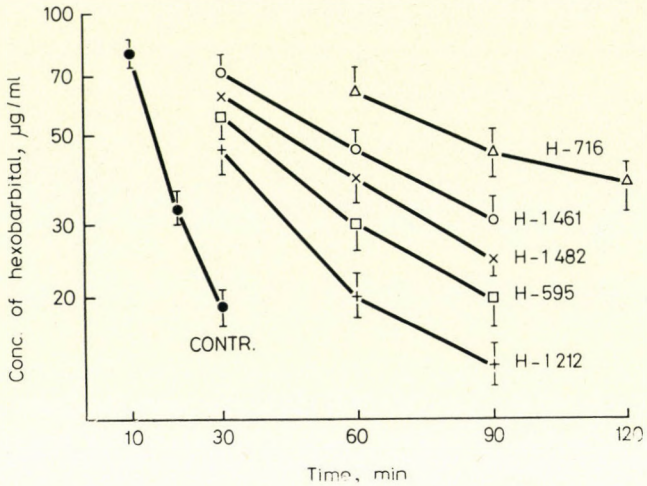


Fig. 1. Decrease of plasma hexobarbital concentration in rats pretreated with various compounds. H-716 (20 mg/kg), H-1461 (30 mg/kg), H-1482 (50 mg/kg), H-595 (100 mg/kg) and H-1212 (100 mg/kg) were injected intraperitoneally 30 minutes prior to the intravenous administration of hexobarbital (75 mg/kg). Each point represents the mean value ±S.D., for eight rats

Table VI

Plasma hexobarbital concentrations in rats pretreated with H-718 or its 3-pyridyl and 2-pyridyl analogues

	Hexobarbital concentration, μg/ml		
	30 min	60 min	90 min
Control	18 ± 4*	10.6 ± 3	6.4 ± 2.1
H-718	54 ± 6	47 ± 5	38 ± 4
3-pyridyl analogue of H-718	35 ± 5	29 ± 4	20 ± 3
2-pyridyl analogue of H-718	24 ± 4	15 ± 4	10.2 ± 3

* Mean ± S.D. for seven rats. The compounds (40 mg/kg i.p.) were administered 30 minutes prior to hexobarbital (75 mg/kg i.v.). Values for plasma hexobarbital concentration measured one and half hour after the administration of hexobarbital differed significantly ($p < 0.05$)

Inhibition of hexobarbital oxidation in the microsomal fraction of the liver

Biotransformation of hexobarbital was inhibited by all 4-pyridyl derivatives. Some pertaining data are shown in Table VII. Pyridine in itself was also inhibitory when used at high concentrations. Thus, pyridine retards the

Table VII

Inhibitor effect of some compounds on hexobarbital metabolism in rat liver microsomes

Compounds	PD_{300}, mg/kg i.p.	Inhibition of hexobarbital oxidation in per cent mol/litre				
		1×10^{-3}	3×10^{-4}	1×10^{-4}	3×10^{-5}	1×10^{-5}
pyridine	> 350	47	25	—	—	—
4-picolylchloride	> 300	61	22	—	—	—
H-552	280	55	37	22	—	—
H-1212	135	68	41	30	—	—
H-950	48	82	54	33	—	—
H-1482	28	—	73	45	29	—
H-1461	19	—	80	44	33	—
H-792	19	—	76	57	22	—
H-716	9.3	—	—	78	45	31

For details, see Methods

decrease of the plasma hexobarbital level and increases the duration of sleeping time by inhibiting the oxidation of hexobarbital. The PD_{300} value of pyridine cannot be determined exactly as the compound not only prolongs but also potentiates the effect of hexobarbital. No close parallelism can be expected between the inhibitory effect measured in vitro and the sleep prolonging potency in vivo, in view of the presumably different distribution, protein binding, elimination velocity, etc., of the compounds. Taking this into account, the parallelism observed in the order of efficiency in vivo and in vitro may be regarded as satisfactory.

Discussion

The pyridine ring plays a decisive part in the inhibitory effect of the compounds investigated on the metabolism of hexobarbital. Substitution of the pyridine ring in some compounds by a benzene or furan ring practically abolishes the activity (Table II). Quaternarization of the nitrogen atom in the pyridine ring has the same consequence (Table III).

Kost et al. (1967) demonstrated considerable differences in the central nervous and autonomic actions of 2-pyridylethylamine and 4-pyridylethylamine. In the experiments of Coper et al. (1968) 250 mg/kg of 2-acetylpyridine prolonged hexobarbital sleeping time by 34%, and 50 mg/kg of 4-acetylpyridine by 100%. In the present experiments, the hexobarbital anaesthesia-prolonging effect of 4-, 3- and 2-pyridyl analogues was compared in seven groups of compounds. In all the cases, the 4-pyridyl derivatives proved to be more

potent prolonging agents than their 2-pyridyl analogues (Table IV). Thus, the peak effect is achieved if the carrier pyridine ring is attached to the haptophore molecular moiety in para position.

The haptophore moiety in itself is ineffective (e.g. benzimidazole or methylmercaptobenzimidazole), and its chemical structure may differ widely (Tables I, IV and V). However, it plays an important part in the inhibitory potency. Certain haptophore groups such as 1-propanol increase only slightly the inhibitory action of pyridine on drug metabolism while other groups, e.g. benzimidazole, augment it considerably. On a molecular weight basis the prolonging effect of H-716 on hexobarbital sleeping time is about 100 times as strong as that of pyridine. Pyridine, nicotinic acid amide and metyrapone were shown to be bound to the microsomal cytochrome P-450, the assumed type II, or aniline-type, binding site [the pertaining literature has been reviewed by MANNERING (1972)]. The compounds presented in this paper probably also exert their effect by being bound to the type II binding site. Still, the function of the haptophore group in the manifestation of the inhibitory action remains unclear.

The potency of the five most active compounds (H-716, H-717, H-718, H-827 and H-1461) was compared in mice, rabbits and dogs. As regards the prolongation of hexobarbital sleeping time, the duration of action, and the toxicity, in these animal species compounds H-718 proved to be somewhat more advantageous and more effective than did the other compounds (unpublished investigations).

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EFFECT OF BETA ADRENERGIC BLOCKING ON THE ARRHYTHMIAS FOLLOWING CORONARY LIGATION IN RAT

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Ligation of the left coronary artery according to SELYE et al. (1960) produced in 19 out of 20 experiments arrhythmia different in severity and duration.

In 15 out of 20 experiments, pretreatment with 200 μg of propranolol per 100 g of body weight normalized heart function within 15 sec. In 11 cases the QRS diminished gradually. Extrasystole was observed in not more than two cases. Ventricular flutter and/or fibrillation occurred in six animals, in three with fatal outcome. In 12 experiments frequency gradually decreased and the heart stopped. In these cases the heart was dilated with an aneurysm-like bulging of the akinetic ischaemic left ventricle.

After pretreatment with 20 μg of pindolol coronary ligation was performed in 24 cases. In three experiments no arrhythmia occurred. With the exception of three animals which died, heart function was normal until the 7th or 8th minute when temporary, spontaneously disappearing ectopic center became manifest. No dilatation of the heart was observed either in the three animals which died or in those examined after 10 minutes and 24 hours, and the ischaemic left ventricle appeared to contract normally.

Twenty coronary ligations were performed after pretreatment with 300 μg of practolol per 100 g of body weight. In the majority of cases the ligation was soon followed by regular sinus rhythm. In seven cases the ECG indicated conduction disorders (in five cases second degree atrioventricular block and in two cases bundle branch block). In four cases irreversible ventricular flutter and/or fibrillation occurred. Three experiments ended also fatally in less than 10 minutes. At the end of the experiment in five cases marked bulging and akinesis of the left ventricle was found and in another three cases only the left ventricle displayed a poor motility.

In a previous study on the ligation of the left coronary artery by means of SELYE's method we have described the types and incidence of arrhythmias occurring in the acute phase (KENEDI and LOSONCI 1973). Pretreatment with small (1 μg per 100 g of body weight) and large (10 μg per 100 g of body weight) doses of isoproterenol enhanced the tendency to arrhythmia without raising lethality. In the present experiments, we have studied the effect of the betalytic agents propranolol, pindolol and practolol.

Method

White rats of both sexes and of 200 g average body weight were subjected under superficial ether anaesthesia to thoracotomy and left coronary ligation according to SELYE et al. (1960). Prior to the opening of the thorax in 20 experiments 200 μg per 100 g of body weight of propranolol (Inderal, ICI), in 24 experiments 20 μg of pindolol (Visken, EGYT—Sandoz) and in 20 experiments 300 μg per 100 g of body weight of practolol were injected into the femoral vein. The effect of the drugs was observed on the ECG taken two minutes after administration.

After thoracotomy the ECG was continuously recorded in the first lead for 10 minutes or till death of the animal. At the end of the experiment the heart was inspected and then subjected to histological examination. Results of earlier experiments and sham operations served as controls (KENEDI and LOSONCI 1973).

Results

In 20 experiments two minutes after propranolol administration, slight bradycardia appeared on the ECG with unchanged waves and a clocklike regularity.

In the first phase following coronary ligation irregular ventricular tachycardia and complete AV block appeared just as in the control experiments, with the sole difference that in the majority of cases the waves of the automatous rhythm were low.

After coronary ligation, closing of the thorax and draining of the pneumothorax, completely regular sinus rhythm appeared in 15 animals in less than 15 sec. In 11 experiments, a gradual lowering of the QRS occurred (Fig. 1). Extrasystole was observed in not more than two experiments. In one case ventricular premature beat was followed by ventricular tachycardia and later by ventricular flutter (Fig. 2); this animal survived for 24 hours. In five experiments, ventricular flutter appeared, in one animal it changed into fibrillation. In addition, in one animal ventricular fibrillation appeared 8 seconds after ligation. Out of the six grave arrhythmias three were lethal. In 12 experiments breathing became superficial then stopped. In the ECG the R–R interval gradually lengthened without a change in QRS size. In the deceased animals, a dilatation of the heart by almost 50% was found. The pale left ventricle was bulging and akinetic.

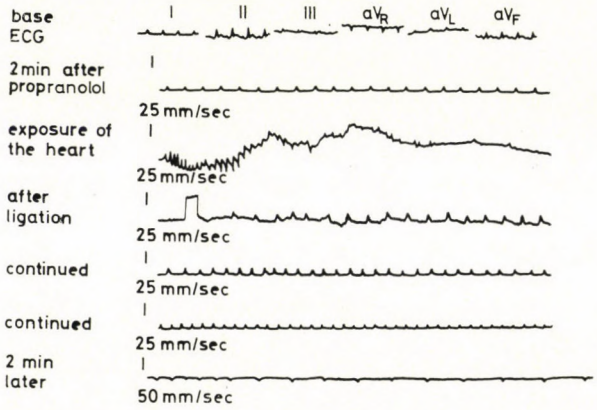

Fig. 1. Regular ECG after 200 μg per 100 g of body weight of propranolol. Irregular ventricular tachycardia after exposure of the heart. After coronary ligation the sinus rhythm is rapidly re-established. Gradual diminution of QRS then slowing of heart function

Three of the five surviving animals were sacrificed in the acute stage when the same bulging of the left heart was found as in the animals which died during the experiment. In two experiments the animals were left alive for 24 hours after coronary ligation and when one day later their thorax was opened the anterior surface of the left ventricle was pale in a circumscribed manner, did not bulge forward and the size of the heart was the same as in the controls.

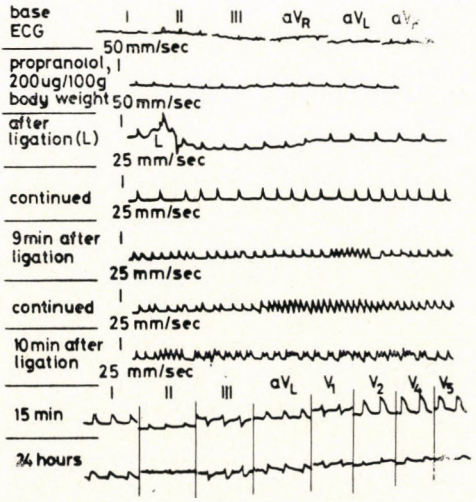

Fig. 2. After pretreatment with 200 μg of propranolol and coronary ligation sinus rhythm is rapidly re-established. 9 minutes later extrasystole, ventricular flutter, then (6th row) ventricular paroxysmal tachycardia and finally (10 minutes) single and serial extrasystole. On the ECG taken 15 minutes and 24 hours after coronary ligation, the ECG signs of an infarction of the anterior wall appear

In 24 experiments pindolol, a drug which has no depressing action on heart contractions was administered intravenously as pretreatment. Two minutes later the ECG became conspicuously rhythmic with slight bradycardia. No decrease in QRS was visible after coronary ligation, neither was the ventricular automatism characterized by small waves (Fig. 3). In most cases, one minute after ligation the sinus rhythm was reestablished, in 16 cases with high, in 8 cases with low QRS.

The continuously recorded ECG remained in three animals regular up to the end. In 18 cases it was regular only for 7 to 8 minutes when the following disturbances were observed: in 13 cases single or short series of extrasystole, in 11 cases paroxysmal ventricular tachycardia (Fig. 4) which changed in 7 cases into extrasystole; in 6 experiments ventricular flutter (changing into fibrillation in two cases); and in three cases, independent ventricular fibrillation. In this arrhythmic period a spontaneously disappearing 2 : 1 AV block

occurred in 4 cases. Of the 21 animals surviving the acute phase 10 minutes after ligation, five showed a Q wave in the first lead and eight a domelike ST elevation. In 24 experiments the animals were allowed to live for 24 hours. In each of these animals a deep Q with an elevated ST appeared in the first, aVL and V_{2-4} leads. The heart was not dilated in the three animals which

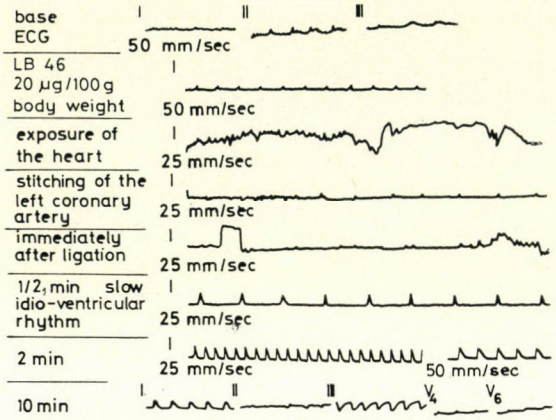

Fig. 3. After administration of 20 µg per 100 g of body weight of pindolol, ECG symptoms of the preparatory phase (rows 3 and 4) appear. Thirty seconds after ligation slow idio-ventricular rhythm with high R wave. From the second minute onward, regular sinus rhythm

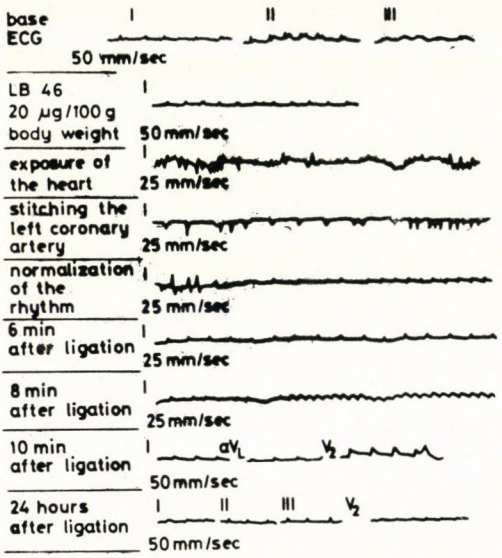

Fig. 4. After the administration of 20 µg of pindolol ECG symptoms related to the exposure of the heart and coronary ligation appear. After ligation, sinus rhythm is rapidly restored. Eight minutes later ventricular paroxysmal tachycardia sets in. On the ECG taken 10 minutes and 24 hours after ligation, signs of an anterior wall infarction

died during the experiment, nor in those surviving 10 minutes and 24 hours, nor was the left ventricle bulging.

In 20 experiments the intravenous administration of practolol was found to abolish spontaneous arrhythmia. Two minutes later the QRS was mostly conspicuously low (Fig. 5). In 12 cases sinus rhythm was restored soon after draining of the pneumothorax. In 13 experiments the QRS was remarkably high after ligation, while in five cases it remained low. Practolol had a marked blocking effect on conduction; in five experiments a second degree AV block with rhythmical P waves (Figs 6 and 7) and in two experiments a reversible bundle branch block (Fig. 5) was observed. Heterotopic stimulation was frequent. In eight experiments single extrasystole and in four, ventricular flutter, viz. ventricular fibrillation occurred. The latter was always of fatal outcome. Thirteen animals pretreated with practolol survived the ligation, but 10 minutes later they displayed signs of a fresh anterior infarction with

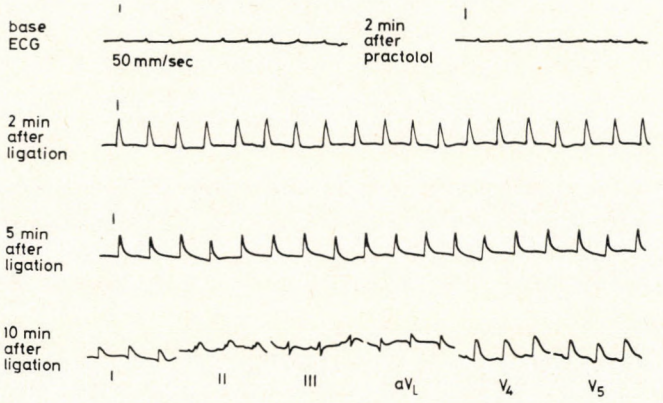

Fig. 5. Coronary ligation after pretreatment with 300 μg per 100 g of body weight of prac tolol. A temporary bundle branch block was produced (2nd row) followed by sinus brady cardia with regular QRS. At the end of the acute phase, signs of an anterior wall infarction appear (deep Q in aV_L, domelike ST elevation in leads I, II and V_{4-5})

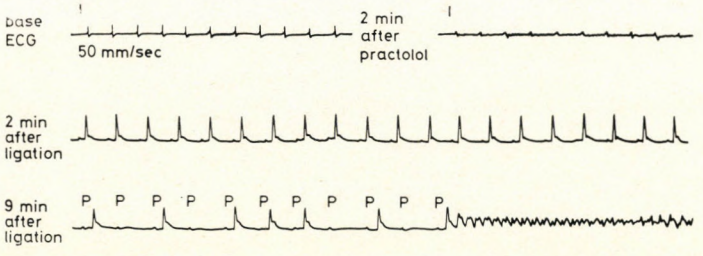

Fig. 6. After the administration of 300 μg per 100 g of body weight of practolol the QRS is conspicuously low. After coronary ligation the sinus rhythm was re-established with high R wave (2nd row) followed in the 9th minute by a 2 : 1 AV block. After extrasystole, lethal ventricular fibrillation developed. Paper speed, 50 mm/sec

deep Q in the aVL lead and dome-like ST elevation in the first, second and left thoracic leads (Fig. 5).

After the experiment with practolol pretreatment, the anterior wall of the left ventricle was consistently paler in a circumscribed area, in five cases it bulged forward and failed to contract. As a manifestation of a milder negative

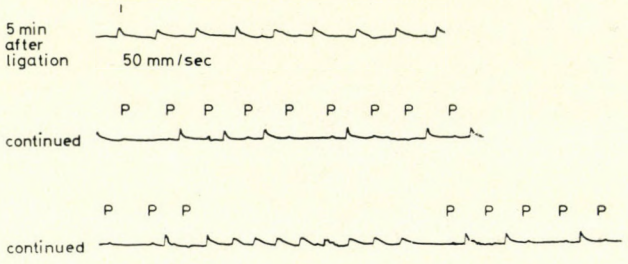

Fig. 7. The coronary was ligated after pretreatment with practolol. Regular sinus rhythm with elevated ST interval. Five minutes later some P waves were blocked towards the ventricle. Beside the conduction disturbance in the 3rd row, transitory ventricular tachycardia with eight waves

inotropic effect in another three cases the left ventricle was akinetic without bulging forward.

The influence of the three beta receptor blocking agents on the arrhythmias following coronary ligation and on survival of the acute phase is summarized in Table I.

It appears from Table I that the incidence of extrasystole following coronary ligation is significantly reduced by pretreatment with some beta blocking agent. According to the x^2 test this reduction is highly significant ($p < 0.01$) for propranolol and practolol, while in the case of pindolol the value of p was 0.03, thus this too was an assessible difference against the control.

As regards the death rate of ventricular arrhythmia, there was no difference between the control group and the group pretreated with pindolol, but

Table I

Effect of beta blocking agents after coronary ligation

Pretreatment	Number of cases	Extra-systole	Ventricular flutter and/or fibrillation	Ventricular arrhythmia, death	Death in acute phase
Control	20	17	8	2	3
Propranolol, 200 μg/100 g	20	2	6	3	15
Practolol, 300 μg/100 g	20	8	4	4	7
Pindolol, 20 μg/100 g	24	13	9	1	3

the difference was strongly significant ($p = 0.001$) in the case of propranolol or pindolol pretreatments.

As to total death rate, there was no difference between the control and the pindolol pretreated groups, while the increase in lethality of propranolol-pretreated animals was highly significant ($p = 0.001$).

It appears further from Table I that, though ventricular flutter and/or fibrillation had a lower incidence after practolol pretreatment than in any other group, this arrhythmia was always fatal.

Histological tests provided the explanation for the different effects of propranolol and pindolol pretreatment.

The gross and microscopic effects of propranolol pretreatment are shown in Fig. 8A and B. In the section perpendicular to the longitudinal axis at the centre of the ventricles, the contracted left ventricle is thickened, has a large volume and the ventricular septum bulges towards the splitlike right ventricle. The vessels are congested and around the coronary, some areas are definitely oedematous. Very marked, mainly venous dilatations are visible subepicardially with haemorrhages among the muscle fibres. Some muscle nuclei hardly take the stain. There is a diffuse slight leucocytic infiltration. The most pronounced signs of infarction appear along the right ventricular septum, in the subepicardial layer of the anterior wall and in a somewhat slighter form in the subepicardial layer of the wall of the left ventricle.

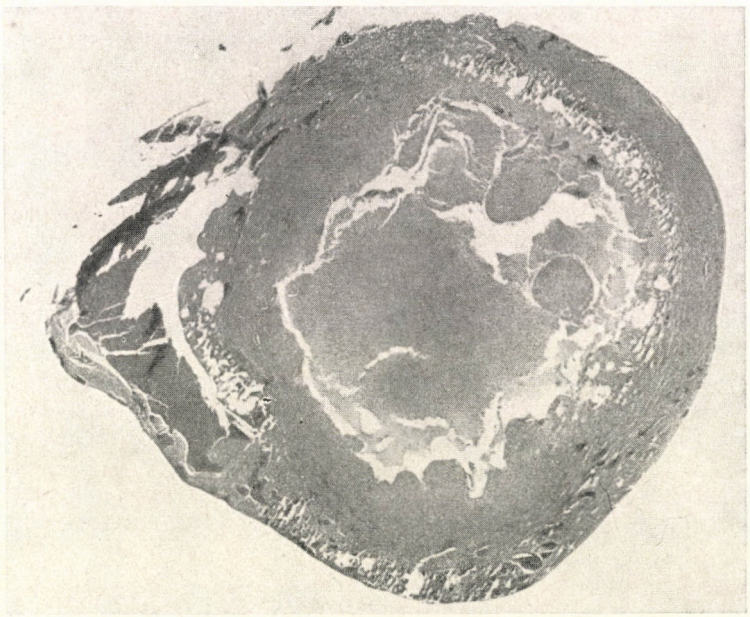

Fig. 8. A: After propranolol pretreatment the dilated left ventricle is seen on the cross-section of the heart

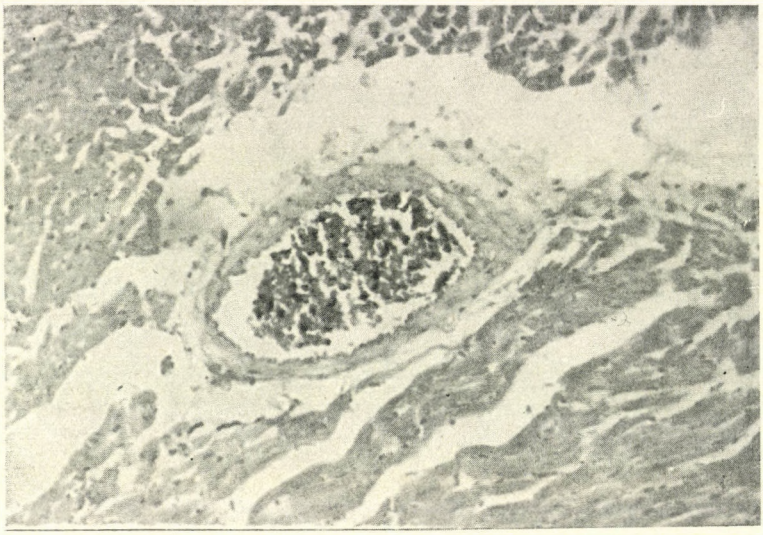

Fig. 8. B: Stasis in the arteries and veins, oedematous areas around the coronary

The picture of the heart after coronary ligation under the effect of pindolol is shown in Fig. 9A and B.

In the section perpendicular to the longitudinal axis at the boundary of the middle and lower thirds, both ventricles are dilated. In the histological picture the vessels are bloodless. The weak staining or its lack, especially in

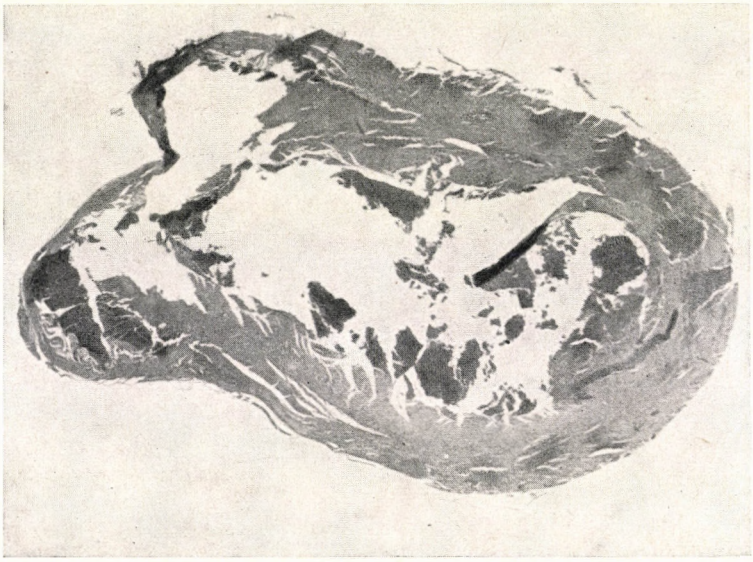

Fig. 9. A: Coronary ligation after pindolol pretreatment. No dilatation of left ventricle appears on the heart's cross-section

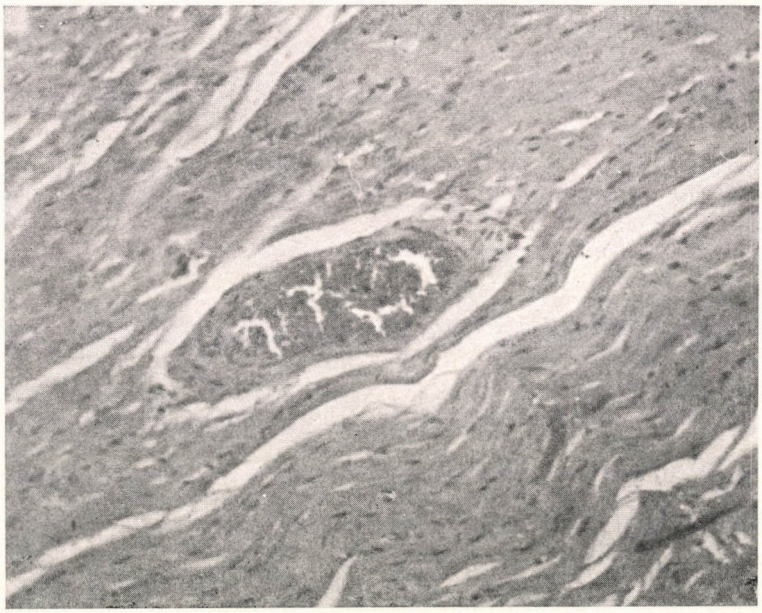

Fig. 9. B: Weak or missing nuclear staining indicates subepicardial infarction

the subepicardial layer, indicates an infarction. Small haemorrhages and leucocytic infiltration are visible.

Discussion

According to our experiments, arrhythmias following ligation of the left coronary and due to enhanced ectopic beats are reduced by beta receptor blocking drugs. Of the three investigated beta blocking agents, propranolol had the strongest antiarrhythmic effect. This drug is, however, unsuited for suppressing the ectopic beats after infarction, because it greatly reduces contractility and causes a paradoxical movement of the left ventricle whose tone has been weakened by ischaemia, so that finally its contracting ability is lost (akinesis) and the ventricular wall bulges passively forward. This process causes first a reduction in systolic output and later heart arrest. The hearts of the three rats which survived the acute stage also displayed an aneurysmlike bulging of the left ventricle. The hearts of the two rats which survived for 24 hours showed only ischaemia, the conspicuous symptoms of the negative inotropic effect of propranolol (bulge, akinesis) had disappeared.

An intracellular electrolyte change might contribute to the heart arrest. According to HIEJIMA et al. (1968), propranolol causes a decrease to almost

one third of the normal value of the myocardial potassium content while the other antiarrhythmic agents have a negligible effect on potassium diffusion. The marked bulging of the ventricular septum towards the right indicates dilatation of the heart. The disturbed electrolyte equilibrium manifesting with an increased sodium content of the cells is supported by the observed oedema.

After pindolol administration, no decrease in the QRS was observed. Sinus rhythm normalized within one minute somewhat slower than in the case of propranolol. The cause of the arrhythmia appearing in 18 experiments in the 7th to 8th minute and which, even in the case of ventricular fibrillation, was spontaneously abolished, is nuclear. In contrast to propranolol, the favourable survival rate found after the administration of pindolol might be explained with the drug's haemodynamic effect. It has been shown in dog experiments (LADDU and SOMANI 1972) that at constant coronary flow, O_2 tension in the coronary sinus rose, hence myocardial oxygen consumption decreased. The same was found in man (DOLDER et al. 1971). The other decisive difference is the complete lack of any depressive effect of pindolol on the myocardium (GIUDICELLI et al. 1969; FENYVESI 1972).

Practolol is a cardioselective beta-blocking agent which causes no bronchospasm and may therefore be applied in pulmonary diseases such as bronchial asthma or chronic bronchitis. In the ischaemic area produced by coronary ligation its negative inotropic effect is somewhat slighter than that of propranolol, but, as shown by our experiments, this unfavourable side-effect must be reckoned with. In human therapy practolol is widely used for correcting arrhythmias after cardiac infarction (RYMASEWSKI et al. 1972). Our experiments have confirmed the findings of FITZGERALD and WALE (1972) who demonstrated in the dog that practolol prolonged conduction time proportionally to its dose. Consequently, even the mildest conduction disorder contraindicates the administration of practolol which might cause a complete block.

On the basis of the favourable survival rate after coronary ligation, the rapid normalization of rhythm, and for the lack of a cardiodepressive effect, pindolol promises to become a valuable drug against the disturbances of rhythm arising in the acute stage of cardiac infarction. Clinicians too apply pindolol without fear to overcome arrhythmias, since due to its negligible inotropic effect it does not threaten with circulatory failure (STORSTEIN 1972).

Despite the antiarrhythmic effect, after pretreatment with betalytic agents survival of coronary ligation was higher only in the pindolol-pretreated group than in the controls. The explanation the higher lethality in the groups pretreated with practolol and propranolol might be found in the negative inotropic effect. Though propranolol was the most efficacious drug, death rate was nevertheless the highest in this group, due to the marked weakening of myocardial contraction by the drug.

Acknowledgement

We are indebted to Dr. LISZKAI for histological tests, Dr. REIMANN for mathematical analysis and Miss E. BÁRDOS for surgical assistance.

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TEMPERATURE REDUCING EFFECT OF OXOTREMORINE IN THE RAT AND THE MOUSE

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The effect of oxotremorine on core temperature of mice and on core and skin temperature and oxygen consumption of rats has been studied.

At room temperature, oxotremorine caused a marked decrease of the core temperature of mice, while it raised the temperature of the skin on the tail and reduced the core temperature of rats and brought about a two-phase increase in oxygen consumption. The hypothermia was presumably due to a higher heat loss.

At an ambient temperature of 16°C, the oxotremorine-induced rise of the skin temperature of rats was less marked and of shorter duration than at room temperature. At this temperature oxotremorine greatly reduced core temperature and temporarily reduced, then raised, oxygen consumption. Reduced heat production contributed to hypothermia.

At an ambient temperature of 30°C, oxotremorine failed to raise skin temperature, had no influence on core temperature, or, if so, raised core temperature and oxygen consumption.

Atropine sulphate blocked these effects of oxotremorine, while methylatropine had no influence on them. Oxotremorine hypothermia has its origin in the central nervous system.

Small doses of urethan blocked the tremor induced by oxotremorine and also both phases in the rise of metabolism in the rat.

During repeated oxotremorine treatment of rats, the core temperature reducing activity of the compound decreased, while pretreatment with tremorine blocked oxotremorine hypothermia in mice.

Oxotremorine caused simultaneously vasodilatation in the skin and a rise of blood pressure in anaesthetized rats. Tachyphylaxis to the former developed rapidly, to the latter, slowly.

The superficial vasodilatory effect of oxotremorine was superior to that of catecholamines, acetylcholine, carbachol, chlorpromazine, ethanol, vasopressin, and amylnitrite.

The temperature reducing effect of tremorine and of its active metabolite oxotremorine, was studied by a number of authors in mice (BLOCKUS and EVERETT 1957; KERANEN et al. 1961; EVERETT 1964; SPENCER 1965; LÉVY and MICHEL-BER 1967; HAMMER et al. 1968; FRIEDMAN and JAFFE 1969; ANKIER et al. 1971; STAIB and SCHRÖDER 1971; LESZKOVSZKY and TARDOS 1971; GERALD et al. 1972; MALATRAY and SIMON 1972) and also in rats (EVERETT 1964; FERRARI and GESSAI 964; MAICKEL et al. 1964; LOMAX and JENDEN 1966; COX and POTKONJAK 1967; KIRPATRICK et al. 1967).

As regards the mechanism of action, there is a certain contradiction in the literature. Several findings suggested a central point of attack, since the

temperature reduction caused by tremorine and oxotremorine is prevented by atropine and other cholinolytics reaching the central nervous system (BLOCKUS and EVERETT 1957; LOMAX and JENDEN 1966; HAMMER et al. 1968; HERMANSON 1968; LESLIE et al. 1972; GERALD et al. 1972), but is not influenced by parasympathetic blocking agents containing quaternary nitrogen (EVERETT 1964; FERRARI and GESSA 1964; SPENCER 1965; LÉVY and MICHEL-BER 1967; FRIEDMAN and JAFFE 1969; STAIB and SCHRÖDER 1971). According to other data, the peripheral effects of tremorine and oxotremorine, if only partly, but nevertheless contribute to the temperature reducing activity, since they are also blocked by quaternary parasympathetic blocking agents (MAICKEL et al. 1964; LESZKOVSZKY and TARDOS 1971; MALATRAY and SIMON 1972).

The influence on body temperature of oxotremorine and of other cholinergic stimulants administered to certain regions of the nervous system was the subject of several investigations. MEETER (1971) showed that intraventricularly administered carbachol reduces heat production in the rat. LOMAX and JENDEN (1966) claimed that when administered into the anterior hypothalamus of the rat both oxotremorine and carbachol increased heat loss and the same effect was observed by ANKIER et al. (1971) after intraventricular injection in mice. AVERY (1970, 1972) on the other hand, found that carbachol administered into the anterior hypothalamus of rats caused hyperthermia. According to MAICKEL et al. (1964), FRIEDMAN and JAFFE (1969), cholinomimetics acting on the posterior hypothalamus will reduce heat production. RUDY and WOLF (1972) studied the effect of carbachol in cats and found that when the compound was injected into the hypothalamus, it caused either a rise or a decrease in temperature, depending on the site of injection. Hence, the central influence of cholinomimetics on body temperature is a complex effect depending on the site of injections and the dose, as well as on ambient temperature. At low ambient temperatures the cholino- mimetics induce hypothermia (FERRARI and GESSA 1964; MAICKEL et al. 1964; HAMMER et al. 1968; AVERY 1972), while at temperatures above 32°C they have no influence or cause a rise in body temperature (COX and POTKONJAK 1967; BLOCKUS and EVERETT 1957; STAIB and SCHRÖDER 1971; HAMMER et al. 1968; AVERY 1972).

Earlier evidence of the temperature reducing effect of tremorine and oxotremorine was indirect and only rarely was their influence on skin temperature tested and their effect on oxygen consumption was never measured.

In our experiments we intended to investigate whether the decrease of temperature caused by oxotremorine was a result of reduced heat production or of enhanced heat loss and, provided this effect was connected with a higher heat loss, whether it was of central nervous or peripheral origin.

In order to find an answer to these questions we investigated at various ambient temperatures 1. whether oxotremorine caused vasodilatation in the

skin; 2. how oxotremorine affected core temperature and 3. the rat's oxygen consumption.

We choose the following three ambient temperatures: room temperature, i.e. 23°C; an indifferent temperature of 30°C (at which heat production is the lowest, see DONHOFFER 1961) and 16°C at which metabolism is markedly enhanced, and at which the depressing activity of a compound on the metabolic centres is definitely manifest.

In view of the contradictory data in the literature, we also investigated the eventual blocking by methylatropine of the oxotremorine-induced hypothermia in mice and rats.

Since acute tolerance, i.e. tachyphylaxis, to several of the central effects of oxotremorine (tremor, analgesia, midriasis, hypertension) might develop (GYÖRGY et al. 1970; 1971; PHAN et al. 1972; PHAN and GYÖRGY 1973), but there is no such tolerance with respect to the peripheral effects (miosis, hypotension), we investigated any eventual blocking of oxotremorine hypothermia by tremorine or oxotremorine pretreatment. So far our experiments seem to indicate that the development of tolerance is an indirect proof of the central origin of the oxotremorine effect.

Methods

In the experiments, Swiss mice of both sexes and of 17 to 21 g body weight and CFE rats, also of both sexes and of 200 to 300 g body weight, were used. Prior to the experiments the animals were kept at 22 to 23°C temperature and were fed a standard semi-synthetic diet.

Core temperature was measured by means of a thermistor led high up into the rectum and connected to an electric instrument. Temperature measurements were performed on randomized groups of rats and mice kept in individual cages. After isolation (in $8 \times 8 \times 8$ cm plastic cages) the core temperature of mice dropped by 1.5 to 1.7°C in 60 minutes and at a somewhat lower rate (0.3 to 0.6°C) in the next 2 to 3 hours. Core temperature of treated mice was always compared with the temperature of physiological saline-treated control animals at the same points of time after treatment, even if in the tables the deviation from the original values has been given ($\triangle$ values). Statistical analysis was performed with Student's *t*-test. Skin temperature on the tail was measured in the case of anaesthetized rats partly with a thermocouple, but always so if the animal was awake and the temperature values were recorded with a Fluvograph or a mirror-galvanometer. In a few individual cases skin temperature was measured by means of a thermistor placed on the tail of the anaesthetized rat and relative changes were recorded by a Hellige-type Multiscriptor apparatus.

Oxygen consumption of rats was measured in two different ways. According to the method of ISSEKUTZ and ISSEKUTZ (1942) the rats were placed in a metal tank immersed into a stable temperature water-bath of 2300 cu.cm connected to a water manometer. Thirty minutes were allowed for adaptation in the tank filled with pure oxygen after which basic oxygen consumption was measured for 30 minutes and the pressure drop read every 5 minutes on the manometer attached to the tank. In these experiments the value for oxygen consumption was calculated in cu.cm/hour:

$$V \times \frac{P}{760} \frac{273}{273+t} \frac{1}{T}$$

where V was the volume of the tank; P, the pressure drop in mm Hg in the system; t, the ambient temperature in °C; T, the duration of the test in hours. The value for O_2 consumption was converted into cu.cm/sq.dm/hour. (Some animals were given prior to the experiment

600 mg/kg of urethan, intraperitoneally, a dose which did not induce sleep.) In these experiments core temperature was not recorded, but the animal was taken out of the tank in the 40th and 90th minute of the experiment and its temperature was measured rectally.

In other experiments oxygen consumption was measured by the method of DONHOFFER et al. (1958) by means of the Noyons-Kipp diaferometer. The rats were starved for 10 to 12 hours immobilized in a wire netting cylinder and so placed into a chamber of 1250 cu.cm volume. Stable temperature in the chamber was ensured by a constant temperature water-bath. Core and skin temperatures were measured with a copper–constantan thermocouple and recorded by a Kipp-type Micrograph.

In some experiments, rats anaesthetized with 1 g/kg urethan intraperitoneally were used whose tail temperature was recorded and in addition their blood pressure was measured by a Statham P 23 Db type pressure transducer in the femoral artery and an electromanometer, and recorded with a Hellige-type Multiscriptor. In these experiments the compounds were injected into the femoral vein. In the case of 5 rats, 48 hours before the experiment a polyethylene cannula was inserted into the jugular vein under pentobarbital anaesthesia. The end of the cannula was led out dorsally under the skin of the neck and through this 0.1 ml of 5% heparin was injected daily. During the experiment the wake animals were given oxotremorine intravenously.

The following compounds were used in the experiments: acetylcholine chloride, isoproterenol (Izolevin-Cilag), methylatropine bromide, adrenaline base, atropine sulphate, dopamine HCl, noradrenaline hydrogen tartrate, oxotremorine oxalate. The doses given in the Tables refer to the salts of the compounds. If not stated otherwise, the compounds were administered intraperitoneally.

Results

1. Effect of oxotremorine on core temperature of mice and rats at room temperature

Oxotremorine in doses of 0.05 mg/kg caused no temperature decrease in mice, while 0.1 and 0.5 mg/kg decreased body temperature in proportion to the dose. In rats, 0.1 to 1.0 mg/kg of oxotremorine caused well measurable, but lesser hypothermia than in mice (see par. 3). In both species, the effect reached its maximum in the 30th minute after administration. In mice the effect of 0.2 mg/kg of oxotremorine lasted one to two hours, that of 0.5 mg/kg doses 7 to 8 hours, while in rats the hypothermia was of somewhat shorter duration.

2. Effect of ambient temperature on oxotremorine-induced hypothermia and on oxygen consumption in the rat

At room temperature (23°C) 0.5 mg/kg doses of oxotremorine caused a marked increase in skin temperature (from 26°C to 34—35°C; Fig. 1).

The maximum of the effect developed in the 10th to 12th minute of treatment and remained unchanged for another 20 minutes. Simultaneously with the rise in skin temperature, or a few minutes later, core temperature began to drop and rose only from the 35th minute of treatment onward. Oxygen consumption of the animals rose immediately after oxotremorine treatment, even before rectal temperature had begun to decrease, and this was followed 35 minutes later by a second rise. (Core temperature began to

increase at the same time as the secondary rise of oxygen consumption had set in.)

Figure 2 shows the mean values for experiments in the Issekutz tank. After administration of 1.0 mg/kg of oxotremorine, oxygen consumption rose from 81 cu.cm/sq.dm/hour to 125 cu.cm/sq.dm/hour.

At an ambient temperature of 16°C, skin temperature was 18 to 19°C, thus considerably lower than at room temperature. Doses of 0.5 mg/kg of oxotremorine caused a far less marked increase in skin temperature; this increase did not exceed 5 to 6°C (Fig. 3).

After the peak had been reached, temperature began to drop immediately and reached its initial level in the 30th minute. At this ambient temperature, core temperature decreased by almost 3°C, while after a significant initial decrease, oxygen consumption rose and later surpassed the initial value.

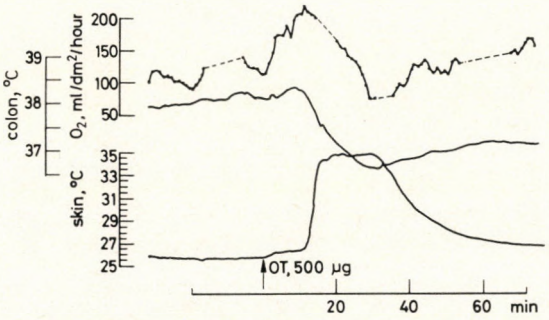

Fig. 1. Male rat, body weight 220 g. Effect of oxotremorine (OT) on oxygen consumption, core temperature and skin temperature of the tail at 23°C ambient temperature. Kipp diaferometer

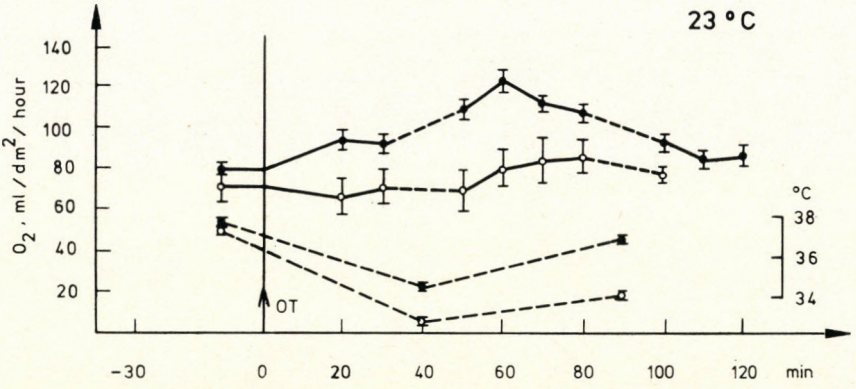

Fig. 2. Effect of oxotremorine (OT) on oxygen consumption at 23°C ambient temperature. Full line: oxygen consumption; dashed line: core temperature. Full circles: 1 mg/kg of oxotremorine; empty circles: 600 mg of urethan + 1 mg/kg of oxotremorine. Average of 10 experiments ± S. E. Issekutz tank

Tests carried out in the Issekutz-tank yielded similar results (Fig. 4). After 1 mg/kg of oxotremorine oxygen consumption dropped from 107 to 80 cu.cm/sq.dm/hour and later rose to 150 cu.cm/sq.dm/hour.

Under the effect of 600 mg/kg urethan, oxotremorine caused a more marked decrease in oxygen consumption which, however, did not surpass the initial value and there was a more marked decrease in core temperature, too (Fig. 5).

At the indifferent ambient temperature of 30°C, skin temperature was 30 to 37°C. This was not raised, but in some cases even slightly reduced by 0.5

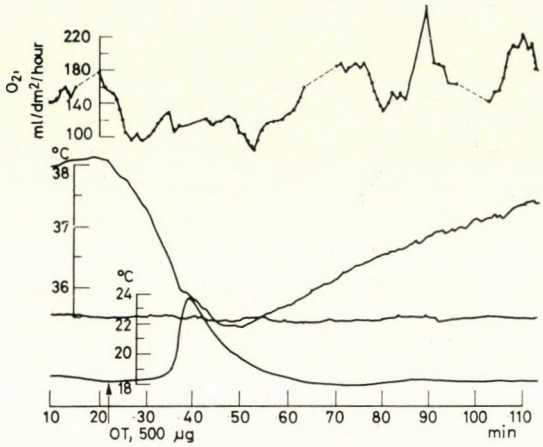

Fig. 3. Male rate, body weight 210 g. Effect of oxotremorine (OT) on oxygen consumption, core temperature and skin temperature at 16°C ambient temperature. 3rd line: chamber temperature. Kipp diaferometer

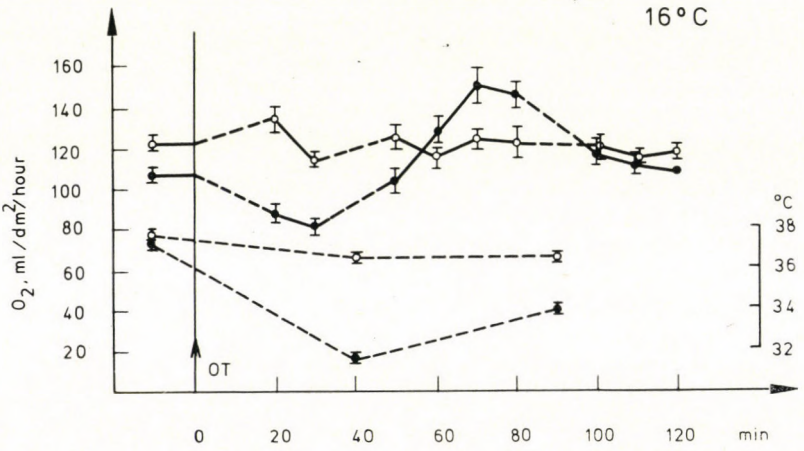

Fig. 4. Effect of oxotremorine (OT) on oxygen consumption at 16°C ambient temperature. Full line: oxygen consumption; dashed line: core temperature. Full circles: 1 mg/kg of oxotremorine (mean of 7 experiments ± S.E.); empty circles: control animals treated with NaCl (mean of 7 experiments ± S.E.). Issekutz tank

mg/kg of oxotremorine (Fig. 6), while at the same time core temperature remained unchanged or rose. Following the administration of oxotremorine, oxygen consumption immediately rose considerably and in the 20th minute approached the initial value, but returned to it only in the 60th minute.

Figure 7 shows the results of similar experiments performed in the Issekutz tank. Under the effect of 600 mg/kg urethan, oxotremorine had no influence either on oxygen consumption or on the core temperature.

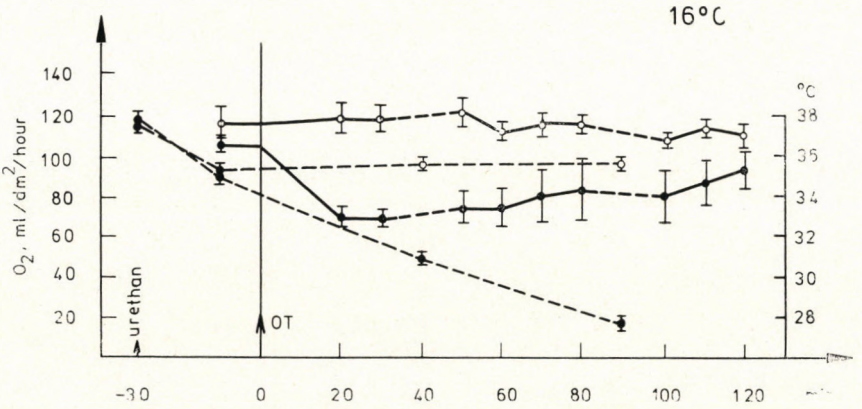

Fig. 5. Effect of oxotremorine (OT) on oxygen consumption of rats treated with 600 mg/kg of urethan at 16°C ambient temperature. Full line: oxygen consumption; dashed line: core temperature. Full circles: urethan + 1 mg/kg oxotremorine (mean of 7 experiments ± S.E.); empty circles: urethan + NaCl treatment (mean of 4 experiments ± S.E.). Issekutz tank

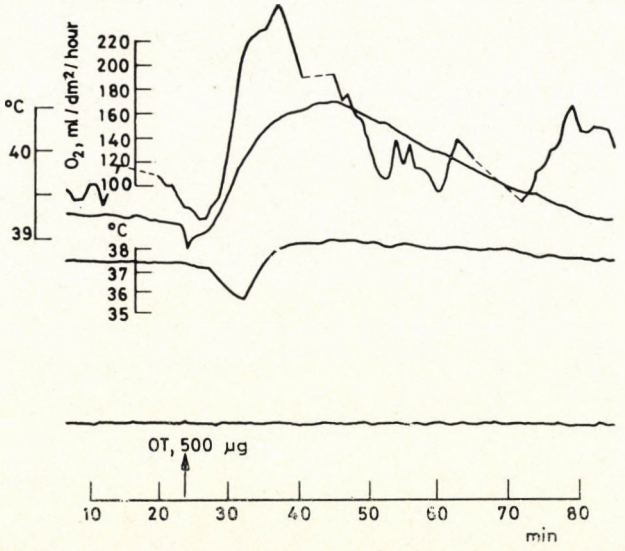

Fig. 6. Male rat of 200 g body weight. Effect of oxotremorine (OT) on oxygen consumption, core and skin temperature at 30°C ambient temperature. 4th line: chamber temperature. Kipp diaferometer

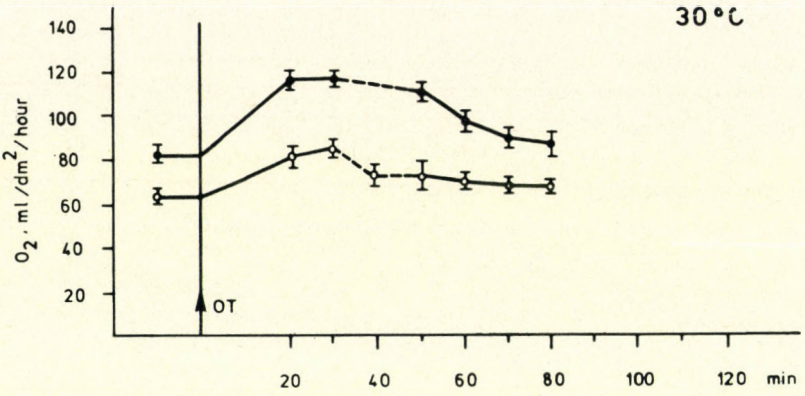

Fig. 7. Effect of oxotremorine (OT) on oxygen consumption at 30°C ambient temperature. Full circles: 1mg/kg of oxotremorine (mean of 9 experiments ± S.E.); empty circles: urethan + 1 mg/kg oxotremorine (mean of 8 experiments ± S.E.). Issekutz tank

3. *Influence of atropine and methylatropine on the oxotremorine effect*

Atropine sulphate in a 5 mg/kg intraperitoneal dose blocked the oxotremorine-induced rise in skin temperature, as well as the drop in core temperature and rise of metabolism at an ambient temperature of 23°C (Fig. 8), just as it blocked the oxotremorine effect at ambient temperatures of 16° and 30°C.

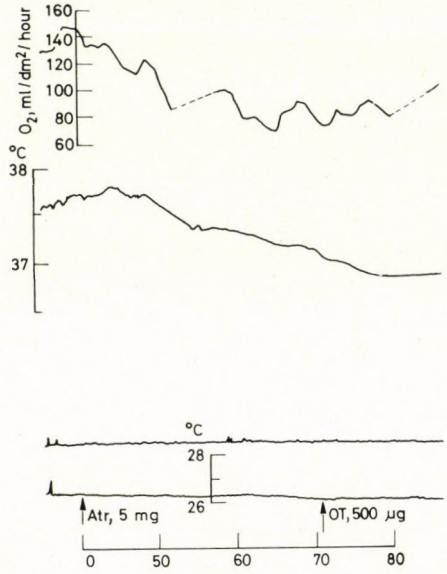

Fig. 8. Female rat of 200 g body weight. Influence of oxotremorine (OT) on oxygen consumption, core and skin temperature of atropine-pretreated (Atr) rat. 3rd line: chamber temperature. Kipp diaferometer

Methylatropine in a dose of 5 mg/kg intraperitoneally had no influence on the vasodilatory effect of oxotremorine in the skin (Fig. 9), neither did it block the decrease of core temperature of rats (Table I) and mice (Table II) induced by oxotremorine.

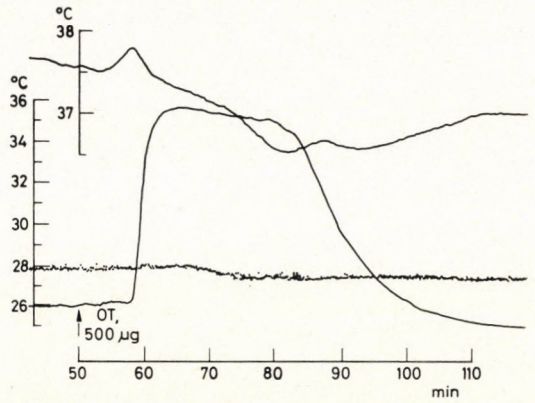

Fig. 9. Male rat of 210 g body weight. Effect of oxotremorine on core and tail temperature. 3rd line: chamber temperature. 30 minutes after administration of 5 mg/kg atropine

Table I

Influence of methylatropine on the temperature reducing effect of oxotremorine in the rat
Rectal temperature, °C ± S.E. Eight animals in each group

Core temperature, 20 min prior to treatment	Treatment, subcutaneously 0 min	Core temperature 20th min	Treatment, intra-peritoneallly 30th min, OT, mg/kg	Core temperature 60th min	Temperature decrease between 20th and 60th min
37.6 ± 0.17	NaCl	37.4 ± 0.17	0.1	36.7 ± 0.35	0.9
37.0 ± 0.14	5 mg/kg MA	36.4 ± 0.17	0.1	35.7 ± 0.35	1.3
37.0 ± 0.17	NaCl	36.9 ± 0.17	0.2	35.5 ± 0.41	1.5
37.1 ± 0.10	5 mg/kg MA	36.8 ± 0.10	0.2	35.8 ± 0.17	1.3
37.0 ± 0.2	NaCl	36.9 ± 0.20	0.5	35.3 ± 0.36	1.7
37.4 ± 0.14	5 mg/kg MA	37.0 ± 0.14	0.5	35.3 ± 0.21	2.1

MA: methylatropine
OT: oxotremorine

4. The role of tremor in certain oxotremorine effects

Urethan in a dose of 600 mg/kg was found to inhibit the tremor induced by 1 mg/kg of oxotremorine in the rat. This dose of urethan inhibited also the increase in metabolism caused by oxotremorine which reached its maximum in the 80th minute, but enhanced oxotremorine hypothermia (Fig. 5, 16°C) and blocked the initial rise in metabolism at 30°C (Fig. 7).

Table II

Influence of methylatropine on the temperature reducing effect of oxotremorine in mice. Rectal temperature, °C ± S.E. Sixteen animals in each group

Core temperature, —60th minute, °C	1st treatment, subcutaneous —30th minute	2nd treatment, intraperitoneal zero minute	Core temperature 30th min, °C
37.4 ± 0.63	NaCl	NaCl	36.6 ± 0.24
37.6 ± 0.15	NaCl	0.2 mg/kg OT	33.2 ± 0.15*
37.2 ± 0.25	5 mg/kg MA	NaCl	36.4 ± 0.31
37.2 ± 0.18	5 mg/kg MA	0.2 mg/kg OT	33.7 ± 0.18*

* The difference between the values marked with an asterisk failed to reach significance

MA: methylatropine
OT: oxotremorine

5. *Acute tolerance to oxotremorine hypothermia*

Rats were given 1 mg/kg of oxotremorine three times daily for three days. This resulted in a tolerance to the temperature reducing effect. After discontinuation of treatment the compound gradually regained its activity (Fig. 10).

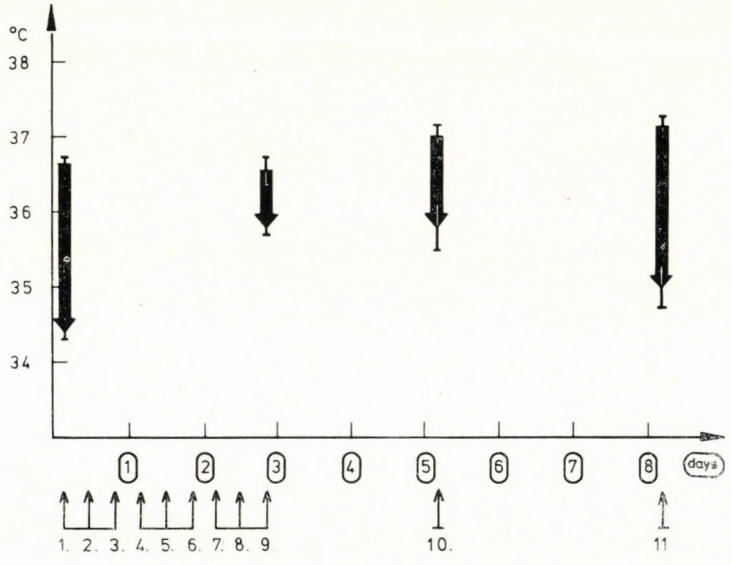

Fig. 10. Temperature reducing effect of oxotremorine during continuous administration to rat. Arrows: 1 mg/kg of oxotremorine. The upper parts of the downward pointing thick arrows represent the initial temperature, their tips the minimum core temperature after oxotremorine treatment (mean values for 10 rats ± S.E.)

When 1 mg/kg doses of oxotremorine were administered three times at 60 to 90 minutes intervals, the skin temperature raising effect disappeared after the third or fourth dose and the same dose failed also to reduce core temperature (Fig. 11).

Earlier experiments have shown that tremorine pretreatment inhibited several of the central nervous effects of oxotremorine (GYÖRGY et al. 1970, 1971; PHAN et al. 1972). Twenty-four hours after a pretreatment with 20 mg/kg of tremorine the temperature decreasing activity of 0.1 mg/kg of oxotremorine was practically abolished and even 0.2 mg/kg had a considerably weaker effect than in the control mice (Table III).

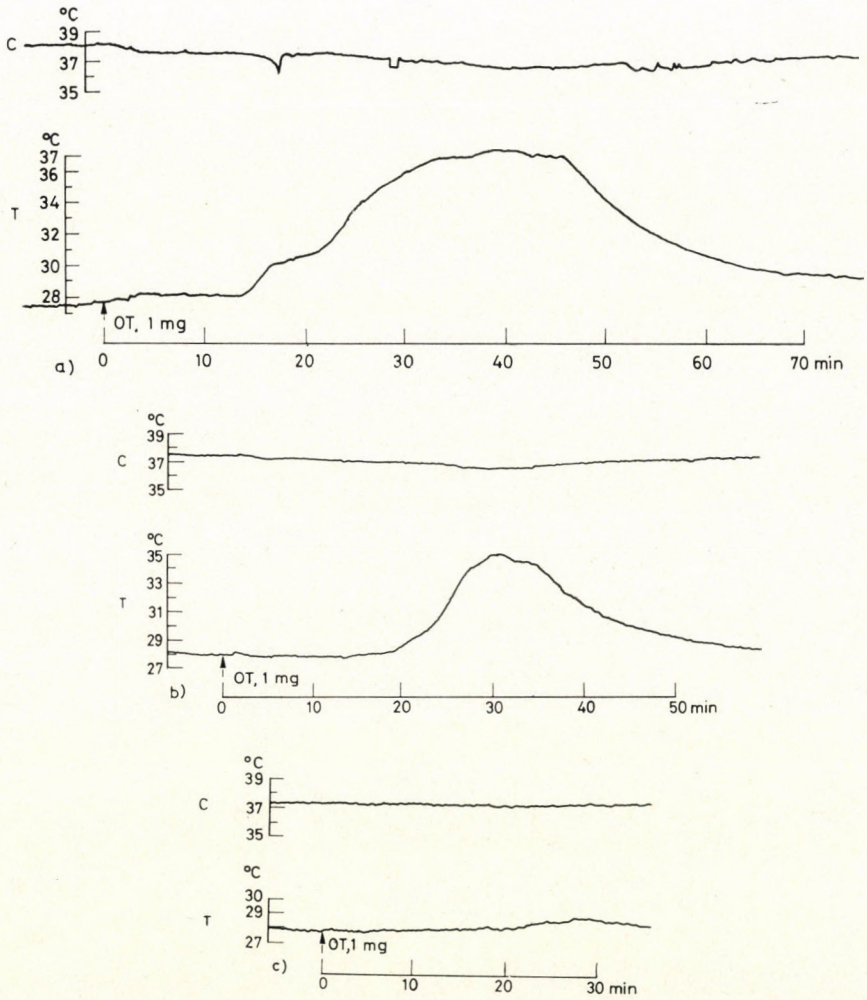

Fig. 11. Female rat of 240 g body weight. Influence of repeated doses of oxotremorine (a, b, c) on core (c) and tail (T) temperature. Fluvograph

Table III

Influence of tremorine on the temperature reducing effect of oxotremorine in mice. Rectal temperature, °C ± S.E. Ten animals in each group

Pretreatment, intraperitoneal —24 hours	Core temperature —30th min, °C	Treatment, intraperitoneal zero min	Core temperature		Temperature drop between the —30th and 30th min, °C
			30th min, °C	60th min, °C	
NaCl	37.4 ± 0.17	0.1 mg/kg OT	34.2 ± 0.36	34.7 ± 0.6	3.2
20 mg/kg TRM	37.2 ± 0.23	0.1 ,, ,,	36.4 ± 0.36	36.9 ± 0.25	0.8
NaCl	37.6 ± 0.23	0.2 ,, ,,	32.0 ± 0.28	31.8 ± 0.52	5.6
20 mg/kg TRM	37.0 ± 0.32	0.2 ,, ,,	35.5 ± 0.37	36.6 ± 0.32	1.5

TRM: tremorine
OT: oxotremorine

6. *Influence of vasoactive compounds on skin temperature and blood pressure of anaesthetized rats*

After an initial hypotension 10 to 50 μg/kg of intravenously administered oxotremorine caused lasting hypertension. The value and duration of the response was proportional to the dose. Doses of 10 to 20 μg/kg had, as a rule, no influence on skin temperature, while 30 to 50 μg/kg caused significant, practically maximum temperature rises: the dose–effect curve was of the "all or nothing" type (Fig. 12).

The effect of oxotremorine on skin temperature was found far superior to intravenously administered 5 to 10 μg/kg of adrenaline, 5 to 10 μg/kg of

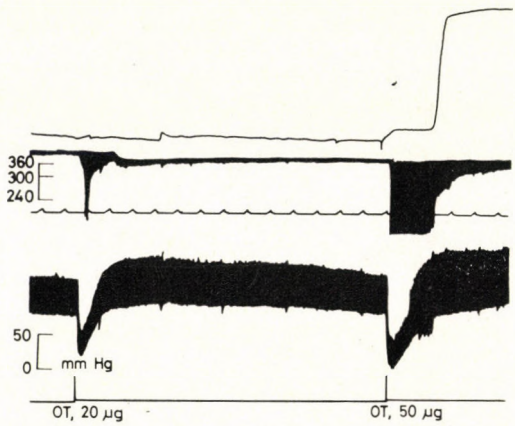

Fig. 12. Male rat of 300 g body weight. 1.5 mg/kg urethan administered intraperitoneally. Influence of intravenous oxotremorine on skin temperature (relative values), pulse rate and femoral blood pressure. Time in minutes. Hellige Multiscriptor

noradrenaline, 10 to 20 μg/kg of dopamine, 2 μg/kg of isoproterenol, 5 μg/kg of acetylcholine, 2 to 5 μg/kg of carbachol, 2 mg/kg chlorpromazine, 10 mg/kg of ethanol or 1 IU/kg of vasopressin, as well as inhaled amylnitrite.

Discussion

The results have unequivocally proved the heat loss enhancing effect of oxotremorine. Rats do not sweat (ADOLPH 1947), hence oxotremorine cannot increase heat loss in this way. Increased salive secretion has neither an important role, since this is completely blocked by methylatropine without any influence on oxotremorine hypothermia. Heat loss could be enhanced by tachypnoea but this was not observed after oxotremorine administration in any of the animals.

During the experiments, temperature of the tail skin was recorded continuously, this being the site where rats lose 20% of their overall heat production (RAND et al. 1965). At room temperature, oxygen consumption did not decrease after oxotremorine administration and vasodilatation in the skin was so marked (manifesting with a temperature rise of 8 to 9°C) that this explained the considerable decrease of core temperature. At an ambient temperature of 16°C, skin temperature was only 18 to 19°C. It was assumed that under such conditions vasoconstriction was so marked that it could not or could only with difficulty be overcome by oxotremorine, since the drug causes only a slight and temporary vasodilatation. In cold ambient temperature this might contribute to the decrease in core temperature, but at 16°C oxotremorine already interferes with oxygen consumption (see below), too. Our data, according to which the temperature decreasing activity of oxotremorine is higher in a cold environment than at room temperature, agree with the findings of FERRARI and GESSA (1964), MAICKEL et al. (1964), and HAMMER et al. (1968). At an ambient temperature of 30°C, tail skin temperature is so high, the vessels so dilated that oxotremorine is unable to cause a further dilatation, hence it will not reduce core temperature either.

At 3°C, oxotremorine causes hypothermia not by reducing heat production since there is no decrease in oxygen consumption. The depressing activity of oxotremorine on the centres of metabolism is slight and noticeable only at an ambient temperature of 16°C at which initial oxygen consumption is high. This fact is in agreement with the law of initial value; neostigmine or chloral hydrate are reducing oxygen consumption the more the higher the initial value (WILDER 1967).

Hence, oxotremorine hypothermia is the result not of reduced heat production, but of enhanced heat loss. The effect of the compound resembles that of chlorpromazine which neither reduces chemical heat regulation (SZEG-

VÁRI et al. 1958), and at neutral temperatures has no influence on oxygen consumption (GIAJA and MARKOVIC-GIAJA 1954), but differs from that of procaine which at an ambient temperature of 30°C still reduces core temperature (ISSEKUTZ and KŐVÁRI 1942—1943). The effect of oxotremorine differs from that of carbachol injected into the hypothalamus (MEETER 1971), or of intravenously administered physostigmine (VARAGIC et al. 1971) which reduce oxygen consumption even at an ambient temperature of 20°C.

Vasodilatation in the tail contributes beyond doubt to the heat loss enhancing effect of oxotremorine. Our attention was drawn to this phenomenon also by the fact that a similar effect was missing in quite a number of vasoactive drugs (catecholamines, vasopressin, chlorpromazine, ethanol, amylnitrite, acetylcholine, carbachol). The vasodilatation is certainly of central origin since it is inhibited by atropine, and the parasympathetic stimulation by oxotremorine does not play a role in it, since it is not blocked by methylatropine. Oxotremorine hypertension (vasoconstriction in the splanchnic area), as well as central sympathetic inhibition have a role in its development (ANKIER et al. 1971). Peripheral vasodilatation is regulated by the central nervous system (THOMPSON and STEVENSON 1965), lowering of the vasoconstrictor tone causing a vasodilatation in the tail and legs (FOLKOW 1955; GRANT 1963). It is not completely understood why tachyphylaxis to the pressor activity of oxotremorine develops slowly, while to vasodilatation in the tail it develops rapidly; in these cases oxotremorine doses of pressor effect do not bring about vasodilatation in the tail of the anaesthetized rat. Hence, the hypertension cannot alone be responsible for vasodilatation.

In the course of the experiments it has been suggested that the cause of tachyphylaxis might be the gradually developing vasoconstriction in the skin, but this hypothesis has not been confirmed. There is no doubt that both the decrease of core temperature and vasodilatation in the tail caused by oxotremorine are resistent to methylatropine, which supports the earlier mentioned fact, namely that oxotremorine hypothermia is the result of an enhanced heat loss.

Why does oxotremorine raise oxygen consumption? An increase in oxygen consumption sets in rapidly at 20° or 30°C ambient temperature (at 23°C even before core temperature begins to decrease) and is inhibited by small doses of urethan. Since urethan does not influence the central amphetamine effect manifesting itself with an enhancement of metabolism (ISSEKUTZ and GYERMEK 1949), but blocks the tremor provoked by oxotremorine, a certain role must be attributed to tremor in the initial increase in oxygen consumption. Tremor developed in rats five minutes after the administration of oxotremorine, lasted for about 20 minutes and disappeared in 30 minutes (COX and POTKONJAK 1969). The possibility of oxotremorine possessing perhaps a certain central stimulating, oxygen consumption enhancing

activity, must not completely be rejected. [Carbachol has such an activity (RUDY and WOLF 1972)].

The time of the first phase in the increase of metabolism caused by oxotremorine sets in coincides with the appearance of the tremorogenic and other effects of the compounds. Oxotremorine, however, causes a secondary rise in oxygen consumption which is noticeable in the 60th to 70th minute of the experiment. This effect appears only at a temperatures of 16° and 23°C when oxotremorine reduces core temperature too and is, in our opinion, of a compensatory nature. This is supported by the observation that in the 60th to 70th minute of treatment, with the exception of hypothermia, the other effects of oxotremorine are already over. Urethan might block the compensatory mechanisms so that after its administration this secondary effect is missing.

Oxotremorine hypothermia is undoubtedly of central origin, since it is blocked by atropine and uninfluenced by methylatropine pretreatment. The central origin is indirectly supported by the development of tolerance after repeated doses, while as a rule no tolerance develops to the peripheral effects of the compound.

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Recensiones

W. HANKE

Vergleichende Wirkstoffphysiologie der Tiere

Reihe: Bausteine der modernen Physiologie. VEB Gustav Fischer Verlag, Jena 1973. 179 pages, with 50 figures and 13 tables. Price: M 19.50

This monograph on the comparative physiology of active substances of animals has been published in the series Building Stones of Modern Physiology. Substances which are regulating and correlating reactions of the organism are now constituting an important part of comparative physiology, studying the response of different species, or of different individuals in a colony.

During the last decade, progress has been significant in this field and beside zoophysiology other disciplines like biochemistry, pharmacology and medicine have been involved in it. The book deals mainly with hormones, less with vitamins, and only superficially with the further active substances and describes the connections between hormones, vitamins, nervous system, interior and exterior milieu.

The book will be interesting for biochemists, physiologists and the readers of every field of medicine.

K. LISSÁK

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Red Cell Shape Physiology, Pathology, Ultrastructure

Springer-Verlag, Berlin, Heidelberg, New York 1973. VIII + 180 pages, with 147 figures. Price: DM 32.40

This volume contains the proceedings of a symposium held June 20 and 21, 1972, at the Institute of Cell Pathology, Hopital de Bicêtre, as part of the scientific exchange program between N.I.H., USA, and I.N.S.E.R.M., France, and published as a special issue of Nouvelle Française d'Hématologie, Vol. 12, No. 6, December, 1972.

The book contains 14 full-length contributions by 20 authors, and 4 short papers; each concludes with a discussion. The contents are divided into four sections. Section I deals with red cell shape, including a rational descriptive nomenclature. Section II discusses the biochemical factors connected with the different transformations of cells, and the plasma factors, the biochemical dynamics of erythrocyte lipids and cellular ATP, calcium aging, and the chemical agents as determinants of the shape of the red cell. Section III discusses biophysical measurements and studies of deformability of cells of different shapes. Section IV deals with ultrastructural studies of normal, sickled, and Heinz body-containing red cells, and discusses the cells injured by antibody and complement. The different shaped red cells termed poikilocytes "become the first living cell permitting us to assess its internal molecular structure on the basis of its external configuration" as M. BESSIS explains in the introductory chapter.

The book gives important information for haematologists and useful reading for every student of medical sciences from physiology to pathology.

K. LISSÁK

J. E. JORPES, V. P. MUTT (Eds)

Secretin, Cholecystokinin, Pancreozymin and Gastrin

Handbook of Experimental Pharmacology. Heffter-Heubner, New Series XXXIV. Springer-Verlag, Berlin, Heidelberg, New York 1973. XII + 376 pages, with 135 figures. Price: DM 165.–

At the beginning of the 20th century, the discoveries of PAVLOV, BAYLISS and STARLING and others resulted in revolutionary changes in the knowledge of digestion. A new concept, the hormonal regulation of the work of the digestive organs, came into prominence. This volume of the classical Handbook of Experimental Pharmacology offers a complete picture from the historical beginnings up to the present points of view, presenting a complete reference work for everybody interested in the hormonal regulation of the work of the digestive organs.

Chapter I discusses Secretin and Cholecystokinin by J. E. JORPES and V. MUTT. Chapter II presents The Synthesis of (Porcine) Secretin by M. BODANSZKY. Chapter III discusses The Secretory Process in the Pancreatic Exocrine Cell: Morphologic and Biochemical Aspects by J. D. JAMIESON. Chapter IV reviews The Secretin Test by M. J. ZIMMERMAN, D. A. DREILING and H. D. JANOWITZ. Chapter V discusses The Use of Cholecystokinin in the Roentgenological Examination of the Extrahepatic Biliary Tract and Intestines by A. TORSALL, M. L. ROMORINO and R. CARRATU. Chapter VI presents the Gastrointestinal Hormones and Islet Function by E. F. PFEIFFER, S. RAPTIS and R. FUSSGÄNGER. Chapter VII discusses The Use of Cholecystokinin in the Roentgenological Examinations and the Clinical Aspects by J. PLESSIER. Each chapter ends with a comprehensive list of references. The volume ends with a subject and author index.

The volume presents fundamental knowledge in physiology, pharmacology and clinical medicine. It is a reference work which must be possessed by every medical library.

K. LISSÁK

H. E. VOSS, G. OERTEL (Eds)

Androgene I

Handbook of Experimental Pharmacology. Heffter-Heubner, New Series XXXV/1. Springer-Verlag, Berlin, Heidelberg, New York 1973. XII + 686 pages, with 117 figures. Price: DM 298.—

This new volume of the classical series of Handbook of Experimental Pharmacology, after a general introduction deals with the history of androgen research, and the physiological and experimental pharmacological regulation of androgen formation in the body, the chemistry of androgens, the correlations with the other endocrine glands in which the androgens play a decisive role.

The eight main chapters offer a through survey of the general significance of androgen hormones. Chapter I: The Regulation of the Functions of Testis by H. E. Voss. Chapter II: The Disconnection of Testis Function by H. E. Voss. Chapter III: Chemistry of Androgen by G. OERTEL. Chapter IV: The Occurrence of Androgen Potency by H. E. Voss. Chapter V: The Connection of Androgens of Testis to Other Endocrine Glands by H. E. Voss. Chapter VI: The Biology of Androgen by H. E. Voss. Chapter VII: The Influence of Androgen on Other Organs, except the Genital Organs and Endocrine Glands by H. E. Voss. Chapter VIII: Different Effects of the Androgens by H. E. Voss. Each chapter ends with a list of pertaining references and the volume with a subject and author index.

The volume merits much interest; in addition to endocrinologists, it will offer important references for physiologists, pharmacologists and for those studying clinical medicine.

K. LISSÁK

H. C. HODGE, J. N. SANNARD, J. B. HURSH (Eds)

Uranium, Plutonium, Transplutonic Elements

Handbook of Experimental Pharmacology. Heffter-Heubner, New Series XXXVI. Springer-Verlag, Berlin, Heidelberg, New York 1973. 995 pages, with 243 figures and 22 Tables. Price: DM 299.—

This new volume of the classical Handbook of Experimental Pharmacology is decicated to the memory of Wright Haskell LANGHAM, who exerted a most significant influence on the field encompassed by this book. In the Preface, J. B. HURSH emphasizes that the approach throughout this handbook is focussed on the control of these hazardous elements in industrial situations and in our environment.

Chapter 1 discusses the History of Uranium Poisoning (1824—1942). Chapter 2 presents the Physical and Chemical Properties of Uranium by J. E. GINDLER; Chapter 3 the Animal Experiments by C. L. YILE; Chapter 4 the Data on Man by J. B. HURSH and N. L. SPOOR; Chapter 5 the Protection Criteria by N. L. SPOOR and J. B. HURSH; Chapter 6 Environmental Monitoring and Personal Protection in Uranium Processing by L. M. SCOTT; while Chapter 7, Uranium Mining Hazards by D. A. HALADAY.

The second part of the book presents the problems of plutonium. Chapter 8 discusses the Biomedical Aspects of Plutonium (Discovery, Development, Projections) by J. N. STONNARD; Chapter 9, the Chemical and Physical Properties of Plutonium by D. M. TAYLOR; Chapter 10, the Distribution, Excretion and Effects of Plutonium as a Bone-Seeker by J. VAUGHON in collaboration with B. BLEENEY and D. M. TAYLOR; Chapter 11, Plutonium in Soft Tissues with Emphasis on the Respiratory Tract by W. J. BAIR, J. E. BALLOU, J. F. PARK and S. L. SANDERS. Chapter 12 reviews the Maximum Permissible Body Burdens and Concentrations of Plutonium: Biological Basis and History of Development by W. H. LANGHAM and J. W. HEALY; Chapter 13 presents the Bioassay of Plutonium by A. G. De LOW-BEER. Chapter 14 discusses the Problem of Plutonium: Industrial Hygiene, Health Physics and Related Aspects by H. M. PARKER. Chapter 15 discusses Plutonium in the Environment by J. N. STANNARD ·

The third part of the book reviews The Transplutonic Elements by A. GHIORSO. Chapter 16 discusses the History of the Transplutonic Elements and Chapter 17, the Chemical and Physical Properties of the Transplutonium Elements by D. M. TAYLOR. Chapter 18 reviews the Metabolism and Biological Effects of the Transplutonium Elements by P. W. DURBIN; Chapter 19, the question of Maximum Permissible Concentrations and Maximum Permissible Body Burdens for Transplutonic Elements by G. W. DOLPHIN. Chapter 20 presents the Bioassay of Transplutonic Elements by A. G. De LOW-BEER; Chapter 21, the Medical Uses: Americium-241, Californium-252 by G. T. SEABORG. Each chapter ends with a very comprehensive list of references and the volume is complete with an author and subject index.

This volume of the Handbook of Experimental Pharmacology is of an exceptional use for those interested in the defence of the environment. It is indispensable not only for pharmacologists but for all those studying medical sciences, hygiene and modern industry.

K. LISSÁK

A. IGGO (Ed.)

Somatosensory System

Handbook of Sensory Physiology. Volume II. Springer-Verlag, Berlin, Heidelberg, New York 1973. XI + 851 pages, with 240 figures. Price: DM 262.—

The volume is introduced by Y. ZOTTERMAN who cites very interesting and colourful historical data and fundamental contemporary facts concerning the development of sensory physiology. The volume has been written by twenty-five prominent research workers from all over the world who give a systematic account of the neuroanatomical and neurophysiological aspects of the somatosensory functions.

The nineteen chapters are as follows. Chapter 1: Morphology of Cutaneous Receptors by K. H. ANDRES and M. v. DÜRING. Chapter 2: Cutaneous Mechanoreceptors and Nociceptors by P. R. BURGESS and E. R. PERL. Chapter 3: Cutaneous Thermoreceptors by H. HENSEL

Chapter 4: Joint Receptors and Kinaesthesis by S. SKOGLUND. Chapter 5: The Concept of Relay Nuclei by G. GORDON. Chapter 6: Control of the Access of Afferent Activity to Somatosensory Pathways by R. F. SCHMIDT. Chapter 7: Distribution and Connections of Afferent Fibres in the Spinal Cord by M. RÉTHELYI and J. SZENTÁGOTHAI. Chapter 8: Dorsal Horn Electrophysiology by P. D. WALL. Chapter 9: The Trigeminal System by I. DARIAN-SMITH. Chapter 10: Ascending and Long Spinal Pathways: Dorsal Columns, Spinocervical Tract and Spinothalamic Tract by A. G. BROWN. Chapter 11: Functional Organization of Spinocerebellar Paths by O. OSCARSSON. Chapter 12: Reticular Formation by O. POMPEIANO. Chapter 13: Convergent Thalamic and Cortical Projections — the Non-Specific System by D. ALBE-FESSARD and J. M. BESSON. Chapter 14: Electrical Recording from the Thalamus in Human Subjects by J. A. V. BATES. Chapter 15: Anatomical Organization of the Somatosensory Cortex by E. G. JONES and T. P. S. POWELL. Chapter 16: Functional Organization of the Somatosensory Cortex by G. WERNER and B. I. WHITSEL. Chapter 17: Somatosensory Cortex: Descending Influences on Ascending Systems by A. L. TOWE. Chapter 18: Somaesthetic Effects of Damage to the Central Nervous System by J. SEMMES. Chapter 19: Electrical Stimulation of Cortex in Human Subjects, and Conscious Sensory Aspects by B. LIBET.

Each chapter is complete with references and the volume ends with an author and subject index.

The book is a very important reference work for neuroanatomy, neurophysiology, neurobiology and clinical neurology. The complete series of the Handbook of Sensory Physiology should find its place in every library of medical and biological sciences.

K. LISSÁK

K. v. FRISCH

Erinnerungen eines Biologen

Dritte, erweiterte Auflage. Springer-Verlag, Berlin, Heidelberg, New York 1973. X + 196 pages, with 41 figures. Price: DM 32.—

This third edition of the autobiography by Karl von FRISCH first published in 1957 has been completed with a chapter on the years of retirement from active professorship (1958) and some poems of the author from the years 1914—1968. The book describes the long, colourful, and in scientific results so sick life of the author who in 1973 was awarded the highest scientific honour, the Nobel Price for Medicine. His outstanding works are: Tanzsprache und Orientierung der Bienen (Springer-Verlag, 1965); Aus dem Leben der Bienen (8th edition, Springer-Verlag, 1969); Duftgelenkte Bienen im Dienste der Landwirtschaft und Imkerei (Springer-Verlag, 1947); Animal Architectonic (Helen and Kurt Wolff Books, New York) and Tiere als Baumeister (Ullstein, Berlin) which is in preparation besides 136 publications in different periodicals.

This autobiography is a very interesting and delightful reading for every scientists whether old or young, vividly recounting the useful experience of a long scientific life.

K. LISSÁK

ABSTRACTS

of the lectures delivered at the 38th Congress

of the

HUNGARIAN PHYSIOLOGICAL SOCIETY

Budapest, May 31—June 3, 1972

INDEX

Anda Erzsébet, Vándor E., Szabó G.: Effect of Muscular Work and Hypoxia on the Lymphatic and Venous Transport of Lactic Acid Dehydrogenase 291
Antal A., Kárpáti F., Petri I.: Electron Microscopic Study of the Regeneration Process (150 Days) of Mast Cells 291
Antal Sára, Gidáli Júlia, Fehér I.: Some Characteristics of the Circulating Primary Cell 292
Apor P., Miklós Márta, Wahlstab Sigrid: Relationship between the Indices of Aerobic Capacity of Sportsmen 292
Avar Z., Monos E., Kurcz M., Nagy I.: Perinatal Reproduction Disturbances Evoked by Parafornical Hypothalamic Lesions in the Rat 292
Ádám G., Pelyhe B. Ilona, Pusztai J.: Conditioned Evoked Potential in Neuronally Isolated Cerebral Cortical Slab 293
Ángyán L., Tóth Brigitta, Czopf J.: Investigation of the Immobility Reflex in the Frog 294
Baintner K.: Effect of the Colostral Trypsin Inhibitor on Digestion in the Newborn Pig 294
Baló J.: The Pulmonary Surfactant 295
Banga Ilona, Bihary-Varga Magdolna: Accumulation in Human Aortic Plaques of a Fluorescent Pigment Resembling Lipofuscin 295
Barankay T., Nagy S., Petri A., Újházi Gyöngyi, Horpácsy G.: Effect of Adrenergic Receptor Stimulants and Inhibitors on the Oxygen Tension of the Lymph 296
Bardóczi Á., Karvaly B.: Some Immunological Problems of the Rat's Yoshida Sarcoma 297
Bartha J.: Effect of Acute and Chronic Sodium Load on the Intrarenal Distribution of Blood Flow 297
Bálint P., Szőcs Éva: The Role of Haemodynamic Factors in the Pathogenesis of Post-Ischaemic Renal Failure 298
Bedő B. Magdolna: Effect of Caffeine on Glucose Metabolism in Different Rat Strains 298
Bencsáth P., Szalay L., Vajda L., Takács L.: Sodium Excretion and Renin Production of the Denervated Kidney During Furosemide Diuresis 299
Benedek G., Turai K., Ozsvár Annamária, Joó F., Zoltán Örs T., Obál F.: Body Temperature in Experimental Cerebral Oedema Induced by Triethyl Tin Sulphate (TET) 299
Bernard A., Zsolnai B., Somogyi J., Menyhárt J., Ikrényi C.: Investigation of Metabolic Products in Toxaemia of Pregnancy 300
Bíró Zsuzsanna, Kovách A. G. B.: Effect of Denervation on Transcapillary Fluid Flow in Haemorrhagic Shock 300
Bite Ágnes, György L.: Influence of Tetrabenazine on the Analgesic Action of Tremorine and Oxotremorine 301
Blazsó G., Koltai M., Ottlecz Anna, Minker E.: Effect of Cyclophosphamide on the Insulin-Induced Sensitization of Anaphylactoid Inflammation 301
Borbola J., Papp G., Szekeres L.: Effect of Lipid Metabolites on the Electrophysiological Parameters of the Heart 302
Boros Ildikó, Zelles T., Keszler P., Gáti T.: Serotonin Level in Resting and Functioning Salivary Glands of the Rat 303
Borvendég J., Hermann Helén, Bajusz S.: Induction of Ovulation by LH-FSH Releasing Hormone in Androgenized Female Rats (Treated with Testosterone Propionate at Newborn Age) 303
Bot G., Kovács F. Edit, Gergely P.: Physiological Significance of the Intermediary Form of Muscle Phosphorylase 304
Brändle K., Székely G.: Movement of Isolated Limb Pairs in the Axolotl 305

Czakó L., Nagy Éva, László F.: Effect of Clofibrate on the Diuresis and ADH-Sensitivity of Rats with Pituitary Stalk Lesion 305
Czéh G.: Some Characteristics of the Action Potential of the Motor Neurone in the Frog 306
Csaba B., Muszbek L., Szokol M., Fésüs L.: Development of Anaphylactoid Hypersensitivity after Adrenocortical Failure Due to *Bordetella Pertussis* Vaccine 307
Csabai A., Szabolcs M., Kövér A.: Effect of Ca and La Ions on Some Characteristics of the Sarcoplasmic Reticular Fraction 307
Csalay L., Csákváry G., Frenkl R.: Effect of Chronic ACTH Treatment on Lipid Metabolism 308
Csákó G., Csernyánszky Hedvig, Tóth S., Szilágyi T.: Endotoxin and Catecholamines .. 308
Csákváry G., Csalay L.: Effect of Nucleotide and Orotic Acid Treatment on the Incorporation of ^{14}C-Stearinic Acid in Animals Kept on Hartroft Diet 309
Csergáti L., Tóth S.: The Effect of ^{75}Se-Methionine on Thrombocytopoiesis in Actinomycin-D-Treated Mice 309
Dankó M., Gesztelyi I., Varga E.: Veratrine-Induced Rhythmic Changes of the Muscle Membrane 310
Dao Van Phan, György L.: Analysis of the Vasopressor Effect of Oxotremorine and Arecoline in the Rat 310
Decsi L., Várszegi-Karmos Mária, Nagy Júlia: Some Effects of Catecholamines after Microinjection into Various Subcortical Regions of Freely-Moving Cats 311
Dévai Gabriella, Széles Margit, Szentiványi M.: The Role of Adrenergic Receptors in the Anaphylactoid Hypersensitivity of the Smooth Muscle of the Upper Respiratory Tract 311
Domonkos J., Vargha M. jr., Heiner L.: Subcellular Distribution of Phospholipids in Tonic and Tetanic Muscle 312
Dóra E., Tulassay T., Strausz J., Kovách A. G. B.: Spontaneous and Evoked Electrical Activity of the Cerebral Cortex, Hypothalamus, Amygdala and Thalamus in Haemorrhagic Shock 313
Dura G., Illés I., Major Magda, Gönczi C.: Neurophysiological Investigations with an Organic Phosphate Compound 313
Eke A., Gyulai L., Sulyok A., Kovách A. G. B.: Blood Flow and Salivary Secretion of the Submandibular Gland in Haemorrhagic Shock 314
Elek S., Borsy J., Maderspach Andrea, Polgári I., Osváth Zsuzsa: Effect of Phenobarbital, Ethyl-p-Chlorophenoxy-Isobutyrate (Clofibrate) and Bis-p-Chlorophenoxy-Acetylurea (M-451) in Experimental Liver Injury of the Rat 315
Elekes K., Kiss T., Rózsa S. Katalin: Ultrastructural Changes in the Heart of *Helix pomatia* L. Caused by Calcium Deficiency 315
Endrényi F., Tardos L.: Effect of Atropine Infusion on Histamine- and Pentagastrin-Induced Gastric Acid Secretion in Anaesthetized Cats 316
Faiszt J., Izsák J.: Effect of Light on the Metabolism of Soluble Cerebral Proteins in the Rat 316
Farkas Gyöngyi, Puskás Mária, Antoni F., Piffkó P.: Lysozyme Activity of the Human Tonsil and Its Role in Local Protection 317
Farkas Ildikó, Sós J.: Effect of p-Chlorophenylalanine on the Spontaneous Activity of Rats 317
Fazekas Á., Fekete Ágnes, Forgács I., Gaál Katalin: Effect of Kidney Denervation on the Renin Activity of Renal Venous Blood of Dogs with Renovascular Hypertension 318
Fazekas S., Kása I.: Derivatographic Analysis of Myosin and Myofibrils 318
Fedina L., Kollai M., Kovách A. G. B.: Activity of the Efferent Sympathetic Nerves of the Heart and Forelimb. II. Effect of Bleeding, Cooling, Asphyxia and Stimulation of the Efferent Nerves 319
Fenyvesi T., Hadházy P.: Effect of Beta-Receptor Blocking Drugs on the Regulation of Systolic Volume 320
Fésüs L., Muszbek L., Csaba B.: Effect of Catecholamines and Shock Mediators (Histamine, 5-Hydroxytryptamine) on the Blood Sugar Level in Rats Treated with *Bordetella pertussis* Vaccine 320
Fóris Gabriella, Füst G., Újhelyi K.: Role of the Adrenals in Endotoxin Hypersensitivity 321
Frenkl R., Szeberényi Sz.: Enzyme Induction in Trained and Non-Trained Animals ... 321
Gaál J., Pogátsa G., Nagy L.: Effect of Surgical Stress on the Glycogen Content of Liver and Skeletal Muscle in the Rat 322
Gaál Katalin, Forgács I., Sallay P.: Effect on Renal Function and Renin Liberation of Beta-Mimetic and Beta Blocking Drugs Infused in the Renal Artery 323

Galgóczy G., Jancsó T., Mándi A.: Bronchodilator Effect of Sympatho-Mimetics and Parasympatholytics in the Human Bronchial System 323
Gallyas Alfréda, Fehér I.: Effect of Phytohaemagglutinin on Thymus Cells 324
Garamvölgyi M.: Structure of Quick and Slow Fibres in Human Muscle 324
Gattyán Erzsébet, Bíró Zsuzsanna, Kovách A. G. B.: Effect of Butylsympathon on the Transcapillary Fluid Transport of the Cat Limb Musculature in Haemorrhagic Shock 324
Gáti T., Farkas Ildikó, Zelles T., Krausz T., Ballya Irén: Effect of Vibration on the Chloral Hydrate and Pentobarbital Sleeping Time of the Rat 325
Gecse Á., Szekeres Lenke, Lózsa A., Lonovics J., Zsilinszky Eleonóra: "Skin Reacting Factor" as Activator of the Bradykinin System 326
Gefferth G.: Incorporation of ^{15}N-Labelled Amino Acids Into the Liver of Starved Rats 326
Gelencsér F., Gáti T., Hideg J.: Role of Adrenergic Receptors in the Regulation of Gastric Motility in the Rat 327
Gerbner M., Ho Duc Duc, Keleti Judit: Individual Variability in the Manifestation of Frontal Signs 327
Gergely P., Varsányi Magdolna, Vereb G., Bot G.: Presence and Ratio of Isophosphorylases in the Heart and Other Organs of Mammals 328
Gesztelyi I., Dankó M. Varga E.: Depolarizing Effect of Veratrine on Skeletal Muscles 328
Gidáli Júlia, Antal Sára, Fehér I.: Regulation of the Level of Circulating Haemopoietic Primary Cells 329
Gibiszer P. Katalin, Pórszász J.: Tolerance and Cross-Tolerance between Clonidine (ST-155) and Indirect Sympathicomimetics 330
Gróf J., Menyhárt J., Somogyi J.: Role of the Urea Cycle in the Development of Uraemic Toxaemia 330
Gulyás G., Szentiványi M.: Alpha- and Beta-Mimetic Interactions on the Rabbit Aorta 331
Hadházy P.: "Anticholinergic" Action of Prostaglandin E_1 (PGE_1) on Isolated Guinea Pig Atrium 332
Hamar J., Kovách A. G. B.: Investigation with ^{133}Xe of the Blood Supply of the Small Intestine in Normal and Hypotensive Dogs 332
Hargita Á., Szentiványi M.: Analysis of the Dynamic Parameters Evoked by Pressure Impulses in Resting Heart Muscle 333
Hartmann G., Fekete M., Vermes I., Lissák K.: Self-Stimulation and Adrenocortical Activity in Young Rats 334
Harza T., Szántó J.: Effect of Adrenaline on Cation Release from the Frog Myocardium 334
Hársing L. jr., Bartha J.: Mechanism of Carotid Polyuria 335
Hermann J., Borvendég J., Bajusz S.: Induction of Ovulation by Synthetic LH-FSH-RH in Mature Rabbits 335
Hiripi L., Salánki J., Nagy I. Zs., Muskó B. Ilona: Subcellular Localization of Biogenic Amines in the Central Nervous System of *Anodonta cygnea* L. 336
Horpácsy G., Farkas G., Barankay T., Nagy S., Petri G.: Enzyme and Metabolite Content of the Perfusion Fluid of the Kidney Preserved According to Collins 336
Horváth B., Kékes-Szabó A.: Influence of Sympathetic Beta Effects on Acid Secretion 337
Hraschek Ágnes, Folly G., Varga B.: Influence of ACTH on Increase in Uterine Weight Evoked by HCG in Infantile Mice 338
Hunyadi J., Dobozy A., Simon M.: Histone Demethylation in Lymphocyte Cultures .. 338
Ikrényi K., Kovách A. G. B., Somogyi J.: Oxidative Ability of Brain Mitochondria Isolated from Animals in Experimental Shock 338
Indi O., Budavári I., Pósch E., Horváth J., Koller O.: Effect of Detergents on Experimental Inflammation Models 339
Jánossy T., Minker E., Koltai M.: Cholinergic–Adrenergic Interactions on Smooth Muscle Organs 340
Juhász G., Kukorelli T.: EEG-Synchronizing Effect of Small Intestinal and Splanchnic Nerve Stimulation 340
Juhász-Nagy S., Szentiványi M.: Adrenergic Responses of the Coronary Circulation after Cardiac Infarction 341
Karády I., Gecse Á., Ottlecz Anna, Lonovics J., Zsilinszky Eleonóra: A So-Far Unknown Inhibitory Effect of a Plasma Component on the Activity of Inflammatory Mediators 341
Karmos-Várszegi Mária, Decsi L., Karmos G.: Some Effects of Intrahypothalamically Injected Cholinolytics in Waking Cats 342
Karvaly B., Bardóczi Á.: Modelling of the Primary Photoelectric Process Arising in the Visual Organ on an Artificial Bimolecular Lipid Membrane 343

Kákonyi G., Koltai M., Minker E.: Investigation on the Excitability of Peripheral Cholinergic Synaptic Structures in Diabetes Mellitus 344
Káldor A., Pogátsa G.: Metabolic Effect of Guanethidine 344
Kárpáti F., Antal A., Gál G., Hézsai Klára: Significance of Mast Cells in Chronic Nephritis 345
Kávai Mária, Zsindely A., Csaba B.: Ezyme Activities in Guinea Pig Macrophages Under the Effect of Antigen–Antibody Reaction 345
Kecskeméti Valéria, Kelemen K., Knoll J.: Effect of Prostaglandin E_1 on the Transmembrane Potential of Mammalian Heart Preparations 346
Kellényi L., Ángyán L.: Application of Heart Rate Analyser in Behavioural Experiments 346
Kenedi I., Losonci A.: Effect of Beta-Receptor Stimulation and Inhibition on the Survival and Arrhythmia after Ligation of the Left Coronary in the Rat 347
Kenessey Ágnes, Huszti Zsuzsanna: Activation of Rat Brain Tyrosine Hydroxylase by Ascorbic Acid in vitro 347
Kertai P.: Role of the Sympathetic–Adrenergic System and Glucagon in Endotoxin-Induced Hyperglycaemia 348
Keszler P., Zelles T., Boros Ildikó, Gáti T.: Role of the Parasympathetic System in the Regulation of Salivary Carboanhydrase Activity in the Rat 348
Kékes-Szabó A., Horváth B.: New Data on the Pathomechanism of the Immobilization and Reserpine Ulcer 349
Király Ildikó, Nagy T., Borsy J., Marosfalvi S., Székely J. I.: Mechanism of Hippocampal Cobalt Epilepsy in the Rat 350
Kiss I., Salánki J.: The Role of Ionic Milieu in the Generation of Spontaneous Electrical Activity of the Identified Giant Neurones of *Lymnaea stagnalis* 350
Kiss R., Gláz Edit, Fodor Éva, Hering Andrea, Morvai Vera, Péteri M., Galavics Rozália, Szabó Mária, Somló P., Szabó D.: Effect of Aminoglutethimide Treatment on Corticosteroid Biosynthesis in the Rat Adrenal 351
Kiss T., Rózsa S. Katalin: Role of Monovalent and Bivalent Ions in Action Potential Generation in the Myocardial Cells of *Helix pomatia* 351
Kocsis Julianna, Csernay L., László F.: Renal Circulation of Hypophysectomized Rats in Hormone-Induced Renal Cortical Necrosis 352
Kollai M., Fedina L., Kovách A. G. B.: Activity of the Sympathetic Afferent Nerves of the Heart and Forelimbs. I. Spontaneous Activity and Baroreceptor Effects .. 352
Koltay Edit, Stoner H. B., Elson M. Patricia: Effect of Hindlimb Ischaemia on the ^{14}C-Tyrosine and ^{14}C-Noradrenaline Content of the Hypothalamus and Brain Stem of the Rat 353
Korányi L., Tamásy Vera, Lissák K.: Multiple Unit Activity in Brain Stem and Limbic System during Habituation to Acoustic Stimulus in the Cat 354
Korényi-Both A., Kelemen Gabriella: Injuries of the Skeletal Musculature Caused by Immunoglobulins in the Rat 354
Kovách A. G. B., Sándor P., Frinak S., Kovách Emma: Investigation by Surface Fluorometry of Oxidative Metabolism, Gluconeogenesis and Redox State of the Perfused Rat Liver during Haemorrhagic Shock 355
Kovács Anikó: Pharmacological Investigations on the Genital Smooth Musculature of *Helix pomatia* 356
Kovács G., Lissák K.: Effect of Corticosteroids on Passive Avoidance Behaviour of the Rat 356
Kovács Irén, Csalay L., Csákváry G.: Antithrombin Effect of Acetylsalicylic Acid ... 357
Kovács S., Vass J., Ferenc P.: Subcellular Distribution of Thyroid Hormones in the Central Nervous System. II. Effect of Thyroid Hormone Treatment at Newborn Age 357
Kovács T., Nagy Mária, Szabó B., Szirmai L.: Kinetic Analysis of the Sodium Transport in Striated Muscle. II. Effect of Veratrine on Sodium Uptake 358
Kónya L., Kövér A., Cseri Júlia, Zsadányi Erzsébet: Effect of La^{+++} on ^{45}Ca Uptake and Release of Frog Skeletal Muscle 358
Kövér A., Szabolcs M., Csabai A., Nagy Z.: The Regulating Role of Membrane-Bound Ca in the Functioning of the Sarcoplasmic Reticulum 359
Kövér G.: Reabsorption Processes in the Proximal Tubule During "Stop-Flow" 359
Kőnig T.: Effect of Quinaldic Acid on Mitochondrial Respiration 360
Kukorelli T., Juhász G.: Control of Cortical Potentials Evoked by Stimulation of Small Intestine and Splanchnic Nerve during Sleep 361
Kürti Marianne, Huszti Zsuzsa, Fekete M., Kasztreiner E.: Studies of Histidine Decarboxylase Inhibitors in Inflammatory Models 361
Lábos E., Láng Eszter: Neuronal Connections in the Suboesophageal Ganglion of *Helix pomatia* 362
Láng Eszter, Lábos E.: Applications of Hill's Subthreshold Stimulus Theory in the Experi-

mental Approximation of the Relationship between Stimulus Intensity and Stimulus Duration 362
László Katalin, Bálint P.: The Role of Haemodynamic Factors in Renal Failure in Dehydration 363
Lehoczky Kornélia, Mészáros I., Sebők J.: EEG and Evoked Potentials in Experimental Organic Mercury Poisoning 363
Leszkovszky G., Tardos L., Szánthó Vilma: Effect of Intracerebrally Administered Diphenyl-Propylamine Derivatives 364
Lonovics L., Szekeres Lenke, Zsilinszky Eleonóra, Gecse Á.: Location of the Subcellular Kininases of the Skin 364
Losonci A.: Effect of Anaemia on the Circulation of Coronary-Ligated Rats 365
Lózsa A.: Sex Differences in the Lipoprotein Subfractions of Rabbits 366
Madarász Emilia, Orosz A., Falus A., Szentpéteri Gabriella: Some Membrane Components of Cerebrocortical Synaptosome Fractions 366
Madarász I., Hunya P.: Dynamics of Human Working Capacity as Evaluated on the Basis of Regulatory Functions 367
Maderspach Andrea, Borsy J., Elekes I., Fischer J., Mikite G., Rákóczi J.: Effect on Lipid Metabolism of N(bis)-p-Chlorophenoxy (Acetyl)-Urea 367
Magda Katalin, Szegi J., Szentjóbi O., Szentmiklósi J.: Experimental Defibrination Evoked by Subtilysin 368
Marosvári I., Schmidt U., Dubach U. C.: Ultramicrochemical Determination of Glucose-6-Phosphatase and Glucokinase in the Rat Nephron 368
Martin J., Pintér I., Kellényi L.: Investigation on the Habituation of Acoustic Stimulus by Means of Evoked Potential Audiometry 369
Mátrai Erzsébet, Dervaderics J., Such G.: Role of Bulbar Cholinergic Processes in Vasomotor Reflex Activities 369
Menyhárt J., Gróf J., Somogyi J.: Relationship of Tissular Metabolic Processes and Cell Division in Regenerating Liver Tissue 370
Miklós Márta, Apor P., Wahlstab-Szabó S.: Measurement of Maximum Oxygen Uptake during Treadmill Exercise 370
Molnár P., Lénárd L., Trinn Csilla, Grastyán E.: Behavioural and EEG Signs of Instrumental Escape from Frustration Situation 371
Monos E., Szücs B.: Dynamics of First-Order and Third-Order Blood Pressure and Pulse Waves in Gradual Hypotension 371
Morava E., Winter M., Tarján R.: Effect of Vitamin D on the Bone of Parathyroidectomized Rats 372
Morava Izabella, Noel Mei: Spinal Afferent Activity Elicited by Potassium Chloride Injected into the Muscle's Nutrient Artery 372
Mózsik G., Kutas J., Nagy L., Tárnok F., Petz Á.: Effect of Drugs Influencing Transport ATPase and Adenylcyclase Systems on the Parietal and Non-Parietal Components of Human Gastric Secretion 373
Muszbek L., Csizér Z., Csongor J., Csaba B.: Disturbance of Insulin Secretion in Rats Treated with *Bordetella Pertussis* Vaccine 374
Nagy A., Sarkadi Á., Tomka I.: Expectancy Wave in Organic Cerebral Processes 374
Nagy Éva, László F.: Effect of Retrograde Degeneration on the ADH Reserve Capacity of Hypophysectomized Rats 375
Nagy Éva, Menyhárt J., Gróf J., Somogyi J.: Citrulline Synthesis of Isolated Mitochondria in Uraemia 375
Nagy I. Zs., Ermini M.: Energy Production through Anoxid Endogenous Oxidation in *Mytillus galloprovincialis* (Mollusca, Pelecypoda) 375
Nagy Júlia, Decsi L.: Effect of Direct Cholinergic and Adrenergic Stimulation of the Hypothalamus, Thalamus and Hippocampus on the Alimentary Conditioned Reflex of the Cat 377
Nagy L., Menyhárt J., Somogyi J.: Effect of Sympatholytics on Liver Associated with Injury Accompanying Hyperthyroidism 377
Nemessuri M., Bretz K.: Mechanical and Electrical Activity of the Muscle during Rapidly Changing Dynamic Effort 378
Németh G., Willig F., Somogyi J., Menyhárt J., Tóth B.: In vitro Activation of the Proteolytic Proenzymes of Pancreas 378
Ormai S., Csáki L.: Blood Flow of Spleen and Mesenteric Lymph Nodes during Lymphocyte Mobilization Caused by Polymetacrilic Acid 379
Ormos Gy., Mányai S., Orosz J., Putton Erzsébet, Süveges M., Végh A.: Apolar Interactions in Extraction Haemolysis 379

Orosz A., Fazekas A., Szentpéteri Gabriella: Immunofluorescent Investigations in Myasthenia Gravis: Antineuronal Activity of the Sera 380
Ottlecz Anna, Jánossy Ágota, Koltai M.: Effect of Hyperlipaemia on the Changes of Vascular Permeability Caused by Vasoactive Substances 381
Papp G., Szekeres L., Resch B.: Electrical Activity and Drug Sensitivity of Early Human Embryonic Hearts 381
Papp M., Varga B., Folly G.: Regulation of Pancreatic Blood Flow: Relationship between Dose and Effect of Intraarterially Injected Secretin, Pancreozymin and Histamine 382
Pavlik G., Hajutyin V. M., Mitsányi A., Lukoskova V. Elena, Erdélyi A.: Effect of Somato-Autonomic Reflexes of Carotid Sinus Pressure Stabilized at Various Levels ... 382
Pellet S., Fehér I.: Haemoglobin of Avian Reticulocytes 383
Pethes G., Winter M.: Effect of Ca-Deficient Diet on Thyroid Function of Chicken ... 383
Pórszász J., Gibiszer P. Katalin: Dependence of the Elementary Phenomena of the Sympathetic Reflex Response on the Functional State of the Central Nervous System 384
Pucsok J., Szollár L., Jáky M.: Fatty Acid Composition of Hyperlipaemic Lipid Fractions 384
Rabloczky G., Jancsó T., Mader R. Mária: Pulmonary Pressure and Cholinesterase Inhibition (Mevinphos Treatment) 385
Rák K.: Effect of Single and Repeated Acute Thrombocytopenia on Thrombocytopoiesis in the Mouse 385
Reivich M., Kovách A. G. B., Harbig K., Sándor P., Spitzer J. J.: Regional Cerebral Blood Flow and Metabolism during Haemorrhagic Shock in the Baboon 386
Resch B., Papp G., Szekeres L.: Functioning of Early Human Embryonic Hearts 386
Rigó J., Tulok I., Pósch E., Sós J.: Effect of Vegetable Amino Acid Concentrates on the Development of Rats Kept on Protein-Deficient Diet 387
Rózsa S. Katalin: Feedback Phenomena in the *Helix pomatia* Heart Showing Diffuse Myogenic Rhythm 387
Sallay P., Forgács I., Gaál Katalin, Bakos L.: The Role of Intrarenal Alpha-Receptors in the Regulation of Renin Secretion 388
Sarkadi Á., Nagypál T., Vörös Mária, Végső L.: Computer Approach to the Relationship between Cortical and Subcortical EEG Signs during Internal Inhibition 389
Sándor P., Kovách A. G. B., Rosell Sune, Spitzer J. J., Spitzer Judy: Effect of Endotoxin Shock on the Blood Flow of Adipose Tissue and on the Metabolism of Isolated Adipose Cells 389
Sárdi F., Andik I.: Alveolar Ventilation and Chronic Dust Inhalation 390
Schäfer Inge, Borsy J.: Investigation of Diuretics by Sequential Analysis in Rats ... 390
Schmidt P., Andik I.: Regulation of Water Intake in Parabiosis 391
Schrameck E. Joan, Székely G.: Changes of Skin Reflexes after Cross-Union of Sensory Nerves in Frogs 391
Sebeszta M., Juhász-Nagy S.: Circulatory Reflex Effects in Myocardial Infarction ... 392
Selmeci L., Pósch E., Farkas T., Goreczky Karola: Lactic Acid Dehydrogenase and Malic Acid Dehydrogenase Activity in Rat Platelets after Splenectomy 392
Simon G., Földvári P., Winter M.: The Role of RES in the Regulation of Serum Pseudocholinesterase Activity 393
Simon M. jr., Ottlecz Anna, Koltai M.: Effect of Hyperlipaemia on the Inflammation-Increasing Effect of Insulin 393
Sipka S., Tóth S., Szilágyi T., Laczkó J.: Effect of Aurothioglucose on Various Types of Skin Reactions 394
Solti F., Krasznai I., Gyertyánffi G.: Regulation of Blood Circulation in Polycythaemia 394
Sólyom J.: Effect of in vivo Sodium Deprivation and Load on the Steroid Production in vitro of the Adrenal Capsule and the Decapsulated Gland 395
Somogyi I., Herpai S.: Interpretation of the Cerebral Activation Phenomenon 395
Somogyi J.: Activation of Transport Adenosine Triphosphatase through Binding Carboxy Groups 396
Sós J.: Pathologic Ageing Induced by Dietary Means 396
Stadler E.: Cardiorespiratory Adaptation 397
Such G., Fehér O.: Microelectrophoretic Investigation on Medullary Neurones 397
Sulyok A., Kovách A. G. B.: Effect of Dibenzyline on Blood Flow of the Thyroid and Salivary Glands during Haemorrhagic Shock 398
Szabó B., Kovács T., Zakar G., Papp Mónika: Sodium Exchange in Striated Muscle .. 398
Szabó G.: Comparative Investigation of the Proteins of Blood Plasma and Lymph ... 399
Szabó I.: Identification of the Hypothalamic Structure Responsible for Reinforcement 399
Szabó T. Mária, Antoni F., Hrabák A.: Biosynthetic Activity of Cells Labelled with ^{51}Cr 400

Szamosi T., Pácz F., Pánczél P.: Citrate Content of Rat Liver Cells 400
Szeberényi M. Judit, Varga Anikó, Pongrácz F., Molnár J.: Temperature-Decreasing Effect of Fenitrothion in the Rat 401
Szekeres L., Udvary Éva, Szurgent J., Papp J.: Mechanism and Drug-Sensitivity of Arrhythmia Following Coronary Ligation 401
Szekeres L., Benedek G., Győri I., Madarász I.: Stability of the Evoked Potentials of Human Visual Cortex 402
Szelényi I., Ermini M., Verzár F.: Metabolic Changes in the White Skeletal Muscles of Aged Rats 402
Szelényi Z., Székely M.: Thermoregulation in the Rabbit Under the Effect of *E. coli* Pyrogen 403
Szentiványi M., Magyar Zsuzsanna, Juhász-Nagy S.: The Physiological Role of the Epicardium 403
Székely M., Szelényi Z.: Changes in Thermoregulation under the Effect of *E. coli* Pyrogen in the Rat 404
Székely O., Arfors K. E.: Microcirculation in Anoxic Hypoxia of the Rabbit 405
Szilágyi T., Tóth S., Laczkó J.: Disseminated Intravascular Coagulation in the Rat after the Simultaneous Administration of Hemocoagulase and Epsilon-aminocaprylic Acid 405
Szirmai I., Czopf J.: Changes in Regional Cerebral Circulation during Focal and Generalized Epileptic Convulsions 406
Szmodis I., Malomsoki M.: Load-Dependent Changes of the Respiratory Quotient ... 406
Szolcsányi J., Jancsó-Gábor Aurelia, Joó F.: The Double Function of Chemosensitive and Pain-Sensitive Nerve Endings 407
Szollár L., Pucsok J., Jáky M.: Serum Triglyceride Pattern of Hyperlipaemic Patients 407
Szőcs Éva, László Katalin, Bálint P.: Role of Haemodynamic Factors in the Pathogenesis of Renal Failure after Sublimate Poisoning 408
Szurgent J., Szekeres L.: A New Type Microelectrode for Continuous Monophasic Action Potential Recording on the Heart in situ 409
Szücs B., Monos E.: Application of Hybrid Computer Technique for Broad-Scale Frequency Spectrum Analysis of Blood Pressure and Blood Circulation 409
Takáts I., Vághy P., Szekeres L.: Comparative Study on Ischaemic Heart Models Used for Analyzing the Effect of Antianginal Drugs on Cardiac Metabolism 410
Tamásy Vera, Korányi L., Lissák K.: Multiple Unit Activity during Habituation to the Environment in the Rat 410
Telegdy G., Robin M., Diczfalusy E.: Synthesis of Steroid Hormones from Cholesterol (7-Alpha-^{3}H) in the Middle and at the End of Pregnancy 411
Tézsla E., Obál F. jr., Obál F.: Nervous Regulation on Initial Body Temperature and its Alterations 412
Thuránszky K., Dénes B., Temesváry Zsuzsa: Electrophysiological Investigation of the Ca^{++}–CS_2 Antagonism on an Isolated Nerve Preparation in situ 412
Tomka I., Nagypál T.: Electric Paroxysms in REM 412
Tost Hilda, Kövér G.: Effect of Cyclic 3'-5'-Adenosine Monophosphate on Tubular Sodium Reabsorption 413
Tóth A., Lázár G., Karády I.: Effect of Histamine and Adrenaline Adaptation on the Hyperfibrinogenaemia Elicited by Histamine and Adrenaline 413
Tóth B., Philemon Msuya: Effect of RNase on Biosynthesis of HbS and HbA in vitro 414
Tóth B., Somogyi J., Németh G., Menyhárt J., Willig F., Farkas I., Ikrényi K.: Proteinase Inhibitors in Serum of Normal Subjects and of Acute Pancreatitis Patients ... 415
Tóth B. L., Irsy G., Szilágyi G., Mosonyi L.: Respiration-Influencing Hypocalcaemia-Sensitive Innervation in the Carotid Artery 415
Tóth M., Medgyesi G., Zsolnai B., Somogyi J.: Immunological Investigation of Serum Proteins in Pregnancy 416
Török A., Lábos E., Szekeres L.: Statistical Analysis of the Activity of Neurones of Permanent and Periodically Changing Frequency 417
Török I., Tóth Ágnes, Szabó Teréz, Faragó Margit: Effect of Acetylcholine on the K-Depleted Frog Heart 417
Tuong Bich Truc, Kertai P.: The Role of the Pituitary in Liver Regeneration 418
Turai K., Benedek G., Ozsvár Annamária, Joó F., Örs T. Z., Obál F.: Effect of Ambient Temperature on Body Temperature of Animals with Cerebral Oedema Induced by Triethylone Sulphate 418
Tury G.: Effect of Spreading Depression on the Unit Activity of Cerebral Cortex 418

Udvary Éva, Csik Veronika, Szekeres L.: Changes after Coronary Ligation in the Electrophysiological Parameters of Myocardial Regions not Directly Affected by the Ligature 419
Ungváry G., Varga B.: Effect of Catecholamines on Blood Flow in the Regenerating Rat Liver 420
Urbanics R., Juhász-Nagy S.: Regional Changes in the Adaptability of Coronary Vessels: the Role of Primary Collateral Branches 420
Vadász I., Elekes E., Salánki J.: Location and Characterization of Br Cell in the Central Nervous System of *Helix pomatia* 421
Varanka I., Svidersky V. L.: Response Types of Wind-Sensitive Receptors of the Interneurones of the Migratory Locust *(Locusta Migratoria Migratorioides)* 421
Varga A., Kónya L., Kövér A.: Determination of Quickly Exchanging Ca Fractions in Frog Skeletal Muscles 422
Varga F., Csáky T. Z.: Estimation of Subepithelial Capillary Blood Flow by Measuring the Outflux of Barbital in the Small Intestine of the Rat 423
Varga P., Durszt F., László F.: Effect of Pituitary Stalk Lesion on the Alteration of Blood Sugar Level and Liver Glycogen Content Induced by Alloxan and Glucagon 423
Varga T., Bujdosó Györgyi, Somogyi E.: Comparative Biochemical and Morphological Investigations in Chronic $HgCl_2$ Poisoning 424
Vass J., Kovács S., Ferenc P.: Subcellular Distribution of Thyroid Hormones in the Central Nervous System. I. Study of the Distribution during Postnatal Development 424
Vándor E., Anda Erzsébet, Szabó G.: Lymphatic Transport of LDH-Isoenzymes 425
Velemir N., Lázár G., Karády I.: Phospholipid Metabolism of Yoshida Sarcoma Tumour Cells 425
Veréb G., Gergely P., Bot G.: Functional Differences in the b⇄a Transformation of Cardiac Iso-Phosphorylases 426
Vermes I., Telegdy G., Lissák K.: Effect of Serotonin on Hypothalamo–Pituitary–Adrenocortical Function in the Rat 426
Vértes Marietta, Vértes Zsuzsa: Binding of Oestradiol in the Hypothalamus of Normal and Androgenized Rats 427
Vértes Zsuzsa, Vértes Marietta, Kovács S.: Effect of Postnatal Oestradiol Treatment on DNA and RNA Synthesis in the Brain 428
Vincze I., Dénes G.: Effect of K Ion on Homoserine Dehydrogenase Activity of *Chlamydomonas reinhardti* 428
Vizi F., Mózsik G., Jávor T.: Inhibitory Effect on Gastric Secretion and Ulceration of Actinomycin-D, Cyclophosphamide and Mannomustine in the Shay Rat 429
Wahlstab S., Apor P., Miklós Márta, Petres Jolán: Cerebral Rheographic Investigations during Pressing Load (Weight-Lifting) 429
Went Mária, Csaba B.: Effect of Cortisone Treatment and Adrenalectomy on 5-HT Metabolism of Mouse Tissues 430
Winter M., Morava E., Szentgyörgyi E., Frank Ágnes, Simon G.: The Role of the Kidney in the Calcium Adsorption Promoting Effect of Vitamin D 430
Wollemann Mária, Farkas T.: Mechanism of the Hormonal Activation of Adenyl Cyclase 431
Zsilinszky Eleonóra, Lonovics J., Gecse Á.: Anti-Inflammatory Effect of Heptyl Esters 431
Zsinka J. Ágnes: Investigation of Pancreatic Excretion by Application of a Chronic Fistula 432

Effect of muscular work and hypoxia on the lymphatic and venous transport of lactic acid dehydrogenase

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Limb ischaemia for 3½ hours significantly increased LDH activity in the serum and in the limb lymph of dogs with thoracic duct fistula. Similarly, muscular work increased the enzyme activity of serum and lymph. Since in these animals the connection between the lymphatic system and the venous vessels was interrupted, the observations were indicative of a direct entry of the enzyme protein into the blood capillaries. The assumption is corroborated by the fact that a significant arterio–venous LDH activity difference develops in the ischaemic limb.

Electron microscopic study of the regeneration process (150 days) of mast cells

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At the 1971 Congress of the Hungarian Physiological Society the authors reported on a so far undiscovered histamine liberating effect of ichthammol, a drug known since more than a hundred years. In addition, they demonstrated this drug to possess an H-substance depleting effect when applied repeatedly. In the present study the biological and light microscopic investigations have been completed by electron microscopic methods. The authors observed the mast cells in the visceral peritoneum of untreated rats to blow up upon the effect of ichthammol and to release their H-substance containing granules. It was found that, in the course of a model experiment similar to a 10-day clinical treatment, the mast cells will be exhausted and disintegrate. Morphological regeneration of the decayed mast cells took about 150 days in the rat under experimental conditions.

In the present experiments it has been attempted to obtain further evidence that ichthammol was a mast cell specific substance and that its empirically discovered antiphlogistic action was due to a depletion of the H-substance content of the mast cells. The lasting clinical symptom and compalint-free period ensured by ichthammol, similarly based on empirical observations, might be ensured by the long regeneration time of the mast cells, and presumably, by the decreased reactivity of the regenerating mast cells.

Some characteristics of the circulating primary cell

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The haematopoietic primary cell occurs in the circulation also under physiological conditions.

The effect of some agents capable of inducing leukocytosis (phytohaemagglutinin, bacterial endotoxin) has been investigated on the number of circulating primary cells. Both agents were found significantly to elevate the primary cell count. The phenomenon was dose-dependent. Maximum outflow occurred 3 to 4 days after the treatment.

The kinetics of primary cell and leukocyte outflow was different, a finding suggesting that the primary cell count is regulated separately from the regulation of the leukocyte level.

Relationship between the indices of aerobic capacity of sportsmen

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Aerobic capacity was measured as the maximum oxygen uptake during vita maxima load on the moving band. The lactacid component of anaerobic energy was measured by the maximum performance (mkp) of running up stairs 35° in steepness. For the measurement of the lactacid component, the time of the standard work leading to exhaustion in about 30 to 60 seconds was used on the one hand and the base deficiency measured during this procedure on the other.

The three physiological energy components were independent in the groups of various training degree, i.e. they did not change in the same direction during the training period, a fact pointing to the high specificity of training adaptation.

Perinatal reproduction disturbances evoked by parafornical hypothalamic lesions in the rat

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It has been shown earlier that an experimental lesion of the parafornical region of the lateral hypothalamus caused severe disturbances in maternal behaviour and in foetal viability during the last third of pregnancy in the rat.

In the present experiments three questions have been investigated. a) How does a reduction by 40 to 50% of the area of the previously effective electrolytic lesions influence the food and water consumption of pregnant rats, the development of maternal behaviour as well as the still-birth ratio and vitality of the newborns? b) How does the prolactin and STH content of the maternal pituitary change? c) How does the light and electron microscopic picture change in the maternal and foetal endocrine organs? The 34 rats investigated were divided in three groups (intact, sham-operated and lesioned in the lateral hypothalamus).

It was found that the previously observed decrease in food and water consumption as well as the disturbance of maternal behaviour manifested themselves also after parafornical lesions of lesser extent. Vitality of the newborns showed a less damage than in the earlier experiments. Lesions in the lateral hypothalamus significantly increased the prolactin content of the maternal adenohypophysis while the STH content was left unchanged. In the lesioned group the histological picture of the maternal adrenals showed an increased, while that of the newborns a decreased, ACTH effect.

Conditioned evoked potential in neuronally isolated cerebral cortical slab

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The possibilities of the building-up of a learnt electric sign, a delayed conditioned evoked potential, have been investigated in cortical slabs isolated from the sigmoid and suprasylvian gyri of the cat.

Delayed successive double stimulation was carried out by means of paired intracortical bipolar electrodes. Records were taken through ball electrodes placed on the cortical surface. It was observed that 1. the stimulus threshold of the isolated cortex slab decreased as compared to that of the intact one, and the amplitude of the evoked potential increased considerably. 2. The conditioned evoked potential developed after 200 pairings in the isolated cortical slab just as it did in the intact cortex. The conditioned evoked potential built up in the intact cortex persisted after isolation. 3. The conditioned evoked potential of the isolated cortical slab could be extinguished and built up again.

Investigation of the immobility reflex in the frog

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In previous experiments the authors analyzed the mechanism of tonic immobility evoked by electrical stimulation of the brain and by conflict situation. The present paper deals with the first results of investigations into the mechanism of immobility reflex ("animal hypnosis") evoked by other procedures. Elicitability and maintenance of the reflex were influenced by several factors. After painting the skin with 0.5% acetic acid it was more difficult to evoke the reflex and the time of immobility was shorter than in the controls. Procaine facilitated the elicitability of the reflex, and the animals remained still for a longer period of time. Upon the effect of adrenaline it was more difficult to elicit the reflex and the immobility was shorter. Acetylcholine had the opposite effect. The immobility period was shortened by atropine and lengthened by prostigmine. Data in the literature contrasting the present findings are explained on the basis of the observation that all the investigated drugs cause, at a toxic dose level, a prolonged but flaccid immobility.

Effect of the colostral trypsin inhibitor on digestion in the newborn pig

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The age-dependent changes in the colostral trypsin inhibitor, the digestive enzymes and the histological picture of the intestinal epithelium were investigated in the small intestine of piglets up to the 8th day of life. The activity of the following enzymes was determined: trypsin, the trypsinlike enzyme insensitive to soya trypsin inhibitor, the soya trypsin inhibitor-sensitive and insensitive chymotrypsins, the enzyme metabolizing caproyl salicylate, amylase and lipase. It was found that during the first two days of life, the amount of colostral trypsin inhibitor rapidly decreased in the intestines and, after two days, it could not be demonstrated any more (period of nil proteolysis). The proteolytic enzymes appear successively in a definite order beginning with those enzymes which are not inhibited by the colostral trypsin inhibitor. At the time when the trypsin inhibitor content of the colostrum decreases to traces, the spectrum of proteolytic enzymes is completed to the set characteristic of suckling animals. At the same time the eosinophilic drops indicative of the adsorption of non-digested protein disappear from the epithelial cells of the intestine. The investigations support the assumption

that the colostral trypsin inhibitor exerts a protective effect on the colostral antibodies to be absorbed. This protective effect is, however, a partial one; certain proteolytic enzymes are able to function in the presence of the colostral trypsin inhibitor.

The pulmonary surfactant

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Electron microscopic investigations revealed the alveoli of the lungs to have a continuous epithelial lining. In this are located the type I flattened pneumocytes intermingled with cubic cells displaying osmiophilic granules in their cytoplasm (type II pneumocytes). The connection between type I and type II pneumocytes manifests itself through desmosomes. The product of the type II pneumocytes forms a layer lining the alveoly; this is the pulmonary surfactant. THANNHAUSER et al. (J. Biol. Chem. **1946**, 166: 669–675) were the first to demonstrate the surfactant to consist of diplamityl lecithin.

The surfactant has an important role in the maintenance of the stability and patency of the alveoli. The layer inhibits fluid flow from the capillaries onto the alveoli, protects against infections and has a significance in the binding and degradation of vasoactive substances.

The tri-complex flocculation procedure has been used for the electron microscopic demonstration of the surfactant. The main principle of the method is that the phospholipid forms an insoluble complex upon the effect of lead nitrate and potassium ferricyanide. By means of this technique the presence of surfactant layer in the lung of young rats has been demonstrated.

Characteristic concentration membranes could be produced in the lumen of the tubular lung adenoma caused by urethan in white mice. This observation shows that surfactant production may not only diminish but also increase.

Accumulation in human aortic plaques of a fluorescent pigment resembling lipofuscin

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In accordance with data in the literature, it was found previously that the amount of fluorescent substances in the vascular wall increases in the old age as well as in the course of atherosclerosis. The authors succeeded in isolating from atherosclerotic aortas a lipoproteinlike substance called atherofluorescent component (AFC; BANGA, Acta morph. Acad. Sci. hung. **1968**,

16: 23; BANGA, Intern. Symp.: Biochemistry of the vascular wall. Fribourg. Part II. **1969**, 18.). Identification of this substance was rendered difficult by two features. First, the fluorescence of the age pigment lies at A/F 290/340 (LABELLA, J. Geront. **1962**, 17: 8.; LABELLA and LINDSAY, J. Geront. **1963**, 18: 111.) though this fluorescence is characteristic of tyrosine. Second, the yellow fluorophloric substance in the elastin is an ampholyte which exchanges a reversible way on dextran cation and anion resin between pH 2.5 and 9.5 (THORNHILL, Connect. Tiss. Res. **1972**, 1: 21.). The present paper reports on the successful isolation by repeated lipid extraction a yellow, strongly fluorescent substance from the elastolysates of human aortic plaques. This substance is not ampholytic and resembles lipofuscin. When dissolved in a mixture of chloroform–methanol (2 : 1) it exhibits maximum fluorescence at 450 nm just as do lipofuscin and it is sudanophilic. A further characteristic of the substance is its strong absorption at 275 nm, similar to that of desmosines. The purified substance is poorly soluble in water.

Effect of adrenergic receptor stimulants and inhibitors on the oxygen tension of the lymph

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In previous experiments it was shown that information could be obtained as to the average pO_2 of the splanchnic region by continuous measurement of the pO_2 of thoracic duct lymph. It was shown further that vasoconstrictor drugs of different site of action are influencing the pO_2 of lymph in different directions. The present experiments deal with the question of how adrenergic receptor stimulating and blocking drugs influenced the pO_2 of the lymph. It was found that a noradrenaline infusion (0.5—1.0 μg/kg/min) increased the pO_2 of the lymph while phenylephrine was practically ineffective. Infusion of isoprenaline augmented the pO_2 of the lymph. When noradrenaline was given after an alpha blocking drug, it retained its pO_2 elevating effect while this effect was considerably decreased after pretreatment with a beta blocking drug. The observation seems to indicate that the increasing effect of noradrenaline on the pO_2 of lymph might be the result, at least in part, of the slight beta-mimetic effect of the drug.

Some immunological problems of the rat's Yoshida sarcoma

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Several authors have made human chorionic gonadotrophin (HCG) responsible for the immunological changes during pregnancy. There are also observations that HCG given to an animal with malignant tumour will cause a rapid proliferation of the tumour and an increase in the metastatic rate.

In the present experiments HCG (2×600 I.U.) was given intramuscularly to rats with Yoshida sarcoma. Sixteen days after the inoculation the tumour was significantly smaller in the treated than in the control group (mean tumour weight of 20 treated rat was 0.815 g, against the control 5.1 g). In contrast, 15 I.U. of FSH applied under identical conditions caused a significant increase in tumour weight (mean, 9.99 g).

No significant difference was found between the individual groups in the enzyme system (NAD^+, MDH, GOT, LDH), in the electrolyte (Na^+, K^+, Cl^-) content of serum and of blood corpuscles, in the total protein level, in the blood counts, in the blood sugar level, in pH and in oxygen saturation. However, significant differences occurred in these parameters as compared with normal values.

Effect of acute and chronic sodium load on the intrarenal distribution of blood flow

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Acute sodium loading was performed in rats by infusing 20 ml/kg or 50 ml/kg of physiological saline. In the case of a chronic salt load the animals were made to drink physiological saline for three weeks, or they were kept on a diet containing 3.5% NaCl with water ad libitum. Renal-, cortical- and medullary blood flow was estimated by means of the isotope fractionation method.

Renal medullary blood flow increased during acute and chronic loads in absolute value as well as in comparing with the cortical flow. The increase in the medullary circulation is considered as a volume regulatory reaction. It can be elicited by minute changes in extracellular (inulin) and sodium space even if it is not to be demonstrated methodically (chronic sodium load).

The role of haemodynamic factors in the pathogenesis of post-ischaemic renal failure

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"Acute oliguric renal failure" was evoked by temporary compression of the renal artery in the dog. The resistance of series-connected vessel segments was measured and calculated on the basis of determining intrarenal pressures and blood flow, while that of parallel connected vessel segments by means of the rubidium fractionation technique. According to the data obtained, the functional organization of the "postischaemic" kidney displays the following characteristics.

1. Total vascular resistance does not change in mild and medium severe cases, it slightly increases in severe cases.

2. The ratio of series-connected vascular segments undergoes a decisive change; the afferent resistance increases and the efferent resistance decreases.

3. In the case of such a change of the afferent–efferent resistance with unaltered or slightly elevated total resistance, the glomerular capillary pressure decreases in the steady state. The extreme diminution of the glomerular filtration rate is a consequence of the effective filtration pressure approaching zero.

4. On the basis of the gross necrotic areas, the diminished Tm_{PAH} values, as well as the rubidium and oxygen extraction, the conclusion has been drawn that a considerable percentage of the blood flows through non-functioning inert renal tissue.

Effect of caffeine on glucose metabolism in different rat strains

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The effect of glucose, caffeine, and glucose plus caffeine loads has been studied in male and female rats of different strains between the age of 6 to 12 months. On the basis of the blood sugar curves obtained in NI (own bred) and R-Amsterdam (R-A) strains, the 6 months old R-A females proved to be the most sensitive to caffeine. The worst glucose tolerance was exhibited by 1 year old R-A females. A combined caffeine and glucose load led to a diabetoic blood sugar curve in all R-A females. No pathological value was found in any of the members of the own bred strain.

The problem is discussed from the point of view of nutritional physiology as well as in respect of the relationship between the manifestation of diabetes and caffeine consumption.

Sodium excretion and renin production of the denervated kidney during furosemide diuresis

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Unilateral splanchnicotomy ("kidney denervation") is followed by considerable natriuresis and decreased renin production without changes in the filtration rate or blood flow both in control and in isotonic volume expanded dogs (BENCSÁTH et al., Europ. J. clin. Invest. **1972**, 2: 422—425). In order to investigate the relationship between sodium transport and renin release, dogs subjected to unilateral splanchnicotomy, were anaesthetized with pentobarbital and the renin secretion of both kidneys was estimated by Boucher's method during diuresis elicited by 5 mg/kg of furosemide injected intravenously.

Filtration rate and blood flow were not different in the intact and the denervated kidney even during furosemide saluresis. The 130—180% increase of sodium excretion seen in the denervated kidney in the control state or in volume expansion, disappeared upon the effect of furosemide, but renin production remained lower than that of the intact kidney. Thus, sodium excretion and renin production of the denervated kidney are not necessarily interrelated.

Body temperature in experimental cerebral oedema induced by triethyl tin sulphate (TET)

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The organic tin compounds used in the synthetic industry may cause severe poisoning accompanied by cerebral oedema, and they not infrequently lead to death. Practically no data are at disposal as to the mode of action of TET; its effect is explained by the development of selective cerebral oedema. The present experiments aimed at obtaining data concerning the mode of action of TET. The body temperature of the experimental animals was observed through 24 hours after the administration of TET. Other parameters (metabolic rate, blood pressure, EEG, etc.) were also recorded during the experiments.

TET considerably decreased the core temperature of the experimental animals in an environment of 20°C, a feature accompanied by changes of other autonomic functions. A close relationship was found between the dose of TET and the extent of the autonomic changes. The effect of TET manifests

itself in two easily distinguishable phases. After intraperitoneal injection (6 to 8 hours), an immediate toxic phase sets in. This is followed by a transient normalization of the autonomic functions between 24 and 72 hours. Thereafter new disturbances appear in the autonomic functions, in addition to the development of cerebral oedema. Impairment of the heat regulation is accompanied by disturbances of other autonomic functions.

Investigation of metabolic products in toxaemia of pregnancy

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Metabolic disturbances in toxaemia of pregnancy can be investigated in humans only, and these investigations should be based on the analysis of body fluids.

Serum lactic acid, pyruvic acid, citric acid, uric acid and carbamide levels were determined in healthy, non-pregnant subjects as well as in healthy and toxaemic subjects with 39 to 47-week pregnancy.

The most characteristic changes during toxaemia of pregnancy were found in the serum uric acid and carbamide level, the uric acid level being 100% and the carbamide level 20 to 30% higher than in normal pregnants. Since the elevated serum uric acid level is a sign of increased nucleic acid destruction, and the elevated carbamide level is mainly the consequence of a beginning renal failure, determination of the ratio uric acid : carbamide allows conclusions as to the pathomechanism of the toxaemia of pregnancy.

It seems from the results that severe disturbances of the most different processes of the intermediary metabolism occur in toxaemia of pregnancy and especially in its severe forms.

Effect of denervation on transcapillary fluid flow in haemorrhagic shock

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Capillary fluid transport during haemorrhagic shock has been investigated by means of measuring the capillary filtration coefficient (CFC) in the limb musculature of cats anaesthetized with chloralose–urethan.

CFC was found to decrease to 57% of its initial value in the second bleeding period of standardized haemorrhagic shock, at a blood pressure value of 35 mm Hg. In order to elucidate the mechanism of this decrease, ex-

periments were performed on denervated muscle preparations in which a polyethylene tubing was inserted between the femoral and popliteal arteries. Under such conditions the CFC did not decrease below the initial value even in the second period of haemorrhagic shock; it averaged 118% during the 90 minutes ($0.05 > p > 0.02$).

Since denervation counteracts the marked and irreversible diminution of CFC in the second period of haemorrhagic shock, it is assumed that the severe alterations of capillary fluid transport are probably the consequences of changes in the functioning of the sympathetic nervous system.

Influence of tetrabenazine on the analgesic action of tremorine and oxotremorine

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The characteristics of the interaction between amine depletors and tremorine and oxotremorine, respectively, has been the subject of much discussions in the literature. Tremorine and its metabolite, oxotremorine, have a strong analgesic action. This effect in the mouse as measured with the hot plate method, was differently influenced by a 1-hour intraperitoneal pretreatment with 10 mg/kg of tetrabenazine; the tremorine effect (5 mg/kg intraperitoneally) was inhibited and the oxotremorine effect (50 μg/kg intraperitoneally) was enhanced. The antagonistic effect of tetrabenazine against tremorine is probably of peripheral origin and due to an inhibition of the metabolic transformation in the liver. This is supported by the finding that proadifen, a drug similarly having liver damaging properties, also antagonizes the effect of tremorine. The amine-depleting effect of tetrabenazine has a part in the synergism between tetrabenazine and oxotremorine. This is proved by the finding that 2 mg/kg of amphetamine, given together with 50 μg/kg of oxotremorine, inhibited the potentiating effect of tetrabenazine.

Effect of cyclophosphamide on the insulin-induced sensitization of anaphylactoid inflammation

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It has been shown previously that the insulin-induced sensitization of anaphylactoid inflammation is counteracted by drugs inhibiting protein synthesis. In the present experiments cyclophosphamide was used, since this

drug, unlike the agents studied earlier (actinomycin D, cycloheximide), did not significantly inhibit dextran oedema even 24 hours after the administration, while it prevented the insulin-induced sensitization of anaphylactoid inflammation. It was found that a single injection of 100 mg/kg of cyclophosphamide caused a long-lasting inhibition of the insulin response. The inhibition ran parallel with the lymphopenia induced by the drug. Administration of cell suspensions prepared from lymph nodes, spleen and thymus of normal rats restored the sensitizing effect of the hormone. Furthermore, it was studied how different doses of insulin influenced the inflammatory response induced by dextran in young and aged rats. It was found that in young rats insulin considerably increased the dextran-induced inflammatory response, while aged animals failed to respond to the hormone. It is concluded that lymphoid organs play an important role in the insulin-induced sensitization of anaphylactoid inflammation.

Effect of lipid metabolites on the electrophysiological parameters of the heart

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It has been shown recently that the free fatty acids and glycerol released during the increased lipolysis accompanying adrenergic stimulation have a direct arrhythmogenic effect and enhance the development of cardiac arrhythmia after coronary occlusion (KURIEN et al., Lancet **1969**, 2: 185.; CARLSTRÖM and CHRISTENSEN, Brit. Heart. J. **1971**, 33: 884.). On this basis it seemed interesting to study the electrophysiological effects on the heart of some fatty acids and of glycerol. According to the results obtained by the application of short carbon-chain fatty acids in the isolated right and left atria of the rabbit, caprylate (octanoate) decreased conduction velocity, cardiac excitability as well as the spontaneous and maximal driving frequency. The duration of extracellularly recorded action potentials was prolonged and their amplitude decreased. Among the long carbon-chain unsaturated fatty acids, oleate similarly decreased the excitability of the heart muscle and the maximum driving frequency. Both caprylate and oleate markedly decreased the contractility of the heart muscle. Glycerol only decreased contractility and failed to influence the electrical activity of the heart muscle. The effective concentration of all three metabolites was about three times higher than the physiological free fatty acid and glycerol level of the serum. It seems that the augmentation of free fatty acids may contribute to the development of cardiac arrhythmia primarily by causing disturbances in conductivity, local blocks and, perhaps, by decreasing sinus dominance.

Serotonin level in resting and functioning salivary glands of the rat

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Serotonin is known to occur in the tissues of several species. As to its role in the fluid secretion of the salivary glands, only assumptions are at disposal. Serotonin probably increases secretion by stimulating adenylcyclase, the enzyme synthetizing cyclic AMP from ATP.

In the lack of pertaining data, the first aim was to determine the 5-HT content of the parotid and submandibular glands, and to observe the 5-HT level under the effect of various stimuli inducing secretion. 5-HT was estimated fluorometrically according to a modification of the method of SNYDER, AXELROD and ZWEIG.

The 5-HT level of the parotid and submandibular glands was found to vary between 2 and 4 μg/g tissue in the resting state. After a 72-hour starvation period a 30-min feeding decreased the 5-HT level in both glands just as did the intraperitoneal injection of 2.5 mg/100 g of pilocarpine 30 minutes following its administration. Isoprenaline was used as sympathetic stimulant (2 mg/100 g of body weight); 45 minutes after its intravenous injection no significant change was found in the 5-HT content of the glands.

It is concluded that serotonin may play an active part in the secretory activity of the glands.

Induction of ovulation by LH-FSH releasing hormone in androgenized female rats (treated with testosterone propionate at newborn age)

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Newborn female rats were treated with testosterone propionate (10 μg, 50 μg, 100 μg/animal) on the second day of life. After the opening of the vaginal orifice vaginal smears were taken daily in order to control the development of constant oestrus. After a 2-week observation period the animals were treated with synthetic LH-FSH-RH [(pyro) Glu-His-Trp-Ser-Tyr-Leu-Arg-Pro-Gly-NH_2] by the subcutaneous route. The doses used were 1.14 nM/kg, 11.4 nM/kg and 111.4 nM/kg.

The number of fresh corpora lutea of ova in the tuba were counted 24 hours after the treatment. The ovarium was in 4% formol for histological investigation.

While the ratio of ovulating rats increased with increasing dose of the decapeptide, the ovulation ratio of animals androgenized with 100 μg and 1000 μg of testosterone propionate decreased.

The observation that ovulation can be induced in the polycystic ovaria in the androgenization syndrome by the subcutaneous administration of synthetic LH-FSH releasing hormone, promises new possibilities in the treatment of the anovulation sterility.

Physiological significance of the intermediary form of muscle phosphorylase

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At previous Meetings of the Hungarian Physiological Society the production and purification by crystallization of the so-called intermediary phosphorylase-a, an enzyme between the inactive b form and the active a form of phosphorylase has been shown.

According to recent investigations, caffeine markedly inhibits both forms of phosphorylase. However, in the presence of AMP and in the case of an appropriate caffeine–AMP ratio only phosphorylase-b is inhibited while phosphorylase-a and the intermediary enzyme are left unaffected. The method allows to study the production of intermediary-a without isolation.

It is shown that the production in vitro of intermediary-a depends on the ratio of phosphorylase-b and phosphorylase-a; its activity is increased by AMP, and inhibited by glucose-6-phosphate and glucose. The most important feature is that its activity is increased by AMP but, unlike phosphorylase-b, it is not inhibited by ATP.

Intermediary-a is produced from phosphorylase-b in the skeletal musculature of the rat under the effect of intraperitoneal adrenaline. Similarly, intermediary-a production was observed in the femoral muscle of the frog upon the effect of electrical stimulation. It seems that, under in vivo conditions, exclusively intermediary-a is produced independently of the extent of stimulation. This intermediary-a too shows a high degree of sensitivity to allosteric affectors.

The physiological significance of intermediary phosphorylase-a is discussed.

Movement of isolated limb pairs in the axolotl

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On the basis of previous experimental data, the brachial segment of the spinal cord has been assumed to have a peculiar structure which is capable of moving one limb in a co-ordinate manner also without afferent information from supraspinal centres and the limb. Control of the bilaterally alternating movement co-ordination characteristic of the limb pair seems to be more intricate. The movement co-ordination of six-legged axolotls developed from longitudinally interlaced embryos suggests that the characteristic diagonal co-ordination of the forelimbs and hindlimbs during walking is controlled by the lumbosacral segments or the supraspinal centres.

To decide this question, axolotl embryos amputated in various degrees were brought into parabiosis with intact embryos so that the parabiotic pair consisted of 1. the brachial segments of the spinal cord and the forelimb pair; 2. medulla, brachial spinal segments and forelimb pair; 3. lumbosacral spinal segments and hindlimb pair; 4. whole spinal cord and forelimb plus hindlimb pair. The results suggest that the lumbosacral spinal segments are by themselves capable of controlling the alternating movement of the hindlimb pair while the brachial spinal segments are capable of governing this limb movement only when they are in connection with the medulla.

Effect of clofibrate on the diuresis and ADH-sensitivity of rats with pituitary stalk lesion

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The therapy of diabetes insipidus is not completely solved. Most recently the oral antidiabetic chlorpropamide has been applied successfully in the treatment of ADH-sensitive diabetes insipidus. However, in view of its hypoglycaemic side effect this treatment has not widely been accepted. Now French authors have reported that the antilipaemic agent clofibrate had antidiuretic properties.

The investigations were performed on ten ADH-sensitive patients possessing ADH reserve. Clofibrate significantly decreased spontaneous diuresis, increased the osmolality of urine and markedly diminished the free water clearance. Endogenous creatinine clearance, serum osmolality and the osmolar

clearance remained unchanged. In one case, clofibrate failed to affect the water household; in this pateint no ADH reserve could be demonstrated.

In rats with pituitary stalk lesion, clofibrate failed to decrease diuresis in the continuous polyuric phase. In contrast, the oliguric interphase which can be explained by an ADH excess, became more marked and prolonged under the effect of the drug.

Two-day pretreatment with clofibrate considerably increased ADH activity together with diuresis in female rats anaesthetized with ethanol.

The results suggest that clofibrate exerts its antidiuretic action by potentiating the ADH effect.

Some characteristics of the action potential of the motor neurone in the frog

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On the basis of previous results obtained with intracellular recording it has been assumed that several trigger zones were initiating the mechanism of after-discharge on the dendrites of the motor neurone in the frog. In the present experiments the mechanism of spreading of the locally initiated discharge process has been analyzed. It seems that, under certain conditions decreasing cellular excitability in a reversible manner, spreading of the discharge is slowed down and its fractionated character is unmasked. The supernumerary deflections appearing at unusual places on the oscillograms seem to prove that the partial asynchronous activation of electrically excitable sites on the cell surface are even if somewhat slowly capable of evoking, through mutual support and addition, the generalized cell discharge.

Since these sites seem to be identical with the trigger zones, the spreading mechanism described supports the earlier assumption that discharge can be initiated on the membrane of these cells not only from one but from several places. According to the data obtained, the motor neurone of the frog might be ranged with those few neurones to which similar abilities have been ascribed by recent data in the literature.

Development of anaphylactoid hypersensitivity after adrenocortical failure due to *Bordetella pertussis* vaccine

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Bordetella pertussis vaccine (BPV) considerably augments the sensitivity of rats against anaphylactoid shock and the shock mediators (histamine, 5-hydroxytryptamine). Previous studies on histamine metabolism raised the possibility that a reversible failure of the adrenal cortex might develop after BPV treatment.

The present experiments revealed that on the 4th and 8th day following BPV administration, i.e. at the time of maximum augmentation of sensitivity, the plasma corticosterone level decreases significantly an alteration ceasing on the 18th day. Pretreatment with dexamethazone decreases the plasma corticosterone level to a very low value both in the control and the BPV-treated animals. In rats pretreated with dexamethazone, ACTH elevated the corticosterone level in BPV-treated animals just like in control rats.

Effect of Ca and La ions on some characteristics of the sarcoplasmic reticular fraction

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The experiments were performed with sarcoplasmic reticular fraction (SRF) prepared from fish *(Aemiurus nebulosus)* muscle. The effect of Ca and La ions has been investigated on native SRF preparations as well as on preparations first treated with desoxycholate (DOC) and filtered on gel.

La ions at concentrations from 10^{-4} to 10^{-3} M considerably decreased the Ca uptake and the cholinesterase activity of the native suspension as well as the Ca-reactivity of ATPase. There was a several-fold increase in the extinction at 280 μm. 10^{-6} to 10^{-5} M of La^{+++} was practically ineffective in all the above tests. Ca ions, even at a concentration of 10^{-1} M, caused only a minimum decrease in cholinesterase activity and a minimum increase in extinction.

In DOC-treated and gel-filtered preparations La^{+++} at concentrations of 10^{-6} to 10^{-4} M increased but at higher concentrations decreased, cholinesterase activity. The maximum increase in extinction under effect of La^{+++} occurred at concentrations from 10^{-3} to 10^{-2} M, where cholinesterase activity decreased to the minimum. 10^{-4} to 10^{-1} M of Ca slightly increased cholinesterase activity. The maximum occurred at 10^{-1} M. An increase in extinction in the presence of Ca was observed only above 10^{-2} M.

Effect of chronic ACTH treatment on lipid metabolism

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It has been shown previously that the serum corticosterone level of ACTH-pretreated animals undergoes a change under the effect of acute ACTH and stress, respectively. The phenomenon was assumed to be caused by an inhibition of endogenous ACTH mobilization from the pituitary, the increased elimination of steroids and an altered reactivity of the tissues.

In the present experiments the mechanism of the above question has further been analyzed by investigating the lipid metabolism of rats pretreated with ACTH.

Rats pretreated 24 hours earlier with a low dose of ACTH have been exposed to ACTH treatment and stress, respectively. The blood fatty acid level was found to exhibit a greater increase in the treated than in normal animals. After pretreatment with a high dose of ACTH neither ACTH nor the stress employed (formalin, histamine, swimming) was increased the serum fatty acid level.

Investigations in progress will have to decide whether or not peripheral changes in lipid metabolism are also figuring in the development of the phenomenon.

Endotoxin and catecholamines

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The vasopressor effect of catecholamines injected rapidly by the intravenous route was investigated in rabbits pretreated with E. coli endotoxin. Low doses of the endotoxin potentiate the response to catecholamines within one hour. Upon the effect of high endotoxin doses one can observe a hyperreactivity to catecholamines, in addition to hypotension. In general hypothermia the blood pressure elevating effect of noradrenaline decreases, while that of adrenaline increases. Hypothermia counteracts both the secondary hypotonic phase which follows the administration of higher doses of adrenaline and the post-endotoxin hypotension. Also cooling counteracts the catecholamine-potentiating effect of the endotoxin.

Proteases are known to play an essential part in endotoxin shock. As it is possible to elicit a cardiovascular shock of the protease type by trypsin, the authors investigated the effect of trypsin on the sensitivity to catecholamines. It was found that the vasopressor effect of both noradrenaline and adrenaline was augmented after trypsin pretreatment.

Effect of nucleotide and orotic acid treatment on the incorporation of ^{14}C-stearinic acid in animals kept on Hartroft diet

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It has been shown previously that the administration of nucleotides and orotic acid, the precursor of nucleic acid, was able to normalize the high serum and liver lipid levels induced by a dietetic lipid load.

The present investigations aimed at elucidating the mode of action. ^{14}C-stearinic acid was given intravenously to non-starving animals. Five minutes later significantly less ^{14}C-stearinic acid was found in the liver of the groups treated with nucleotide. The incorporation of activity decreased in the neutral lipid fractions (TG, ChE). A DNS increase proportional to the enlargement of the liver was found.

The phenomenon is explained by active cell proliferation and by increased lipid catabolism.

The effect of ^{75}Se-methionine on thrombocytopoiesis in actinomycin-D-treated mice

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The platelet count of mice undergoes a marked diminution upon the effect of 15 μg actinomycin-D given subcutaneously. The thrombocytopenia reaches its deep point on the 5th day after the injection, being 80 to 90% lower than the initial value. When serum taken from the animals at this point of time is injected into normal mice, this intervention results in a considerable increase of the platelet count with a maximum similarly appearing on the 5th day following the injection.

In the present experiments the incorporation of ^{75}Se-labelled methionine into the platelets of the mouse has been investigated by the original method of PENNINGTON, described in 1969—1970. The ^{75}Se-methionine uptake of the platelets of animals treated with actinomycin-D increased considerably after an initial diminution, and was significantly higher than that of the control animals. A similarly increased activity was found under the effect of the serum of actinomycin-D-treated mice as compared with the incorporation found in animals treated with normal mouse serum. It is essential to choose the right interval between the intervention aiming at changing the blood count (actinomycin-D or the serum of mice treated with this drug), the labelling with ^{75}Se-

methionine and the measurement of the activity. In general, the most suitable time for measuring the incorporation rate of the radioisotope seemed to be the 48th and 72nd hour as well as the 5th day after the injection.

Veratrine-induced rhythmic changes of the muscle membrane

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In the course of experiments studying the depolarizing effect of veratrine it has been observed that under the effect of the drug, the membrane potential of the muscle shows distinct rhythmic changes, the peak-to-peak value of which is about 20 to 30 mV. In the present experiments the mechanism of the rhythmic potential changes has been investigated. Tetrodotoxin, at a concentration of 10^{-7} g/ml, causes a nearly complete repolarization of the already depolarized membrane exhibiting rhythmic oscillations. At the same time, it also abolishes the oscillations in a reversible manner. Lowering the sodium concentration of the Ringer's solution leads to decreased oscillation frequency. When the total sodium content is replaced by choline, the membrane will be repolarized and the oscillation will cease even in spite of the presence of veratrine. All these findings unequivocally point to the sodium-dependence of the phenomenon.

The veratrine-induced rhythmic oscillation of the membrane potential is abolished by treatment with monoiodoacetic acid (1 mM), sodium fluoride (10 mM) and ammonium chloride (10 to 20 mM), as well as by cooling the incubating Ringer's solution below 10°C. It is assumed that the oscillation of the membrane potential might bear some connection with the metabolic processes active in the inside of the fibre.

Analysis of the vasopressor effect of oxotremorine and arecoline in the rat

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The parasympathicomimetic drugs arecoline and oxotremorine lower the blood pressure of rats anaesthetized with urethan. After pretreatment with the peripheral parasympathicolytic atropine methylbromide, they cause a rise of blood pressure.

The pressor effect of both compounds is of central origin. It can be prevented by lesioning the spinal cord or by cervical transection, but not by previous removal of the adrenals. Atropine sulphate inhibits the pressor

effect of both compounds, that of oxotremorine in lower doses than that of arecoline.

The pressor reflex is mediated via the sympathetic nervous system; it is inhibited by the ganglionic blocking agent mecamylamine and by the alpha receptor blocking drug phentolamine.

The mode of action of the two compounds in the central nervous system is not identical. Morphine, given intravenously, inhibits only the oxotremorine-induced vasopressor response but not that evoked by arecoline. Hexamethonium and atropine methylbromide injected into the lateral ventricle inhibit the oxotremorine effect more strongly than the arecoline effect. It is assumed that central nicotinic and muscarinic receptors are also figuring in the development of the pressor response.

Some effects of catecholamines after microinjection into various subcortical regions of freely-moving cats

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An adrenergic modulatory system was demonstrated to be present in certain subcortical brain areas of the cat. Microinjection of noradrenaline, dopamine, isoprenaline, methoxamine, phenylephrine as well as of various adrenolytics in various parts of the brain revealed this system to exert a profound modulatory influence on the characteristic emotional-behavioural (rage) reaction which can be evoked by direct cholinergic stimulation of certain subcortical brain areas. This modulatory influence means, in all cases, an inhibition either locally or from some more remote, but functionally involved, region. Also, the autonomic and EEG effects of cholinergic stimulation are partially or fully abolished by simultaneous adrenergic stimulation. The modulatory system consists of beta-type adrenergic receptors and involves, in addition to certain parts of the limbic system, the intralaminary cell group of the thalamus, the red nucleus and probably also the caudate nucleus.

The role of adrenergic receptors in the anaphylactoid hypersensitivity of the smooth muscle of the upper respiratory tract

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It has been demonstrated in previous experiments that the responsiveness to adrenergic and cholinergic stimulants of the smooth muscle of the upper respiratory tract is altered during anaphylactoid hypersensitivity. Namely,

alpha-receptor excitation appears in addition to the beta-adrenergic response, and the sensitivity to acetylcholine (ACh) also increases. It was shown that ACh inhibited the effect of beta stimulants. In the present experiments the histamine–beta-adrenergic interaction has been compared with the ACh–beta-adrenergic interaction.

Dose–effect curves were drawn simultaneously from two tracheal rings of the same animal (guinea pig) with isoprenaline (Iso) during ACh contraction and during histamine contraction which was roughly identical in magnitude with the ACh contraction. The investigations were repeated after the administration of 0.1 μg/ml, 0.2 μg/ml and 0.5 μg/ml of propranolol. Propranolol was found to inhibit the Iso effect significantly stronger during ACh than during histamine contraction. These changes were more marked in animals with anaphylaxis than in normal ones.

A specific connection can be assumed to exist between the cholinergic and beta-adrenergic systems (receptors). This interaction may figure in the development of the anaphylactoid reaction type. With the decrease in number of the beta receptors in anaphylaxis the predominance of the cholinergic system becomes even more conspicuous.

Subcellular distribution of phospholipids in tonic and tetanic muscle

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There is no marked difference in the percentage distribution of the phospholipids in the muscle "stroma", myofibrillar protein or mitochondria, and the sarcoplasma-mitochondrial subcellular fractions of tonic and tetanic muscle in spite of the finding that the P-lipid content is different in the various fractions. In the united sarcoplasma-microsomal fraction both the choline-containing and the non-choline-containing phosphatide contents are higher in tonic than in tetanic muscle. However, in the three other subcellular fractions, only the non-choline phosphatide level is higher in the tonic than in the tetanic muscle, just as is the case with the whole muscle.

The fatty aldehyde content of the choline-phosphatides is roughly identical in all subcellular fractions of both muscles except the mitochondrial fraction. Within the muscle the fatty acid content of the non-choline phosphatides is the same in all subcellular fractions. There is, however, an essential difference between the two kinds of muscle: the fatty aldehyde content is higher in the subcellular fractions of the tetanic muscle whereas the noncholine phosphatide level is considerably lower.

Spontaneous and evoked electrical activity of the cerebral cortex, hypothalamus, amygdala and thalamus in haemorrhagic shock

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The following questions have been studied.

1. Is it due to the injury of the ventral posterolateral thalamic nucleus that the cortical potentials evoked by sciatic stimulation disappear in the early stage of irreversible haemorrhagic shock?

2. How does the spontaneous and evoked electrical activity change in the primary sensory cortex, in the VPL thalami, in the nucl. ventromedialis hypothalami and in the nucl. basomedialis amygdalae, respectively, during irreversible haemorrhagic shock?

The experiments were performed on 15 cats anaesthetized with 60 mg/kg of chloralose and immobilized with gallamine. Haemorrhagic shock was induced according to the modified technique of LAMSON.

On the basis of the results it is the first third of the second phase of bleeding (blood pressure, 30 to 40 mm Hg) which seems to be the critical period. At this time the amplitude of all evoked potentials is lower than their initial amplitude and also their forms differ from the initial wave form. In the middle third of the second phase there is no response to sciatic stimulation in either structure. At this stage, however, the sensory cortex yet responds to stimulation of the VPL thalami, and the hypothalamus to that of the amygdala. At the end of the bleeding all responses disappear and are not restored by reinfusion.

Neurophysiological investigations with an organic phosphate compound

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EEG and EMG investigations were performed with the widely used potent pesticide, malathione.

Acute malathione poisoning (300 mg/kg) causes a strong desynchronization of the EEG within the first 24 hours but high amplitude spike potentials also appear. These spike discharges become more marked upon the effect of photostimulation. The results were evaluated with a bioanalysator integrator; this revealed the integrated value of the amplitude of the EEG records to diminish under in malathione poisoning.

Electromyographic studies revealed the motor unit potentials of the gastrocnemius muscle to augment in duration without any significant change in discharge frequency. Biointegrator evaluation of the electrogram showed an increased electric activity of the muscle.

The degree of inhibition of cholinesterase activity in various parts of the brain and in blood was determined.

Summing up, poisoning with the cholinesterase inhibitor malathione causes an increased excitation of the central nervous system and lengthens the duration of the motor unit potential.

Blood flow and salivary secretion of the submandibular gland in haemorrhagic shock

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It was shown previously that during haemorrhagic shock the blood flow of the submandibular gland decreased parallel with the decreasing blood pressure down to 60 mm Hg, with no further change during further bleeding. In the present experiments the relationship between blood flow and salivary secretion of the submandibular gland has been investigated in control animals, during gradual bleeding as well as in haemorrhagic shock.

The resting blood flow of the gland as well as the changes caused in flow and secretion by stimulation of the chorda tympani (2 V, 16 Hz, 2 msec) were studied in dogs under chloralose anaesthesia. Venous outflow was measured with a drop counter and tissular circulation with a heated thermistor.

In the control period, venous outflow (20 ± 3 ml/min/100 g) exhibited an 8-fold and the tissular circulation a 4-fold increase. There was not measurable salivary secretion without stimulation; on stimulation, saliva production averaged 28 ml/min/100 g. During bleeding the circulatory responses to stimulation, as measured by both methods, decreased more markedly than did salivary secretion. After retransfusion, blood flow of the salivary gland returned to the control level and arterial blood pressure to 67% of the control. The flow change caused by stimulation of the chorda tympani reached 50% and salivary secretion 65% of the control, as measured by both methods.

Effect of phenobarbital, ethyl-p-chlorophenoxy-isobutyrate (clofibrate) and bis-p-chlorophenoxy-acetylurea (M-451) in experimental liver injury of the rat

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It was investigated how experimental liver injuries are influenced by phenobarbital (a drug inducing microsomal enzyme synthesis in the liver) as well as by drugs influencing lipid metabolism mainly through an effect on the liver.

Liver damage was induced by carbon tetrachloride, ethionine and alfa-naphthylisothiocyanate (ANIT), a drug causing intrahepatic cholestasis. For ten days before the liver damaging agent, the animals received phenobarbital (10 and 30 mg/kg intraperitoneally) or compounds decreasing the cholesterol level. These were Clofibrate ® (100 and 300 mg/kg, orally) and M-451 (10 and 30 mg/kg, orally). The extent of liver injury was judged by laboratory and morphological methods. In addition, the triglyceride and cholesterol content of the liver as well as the free fatty acid (FFA) and cholesterol level of the serum were determined.

The results showed phenobarbital to increase, while Clofibrate ® and M-451 to decrease the extent of liver injury. The effects obtained depended on the doses applied and the character of the injury.

The results are discussed in connection with the effect on lipid metabolism of the compounds investigated. Further investigations of the liver-protective effect of lipid-decreasing agents seem to be indicated.

Ultrastructural changes in the heart of *Helix pomatia* L. caused by calcium deficiency

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An important role in the mechanism of contraction is ascribed to the Ca-storing sarcotubular system of various muscles. This system has been investigated in the heart muscle of *Helix pomatia*. It was studied what physiological and ultrastructural alterations would appear under the effect of a Ca-free medium. Both electrical and contractile activity were found to cease in a Ca-free milieu. In addition, essential alterations appeared in the ultrastructure of the *Helix* heart muscle cells after perfusion with Ca-free solution for 30 minutes and two or four hours. The elements of the sarcotubular system (and the subsarcolemmal cistern in particular) are swollen, the matrix of the mito-

chondria is loosened, and the membrane structure undergoes desctruction. On the basis of the data obtained the above cell constituents are assumed to play a decisive part in intracellular calcium metabolism, and also in the connection of excitation-contraction processes in *Helix* heart muscle.

Effect of atropine infusion on histamine- and pentagastrin-induced gastric acid secretion in anaesthetized cats

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Data are contradictory concerning the effect of atropine on gastric acid secretion elicited by histamine (H) and pentagastrin (Pe). The reason for the discrepancy might be in the different manners of administration of the antagonists and the agonists, the secretion-stimulating compound being generally applied by infusion while the antagonist in the form of a single intravenous injection.

In the course of the experiments the effect of atropine has been investigated on gastric acid secretion stimulated by pilocarpine, H and Pe, respectively, in cats anaesthetized with pentobarbital. Increasing doses of the agonists were infused simultaneously with an atropine solution of standard concentration.

The dose–effect curve of all three agonists showed an inhibition under the effect of atropine infusion, although the course of the curves was different Much higher doses of atropine were necessary to inhibit the H and Pe than the pilocarpine effect.

The results indicate that vagal regulation plays a part in the effect of H and Pe, agents directly influencing the parietal cells, for its abolition alters hydrochloric acid secretion.

Effect of light on the metabolism of soluble cerebral proteins in the rat

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It has been reported at the 1971 Congress of the Hungarian Physiological Society that, with certain quantitative differences, the acid soluble proteins take up the composition characteristic of the adult rat on the 20th day of life. In the present experiments the influence of enucleation performed on the 4th day and that of photic stimulation from the time of the opening of the eyes were investigated on the cerebral protein map constructed on the basis of two-step protein fractionation (DEAE cellulose anion exchange, gel electro-

phoresis). According to the observations, the quantitative composition of proteins in the occipital region of the brain was markedly influenced by enucleation as compared with either 20-day-old or adult controls; the amount of certain more acid components was considerably decreased. No similar changes were observed in the frontal brain region. Photic stimulation at the time of eye opening markedly increased the amount of some acid proteins in the occipital region as compared with 20-day-old controls. Photic stimulation of adult animals failed to cause any changes of similar character.

Lysozyme activity of the human tonsil and its role in local protection

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In previous investigations the authors have described the ability of tonsillar lymphocytes to synthetize nucleic acid and protein and, in this connection, the inhibitory effect of emetine on protein synthesis.

The present investigations deal with the lysozyme activity of human tonsils and tonsillar lymphocytes. Lysozyme and tonsillar lymphocytes were isolated and purified from human tonsils according to the procedure of JOLLES.

The authors succeeded in a 15,700-fold purification of the enzyme on Bio-Gel 10 column. The purity of the preparations was checked by polyacrylamide gel electrophoresis.

In the course of the investigations lysozyme synthesis was studied in cell cultures.

On the basis of the results it is assumed that the lysozyme activity of tonsillar lymphocytes plays a part in local protection under conditions in situ.

Effect of p-chlorophenylalanine on the spontaneous activity of rats

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The drug p-chlorophynlalanine inhibits the biosynthesis of serotonin. According to data in the literature a dose of 100 mg/kg given for three consecutive days considerably decreases the serotonin level of the central nervous system with a return to normal on the 10th day. This point of time and the following periods were chosen to observe the behaviour of the animals.

Spontaneous motor activity was measured each morning for half an hour; no significant difference was found between the treated and the control groups.

The maze running time of the animals decreased and the number of errors increased. When the results obtained in animals in the active and passive group were evaluated separately, the active animals were found hardly to change during and after the treatment, while passive animals exhibited a significantly worse performance under the effect of the drug.

Effect of kidney denervation on the renin activity of renal venous blood of dogs with renovascular hypertension

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Ligation of the right renal artery in the dog led to the development and persistence of hypertension irrespective of the fact whether the contralateral kidney was innervated or denervated. According to previous investigations the renin production of the intact kidney plays an important part in the maintenance of hypertension after removal of the ischaemic kidney, the renin activity of the venous blood being constantly elevated during a 360-day observation period.

In the present experiments the splanchnic nerve running to the contralateral kidney was transected simultaneously with the hypertension inducing intervention and the renin production of the denervated kidney was continuously followed. Renin content in the venous blood of the denervated kidney was significantly lower in the early phase of the hypertension than the value found in the venous blood of the innervated kidney. In more advanced phases of hypertension, the renin activity increased in a manner similar to that seen in the innervated group. The authors are of the opinion that both intra- and extrarenal mechanisms influencing renin production are figuring in the maintenance of chronic hypertension.

Derivatographic analysis of myosin and myofibrils

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The derivatograph allows to perform thermoanalytic investigations on various compounds. Under the effect of gradually increasing temperatures, organic compounds undergo thermal disintegration at definite temperature points depending on the strength of their chemical bonds. Various groups of compounds, such as carbohydrates, lipids and proteins, undergo thermal disintegration corresponding to the character of their chemical bonds. Therefore,

the maxima of thermal disintegration are characteristic of a large group of compounds. The apparatus is capable of measuring and recording weight loss, the speed of thermal disintegration as well as the direction (endothermic, exothermic) and extent of the reaction in the course of thermal change. Myosin prepared from striated muscle as well as its chromatographically separated fractions were subjected to thermoanalytic investigations. The myosin fraction was found to have an endothermic maximum indicative of water loss at 70 to 75°C, and other ones at 185 and 215°C introducing the thermal disintegration where the protein so to say melts (lyotropic mesomeric state); thereafter, the disintegration becomes exothermic with great exothermic maxima at 280 to 285 and 310 to 320°C. In addition, maxima appear in the 2nd chromatographic fraction at 490°C and in the 3rd and 4th fractions at 505 to 510°C. The thermal data for the separate myosin fractions differ considerably from those of non-separate control myosin. The thermoanalytic maxima of the myofibril correspond to the temperature points characteristic of protein; however, owing to their complex structure, the course and form of the curves greatly differ from those of purified protein preparations.

Activity of the efferent sympathetic nerves of the heart and forelimb. II. Effect of bleeding, cooling, asphyxia and stimulation of the efferent nerves

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In previous experiments a difference has been demonstrated to exist in the central regulation of the activity of vertebral and cardiac nerves. In the present experiments the effect of various factors influencing efferent sympathetic activity has been investigated. The methods have already been reported.

During step-wise bleeding the activity of the vertebral nerve increases more markedly than that of the cardiac nerve. After retransfusion, the activity of both nerves returns to normal.

Cooling the ambient air from 30°C to 15°C increased vertebral nerve activity to 125% and cardiac nerve activity to 118%. Further cooling caused the activity of the vertebral nerve to increase considerably more than did that of the cardiac nerve (178 and 117%, respectively). Rewarming the ambient air (30°C) restored the activity to the control level in both nerves.

In asphyxia, the activity of the vertebral nerve gradually increased and reached the maximum (300%) at the 60th second. Activity of the cardiac nerve began to increase later and reached 160% at the 60th second.

On sciatic stimulation, an increase or decrease in activity, depending on the parameters used, were observed more frequently in the vertebral than in the cardiac nerve.

The results show the activity of the sympathetic nerves innervating the two regions to change in a different extent under the effect of various factors.

Effect of beta-receptor blocking drugs on the regulation of systolic volume

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Cardiac output remains constant when the pulse rate is altered by pacemaker between 100 and 200 in the dog and between 60 and 120 in human; thus, the systolic output decreases. The augmentation of diastolic filling in load-induced tachycardia and the increase of contractility in the resting state by themselves do not lead to an increase of the volume.

In experiments of heart–lung preparation of 22 dogs, isoprenaline slightly increased stroke volume in the control state, but the extent of this increase did not depend on the dose applied. The effect of three beta-receptor blocking drugs (propranolol, practolol and pindolol) was investigated in three series of experiments. The increasing effect of isoprenaline on stroke volume became more and more dependent upon the effect of increasing doses of the antagonists. Quantitative analysis of the inhibition of inotropic and chronotropic effects revealed the beta-blocking drugs applied to inhibit the positive inotropic effect of isoprenaline in a significantly lesser degree than the positive chronotropic effect. As a consequence, the cardiac output increasing effect of isoprenaline depends, under intact conditions, mainly on the heart rate and becomes, after beta inhibition, dependent on the stroke volume.

Effect of catecholamines and shock mediators (histamine, 5-hydroxytryptamine) on the blood sugar level in rats treated with *Bordetella pertussis* vaccine

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In previous experiments, *Bordetella pertussis* vaccine (BPV) evoked considerable alterations in the regulation of glycogenolysis in the rat, an effect presumably connected with the appearance of an increased sensitivity to anaphylaxis.

In the present experiments this mechanism was further investigated with the result that neither adrenaline nor isoprenaline caused hyperglycaemia in rats pretreated with BPV. Noradrenaline failed to affect the blood sugar level in BPV-treated animals just as it did in control rats. Ten mg/kg of 5-hydroxytryptamine given intravenously markedly increased the blood sugar level in the control animals, but decreased it on the 4th day of BPV pretreatment. BPV also altered the blood sugar effect of histamine. These effect of BPV are assumed to play an important part in the development of the increased sensitivity to shock mediators and to anaphylactoid shock.

Role of the adrenals in endotoxin hypersensitivity

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According to previous results, the toxicity of *Serratia marescens* endotoxin increases in the adrenalectomized rat after a few minutes incubation with normal rat serum.

However, no post-incubation increase of the toxicity was seen in normal or hypophysectomized rats, or in animals sensitized with Actinomycin-D.

The extent of the increase in toxicity depends on the C'3 complement level of the serum used for incubation. No increase in toxicity was observed in the case of sera inactivated at 56°C or exhausted by zymosan.

Thus, adrenalectomized rats exhibit special sensitivity against the toxic substances (anaphylatoxin, chemotaxic agents) produced during activation of the C'3 complement component.

The serum level of the C'3 complement component is high in adrenalectomized rats. In addition, the C'3 content of the serum of such animals is more rapidly and more perfectly activated under in vitro conditions than that of the serum of normal animals. These findings may offer one of the possible explanations of the endotoxin hypersensitivity of adrenalectomized animals.

Enzyme induction in trained and non-trained animals

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Previous investigations have shown that steroids are connected with muscular work and enzyme induction at several points. In the trained organism, both the pituitary–adrenocortical system and steroid metabolism undergo alterations. The steroid hormones are substrates of the microsomal enzyme system(s) of the liver playing a key role in the elimination of xenobiotic sub-

stances. This connection called the authors attention to the question of whether the hexobarbital oxidase activity of the liver changed in animals trained by swimming. A further question was whether the experimental training affected the effect of catatoxic steroids.

Hexobarbital oxidase activity determined in vivo by measuring hexobarbital sleeping time considerably increased in the initial phase (first week) of swimming, and gradually diminished at the 4th to 6th week. (According to previous experiments this is the time of the development of the trained condition.) The inducing effect of metandienone and spironolactone was weaker in the trained than in the non-trained animals.

Effect of surgical stress on the glycogen content of liver and skeletal muscle in the rat

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Surgical stress is a consequence of the simultaneous effect of several factors. Of these factors the effect of anaesthesia, laparotomy and manipulation of the intestines has been investigated on the glycogen content of the liver and skeletal musculature of rats. The experiments were performed on a total of 36 rats; each animal served as its own control. Laparotomy was made under pentobarbital (40 mg/kg) or superficial ether anaesthesia. Glycogen was determined in samples excised from the liver and from the quadriceps femoris muscle. The abdomen of six animals anaesthetized with pentobarbital and six animals anaesthetized with ether was closed. Of the remaining rats, in six the abdomen was kept open for one hour and in another six for two hours. In 8 animals the abdomen was kept open for one hour during which a piece of gauze soaked in physiological saline was placed on the mobilized intestinal loops for 5 seconds at ten minutes intervals. After 1 and 2 hours, a new sample was excised from the liver as well as from the contralateral quadriceps femoris. The abdomen of the first two groups of rats anaesthetized with pentobarbital and ether was opened again and new liver samples were excised. Glycogen was determined according to GOOD, KREMAR and SOMOGYI.

The glycogen content of the liver underwent a considerable diminution upon the effect of laparotomy.

Manipulation of the intestines accentuated this diminution even more. No significant difference was found between the animals anaesthetized with pentobarbital and those with ether. The glycogen content of the liver at the end of the surgical intervention depended on the initial level; it was directly proportional to the latter. Diminution of the glycogen content of the skeletal muscle was considerably less marked.

Effect on renal function and renin liberation of beta-mimetic and beta blocking drugs infused in the renal artery

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The effect of beta-mimetic and beta blocking drugs on renal function and renin liberation has been investigated in dogs anaesthetized with pentobarbital.

1. Infusion of 1 μg/kg/min of isoprenaline decreased blood pressure and R_{ren}; renin activity in the peripheral arterial and in the renal venous blood was increased. 2. Intrarenal infusion of 10 μg/kg/min of oxprenolol decreased blood pressure and left R_{ren} unchanged. Renin activity of renal venous blood was increased. 3. When the beta-mimetics were applied under alpha inhibition, no elevation was seen in the renin activity of the peripheral arterial and renal venous blood in spite of the diminution of blood pressure and of R_{ren}. A beta blocking drug applied during alpha inhibition failed to cause any change in either the haemodynamic parameters or renin secretion.

It is concluded that no specific role can be ascribed to the intrarenal beta-receptors in the regulation of renin secretion. The augmentation of plasma renin activity after selective stimulation or inhibition of the beta-receptors is considered consequence of the haemodynamic alterations, the manifestation of which is bound to the alpha-receptors.

Bronchodilator effect of sympatho-mimetics and parasympatholytics in the human bronchial system

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The broncholytic effect of inhaled atropine, isoprenaline and orciprenaline as well as of atropine injected intravenously has been investigated in ten patients with chronic obstructive bronchitis. In the applied doses all drugs decreased total respiratory air flow resistance (R_1) and intrathoracic gas volume (ITGV) to an equal extent, while specific conductance was considerably augmented. In the case of all the drugs investigated, the extent of the broncholytic action depended, in an equal degree, on the initial predrug value. Thus the authors regard the "law of initial value" confirmed for the circulatory system also valid for the bronchial system. The conclusion is drawn that the dysfunction of the autonomic nervous system manifesting itself with increased cholinergic activity plays an important part in the pathomechanism of chronic obstructive bronchitis.

Effect of phytohaemagglutinin on thymus cells

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Two functionally different kinds of lymphocyte occur in adult animals, the long-life and the short-life subpopulations. No long-life lymphocyte occurs in newborns, this cell type appears with age, parallel to growth. The development and amount of this fraction are regulated by the thymus and by a humoral factor of thymic origin. Long-life and short-life lymphocyte subpopulations are known to respond in a different manner to a given stimulus; their behaviour is different after the administration of corticoids or phytohaemagglutinin and their sensitivity to radiation is also different.

In the present experiments the post-irradiation regeneration of the long-life and short-life fractions was investigated in animals of both sexes and different ages, treated with phytohaemagglutinin.

Structure of quick and slow fibres in human muscle

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Two types of fibres have been distinguished in normal human abdominal muscle. When compared with data obtained in other mammals these fibres correspond, with great probability, to quick and slow fibres. Differences can be demonstrated in them in the number of mitochondria as well as in the amount of the triads. The myofibrils of the two fibre types are also different. The Z-lines of the slow fibres are more than twice as thick as those of the quick ones. However, the classical "Fibrillenstruktur-Feldstruktur" classification does not seem a reliable criterion in human muscle, owing to the regular position of the mitochondria. It is important to distinguish between the two fibre types in clinical examinations.

Effect of butylsympathon on the transcapillary fluid transport of the cat limb musculature in haemorrhagic shock

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The influence of Butylsympathon, an anti-shock drug, has been investigated on the capillary filtration coefficient (CFC) in the limb musculature of cats under chloralose–urethan anaesthesia.

1. In non-anaesthetized cats, 15 mg/kg of Butylsympathon increased the CFC from 0.015 to 0.0205 $\pm$ 0.0031 ml/min/mm Hg/100 g ($p < 0.05$); the effect persisted for 80 to 90 minutes. 2. According to previous experiments, the CFC decreases to 57% of its initial value in standardized haemorrhagic shock at a blood pressure level of 35 mm Hg. In animals treated with Butylsympathon, CFC increased to 142% of the original (0.028 ml/min/mm Hg/100 g; $p < 0.02$).

The marked and irreversible diminution of the capillary filtration coefficient in the second phase of standardized haemorrhagic shock was counteracted by Butylsympathon. On the basis of this finding it can be assumed that the effect on fluid transport plays a part in the anti-shock activity of the compound, probably by decreasing the tone of the precapillary sphincters.

Effect of vibration on the chloral hydrate and pentobarbital sleeping time of the rat

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Vibration exerts a general effect by influencing the functional state of the central nervous system. In higher animals it is the psychogenic effect which dominates, though a somatosensory mode of action is also probable. Previous investigations revealed the deafferentation of the hypothalamus to prevent the vibration-induced elevation of the 17-hydroxycorticosterone and adrenaline content. The impulses evoked by vibration in the whole body affect the central nervous system mostly via the reticular formation. This initial activation is followed by exhaustion.

Horizontal vibration of 3 and 5 Hz and 20 mm amplitude did not influence the pentobarbital sleeping time while increased the chloral hydrate sleeping time when compared with the controls.

When the animals were subjected to horizontal vibration for two hours twice daily for a period of 6 weeks, the chloral hydrate sleeping time was considerably prolonged as compared to the control; thus, in this respect, no adaptation to vibration developed.

The results suggest the anaesthesia-prolonging effect of vibration to have a cortical site of action.

"Skin reacting factor" as activator of the bradykinin system

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Lymphocytes from Mantoux hyperergic subjects were incubated in Parker's 199 medium in the presence of homologous antigen (PPD) at 37°C for 24 hours. The supernatant of the cell culture was filtered on Sephadex G-200 and further purified on DEAE column. The samples obtained in this manner were separated by polyacrylamide gel electrophoresis. The vascular permeability-increasing effect of the samples obtained was determined in R Amsterdam rats after each step of the purification.

Materials causing haemorrhage and inflammation were found in two molecular weight ranges, one with a molecular weight of 60,000 to 70,000 and another with a molecular weight of 30,000. The increase in vascular permeability can be observed as soon as 15 minutes following the administration of the substance, with a maximum at the 60th minute. The amount of the constituents of the bradykinin system was determined in the inflammatory oedema fluid. The enzyme-producing bradykininogen and kinin reaches the maximum 30 minutes after the appearance of the inflammatory reaction. Total content and bradykininase activity were highest at the 60th and 120th minutes, respectively, after the inflammation. The effect of the two inflammation-inducing substances could not be inhibited by either SBTI or incubation at 56°C for 15 minutes.

On the basis of the results the sensitized lymphocytes seem to produce, in the presence of homologous antigen, substances causing inflammation of early type and to induce an extravascular activation of the bradykinin system.

Incorporation of ^{15}N-labelled amino acids into the liver of starved rats

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Amino acids were labelled with ^{15}N, the stable isotope of nitrogen which occurs also in nature. The presence of the labelled isotope in the samples investigated was demonstrated after liberation of the nitrogen. For this purpose the nitrogen was kept in vacuum in the presence of a mixture of CaO and CuO at 500°C for three tours. The nitrogen gas developed was then excited in a high-frequency power field. The occurrence ratio of the nitrogen isotopes was determined by spectrophotometric analysis of the emission spectrum. The determination requires about 10 μg of nitrogen.

With the method described the matabolism of the amino acids applied was investigated in control and starved rats.

Role of adrenergic receptors in the regulation of gastric motility in the rat

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The effect of adrenergic receptor agonists and antagonists on gastric motility has been investigated in male albino rats weighing 150 to 200 g. Gastric emptying was studied by X-rays taken after the oral administration of 2 ml/100 g of Novobarium ®.

Adrenaline was found to inhibit gastric motility. Phenylephrine, a drug with a predominant alphamimetic action, was ineffective, while the alphalytic phenoxybenzamine slightly inhibited gastric emptying.

Isoprenaline was strongly inhibitory while propranolol slightly enhanced gastric emptying. From these data it has been concluded to a small extent of beta-receptor tone as far as gastric motility is concerned.

Adrenaline was fully effective also in animals pretreated with phenoxybenzamine, while propranolol pretreatment abolished the motility-inhibiting effect.

It is concluded that the inhibitory effect of adrenaline on gastric motility is brought about through beta-receptors.

Individual variability in the manifestation of frontal signs

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Remembrance of instrumental discrimination changes in the dog after dorsal prefrontal (DPF) and lateral premotor (LPM) lesion. The number of reactions increases after DPF lesion, for the animals react more often to non-awarded stimuli and during intersignal periods. The latency, which had been decreased during learning the reaction, usually undergoes a further diminution. All these point to an increased reactivity. The number of reactions decreases after LPM lesion, for the animals do not react to part of the awarded stimuli, and the latency of the responses is prolonged. There are individual differences in the extent of these alterations. In animals in which the DPF lesion had caused great changes, the LPM lesion usually had a slight effect. If the effect of the DPF lesion was medium, also the alteration after LPM lesion was of a medium degree. If the consequence of the DPF lesion is small, after LPM lesion one generally sees a great change. In intact animals changes of analogous direction and extent as after DPF lesion were seen when the dogs had starved, while in satiated animals changes not unlike those seen after LPM lesion were observed.

The data are interpreted in such a way that it is the site of the lesion which determines whether the reactivity will increase or decrease. The individual variations in the quantitative changes can be brought into connection with the individual extent of the "drive level". The characteristic variability of the frontal syndrome might be accounted for by the site of the lesion and by the interaction of the individual reactivity levels.

Presence and ratio of isophosphorylases in the heart and other organs of mammals

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Blood serum of cocks immunized with crystalline phosphorylase prepared from the skeletal muscle of rabbits completely inhibits the skeletal muscle type phosphorylases.

It has been reported at the 1968 Congress of the Hungarian Physiological Society that a heart-specific isophosphorylase immunologically different from the phosphorylase of skeletal muscle could be demonstrated in the heart of some mammals. The activity of this isophosphorylase was slightly inhibited by skeletal muscle antiphosphorylase. On the basis of the immunological differences of cardiac isophosphorylases, a method has been developed for the parallel determination of heart and skeletal muscle-specific phosphorylases. The method is based on the observation that the heart extracts containing the two isophosphorylases exhibit, upon titration with antiphosphorylase, a biphasic inhibitory curve. From this, the amount of isophosphorylases can be determined. The method was controlled with mixtures containing chromatographically separated cardiac isophosphorylases in different ratios.

The ratio of isophosphorylases present in the heart of various mammals (human, rabbit, pig, ox, sheep, etc.) was investigated with the above method.

The development of cardiac isophosphorylases and their ratio have been studied. The individual and sexual phosphorylase types of various organs (smooth muscle, brain, kidney, spleen) have also been investigated with skeletal muscle antiphosphorylase.

Depolarizing effect of veratrine on skeletal muscles

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According to previous investigations the depolarizing effect of veratrine on skeletal muscles is due to increased sodium permeability and increased sodium influx (Acta physiol. Acad. Sci. hung. **1967**, 32, 60 and 69). The

authors' recent results concerning the mechanism of veratrine depolarization are as follows.

1. Cl^- ions are not indispensable in the mechanism of depolarization since the depolarizing effect of veratrine is manifest also in Cl^--free (sulphate) Ringer's solution.

2. Veratrine does not depolarize in K-free Ringer's solution.

3. Veratrine does not depolarize in hypertonic Ringer's solutions of various composition, even though an action potential can be generated in the muscle membrane, in accordance with data in the literature. It is concluded that two different Na-channels are figuring in the depolarizing action and in the action potential-evoking effect of veratrine.

4. As described previously, veratrine depolarization of the aneural muscle membrane occurs with a latency of 20 minutes on the average. According to the present results this long latency is shortened to a quarter if the muscle incubated in veratrine is stimulated electrically at appropriate times.

Regulation of the level of circulating haemopoietic primary cells

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The physiological function of the circulating primary cells (colony forming cells, CFU) and their role in haemopoietic regeneration are unelucidated. Nor is known the regulation of the level of the circulating primary cells.

The role of the spleen in the regulation of the peripheral CFU level has been investigated in BALB/c$\times$CBA/F_1 hybrid mice. Previous findings showed that the CFU released from the bone marrow into the blood stream are taken up by the spleen. The elevation of the peripheral CFU level upon appropriate stimuli is significantly higher in splenectomized than in control mice. The disappearance rate of transplanted CFU from the blood stream also changes after splenectomy. The spleen may be assumed to play an important part in the removal of the primary cells from the circulation.

The turnover rate of the bone marrow primary cells and that of the peripheral primary cells were found to be different. The disappearance from the circulation is more rapid in the case of peripheral CFU than in the case of CFU of bone marrow origin. It is concluded that the two populations are not completely identical.

Tolerance and cross-tolerance between clonidine (ST-155) and indirect sympathicomimetics

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Clonidine is known to possess a long-lasting hypotensive effect (HOEFKE and KOBINGER, Arzneimittelforsch. **1966**, 16: 1038—1050.); a depression of the vasomotor centre has been assumed to figure in this action (SCHMITT et al., Europ. J. Pharmacol. **1967**, 2: 147—148.). Experiments have been made on cats to elucidate more precisely the site of action of the compound.

1. An acute tolerance develops against the vasodepressor effect of Clonidine; in this tachyphylactic state the compound does not lower but elevates blood pressure.

2. The hypotensive action can be counteracted by the previous administration of amphetamine, ephedrine, tyramine and cocaine; in fact, the effect will be reversed. This blood pressure elevation can be inhibited by alpha blocking drugs.

3. Clonidine fails to decrease blood pressure in animals pretreated with reserpine.

4. Clonidine inhibits efferent sympathetic activity. An acute tolerance can be demonstrated in the case of this effect, too.

5. In experiments in vitro the drug depletes the catecholamine content of the sympathetic fibres of the rat iris.

6. It is assumed that an intact catecholamine metabolism is necessary for the vasodepressor effect of Clonidine.

Role of the urea cycle in the development of uraemic toxaemia

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Both clinical and experimental observations have revealed the blood urea level to change asymptotically and not linearly in renal failure. The causal role of urea in the development of the phenomenon is evidenced by the observation, according to which the concentration of urea and its synthesis rate are inversely proportional. This finding also points to the dependence of the velocity of the urea cycle on the concentration of urea.

Serum concentration of the guanidino derivatives undergoes a considerable augmentation in chronic renal failure. Of these derivatives, guanidino-

succinic acid (GSA) and methylguanidine (MG) may be responsible for certain symptoms of uraemic toxaemia.

In the present experiments, the blood urea level of bilaterally nephrectomized rats was elevated to 30 to 150 mM by the intravenous administration of urea. Twenty-four hours later the animals were bled and after separation by column chromatography the blood GSA and MG levels were determined.

According to the results, the serum urea level plays a key role in the alterations of guanidino derivatives of toxic character; the changes in urea concentration are accompanied by characteristic changes of the GSA and MG concentrations.

Alpha- and beta-mimetic interactions on the rabbit aorta

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It has been shown previously that only one common adrenergic receptor system existed which behaved either as alpha or as beta receptor, depending on the circumstances. In the present experiment it was investigated how the alpha- and beta-mimetic drugs influenced each other effect.

The contractions of strips of the rabbit aorta in Krebs-bicarbonate organ bath were recorded isotonically and isometrically. Separate dose–effect curves were taken up for noradrenaline and isoprenaline. Under such conditions only the weak alpha effect of isoprenaline was manifest; the drug caused contractions in doses 2—3 orders of magnitude higher than did noradrenaline. These concentrations were inhibited by phentolamine.

Experiments in which the dose–effect curve was taken up in such a way that increasing doses of noraderenaline and isoprenaline were applied alternatively, yielded the following results. 1. Low doses of isoprenaline exhibited a betalytic effect after the administration of small amounts of noradrenaline. 2. The dose–effect curve of noradrenaline taken up in combined experiments revealed an increase in noradrenaline sensitivity. 3. On combined application, both alpha- and beta-blocking drugs inhibited the effect of both noradrenaline and isoprenaline. The receptor behaved as if it were of alpha and beta nature at the same time. 4. All these effects were specific catecholamine effects; KCl, through eliciting strong contractions, induced the betalytic effect of isoprenaline to a considerably lesser extent. The mutual induction of alpha and beta properties speaks for a uniform receptor system.

"Anticholinergic" action of prostaglandin E_1 (PGE_1) on isolated guinea pig atrium

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Some effects of PGE_1 have been studied on guinea pig atrium preparations isolated together with the right vagal nerve. Part of the preparations functioned spontaneously and part was driven by constant frequency stimulation (210/min). Krebs solution of 31°C oxygenized with a gas mixture of 95% oxygen and 5% CO_2 was used as organ bath. Contractile force was recorded isometrically, and heart rate by means of a tachymeter. The vagal nerve was stimulated through a platinum electrode pair at supramaximum voltage, 0.5 msec impulse width and 4 Hz, at 30 and 60 sec intervals.

PGE_1, in concentration ranges from 0.1 to 10×10^{-9} g/ml, increased heart rate in a dose-dependent way in the majority of the preparations. At a concentration of 2.5×10^{-9} g/ml, PGE_1 decreased the negative chronotropic effect of vagal stimulation to about 1/5 of its original value. In doses of 5 to 10×10^{-9} g/ml the drug markedly inhibited the negative chronotropic effect of exogenous acetylcholine (5 to 75×10^{-8} g/ml). At a concentration of 20×10^{-9} g/ml, PGE_1 failed to influence either the contractile force of preparations driven at constant frequency or the negative inotropic effect of exogenous acetylcholine.

It is concluded that PGE_1 inhibits the cholinergic responses investigated by a direct effect on the pacemaker cells. This effect is inasmuch "specific" that it is not manifest on the working muscle cells of the atrium.

Investigation with ^{133}Xe of the blood supply of the small intestine in normal and hypotensive dogs

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Blood flow in the jejunum and ileum of dogs anaesthetized with pentobarbital has been studied my means of ^{133}Xe with simultaneous recording of the venous outflow of an intestinal loop.

A small intestinal loop weighing about 10 to 15 g was isolated. ^{133}Xe was injected in one of the supplying arteries and the decrease of the beta and gamma activity of the isotope was measured over the given intestinal area. Scintillation detectors, ratemeter and compensograph were used for the measurement. The multicomponent curves obtained were resolved into vectors, and the flow values characteristic of the individual components were computed.

In the course of the evaluation it was checked how the flow values calculated and those measured by direct outflow agreed. The beta curves consisted of 2 or 3 while the gamma curves of 3 components. Only those curve pairs were taken into account in which the corresponding components were in agreement.

Various flow levels were produced by hypotension or by the infusion of isopropylnoradrenaline. In addition to measuring the flow in the intestinal wall layers, the limits of the applicability of the methods used were checked. Total blood flow of the two intestinal segments was different. It was mainly the blood flow of the mucosa which changed at various flow levels.

Analysis of the dynamic parameters evoked by pressure impulses in resting heart muscle

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The alterations in cardiac volume elicited by impulselike pressure changes were investigated in frog hearts stopped by Stannius II ligature. The pressure and volume curves were fed into an analogous computer and the first derivates of the curves were continuously recorded on an oscilloscope. Conclusions as to the inner dynamic resistance of the heart muscle were drawn from the difference in the velocity constants of the two curves during the duration of the pressure impulse delivered to the cavity of the heart.

The resistance of the heart muscle to impulselike pressure changes was decreased by 0.5 to 1 μg of adrenaline (the V/P increased). Higher doses (5 to 10 μg) caused changes of opposite direction. The changes of both directions increased proportionally when the initial inner pressure (preload) increased. Since it is the length of the sarcolemma which changes upon the effect of preload, one may assume the changes of both directions to manifest themselves on the sarcomers.

Under static conditions the V/P curve either remained unaltered or was shifted to the right upon the effect of adrenaline. Since the increase of the V/P curve can be observed only in moving heart muscles, small doses of adrenaline can be assumed to increase the disruption velocity of the actomyosin cross bridges. Higher doses of the drug increase the interaction between cross bridges and reactive parts.

Self-stimulation and adrenocortical activity in young rats

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Bipolar electrodes were implanted into the region of the medial forebrain bundle in 12 to 17-day-old rats. Under appropriate conditions the animals performed active self-stimulation from the 14th day of life on. No similar behaviour was observed in the 12 to 13-day-old group; therefore, these animals were stimulated externally with stimulus parameters corresponding to those of self-stimulation. The plasma corticosterone level was significantly increased after 20-min stimulation in both self-stimulating and passively stimulated animals. Histamine or surgical stress failed to increase the plasma corticosterone level in 12 to 14-day-old animals. The appearance of active self-stimulation and the manifestation of the stress effect occurred at the same postnatal age. This raises the possibility that identical central nervous mechanisms are figuring in both functions.

Effect of adrenaline on cation release from the frog myocardium

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Sixteen to twenty hours after the intraperitoneal injection of 80 to 100 μCi of ^{42}K, ^{24}Na or ^{86}Rb, a 30 to 50 mg strip was excised from the basis of the frog heart ventricle and perfused with Ringer's solution. The activity of the preparation was measured for 180 minutes, and the K and Na content was determined at the end of the perfusion.

The amount of cations released from various fractions into the perfusion fluid was calculated on the basis of the washout curves. Perfusion with Ringer's solution containing 0.1 μg/ml of adrenaline failed to cause any change in Na release as compared with the control; the release of K and Rb decreased to an identical extent. The coefficient of K release was, on the average, 0.0052 min^{-1} in the control groups and 0.0042 min^{-1} in the presence of adrenaline. The same values for Rb were 0.0025 min^{-1} and 0.0021 min^{-1} respectively.

The tissular K concentration was 20% higher after adrenaline perfusion than in the control group.

It has been concluded that adrenaline, though decreasing K release, does not influence the K–Na exchange in the ventricular musculature.

Mechanism of carotid polyuria

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The observations of THURAU and DEETJEN concerning the pressure-dependency of the renal medullary circulation have been extended to explain the mechanism of carotid polyuria. Since other authors failed to confirm the original observations, in the present experiments the problem was reinvestigated by using the Rb isotope fractionation technique.

It was stated that in the intact innervated kidney not only the cortical circulation but also the medullary circulation is relatively independent of arterial blood pressure. During vasopressor response of carotic sinus origin, the medullary circulation does not increase as it had been assumed, but decreases to an even greater extent than does the cortical blood flow. That means that medullary resistance increases more rapidly than does the cortical one. The results did not support the above-outlined explanation of the mechanism of carotid polyuria.

Investigation of the intrarenal distribution of resistance changes revealed the cortical resistance to increase in the preglomerular segment and to remain unchanged in the postglomerular one. In contrast, resistance in the postglomerular segments considerably increases in the medulla, in addition to a slight increase in the resistance of the preglomerular vessels. Therefore, filtration does not change or slightly decreases in the cortical glomeruli in spite of the blood pressure elevation during the pressor response, while it increases in the juxtamedullary glomeruli corresponding to the increase in pressure. The intrarenal redistribution of glomerular filtration may explain the increased diuresis and sodium excretion.

Induction of ovulation by synthetic LH-FSH-RH in mature rabbits

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In the present years, SCHALLY and his team described the amino acid sequence of the LH-FSH releasing hormone of the pig: (pyro) Gly-His-Prp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2.

The decapeptide was synthetized in the authors' laboratory by means of the fragment condensation method. Purity of the preparation was controlled by layer chromatography and its composition by amino acid analysis.

The ovulation-inducing effect of the decapeptide was investigated in adult, virginal New-Zealand rabbits anaesthetized with pentobarbital (35 mg/kg) and pretreated with oestradiol (150 μg/animal).

Increasing doses of the preparation were infused into the carotic artery of the animals by means of a Braun syringe. Fourty-eight hours later the ovulation points in the ovaria were counted and the presence of ova in the tubae checked.

The ovulation index was 6/1 after the administration of 1.14 nMol/kg of the decapeptide, 4/3 after 5.7 nMol/kg, 6/3 after 28.5 nMol/kg and 4/4, i.e. 100%, after 142.5 nMol/kg or more.

Subcellular localization of biogenic amines in the central nervous system of *Anodonta cygnea* L.

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Of the ganglia of lake shell-fish, a 10% homogenate in isotonic sucrose was separated into four primary fractions by means of differential centrifugation. The fractions were analyzed for 5-hydroxytryptamine, dopamine, noradrenaline and protein.

According to the results, 90% of all three amines are particle-bound and 10% can be found in the supernatant. 65 to 70% of all three monoamines is located in the mitochondrial and 15 to 20% in the microsomal fraction. The relative specific activity (RSA) calculated for protein content of the amines investigated did not exhibit any significant difference in the two fractions.

The mitochondrial fraction containing the bulk of the amines is heterogeneous morphologically. Separation with sucrose gradient of this fraction into seven subfractions revealed no strikingly high RSA for any amine. Sixty per cent of the three amines were mainly located in the three subfractions rich in synaptic elements and containing considerable amounts of various membrane profiles.

Treatment of the mitochondrial fraction with distilled water (osmotic shock) brought about significant changes only in the distribution of serotonin in the subfractions.

Enzyme and metabolite content of the perfusion fluid of the kidney preserved according to Collins

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Unilateral nephrectomy was performed in dogs under pentobarbital anaesthesia. The kidney was preserved according to COLLINS, and the animals were bled into an open reservoir till an arterial blood pressure of 40 mm Hg.

After a 60-min hypotensive period the other kidney was removed too and preserved in the above manner.

The enzyme and metabolite content of the perfusion fluid was determined 1, 2, 4, 6, 8, 24 and 72 hours after nephrectomy. Spectrofluorometric methods were used to determine the activity of acid phosphatase, beta-glucuronidase, leucinaminopeptidase, arylsulphatase A, and LDH, as well as the level of lactic acid and pyruvic acid.

1. The activity in the perfusion fluid of the enzymes investigated increased parallel with the duration of preservation. The increase was especially marked after 48 and 72 hours.

2. The activity of arylsulphatase A and LDH differed significantly in the two groups even at the beginning of preservation.

3. In the case of the lysosomal enzymes (acid phosphatase, beta-glucuronidase, arylsulphatase A) a gradually increasing difference was observed between the two groups.

4. The values for LDH and lactic acid exhibited considerable differences between the two groups at the beginning of preservation. At the end of the experiment no difference could be demonstrated even though the absolute values were high in both groups.

Influence of sympathetic beta effects on acid secretion

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The influence of the sympathetic nervous system on gastric acid secretion has been the subject of many investigations. However, owing to the contradictory data, no unequivocal answer has been obtained. Similarly, the effect of sympathetic excitation is also contradictory. It seems on the basis of recent investigations that sympathetic excitation decreases the volume of secretion and total acidity. In GÁTI's opinion, adrenergic beta-receptors are responsible for this effect.

The present experiments aimed at studying how the beta effect influenced the secretion of acid in the rat. As beta blocking agents, propranolol, pindolol and practolol, and as beta-mimetics, isoprenaline, salbutanol and hexoprenaline were tested.

Beta blocking agents were found to influence gastric acid secretion (volume and total acidity) of the rat to a very small extent. Beta-mimetics, on the other hand, considerably modified it and unequivocally decreased the volume and total acidity of gastric secretion. It is concluded that the beta action is the decisive one among the beta mimetic effects.

Influence of ACTH on increase in uterine weight evoked by HCG in infantile mice

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It was investigated in CFLP white mice weighing 10 to 12 g whether the uterine weight increasing effect of HCG would be influenced by ACTH (Synacthen depot, Ciba) administered simultaneously.

The treatment consisted of administering a single dose daily of either HCG (Richter) or HCG + ACTH intramuscularly for three days. The animals were killed on the fourth day, and the wet and dry weight of the uterus was measured.

Analysis of the dose–effect curves revealed low doses of ACTH (1.25 μg/day) to potentiate the effect of low doses of HCG (0.05 to 0.2 I.U.). On increasing the dose of the two hormones either separately or together, the potentiating effect disappeared, in fact, an inhibition of the HCG action was observed.

ACTH given alone, or dexamethasone (Oradexon, Organon) given together with HCG did not alter uterine weight.

Histone demethylation in lymphocyte cultures

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The kinetics of histone methylation have been studied in human lymphocyte cultures stimulated by phytohaemagglutinin and specific antigens. Labelling of the histones by $^{14}CH_3$ was found to undergo a marked increase 15 minutes after the stimulation.

Further investigations revealed the presence in human peripheral lymphocytes of an enzyme which liberates a methyl group from $^{14}CH_3$-histone. The activity of this enzyme does not change during the first three hours of phytohaemagglutinin stimulation. The kinetics, pH optimum and heat inactivation of the enzyme have been investigated.

Oxidative ability of brain mitochondria isolated from animals in experimental shock

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The oxygen consumption of isolated brain mitochondria was measured polarographically at 37°C. Their basal respiration as well as the respiration

stimulated by ADP or DNP were estimated in the presence of various substrate pairs, and the value for the respiratory control was calculated.

Shock was induced in rats by freezing the hindlimbs. The basal respiration of the mitochondria isolated from the brain of these animals was slightly decreased, and their respiration stimulated by either ADP or DNP diminished significantly as compared to the control. The values for the respiratory control were also considerably lower. The most marked diminution was observed with beta-hydroxybutyric acid + malic acid as substrate while the slightest one in the course of succinic acid oxidation.

When the brain of the control animals was kept in the homogenizing medium at 0°C one hour prior to the preparation of the mitochondrial fraction, the parameters changed in a way similar to those of the shocked group. The ADP-stimulated respiration of the brain mitochondria of shocked animals or that of the brains kept standing for one hour considerably increased in the presence of 2.5 mg/ml of serum albumin. However, the increase in the oxidation of various substrates was of a different extent.

The diminution of the oxygen consumption of brain mitochondria can be assumed to be due to the fatty acids liberated under the effect of hypoxia.

Effect of detergents on experimental inflammation models

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It has been shown previously that local cell injury plays an important role in the development of the "acute phase reaction" accompanying acute inflammation. The membrane labilizing effect of surface-active detergents is well-known. Starting from this consideration the effect of Triton WR 1339 and Tween 80 on some local and general manifestations of turpentine inflammation has been investigated.

The experimental animals were divided into 6 groups: 1. Control; 2. Turpentine abscess with SELYE's granuloma pouch method; 3. Turpentine abscess + intravenous Triton treatment with 1 ml of a 2.5% solution every second day; 4. Turpentine abscess + local Triton treatment with 1 ml of a 2.5% solution into the granuloma pouch once daily; 5. Turpentine abscess + intravenous Tween treatment with 1 ml of a 2.5% solution every second day; 6. Turpentine abscess + local Tween treatment with 1 ml of a 2.5% solution into the granuloma pouch once daily.

At the end of the 10 day experimental period, white blood cell count, phagocytosis index and the acute phase glycoproteids (coeruloplasmin, haptoglobin and alpha^2-macroglobin) were determined in the blood serum and in the exudate.

The investigated parameters were not significantly influenced by intravenous Triton or Tween treatment in either the blood serum or the exudate. Local detergent treatment increased the leucocyte content of the exudate and also the phagocytosis and the level of the acute phase glycoproteids in both the blood serum and the exudate.

Cholinergic–adrenergic interactions on smooth muscle organs

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It has been observed previously that in smooth muscle organs responding with contraction to both adrenergic and cholinergic agonists (nictitating membrane of the cat, isolated aortic strip of the rabbit) one single acetylcholine (ACh) exposition applied between serially evoked noradrenaline reactions inhibited the forthcoming adrenergic response. This inhibition persisted in the isolated rabbit aortic strip after washing out the ACh from the organ bath. In the present experiments the effect of ACh on the phenylephrine (PhE)-induced contractions of the isolated terminal ileum of guinea pigs was investigated. ACh was found to inhibit the PhE response also on this preparation. The inhibition persisted after washing out the ACh, it was reversible and depended on the dose of the drug. It could not be brought into connection with the mere fact of the contraction since it cannot be evoked by either histamine or KCl both causing maximum contraction. The possibility of counteracting the inhibitory effect exhibits seasonal variations. Under mild weather conditions it can be antagonized with propranolol and, in the winter, with methylhomatropine. Similar phenomena have been observed also on the isolated uterus of rats pretreated with stilboestrol. Since ACh inhibits adrenergic reaction after having been washed out from the organ bath, the inhibition is assumed to be accounted for by a conformation change of the adrenergic motor receptor.

EEG-synchronizing effect of small intestinal and splanchnic nerve stimulation

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Experiments were performed on cats bearing Thyri-Vella loop with chronic splanchnic electrodes. The intestinal epithelium was stimulated by mechanical as well as electrical stimuli of various parameters, and the changes

in the EEG pattern were followed. The stimulation was performed in various phases of the sleep–wakefulness cycle.

Low frequency (1 to 8 cps) stimulation of the small intestine and splanchnic nerve at intensities below the threshold of the viscerosomatic reflex, or the application of habituated stimuli, resulted in synchronization and desynchronization, respectively, of the EEG when the stimulation was performed during drowsiness or slow wave sleep. In contrast stimulation over the threshold of the viscerosomatic reflex caused arousal when applied during sleep.

Adrenergic responses of the coronary circulation after cardiac infarction

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Myocardial infarction was produced by ligation at various levels of the descending branch of the left coronary artery in the dog. Adrenergic reactivity of the collateral coronary flow supplying the area of the infarction was studied after the operation under acute experimental conditions and compared with the behaviour of the vessels supplying the intact myocardial region. Local tissular blood flow was measured by means of a flexible thermocouple.

A quantitative alteration was demonstrated in the vascular response of the myocardium belonging to the occlused coronary trunk for 10 to 15 or even more days. Adrenergic excitation elicited by either nervous stimulation or pharmacological means resulted in strong and unequivocal local vasoconstriction, while the physiologically dominant beta-adrenergic dilatory effect disappeared fully, or nearly fully. At the same time the circulatory response of the "intact" myocardial segment exhibited the usual pattern. From the third week on following coronary ligation the adrenergic responses of the collateral flow gradually approached the reactions of the intact region.

A so-far unknown inhibitory effect of a plasma component on the activity of inflammatory mediators

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It has been demonstrated previously that the histamine spray time of guinea pig was considerably prolonged six hours after the intraperitoneal injection of serum obtained from rats in the state of non-specific stress resistance.

In the present experiments the plasma of normal rats and that of rats in the state of non-specific stress resistance was administered in the form of aerosol to guinea pigs. If the plasma spray was applied prior to the administration of histamine the bronchial spasm developed later in the case of normal rat plasma than in untreated control animals. The plasma of the resistant animals exhibited an even stronger protective effect. To determine what plasma component might be responsible for the effect, the plasma of both normal and resistant rats was separated on Sephadex G-200. In the case of normal rat plasma a protein fraction with a molecular weight between 100,000 and 200,000 was found to inhibit the histamine-induced bronchial spasm in the guinea pig. In the case of the plasma of resistant rats the protective protein component had a molecular weight under 100,000. The plasma protein component of low molecular weight obtained from resistant rats was considerably more effective.

It seems that the plasma protein fraction of normal rats inhibits the development of histamine-induced bronchial spasm and that the protective effect can be augmented by subjecting the rats to stress.

Some effects of intrahypothalamically injected cholinolytics in waking cats

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Injection of carbachol into the anterior hypothalamus of freely moving waking cats has previously been shown to evoke a characteristic rage reaction.

The present paper deals with the possibility of inhibiting this emotional reaction and with the EEG background of the inhibitory effects. Since the rage reaction was evoked by a cholinergic stimulatory drug, the effect of atropine and scopolamine applied intraperitoneally or intrahypothalamically was investigated on the spontaneous behaviour of the animals, the inhibition of the rage reaction and the EEG activity.

The rage reaction was markedly inhibited after the intraperitoneal administration of both anticholinergic substances, scopolamine being about twice as effective than atropine in this respect. Upon intrahypothalamic administration, scopolamine exerted by itself a sedative action while atropine produced a slight excitation. In spite of the opposite influence on general behaviour, the EEG effects were identical in both cases and also the carbachol-induced rage reaction was partially or fully inhibited by both drugs.

Since both atropine and scopolamine have a postsynaptic site of action, a compound with a presynaptic site of action was also investigated as to its

effect on the carbachol-induced rage reaction. Hemicholine-3 injected into the hypothalamus failed to influence the spontaneous behaviour of the cats and did not inhibit the rage reaction even in doses 50-times higher than the effective dose of atropine.

The time-relations as well as the dose–effect relations of the behavioural and electric alterations have been analyzed in detail.

Modelling of the primary photoelectric process arising in the visual organ on an artificial bimolecular lipid membrane

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An artificial bimolecular lipid membrane (BLM) was prepared on a 1.5 cm hole of a teflon cup from cholesterol (Δ^5-cholesten-3-ol) oxidized in octane:

calomel electrode	aqueous $FeCl_3$ solution	BLM	aqueous iodine solution	calomel electrode

Illumination through the aqueous iodine phase resulted in the appearance of a biphasic reversible photovoltage of 10 mV order of magnitude consisting of a quick and a relatively slow component. The condition for the reversible, biphasic photo response was the simultaneous presence of the two redox systems Fe^{3+}/Fe^{2+} and I^2/I^-. The photo effect observed can be ascribed to the photochemical reaction

$$2\ Fe^{2+} + I^2 \overset{h\nu}{\rightleftarrows} 2\ Fe^{3+} + 2\ I^-$$

taking place on the membrane phase, as well as to the electrochemical electrode reaction on the intersurfaces of the membrane–aqueous phase. It is assumed that the primary process of vision is the injection of electric charge (electrone or hole) produced by the absorbed photon and the accompanying electrochemical reaction. This latter is of low energy demand and also ensures the reversibility. Under conditions in vivo this electrode reaction is probably governed enzymatically. The model suggested assumes both electronic and ionic conduction process through the membrane and emphasizes the electronic character of the primary effect of light.

Investigation on the excitability of peripheral cholinergic synaptic structures in diabetes mellitus

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It has been shown previously that competitive inhibitors (hexamethonium, d-tubocurarine) exhibited a diminished paralyzing effect in vivo on the peripheral cholinergic synaptic structures (ganglion, motor end plate) in cats with pancreatic diabetes or in rats with alloxan diabetes. Since no difference in pharmacological sensitivity was found between the normal and diabetic end plates, the phenomenon was assumed to be due to the presence of humoral factors. In the present experiments the serum of diabetic cats was found to increase the asynchronous electrical activity of the renal sympathetic in normal cats, as well as the spontaneous electrical activity of the denervated m. tibialis anterior in healthy rats. The activating effect was abolished by heat treatment of the serum, or by pretreating the diabetic donor animal with cyclohexemide. Gel filtration revealed the activity-increasing factors to be present in the high molecular weight fraction of the diabetic serum. It was further observed that chronaxie in the in vivo nerve-muscle preparation diminished in diabetic rats. Thus an increased excitability develops on the structures in question during diabetes, in addition to the altered pharmacological sensitivity, a feature presumably due to the accumulation of factors of protein nature.

Metabolic effect of guanethidine

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The effect of adrenergic neurone blocking drugs on carbohydrate metabolism is not clear. According to some authors guanethidine may cause hypoglycaemia, and, a beneficial effect in diabetes of the drug was also described. Others observed hyperglycaemia after the acute administration of guanethidine.

In the present experiments the effect of the drug on the carbohydrate metabolism of the liver has been studied. Perfusion of isolated rat livers with guanethidine resulted in a considerable increase of the glucose release at the beginning of the perfusion, which was then followed by a glucose release lower than that of the controls. Administration of guanethidine in combination with the hypoglycaemic agent chlorpropamide, left the glucose release-increasing effect unaltered, a fact pointing to different sites of action of the

two compounds. The hyperglycaemia-inducing acute effect of guanethidine is presumably the consequence of an effect on the liver, while the "antidiabetogenic" action seems to manifest itself by some other way.

Significance of mast cells in chronic nephritis

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The kidneys belong to the parenchymal organs poor in mast cells under normal conditions. However, SLICH (1956), PELLEGRINI (1958) as well as PAVONE-MACALUSO (1960) observed that in chronic nephritis and chronic pyelonephritis the number of mast cells in the kidney was pathologically augmented.

The present authors have shown that, in addition to an increase in number of the mast cells, different variants occurred in the histological sections, such as cells with vacuolized, or homogeneous or "exploding" cytoplasm. These cell changes are interpreted functionally, and in a way adaptable to EHRLICH's concept (1877):

1. "The mast cells are characteristic cells of chronic inflammation. They accumulate especially in cases with lymphatic obstruction and disappear once the definite state, the scar tissue, has developed."

2. It is considered a part-phenomenon of EHRLICH's concept that the mast cells, by means of their known H-substances, participate in the maintenance of inflammatory processes with an immunological basis which always has a part in the diseases mentioned; and

3. by means of their liberated histamine and heparin content they induce fibroplasis which leads to a destruction of the functioning parenchyma through proliferation and shrinking of the connective tissue.

Enzyme activities in guinea pig macrophages under the effect of antigen–antibody reaction

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Phagocytosis of the rheumatoid factor and IgG complex in polymorphonuclear leukocytes and macrophages has been investigated by WEISSMANN et al. They stated that the uptake of the complex went together with a selective liberation of lysosomal enzymes.

In the present experiments, macrophage changes due to ovalbumin–rabbit-antiovalbumin–IgG complex were observed after a 30 min incubation

period. The changes mainly comprised an outflow of cytoplasmic elements, and were not influenced by the presence of complement.

Various cytoplasmic and lysosomal enzymes were studied in the supernatant of the solution containing the cells. The cytoplasmic lactic acid dehydrogenase level did not change in the supernatant as compared to the control. Among the lysosomal enzymes investigated, acid phosphatase remained unchanged, beta-glucuronidase activity exhibited a slight augmentation and ribonuclease a marked augmentation.

The cellular alteration accompanying the initial phagocytosis of the immune-complex might be connected with the liberation of the lysosomal enzymes of the macrophages.

Effects of prostaglandin E_1 on the transmembrane potential of mammalian heart preparations

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The effect of prostaglandin E_1 (PGE_1) (Chinoin, Budapest) was investigated on the transmembrane potential of mammalian heart preparations (rabbit, cat, guinea pig left auricle and rat ventricle). Two concentration levels were used: 5 ng/ml (1.4×10^{-8} M) and 20 ng (5.6×10^{-8} M).

Five ng of prostaglandin did not cause any change in the magnitude of the resting potential in any of the preparations. It increased (10 to 54%) the depolarization velocity of the action potential, in addition to a slight augmentation of the reverse potential (overshoot).

At higher PGE_1 concentration (20 ng/ml) there appeared effects of opposite direction. This dose did not influence the magnitude of the resting potential but decreased that of the reverse potential. At the same time, it caused a significant (19 to 34%) diminution of the depolarization velocity in all species.

The repolarization phase was differently influenced by PGE_1 in various species; however, the dose-dependent biphasic effect observed in connection with depolarization velocity and reverse potential was not manifest in connection with the repolarization.

Application of heart rate analyser in behavioural experiments

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During behavioural–physiological experiments there is a need of the simultaneous recording of visceral changes and of cardiac function. For a reli-

able evaluation of the heart rate changes accompanying rapid behavioural alterations (e.g. self-stimulation, motor rebounds) a special analyser has been developed. After pre-amplification the ECG sign is fed into the input of the analyser which transmits it through a form-recognizing system to a measuring circuit. On the output of the measuring circuit the actual sign appears in relation to the present heart rate value and is continuously recorded. In addition to animal experiments the apparatus seems suitable for clinical purposes.

Effect of beta-receptor stimulation and inhibition on the survival and arrhythmia after ligation of the left coronary in the rat

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The descending branch of the left coronary was ligated at its origin according to SELLYE (1960).

In the 26 basic experiments, 9 animals survived the first ten minutes after ligation. The type and duration of the arrhythmia appearing during the first phase of the intervention, as well as the alteration after the ligation were followed by means of continuous ECG recording.

All of the 20 sham-operated animals survived the intervention with transitory ECG changes seen during the first phase.

Twenty animals were pretreated with isoprenaline (10 μg/100 g body weight); 16 of them survived the coronary ligature, with frequent heterotopic manifestations (ventricular tachycardia, extrasystole, transient ventricular flutter and fibrillation).

Ten animals were pretreated with propranolol (200 μg/100 g body weight); only two of them survived the ligation. In all cases, the ECG pattern was characterized by a depression of the QRS complex and low voltage. Cardiac activity was remarkably rhythmic till death.

Activation of rat brain tyrosine hydroxylase by ascorbic acid in vitro

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Since the discovery of tyrosine hydroxylase (NAGATSU et al., J. Biol. Chem. **1964**, 239: 2910), a number of authors studied the inhibition of its activity (MCGEER, Can. J. Biochem. **1967**, 45: 115—131; TAYLOR et al., Biochem. Pharm. **1968**, 17: 1779—1788; LEVITT et al., Biochem. Pharm. **1967**,

16: 1313—1321); the problem of the activation of the enzyme only arose during the last years. PETRACK et al. (J. Biol. Chem. **1968**, 243: 743) were the first to observe that the tyrosine hydroxylase of the ox adrenal is activated by ascorbic acid in vitro. The present authors have observed the ascorbic acid-induced activation of the rat brain tyrosine hydroxylase. The optimum concentration of ascorbic acid in the case of partially purified rat brain tyrosine hydroxylase is 3 to 5 μmol/mg protein. Alpha-methyl-p-tyrosine fails to inhibit the activity of the enzyme in the presence of high concentration of ascorbic acid. In experiments in vivo, oral treatment with ascorbic acid did not alter the enzyme activity in control- and in α-methyl-p-tyrosine-treated rats.

Role of the sympathetic adrenergic system and glucagon in endotoxin-induced hyperglycaemia

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Injection of the endotoxin of Gram-negative bacteria is followed by hyperglycaemia. Some authors ascribed this phenomenon to adrenaline mobilization, while some others to a glucagon effect. Experiments were performed on dogs divided into four groups. Endotoxin injected into sham-operated animals produced marked hyperglycaemia. This hyperglycaemia was much slighter in dogs subjected to bilateral transection of the splanchnic nerves one week prior to the endotoxin administration. Similarly, the hyperglycaemia decreased in animals in which the pancreas had been removed one week earlier; the diabetes of these dogs was treated with insulin. No hyperglycaemia developed under the effect of endotoxin one week after simultaneous splanchnic transection and extirpation of the pancreas; on the contrary, a transient diminution of the blood sugar level was observed in these animals. It is concluded that catecholamine mobilization and glucagon liberation are equally responsible for the endotoxin hyperglycaemia.

Role of the parasympathetic system in the regulation of salivary carboanhydrase activity in the rat

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It was reported at the 1971 Congress of the Hungarian Physiological Society that alpha- and beta-receptors participated in the regulation of salivary carboanhydrase activity in the rat.

In the present experiments the role in this process of the parasympathetic system has been investigated. For parasympathetic stimulation,

various doses of pilocarpine and carbachol were infused intravenously. The amount of the saliva secreted by the submandibular gland was measured by the colorimetric method of WILBUR and ANDERSON. Enzyme activity in the secreted saliva was in no relationship with either the dose applied or the velocity of secretion. Similarly, no difference was found in the effect on carboanhydrase activity of pilocarpine, a drug possessing a slight sympathomimetic side-effect and that of the purely cholinomimetic carbachol. Enzyme activity in the saliva produced under the effect of parasympathetic stimulation was considerably lower than that of saliva obtained after sympathetic stimulation. In self-control experiments, parasympathetic stimulation for 1 to 2 hours failed to decrease enzyme activity in the submandibular gland, in contrast with e.g. the beta-mimetic isoprenaline.

Enzyme activity in the saliva produced by the submandibular gland seems to be regulated by the sympathetic system. Also, the alterations measured after parasympathetic stimulation can be explained by a slight activation of the sympathetic system.

New data on the pathomechanism of the immobilization and reserpine ulcer

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The role of the autonomic nervous system in the pathomechanism of experimental gastric ulcers is not clear. While the deteriorating effect of parasympathetic excitation and the beneficial effect of parasympathetic blockade are unequivocally accepted, opinions differ concerning the role of the sympathetic nervous system and its mediator substances.

In view of this the effect of beta-mimetic and blocking drugs has been studied on the experimental gastric ulcer produced by immobilization and reserpine in the rat.

Beta blocking agents did not significantly influence the ulcers, while beta-mimetics decreased the extent of ulceration in both models. It is probably the beta^2 effect which is responsible for the moderation of the pathological changes.

It is concluded that sympathetic effects play a non-negligible part in the pathomechanism of immobilization and reserpine ulcer.

Mechanism of hippocampal cobalt epilepsy in the rat

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In previous experiments the characteristics of experimental epilepsy elicited by the intrahippocampal application of cobalt powder in the rat have been studied. In the present experiments, the changes in electrical activity of the bilateral hippocampus and mesencephalic reticular formation have been observed on the same model. Spike potentials were found to appear around the cobalt focus and then in the contralateral, intact hippocampus, i.e. in the mirror focus.

Motor fits only develop when spike potentials of high voltage and frequency appear in the lesioned region or in the mirror focus, and high frequency spike potentials in the reticular formation and cortex. In the case of low frequency spike activity of the reticular formation and cortex, no seizure develops. Consequently, the electroclinical seizures manifest themselves in the model described when the convulsive potential spread from the hippocampus over the ipsilateral reticular formation and then to the cortex.

The role of ionic milieu in the generation of spontaneous electrical activity of the identified giant neurones of *Lymnaea stagnalis*

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The effect of changes in extracellular Na^+ and Ca^{++} concentration were investigated on the identified giant neurones of *Lymnaea stagnalis*. In Na^+-free solution, spontaneous activity creased in 5 to 10 minutes. During this period there was a transient hyperpolarization of the membrane. At the same time, excitability of the membrane persisted for a long period, and the neurone could be activated by depolarization.

In Ca^{++}-free medium the frequency initially increased. This was followed by a gradual deterioration of action potential generation, and the membrane lost its excitability. In the meantime, a transient change could be observed in the membrane potential.

The different neurones showed marked individual variations. The sensitivity of various identified neurones to deprival of the ions investigated was compared.

On the basis of the results, conclusions have been drawn as to the ionic mechanisms of action potential generation.

Effect of aminoglutethimide treatment on corticosteroid biosynthesis in the rat adrenal

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Aminoglutethimide (A-G, Elipten, CIBA) is known to inhibit steroid biosynthesis, i.e. the transformation of cholesterol into delta-5-pregnenolone. In addition, the data in the literature speak for a striking inhibitory effect of the drug on aldosterone secretion (KÜCHEL et al. **1967–71**; GLÁZ et al. **1970–71**). The mechanism of the inhibition of aldosterone biosynthesis is not clear as it is not known whether the effect was produced by influencing just one, or several, steps of corticosteroid biosynthesis. To study this question, the effect of A-G treatment on corticosteroid biosynthesis in the rat adrenal has been investigated in the presence of ^{3}H-pregnenolone.

A-G treatment significantly decreased both the absolute amount of 18-OH-corticosterone and aldosterone synthetized from the endogenous precursor, and the percentage incorporation of ^{3}H-pregnenolone from the exogenous precursor. In contrast, these parameters were unequivocally augmented in the case of 18-OH-DOC and corticosterone. This latter observation points to the inability of A-G to counteract the corticosterone–ACTH feed-back effect. Since in spite of the augmented corticosterone values the synthesis of both aldosterone and 18-OH-corticosterone was significantly decreased it may be assumed that aminoglutethimide inhibits the activity of 18-hydroxylase. A generalized inhibition of corticosteroid synthesis by A-G could also be achieved in the case of simultaneous dexamethasone administration to eliminate the corticosterone–ACTH feed-back effect. The studies have been completed by morphological methods.

Role of monovalent and bivalent ions in action potential generation in the myocardial cells of *Helix pomatia*

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Experiments with microelectrodes revealed the resting potential (RP) to be mainly potassium-dependent in the edible snails. However, Na, Ca and presumably also Cl ions are participating in the determination of RP.

Low Na and high Ca concentrations caused a hyperpolarization of the membrane. Action potential generation ceased in both Na-free and Ca-free

medium. If the Na ions were replaced by Li ions the velocity of the ascending branch of the action potential increased. This was followed in some cases by an inhibition of the generation of spontaneous activity, but the excitability of the membrane persisted. A similar effect was observed in solutions with a high extracellular Ca content.

The generation of action potential was inhibited by tetradotoxin, ouabain and $MnCl_2$.

Renal circulation of hypophysectomized rats in hormone-induced renal cortical necrosis

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It has been shown earlier that in rats hypophysectomized a few weeks earlier vasopressin administration after oestrogen pretreatment failed to induce renal cortical necrosis or, if so, in a slight degree only, in contrast to non-operated control animals.

In the present experiments, HAMILTON's dye dilution method was combined with SAPIRSTEIN's ^{86}Rb technique. It was shown by means of this combined method that renal blood flow decreased in untreated rats subjected to hypophysectomy a few weeks earlier. Renal blood flow diminished to an even greater extent one hour after the administration of vasopressin to oestrogen-pretreated hypophysectomized animals. In accordance with this, angiorenography revealed a high degree of vasoconstriction to develop one hour after the injection of vasopressin in oestrogen-treated hypophysectomized rats as compared to non-operated control animals.

It seems that the oxygen demand of the kidney is diminished after hypophysectomy. Oestrogen and vasopressin treatment does not lead to renal cortical necrosis because the oxygen deficiency due to the renal vascular spasm is better tolerated under such conditions.

Activity of the sympathetic afferent nerves of the heart and forelimbs. I. Spontaneous activity and baroreceptor effects

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Only few investigations have been performed into the regional differences in efferent sympathetic activity even though the question is of importance from the point of view of the regulation of circulation.

Activity of the sympathetic postganglionic nerves running to the heart (cardiac nerve) and forelimbs (vertebral nerve), the integram of the activities, arterial blood pressure as well as respiration were recorded in immobilized and artificially breathed cats under chloralose–urethan anaesthesia.

Spontaneous activity: pulse and respiration synchronous groups appeared on both nerves. The active and inhibited periods were more sharply separated in the cardiac nerve.

Baroreceptor effects: upon bilateral compression of the common carotid arteries an abrupt augmentation was observed in the activity of both nerves during the first 5 seconds (176% in the case of the vertebral nerve and 157% in the case of the cardiac nerve). The activity of the vertebral nerve remained elevated (196%) for the duration of the compression, while that of the cardiac nerve rapidly decreased and returned to normal around the 20th second. The changes in blood pressure during compression ran parallel with the changes of the activity of the vertebral nerve. When blood pressure was kept at a constant level by means of a buffer system, the activity of the cardiac nerve remained elevated for the duration of compression.

The results point to a different central regulation of sympathetic activities running to the two regions.

Effect of hindlimb ischaemia on the ^{14}C-tyrosine and ^{14}C-noradrenaline content of the hypothalamus and brain stem of the rat

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According to previous experiments (STONER and ELSON, J. Neurochem. **1971,** 81: 1837.), noradrenaline concentration in the hypothalamus decreases under the effect of hindlimb ischaemia, a feature to be ascribed to an increased noradrenaline utilization. Similar changes were found in the brain stem after treatment with alpha-methyl-p-tyrosine, a drug inhibiting noradrenaline synthesis.

In the present experiments, noradrenaline turnover in the hypothalamus and brain stem was investigated after hindlimb ischaemia. ^{14}C-tyrosine was used as precursor. The control animals were investigated 1, 2 and 5 hours after the administration of tyrosine, and the shocked animals 1 and 4 hours after injection of the precursor. The level of newly synthetized ^{14}C-noradrenaline and that of ^{14}C-tyrosine and unlabelled tyrosine were determined in the tissues mentioned above and in blood. The incorporation rate of ^{14}C in both hypothalamus and brain stem was higher after limb ischaemia than in control animals.

Multiple unit activity in brain stem and limbic system during habituation to acoustic stimulus in the cat

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Semi-microelectrodes were chronically implanted into the brain stem and forebrain structures of freely moving cats by the standard stereotactic technique. The changes in multiple unit activity (MUA) were studied according to the modified method of GUZMAN and ALCARAZ. The method allows the simultaneous and continuous recording of EEG and MUA of several cerebral regions. Electrical activity was simultaneously recorded in a minimum of four subcortical regions. In addition, a programmed switching of the EEG apparatus and of the MUA analyser allowed the alternate analysis of 6 or 8 brain regions in the same animal.

Prior to the experiments the animals were habituated to the experimental environment for several hours a day, always at the same time, for weeks. This was followed by habituation to the acoustic stimulus. The cats habituated to the environment showed an attentive reaction at the beginning of the serial presentation of the acoustic stimulus. Later, this changed to a slow wave and paradoxical sleep phase.

The results of EEG and MUA analyses unequivocally showed that the diminution in reactivity to the acoustic stimulus of the central nervous system developed significantly later than did the disappearance of the EEG arousal reaction. The paradoxical sleep phase was followed, with great regularity, by a transient augmentation of the reactivity of all the brain regions investigated.

Injuries of the skeletal musculature caused by immunoglobulins in the rat

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An experimental model was developed to test the effect of immune sera produced against various muscular elements (myofibril, myosin and actin).

The fine structure of the muscle fibre was damaged at an early stage of the intervention, i.e. in specimens obtained on the 15th day; the sarcometric arrangement and the fibrillar organization of the filaments disappeared.

In specimens obtained on the 30th day of the experiment the damage to the fine structure was more extensive and autophagous vacuoles appeared. The synaptic cleft in the neuromuscular junction was widened and basal membranelike material had accumulated in it. The postsynaptic apparatus was distorted.

In specimens obtained on the 60th day the interfibrillary substance was increased and many fibroblasts rich in collagen fibres appeared. Lymphocytes and plasmocytes could be observed in the blood vessels.

The injuries involved all organelles of the muscle fibre and became increasingly more severe with the duration of the experiments.

Investigation by surface fluorometry of oxidative metabolism, gluconeogenesis and redox state of the perfused rat liver during haemorrhagic shock

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Previous investigations and data in the literature led to the conclusion that the oxidative system of isolated mitochondria is damaged in the terminal phase of haemorrhagic shock. The problem arose whether the damage was a consequence of preparation inasmuch as shocked mitochondria would tolerate the trauma caused by their isolation to a lesser degree than do normal mitochondria. To decide this question as well as to clarify the essence of the hepatic injury, the liver was removed at various stages of the haemorrhagic shock and perfused with Krebs-Ringer solution + 4% albumin. The pH of the perfusion fluid was brought to 7.4 by a gas phase of 95% O_2 5% CO_2.

Glucose production and oxygen consumption by the liver were determined and the redox state of the flavoprotein and pyridine nucleotides, the kinetics of the post-hypoxic restitution as well as the effect of activators and inhibitors were measured by means of surface fluorometry. A special advantage of this new optic method is its allowing a continuous study of the cellular energy level and the kinetics of the cells without disrupting them.

Gluconeogenesis in the perfused liver of rats in haemorrhagic shock decreased significantly in the phase when oxygen consumption was still normal. Thereafter, the flavoproteins and pyridine nucleotides of the liver cells underwent a reduction with the oxygen consumption, unchanged. The changes were counteracted by phenoxybenzamine.

According to the results, it is the extramitochondrial system which is first damaged in shock. This is followed by uncoupled respiration and, finally, by an inhibition of mitochondrial oxidation.

Pharmacological investigations on the genital smooth musculature of *Helix pomatia*

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The pharmacological sensitivity of the longitudinal musculature of the male genital tract of *Helix pomatia* has been studied. Spontaneous contractions were recorded by means of an auxotonic lever. Spontaneous contractions were augmented by serotonin (10^{-8} M, increase in amplitude, frequency and tone) and left unaltered by histamine and tyramine.

The preparation exhibited a remarkable sensitivity to cellulin-A. The lyophilized powder of 0.5 to 1 U/ml cellulin-A activity prepared from frog skin considerably increased the activity in both normal and low-Ca medium.

It was characteristics of the investigated organ that cellulin preparations from frog liver and urinary bladder, acting like cellulin-A on the frog heart, were ineffective.

Prostaglandins (PGE_1 and PGE_2) at concentrations from 5 to 10 ng/ml exerted a cellulin-A-like enhancing effect.

According to the results, the genital smooth muscle of *Helix pomatia* represents a preparation of special pharmacological sensitivity, suitable for testing substances of biological origin.

Effect of corticosteroids on passive avoidance behaviour of the rat

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The effect of corticosterone, 11-desoxycorticosterone (DOC) and of synthetic 6-dehydro-16-methyl-hydrocortisone (6dh-16m-hydrocortisone) has been studied on the thirst-driven passive avoidance behaviour of the rat.

The latency of passive avoidance behaviour elicited by electric shock decreased on the systemic administration of corticosterone and 6dh–16m hydrocortisone immediately after the shock and 24 hours later. A similar effect was observed after the implantation of corticosterone in the anterior hypothalamus, rostral septum, dorsal hippocampus or midline thalamic nuclei. Implantation into most of these structures of 6dh–16m-hydrocortisone and DOC proved to be ineffective.

According to the results, the direct central action of the hormones is more important in the development of their behavioural effects than are the effects due to the decreased ACTH secretion. In addition to the forebrain structures, the midline thalamic nuclei are responsible for the action.

Antithrombin effect of acetylsalicylic acid

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Acetylsalicylic acid is known to exert a marked and long-lasting inhibitory effect on the aggregation and adhesion of blood platelets. On this basis, the possibility arose of a clinical application of the compound in the prevention of thrombosis.

The data on the inhibitory effect of acetylsalicylic acid on experimental thrombosis are contradictory. According to some authors, the compound exerts an antithrombotic action in vivo, while others failed to confirm these results (laser-thrombosis test, ARFORS et al. 1971).

Among the in vivo methods it is the thrombosis induced by laser which is today the most precise experimental method, and the most similar to human thrombosis in its mechanism. The experimental equipment using gas-laser, and the technique employed is basically different from the laser-thrombosis method described earlier for the investigation of drug effects. The experimental parameters are demonstrated on the example of acetylsalicylic acid, and the effectiveness of the compound is evaluated.

Subcellular distribution of thyroid hormones in the central nervous system. II. Effect of thyroid hormone treatment at newborn age

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The effect of unlabelled triiodothyronine (25 μg subcutaneously) given to female albino rats on the day of birth has been investigated on the ^{125}I-triiodothyronine uptake of the brain (cerebrum, cerebellum, hypothalamus), and on the subcellular distribution of the hormone during postnatal development.

The uptake of the labelled hormone was considerably higher in all three brain regions investigated when the animals had been treated with triiodothyronine at newborn age. Significant differences were found in the subcellular distribution of the labelled hormone between the treated and control animals. Upon the effect of thyroid hormone treatment, activity decreased in the cell nuclei but markedly increased in the mitochondrial fraction. The difference was slighter in the microsomal fraction, while the radioactivity found in cellular fluid (supernatant of 105,000 g) was considerably diminished in the treated animals.

As to the effect of thyroid hormone on the subcellular distribution of labelled thyroid hormone, there were differences between the individual brain regions.

The differences observed between treated and control animals were most marked in the young age group.

Kinetic analysis of the sodium transport in striated muscle. II. Effect of veratrine on sodium uptake

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It has been shown at the 37th Congress of the Hungarian Physiological Society that the ^{24}Na uptake of isolated frog sartorius muscle consisted of a quick and a slow component. In the present experiments two questions have been investigated. 1) How does the alteration of external Na concentration influence the parameters of Na uptake? 2) How does veratrine influence the quick and slow components of Na uptake? (Veratrine was demonstrated to increase Na influx at an external Na concentration as low as 2 mM.)

The quick and slow components of Na uptake are based on two different mechanisms. Influx of the quick phase followed Michaelis–Menten's kinetics and proved to be a carrier-mediated process ($V_{max} = 5.6$ pM $\cdot$ cm^{-2} $\cdot$ sec^{-1}; $K_m = 10$ mM).

Veratrine only accelerated the quick component of Na influx at an external Na concentration higher than 10 mM. In contrast, it augmented the Na influx of the slow component by more than 100% in the case of $Na^0 =$ $= 2$ mM. ($V_{max} = 9.2$ pM $\cdot$ cm^{-2} $\cdot$ sec^{-1}) and decreased the K_m ($K_m =$ $= 75$ mM).

Veratrine is assumed to increase either the concentration of carrier molecules, the binding affinity of Na ions, or the rotation velocity of the carrier molecules.

Effect of La^{+++} on ^{45}Ca uptake and release of frog skeletal muscle

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According to data in the literature, 1 mM of La^{+++}, when given to Ringer's solution, decreases ^{45}Ca uptake of the muscle and uncouples its electrical and mechanical processes. Thus, the effect of La ions on the magnitude of Ca fractions and on their exchange rate has been investigated. Fibre preparations of frog semitendinosus muscle were incubated in La^{+++}-free

and La^{+++}-containing (1 mM) Ringer's solution for various periods of time. Thereafter, the ^{45}Ca fractions were analyzed by means of desaturation curves.

In the control muscle the Ca fraction (which fraction amounts to 17 $\pm$ $\pm$ 3 μM/g fresh weight, has a half-life of 5.8 $\pm$ 1.2 min and is presumably loosely bound to the surface membrane) could not be demonstrated in the presence of La^{+++}. Parallel with this there was a significant augmentation in the intracellular ^{45}Ca space, while the exchange of intracellular Ca decreased during the wash-out period. Incubation for less than two hours decreased the La^{+++} effect, while longer incubation increased the total Ca content of the muscle.

The regulating role of membrane-bound Ca in the functioning of the sarcoplasmic reticulum

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The sarcoplasmic reticular fraction (SRF) was prepared from the white skeletal muscles of *Ameiurus nebulosus*, suspended in 0.1 M KCl and, after the addition of EGTA solution in a final concentration from 0.1 to 1.0 mM, the pH of the suspension was adjusted to 6, 7 or 8, by means of 50 mM KOH solution. Thereafter, the SRF samples were diluted with an appropriate amount of 0.1 M KCl solution containing 5 mM of histidine, and their Ca uptake, ATPase and cholinesterase activities were determined. Ca uptake was measured by means of ^{45}Ca and the MILLIPOE technique. In some instances, the Ca content of the fractions was checked by spectrographic analysis. It was found that adjusting the pH in itself, (i.e. without EGTA treatment) did not influence the characteristics of the fractions. EGTA treatment (final concentration from 0.2 to 1.0 mM, pH 7 and 8) removed most of the bound Ca, as shown by spectrographic analysis.

Ca uptake was decreased to 1 to 2% of the control value and, at the same time, a 2 to 3-fold increase occurred in the activity of ATPase and cholinesterase. A partial or complete restitution could be achieved by applying an equimolar amount of Ca together with EGTA.

Reabsorption processes in the proximal tubule during "stop-flow"

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The reabsorption processes in the proximal tubule closed for 5 and 10 minutes were studied in anaesthetized rats during mild diuresis produced by physiological saline or mannitol.

1) Osmolarity, Na and K concentration of the tubular fluid did not change during stop flow under saline or mannitol diuresis. F/P_{osm}, F/P_{Na} and F/P_K values were the same as during free urine flow.

2) Inulin concentration (F/P_{in}) in the tubular fluid increased as a function of time in the closed tubule, a finding indicative of a continuous filtration during stop flow.

3) The data obtained during mannitol diuresis indicated that mannitol was easily rediffusing in the closed proximal tubule.

4) Due to the developed pressure gradient, the tubular fluid in the proximal tubule also rediffused passively while the reabsorption process involved an active cell function.

Effect of quinaldic acid on mitochondrial respiration

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According to HANSON et al. (J. biol. Chem. **1969,** 244: 4351.), quinaldic acid inhibits gluconeogenesis by blocking pyruvate oxidation in liver mitochondria. The present work performed with oxygen electrodes showed quinaldic acid instantaneously to inhibit the oxidation of a number of substrates in the rat kidney and liver mitochondria. In the liver mitochondria, a 50% inhibition of oxygen consumption activated by ADP was induced quinaldic acid at the following concentrations, 1 mM with pyruvate, 2-oxoglutarate (in the presence of malonate) or 3-hydroxybutyric acid; 2 mM with glutamate + + malate; 5 mM with succinate (in the presence of malate and arsenite). The corresponding values in the kidney mitochondria were as follows: 2 mM with glutamate + malate as substrate; 4 mM with 2-oxoglutarate or isocitrate, and 8 mM with succinate. Practically identical K_i values were obtained in the presence of uncoupling agents (instead of ADP) or after previous freezing–thawing of the mitochondria. Quinaldic acid also inhibited the resting and ADP-activated endogenous respiration of liver mitochondria, at the concentration levels investigated the compound did not uncouple nor inhibit either the oxidative phosphorylation or the respiratory chain. It is concluded that quinaldic acid inhibits mitochondrial respiration at the dehydrogenase level in dependence on the sensitivity to quinaldic acid of the various dehydrogenases.

Control of cortical potentials evoked by stimulation of small intestine and splanchnic nerve during sleep

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The changes in evoked cortical potentials during sleep have been studied in the cat with small intestine fistula and chronically implanted splanchnic electrodes. By means of the latter the epithelium of the small intestine and the splanchnic nerve were stimulated at varying intensities. The potential evoked in the cortex during slow wave sleep spindles and spindle-free periods as well as those observed during the phasic (REM) and tonic periods of paradoxical sleep were compared.

The control patterns of the viscerally evoked potentials during the periods mentioned above depended on the condition whether weak stimuli under the threshold of somato-visceral reflexes or strong ones activating the above reflexes were applied in order to evoke the cortical responses.

Studies of histidine decarboxylase inhibitors in inflammatory models

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The effect of two specific histidine decarboxylase inhibitors, 2-hydroxy-5-carbomethoxy-benzyloxyamine and O-3'-pyridyl-methyl(-hydroxylamine), and that of the enzyme inhibitors brocrezine and tritoqualine were compared. The enzyme inhibitory action as well as the histamine level decreasing effect of the compounds were analyzed. In addition, the types of inflammation inhibited by the above compounds were studied.

Carragenan oedema, cotton-wool granuloma, adjuvant arthritis as well as systemic and cutaneous anaphylaxis served as experimental models.

The compounds, when used in oral doses from 150 to 450 mg/kg in form of a subacute treatment, mainly inhibited the processes accompanied by proliferation. In the guinea pig they also inhibited the allergic manifestations.

The results support the assumption that histamine does not always play a primary role in inflammatory processes.

Neuronal connections in the suboesophageal ganglion of *Helix pomatia*

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Action potentials were simultaneously recorded in 20 instances from three suboesophageal neurons of *Helix pomatia*. The synchronous frequency-distributions obtained from the activity of the individual neurons were analyzed. The following type-characteristics could be distinguished.

1) Monomodal triplet: two or three frequency maxima at the same site.

2) Monomodal or bimodal neurons differing in main frequency.

3) A neuron of trimodal frequency distribution with two other neurons, the main frequency of which coincides with the rarest frequency of the first neuron (i.e. with the minimum points of the distribution).

4) At least one of the maxima of the bimodal activities is identical in all three neurons.

5) Mixed, and intrically connected types. E.g. the second neuron is active at the minimum place of the first neuron, or, the minimum place of the first neuron is found at the main frequency of the first neuron.

It is concluded that the investigated neurons of the ganglion represent activities of certain favoured frequencies. The maximum and minimum places of the frequency distributions are interconnected.

Applications of Hill's subthreshold stimulus theory in the experimental approximation of the relationship between stimulus intensity and stimulus duration

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Investigation of the validity of HLIL's theory (1936) of subthreshold stimuli meets with numerical difficulties. The theory has not lost its actuality since it interprets the time duration curves of stimulus intensity (SDR) and the accommodation in a simple way only its accuracy is not satisfactory (LÁBOS and FAZEKAS, 1969). The consequences of the theory have been analyzed as follows.

1) The theoretical SDR curves were tabulated according to three variants: the time constant t, the accommodation constant λ, and duration d. About 10,000 threshold values were obtained with the aid of a computer.

2) The authors' experimental data cannot be reconstructed either by closing or opening SDR curves.

3) Marked differences occur in large and small d stimulus durations. In addition, HILL's theory is not correctly interpreting the closing threshold

of prolonged stimuli. The theoretical opening curve was found better for the approximation of the closing stimulus.

4) With the sufficiently detailed HILL's table the accommodation constant can already be estimated on the basis of the SDR curves. However, the accuracy of this estimation is limited by the accuracy of HILL's theory.

The role of haemodynamic factors in renal failure in dehydration

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The parameters of renal function have been studied in dogs during dehydration azotaemia induced by pyloric ligation. On the basis of the resistance of parallel-connected and series-connected intrarenal vascular sections, the functional organization of the dehydrated animals changes as follows.

1) Arterial pressure and renal blood flow change slightly and nearly proportionally. Vascular resistance of the kidney is somewhat increased.

2) A significant elevation of pressure occurs in the afferent vascular sections; as a consequence, glomerulocapillary pressure is considerably decreased.

3) Glomerular filtration rate decreases in proportion to the effective filtration pressure. The result is a diminution of glomerulocapillary pressure and a haemoconcentration due to the increased colloid-osmotic pressure.

4) The extraction of PAH, oxygen and Rb allows to conclude to a preferential diminution of blood flow in the medulla during dehydration azotaemia. The decrease in cortical circulation is less pronounced.

5) The above changes point to an adjustment of renal function. The increase in afferent resistance caused by the stimulus of dehydration as well as the decreased medullary circulation create the conditions necessary for the production of a small amount of maximally concentrated urine.

EEG and evoked potentials in experimental organic mercury poisoning

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It was shown previously that methoxy-ethylmercuri chloride (MEMC) diminished the conduction velocity in the sciatic nerve of the rabbit (35th Congress of the Hungarian Physiological Society, **1969**). This could be explained by a demyelinization of the nerve.

In the present experiments, EEG and cortical evoked potentials have been studied in the course of subacute MEMC poisoning. EEG frequency changed considerably, revealing a significant increase in alpha, $beta_1$ and $beta_2$ frequency, in addition to a slightly increased total energy level.

Amplitude of the evoked potentials recorded from the somatomotor cortex after sciatic stimulation decreased during the poisoning. The duration of the first positive wave increased considerably to about double the original. In contrast with expectations, all this indicated that MEMC induces a grave nervous system injury similar to those caused by methyl-mercuric compounds.

Effect of intracerebrally administered diphenylpropylamine derivatives

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In the authors' previous experiments the compound 174. TK [1,1-bis-(4-ami-nophenyl)-propylamine-(3)] exhibited a cocainelike noradrenaline potentiating effect. The compound devoids, however, of a central nervous action since it does not penetrate across the blood–brain barrier.

In the present experiments the drug was administered intracerebrally to mice. A dose of 120 μg/animal evoked an excitation state accompanied with signs of sympathetic excitation (exophthalmus, mydriasis, piloerection). This lasted for hours and could be inhibited by chlorpromazine, tetrabenazine and urethane. Higher doses caused convulsions and death; $DL_{50} = 294$ μg/animal.

174. TK applied intracerebrally in a dose of 120 μg/mouse caused a 2°C fall in body temperature lasting 2 to 3 hours. This could be inhibited by chlorpromazine and phenoxybenzamine.

The concentration of intracerebrally applied 174. TK in the brain was as follows: in the first minutes after the injection of 48 μg it was 5.2 μg/g; while after the injection of 120 μg it was 20.1 μg/g. The two doses disappeared from the brain in 180 and 300 minutes, respectively. Thus, 174. TK exerts its sympathomimetic, noradrenaline-potentiating effect also after intracerebral injection.

Location of the subcellular kininases of the skin

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Human skin as well as the skin of R-Amsterdam and CFY rats were used in the experiments. From the blood-free cutis and subcutis, a 10% per cent homogenate in 0.25 M sucrose was prepared with a high-speed homogenizer

and centrifuged at 700 g. Total kininase activity was estimated in the supernatant. Mitochondrial, microsomal and lysosomal fractions were separated. The kinin-splitting enzymes were set free from the subcellular fractions by repeated freezing, ultrasonic treatment and Potter homogenizer.

The mitochondrial fraction contained insignificant amounts of kininase. Of the subcellular elements, the microsomal and lysosomal fractions contained nearly all of the bradykinin-splitting enzymes. The kininase obtained from the subcellular elements (microsomes, lyosomes) was separated on Sephadex G-200 column. Three bradykinin-splitting enzymes different in molecular weight were separated.

The free bradykininase level of the skin was high, irrespective of the species.

It seems from the results that a considerable amount of active kininase is present in human and rat skin, in addition to subcellular particles.

Effect of anaemia on the circulation of coronary-ligated rats

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The effect of anaemia on systemic and regional circulation was investigated in coronary-ligated rats twenty-four hours after ligation of the left descending coronary artery, under pentobarbital anaesthesia. Anaemia was induced by a stepwise withdrawal of arterial blood for 20 to 25 minutes and by replacing of an equal amount of rat plasma with intravenous injection. Mean haematocrit was 29%. This was followed by determining cardiac output by the dye dilution method and the organ fractions of cardiac output by SAPIRSTEIN's ^{86}Rb and ^{125}J-antipyrine isotope indicator fractionation technique.

In the coronary-ligated and anaemic rats was found to increase cardiac output and total vascular conductance (Q/P) as compared to the control coronary-ligated rats, and organ blood flow and vascular conductance were elevated. The shifting of cardiac output, such as found in the control coronary-ligated rats failed to occur in the coronary-ligated and anaemic ones.

As compared with anaemic (without coronary ligation) controls to the coronary-ligated and anaemic rats, the circulatory changes were qualitatively similar, but quantitatively less. Thus the same compensatory mechanisms will be activated in the coronary-ligated rats under the effect of anaemia as in anaemia alone, and the circulatory effect of anaemia is manifested in spite of the coronary ligation, even though to a lesser degree.

Sex differences in the lipoprotein subfractions of rabbits

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In previous investigations it has been shown that the serum lipoprotein spectrum of normal rabbits was characterized by sex differences. Beta-lipoprotein, forming an intense band in the case of females, was practically lacking in male animals. Agar electrophoresis revealed a relatively high pre-beta-lipoprotein and a very low beta-lipoprotein level in the serum of male rabbits while the opposite ratio was observed in females. In further experiments the fine structure of the serum lipoprotein spectrum of normal rabbits was studied by acrylamide electrophoresis. Gradual and continuous, linear and exponential pore gradients as well as various buffer systems were checked. As a result, a method was developed which distinguished 3—5 subfractions in the alpha-lipoprotein and 5—7 subfractions in the beta-lipoprotein group. Some 12 subfractions different in mobility were obtained in one run. Some bands were present only in the male, others only in the female serum. In addition, quantitative differences were found between subfractions of identical mobility occurring in both sexes. The results indicate that sexual hormones are implicated in the regulation of the whole lipoprotein system of the rabbit.

Some membrane components of cerebrocortical synaptosome fractions

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At the 37th Congress of the Hungarian Physiological Society it has been reported that antibodies could be produced in the rabbit against antigens isolated from the cerebral cortex homogenate of cats. The immunoglobulins separated from rabbit serum reacted with the soluble membrane subfractions, as well as with the membrane subfraction prepared from the synaptosome fraction. In the present experiments purified membrane subfractions were used for immunization, and antibody activity could be demonstrated in the immune sera by means of immunodiffusion. Intracellular location of the membrane antigen was studied electron microscopically. Comparative disc-electrophoresis of cerebrocortical subcellular preparations as well as other chemical analytical methods yielded data concerning the chemical characteristics of the antigen.

Dynamics of human working capacity as evaluated on the basis of regulatory functions

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For human work performance a co-ordinated functioning of interrelated physiological regulatory systems is necessary. The complex systems cannot be investigated at the level of present knowledge but it is always possible to study some partial systems as "elementary" regulatory systems. With a sufficient number of well-chosen "elementary systems" a comparatively true picture can be obtained by means of combined mathematical analysis of elementary functions. In the present experiments on volunteers, the effect of a known work load was studied by estimating static, intentional and autonomic tremor, simple optomotor reaction time, disjunctive activity and error times as well as blood pressure. The data of individual tests were evaluated according to identical points of view furnished by the regulation theory. The results could be therefore represented by homogeneous co-ordinates (generalized distance) in a multi-dimensional space. In this way, a number of characteristics of the dynamics of complex regulation could be investigated in spaces of reasonable dimension number through conjunction of the various subspaces. The dynamics of complex regulation was found considerably to depend on the nervous reaction-type of the individual as well as on the starting situation of the regulatory system. Therefore, an approximate knowledge of the amount of work-load is necessary for measuring and evaluating the consequences of a given load.

Effect on lipid metabolism of N(bis)-p-chlorophenoxy(acetyl)-urea

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The effect of several bis-p-chlorophenoxyacetic acid derivatives was investigated on the cholesterol, triglyceride and free fatty acid level in normolipaemic rats.

The most active compound was N(bis)-p-chlorophenoxy(acetyl)-urea (M-451). Its lipid-decreasing effect surpassed that of clofibrate. Subacute treatment with 3, 10 and 30 ng/kg doses of the compound considerably decreased the level of serum lipid components and inhibited the incorporation of ^{14}C-acetate into the free cholesterol fraction of the serum and liver of the

animals. In the same animal species the drug diminished the hyperlipaemia caused by tyloxapol and fructose pretreatment.

The drug increased the liver parenchyma to a greater extent than did clofibrate without causing any change in the cholesterol content of the liver.

Experimental defibrination evoked by subtilysin

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Subtilysin, produced by *B. subtilis* var. proteolytic given to rats intravenously in doses of 5 to 10 mg/kg causes defibrination in one hour and lasting 10 to 24 hours. The changes due to subtilysin in the blood clotting and fibrinolytic system were characterized by an extreme lengthening of clotting time, the disappearance of fibrinogen, and by thromboelastographic alterations characteristic of a fibrinogaemia.

The blood of rats treated with subtilysin failed to show fibrinolysis on the Astrup fibrin-agar plate in vitro. From this it has been concluded that the lack of clotting is not due to an increased fibrinolysis but to the proteolytic effect of the drug on fibrinogen and other clotting factors. This was corroborated by the finding that subtilysin decomposes 100-times more fibrinogen than thrombin, and that its effect is not inhibited by EACA in vivo.

Ultramicrochemical determination of glucose-6-phosphatase and glucokinase in the rat nephron

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Quantitative histochemical methods were applied to determine the activity of the gluconeogenetic key-enzyme (glucose-6-phosphatase, G-6-Pase) and that of the glycolytic key-enzyme (glucokinase, GK) in various parts of the rat nephron. The following G-6-Pase/GK ratios were found in various part of the nephron:

Glomerulus	1.50
Convulated part of proximal tubule	2.80
Straight part of proximal tubule	1.75
Ascending limb of Henle	0.28
Convulated part of distal tubule	0.46

According to these results, the decrease of the ratio of the two enzymes in the distal nephron is indicative of a relative predominance of glycolysis.

The various parts of the nephron (proximal and distal convulated tubules) may exhibit different enzymatic and metabolic characteristics within the renal cortex.

Investigation on the habituation of acoustic stimulus by means of evoked potential audiometry

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Application of the audiometric method based on vertex potential detection has been reported at the 37th Congress of the Hungarian Physiological Society. Habituation to acoustic stimuli has, however, made it difficult to determine the acoustic threshold.

In the present experiments the effect of acoustic stimuli of different frequency applied in a random or periodic manner was investigated with special regard to habituation. The amplitude of the vertex potential was found to increase in proportion to the diminution of repetition frequency. There was no significant increase in the amplitude of responses to random stimuli. Also, the extent of habituation depended mainly on the frequency of repetition, the acoustic stimuli applied at random having been habituated in the same way as the stimuli applied periodically.

Role of bulbar cholinergic processes in vasomotor reflex activities

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It has been shown at the 37th Congress of the Hungarian Physiological Society that the bulbar application of curare inhibits or reverses the depressor reflexes elicited by stimulation of the central stump of the vagal and tibia nerves, while it facilitates the originally pressor-type reflexes. Curare is known to abolish monoaminergic inhibitions, in addition to the abolition of cholinergic ones. In the present study it was investigated to what extent the described phenomena could be brought into connection with the effect of curare on cholinergic processes.

Various drugs, selectively influencing cholinergic mechanisms (eserine, carbamoylcholine, hemicholine), were microinjected into the medial regions of the oblong medulla, and the changes in vasomotor reflex activity were followed. From the results, conclusions have been drawn as to the role of cholinergic processes in central vasomotor activity.

Relationship of tissular metabolic processes and cell division in regenerating liver tissue

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According to previous experiments, the energy household of regenerating liver tissue considerably differs from that of the control tissue at the time of high-amplitude mitotic activity after partial hepatectomy. It was characteristic of these alterations that both the anaerobic and aerobic glycolytic capacities of the tissue were elevated in the early, high-mitotic activity period of liver regeneration. A relationship was found to exist between mitotic activity and the increase of glycolysis in the tissue.

Glycolysis consists of a gluconeolytic and a glycolytic component. The ratio of the participation of the two components in total glycolysis is a function of and expresses the actual metabolic state of the tissue. Through selective inhibition of the glycolytic component by 2-D-glucose the glycolysis of mitotically resting tissue was shown to occur mainly at the expense of the gluconeolytic component, while the glycolytic component became dominant in the glycolysis of mitotically activated tissue, depending on the extent of mitotic activation.

Measurement of maximum oxygen uptake during treadmill exercise

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There are several load-patterns of the vita maxima run on the treadmill and the aim of the present investigations was to find the most appropriate one.

Ventillation, VO_2, VCO_2, RQ, heart rate, and gas exchange values were measured during four kinds of load different in running velocity, degree of elevation and duration. A Jaeger spiroergometer equipment was used and data were evaluated by means of an on line computer and digital printer.

The values for maximum oxygen uptake measured during the four types of load were practically the same.

Reproducibility of the parameters measured during 2 minutes running at a speed of 12 km/hour and increasing the elevation by 5 or 2.5% every two minutes are presented. Data are shown for sportsmen of varying endurance fitness.

Behavioural and EEG signs of instrumental escape from frustration situation

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Cats implanted with hippocampal electrodes were placed into a restraining cage after a 24—36-hour starving period. A piece of meat was put at a well-visible place 1.5 m apart from the plexiglass door of the cage. The door was opened, and the meat became accessible to the animals whenever the cat had touched an object of matchbox size placed in the cage. The animals exhibited a chaotic, excited behaviour in the cage (frustration phase accompanied by hippocampal desynchronization). After escape, perseverative motor manifestations, long-lasting orientation activity accompanied by continuous theta activity in the hippocampus as well as a transient suppression of the conditioned approach reactions were observed. These transient phenomena disappeared after a few repetitions but, after changing the place of the animal, they transiently reappeared.

The frustration arrangement is considered to represent a suitable method for elucidating the relationship between orientation and reinforcement mechanisms and the reproduction under natural environmental conditions of the crucial neuronal mechanism of reinforcement. This conclusion has been drawn from the present experiments as well as from the similarity of motor and bioelectric phenomena previously observed after electrical stimulation of subcortical regions (see: GRASTYÁN, SZABÓ, MOLNÁR and KOLTAI, 1968).

Dynamics of first-order and third-order blood pressure and pulse waves in gradual hypotension

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In dogs anaesthetized with chloralose a study was made of the pressure and flow in the iliac artery, central venous pressure, and pressure in the ascending aorta. Under artificial respiration and immobilization with gallamine, mean arterial pressure was stabilized by means of a buffer system between 150 and 170 mm Hg at 20 mm Hg steps in 30 min periods.

The stationary character of the processes investigated was checked by continuous recording and computing of the square mean value in the pulsative range and by that of the mean value in the third-order range. The periodic and stochastic components of circulatory characteristics were determined on the basis of autocorrective functions. Interactions of the signals were investigated by cross-correlation functions and frequency spectra.

The square mean value for the four pulsative characteristics of systemic circulation changed in a different way as a function of mean pressure. The relative value of the stochastic component showed a more uniform change, being the smallest at normotensive (110—130 mm Hg) and 70 mm Hg levels. Resonance phenomena could be observed in the supersonic domain of the pulse waves.

Effect of Vitamin D on the bone of parathyroidectomized rats

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The effect of Vitamin D on bone metabolism has been investigated in parathyroidectomized and sham-operated rats.

Vitamin D was administered by the intravenous route 24 hours before sacrifice.

The metaphyses of the tibia and femur were removed, and in their fragments glucose and hydroxyproline metabolism was studied.

Parathyroidectomy decreased glucose uptake and hydroxyproline liberation of rachitic bone, but did not inhibit the effect of Vitamin D on bone metabolism. Vitamin D increased glucose uptake and hydroxyproline production in the bones of parathyroidectomized rats just as it did in sham-operated rachitic rats.

Spinal afferent activity elicited by potassium chloride injected into the muscle's nutrient artery

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Previous studies allowed indirect conclusions as to the afferent structures implicated in the blood pressure reactions evoked by the injection of potassium ions into the popliteal artery.

In the present experiments, the afferentation of the said reflexes have been investigated by electrophysiological methods.

Afferent activity was recorded by means of extracellular microelectrodes in the spinal ganglion of immobilized cats anaesthetized with chloralose–urethan. The changes of the afferent activity were studied after injecting various amounts of potassium chloride in the ipsilateral popliteal artery. The type of fibre investigated was determined by electric stimulation and by measuring threshold conductance velocity.

Fibres of slow conductivity could be activated by small amounts of KCl, while the activity of fibres of the highest velocity changed only after the highest doses of potassium. The results are indicative of a physiological role of slow-conductivity fibres of muscular origin.

Effect of drugs influencing transport ATPase and adenylcyclase system on the parietal and non-parietal components of human gastric secretion

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The effect of histamine (0.5 mg, subcutaneously), pentagastrin (6 μg/kg, subcutaneously), atropine (1.0 mg, subcutaneously), theophylline (480.0 mg, intravenously) and of the beta blocking drug oxprenolol (2 mg, subcutaneously) has been studied on the parietal and non-parietal components of human gastric secretion. These components were determined on the basis of the amount, acidity, further the sodium, potassium, calcium and chloride content of the gastric juice.

1) The absolute amount of the parietal component (mEq/h) increased under the effect of histamine ($p < 0.01$), pentagastrin ($p < 0.01$) and theophylline ($0.05 > p > 0.01$), and decreased after the administration of oxprenolol ($p > 0.05$) and atropine ($p < 0.01$). 2) The amount of the non-parietal component decreased under the effect of histamine ($0.05 > p > 0.001$), atropine ($p < 0.01$) and oxprenolol ($p > 0.05$), and remained unaltered after theophylline and pentagastrin. 3) Concentration of the parietal component (mEq/litre) increased after the administration of histamine ($p < 0.01$), theophylline ($p < 0.02$) and pentagastrin ($p < 0.01$), and decreased after oxprenolol ($p > 0.05$). Atropine had no effect. 4) Concentration of the non-parietal component decreased after histamine ($p < 0.01$) and pentagastrin ($p > 0.05$), remained unaltered after oxprenolol and theophylline, and increased after the administration of atropine ($p > 0.05$).

Conclusions. 1) The hormonal and pharmacological regulations of the parietal and non-parietal components of human gastric secretion are different. 2) Hormones and drugs increasing the activity of the adenylcyclase system (histamine, pentagastrin, theophylline) cause a selective increase in the parietal component. 3) Beta blockade causes a moderate diminution of basal secretion. 4) Atropine, a drug inhibiting transport ATPase, preferentially decreases the non-parietal component of gastric secretion.

Disturbance of insulin secretion in rats treated with *Bordetella pertussis* vaccine

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The effect of adrenaline and noradrenaline on insulin secretion is reversed after pretreatment with *Bordetalla pertussis* vaccine (BPV), i.e. the two catecholamines decrease the plasma insulin level in control animals but considerably increase it after BPV treatment. Isoprenaline, theophylline and phentolamine augment insulin secretion in control rats, but considerably more in BPV-treated animals. The effect of glucose on insulin secretion is also enhanced by BPV treatment, but much less than in the case of the said substances. The disturbance in insulin secretion caused by BPV may play an important part in the pathologic regulation of carbohydrate metabolism as well as in the increased anaphylactoid sensitivity subsequent upon BPV treatment.

Expectancy wave in organic cerebral processes

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Expectancy waves have been recorded partly as a completion of the routine EEG of neurosurgical patients.

1) The expectancy waves can be ranged in characteristic morphological groups according to the points of view known from literature. There is no relationship between the shape of the expectancy wave and the physiological or pathological nature of background activity.

2) Appearance of the expectancy wave depends primarily of psychic factors.

3) Expectancy wave may develop after in the case of a severe cerebral damage, if otherwise the patient is capable of performing the paradigm.

4) The expectancy wave is considered an electrophysiological manifestation of a special psychic state ("conation") connected with a group of stimuli. The slow electronegative change is probably independently the frontal cortex.

Effect of retrograde degeneration on the ADH reserve capacity of hypophysectomized rats

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In previous experiments synthetic ACTH was shown to increase diuresis and to normalize the diuretic reaction induced by a water load in the hypophysectomized rat. This was assumed to be due to the fact that hypophysectomy is not followed by retrograde degeneration of the magnocellular portion of the hypothalamus.

In the present experiments prior to hypophysectomy electrolytic lesions were placed in the pituitary stalk by means of a Horsley–Clarke apparatus in order to elicit retrograde degeneration. In addition, the animals were treated with highly purified pig STH, and with a synthetic ACTH preparation of prolonged action containing 24 amino acids.

STH failed to influence urine excretion in the stalk-lesioned, hypophysectomized rats. On the other hand, a moderately increased diuretic response was observed after the administration of ACTH. In order to determine how the stalk lesion influenced the disturbances in water metabolism of hypophysectomized animals, the ADH content of the hypothalamus as well as the ADH reserve capacity were determined by the hyperosmosis method. The ADH content of the hypothalamus decreased moderately after the two surgical interventions but showed no further depletion after dehydration. Correspondingly, the amount of ADH in urine failed to increase to any significant extent upon the effect of hyperosmosis.

The results indicate that the stalk lesion prevented the regeneration of the stalk stump and inhibited by the retrograde degeneration the ADH outflow elicited by physiological stimuli. This mechanism may have a part in the phenomenon that ACTH administration causes polyuria in rats subjected to hypophysectomy after stalk lesion.

Citrulline synthesis of isolated mitochondria in uraemia

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Two of the five enzymes of the urea cycle, i.e.:

1) carbamylphosphate synthetase (CPS) and

2) ornithine-carbamyl transferase (OCT) are located intramitochondrially. In the intramitochondrial phase of urea synthesis, permeability factors

are involved in addition to the enzymatic steps. The substrate of OCT, i.e. the ornithine produced extramitochondrially, has to enter into the mitochondria the substrate of arginosuccinate synthetase (ASS), i.e. the citrulline produced intramitochondrially, must leave the mitochondria.

In addition, there is a high energy demand in the first intramitochondrial step of urea synthesis; it requires 2 mol of ATP. Owing to the metabolic disturbances characteristic of uraemic toxaemia (acidosis, disturbances in ion distribution, appearance of toxic products, etc.), many mitochondrial functions including citrulline synthesis, will be disturbed. This is shown, among others, by the finding that the citrulline-forming capacity of the mitochondria isolated from the liver of healthy animals decreases in the presence of uraemic serum as compared with the control.

Factors of energy production, permeability and enzymic functioning can equally be made responsible for the disturbances of intramitochondrial citrulline synthesis under uraemic conditions.

Energy production through anoxid endogenous oxidation in *Mytillus galloprovincialis* (Mollusca, Pelecypoda)

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It has been found that an ethanol or propanol extract prepared from the whole body of *M. galloprovincialis*, and containing the lipochromic pigment, is capable of oxidizing $NADH_2$ in vitro in a reversible way. This oxidative capacity gradually decreases after the animals had been in a state of permanent anoxia. As calculated on the basis of the pigment's redox potential, a mechanism in which the electron-acceptor role of the oxygen has been taken over by the lipochromic pigment, is capable of liberating about 57% of the energy produced during normal respiration. In animals under normal and permanently anoxic conditions, ATP production under anoxic conditions amounted to at most 57—60% of the control value. This suggests the existence in mollusc of such an energy producing mechanism which can supply energy without oxygen, at the expense of the oxidative capacity of the lipochromic pigment, the animal's environment has been gradually depleted of oxygen. This mechanism is termed anoxic endogenous oxidation.

Effect of direct cholinergic and adrenergic stimulation of the hypothalamus, thalamus and hippocampus on the alimentary conditioned reflex of the cat

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In connection with the study of the receptor structures of the brain regions mentioned in the title the effect of direct intracerebral application of carbachol, nicotine and noradrenaline were investigated on the conditioned alimentary reflex performance of the cat. Noradrenaline, up to a dose of 50 μg, was ineffective in all the investigated areas. Carbachol, in doses from 0.2 to 10 μg, reversibly blocked the conditioned reflex when injected into the anterior hypothalamic region, the dorsal and ventral hippocampal formations as well as into the intralaminary cell groups of the thalamus. Nicotine was ineffective in all these regions.

The deconditioning effect of carbachol was counteracted by small doses of atropine applied topically. These observations, together with the ineffectiveness of nicotine, indicate that the stimulation of muscarinic receptors was responsible for the effects observed. In all probability, stimulation by carbachol releases an increased functional activity. This may explain the ineffectiveness of noradrenaline, a drug playing only a modulatory role and being mainly depressor in character.

Effect of sympatholytics on liver associated with injury accompanying hyperthyroidism

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Intraperitoneal pretreatment of adult male rats with 0.5 mg/kg of triiodothyronine daily for ten days significantly decreased glucuronide conjugation and excretion both in the resting state and after a bilirubin load, as compared with the control group. Bilirubin excretion and conjugation in the treated group were higher before loading, and significantly lower after loading.

Without loading, the distrubance of glucuronide conjugation and excretion due to thyroid hormone treatment was not influenced by dibenzyline, propranolol or reserpine given simultaneously with the hormone. During bilirubin load, dibenzyline and reserpine treatment improved, while propranolol did not influence the extent of injury caused by triiodothyronine.

Without load, the degree of augmentation of bilirubin excretion and conjugation due to thyroid treatment was further increased by additional

reserpine administration. Dibenzyline had no effect, and propranolol influenced the augmentation only insignificantly. After bilirubin load, excretion and conjugation improved in the reserpine group, remained unaltered in the dibenzyline group, and diminished further upon the effect of propranolol.

Thus, the sympathico–adrenergic system is very likely involved in the development of hepatic injury associated with hyperthyroidism.

Mechanical and electrical activity of the muscle during rapidly changing dynamic effort

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Electrical activity of muscle was recorded in 30 junior sportsmen. It was possible to derive from the transformed EMG the force–time diagram with good approximation during both isometric and dynamic muscular works.

The record can be used to describe the mechanical activity of the muscle, provided the strict requirements connected with electrode positioning and calibration are compiled with. The results were evaluated by computer analysis; it was demonstrated that, with individual calibration, the method is highly reliable in a telemetric system, too.

The histograms determined from the discrete values belonging to the time-scaling gave correlations of $r = 0.93 - 0.98$.

The delay values for the electromyogram–dynamogram were determined by simultaneous multichannel equipment.

In vitro activation of the proteolytic proenzymes of pancreas

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Both normal and inflamed pancreases contain various proteinases in active and inactive form; these proenzymes are activated under different conditions.

In the normal pancreas homogenate active trypsin could not be demonstrated after 6-hour incubation at pH 6.0 or higher. In contrast, 3-hour preincubation at pH 4.2 resulted in the appearance of a considerable trypsin activity. Preincubation of normal pancreas homogenate with enterokinase at neutral pH induced the production of chimotrypsin with a maximum activity at the 50th minute. Trypsin activity began to augment considerably after the activity of chimotrypsin has declined.

The activity of trypsin, chimotrypsin, elastase as well as the azocasein splitting activity of the normal pancreas homogenate decreases by about 78% after 10-min incubation at 60°C. A similar degree of inactivation occurred when heat-treatment of the homogenate was performed in the presence of chimotrypsin or elastase. On the other hand, incubation of the homogenate in the presence of trypsin at 60°C for 10 minutes caused an about 25% increase in activity as compared with the control. Accordingly, thermostable proteinases are produced from proenzymes upon the effect of trypsin. Similar processes can be observed in the inflamed pancreas without trypsin; here, proteolytic activity rapidly decreased in the first minutes of incubation at 60°C, followed by an augmentation in activity to above the control value.

Blood flow of spleen and mesenteric lymph nodes during lymphocyte mobilization caused by polymetacrylic acid

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Increased invasion into blood by reserve lymphocytes was produced by means of polymetacrylic acid (40 mg/kg, intravenously). Blood flow in spleen and mesenteric lymph nodes was measured with the ^{86}Rb technique and arterial blood pressure was recorded. It was investigated whether the mobilization of reserve lymphocytes was brought about by the haemodynamic changes (blood pressure elevation, increased blood flow in lymphatic organs) caused by polymetacrylic acid.

It was found that neither spleen or mesenteric lymph node flow nor arterial pressure had changed upon the effect of the mobilizing agent.

It has been concluded that the reserve lymphocytes in lymphoid organs enter the lymph circulation and systemic circulation by their active movements.

Apolar interactions in extraction haemolysis

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The relationship between the lipophilia of bencyclane and analogous compounds and the intensity of haemolysis occurring during the extraction of these drugs from red cell suspensions has been investigated. The degree of lipophilia was estimated on the basis of the distribution of the drugs between n-octanol and the water phase. A total of 19 compounds was investigated;

their distribution coefficients (K) ranged over more than three orders of magnitude. Haemolytic activity was characterized by that concentration (C) of the analogue which caused 50% haemolysis in a 25% red cell suspension in Krebs-PO_4 buffer pH 7.4, incubated at 37°C and than extracted with paraffin oil. A close relationship was found between the two parameters in the case of analogues containing a dialkylamino group. These analogues also caused haemolysis in the case of cooled red cells or their dilution with the medium. The degree of lipophilia was related to the concentrations causing 50% haemolysis also in this case.

Regression lines were computed from the measurements according to the relation, $\lg 1/C = a + b \lg K$. The parameters of the equations in the order of extraction, cooling and dilution haemolysis were, $a = -0.25; +0.64; +0.35$; $b = 10.8; 0.80; 0.86$ and the correlation coefficients $r = 0.93; 0.91; 0.93$. On the basis of the Hansch model the results point to an apolar character of the interaction between red cell membrane and drugs.

Immunofluorescent investigations in myasthenia gravis: antineuronal activity of the sera

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Immunoglobulins were isolated from the serum of healthy persons and of patients suffering from myasthenia gravis. The immunoglobulins were labelled with fluorescein-isothiocyanate. Binding of the labelled antibodies was investigated in tissue sections of various mammals by direct and indirect immunofluorescence. The serum of myasthenic patients reacted with certain neurones of the cerebral cortex, brain stem, cerebellar Purkinje cells and the nuclei of the spinal motoneurones. The immunoglobulins of the myasthenic patients were bound to the nuclei of spermatogonia, and some sera also reacted with the nuclei of the epithelial cells of the thyroid acini. Antibodies from healthy and sick persons were not bound to the nuclei of muscle liver, spleen, ovarium and kidney tissue. On the central nervous factors are assumed to have a role in the pathogenesis of myasthenia gravis.

Effect of hyperlipaemia on the changes of vascular permeability caused by vasoactive substances

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It was shown earlier that artificially evoked hyperlipaemia inhibited the early vascular phase of acute inflammation in various inflammatory models. In the present experiments, the changes in vascular permeability evoked by various vasoactive substances (dextran, 48/80, histamine, serotonin) have been studied in hyperlipaemia induced by sunflower oil or Intralipid pretreatment, as well as in triglyceridaemia caused by tyloxapol pretreatment. Administration of sunflower oil or Intralipid diminished considerably the permeability increasing effect of dextran, histamine and serotonin, and moderately that of 48/80. Intravenous Intralipid exhibited a typical dose–effect relationship. Serum lipid determinations showed the inhibition to increase parallel with the increase of the triglyceride level. In hyperlipaemia induced by tyloxapol a moderate decrease was only seen in the effect of the vasoactive substances which did not bear any relation to the marked elevation of the triglyceride level.

It has been concluded that development of the antiinflammatory action depended greatly on the mode of induction of the hyperlipaemic state. Exogenous triglycerides produce a well-marked effect, while endogenous triglycerides only a moderate one. The phenomenon suggests that metabolic processes have a significant role in the effect of hyperlipaemia on inflammation.

Electrical activity and drug sensitivity of early human embryonic hearts

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The electrophysiological characteristics of the human embryonic heart have not been investigated before the 8th week of pregnancy. Similarly, few data are available as to the electrical activity of the heart of older embryos. Therefore, the heart of 5 – 20-week-old human foetuses obtained from interruption, has been studied by the intracellular microelectrode technique.

It was found that the duration of the ventricular action potential increases markedly with age. On hearts driven artificially the change was independent of the heart rate. The action potentials become typically human in shape at an early age and can easily be distinguished from those of other species, especially in certain atrial fibres. Sensitivity to beta-mimetic drugs

(isoprenaline, adrenaline, noradrenaline) increases with age. These drugs were already effective in the early stage when in the hearts no adrenergic nerve could be revealed histochemically and in which tyramine failed to cause an increase in frequency or contractility. Of the alpha-mimetic drugs, phenylephrine had to be given at high concentrations to cause a slight tachycardia, and this effect was practically similar on hearts of various sizes. Sensitivity to cholinomimetics (acetylcholine, carbachol) increased with age. On 5 to 8-week-old hearts low concentrations of acetylcholine sometimes evoked an initial tachycardia. The results showed that electrical activity and drug sensitivity of the human heart are the results of a developmental process.

Regulation of pancreatic blood flow: relationship between dose and effect of intraarterially injected secretin, pancreozymin and histamine

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Secretin, pancreozymin and histamine were injected into the duodenal side branch of the superior pancreaticoduodenal artery in dogs under chloralose anaesthesia, and the relationship between the dose of the drugs and their effects on blood flow was investigated.

1) It was shown by means of an electromagnetic flowmeter that both secretion (BOOTS, NOTTINGHAM) and histamine increased blood flow in the superior pancreaticoduodenal artery. A linear relationship was found between the doses of the drugs and the effects obtained (secretin: 0.05, 0.5, 1.0 U/kg; histamine: 0.5, 1.0, 2.0, 4.0 μg/kg).

2) It was demonstrated by means of a thermocouple inserted into the pancreas that both secretin and pancreozymin (BOOTS, NOTTINGHAM) increased the blood flow of the pancreas. A linear relationship was found between the logarithm of the doses and the effects obtained (doses, 0.125, 0.25, 0.5, 1.0 U/kg).

Effect on somato-autonomic reflexes of carotid sinus pressure stabilized at various levels

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The effect of the pressure of the perfused carotid sinus was investigated on the components of the autonomic reflex responses evoked by electrical stimulation of the sciatic nerve (blood pressure, pulse rate, contractions of

the nictitating membrane, and sympathetic efferent activity). Cats under urethan and urethan + chloralose anaesthesia were used; the animals were immobilized and subjected to bilateral vagotomy.

Low carotid sinus pressure facilitated the somato-autonomic reflexes. Direction and extent of the effects were, however, determined by the actual equilibrium of the excitatory and inhibitory processes of the central nervous system. In the case of a high carotid sinus pressure, inhibition was characteristic not only of the background activity but also of the reflex responses.

Haemoglobin of avian reticulocytes

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Erythropoietin plays an important part in the regulation of erythroid maturation. In the present experiments, haemoglobin synthesis has been studied in red cells and erythropoietin-containing serum of anaemic ducks. Haemoglobin synthesis was estimated on the basis of ^{59}Fe incorporation; its increase went parallel with an increase in RNA synthesis. The increase of RNA synthesis measured on the basis of ^{3}H-uridine incorporation was inhibited by actinomycin-D.

The presumably haemoglobin m-RNA was separated by means of acrylamide electrophoresis.

Effect of Ca-deficient diet on thyroid function of chicken

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The effect of normal and Ca-deficient diet was investigated in chicken by means of thyroid function tests. The animals were kept on a semi-synthetic diet up to the 21st day of life. Daily food intake was identical in the control animals and in chicken kept on a Ca-deficient diet.

The animals received 3 μCi of ^{131}I on the 21st day by the intramuscular route and were investigated 8, 24 and 48 hours later.

Iodine uptake and storage and plasma protein-bound radioactivity indicated that thyroid function was increased in the animals kept on the Ca-deficient diet.

Experiments are in progress to decide whether an increased peripheral consumption or a decrease in hypothalamic inhibition was responsible for the phenomena observed.

Dependence of the elementary phenomena of the sympathetic reflex response of the functional state of the central nervous system

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The elicitability of the two elementary phenomena of the sympathetic reflex response, i.e. the late response and the silent period, has been investigated by single and serial stimuli applied to the autonomic and somatic afferents in cats under chloralose, urethan, or chloralose + urethan, anaesthesia.

The results revealed that the anaesthesia were profoundly modifying or even qualitatively altering, the reflex responses evoked by both single and serial stimulation. Under urethan anaesthesia, the excitatory process will be dominant; in this case there is a considerable excitatory after-discharge. However, under chloralose urethan anaesthesia, the inhibitory processes will dominate; this manifests itself in the summation of the silent periods and in prolonged inhibitory after-discharge.

It is concluded that the elementary processes of the sympathetic reflexes manifest themselves as the resultants of complex interactions of the medullary or higher central nervous structures. The late response and the silent period can only be demonstrated under well-defined conditions.

Fatty acid composition of hyperlipaemic lipid fractions

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The changes in lipids and fatty acids have been investigated in hyperlipaemic patients of Frederickson's type II/A, II/B, III, IV and V. The lipoproteins were separated by gel electrophoresis. Thereafter, the amount of serum lipids was determined according to FOLCH, and the lipid fractions were separated qualitatively by thin-layer chromatography. The fatty acid composition of the serum lipid components was determined by gas chromatography. In hyperlipaemias accompanied by accumulation of cholesterol (II/A–II/B), an increase was observed in the ratio of unsaturated fatty acids, in addition to an increase in amount of cholesterol ester.

A decrease of the unsaturated fatty acids was observed in the composition of the triglyceride fraction in the so-called endogenous and exogenous hyperlipaemias accompanied by triglyceride accumulation. The above methods allowed to distinguish between the various types of hyperlipaemia: for this purpose, the composition of phosphatides and free fatty acids, as well as the qualitative and quantitative changes of triglycerides and cholesterol ester have to be taken into account.

Pulmonary pressure and cholinesterase inhibition (mevinphos treatment)

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As known from DE BURGH DALY's investigations, some cholinesterase inhibitors are eliciting vasoconstriction in the pulmonary circulation. This effect could be evoked also by eserine and mevinphos and effect of the latter drug has been studied in detail.

1) Considerable differences were found in the influence of drugs on the systemic and pulmonary circulatory effects.

2) Mevinphos evoked a pulmonary pressor response in intravenous doses as low as 10—50 μg/kg which were devoid of any other action.

3) With increasing doses this pressor effect showed a linear log-dose relationship while in the case of andrenaline, no dose–effect relationship could be demonstrated in the pulmonary arterial pressure.

4) The effect of mevinphos on pulmonary circulation was not influenced by vagotomy, adrenalectomy, atropine, or by hexamethonium or propranolol pretreatment.

5) Only phentolamine (3 mg/kg) exhibited an inhibitory effect.

Thus, the pulmonary pressor effect of mevinphos is considered to represent a direct vascular action independent of the enzyme inhibitory effect. The phenomenon is discussed from the point of view of poisonings and therapeutic possibilities.

Effect of single and repeated acute thrombocytopenia on thrombocytopiesis in the mouse

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The effect of acute thrombocytopenia has been investigated on the reappearance of platelets in peripheral blood and on the incorporation of ^{75}Se-methionine into thrombocyte precursors in C57 Bl mice. Mouse anti-platelet rabbit serum (APS) was administered to group A on one occasion, to group B on two occasions at seven-day intervals, and to group C on three occasions at seven-day intervals. The platelet count as well as the activity in circulating platelets of ^{75}Se-methionine given 24, 48 and 72 hours after the last APS dose were determined.

1) Duration of the APS-induced thrombocytopenia was different in the three groups; it was longest in group A (72 hours) and shortest in group C (42 hours). 2) The degree of reactive thrombocytosis increased with the increasing number of APS doses: it averaged 1.1 M after a single ASP dose,

1.7 M after two, and 1.86 M after three doses (in untreated mice, mean platelet count was 0.67 M). 3) Incorporation rate of ^{75}Se-methionine into precursors and, thus, the activity appearing in the circulating platelets was also influenced by the interval between the injection of ASP and that of the isotope. Incorporation was highest with an interval of 72 hours in group A, 48 hours in group B, and 24 hours in group C. The results allowed conclusions as to the kinetics of thrombocytopoiesis.

Regional cerebral blood flow and metabolism during haemorrhagic shock in the baboon

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The aim of the investigations was to elucidate the significance of central nervous alterations due to haemorrhagic shock in primates.

The experiments were performed on baboons weighing 12—14 kg, under sarnylan anaesthesia (1 mg/kg). The animals were immobilized with gallamine and kept under artificial respiration, the depth of which was controlled by continuously recording the CO_2 pressure of the expired air.

Regional cerebral blood flow was determined in 8 brain areas on the basis of ^{133}Xe clearance, by means of NaI crystal 5 mm in diameter. Blood flow of smaller areas was estimated autoradiographically, by means of ^{14}C-antipyrine administered at the end of each experiment.

Blood flow in the diencephalic region already decreased at bleeding to 55 mm Hg. In shock, CO_2 sensitivity of the cerebral vessels decreased and the gas failed to increase blood flow. Oxygen consumption significantly decreased at a blood pressure of 35 mm Hg with no change in cerebral glucose utilization. This was related probably to the diminution of brain material participating in oxidation, as indicated by studies with ^{14}C-antipyrine which revealed spotlike flow defects in the frontal cortex and the hypothalamus.

Functioning of early human embryonic hearts

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The earliest indirect ECG recordings of human embryos were made in the 12—14th weeks of pregnancy. With ultrasonic procedures, embryonic heart frequency could be recorded 5—6 weeks earlier. This method now allowed to determine the frequency of the heart (weighing about 3 mg) of a

52-day-old embryo. The success of ultrasonic recording depends on the age of the embryo and increases from 5% to practically 100% from the 7th to the 12th week of pregnancy. Heart rate was often as high as 190/min in the 7-week embryo. Subsequently by the 130th day it gradually decreases to 140/min, the rate characteristic of the human foetus. Development of vagal and sympathetic tone could also be followed on the basis of the heart rate, after treatment of the mother with atropine and beta blocking drugs. Since procedures in vivo yield information only on heart rate in vitro micromethods had been developed to analyze the function of embryonic hearts. With special extracellular electrodes, even the electric threshold and maximum driving frequency of a heart weighing 2.7 mg could be determined. In addition, an intracellular microelectrode technique was applied for recording electrical activity in various parts of the heart. In hearts weighing 10 mg or more, conduction velocity and contractility were measured regularly. The method yielded comprehensive information on the increased tolerance to hypoxia and the sensitivity to drugs of the embryonic heart.

Effect of vegetable amino acid concentrates on the development of rats kept on protein-deficient diet

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Enzymatic digestion of yellow lupin yields a concentrate containing 25% free amino acids. One kg of the concentrate contains 5.2 g lysine, 12.8 g histidine, 22.8 g arginine, 4.0 g proline, 2.8 g glycine, 8.8 g alanine, 24.8 g valine, 8.4 g isoleucine, 9.6 g leucine, 25 g glutamic acid and 7.0 g tyrosine. In addition, it is rich in free aspartic acid.

The effect of such concentrates was investigated in rats on weight gain, serum protein and individual protein fractions as well as on the blood counts.

Development of the animals was accelerated, and the hypoproteinaemia elicited by a 9% casein diet were counteracted by the concentrate.

In certain circumstances, use of the concentrate might favourably influence hypoproteinaemic conditions in humans.

Feedback phenomena in the *Helix pomatia* heart showing diffuse myogenic rhythm

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The nature of afferent signals has been investigated in the heart-extracardial nerve preparation of *Helix pomatia*. Under control conditions, afferent

signals of three different amplitudes go to the central nervous system through the cardiac branch of the intestinal nerve. Two of these signals are of cardiac origin. When the heart is exposed to tactile, pressure, osmotic or chemical stimuli, series of potentials of all or none character start towards the brain. The greatest increase in frequency to about 500% was observed in the case of tactile receptors. The response amounted to about 400% with chemoreceptors, about 300% with osmoreceptors and to about 200% with pressure receptors. The tactile, chemo- and osmoreceptors belong to the group of slowly adapting receptors while the pressure receptors to that of quickly adapting ones. At least two different responsive apparatuses are assumed to be present in the heart of *Helix pomatia*. The reflexogenic zones of this heart are to be considered analogous with similar structures of higher animals and to participate in the feedback system of the central regulation of cardiac function.

The role of intrarenal alpha-receptors in the regulation of renin secretion

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The effect of sympathetic stimuli transmitted to the intrarenal alpha-receptors was investigated on the haemodynamics and renin secretion of the kidney of anaesthetized dogs.

Phenoxybenzamine infused in a dose of 0.15 mg/min into the renal artery evoked a significant fall in blood pressure and resistance. However, instead of the expected increase of renin secretion, its significant diminution was observed. From this it has been concluded that the intactness of intrarenal alpha-receptors is necessary for the manifestation of stimuli promoting renin secretion.

A normotensive hypervolaemia was produced in the upper part of the body by partial compression (80 mm Hg) of the aorta above the origin of the renal arteries. As a consequence, inhibitory stimuli reached the juxtaglomerular apparatus and a significant diminution of renin secretion followed. This diminution of renin secretion was not observed after infusion of phenoxybenzamine under the above conditions, since the intrarenal alpha-receptors had been blocked.

Computer approach to the relationship between cortical and subcortical EEG signs during internal inhibition

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The characteristics of activities appearing in acoustic and "association" cortex, corpus geniculatum mediale, nucleus ventralis lateralis thalami, putamen and nucleus caudatus of the cat were compared by means of stochastic integrals during various phases of delay inhibition and discriminative inhibition as well as during sleep. The patterns characteristic of identical behavioural states are discussed.

Effect of endotoxin shock on the blood flow of adipose tissue and on the metabolism of isolated adipose cells

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It has been shown earlier that during haemorrhagic shock blood flow decreased to a greater extent in fat tissue than in any other organ, and that the arterial free fatty acid level remained low in spite of an increase in the blood glycerol level. All these changes were counteracted by phenoxybenzamine, an alpha-receptor blocking drug. Isoprenaline, a beta-receptor stimulatory agent, counteracted the diminution of blood flow in haemorrhagic shock and augmented the release of free fatty acid.

Injection of endotoxin evoked extensive circulatory and sympathicomimetic changes. Since the amount of adipose tissue comprises 20% of the whole organism, and about 15% of the cardiac output flows through it, its circulatory alterations must have general haemodynamic consequences.

Fat cells isolated with collagenase were prepared from the subcutaneous, omental and mesenteric adipose tissue before and after endotoxin administration.

It was found that blood flow in adipose tissue decreased less in endotoxin than in haemorrhagic shock. The isolated fat cells prepared from the omental and mesenteric regions responded to noradrenaline with a greater fatty acid and glycerol release than did the cells isolated from subcutaneous adipose tissue. It was further established that endotoxin increased the noradrenaline-induced lipolysis of fat cells in all three regions. When measured in glucose-containing medium, the endotoxin definitely decreased re-esterification.

Alveolar ventilation and chronic dust inhalation

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Evaluation of spirometric results of subjects exposed to chronic dust inhalation revealed that those parameters will first become pathologic which are indicative of an uneven alveolar ventilation.

The unevenness of alveolar ventilation has been investigated in patients exposed to chronic dust inhalation. The helium-dilution method used for measuring FRC was applied, and the changes in CO_2 concentration measured during one expiration were analyzed. A close relationship was found between the unevenness of alveolar ventilation measured by change of CO_2 concentration during one expiration and the spirometric data.

The unevenness of CO_2 concentration during expiration was related more closely to the spirometric result than to that of the helium-dilution method. The former procedure is more simple to perform and yields more exact data.

The investigations showed unequivocally that chronic dust inhalation considerably increases the unevenness of alveolar ventilation and may lead to respiratory insufficiency.

Investigation of diuretics by sequential analysis in rats

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In order to rationalize the screening program, WALD's sequential analysis has been applied to analyze the diuretic effect of some new compounds.

The criteria of sequential analysis were computed on the basis of 56 investigations corresponding to the given experimental conditions. The reliability of the established schemes was checked by the use of chlorothiazide, hydrochlorothiazide, furosemide, clopamide and acetazolamide. Dose–effect curves were prepared with these compounds concerning their diuretic as well as the natriuretic, kaliuretic and chloruretic effects. On the basis of the curves, two doses were applied one of which was slightly and the other one considerably saluretic in action. A reliable evaluation was obtained in both cases, and placebo experiments made for control purposes were recognized as ineffective.

The use of sequential analysis is recommended in routine screening, especially in view of its unequivocality, rapidity, narrow limits of error and simplicity of the calculations involved.

Regulation of water intake in parabiosis

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Total water intake of the parabiotic pair decreases by 20—30% if water is allowed only for 1—2 hours daily. The same is the case with normal rats. While kept on a dry diet for 20 hours a day, one member of the parabiotic pair was allowed to drink water one hour earlier than its partner in the morning and afternoon for one hour during the 10-week experimental period. Water intake of the animal allowed to drink later was significantly below that of the partner.

When one member of the parabiotic pair was kept thirsting for 6 days, water uptake of the other animal significantly increased. Subsequently, water consumption of the thirsting animal was significantly higher than the original for 1—2 weeks, while water uptake of the partner returned to the control level during this period. When to one member of the pair a hypertonic sodium chloride solution was given by a gastric tube (2×5 ml of 5% sodium chloride daily for two weeks), water uptake significantly increased in this animal without any change in that of the partner.

The connection between the members of the parabiotic pair was investigated by means of ^{131}I-albumin. Activity was roughly identical in the parabiotic pair 1.5— 2 hours after intravenous application of the labelled material.

The results indicated that, unlike food uptake (SCHMIDT and ANDIK, 1969), water uptake of one parabiotic rat has a certain influence on the water uptake of the partner.

Changes of skin reflexes after cross-union of sensory nerves in frogs

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When the skin of tadpoles is excised at the level of the trunk and retransplanted after 180° rotation, an inverse wiping reflex develops. In the present experiments the dorsomedial skin area was innervated by cross-union of the nerves of the ventrolateral skin area and vice versa. After regeneration the animal responded to stimulation of the dorsomedial skin area with a wiping reflex directed to the site of stimulation. Wipings directed to the ventrolateral skin area could also be elicited from certain points. From the region of the sensory nerves left at the original site of the ventrolateral skin

area, wipings directed to the abdomen could be evoked. Such stimulation in the intact frog causes wiping of the side of the body. The results are in contrast to those obtained after inverse transplantation of the given skin areas and do not support the assumption that the embryonic development of certain reflex arches would be governed by a biochemical specificity of the nerve cells.

Circulatory reflex effects in myocardial infarction

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It has been shown previously that a decisive role is played by the selective inhibition of myocardial adrenergic tone in the effect of circulatory reflexes elicited from the coronary veins, while the reflectory changes in the vasomotor tone of the vessels of systemic circulation are usually insignificant. The role of the above components is exactly the opposite in the carotid sinus depressor reflex.

In the present experiments the changes occurring in these circulatory reflex actions have been studied in ventricular infarction produced by coronary ligation in anaesthetized dogs. The extent of the infarction was chosen so that neither blood pressure nor cardiac output should considerably be affected.

The characteristic circulatory effects of the venous coronary reflexes were found to increase after myocardial infarction. At the same time, the character of the effect of the carotid sinus reflex approached that of the venous coronary reflex. In the latter reflex response, a previously latent negative ventricular inotropic effect appeared.

Lactic acid dehydrogenase and malic acid dehydrogenase activity in rat platelets after splenectomy

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Six days after splenectomy platelets were obtained by differential centrifugation and their lactic acid dehydrogenase (LDH) and malic acid dehydrogenase (MDH) activities were determined by ultraviolet spectrophotometry. The LDH isoenzymes and protein fractions of the platelets were separated on agar and polyacrylamide gel, respectively. Platelets of sham-operated rats served as control.

LDH and MDH activities calculated per unit number of isolated platelets, were significantly lower in the splenectomized group. It has been concluded that the platelets, increased in number after splenectomy, differ in composition from normal ones.

The role of RES in the regulation of serum pseudocholinesterase activity

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Though serum cholinesterase is produced by the parenchymal cells of the liver, LABORIT suggested that the RES cells too might play a part in the synthesis of pChE.

In order to elucidate this question, RES activity was inhibited by Indian ink in male mice. RES clearance as well as the activity of pChE in serum and liver were determined. One day after Indian ink administration, at the time of the strongest inhibition of RES, pChE activity was increased. Three days later when the inhibition of RES was slight, pChE activity hardly differed from the normal. After 4 days when no RES inhibition was present, pChE activity decreased. After 7 days when RES function increased, a considerable diminution of pChE activity was found in both serum and liver.

The results are in accordance with the view that pChE is eliminated in the RES and that it was the inhibition of RES which affected pChE activity. In view of the rapid changes, the possibility cannot, however, be excluded that the RES had some part in the synthesis or liberation of the enzyme.

Effect of hyperlipaemia on the inflammation-increasing effect of insulin

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The influence on the anaphylactoid inflammation-increasing effect of insulin of hyperlipaemia elicited by oral sunflower oil or intravenous soybean oil plus lecithin, or of triglyceridaemia elicited by tyloxapol bean oil administration have been investigated. Both sunflower oil and soy in doses not significantly influencing dextran oedema were inhibiting the sensitizing effect of insulin on the anaphylactoid reaction. The serum lipid level revealed a definite elevation of FFA after low doses of sunflower oil and various doses of soybean oil, in addition to a slight augmentation of the serum triglyceride level. Tyloxapol pretreatment induced a marked triglyceridaemia and hypercholesterinaemia with a slight increase in the FFA level, but failed to influence the inflammation-increasing effect of insulin. It is concluded that the inhibition of the anaphylactoid inflammation-enhancing effect of insulin under hyperlipaemic conditions was in close connection with the elevated serum FFA level.

Effect of aurothioglucose on various types of skin reactions

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It has been shown previously that infiltration of the site of preparation with aurothioglucose inhibited the local Shwartzman phenomenon.

In the present experiments the effect of aurothioglucose was investigated on various types of inflammatory reactions. The acute inflammation induced by xylene on the rabbit ear was not influenced by aurothioglucose which also failed to decrease the carrageenan inflammation of the rat paw in the early phase. In a later phase, when leucocytes were already present, the administration of aurothioglucose decreased the inflammation as compared with the contralateral paw. Aurothioglucose inhibited or diminished also the late type inflammation induced by an inverse Arthus reaction or by repeated administration of ovalbumin into the skin of the guinea pig.

Thus, topically applied aurothioglucose seems to diminish only those types of inflammatory skin reactions in which leucocytes appear early and play a decisive part.

Regulation of blood circulation in polycythaemia

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The effect of polycythaemia on blood circulation was investigated in eighteen dogs of both sexes. Polycythaemia was induced by repeated administration of dog erythrocyte suspensions of a mean haematocrit of 55%. Forty healthy dogs served as control. Cardiac output, circulating blood volume and circulatory time were estimated by the Evans blue dilution method. Nutritive blood flow of brain, liver, kidneys, muscle and skin was determined by the ^{86}Rb and ^{131}I-antipyrine fractionation technique.

In artificial polycythaemia circulating blood volume increased, flow velocity decreased and total vascular resistance increased. Organ blood flow decreased; its decrease was significant in the kidneys, liver, muscle and skin. Cardiac output fraction of the organs investigated did not significantly change under the effect of polycythaemia.

The effect of polycythaemia on blood circulation is discussed on the basis of the present findings and of investigations into the cerebral and limb circulation of polycythaemic patients.

Effect of in vivo sodium deprivation and load on the steroid production in vitro of the adrenal capsule and the decapsulated gland

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Rats were kept on a low sodium diet for two weeks. The adrenals were removed, cut into four, and their hormone production was investigated in vitro. Corticosterone production was significantly lower than the control, but it was considerably increased in the adrenals of animals loaded with sodium. The phenomenon is probably to be accounted for by an altered activity of the zona glomerulosa, since (i) changes of opposite direction occur in the aldosterone production of adrenals cut into four; (ii) corticosterone production of the decapsulated adrenal containing the zone glomerulosa is not influenced by the sodium supply in vivo; (iii) the adrenal capsule containing the zona glomerulosa exhibits an increased aldosterone production and a decreased corticosterone production in sodium depletion, while opposite changes occur after a sodium load.

Interpretation of the cerebral activation phenomenon

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The effects of visual, acoustic and electric stimuli, and in particular the phenomenon of desynchronization were studied in human and animal experiments by means of frequency analyser and biointegrator.

In human experiments the 10-sec total territory of the various bands of the resolved wave spectrum, the total wave number in 10 sec of the individual wave forms, their frequency distribution as well as the one-wave-area average were investigated before and during the alpha blocking which follows the opening of eyes. In cats, the values obtained before and during acoustic stimuli as well as those recorded before and after electrical stimulation of the reticular formation were studied in a similar way.

It is concluded that the above stimuli are altering the amplitude and not the frequency. The frequency was neither increased nor decreased; it was the one-wave-area and the total area which changed. There was no significant alteration in the delta, theta and alpha frequency values, but the amplitudes were markedly reduced. Similarly, no essential alteration occurred in the gamma range.

Activation of transport adenosine triphosphatase through binding carboxy groups

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The $Na^+ + K^+$ activated ATPase isolated from the rat is inhibited by N,N-dicyclohexylcarbodiimide (DCCD), an agent, blocking carboxy groups. The extent of inhibition depends on the concentration of DCCD, the time of preincubation and on temperature. Inhibition is weakened by preincubation in the presence of Na^+ or K^+. ATP has no protective effect, and not even the protective action demonstrable with Na^+ or K^+ is manifest in the presence of ATP.

When the enzyme preparation was treated with mersalyl prior to preincubation with DCCD, the loss of ATPase activity after reactivation of SH groups was greater than the sum of the separately caused inactivations. ATP did not show any protective effect under these conditions either. If the DCCD-treated enzyme preparation was incubated with trinitrobenzene sulphonic acid (TNBS) the decrease in activity was identical with the sum of the separately measured inhibitions, yet the protective effect of ATP against TNBS, was not manifest under these conditions.

The results described can be interpreted in two ways. 1. DCCD has a considerably greater affinity to the corresponding carboxy group of the active centre of ATPase than has ATP. 2. The inhibition by DCCD is not brought about through the free carboxy group of the glutamic acid producing the phosphorylated intermediate, but through the carboxy groups binding monovalent cations.

Pathologic ageing induced by dietary means

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Rapid changes in the diet, from exaggerated richness to deficiency are known to elicit pathological alterations. To study the problem in detail, one-year-old rats were fed diets of different composition on certain days of the week, but in such a manner that the average weekly consumption remained within normal limits.

On such a diet, the weight of the rats surpassed the normal average. Moderate hypertension developed in 8—10 weeks. After the 12th week, slight liver and kidney injury could be demonstrated in every animal. The wall of the mesenteric and cerebral small arteries was thickened. Myelopathy developed in some cases. The creatine phosphate content of the skeletal muscu-

lature decreased from 1.05 mg/g to 0.38 mg/g, a value characteristic of aged (3-year-old) rats. There was a diminution in the activity of liver succinic dehydrogenase and acid phosphatase. The total serum lipid content was doubled. Within this the amount of triglyceride, fatty acid methylester and cholesterol, as well as the ratio of saturated fatty acids were elevated. All these changes are signs of an early pathologic ageing.

Cardiorespiratory adaptation

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The effect of respiratory rhythm and volume on heart function has been investigated to clarify how voluntary alterations of breathing are influencing the heart revolutions in rest and after loading.

The effects of spontaneously on voluntarily altered breathing were continously recorded on the ECG. The records revealed that 1. respiratory arrhythmia increased in the resting state, and gave rise to variable heart cycles. 2. After loading, heart function returned to normal earlier upon the effect of voluntary respiration that after spontaneous respiration.

Under the effect of hyperventilation, restitution set in earlier in trained persons, due to their increased ability to adaptation.

Cardiorespiratory adaptation may be enhanced by trained ventilation.

Microelectrophoretic investigations on medullary neurones

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The reactivity of caudal medullary neurones of the cat to biologically active substances and drugs has been studied by use of five barrel iontophoresis. The substances examined were: acetylcholine, glutamic acid, gamma-aminobutyric acid, glycine, d-tubocurarine and strychnine.

Typifying of the neurones studied on the basis of their reactivity was a priori handicapped by the fact that the actions of the substances employed seemed to depend on the polarity of the iontophoretic current. Yet, making use of appropriate control injections of sodium chloride, it could be stated that the actions of glycine and gamma-aminobutyric acid largely depend on the ionic form in which they are present in the buffer solution.

Effect of dibenzyline on blood flow of the thyroid and salivary glands during haemorrhagic shock

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During gradual bleeding, blood supply to the thyroid decreases considerably in the case of a fall in arterial blood pressure as small as 20 mm Hg. The decrease in venous outflow then amounts to 70%, that of tissular circulation to 55%. Further blood loss causes no further decrease. Blood flow in the salivary glands changes parallel with the fall of blood pressure up to 60 mm Hg. When arterial blood pressure is kept at 55 and 35 mm Hg for 1.5 and 2 hours, respectively, a moderate shifting cardiac output occurs.

In the present experiments the influence of the adrenergic mechanism was studied on thyroid and salivary gland flow during haemorrhagic shock in dogs under chloralose anaesthesia. Venous outflow of the glands was measured by direct cannulation and a drop counter, and tissular circulation by means of thermocouples. Under the effect of 3 mg/kg of dibenzyline administered two hours prior to bleeding, venous outflow of the thyroid which in the controls was 207 ± 64 ml/min/100 g decreased by 40%, parallel with the diminution of arterial blood pressure. The decrease in tissular circulation was 6%. After retransfusion, venous outflow increased to 70% and tissular circulation to 90% of the control value. Venous outflow of the salivary gland (24 ± 5 ml/min/100 g in controls) increased to 110% and tissular circulation to 130% upon the effect of dibenzyline. When arterial pressure was lowered to 35 mm Hg, venous outflow decreased to 65% and tissular circulation to 80% of the control. No alteration was observed in the circulatory parameters. After retransfusion, blood flow of the salivary gland increased to the original value.

Sodium exchange in striated muscle

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The exchangeability of Na was studied in isolated frog's sartorii. The muscles were exposed to 24-Na-Ringer for different period of time and then transferred to Na-free choline-Ringer for 10 minutes. The total Na content of muscles was on average 14.5 mmoles Na/kg ww and during exposure to radioactive Na-Ringer, for 1—5 hours, only about 6 mmoles Na/kg ww was found to be exchanged. The remaining Na in muscle had a very slow exchange rate and therefore it was treated as a "non-exchangeable" fraction under the experimental conditions. There was a significant decrease in the amount of "non-

exchangeable" Na if muscle was exposed to 10^{-4} M ouabain, while the total Na content was increased by the drug.

Soaking muscles in a hypotonic Ringer's solution (50 mM Na) resulted in a considerable decrease in i.c. Na concentration as a consequence of net Na-loss and water gain under unbalanced osmotic conditions. A several-fold increase in the external K^+-dependent Na-pump activity was found in muscles with low i.c. Na^+, kept in hypotonic media. It seems to contradict earlier observations of many authors, that the Na-pump activity increases with i.c. Na concentration. However, we have found that the decrease in internal Na content, during hypotonic treatment, was mainly due to the decrease of the "non-exchangeable" fraction of i.c. Na. It is suggested that the Na-pump activity is not directly governed by the total intrafibral Na but rather by the "exchangeable fraction", while it may be supposed that the "non-exchangeable" Na plays an important role in maintenance of internal osmotic pressure.

Comparative investigation of the proteins of blood plasma and lymph

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Recent morphological findings have cast doubt on the concept that the capillary wall is a semipermeable membrane having pores of definite size. In addition, considerable differences in the structure of the vascular wall have been found in different regions. The lymph, being a product of capillary filtration, may supply information concerning permeability conditions in individual capillary regions and also on the mechanism of permeability.

Various methods (zone, disk and immune electrophoresis, enzyme determinations, etc.) were used to study the composition and specific components of the protein in lymph obtained from the thoracic duct, heart, liver, intestines, kidney and limb. Permeability of the capillaries of individual regions was found to be different even though the functional results did not completely agree with the morphological findings. The observations were consistent with the concept that the transport of macromolecules through the capillary wall is determined by physicochemical forces.

Identification of the hypothalamic structure responsible for reinforcement

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MILLHOUSE (1969) has shown that the dendrites of the "pathway-neurones" of the medial forebrain bundle were mainly directed laterally in the

lateral hypothalamus and preoptic area, that their medial axon is divided into descending and ascending branches and, after giving collaterals to the neighbouring pathway-neurons, it forms reverberation circuits. The results of self-stimulation experiments proved that it is the excitation of the pathway-neurones which is responsible for the reinforcing effect evoked from the lateral hypothalamus.

1. It has been shown with conditioning–test stimulation technique that those cells will be excited by self-stimulation which activate each other by axon-collaterals, with a latency of 0.6 msec. Correspondingly, a change in excitability occurs the pattern of which resembles the amplitude and time relationships of the excitatory postsynaptic potentials (EPSPs). The poststimulatory increase in excitability following more intense conditioning pulses, as well as the rebound-excitation responsible for the reinforcing effect, are indicative of repeated synaptic activations and reverberations.

2. The elements of the reverberating system responsible for self-stimulation, just like the reverberating circuits consisting of pathway-neurones, are oriented sagittally, since stimulation with electrodes of antero-posterior pole alignment is less suitable for eliciting self-stimulation, owing to an anodal block.

Biosynthetic activity of cells labelled with ^{51}Cr

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Lymphocytes isolated from human tonsils were labelled with ^{51}Cr, and the time and temperature dependence of radioactivity was studied. ^{51}Cr released from the surface of the cells was studied. $^{51}Chromium$ was firmly attached also to proteins which were synthetized within a short period during the in vitro incubation of ^{51}Cr-labelled cells in the presence of ^{14}C-labelled amino acids.

Citrate content of rat liver cells

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Citrate content and intracellular citrate distribution were investigated in the liver cells of old and hyperlipaemic adult rats. Adult, non-hyperlipaemic rats served as control. Hyperlipaemia was induced by neurotization or intravenous alloxan administration. After the development of hyperlipaemia, the animals were bled to death and the serum-free fatty acid and total lipid levels were determined. The citrate content in the liver cells as well as in their

mitochondrial and supernatant fractions were estimated. In addition, the permeability of liver mitochondria was determined in the isoosmotic solution of certain ions, by means of recording the extent of swelling. Significant differences were found in the intracellular distribution of citrate between adult and old animals. These differences were ascribed to differences observed in permeability.

Temperature-decreasing effect of fenitrothion in the rat

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In poisonings with insecticides of the phosphoric acid type it is important to measure the efficacy of the antidote. Estimation of the influence of various drugs on the hypothermia caused by cholinesterase inhibitors is a method suitable for the purpose (MEETER, 1971). The temperature-decreasing effect of fenitrothion administered by the oral route has been studied in male and female rats.

Depending on the dose applied, fenitrothion transiently decreased body temperature; the maximum of the decrease was reached in the second hour. Male animals reacted with greater sensitivity than did female ones. The hypothermia was accompanied by a definite and prolonged decrease of blood cholinesterase activity. The fall in body temperature was inhibited by atropine sulphate but was not influenced by atropine methylbromide. Sixty mg/kg of obidoxime given subcutaneously one hour after the administration of fenitrothion prevented the hypothermia. The fall in body temperature was augmented by 120 mg/kg and hardly influenced by 30 mg/kg of obidoxime. However, body temperature returned to normal earlier after the administration than without obidoxime.

Mechanism and drug-sensitivity of arrhythmia following coronary ligation

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In intact, anaesthetized dogs, ligation of the descending branch of the left coronary near its origin induces ventricular arrhythmia and even ventricular fibrillation, parallel with a deterioration of heart functioning. Coronary occlusion of a similar extent on the isolated heart perfused with donor blood similarly caused a marked deterioration of heart function but arrhythmia developed rarely, if at all. In order to eliminate the influence of the autonomic nervous system, 0.4 mg/kg of atropine and 4 mg/kg of practolol, a drug selec-

tively blocking the beta-receptors, were administered to anaesthetized dogs. In untreated animals, ligation for 5 minutes (allowing for full restitution after relieving the ligature) elicited marked ventricular arrhythmia and sometimes ventricular fibrillation. In contrast, in the treated animals extrasystole due to ligation was unfrequent and no ventricular fibrillation developed in any case.

When the effect of chemical denervation wears off, arrhythmia and fibrillation reappear in their original severe form. Abolition of the cholinergic tone by itself or of adrenergic tone by itself failed to ensure satisfactory protection. In two cases ventricular fibrillation appeared even after atropinization in a heart responding to previous coronary occlusion with slight arrhythmia only.

Stability of the evoked potentials of human visual cortex

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In 20-year-old males (n = 20) averaged responses were recorded from the visual cortex under standard stimulation and recording conditions with 200—200 flashes applied every other day for 20 days. The data were analyzed with computer and evaluated from the point of view of stability. From the roughly 150,000 data, the computer performed a smoothing and calibration of the curves and recognized the positive and negative components. In such a way data were obtained concerning 1. the time and amplitude stability of individual components, and 2. the stability of the whole curve in the case of each individual subject and the whole group. Stability relationships were expressed in objective terms, by using deviation square, confidence interval, time row analysis and regression calculatory processes. Five to eight components of different stability could be distinguished in the evoked responses of the human visual cortex. The individuals could be grouped on the basis of the stability data of the complete evoked responses. Individual differences were greater than the oscillations of the same person within one month, yet the limits were not sharp. Physiological standard values could be established for at least 3 main components of the whole population.

Metabolic changes in the white skeletal muscles of aged rats

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The white skeletal musculature (quadriceps femoris muscle and the peripheral part of the gluteus) of albino rats older than 20 months, was investigated and compared with that of 6—8-month-old animals.

1. Like in previous experiments (ERMINI, M., Gerontologia, **1970**, 16: 65.) the creatine phosphate content was decreased.

2. There was no change in the total adenosine nucleotide content, yet the ATP level decreased with a parallel increase of the ADP content.

3. The acid-soluble glycogen content decreased with increasing age.

4. The succinic acid dehydrogenase activity diminished.

5. Aldolase activity did not change.

In agreement with previous findings, the decrease in creatine phosphate content of the white musculature is ascribed to the diminution of aerobic metabolic processes, and to the consecutive diminution of ATP generation.

Thermoregulation in the rabbit under the effect of *E. coli* pyrogen

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Heat production (oxygen consumption) and the individual mechanisms of heat loss (ear temperature respiratory rate) were investigated after the intravenous administration of *E. coli* pyrogen at various ambient temperatures.

In an environment warmer than neutral (30°C) heat loss manly decreases through the inhibition of thermal hyperpnoea at the development of fever, with hardly any vasomotor change. In a neutral environment (15—20°C) it is the vasoconstriction (decrease of ear temperature) which dominates in the diminution of heat loss, while the respiratory rate decreases but slightly. Elevation of colonic temperature is usually accompanied by an increase of the energy household.

The physiological role of the epicardium

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Starting from the assumption that the behaviour of the cardiac connective tissue can be studied on the epicardium, permeability of the epicardium to various substances has been studied.

1. ^{125}I-polyvinylpyrrolidone (PVP) molecular weight 35,000 injected into the inner layers of the myocardium in a volume of 0.1 ml appears in cardiac lymph and blood and also in the pericardial fluid. PVP applied on a filter paper placed at the surface of the epicardium soon appears in the deeper layers of the myocardium and in blood.

2. Application on the surface of the epicardium of as little as 0.1 μg of noradrenaline or isoprenaline causes a significant increase in coronary flow.

3. Propranolol applied on the epicardium by means of filter paper markedly decreased the coronary effect of intravenously administered noradrenaline or isoprenaline in doses as low as 0.005—0.01 mg/kg, without affecting the heart rate or blood pressure. Higher doses (0.05—0.1 mg/kg) inhibited the effects on blood pressure and heart rate. This means that the effect of agents absorbed from the pericardium is more marked on the coronaries than on the myocardium, in contrast with the case after intravasal application. It is assumed that the intracardiac distribution of substances absorbed through the epicardium differs from that of substances injected into the systemic circulation. Molecules in the extracellular space can be excreted through the epicardium and, at the same time, substances can enter the heart through the epicardium.

Changes in thermoregulation under the effect of *E. coli* pyrogen in the rat

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The changes in the energy household and in the temperature of the colon brown adipose tissue and tail were recorded continuously at various ambient temperatures, after the intravenous administration of 1 μg/100 g of *E. coli* pyrogen.

Oxygen consumption as well as the temperature of the colon and brown adipose tissue began to increase about 30—35 minutes after administration of the pyrogen, in both neutral and cool environment. During the quick (10—15 min) augmentation of energy household the brown adipose tissue was warmed up more rapidly than the colon, a fact indicative of a local increase of heat production. In experiments performed at neutral ambient temperature the tail temperature decreased in parallel. The oxygen consumption in the new equilibrium, produced by a higher core temperature, decreased to the pre-pyrogen level, the original relationship between the temperature of brown adipose tissue and colon was restored and tail temperature increased again.

In neutral environment a new pyrogen dose given after body temperature had become normal, elicited roughly the same temperature elevation as did the first dose. In this case, however, the metabolic rate was increased less or not at all.

Microcirculation in anoxic hypoxia of the rabbit

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The microcirculation was studied in wake rabbits by the ear-chamber technique after breathing various mixtures of O_2 and CO_2. The diameter of arterioles was estimated, the circulatory velocity of red corpuscles was measured with a rotating-prism velocity meter and flow in the given vascular sections was calculated. Htc, pH, paO_2 and $paCO_2$ values were estimated.

In group I the parameters decreased significantly on inhaling 8% O_2 + 92% CO_2. The $paCO_2$ diminished considerably due to the hyperventilation induced by hypoxia.

In groups II and III gas mixtures of 8% O_2 + 5% CO_2 + 87% N_2, and 8% O_2 + 8% CO_2 + 84% N_2, respectively, were administered. In this way $paCO_2$ was normalized, or hypercapnia was produced. The parameters studied increased or remained unchanged.

In groups IV and V gas mixtures of 21% O_2 + 5% CO_2 + 74% N_2, and 21% O_2 + 8% CO_2 + 71% N_2, respectively, were administered. No significant change was observed in these animals.

Thus blood flow in the arterioles of the rabbit ear decreased in anoxic hypoxia. In the case of a simultaneous hypocapnia, when the $paCO_2$ was adjusted to normal or a hypercapnia was produced, blood flow had become normal or was even augmented in spite of the unchanged hypoxia.

Disseminated intravascular coagulation in the rat after the simultaneous administration of hemocoagulase and epsilon-aminocaprylic acid

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In female rats weighing 90—95 g, the simultaneous intravenous injection of 0.5 Klobusitzky unit of hemocoagulase and 200 mg of epsilon-aminocaprylic acid induced disseminated intravascular coagulation. The fibrinogen level decreased and severe haemorrhagic pulmonary changes, characterized histologically by multiple thromboses of the small vessels developed in 2 to 24 hours. Hemocoagulase in itself did not elicit pulmonary alterations, despite the decrease of the fibrinogen level.

Injection of hemocoagulase and aminocaprylic acid into the left ventricle of the heart failed to elicit haemorrhagic pulmonary thrombosis. In some cases, however, a cortical necrosis reminding of a generalized Schwartzman reaction was observed in the kidneys.

According to the findings, disseminated intravascular coagulation develops in the rat only after the simultaneous administration of hemocoagulase which has a thrombinlike activity, and epsilon-aminocaprylic acid, which is inhibiting fibrinolysis. It depends on the route of administration whether the thrombobaemorrhagic alteration manifests itself in the lungs or in the kidney.

Changes in regional cerebral circulation during focal and generalized epileptic convulsions

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Waking rabbits immobilized with gallamine and subjected to artificial respiration were used in the experiments. Focal fits were evoked by intracortical or intrahippocampal injection of acetylcholine or pentamethylentetrazole, and generalized ones by means of intravenous pentamethylentetrazole. Blood flow was measured with SEYLAZ's thermoclearance method in the cerebral cortex, thalamus and hippocampus. Blood flow in the focus decreased after the topical application of acetylcholine and pentamethylentetrazole, a finding in agreement with previous observations made in freely-moving animals. During spreading convulsions, blood flow in regions remote from the focus increased at the beginning of the convulsions but, during the intense convulsive activity, it decreased also in the later firing territories. During convulsions evoked by intravenous pentamethylentetrazole, blood flow always increased, a finding in acccordance with data in the literature.

Accordingly, regional cerebral blood flow changes in an opposite way during local and generalized fits. The diminished blood flow during local convulsions of the firing regions is primarily an effect of the metabolic changes accompanying the convulsions. During a generalized convulsion, the autoregulation of cerebral circulation changes in a way different from that in focal fits.

Load-dependent changes of the respiratory quotient

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The changes in the respiratory quotient during loading were investigated by means of 113 gas metabolism analyses performed in sporting and non-sporting groups under various conditions. The individual bicycle ergometric loads were between 0 and 400 watts for two minutes (no steady-state). The characteristic points and sections of the RQ curve were connected to the appropriate sites of the time-curve of percentage O_2 utilization and CO_2 pro-

duction, and partly to the values for respiratory volume and respiratory minute volume. The mechanisms of respiratory function and regulation underlying the individual sections of the RQ curve, as well as the connection of CO_2 to respiratory volume, are analyzed in detail.

The double function of chemosensitive and pain-sensitive nerve endings

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Antidromic stimulation of sensory nerves, or the application of pain-inducing chemical agents, evokes a neurogenic inflammation. This reaction can be blocked by capsaicin desenzitization. The inflammation produced by pain-inducing chemicals cannot be inhibited by local anaesthetics, a fact proving that the mediator which increases permeability, can be liberated without an axon reflex.

The following findings indicate that the mediator is released from the activated sensory receptors and that the sensory and mediator-liberating functions of these receptors are closely interconnected.

1. Not only the classical local anaesthetics, but even tetrodotoxin fails to inhibit the neurogenic inflammation produced by chemical agents (capsaicin, xylene, mustard oil).

2. The extent of the inflammatory reaction evoked by various pain-inducing pungent agents is proportional to their pain-inducing potency even in tetrodotoxin local anaesthesia.

3. The free nerve endings contain vesicles and accumulated mitochondria (rat cornea) just as do the presynaptic nerve endings.

4. General capsaicin desensitization causes a long-lasting swelling of the mitochondria in certain cell types of the sensory ganglia, parallel with the sensory outfall. Local capsaicin desenzitization causes a similar mitochondrial swelling and diminution of the number of vesicles in certain sensory nerve endings.

Serum triglyceride pattern of hyperlipaemic patients

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The sera of 30 patients with hyperlipaemia accompanied by triglyceride elevation were studied. The patients belonged to hyperlipaemic classes of type IIb, III, IV and V according to the classification of FREDERICKSON and the WHO.

After extraction according to FOLCH the serum triglycerides were isolated by thin-layer chromatography and separated on $AgNO_3$-impregnated plates according to the saturation degree. The triglyceride fractions of identical saturation were measured gravimetrically, and the individual glycerides separated on paraffin-impregnated plates by means of reversed-phase thin-layer chromatography according to the length of the carbon chain. Quantitative evaluation was performed on the basis of the fatty acid composition determined by gas chromatography.

It was found that in the various types of hyperlipaemia the distribution of individual triglycerid isomers was different, with identical average fatty acid composition of the total glyceride. The difference was due primarily to the uneven distribution of saturated fatty acids (myristic acid, palmitic acid, stearinic acid) at the alpha and beta site of the glyceride molecule. Accordingly, in these states, the principle of the so-called restricted random distribution is valid to a limited extent in endogenous triglyceride synthesis.

Role of haemodynamic factors in the pathogenesis of renal failure after sublimate poisoning

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The parameters of renal function were investigated 12, 24, 48 and 72 hours following the intravenous administration of 2 mg/kg of $HgCl_2$ in acute experiments on dogs. On the basis of the series-connected and parallel-connected intrarenal vascular resistances, the functional organization of the sublimate kidney is as follows.

1. At 12 and 24 hours after the application of sublimate total vascular resistance of the kidney increases. The increase is exclusively due to the augmented resistance of the afferent arterioles.

2. As a consequence of the afferent spasm, the glomerular, capillary and effective filtration pressures decrease, causing a diminution of the glomerular filtration rate.

3. At 48 and 72 hours after poisoning total vascular resistance of the kidney decreases thus a well-defined renal hyperaemia ensues. The phenomenon is related to the extreme diminution of resistance in the postglomerular (afferent + venular) vascular sections. The equilibrium is characterized by a moderate increase in afferent resistance and glomerulo-capillary pressure and an extreme diminution of the glomerular filtration rate.

4. Measurements of PAH, oxygen and Rb extractions revealed that the primary cause of the "collapse" of renal function is dilatation respectively a rearrangement of the microcirculatory system of the peritubular capillaries, an event resulting in the cessation of glomerular filtration.

A new type microelectrode for continuous monophasic action potential recording on the heart in situ

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In the case of an appropriate recording, the shape and course of the monophasic action potential of heart muscle are identical with those of the intracellular potential only the amplitude of the membrane potential and of the action potential is lower. This allows to follow the selective changes in ion permeability of the heart muscle membrane of the heart in situ even simultaneously at different sites. Such investigations with intracellular microelectrodes are technically difficult due to the difficulties of immobilization and fragility of the electrodes. Even the procedure using suction electrodes is not free of shortcomings as the suction needed for ensuing the sufficiently high potential of 40—50 mV is damaging the heart muscle. On the other hand, the amplitude of the potential rapidly decreases, a fact causing uncertainties in evaluation.

The new electrode is a combination of suction electrode needing mild suction and a depolarizing electrode filled with saturated KCl. Its main advantages are: 1. Slight vacuum, less damaging to the heart, is needed to produce the necessary tissue injury. 2. The amplitude of the action potential hardly decreases even during a longer period of time; thus, not only the changes caused by the interventions but also the restitution can reliably be evaluated.

Application of hybrid computer technique for broad-scale frequency spectrum analysis of blood pressure and blood circulation

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The components of blood circulatory waves occupy a broad frequency band from about 0.01 to 100 Hz. The components show partly periodic and partly random properties, their power density comprising a range of 60dB. Analysis of the total store of circulatory signs, complete from the point of view of both amplitude and frequency, faile to ensure a sensitive analysis of the sign.

The experiments were performed on anaesthetized dogs. Four circulatory characteristics were resolved by means of an autonomic real-time computer and recorded simultaneously on magnetic tape and by a polygraph. Statistic structure and dynamic relation of primary and tertiary sign components were studied separately at various mean arterial pressures. The relationship between

pulsatile component parameters (pulse rate, pulse pressure, changes in steepness) and the tertiary waves has also been studied by hybrid computer technique. The methodology of the investigation is discussed.

Comparative study on ischaemic heart models used for analyzing the effect of antianginal drugs on cardiac metabolism

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When evaluating antianginal drugs, their effect of cardiac metabolism has also to be taken into account, in addition to the coronary dilator action. No model of human angina could yet been produced in experimental animals. Therefore, several less adequate methods are used for this purpose, such as arterial hypoxia produced by inhalation of gas mixture of low O_2 content, administration of metabolism-increasing sympathomimetics, or the combined administration of both procedures. All these interventions affect the whole organism and influence heart function and metabolism in a secondary way, instead of loading primarily the heart. In addition, as an effect of the various factors not easy to control and standardize, the results obtained during a cardiac load exhibit great variations, a fact which makes it difficult to evaluate the protective effect of antianginal drugs. In order to eliminate these shortcomings, three simple procedures primarily causing myocardial ischaemia are recommended, and they are compared on the basis of their effect on the high energy phosphate contents of the heart muscle. The procedures are as follows. 1. Determination of the ATP and CrP level at various periods of time following excision of the heart and comparison of the values with those obtained on hearts of animals pretreated with the antianginal drug. 2. After excision, the heart is made to function in Ringer's solution at 37°C for a definite period of time. 3. The heart is made to function in situ for a definite period of time after compression of the ascending aorta. Dominant in the first two models is the ischaemia due to the cessation of circulation and, in the third model, the collapse of the equilibrium between mechanical overload of the heart and coronary circulation, i.e. between O_2 demand and supply.

Multiple unit activity during habituation to the environment in the rat

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Multiple unit activity (MUA) of forebrain and brain stem structures during habitation to the environment has bean studied in freely moving rats under chronic experimental conditions.

Subcortical semi-microelectrodes were implanted by a standard stereotactic technique. The experiments were started 1—3 weeks following the operation. Electrical activity (EEG) and MUA of the brain were recorded by means of the modified method of GUZMÁN and ALCARAZ. The modification allowed the simultaneous recording of a minimum of four or, when switched according to an appropriate pre-set program, more than four, brain areas alternately, in addition to the digital analysis of cell firings discriminated according to amplitude. The animals spent two hours daily in the new environment and 22 hours in the home cage. On consecutive days, continuous MUA recordings were taken both in the environment and the home cage, for two hours each, during orientation reaction, during slow wave phase of sleep and during paradoxical sleep.

MUA activity of all investigated regions exhibited a several-fold increase in the new environment. This was the case in both the slow wave and the paradoxical phase of sleep. With progressing habituation the activity gradually decreased to a stable level which approached the sleep level recorded in the home cage.

Synthesis of steroid hormones from cholesterol (7-alpha-^{3}H) in the middle and at the end of pregnancy

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It has been shown previously that the human placenta is not capable of a de novo synthesis of steroid hormones and uses maternal and foetal cholesterol as precursor. In the present experiments the quantitative and qualitative relationships of hormone production from cholesterol have been studied. Placentae from pregnancy and those obtained after delivery were perfused in vitro with 6 mCi of ^{3}H cholesterol containing Rh "O" blood under controlled pH, pO_2 and CO_2 conditions at 37°C for two hours. After perfusion the steroid content of the perfusate and of the placental tissue was measured quantitatively. For correction, a so-called inner standard labelled with ^{14}C was used.

It was found that the placenta was capable of synthesizing 7—8-times more pregnenolone and progesterone than dehydroepiandrosterone and 17-beta oestradiol. In mid pregnancy placentae 0.01% and in the placentae after delivery, 0.015% of the perfused cholesterol was transformed into the hormones investigated.

These data, together with the results of previous investigations, point to the ability of the placenta to produce dehydroepiandrosterone directly from cholesterol in a so far unclarified way.

Nervous regulation of initial body temperature and its alterations

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Changes were produced in the body temperature of rats and guinea pigs by conditioning-like, repeated and regular administration of drugs. Body temperature was continuously recorded by a thermometer fixed in a skin fold for several weeks and compared with the value measured rectally in other animals. It was determined to what extent did these modes of temperature measurement represent such a non-specific stimulus which would alter the initial value. Habituation to the effect of rectal measurement was complete in 3—5 days. The continuous recording permitted the limits of error of temperature regulation to be recorded in waking animals, during sleep, in stimulus-poor environment and under conditions of attention. The continuous recording also allowed to recognize the point of time of the early changes in the initial value. The effect of the drugs acting on body temperature underwent alterations during repetitions. This alteration was manifest also independently of the initial value and did not follow Wilder's rule quantitatively. The continuous, moderate changes of the initial value may evoke a qualitative alteration in the drug effect, in accordance with the rule of initial values.

Electrophysiological investigation of the Ca^{++}–CS_2 antagonism on an isolated nerve preparation in situ

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In the in situ isolated saphenous nerve preparation of the rat, CS_2 antagonized the membrane-stabilizing effect of Ca^{++}. The effect of CS_2 was studied in Tyrode's solution containing $CaCl_2$ at $0—10^{-4}$ g/ml. The two substances were found to exhibit a mutual antagonism in a near-equimolar concentration. The "membrane-labilizing" effect of CS_2 was manifest in the augmentation of the total electric activity led off from the isolated saphenous nerve.

Electric paroxysms in REM

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Epileptic patients were studied during natural sleep. With rare exceptions, the epileptic discharges markedly decreased during REM as compared with the discharges observed under slow wave sleep.

1. The generalized bilateral discharges (patients with PM and GM fits) could be grouped as follows. a) Complete inhibition of discharges during all REM; b) marked depression but persistence of discharges; c) augmentation of discharges as compared to the value seen during slow wave sleep; d) status electricus without clinical manifestation, e) clinical fit with the corresponding ictal electric signs. The generalized epileptic discharges during REM corresponded morphologically to those during wakefulness and differed considerably from the degraded discharges appearing during slow sleep.

2. The electric discharges during REM of patients with encephalopathic epilepsy were considerably more abundant than in the previous group. Sometimes a slight reduction was only observed as compared to the value obtained during slow sleep. Morphologically they approached the waking state discharges.

3. In temporal epilepsy, the cortical foci exhibit an increased activity during REM, while the discharges of the so-called secondary temporal foci are often completely depressed.

Effect of cyclic 3'-5'-adenosine monophosphate on tubular sodium reabsorption

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Administration of 1 mg/min of cyclic 3'-5'-adenosine monophosphate into the left renal artery did not change either renal haemodynamics or glomerular filtration, but decreased sodium excretion.

Infusion into the renal artery of 1 mg/min of theophylline led to increased glomerular filtration and increased water and sodium excretion, due to the vasodilatation.

Simultaneous administration of the two substances resulted in a decrease of sodium excretion with unaltered water excretion.

It is concluded that cAMP increases tubular sodium reabsorption, and seems to play a part in the regulation of the tone of renal vessels.

Effect of histamine and adrenaline adaptation on the hyperfibrinogenaemia elicited by histamine and adrenaline

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According to previous investigations, administration of histamine (0.3 mg/100 g, subcutaneously) and adrenaline (0.2 mg/animal, subcutaneously)

leads to marked fibrinogenaemia 24 hours following the injection. After the development of histamine adaptation, adrenaline still caused a marked hyperfibrinogenaemia while histamine failed to do so. On the other hand, adaptation to adrenaline counteracted the development of histamine and adrenaline hyperfibrinogenaemia.

In the present study histamine adaptation abolished or markedly decreased the liberation of catecholamine together with the consecutive hyperfibrinogenaemia. It is concluded that the hyperfibrinogenaemia observed in control animals after histamine administration was based on catecholamine release.

Effect of RNase on biosynthesis of HbS and HbA in vitro

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HbS-synthetizing cell-free reticulocyte systems isolated from the blood of young homozygote sickle-cell anaemia patients have been studied in vitro. It was found that HbS synthesis was blocked by RNase I much slower than expected.

The hydrolysis of highly polymeric exogenous (yeast) RNA was not inhibited by the presence of the HbS-synthetizing system. This finding was interpreted in the sense that no natural RNase I inhibitor was present in the system.

The same experiments were performed with reticulocytes isolated from the blood of healthy Africans. It was found, without exception, that RNase I treatment blocked the biosynthesis immediately.

The RNA fractions participating in the biosynthesis of HbS were assumed to be under different degrees of "membrane protection" due to binding of various membranes, an event allowing them to survive the immediate effect of RNase.

To check the "membrane-protection" the following experiment was carried out. Non-purified t-RNA, ribosome and membrane-bound polysome fractions were prepared by cell fractionation and treated separately with RNase I. The remaining RNA fractions were prepared with phenol and analyzed with sucrose density gradient centrifugation. The t-RNA and free r-RNA fractions underwent complete hydrolysis, while about 50% of the m-RNA and r-RNA of ribosomal origin survived.

Proteinase inhibitors in serum of normal subjects and of acute pancreatitis patients

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The serum proteinase inhibitor is capable of inhibiting the activity of trypsin, chimotrypsin, elastase and plasmin. The extent of the inhibition is different with the different proteinases. One ml of human serum can neutralize the effect of about 1 mg of crystalline trypsin. The proteinase inhibitor can be demonstrated in several fractions of the serum, the most marked activity being attached to alpha^1 and alpha^2 globulins. Preincubation of the serum at 65°C caused an inactivation of the inhibitor, the extent of which depended on the duration of preincubation. The diminution of the inhibitory effect against various proteinases was, however, different.

The presence of serum considerably decreases the activity of pancreatic proteinases activated by either in vitro autolysis or in vivo injection of olive oil into the pancreatic duct. In contrast, the serum had hardly any effect on the activity of proteinases of normal pancreatic tissue.

According to previous investigations, the permeability of the pancreatic cells was increased in acute pancreatitis. In this case not only the activity of pancreatic proteinases increased but also the amount of proteinase inhibitors. Therefore, the inhibitory action of the serum of control rats and rats with pancreatitis was compared on crystalline trypsin and chimotrypsin. The inhibitory capacity of pancreatitic sera did not significantly differ from that of the control sera.

Respiration-influencing hypocalcaemia-sensitive innervation in the carotid artery

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In 21 dogs under chloralose anaesthesia, one of the common carotid arteries was isolated, immediately from above its origin up to the carotid body. The isolated vascular section was perfused with physiological saline, sodium citrate, calcium citrate, calcium acetate and EDTA solutions, as well as with the blood of dogs thyroid–parathyroidectomized on the 5th postoperative day in cross experiments.

Infusion of sodium citrate and EDTA caused changes in respiration which changes could be prevented by denervation of the vascular section. This phenomenon failed to appear in isolated sections of the femoral artery. A decrease in serum calcium level was observed after the infusion of thyreocalcitonin. This effect was not manifest after previous bilateral thyroid–parathyroidectomy.

On the basis of the results, a nervous reception has been assumed to be present in the common carotid artery which exerts an influence on respiration. The afferent impulse may be due to the decalcification of the membrane of the receptor.

Immunological investigation of serum proteins in pregnancy

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Various serum protein components were investigated with immunological methods in normal pregnancy, in the nephrotic syndrome and in toxaemia of pregnancy.

The serum IgM concentration increased in toxaemia of pregnancy and the transferrin level decreased as compared to the values in normal pregnant women. In nephrosis, the serum IgM fraction was higher, and the transferrin level lower, than in the controls.

A "pregnancy protein" giving one or two arches appears in the alpha^2 and beta-zones of the immunoelectrophoretic pattern of pregnant serum and urine. This protein gives an immune reaction with several tissue elements during pregnancy. Since neither the pathophysiological nor the clinical significance of this "pregnancy protein" are known, preparation of this protein in pure form is needed. An attempt has been made at purifying it as follows.

To the serum ammonium sulphate was added to a saturation of 0.4. The precipitate was dissolved in 0.9% NaCl, dialyzed, and fractioned on Sephadex G-200 column. The "pregnancy protein" was contained in the first large fraction. This fraction was concentrated by dialysis under reduced pressure. This was followed by gradient elution 0.003—0.3 M phosphate buffer pH 8.0 on DEA cellulose column which resulted in the separation of the fraction containing the pregnancy protein. This fraction was not yet homogeneous, since it gave 6—7 arches in the immunoelectrophoretic pattern. Further purification was made by immune-absorption, with the aid of monovalent antisera.

Statistical analysis of the activity of neurones of permanent and periodically changing frequency

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Spontaneous activity of some well-localizable neurones of the suboesophageal ganglion of *Helix pomatia* was analyzed by mathematical methods. The ganglion excised was kept in snail Ringer solution during the experiment, at room temperature. Electrical activity of the soma was recorded by means of glass microelectrodes.

In all cases continuous recordings lasting several hours were made with magnetic data storing. During slow play-back the results of measurements were transmitted by means of a converter to an 8-channel punch card for further digital treatment. Statistical analysis was performed with a 512-channel analyser (Central Institute for Physical Research) and partly with a computer of the MINSK 22 type.

The spontaneous activity of neurones functioning in a periodically changing rhythm on the one hand and that of the continuously functioning neurones of permanent frequency on the other. Relationships were found in impulse width, time, frequency and length of the spike series and, within this, between the number of firings. The relationships within the given parameters were investigated not only from a static (histogram) but also from a dynamic (mirroring temporal changes) point of view.

Effect of acetylcholine on the K-depleted frog heart

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ERNST et al. (1966) observed an increase in the acetylcholine sensitivity of the frog heart under the effect of K-depletion. The phenomenon was interpreted as an antagonism between acetylcholine and K ions.

In the present experiments, muscle strips from the frog heart ventricle were incubated in K-free Ringer's solution. The membrane potential of the ventricular musculature as well as the action potentials were recorded by intracellular microelectrodes. In addition, the mechanical response of the muscle strips was recorded by means of an electronic transducer. Upon the effect of K deprival, the muscle membrane underwent a depolarization from 79.4 ± 7.1 mV to 62.58 ± 4.4 mV, in the average of 21 experiments. The duration of the action potential markedly increased, to about 180% on the average in 8 experiments in normal Ringer's solution. At the same time, the

amplitude of the concentrations decreased. When a 10^{-6} M threshold dose of acetylcholine was applied under such conditions, the duration of the action potential diminished by 30% on the average of measurements on 7 fibres.

The significance of the changes is discussed with respect to the mechanism of the increased acetylcholine sensitivity occurring in K-free Ringer's solution.

The role of the pituitary in liver regeneration

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The extent of liver regeneration was investigated on Wistar A/RXLE F^1 hybrids. After hypophysectomy the weight of the regenerated liver tissue as well as the number of mitoses were considerably decreased. In parabiotic pairs the number of mitoses increased also in the intact partner when the other animal had previously been subjected to partial hepatectomy. The phenomenon was not manifest in the case when the pituitaries had previously been removed from the animals. Thus, the presence of the pituitary is necessary for the action of the unknown humoral factor which promotes liver regeneration.

Effect of ambient temperature on body temperature of animals with cerebral oedema induced by triethylone sulphate

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It was shown previously that after inducing cerebral oedema by thiethylone sulphate the animals' body temperature decreased considerably at an ambient temperature of 20°C. In order to elucidate the mechanism underlying the temperature regulation disturbance the effect of TET on core temperature has been investigated at different ambient temperatures.

Higher ambient temperatures markedly decreased the TET-induced diminution of body temperature, a fact also influencing other autonomic changes. The results pointed to a primary disturbance in heat loss.

Effect of spreading depression on the unit activity of cerebral cortex

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In previous experiments, the drug-induced inhibition of the development and propagation of spreading depression was investigated by means of a

superficial DC lead. Most drugs were tertiary anticholinergic compounds. In the present experiments the correlation between superficial depolarization and unit activity has been studied with extracellular microelectrodes in the cerebral cortex of the rat. Cells exhibiting different spontaneous activity were found to react differently to the effect of spreading depression. The depression propagates on the cerebral cortex in the form of a concentric wave. In the depth of the cerebral cortex it reaches the external pyramidal layer (600—800 μ) but the effect does not spread deeper. The front of the depression in the depth of the cortex occupies a spherical section, with its deepest point in the epicentre. The spreading velocity of the depression is 2—3 mm/sec on the surface but only 100—200 μ/sec in the depth. Its extension is influenced by the columnar organization of the cortex.

Changes after coronary ligation in the electrophysiological parameters of myocardial regions not directly affected by the ligature

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At the 37th Congress of the Hungarian Physiological Society the importance of heart muscle not directly affected by coronary ligation has been stressed in the genesis of arrhythmias. The aim of the present experiments was to elucidate whether changes could be demonstrated in the electrophysiological parameters of this region to explain the increased susceptibility to arrhytmia of the heart muscle not directly affected by the occlusion. The experiments were performed on the heart in situ of anaesthetized cats under artificial respiration. The left descending branch of the coronary artery was ligated and the fibrillation threshold of the region not affected by the occlusion was continously recorded. No significant change occurred as compared to the initial value. Neither did the coronary ligation elicit a change in the excitability of intact heart muscle of anaesthetized dogs, as judged on the basis of the diastolic electrical stimulus threshold which was recorded continuously. A special electrode was constructed to record the monophasic action potential of the intact heart muscle in four points 9 mm apart. The slight differences in repolarization half time of the action potentials under normal conditions were, however, augmented after coronary ligation. The amplitude of the action potentials generally decreased during ligation but to different extents in the four points. The increased inhomogeneity in selective ion permeability of the membrane of near-lying myocardial fibres gives an explanation for the increased susceptibility to arrhythmia of regions not affected by the occlusion.

Effect of catecholamines on blood flow in the regenerating rat liver

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Removal of 2/3 of the liver (partial hepatectomy) is followed by a rapid regeneration of the residual liver tissue. In the rat, restitution of the liver to its preoperative weight takes about three weeks. In the present experiments the rate of regeneration of the portal and hepatic vessels has been studied in comparison with the restoration of the liver parenchyma. The effect of catecholamines on hepatic blood flow during regeneration has also been studied.

In rats, regeneration of the portal vein and of the hepatic artery was investigated at various points of time following partial hepatectomy, by means of a quantitative injection-corrosion procedure (UNGVÁRY et al., 1969). The capacity of the afferent vessel systems was found to be below the control value during the first days after partial hepatectomy both in absolute value and calculated per g of liver tissue. The effect of catecholamines injected intravenously and intraportally on tissular blood flow of the liver was measured and recorded by means of a fluvograph through thermocouples placed in the hepatic lobe. In most animals, noradrenaline and isopropylnoradrenaline exerted an effect opposite to that seen in control rats. The phenomenon is assumed to be due to the altered characteristics, or amount of the receptors in hepatic vessels, or to an imperfect innervation.

Regional changes in the adaptability of coronary vessels: the role of primary collateral branches

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In dogs under chloralose anaesthesia a hyperaemic reaction was produced by the transient occlusion of various branches of the left coronary system. The extent of reactive hyperaemia, calculated from total flow, was the greater the smaller was the size of the myocardial segment directly exposed to hypoxia, in relation to the whole left ventricle. Myocardial blood flow measurements by the heat clearance method revealed that the local peak value of vasodilation was, in fact, considerably greater in the case of circumscribed reactive hyperaemia than during the hyperamic response involving the whole left coronary system, even though the extent of the local ischaemia evoking the vasodilation was considerably greater in the latter case. The phenomenon is ex-

plained by the equilibrating function of the primary collateral branches connecting various sections of the left coronary system.

A segmental disturbance in coronary adaptability was produced by topical application of a small amount of hexamethonium or by repeated hypoxic loads. Under these conditions, no increase in blood flow was elicited by a circumscribed ischaemia in the vascular region made incapable of adaptation while an involvement of the hyperaemic reaction of the intact coronary segments caused a diminution in the absolute level of local flow in the myocardial regions supplied by the coronaries with damaged adaptability.

Location and characterization of Br cell in the central nervous system of *Helix pomatia*

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The Br cell (ARVANITAKI and CHALAZONITIS, 1955) characterized by the periodic appearance of burstlike spike series, has been described in the central nervous system of several gastropods. In the present experiments, the exact location of such a giant cell has been determined in the right parietal ganglion of *Helix pomatia* after intracellular dye application. The cell is unipolar, its axon after a short course gives off branches and is divided in thin fibres in the region of the two separate cell groups of the ganglion.

Physiological investigations have verified the Br character of the cell. Characteristic of cellular activity were the consecutive interspike intervals in the function of time as well as the distribution of the time interval, the impulse width of consecutive spikes in the function of time and potential, the time interval between the potential series as well as the ratio of activity and resting sections.

The data obtained present a basis for the investigation of the mechanism of potential generation and the physiological role of the Br cell.

Response types of wind-sensitive receptors of the interneurones of the migratory locust *(Locusta migratoria migratorioides)*

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Spontaneous activity of interneurones in the connectives of *Locusta migratoria* was recorded extra- and intraaxonally. Also recorded were the responses of the neurones to ipsi- and contralateral stimulation of the wind-

sensitive receptors of the head; the course of the interneuronal pathway was determined by transecting the connectives.

The interneurones in the connectives were either silent or exhibited a regular or irregular activity. Some of them responded to the stimulation by air-movement of the wind-sensitive receptors of the head.

From these structures, the following responses were obtained, in addition to the slow-adaptation (phasic-tonic) and quick-adaptation (phasic) interneurone responses already known.

1. An inhibitory response which occurs with either slow or quick adaptation types.

2. "On–off" responses manifest at the beginning and at end of air stimulation. Adaptation of the "off" responses is always rapid while that of the "on" responses may take different times.

3. Transection experiments revealed a straight course of the pathways crossing in the brain, subeosophageal ganglion and in the first and second thoracic ganglia.

Determination of quickly exchanging Ca fractions in frog skeletal muscles

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Calcium bound to the surface membrane has an important role in the preservation of the normal function of muscles; yet, there are few experimental data concerning its amount and binding characteristics. To study this problem, ^{45}Ca wash-out experiments were performed. Fibre boundles of frog m. semitendinosus were incubated for two hours in frog Ringer solution containing ^{45}Ca. Thereafter, the activity was washed out, determined, and desaturation curves were constructed. Their graphic analysis revealed the presence of 5 Ca fractions with half-time (in minutes) as follows: 0.25 ± 0.1 (1); 1.5 ± 0.9 (2); 5.8 ± 1.2 (3); 19.6 ± 4.2 (4); 136 ± 38.6 (5). The separation of the individual fraction was significant and corresponded probably to the ^{45}Ca originating from the muscle surface, the extracellular space, the membrane (fractions 3 and 4) and the intracellular space, respectively. The amount of the loosely bound membrane Ca (3) was 0.34 ± 0.06 μM/g, while the strongly bound one (4) 0.5 ± 0.05 μM/g of wet muscle weight. Together they approached to about 40% of the total Ca content of the muscle.

Estimation of subepithelial capillary blood flow by measuring the outflux of barbital in the small intestine of the rat

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A loop of the small intestine of Sprague–Dawley rats under urethan anaesthesia was perfused with bicarbonate buffer of pH 9.5, and the outflow of intravenously administered ^{14}C-barbital in the loop was measured. In parallel experiments, total blood flow of the intestine was determined by SAPIRSTEIN's ^{86}Rb method, and the distribution of blood flow in various layers of the gut was estimated by the microsphere technique. If blood flow in the mucose determined with the microsphere technique is taken for 100% barbital clearance is only 80% in the ileum and 47% in the duodenum.The clearance of barbital depends on the perfusion procedure; slow and one-way perfusion results in a lower clearance than a technique ensuring a better intraluminar mixing.

It has been concluded that the filtering surface of the mucous membrane or, in the case of drug absorption, the actual absorbing surface, is considerably smaller than the anatomical total surface of the mucosa. The ratio of filtering surface : total surface is smaller in the duodenum than in the ileum. No gradient could be observed in the duodenum–ileum direction in either barbital clearance or in drug absorption.

Effect of pituitary stalk lesion on the alteration of blood sugar level and liver glycogen content induced by alloxan and glucagon

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In previous investigations it has been shown that pituitary stalk lesion considerably modified the alloxan-induced disturbance in carbohydrate metabolism. The initial hyperglycaemia following alloxan administration was absent or very slight, the hypoglycaemic phase, attributed to insulin mobilization, was prolonged and the blood sugar elevation during the permanent hypoglyceamic phase was of a smaller extent.

In the present investigations the alteration observed during the transient hyperglycaemic phase has been studied. The changes of liver glycogen content were investigated in non-operated control rats as well as after the administration of alloxan and glucagon in animals with pituitary stalk lesion. The blood

sugar level of control animals was found considerably to increase after the administration of alloxan and glucagon, with a concomitant significant decrease of the hepatic glycogen level. In animals with pituitary stalk lesion the blood sugar level increased moderately and no significant change occurred in hepatic glycogen content.

A role may be ascribed to the inhibition of liver glycogen mobilization in the absence of alloxan-induced transient hyperglycaemia in pituitary stalk-lesioned rats. Clarification of the pituitary hormone(s) involved into the phenomenon requires further experiments.

Comparative biochemical and morphological investigations in chronic $HgCl_2$ poisoning

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It was shown previously that respiration of isolated liver mitochondria was inhibited by $HgCl_2$ added to the incubation medium. At the same time, oxidative phosphorylation was uncoupled. It was shown that ^{202}Hg was bound to the inner membrane of the mitochondria. Electron microscopic investigations on rats with renal failure induced by $HgCl_2$ revealed no structural alteration in the liver mitochondria.

The ultrastructure of the liver and kidney as well as the ATPase activity of isolated liver and kidney mitochondria were investigated after oral administration of subtoxic doses of $HgCl_2$. No significant alteration was found in the ATPase activity of kidney mitochondria. After the application of 0.5 mg/100 g of $HgCl_2$, ATPase activity of liver mitochondria exhibited a slight decrease followed by a marked increase by the 3rd—5th week of the treatment. This finding can be explained by an increased detoxicating activity of the liver. After the administration of 2.5 mg/100 g of $HgCl_2$, ATPase activity of liver mitochondria decreased by the 4th—5th week of treatment.

The morphological picture of liver mitochondria was unaffected.

Subcellular distribution of thyroid hormones in the central nervous system, I. Study of the distribution during postnatal development

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^{125}I-triiodothyronine and thyroxine uptake of the cerebrum, cerebellum and hypothalamus as well as the subcellular distribution of these hormones have been investigated in male albino rats during postnatal development.

Concentrations of both hormones were highest in all the three regions, one or two days after birth. Hormone uptake of the brain decreased markedly with age, being 25—30% of the newborn value at the 14th day, and 3 to 5% in adults. Considerable differences were observed in the subcellular distribution of the two hormones during development.

Cerebrum. Activity in the nuclear fraction increased till the 7th postnatal day to more than 30% of the total activity. Then by the 14th day it decreased to 25% and stayed at this level till the 21st day. In older animals (50—90 days) a new significant elevation occurred and 35—40% of the total activity was found in the nuclei. Activity in the mitochondria increased considerably up to the 21st day, when it amounted to more than 30% of the total activity. Thereafter the activity decreased, then slightly increased to 21—26% by the 75th—90th day. Activity detectable in the microsomal fraction was considerably lower (8—13%) and behaved as that of the mitochondrial fraction. Activity in cellular fluid (supernatant of 105,000 g) decreased with age.

Similar significant differences were found in the distribution of thyroid hormone in the hypothalamus and cerebellum in the animals of various ages. There were differences between these two regions and the cerebrum.

Lymphatic transport of LDH-isoenzymes

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The LDH activity and LDH-isoenzyme spectrum were investigated in the lymph of the heart, intestine, kidney and limb of the dog. LHD activity (U/ml and U/g of protein) in lymph samples taken from various regions was similar as in serum, only in heart lymph and limb lymph was it considerably higher.

The isoenzyme spectrum (ratio of H : M subunits) of the lymph samples differed significantly from that of the serum, with the exception of liver lymph. The ratio of H : M of the intestinal and cardiac lymph did not significantly differ from the values found in the corresponding tissue homogenates.

The results are in accordance with the view that under normal conditions the lymph vessels transport tissular LDH into the circulation, particularly from the skeletal and heart muscles.

Phospholipid metabolism of Yoshida sarcoma tumour cells

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Ascites fluid taken from rats treated intraperitoneally with 10×10^6 Yoshida sarcoma cells as well as the tumour tissue of rats treated with ascites

cells were found to contain considerable amounts of phospholipids. These mainly consisted of phosphatidyl choline and phosphatidyl ethanolamine in the ascites cells and tumour tissue, and of phosphatidyl choline in the ascites fluid. Concentration of sphyngomyelin, phosphatidyl serine, phosphatidyl inosit, phosphatidyl acid and the lyso components, was much lower. The fatty acids of the phospholipids investigated were determined by gas chromatography.

Functional differences in the b $\rightleftarrows$ a transformation of cardiac isophosphorylases

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It has been assumed that the transformation of isoenzymes from the inactive b-form into the active a-form occurred in more than one step. In our study this simultaneous determination of isophosphorylases was performed by means of antibody inhibition as described previously. According to the first observations, part of the phosphorylases present in the heart are found in a-form, but this contains only one isoenzyme, the P^3 of cardiac type.

When either in vivo or in vitro a phosphorylase transformation b $\rightleftarrows$ a is induced by adrenaline, transformation of P^3 isophosphorylase into a-form always precedes the activation of the other (muscular type) P^1 fraction.

Similar results were obtained when P^1 and P^3 isoenzyme preparations were activated by phosphorylase kinase; the transformation of fraction P^3 into form-a preceded the transformation of fraction P^1 also in this case. On this basis phosphorylase kinase may be assumed to have a different affinity to the two isophosphorylase fractions, and this is the cause of the temporal shift of activation. (The authors thank M. DANKÓ, Institute of Physiology, University Medical School, Debrecen, for help in making heart muscle preparation.)

Effect of serotonin on hypothalamo-pituitary-adrenocortical function in the rat

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The effects of serotonin were studied in vitro and in vivo on the functions of the pituitary–adrenocortical system in the resting state and during load. Twenty μg of serotonin, injected into the lateral ventricle, counteracted

pituitary–adrenocortical activation by surgical stress. Administration of crystalline serotonin (20 μg) into the ventromedial hypothalamus decreased the extent of pituitary–adrenocortical activation by ether stress, with the basal secretion unaltered. A similar amount of serotonin implanted into the anterior or posterior hypothalamus had no effect on either basal secretion or load-induced activation of the pituitary–adenocortical system.

Under conditions in vitro, serotonin (10^{-7} M/l) slightly increased adrenal corticosterone production but had no influence on the increase in the corticosterone production induced by exogenous ACTH or anterior lobe tissue. In addition, it inhibited the stimulatory effect of hypothalamic tissue on the pituitary–adrenocortical system.

It is concluded that at the hypothalamic level serotonin inhibits the load-induced activation of the pituitary–adrenocortical system.

Binding of oestradiol in the hypothalamus of normal and androgenized rats

MARIETTA VÉRTES and ZSUZSA VÉRTES
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Binding of (6,7 ^{3}H)-oestradiol-17-β in the hypothalamus has been studied at various stages of postnatal development in normal and androgenized female albino rats (1 mg of testosterone propionate subcutaneously at the second postnatal day).

No specific oestradiol binding was observed in the hypothalamus at the age of 14 days. By the 21st day, specifically bound ^{3}H-oestradiol appeared in the nuclei, as a sign of the development of oestradiol reception. Binding in both the cytoplasm and the nucleus increased with age, reaching the highest value after sexual maturation.

Androgenization had a profound influence on oestradiol binding in the hypothalamus. The marked augmentation of binding in the cell nucleus seen under normal conditions on the 21st day of life failed to occur in androgenized animals, and the binding of oestradiol in the cell nuclei was far below the control also in older animals. Binding in the cytoplasm increased in immature animals under the effect of androgenization, i.e. the amount of ^{3}H-oestradiol specifically bound in the cytoplasm was higher than in the controls. In mature animals, however, binding in the cytoplasm was decreased by androgenization.

Effect of postnatal oestradiol treatment on DNA and RNA synthesis in the brain

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The effect of 500 μg of oestradiol, administered subcutaneously on the second postnatal day, was investigated on DNA and RNA synthesis in the brain (cerebrum, cerebellum, hypothalamus) at various points of time of postnatal development in female albino rats.

After oestradiol administration incorporation of ^{3}H-thymidine into DNA decreased considerably in all brain regions. The decrease was especially marked during the first week. After the second week, however, no difference could be demonstrated between treated and control animals.

Incorporation of ^{14}C-orotic acid into RNA decreased considerably after postnatal oestradiol treatment. The diminution was most marked in the anterior hypothalamus and mainly in the younger age groups. At the age of 60 days no significant difference in incorporation could be found between treated and control animals.

Effect of K ion on homoserine dehydrogenase activity of *Chlamydomonas reinhardti*

I. VINCZE and G. DÉNES
INSTITUTE OF MEDICAL CHEMISTRY, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL, BUDAPEST, AND BIOLOGICAL CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, SZEGED

Homoserine dehydrogenase is an allosteric enzyme at the branching point of amino acid biosynthesis starting from aspartic acid. The enzyme catalyzes the transformation of aspartic semialdehyde into homoserine in the presence of NADH and NAD. The reaction is inhibited by one of the end products, i.e. threonine on the one hand and by cysteine on the other. Of the monovalent cations, potassium increases the activity of the enzyme, decreases or abolishes the inhibitory effect of threonine, but has no influence on that of cysteine. A competition with threonine could be demonstrated in the effect of K ion. The kinetically determinable dissociation constant of threonine exhibited a 45-fold augmentation, from 1.5×10^{-5} to 7.10^{-4}, in the presence of 100 mM of KCl. The homoserine dehydrogenase of *Chlamydomonas reinhardti* was inactivated at 50°C. Threonine protected the enzyme against this inactivation. This phenomenon was used for determining the dissociation constant of threonine, independent of kinetic conditions. Potassium ion had no influence on the dissociation constant of threonine determinable in this way. It is assumed that the threonine–enzyme is a kinetically inactive complex which is transformed into the kinetically active complex threonine–enzyme–K.

Inhibitory effect on gastric secretion and ulceration of actinomycin-D, cyclophosphamide and mannomustine in the Shay rat

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The effect of mannomustine, cyclophosphamide and actinomycin-D was investigated on gastric secretion, hydrochloric acid secretion and ulcer development in Shay rats, 7 and 24 hours after pyloric ligation.

1) Mannomustine (0.1, 1.0, 10.0 and 25.0 mg) significantly decreased gastric secretion, acid secretion and ulceration in both groups of animals; 2) actinomycin-D (100, 200 and 250 μg) significantly decreased gastric secretion, acid secretion and ulceration in both groups of animals; 3) cyclophosphamide (1.0—10.0 mg) significantly decreased gastric secretion, acid production and ulceration in both groups of animals.

The following conclusions have been drawn. 1) The inhibitory effect of drugs applied on gastric secretion and acid secretion is manifest within 0—7 hours after pyloric ligation, while the inhibitory effect on ulceration appears between the 7th and 24th hours. 2) The inhibitory effect on gastric secretion and ulceration can be separated in time by means of the cytostatic drugs. 3) Augmentation of nucleic acid metabolism plays an important part in the development of gastric hypersecretion and ulceration, while its inhibition counteracts both gastric hypersecretion and ulceration.

Cerebral rheographic investigations during pressing load (weight-lifting)

S. WAHLSTAB, P. APOR, MÁRTA MIKLÓS and JOLÁN PETRES
NATIONAL INSTITUTE OF SPORTS MEDICINE, BUDAPEST

The fits of dizziness and unsteadiness occurring during pressing raise the possibility of a functional disturbance of the central nervous system. It was therefore studied what changes occur in the rheographic parameters of the head, the CO_2 and O_2 content of expired air, and some circulatory parameters during weight-lifting of nearly 100% intensity.

By the 10th—20th second after pressing, relative pulse volume increased. The relationship between rheographic changes, gas content of expired air, cardiac work and other parameters is discussed.

Effect of cortisone treatment and adrenalectomy on 5-HT metabolism of mouse tissue

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It is known that mice become sensitive to shock mediators after adrenalectomy and that this sensitivity decreases after cortisone treatment.

In the present experiments, cortisone treatment significantly decreased the 5-HT level in the blood, liver, lung, pylorus and small intestine of the mouse. After adrenalectomy, the 5-HT level decreased in the blood but increased in other tissues.

Activity of 5-hydroxytryptophan decarboxylase an enzyme participating in 5-HT synthesis, did not change after adrenalectomy or cortisone treatment. Monoamine oxidase activity was unaltered after adrenalectomy and decreased after cortisone treatment in all the investigated organs.

Thus, although adrenalectomy or cortisone treatment were considerably affecting tissular 5-HT level and, essentially in opposite directions, their effect is not brought about at the level of the enzymes immediately involved into the metabolism of 5-HT.

The role of the kidney in the calcium adsorption promoting effect of Vitamin D

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INSTITUTE OF PATHOPHYSIOLOGY, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL, AND INSTITUTE OF NUTRITION, BUDAPEST

The role of the kidney in enteral calcium adsorption has been studied in the rat. In animals kept on a normal diet, renal failure did not influence calcium adsorption, irrespective of the fact whether the uraemia had been brought about by bilateral nephrectomy or bilateral ligature of the ureters. In accordance with previous results, physiological doses of vitamin D markedly increased enteral calcium adsorption in vitamin D deficient animals. No such effect was seen in animals suffering from renal failure. The phenomenon was more marked in rats with bilateral nephrectomy than in those with ureteric ligature, although the degree of azotaemia was similar. Thus, intact kidney tissue is necessary for the action of vitamin D.

Mechanism of the hormonal activation of adenyl cyclase

MÁRIA WOLLEMANN and T. FARKAS
SECTION OF BIOCHEMISTRY, BIOLOGICAL CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, SZEGED

Adenyl cyclase activity was investigated in the particulate fraction of rat heart and brain in the presence of noradrenaline, isoprenaline, acetylcholine and carbamylcholine. The activating effect of catecholamines was inhibited by receptor blocking agents and left uninfluenced by 3×10^{-3} M of theophylline. In the rat heart fractions, the cholinesters applied at the same concentration counteracted the effect of catecholamine and inhibited the basal activity of adenyl cyclase. This effect of catecholamines is probably brought about partly through an activation of phosphodiesterase.

In same cellular fractions, catecholamine inhibited the incorporation of ^{32}P into various lipid fractions. In contrast, incorporation was enhanced by acetylcholine. It is concluded that the metabolism of membrane lipids plays an important part in the hormonal regulation of adenyl cyclase activity.

Anti-inflammatory effect of heptyl esters

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INSTITUTE OF PATHOPHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, SZEGED

Phenylalanine and arginine are known to play a decisive role in the binding of bradykinin to receptors. It seemed thus interesting to investigate the antagonistic effect of heptyl esters of these amino acids against inflammatory mediators.

The experiments were performed on guinea pigs as well as on R-Amsterdam and CFY rats. The heptyl ester of phenylalanine (PHE) markedly inhibited the increase in permeability caused by bradykinin, carrageenin, serotonin and dextran. The effect was considerably weaker on inflammation induced by histamine. The effect of arginine heptyl ester (AHE) was similar to that of PHE, except that it inhibited histamine to a greater extent and serotonin to a lesser extent.

An attempt was made to inhibit by AHE and PHE the bronchial spasm elicited in the guinea pig by vasoactive mediators. Simultaneous application of the esters and mediators in aerosol prevented the bronchial spasm. When the concentration of AHE and PHE was above 0.25%, the bronchial spasm previously evoked by the vasoactive substance ceased and respiration became normal.

It seems that the amino acid esters prepared by the authors possess a favourable anti-inflammatory effect and may be used for therapeutic purposes.

Investigation of pancreatic excretion by application of a chronic fistula

ÁGNES J. ZSINKA
INSTITUTE OF NUTRITION, BUDAPEST

For studying pancreatic excretion, the physiologically most appropriate method involving a chronic fistula has only been used in large animals, owing to the technical difficulty of the surgical procedure. However, under certain conditions, for instance, in nutrition studies, the omnivorous rat must be used. For this purpose, method has been developed for creating a chronic fistula. For checking the applicability of the chronic fistula, the secretin–pancreozymin test was used. The experiments were performed on two groups of animals. In one group a chronic fistula was created and the investigations were performed on waking animals. In the second group an acute fistula was prepared in anaesthetized rats. The amount of pancreatic juice secreted spontaneously, or under the effect of secretion pancreozymin, as well as the total protein content, trypsin, lipase and amylase activity were determined and the mean values compared. The relationship between secretion, enzyme activities and histological picture was also investigated. The method seems suitable for studying in vivo the excretion of the intact and the injured pancreas.

INDEX

PHYSIOLOGIA

Nikolásev V., Resch B. A., Mészáros J., Szontágh F. E., Karády I.: Lipid Content of Human Embryonic Tissues 201
Radev A. I.: Effect of Simultaneous Oestriol and Progesterone Administration on Sodium and Potassium Excretion and on their Tissue Content in Female Rats 209
Баклавaджян О., Бужаки Г., Андьян Л.: К электрофизиологическому анализу влияния атропина на реакцию самораздражения у кошек 217

PHARMACOLOGIA

Varga F., Fischer E., Szily Terézia: Inhibition of Microsomal Drug Metabolism by 2-{[2-(4-Pyridyl)-Methyl]-Thio}Benzimidazole (H-718) 227
Varga F., Hankovszky H. Olga, Hideg K., Fischer E., Szily Terézia: Inhibition of Hexobarbital Metabolism by Pyridyl Derivatives 235
Kenedi I., Losonci A.: Effect of Beta Adrenergic Blocking on the Arrhythmias Following Coronary Ligation in Rat 247
Phan D. V., György L., Varga B., Szelényi Z.: Temperature Reducing Effect of Oxotremorine in the Rat and the Mouse 259

RECENSIONES

Hanke W.: Vergleichende Wirkstoffphysiologie der Tiere. VEB Gustav Fischer Verlag, Jena 1973. (K. Lissák) 277
Béssis M., Weed R. I., Leblond P. F. (Eds): Red Cell Shape Physiology, Pathology, Ultrastructure. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 277
Jorpes J. E., Mutt V. P. (Eds): Secretin, Cholecystokinin, Pancreozymin and Gastrin. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 278
Voss H. E., Oertel G. (Eds): Androgene I. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 278
Hodge H. C., Sannard J. N., Hursh J. B. (Eds): Uranium, Plutonium, Transplutonic Elements. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) .. 279
Iggo A. (Ed.): Somatosensory System. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 279
Frisch K. v.: Erinnerungen eines Biologen. Springer-Verlag, Berlin, Heidelberg, New York 1973. (K. Lissák) 280

ABSTRACTS of the lectures delivered at the 38th Congress of the HUNGARIAN PHYSIOLOGICAL SOCIETY, Budapest, May 31—June 3, 1972 281

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All correspondence related to the meeting should be addressed to:

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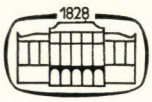

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Symposion der Deutschen Akademie der Naturforscher Leopoldina vom 18. bis 19. Oktober 1971 in Halle (Saale)
Herausgegeben von Prof. Dr. B. Lüeken, Halle/S., und Prof. Dr. Dr. J.-H. Scharf, Halle/S.

(Nova acta Leopoldina. Neue Folge. Nr. 208 Bd. 37/2)
1973. 331 Seiten mit 229 Abbildungen und 21 Tabellen
Broschiert 46,— M

Da die Jahresversammlung 1971 der Leopoldina das Gesamtgebiet der „Informatik" behandelte, entstand der Wunsch, den speziellen Problemen aus dem Grenzgebiet zwischen Informatik und Sinnesphysiologie ein Spezialsymposion in unmittelbarem Anschluß an die Jahresversammlung zu widmen. Der vorliegende Band legt 20 Vorträge international anerkannter Experten vor, die aus Europa und Übersee nach Halle gekommen waren. Berücksichtigt sind Wirbellose und Wirbeltiere.

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Festschrift für Bernd Lueken anläßlich seines 65. Geburtstages am 8. August 1973

Herausgegeben von Prof. Dr. Dr. h. c. mult. K. MOTHES, Halle/S., und Prof. Dr. Dr. J.-H. SCHARF, Halle/S.

(Nova acta Leopoldina. Neue Folge. Nr. 211, Bd. 38)
1973. 595 Seiten mit 229 Abbildungen und 23 Tabellen
Broschiert 90,— M

Dem bekannten halleschen Herz- und Neurophysiologen Bernd Lüeken ist die Festschrift anläßlich seines 65. Geburtstages gewidmet worden, um seine hervorragenden Verdienste als Wissenschaftler und als langjähriger Senator der Deutschen Akademie der Naturforscher Leopoldina zu würdigen. Die Festschrift enthält Beiträge zur Herzphysiologie, u.a. von K. Wezler, zur Sinnesphysiologie, u. a. von H. Drischel, W. D. Keidel und R. Thauer sowie weitere Arbeiten zur normalen bzw. pathologischen Physiologie und mathematische Beiträge, u. a. über biologische Modellbildungen und quantenmechanische Messungen in der Biologie.

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РЕЗЮМЕ

СОДЕРЖАНИЕ ЛИПИДОВ В РАННИХ ЧЕЛОВЕЧЕСКИХ ЭМБРИОНАЛЬНЫХ ТКАНЯХ

В. НИКОЛАШЕВ, Б. А. РЕШ, Й. МЕСАРОШ, Ф. Э. СОНТАГ и Ш. КАРАДЫ

Исследовалось содержание липидов в последе и в различных органах (мозг, печень сердце, легкие, почка) 12-недельных человеческих эмбрионов, полученных при прерывании беременности. Определялось количество холестерина, холестерин-эстера, свободной жировой кислоты, моноглицерида, 1, 2-диглицерида, 1,3-диглицерида и триглицерида и изучался состав жировых кислот этих липидных фракций. Результаты дают прежде всего информацию о стандартных величинах, имеющихся в ранней беременности.

ДЕЙСТВИЕ ВВЕДЕННЫХ ОДНОВРЕМЕННО ЭСТРИОЛА И ПРОГЕСТЕРОНА НА ЭКСКРЕЦИЮ НАТРИЯ И КАЛИЯ И НА ИХ ТКАНЕВОЕ СОДЕРЖАНИЕ В ПОЧКЕ У КРЫС-САМОК

А. И. РАДЕВ

Интактным крысам-самкам вводилось подкожно 2 Мг эстриола и 0,002 г прогестерона ежедневно в течение 10-и дней. В этот период каждый день определялись мочеопорожнение, уровень натрия, калия и уровень осмотически активных веществ в моче и концентрация эндогенного креатинина в последней порции мочи. По окончании экспериментального периода измерялись содержание натрия, калия и воды в коре, медулле и соске почки, клиренс натрия, калия, осмотически активных веществ и эндогенного креатинина. Одновременное введение двух гормонов повышало гломерулярную фильтрацию (GFR) и реабсорбцию натрия и калия. Содержание натрия и калия в коре и медулле повышалось, а в области соска не изменялось.

К ЭЛЕКТРОФИЗИОЛОГИЧЕСКОМУ АНАЛИЗУ ВЛИЯНИЯ АТРОПИНА НА РЕАКЦИЮ САМОРАЗДРАЖЕНИЯ У КОШЕК

О. БАКЛАВАДЖЯН, Г. БУЖАКИ и Л. АНДЬЯН

На русском языке.

ТОРМОЖЕНИЕ МИКРОСОМАЛЬНОГО МЕТАБОЛИЗМА ЛЕКАРСТВ 2-{[2-(4-ПИРИДИЛ)-МЕТИЛ]-ТИО}БЕНЗИМИДАЗОЛОМ (Н-718)

Ф. ВАРГА, Е. ФИШЕР и Т. СИЛИ

Величина $ЛД_{50}$ Н-718 при внутрибрюшинном введении у крыс была 314 мг/кг. Период сна, вызванного гексобарбиталом, продлевался в 4—5 раз и время полураспада концентрации гексобарбитала в плазме увеличивалось после внутрибрюшинного введения 10—15 мг/кг Н-718. Этот препарат предотвращал тремор, вызванный треморином, но не тормозил тремора, вызванного оксотреморином. Он удлинял время выживаемости крыс,

обработанных летальной дозой паратиона, но не влиял на токсичность параоксона. Печеночное микросомальное окисление гексобарбитала, гидроксиляция анилина и N-деметиляция аминофенорона значительно тормозились H–718 при его концентраци 10^{-4} М, но при этой концентрации активность прокаин-эстеразы уменьшалась только умеренно. Препарат, даже в дозе 50 мг/кг, при внутрибрюшинном введении, не оказывал влияния на связывание бромсульфофталеина с глутатионом. Данные показывают, что H–718 тормозит микросомальную, зависящую от NADPH и цитохрома P_{450}, энзимную систему печени, метаболизирующую лекарство.

ТОРМОЖЕНИЕ МЕТАБОЛИЗМА ГЕКСОБАРБИТАЛА ПРОИЗВОДНЫМИ ПИРИДИЛА

Ф. ВАРГА, О. Х. ХАНКОВСКИ, К. ХИДЕГ, Е. ФИШЕР и Т. СИЛИ

У крыс сравнивались эффекты 34 производных пиридила на продление времени сна, вызванного гексобарбиталом. Было найдено, что эти вещества увеличивали продолжительность действия гексобарбитала, торможением его микросомального окисления. Носителем эффекта является пиридиновое кольцо. N-метиляция пиридинового кольца уничтожала тормозное действие. Производные 4-пиридила были всегда более эффективными, чем производные 3-пиридила, а они были эффективнее, чем производные 2-пиридила. Сама гаптофорная часть молекулы не эффективна и может иметь различные химические структуры. Гаптофорная группа усиливает слабое тормозное действие пиридина в 10—100 раз.

ВЛИЯНИЕ БЕТА-АДРЕНЭРГИЧЕСКОГО ТОРМОЖЕНИЯ НА АРИТМИЮ, ВОЗНИКАЮЩУЮ ПОСЛЕ ПЕРЕВЯЗКИ КОРОНАРНОЙ АРТЕРИИ

И. КЕНЕДИ и А. ЛОШОНЦИ

После перевязки левой коронарной артерии по методу Selye, в 19-и из 20-и экспериментов возникала более или менее тяжелая аритмия, с различной продолжительностью.

У 15-и из 20-и животных, обработанных предварительно 200 мг на 100 г веса тела пропранолола (Inderal, ICI), деятельность сердца стало ритмической в течение 15-и сек. В 11-и случаях зубец QRS постепенно уменьшался. Только в двух опытах наблюдалась экстрасистола. У 6-и животных возникало трепетание или мерцание желудочка, из них 3 погибли. В 12-и опытах частота постепенно замедлялась и сердце останавливалось. В этих случаях была найдена тяжелая дилатация сердца, ишемический левый желудочек был акинетическим и сводчатым, как аневризма.

После введения 20 мг на 100 г веса тела пиндолола (Visken, Egyt-Sandoz) у 24-ех животных перевязывалась коронарная артерия. В 3-ех опытах аритмия не возникала. 3 крысы погибли, у других деятельность сердца была ритмической до 7—8-ой мин. Тогда появились переходные, спонтанно исчезающие гетеротропные расстройства выработки импульса. Сердце ни у 3-ех погибших животных, ни у животных, контролируемых через 10 мин или 24 часа, не было дилатировано и сокращение ишемического левого желудочка казалось нормальным.

После обработки практололом было сделано 20 перевязок. В большинстве случаев очень быстро начиналась регулярная синусовая деятельность. В 7-и случаях ЭКГ показывала расстройство в проведении возбуждения (в 5-и случаях атриовентрикулярный блок второй степени и в 2-ух случаях блок ножки Tawara). В 4-ех случаях возникало трепетание или мерцание желудочка, которое было ирреверсибильным. Кроме этого 3 опыта закончились летально в течение 10-и мин. В конце опыта в 5-и случаях были найдены значительная сводчатость левого желудочка и акинез, в 3-ех случаях — только недостаточное движение левого желудочка.

АНАЛИЗ СНИЖАЮЩЕГО ТЕМПЕРАТУРУ ЭФФЕКТА ОКСОТРЕМОРИНА НА КРЫСАХ И МЫШАХ

Д. В. ФАН, Л. ДЬЕРДЬ, Б. ВАРГА и З. СЕЛЕНЬИ

Исследовалось влияние оксотреморина на температуру тела мышей и на температуру тела и кожи и потребление кислорода у крыс.

При комнатной температуре оксотреморин значительно уменьшал температуру тела мышей. У крыс повышал температуру кожи хвоста, уменьшал температуру тела и вызывал двухфазное повышение потребления кислорода. Можно предполагать, что причиной гипотермии является повышение теплоотдачи.

При окружающей температуре в 16°С у крыс оксотреморин повышал температуру кожи, но в меньшей степени и в продолжении более короткого времени, чем при комнатной температуре. Значительно уменьшал температуру тела, и вначале уменьшал, потом повышал потребление кислорода. В гипотермии играло роль и уменьшение теплопродукции.

При окружающей температуре в 30°С оксотреморин не повышал температуру кожи, не оказывал влияния или повышал температуру тела и усиливал потребление кислорода.

Сульфат атропина тормозил вышеупомянутые действия оксотреморина, а метилатропин не влиял на них. Гипотермия, вызванная оксотреморином, исходила из центральной системы.

Небольшая доза уретана тормозила тремор и обе фазы повышения обмена веществ, вызванные оксотреморином.

При повторном введении оксотреморина ослабевал его уменьшающий температуру эффект на крысах. На мышах предварительная обработка треморином тормозила гипотермию, вызванную оксотреморином.

Дилатация сосудов в коже и повышение кровяного давления, вызванные оксотреморином, возникают одновременно на наркотизированных крысах. Против первичного эффекта быстро, против вторичного медленно возникала тахифилаксия.

Активность некоторых из изученных веществ (катехоламины, ацетилхолин, карбохол, хлорпромазин, этанол, вазопрессин, амилнитрат) не достигала расширяющей сосуды активности оксотреморина.

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