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and
COMMISSION FOR STUDY OF MULTIPLE SCLEROSIS

INTERNATIONAL SYMPOSIUM ON BIOCHEMISTRY AND HISTOCHEMISTRY OF MYELIN AND DEMYELINATION

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NEUROPAT. POL. 1969, VII, 3

EDITORIAL

DEVELOPMENT OF NEUROPATHOLOGY IN PEOPLE'S POLAND

On the 25-th anniversary of Polish People's Republic

The history of Polish neuropathology is as long as the history of Polish neurologists. This statement can be easily verified by the readers of the book: Polish Neurologists written by E. Herman edited in 1958 by PZWL. This book as well as the papers published by this author in Neuropatologia Polska in the years 1967 and 1968 shows how significant was the contribution of Polish scientists to the world neuropathology. This is, however, the history of the life and work of scientists.

On the other hand, the history of neuropathology as a separate speciality cultivated by special institutions has begun and has been developing in Peolpe's Poland. This development has been furthered by the Polish Academy of Sciences and particularly by Secretaries of the Committee and then of the Section of Medical Sciences: professors Ludwik Paszkiewicz, Witold Zawadowski, Andrzej Biernacki. During their tenures the foundations of the institutions of this newly-isolated discipline have been laid: Department of Neuropathology of the Polish Academy of Sciences (called formerly Department of Histopathology of the Nervous System) has been founded in 1954 (its name was changed in 1959), the periodical Neuropatologia Polska was founded in 1963 as a paper of this Department, Society of Polish Neuropathologists was organized in 1964 — this society was founded by a group of enthusiasts of neuropathology participating in the scientific sessions of Department of Neuropathology and it is working under the auspices of the Polish Academy of Sciences.

There is, obviously, a close connection between the history of Polish neuropathologists and the new history of neuropathological institutions. If these scientific traditions had not been existing and especially if in 1954 professor Adam Opalski had not been in Warsaw, the Polish Academy of Sciences would have no ground to organize the new institution which began first the training of future neuropathologists and then scientific research work in this officially new speciality.

It would be wrong to claim that no research work in neuropathology had been conducted in Poland prior to the organization of the Department of Neuropathology, Polish Academy of Sciences. Laboratories of histology existed at the Institute of Psychoneurology and at numerous departments of neurology of Medical Academies in Kraków, Wrocław and — in the first place — in Warsaw. Department of Neuropathology was developed from laboratory of neurohistology in Department of Neurology, Medical Academy in Warsaw. These laboratories had been organized and worked because certain clinicians showed a more or less deep interest in neuropathology, some of these clinicians were heads of departments (as it was the case with professor Adam Opalski) others were neurologists working in these departments. Histopathology of the nervous system was then the object of studies of neurologists, neurosurgeons and, less frequently, pathologists who treated them more or less as a hobby. After organization of Department of Neuropathology research work in this field has become the main and basic object of interest of these scientists.

The Warsaw institution existing in the time — period from 1954 to 1967 was headed by Adam Opalski (1954—1958), Adam Kunicki 1959—1962), Ewa Osetowska (1962—1967). In the second half of the year 1967 Department of Neuropathology has been incorporated (losing its former name) into the Center of Experimental and Clinical Medicine, Polish Academy of Sciences. Up to this time over 250 papers had been published by the staff of the Department, 23 doctor's degrees and 5 docents degrees have been conferred.

Already in 1957 a Laboratory of Neurosurgical Pathology, Polish Academy of Sciences has been called into existence in Kraków, initially it was subordinated to the Department of Neuropathology in Warsaw and then it has become an independent institution headed by professor Adam Kunicki.

In the years of the activity of Department of Neuropathology in Warsaw the laboratories of various Medical Academies have been developing, either independently of the Warsaw institution or with its help in the form of training courses and work on the preparation of papers to be presented for the degree of a doctor or a docent. First, when the Department of Neuropathology had moved from the Department of Neurology, Medical Academy to No 3 Pasteur Street in Warsaw, the Laboratory of Neuropathology of the latter Department began its independent development under the direction of Lech Iwanowski M.D., Danuta Markiewicz M.D. after a training period in Department of Neuropathology has founded a Laboratory of Neuropathology in the

Abramowice Psychiatric Hospital in Lublin. The greater part of his paper for the doctor's degree was prepared by Jerzy Kulczycki M.D. in the Department of Neuropathology in Warsaw. Dr. Kulczycki was appointed then head of Laboratory of Neuropathology at the Department of Neurology, Pomeranian Medical Academy in Szczecin. Research work for the degree of a doctor and then of a docent was done in this Department by Jerzy Dymecki head of the Laboratory of Neuropathology, Institute of Psychoneurology in Pruszków.

Independently of Department of Neuropathology, but perhaps following somewhat its example the Laboratory of Neuropathology has been developing at the Medical Academy in Łódź, headed by docent A. Głuszcz M.D. Similarly independetly of the Warsaw Department a large Laboratory of Neuropathology has been developing at Department of Neurology, Medical Academy in Poznań. The Warsaw institution is continuing further training courses for heads of the neuropathological laboratories of the Silesian Medical Academy (Stefan Kasperek M.D.) and Department of Neurosurgery in Lublin (dr. Zofia Muszyńska).

Members of the Society of Polish Neuropathologists come from these recently founded or former centers. The greatest group among them is formed by neuropathologists from the Warsaw and Kraków neuropathological centers under the auspices of the Polish Academy of Sciences. They have been the first Polish "full-time" neuropatologists for whom neuropathology has become the only medical speciality. The Society supported by the Polish Academy of Science pays systematically the fares of members coming every month for sessions of the Society. The number of neuropathologists members of the Society exceeds now 50.

The development of Polish neuropathology is evidenced not only by the activities of the Society of Polish Neuropathologists but also by the periodical Neuropatologia Polska which has been founded as an organ of only one neuropathological center (it was founded before the official foundation of the Society) but it is publishing now papers on neuropathology from the whole country. It is an evidence of the widespread scientific movement which has reached all greater medical centers in Poland, all neurological departments and divisions of neurology, neurosurgery and psychiatry. Trainees from the provincial towns coming for short-term or long-term training courses to the Warsaw or Kraków centers or to other greater laboratories connected with Departments of Neurology or Institutes either attempt afterwards to set up own laboratories or remain in contact with the training center

sending material or participating as partners in the work on sporadic cases or more extensive studies done for the doctor's degree.

The development of neuropathology in Poland is correlated with the place occupied by the Polish neuropathology in the world. At the IV Congress of Neuropathology in Munich in 1961 the Polish neuropathology was represented still by the Polish Neurological Society and only one paper on neuropathology was read by the Polish participants. In 1965 at the V Congress of Neuropathology in Zürich the Polish neuropathologists had already a delegate of the Polish Society of Neuropathology who was also the so-called country vice-president of the Congress and five papers were read by the Polish delegation. At the VI Congress of Neuropathology to be held in Paris in 1970 the Polish delegate is chairman of one of four main problem sections of the Congress.

It has been stressed in the introduction that this enormous development of Polish neuropathology is a continuation of the tradition of great individual achievements in this discipline. It is doubtlessly a great merit of the organizators of scientific research in People's Poland that these traditions have not only been continued but provided with a plan for further development of this speciality based on organizational and other means.

E. Osetowska

HISTOCHEMISTRY OF MYELIN AND NEUROGLIA

NEUROPAT. POL. 1969, VII, 3

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LIPID-PROTEIN RELATIONSHIPS IN NORMAL AND DEGENERATING MYELIN *)

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We propose to discuss myelin and demyelination from the following aspects:

a) The proteins of the myelin sheath
b) The *in vitro* release of lipids engendered by exposure of myelin to proteolytic enzymes
c) Proteolytic activity in Wallerian degeneration and around plaques of multiple sclerosis
d) The location of certain proteolytic enzymes within myelin.

The hypothesis to be considered is that proteolytic enzymes are responsible for the initial breakdown of the myelin sheath both in demyelinating conditions and Wallerian degeneration.

A. PROTEINS OF MYELIN SHEATH

By histochemical techniques two protein-types can be distinguished within the normal myelin sheath. The first sort is akin to proteolipid--protein and neurokeratin (Folch and Lees 1951, LeBaron and Folch 1956) and can be displayed histochemically by its strong reaction with the DMAB technique for tryptophane (Fig. 2; Adams 1957, 1958). In conformity with biochemical observations, this protein cannot be removed from the section by either trypsin or dilute sulphuric acid (Adams and Bayliss 1968a). The other protein resembles the various basic myelin proteins that have been isolated and characterized by several groups of biochemists (e.g. Lowden et al. 1966, Lumsden et al. 1966, Martenson and LeBaron 1966, Nakao et all. 1966). Histochemically, this basic protein component can be demonstrated by its affinity for

*) Supported by the Multiple Sclerosis Society (U.K.).

the acidic dye trypan blue (Fig. 3; Adams and Bayliss 1968a). This trypanophilia is extinguished by treatment with cold nitrous acid, indicating that protein amino-groups are responsible for such staining. In accord with the above-mentioned biochemical studies, the basic protein is digested by trypsin and can be extracted from the section with dilute sulphuric acid.

B. *IN VITRO* RELEASE OF MYELIN LIPIDS BY PROTEOLYTIC ENZYMES

Myelin in sections of formol-calcium-fixed tissue is relatively resistant to trypsin and other proteolytic enzymes: only a small part of the myelin lipids appear to be released (Fig. 1; Adams and Bayliss 1968 b). However, digestion of the basic protein appears to unmask acidic phospholipid groups (Fig. 1), because the sheath is then more readily stained by basic dyes and other cationic substances. This unmasking of phospholipids is more pronounced after extraction of fixed myelin with acetone, an effect that can be attributed to the selective removal of cholesterol previously hydrogenbonded to phospholipids (Fig. 1; Adams and Bayliss 1968 b, c).

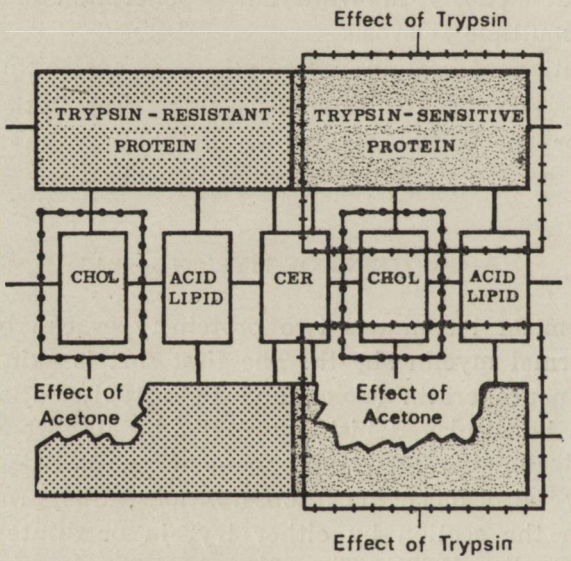

Fig. 1. Myelin molecular structure.

In unfixed sections of brain or peripheral nerve, most of the lipids are released from myelin by the action of trypsin, pepsin or

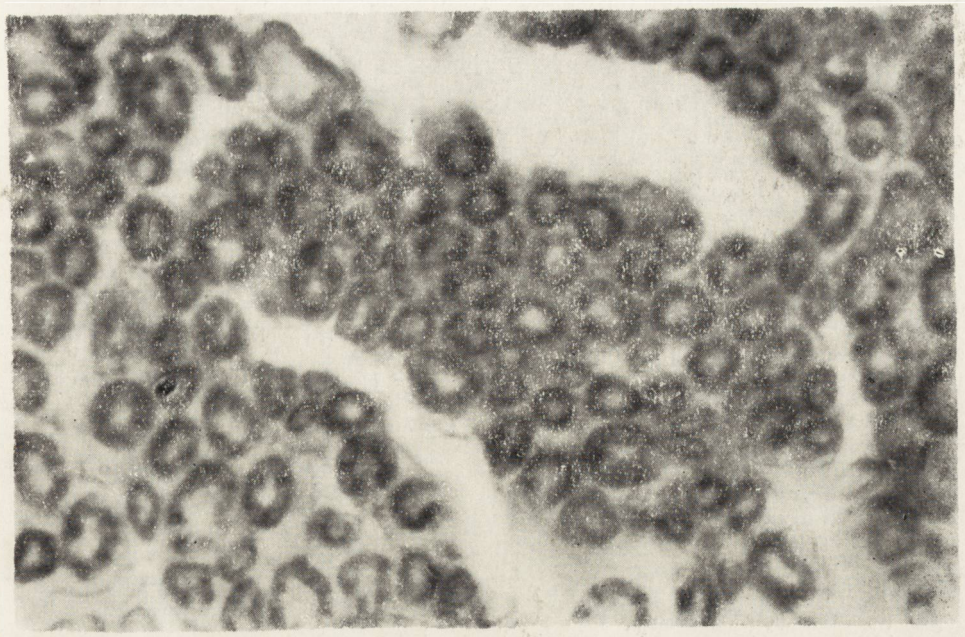

Fig. 2. Tryptophan in neurokeratin of rat peripheral nerve myelin. DMAB method, × 2260.

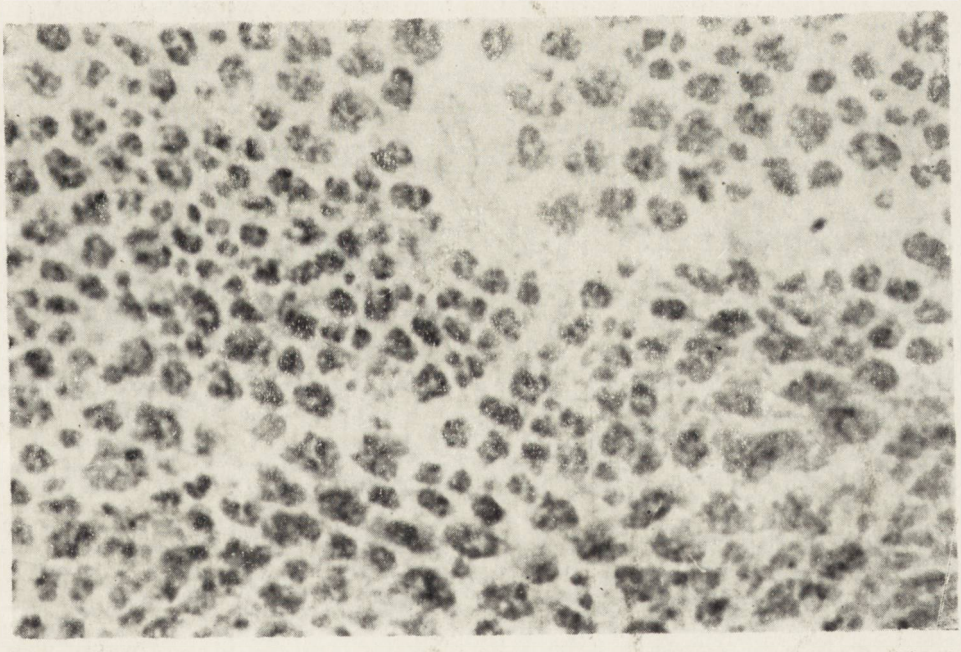

Fig. 3. Basic protein in rat peripheral nerve myelin. Trypan blue, pH 5, × 960.

Fig. 4. Effect of dilute trypsin applied to white matter of human brain, cryostat section post-stained with Sudan black, × 7. Note gross loss of lipid from trypsin-digested white matter.

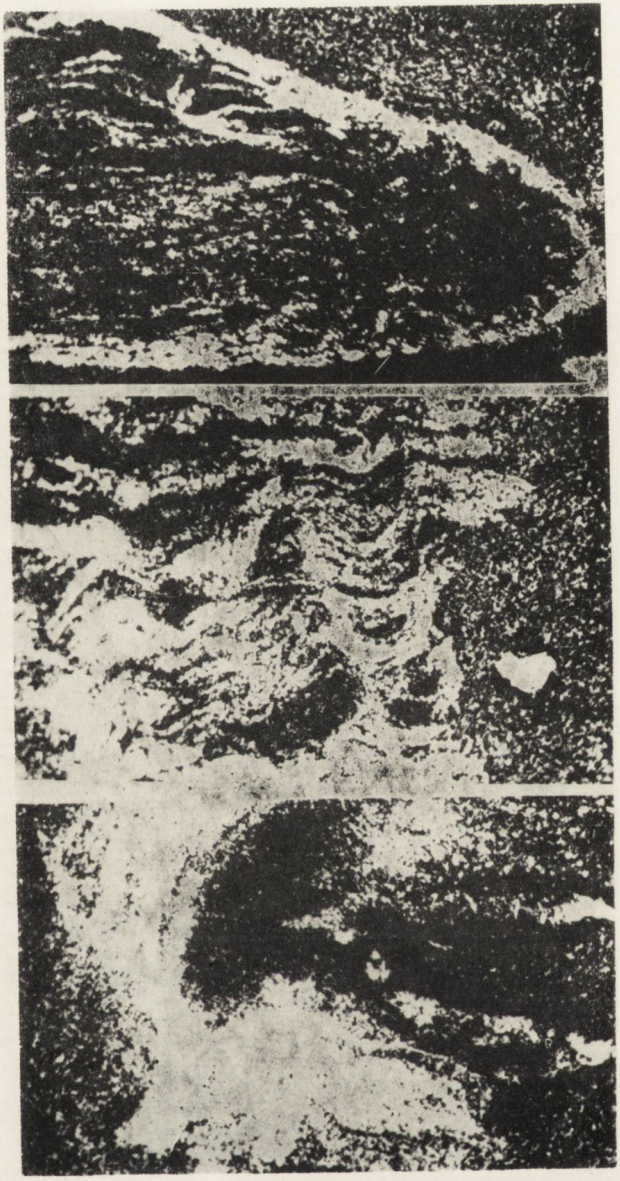

Fig. 5. Increased proteolytic activity (translucency) during myelin breakdown in rat sciatic nerve. Top, normal nerve; middle 4 days after nerve section; bottom, 6 days after nerve section. Gelatin-silver autogram, × 96.

Fig. 6. Increased proteolytic activity at the edge of an active plaque of multiple sclerosis. Demyelinated centre of plaque at top. Gelatin-silver autogram, × 550.

elastase (Fig. 4; Wolman and Hestrin-Lerner 1960, Tuqan and Adams 1961, Adams and Bayliss 1968 b). Presumbaly a large part of these lipids is attached to the trypsin-sensitive basic protein, and digestion of this protein removes the molecular skeleton to which these lipids are partly attached and on which they are stabilized.

C. PROTEOLYTIC ACTIVITY IN MYELIN BREAKDOWN

The question that now arises is whether the *in vitro* observations presented in the preceding paragraph have any relevance to myelin breakdown *in vivo*. If proteolysis is responsible for the release of myelin lipids in the initial stages of the sheath's breakdown, a ready explanation becomes available for Rossiter's observation (Johnson et al. 1950) that the myelin lipids remain chemically normal for the first week after nerve-section-even though the sheath and its constituent lipids physically fragment and disrupt during this initial period of Wallerian degeneration.

In fact, proteolytic activity does increase in this initial stage of myelin breakdown. Porcellati and Curti (1960) showed that such acivity is increased at the end of the first week after nerve-section but, by relatively crude biochemical techniques, our previous work showed that it increases within the first few days after neurotomy (Adams and Tuqan 1961a). Our biochemical observation were supported by the silver-gelatin film technique (Adams and Tuqan 1961 b), where sections are mounted on blackened photographic plate and where proteolytic activity is revealed as a translucency in the film. This technique showed a very substantial increase in neutral proteolytic activity during the first week of myelin breakdown (Fig. 5; Adams and Tuqan 1961 a). Furthermore sophistocated biochemical and histochemical studies on this problem are reported in the following paper (Hallpike and Adams 1969).

During the last year we have been able to obtain 5 brains from cases of multiple sclerosis. The active plaques in these cases showed markedly increased acidic and neutral proteolytic activity around them, as revealed by the gelatin-silver film technique (Fig. 6). Activity was adjudged on the basis proposed by Ibrahim and Adams (1963), namely that the neuroglial (oligodendroglial) population and oxidative enzyme activity are increased at the edge of such lesions. Moreover, the presence of Marchi-positive (OTAN black) lipids (cholesterol esters) within gitter-cells indicates the presence of active demyelination.

From the foregoing observations, it is clear that proteolytic activity increases in the early stages of the process of myelin breakdown.

We infer that such activity is responsible for the initial fragmentation of the myelin sheath, at a time when the myelin lipids are chemically normal and have not yet been degraded to cholesterol esters (for general reviews, see Adams et al. 1965, Adams and Leibowitz 1969).

In the next paper and in future work we propose to consider the origin of these proteolytic enzymes that are involved in myelin breakdown. The question to be considered is whether they are derived from lysosomes, other subcellular particles or the myelin membrane itself. In the last part of this paper, we wish to discuss the localization of a proteolytic enzyme and a peptidase actually within the myelin sheath itself.

D. NEUTRAL PROTEINASE AND LEUCINE-AMINOPEPTIDASE IN MYELIN

After extracting rat peripheral nerve myelin with chloroform--methanol and washing it (Folch et al. 1957), about half of the neural neutral proteinase was recovered from the interfacial fluff (proteolipid) and chloroform phase (lipid). The other half was recovered from the aqueous phase and the unextractable residue (Table 1; Adams and

Table 1. Distribution of peripheral nerve neutral protease after „Folch-Wash"

Chloroform phase-lipid	17·2%	54·3%
Interface-proteolipid	37·1%	
Water phase + residue	45·7%	

Bayliss 1961). Enzyme activity in the lipid phase is presumably an artefact due to contamination during removal of the interfacial proteolipid fluff. These observations suggest that about half the neutral proteinase activity in peripheral nerve is associated with myelin constituents. This conclusion is in accord with Marks and Lajtha's (1963) subcellular fractionation study on brain, where they found that a substantial amount of cerebral neutral proteinase activity was present in the myelin subfraction of the mitochondrial fraction.

Leucine aminopeptidase (LAP; L-leucyl-β-naphthyl-amidase) activity is more concentrated in white matter than grey; it is present in rat myelinated peripheral nerves, but absent from unmyelinated leg nerves of the lobster (Table 2; Adams and Glenner 1962). Moreover, in contrast to various respiratory enzymes, LAP activity in the subcellular myelin fraction of rat brain accounts for as much as 17 per cent of that in whole brain (Table 3; Adams et al. 1963). Myelin fractions obtained from peripheral nerves — a new technique that involves softening of collagen with 0.7 M glycine — contain betwen 63 and 100 per cent

of total nerve LAP activity (Table 4; Adams et al. 1968). It is not really surprising that the compacted cell-surface (Schwann or oligodendroglial) membranes of myelin should contain so much LAP, for this enzyme appears to be a characteristic component of the plasma membrane (Coleman and Finean 1966, Emmelot et al. 1968).

Table 2. L-leucyl-β-naphthyl-amidase activity in neural tissue

	Naphthylamine μg/mg wet wt/hr
Sciatic nerve, rat	1·53
Unmyelinated nerve, lobster	0·00
Optic nerve, rat	1·12
Corpus callosum, rat	2·73
Frontal cortex, rat	1·59

Table 3. Enzymic activity in myelin preparation of rat brain

	Myelin enzyme activity as % of total recovery
Succinic dehydrogenase	2·3
ATPase (total)	2·16
ATPase (Na+)	2·19
Acid phosphatase	3·2
Alk. phosphatase	2·9
G-6-P dehydrogenase	2·7
Aminopeptidase	17

Table 4. Enrichment of peripheral nerve myelin fraction compared with whole homogenate

	Whole homogenate	Human dorsal root	Rabbit sciatic
Cerebroside	1	2·20	1·58
Cholesterol	1	2·05	1·61
Succinic dehydrogenase	1	0·0013 (0·06%)*)	0·0018 (0·11%)*)
Leucine aminopeptidase	1	1·42 (64%)*)	1·63 (103%)*)

*) Enrichment compared with cerebroside

C. W. M. Adams, F. Hallpike

STOSUNEK LIPIDOWO-BIAŁKOWY W PRAWIDŁOWEJ I DEGENERUJĄCEJ MIELINIE

Streszczenie

A. Mielina zawiera dające się wykryć histochemicznie bogate w tryptofan, oporne na trawienie trypsyną białko (neurokeratyna), lipoproteidy oraz trypanofilne, wrażliwe na trypsynę białko zasadowe.

B. Trawienie trypsyną *in vitro* białka zasadowego daje w wyniku uwolnienie lipidu z mieliny.

C. Aktywność proteolityczna wzrasta we wczesnych stadiach rozpadu mieliny (degeneracja Wallera) i na brzegach świeżych plak stwardnienia rozsianego. Trawienie białka zasadowego w mielinie może wyjaśnić początkowe rozerwanie osłonki, w czasie gdy lipidy pozostają chemicznie niezmienione.

D. Obojętna proteaza oraz aminopeptydaza leucynowa wydają się być stałymi składnikami enzymatycznymi prawidłowej mieliny.

Ц. В. М. Адамс, Ф. Холлпайк

ЛИПИДНО-БЕЛКОВОЕ ОТНОШЕНИЕ В ПРАВИЛЬНОМ И ДЕГЕНЕРИРУЮЩИМ МИЭЛИНЕ

Содержание

А. Миэлин содержит гистохимически выявляемый, богатый триптофаном, резистентный на переваривание трипсином белок (нейрокератин), липопротеиды и трипанофильный чувствительный к трипсину основной белок.

Б. Переваривание трипсином *in vitro* основного белка дает в результате освобождение липида из миэлина.

В. Протеолитическая активность возрастает в ранних стадиях распадения миэлина (дегенереция Валлера) и на краях активных бляшек рассеянного склероза. Переваривание основного белка в миэлине может выяснить начальный разрыв оболочки, в то время когда либиды остаются химически неизмененные.

Г. Нейтральная протеаза и лейцин-аминопептидаза, кажется постоянные энзиматические составные правильной миэлины.

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PROTEOLYTIC ENZYMES IN MYELIN BREAKDOWN *)

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Proteins are important constituents of the myelin sheath and, according to present views (Finean, Robertson 1958) serve to maintain its radial stability by polar linkages with adjacent phospholipids. The problems to be considered are the relationship between proteolytic enzyme action and myelin breakdown and the sources of these enzymes in both experimental and human demyelinating disorders.

Histochemical observations (Wolman, Hestrin-Lerner 1960, Tuqan, Adams 1961) have shown that the effect of trypsin on unfixed myelin is the removal of a trypsin-digestible protein accompanied by lose of a lipid moiety. Proteinases have been isolated from normal brain (Ansell, Richter 1954, Guroff 1964, Marks, Lajtha 1965) and increases in endogenous protease activity in pheripheral nerve undergoing Wallerian degeneration have been found by Porcellati and Curti (1960) and by Adams and Tuqan (1961). The link between proteolysis and demyelination has also been strengthened by the finding of increased cerebral protease activity in experimental allergic encephalomyelis (Gabrielescu 1968) where some of the earliest morphological changes appear to be due to splitting of the protein layers forming the intraperiod line (Lampert 1965). Histochemical evidence of increased proteolytic activity around active plaques of multiple sclerosis has been referred to elsewhere at this Symposium (Adams and Hallpike, 1969).

A. WALLERIAN DEGENERATION

In spite of the complexity of the changes accompanying Wallerian degeneration, this model remains the most convenient way of studying changes occurring after specified periods of myelin breakdown.

*) Supported by the Multiple Sclerosis Society (U.K.).

Rats were subjected to unilateral or bilateral sciatic nerve section. The gelatin-silver autogram technique (Adams, Tuqan 1961) was used for the histochemical demonstration of protease activity at pH — 3 · 5 and 7 · 4. Biochemical determinations of proteinase activity in whole nerve homogenates were carried out at pH 7 · 4 using casein as substrate (U.V. absorption at 280 mμ) and at pH 3 · 5 using gelatin in the incubation mixture and measuring the release of ninhydrin-reacting products colorimetrically (Matthews, Muir, Baron 1964). Enzyme activities are expressed as a percentage of control nerve activity and wet weight changes in degenerating nerves allowed for by relating results to unit length of normal nerve. Acid phosphatase was demonstrated histochemically in cryostat sections, post-fixed in acetone, using β-glycerophosphate, 1. naphthylphosphate and naphthol AS. TR phosphate as substrates and biochemical estimations in whole nerve homogenate supernatants using disodium phenyl phosphate (King, Wootton 1956). L-leucyl-β-naphthyl amidase activity was measured in the homogenate supernatants (Adams, Glenner 1962).

Histochemical evidence was obtained of increases in protease and acid phosphatase activity from the second day after nerve section. These changes were well developed by the sixth day and the distribution of enzyme activities appeared comparable (Fig. 3 and 4). Biochemical results (Fig. 1) confirmed the early rise in acid and neutrally-

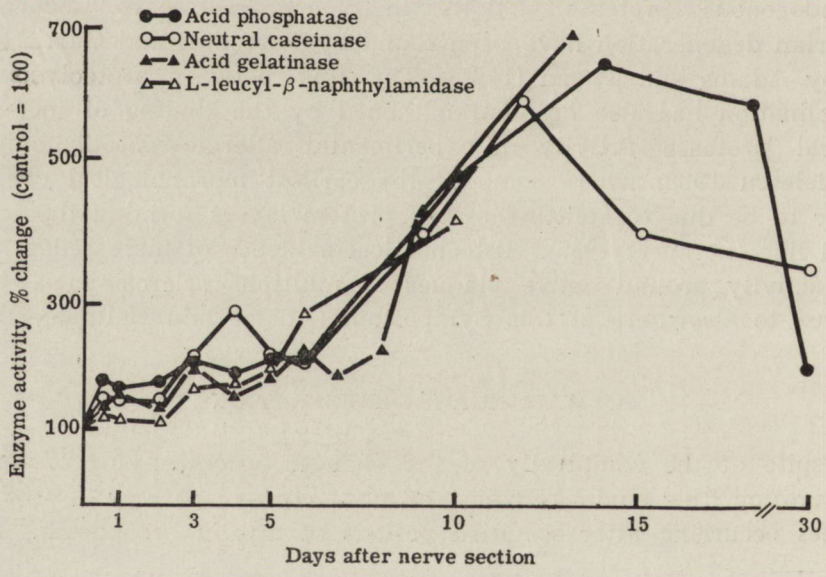

Fig. 1. Wallerian degeneration.

-acting proteinases with changes by 12 hours after nerve section. Acid phosphatase activity also increased at an early stage and the proteinases and acid phosphatase showed a second more pronounced rise after 6 days. Leucine aminopeptidase activity did not show appreciable increase until 3 days after section. The relative changes in these enzymes in the first 6 days of degeneration are shown in Fig. 2.

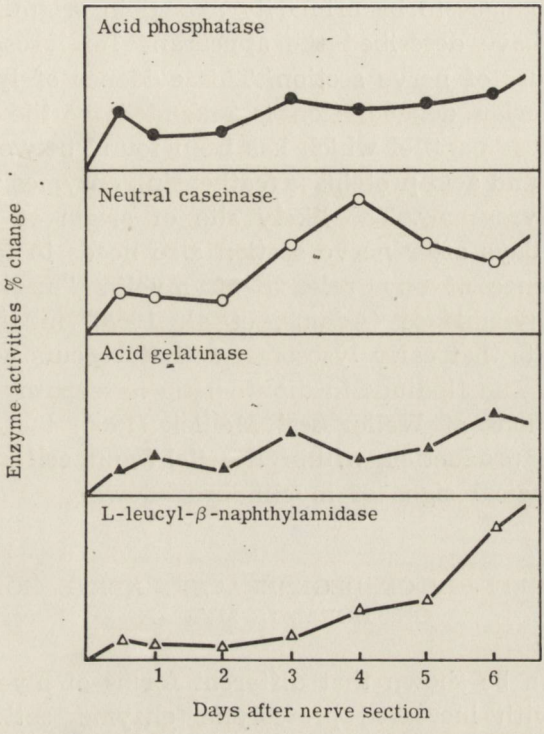

Fig. 2. Wallerian degeneration (early changes). Activity scale for each enzyme 100—300%; control = 100.

B. DIPHTHERITIC NEUROPATHY

Intraneural injection of an aqueous dilution of dialysed diphtheria toxin (Jacobs 1967) was used to produce a localised area of segmental demyelination in rat sciatic nerves. Histochemical observations were concentrated on the period between injection of the toxin and the onset of foot paralysis. Increased proteinase (Fig. 5) and acid phosphatase activity (Fig. 6) were evident within a week of injection and before myelin breakdown was advanced.

C. DISCUSSION

The histochemical findings in Wallerian degeneration and diphtheric neuropathy show that increased activity of proteinases and acid phosphatase occur at early stages of these two pathologically distinct forms of myelin breakdown. Previous workers (Gould, Holt 1961, Bubis, Wolman 1965) found histochemical evidence of acid phosphatase activity during the first week of peripheral nerve Wallerian degeneration and considered the changes to be primarily axonal in origin. Holtzman and Novikoff (1965) have described the appearance of lysosomes in axons within a few hours of nerve section. This evidence of lysosomal participation in Wallerian degeneration is extended by the present work because of the close parallel which has been found between the rises in acid phosphatase and acid proteinase (cathepsin), enzymes which are both identified with lysosomes. The likely site of origin of these enzymes in the first few days after nerve section also needs to take account of the virtual absence of any release of myelin "marking" enzyme, L-leucyl-β-naphthylamidase (Adams, et al. 1968), in the first three days. This suggests that early lysosomal activity occurs within the axon or Schwann cell. The findings in diphtheritic neuropathy are consistent with the conclusions of Weller and Mellick (1966) that lysosome and acid phosphatase production within the Schwann cell accompany the earliest morphological signs of myelin breakdown.

D. *IN-VITRO* EFFECT OF DEGENERATING NERVE HOMOGENATE ON NORMAL MYELIN

Although it can be shown that different forms of myelin breakdown are associated with increased proteolytic enzyme activity a causal relationship between the two processes is difficult to establish and it was therefore decided to investigate the effect of enzymes released during Wallerian degeneration on intact myelin.

Distal portions of rat sciatic nerves, ten days after section, were homogenized by hand in ice in O,05 M sodium acetate buffer at pH 3 · 5 (25 mg. nerve/ml). Supernatants were prepared from degenerating and control nerves by centrifuging at 3000 r.p.m. for 20 min. and kept at 0°C until used on the same day. Unfixed cryostat sections of normal rat brain and sciatic nerve were incubated at 37°C with supernatants from degenerating and control nerves and the buffer alone. Sections were examined under polarized light at intervals and also stained with 0·1% trypan blue (pH 5·0).

The effect of degenerating nerve homogenate-supernatants in reducing peripheral nerve trypanophilia is shown in Fig. 7. Comparable effects were observed in brain white matter after shorter incubation periods. Evidence of physical disintegration of myelin was provided by the rapid appearance of large numbers of myelin buds with birefringent properties under polarized light (Fig. 8) in supernatant-treated sections. The provisional conclusions drawn from this experiment are that some of the effects of trypsin on unfixed myelin can be reproduced by degenerating nerve homogenate-supernatants to a greater extent than by supernatants derived from normal nerve or by buffer alone. These supernatants are known to be rich in proteolytic enzymes and demonstration of their ability to damage normal myelin would clearly be pertinent to any causal role in demyelination. Myelin bud formation has been recognized (Leathes 1925, Wolman 1965) as a reliable guide to myelin breakdown but critical factors are known to include the nature and concentration of certain cations in the medium. A more detailed account of this work (Hallpike and Adams, to be published) will include fuller mention of these aspects and the results of enzyme inhibition studies.

E. CONCLUSION

There is now sufficient evidence to associate the activity of proteolytic enzymes with myelin breakdown. In Wallerian degeneration and in diphtheritic neuropathy increased enzyme activity which seemed to be wholly or partly lysosomal in origin was observed and in the former the earliest enzymic changes seemed to take place in the axon or Schwann cell rather than the myelin sheath. Further work using enzyme "markers" for certain organelles and combining quantitative studies with subcellular fractionation should add to our knowledge of the source of digestive enzymes in myelin breakdown — whether they are derived from lysosomes, myelin membrane or some other subcellular particle. Ideas thus obtained may well find application to the wider problems of the events leading upto demyelination and of human demyelinating disease.

J. F. Hallpike, C. W. M. Adams

ENZYMY PROTEOLITYCZNE A ROZPAD MIELINY

Streszczenie

1. Przedstawia się pokrótce dane łączące proteolizę z rozpadem mieliny.

2. Wrost aktywności enzymów proteolitycznych oraz kwaśnej fosfatazy pojawił się w zwyrodnieniu Wallera w 12 godzin po przecięciu nerwu poprzedzając

przypuszczalne uwolnienie z mieliny L-leucyl-β-naftylamidazy.

3. Wzrost aktywności enzymów proteolitycznych oraz kwaśnej fosfatazy były badane histochemicznie we wczesnych stadiach segmentowej demielinizacji.

4. Niektóre z efektów działania trypsyny na nieutrawaloną, prawidłową mielinę mogą być powtórzone przez homogenaty z wyrodniejących nerwów obwodowych.

5. Dyskutuje się znaczenie niniejszych badań dla wyjaśnienia przypuszczalnej roli lizozomów w procesie rozpadu mieliny.

Ф. Холлпайк, Ц. В. М. Адамс

ПРОТЕОЛИТИЧЕСКИЕ ФЕРМЕНТЫ В РАЗЛОЖЕНИИ МИЭЛИНА

Содержание

1. Кратким образом излагаются данные соединяющее протеолиз с разложением миэлина.
2. Увеличение активности протеолитических ферментов и кислой фосфатазы появились в валлеровском вырождении в 12 часов после перереза нерва предшествуя вероятное уволение из миэлина L-лейцин-β-нафтиламидазы.
3. Увеличение активности протеолитических ферментов и кислой фосфатазы исследовались гистохимическими методами в ранних стадиях сегментной демиэлинизации.
4. Некоторые эффекты действия трипсина на нефиксированный, нормальный миэлин можно повторить действием гомогенатов из дегенерирующих периферических нервов.
5. Обсуждается значение проведенных исследований для понимания предполагаемой роли лизозомов в процессе разложения миэлина.

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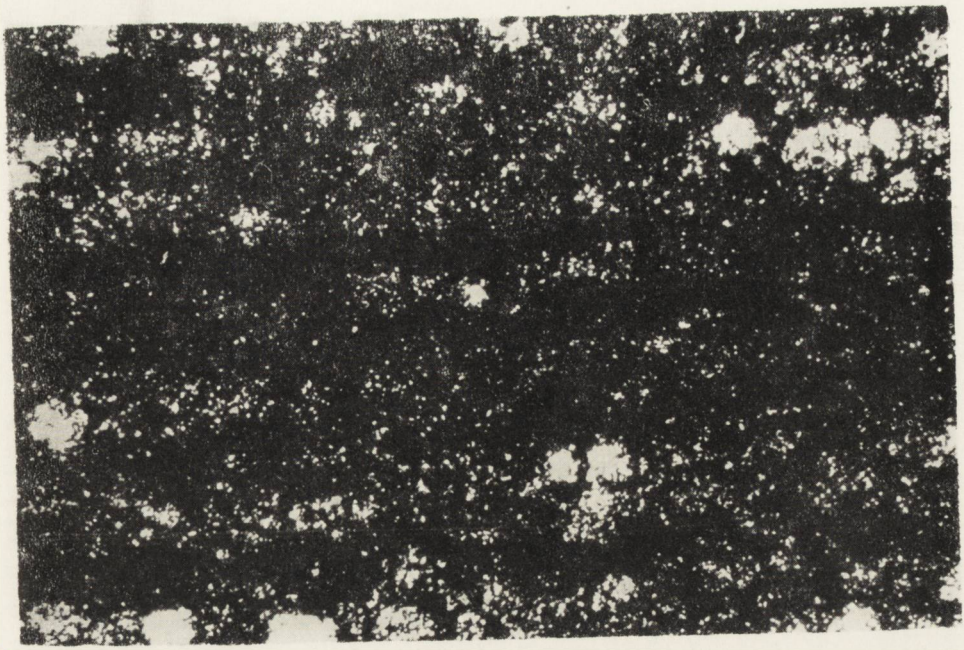

Fig. 3. Protease activity in Wallerian degeneration, 8 days. Gelatin-silver method, pH 3·5 × 620.

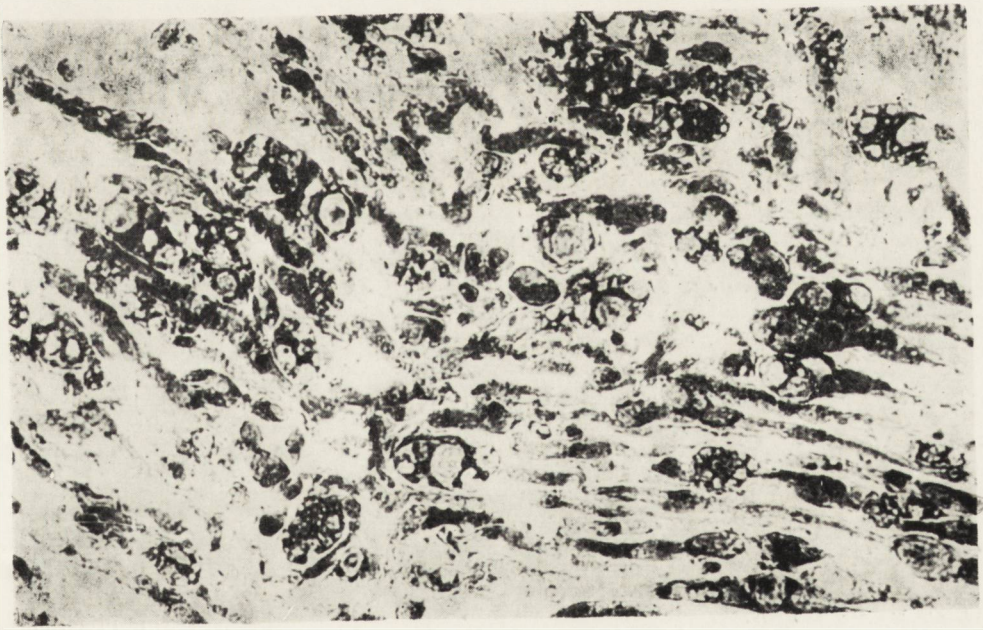

Fig. 4. Acid phosphatase activity, Wallerian degeneration, 8 days. Gomori, × 620.

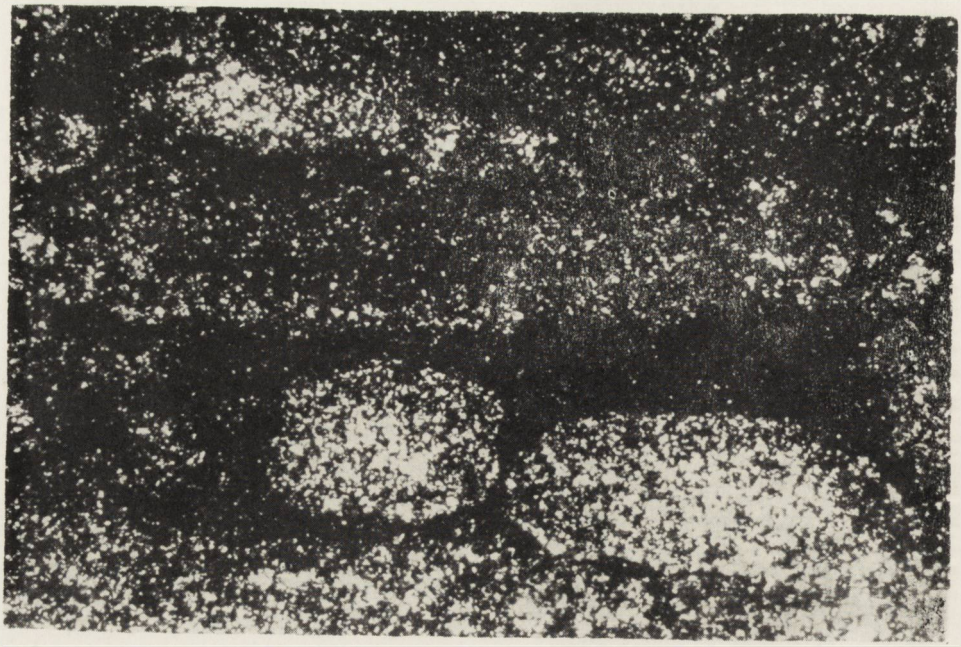

Fig. 5. Protease activity one week after injection of diphtheria toxin. Gelatin-silver method, pH 3·5, × 940.

Fig. 6. Acid phosphatase activity one week after diphtheria toxin injection. 1-Naphthylphosphate/GBC, × 940.

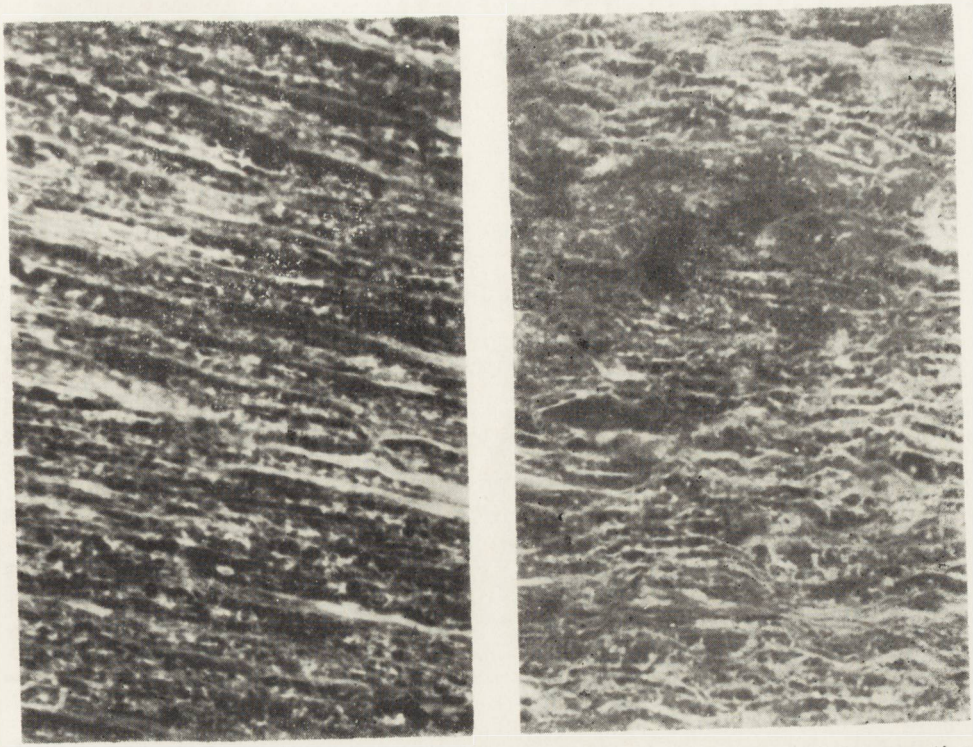

Fig. 7. Peripheral nerve trypanophilia after buffer (left) and buffered degenerating nerve homogenate supernatant (right). Myelin changes and reduced trypanophilia on right, × 620.

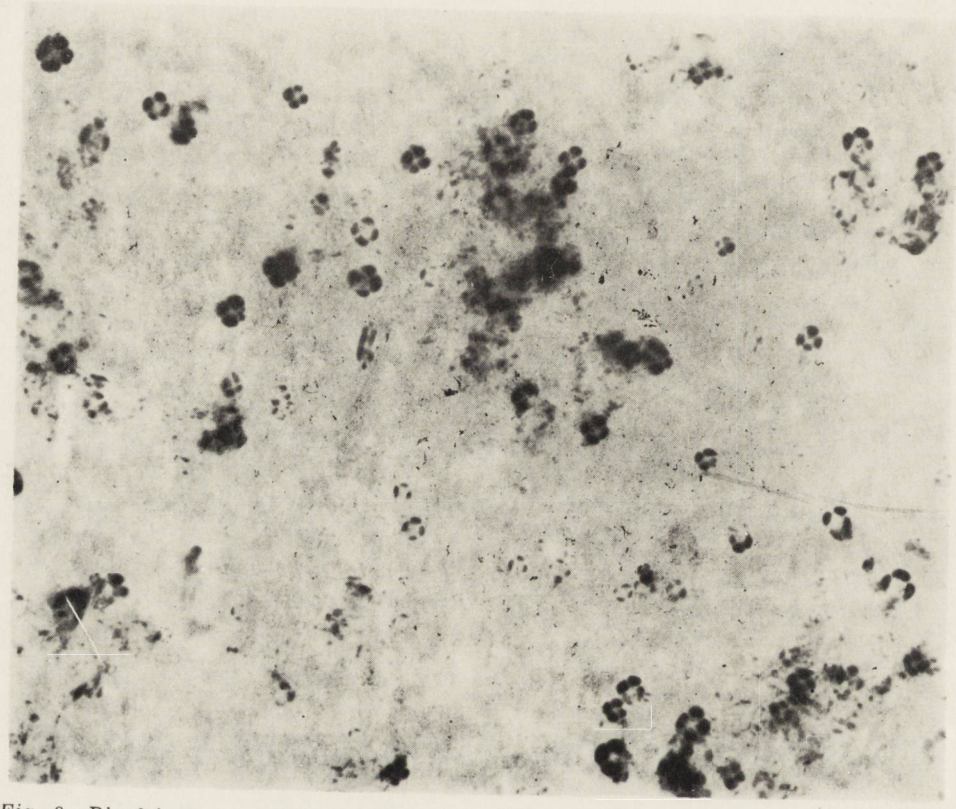

Fig. 8. Birefringent myelin buds in brain treated with degenerating nerve homogenate supernatant. Polarised light, × 620.

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I. N. ULYBINA

HISTOCHEMICAL DEMONSTRATION OF PHOSPHOLIPIDS IN THE NERVOUS TISSUE

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Histochemical methods for demonstration of tissue phospholipids, including the phospholipids of nervous tissue have a number of essential shortcomings, and results obtained with their help are difficult to interpret (Bourgeois, Hack 1962). None of the existing methods of demonstration of phospholipids guarantees even relative specificity for phospholipids. Moreover not one of them is reliable as regards quantitative preservation of the content and subcellular distribution of phospholipids in the tissue.

Many stages of tissue preparation for histochemical study have no satisfactory chemical explanation both qualitatively and quantitatively. Further development of histochemical methods of study of tissue phospholipids can be based only on a more detailed knowledge of the chemical processes taking place in tissue under the influence of various reagents.

Histochemical demonstration of phospholipids both by light and electron microscopy is connected with the necessity of using organic solvents for the treatment of tissue mainly alcohols which extract phospholipids from tissue (Morgan, Huber 1967).

According to Curri (1965) xylene or toluene used for embedding of tissue sections in paraffin and for the following deparaffination, also remove from the tissue considerable amounts of phospholipids. To prevent the loss of lipids from the tissue such fixatives have been used which stabilize lipids in the tissues. In experiments with freezing sections mostly Baker's fixative (10% neutral formol with 1% calcium chloride) is used. Most probably calcium ions induce complex formation of phospholipids with proteins and other substances, thus preventing diffusion of phospholipids into the fixative solution or into other solutions in the subsequent treatment of tissue (Pearse 1960). The process of chromation that follows the fixation is used in a number

of methods for demonstration of phospholipids and is based on the fact that phospholipids combine specifically with chromium salts; it is supposed that this process reduces the possibility of phospholipids being extracted by organic solvents (Elftman 1954). In cases when it is necessary to embed the tissue in paraffin, besides longer post-chromation, fixatives containing chromium are also used such as: formol-saturated Reinecke salt, Lewitsky, Zenker, Helly and other fixatives, which from the very beginning play an active role in forming difficultly soluble complexes of phospholipids with chromium salts. It is quite possible that each stage of treatment of tissue can lead to quantitative and qualitative changes of phospholipid content in the structures under study (Brante 1949, Heslinga, Deierkauf 1961, Pulido 1966).

The aim of this work was to estimate the changes in the content of phospholipids which take place in the nervous tissue at different stages of its preparation for histochemical demonstration of phospholipids. We also wanted to find out whether this procedure influences the phospholipid content in the tissues or their extractability by organic solvents. With this purpose in view we carried out the chemical analysis of the content of the lipid phosphorus in the nervous tissue at various stages of routine treatment for histochemical demonstration of phospholipids.

MATERIAL AND METHODS

The cerebellum of white Wistar rats was used. Half of the cerebellum weighing 100—120 mg was taken for each sample since it is aproximately the volume of the tissue sections usually taken for histochemical treatment. Extraction of lipids both from intact tissue and from that preliminarilly treated with various reagents was carried out after Folch et al. (1957); lipid phosphorus was determined after mineralization according to the method of Fiske-Subbarow. The content of lipid phosphorus in fresh tissue expressed in μg per gram of tissue was taken as 100% (control). In all other series of determinations tissue samples were either fixed by different methods used in histochemical demonstration of phospholipids or treated by the reagents used at various stages of histological procedure for embedding in paraffin: alcohols, xylene and its mixture, on fresh and fixed tissue. At each stage the amount of extracted lipid phosphorus was determined. Its content was also measured at different stages of tissue treatment by the Baker test after fixation and chromation for different periods

at room temperature and at 60°. We studied the effect of pyridine which is usually used with the purpose of control of the specificity of Baker's method. Fresh tissue as well as the tissue after fixation and during embedding in paraffin was extracted with pyridine.

RESULTS

The effect of various fixatives on the content of phospholipids is similar and the lipid phosphorus extracted by a chloroform-methanol mixture from the fixed tissue accounts for approximately 80—90% of the control (Fig. 1). Neither Baker's fixative, considered to be the best

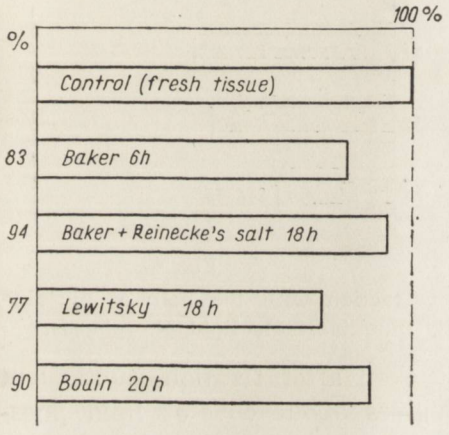

Fig. 1

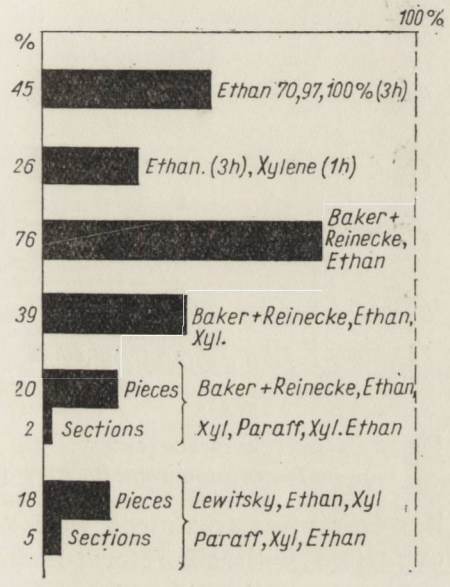

Fig. 2

Fig. 1. Effect of fixation on quantity of lipid phosphorus extracted.
Fig. 2. Lipid phosphorus extractable after histological treatment.

for phospholipids, nor fixatives containing chromium differ in this respect from the standard histological fixative — Bouin solution. It would seem that in the course of fixation a part of the phospholipids loses its extractability by the chloroform-methanol mixture.

If fresh tissue is placed in alcohols of increasing concentration for 3 hrs a high loss of lipid phosphorus is observed (chloroform-methanol extracts 45%) (Fig. 2). When the tissue treated with alcohol is subjected

to a mixture of equal amounts of 100% alcohol with xylene and to the effect of xylene only, then from the minced tissue only 26% of the lipid phosphorus content of the control can be extracted. In this series of experiments when the tissue was initially fixed by formol with calcium chloride or by the same mixture saturated with Reinecke salt, 3-hr treatment by the alcohols led to a smaller loss of phospholipids. It is probable that fixation plays a stabilizing role in this case.

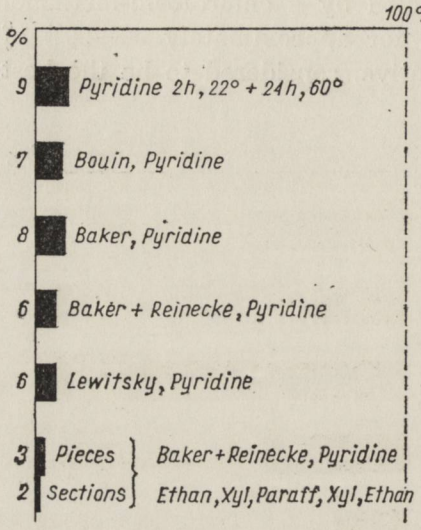

Fig. 3

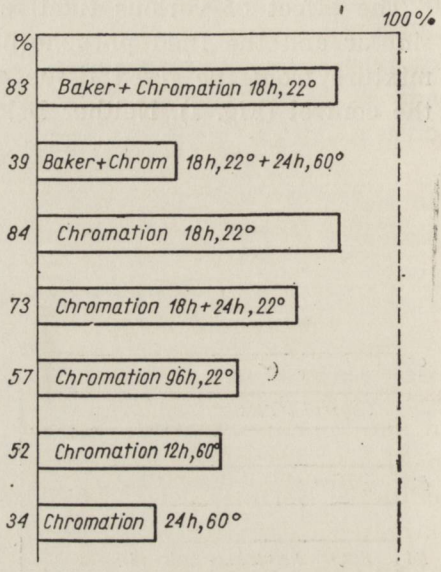

Fig. 4

Fig. 3. Lipid phosporus extractable after extraction with the help of pyridine.
Fig. 4. Effect of chromation on quantity of extractable lipid phosphorus.

However after treatment with xylene even after fixation, subsequent extraction with chloroform-methanol gives about 40% of lipid phosphorus from the minced tissue. After complete histological treatment with embedding in paraffin and releasing the sections from paraffin, about 20% of lipid phosphorus extracted with chloroform-methanol, remains in the tissue. In the experiments when releasing from paraffin with xylene and treatment in alcohols of decreasing concentration took place in sections of 10 μ only as little as 2—5% of lipid phosphorus, of its amount in the fresh tissue, can be extracted from them (as in standard histological investigations). Thus, a great difference is observed in the content of lipid phosphorus depending on whether the pieces or the sections of the tissue were treated with xylene and alcohols.

Extraction of fresh tissue with pyridine gives rise to approximately the same results as extraction of tissue fixed by different mixtures,

nonextracted lipid phosphorus accounts for 5—9% in all cases (Fig. 3). The complete histological treatment with embedding into paraffin and removal from it results in still greater losses of lipid phosphorus (the remainder forms only 2—3% of the initial quantity).

The amount of lipid phosphorus determined in brain tissue treated by Baker's test for histochemical demonstration of phospholipids accounts for about 40% of its content in intact tissue. The process of chromation at high temperature proceeds faster than at room temperature (Fig. 4). Apparently in this process temperature plays a more essential part than time, since even long chromation (96 hrs at room temperature) does not give such an effect as does chromation for 24 hrs at 60°.

It was of interest to elucidate at which stages of the Baker acid haematein test, such a considerable loss of lipid phosphorus takes place. The loss at fixation with formal with calcium chloride was 17%. Investigation of the fixing mixture and dichromate potassium solution used for the chromation showed the absence of lipid phosphorus in them. To achieve a more complete extraction of phospholipids from chromated tissue, that is to extract phospholipids firmly bound to protein, we used an additional extraction with hot neutral and then acidic chloroform--methanol (20 : 100 : 1). On hot extraction from chromated tissue an additional 6% of lipid phosphorus was extracted and the same after extraction with the acidic mixture of chloroform-methanol. Thus, it became clear that about 30% phosphorus bound to lipid remains in the tissue in the form of a stable complex with chromium salts and that this complex is extractable neither by a neutral cold or hot, nor an acidic chloroform-methanol mixture. Such a stabilization of phospholipids in the tissue by means of its treatment with chromium containing reagents is a chemcial base for Baker's, Elftman's and other methods suggested for the histochemical demonstration of phospholipids.

It was also interesting to elucidate which groups of phospholipids are stabilized in course of chromation to a greater or lesser degree. The data obtained in preliminary experiments indicate that the firmest bonds with the chromium salts as the result of the chromation are formed by phosphatidylethanolamine.

We believe that one of the lines along which neurohistochemistry should develop is the elucidation of closer correlations between the histochemical pictures in the nervous tissue and their biochemical bases, in order to substantiate the so far empirical demonstration of phospholipids in various structures of the nervous tissue.

I. M. Ulybina

NIEKTÓRE ZAGADNIENIA HISTOCHEMICZNEGO WYKRYWANIA FOSFOLIPIDÓW W TKANCE NERWOWEJ

Streszczenie

W czasie utrwalania, chromowania oraz histologicznego przygotowania tkanki dla histochemicznego wykazania fosfolipidów, spada znacznie zawartość fosforu w lipidach, który jest ekstrahowany mieszaniną chloroform-metanol (2 : 1).

Przeprowadzono badania chemiczne nad zawartością fosforu lipidowego w móżdżku szczurów po zadziałaniu na tkanki odczynnikami na różnych etapach metod służących wykryciu fosfolipidów.

Po utrwaleniu tkanka traciła 80—90% fosforu lipidowego w porównaniu z tkanką świeżą. Działanie na świeżą tkankę etanolu i ksylenu spowodowało spadek ilości fosforu lipidowego do 26%. Jednakowoż, uprzednie utrwalenie tkanki prowadzi do mniejszych strat fosfolipidów w etanolu i ksylenie. W czasie chromowania wg Bakera, około 30% fosforu związanego z lipidami pozostaje w tkance w trwałym związku z solami chromu. Kompleks ten nie podlega ekstrakcji ani obojętną ani zakwaszoną, ani też gorącą mieszaniną chloroformu z metanolem.

Stabilizacja fosfolipidów przez zadziałanie na tkankę odczynnikami zawierającymi chrom stanowi podstawę szeregu histochemicznych metod wykrycia fosfolipidów.

И. Н. Улыбиной

НЕКОТОРЫЕ ВОПРОСЫ ГИСТОХИМИЧЕСКОГО ВЫЯВЛЕНИЯ ФОСФОЛИПИДОВ В НЕРВНОЙ ТКАНИ

Содержание

При фиксации, хромировании и в процессе гистологической обработки ткани для гистохимического выявления фосфолипидов значительно снижается количество липидного фосфора, экстрагируемого смесью хлороформ-метанола (2 : 1).

Производилось химическое определение липидного фосфора в навесках мозжечка крыс после обработки ткани реактивами на различных этапах методов выявления фосфолипидов.

После фиксации из ткани извлекалось 80—90% липидного фосфора от содержания его в свежей ткани. Обработка свежей ткани спиртом и ксилолом вызывала снижение количества липидного фосфора до 26%. Однако предварительная фиксация ткани приводит к потере меньших количеств фосфолипидов в спирте и ксилоле. При хромировании по методу Бэкера около 30% фосфора, связанного с липидами, остается в ткани в прочном соединении с солями хрома. Этот комплекс не экстрагируется нейтральной, кислой и горячей смесями хлороформ-метанола.

Стабилизация фосфолипидов путем обработки ткани хромсодержащими реактивами является химической основой ряда гистохимических методов выявления фосфолипидов.

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J. DOBBING

THE INFLUENCE OF GROWTH RETARDATION ON MYELINATION

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The purpose of the experiments to be described was to detect any long term effects in the adult brain of growth restriction during the fastest period of the brain's early growth.

METHODS

The growth rate of rats is varied during the suckling period by varying the litter size. Maximum nutritional rehabilitation of small weanlings from large litters is achieved by allowing *ad libitum* food from weanling onwards. In rats the period of the brain's fastest growth is entirely postnatal and within the suckling period. By this procedure, large litter animals are therefore having their milk supply quite moderately restricted at this time. In humans the comparable period of brain development is from about eight weeks before birth to about one year afterwards.

The growth of the brain and its ultimate adult state is assessed by using neurochemical indices of cell number, degree of myelination etc., since the techniques of quantitative histology are much too laborious at present.

It is planned to investigate some behavioural correlates in animals which have been subjected to the same experimental conditions.

FINDINGS

Severe and prolonged starvation of adult animals produces no detectable reduction in brain weight nor in the brain's detailed chemical composition. By contrast, the mild regimen described above imposed during the brain's "growth spurt" results in permanent reduction of brain weight and brain cell numbers. These reductions,

however, may not have functional significance since they are accompanied by a corresponding permanent deduction in body size.

Of more possible significance is the finding that the degree of myelination as measured by myelin lipid concentration (per gram wet weight) is found to be permanently reduced in surviving adults to an even greater extent than would be expected from the reduced brain size.

These findings are not regarded as being specifically relevant to behaviour. The role of myelin is largely unknown, and it is of course equally uncertain which alternative substances should be estimated as indices of mental function. However, this easily demonstrable model provides a tangible example of permanent restiction of one developmental process in the brain which can be achieved by comparatively mild physical stress, provided it is imposed during a vulnerable or critical period of growth. It is probable that many other components of physical brain development can be similarly affected, possible including some which have behavioural significance.

J. Dobbing

WPŁYW ZAHAMOWANIA WZROSTU NA PROCES MIELINIZACJI

Streszczenie

Ostra głodówka prowadząca do śmierci, zastosowana wobec dorosłych zwierząt nie wywiera żadnego wpływu na rozmiar czy też skład mózgu, jak i na skład mieliny. Przeciwnie niewielki stopień ograniczenia żywienia, zastosowany w czasie gwałtownego wzrostu mózgu powoduje stałe zmniejszanie się rozmiaru, liczby komórek i zawartości lipidów w mielinie, które to zmiany pozostają pomimo następczego odżywiania *ad libitum* (Dobbing, 1968).

Powody tej niemożności wyrównania niedoborów nabytych w okresie wrażliwości mózgu związanym z jego rozwojem są przedmiotem niniejszych badań.

Дж. Доббинг

ВЛИЯНИЕ ЗАМЕДЛЕНИЯ ВОЗРАСТА НА ПРОЦЕСС МИЭЛИНИЗАЦИИ

Содержание

Острое голодование ведущее к смерти взрослого животного не производит никакого влияния на размер или состав головного мозга и на состав миэлина. Противоположно небольшой степени ограничение питания примененное во время бурного возраста головного мозга вызывает постоянное падение величины, количества клеток и содержания миэлиновых липидов, при чем эти изменения остаются помимо последующего питания без ограничений. (Доббинг, 1968).

Причины ведущие к невозможности корректуры этих дефицитов приобретенных во время периода развития головного мозга являются предметом настоящей работы.

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ENZYME HISTOCHEMISTRY OF THE MYELINATION GLIOSIS

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The myelinogenesis of nervous fibres, one of the basic developmental events in the central nervous system is causatively connected with the entirety of maturation phenomena of the neuroglia cells, commonly designed as myelination gliosis (Roback, Scherer 1935; de Robertis et al. 1958; Mitrova 1963; Hillebrand 1966).

The problem of metabolic alterations in the neuroglia in course of myelination gliosis was raised by the histochemical studies of Friede (1961) and Yonezawa et al. (1962). The above mentioned process was investigated with respect to changes in the activities of some phosphatases and esterases of the neuroglia, in our three subsequent papers, using white mice (Wender and Kozik — 1968), rabbits (Wender et al. 1969) and human infants (Wender et al. 1969).

The aim of this paper is a comparison of results obtained in these different species, which should provide more information and thus allow to draw a more generalized significance of these findings.

MATERIAL AND METHODS

Studies were performed on 30 white mice, 42 rabbits and 16 cases of human infants. The material of mice was divided into 5 age-groups with 6 animals in each: 5, 10, 14, 20 and 70 days and of rabbits into 7 age-groups: foetuses in 25th day of uterine life, and 1, 3, 12, 30, 48, 120 days and 1 year. The human material comprises 16 infants 22 to 37 weeks-old, who died in first three days of life.

The brains were removed between 10 and 60 minutes after the death, and immediately thereafter fixed in 10% formol-calcium at 4°C for 18 hours. The same fixation was used for animal material.

The activities of following enzymes were studied: acid and alkaline phosphatases, thiaminepyrophosphatase, adenosinetriphosphatase, non-

-specific esterase, 5'-nucleotidase, acetylcholinesterase, non-specific cholinesterase and aryl sulphatase.

For details concerning methodology, the following references are given for the individual enzymes; acid and alkaline phosphatases (Gomori 1953); TPP-ase (Novikoff, Goldfischer 1961); ATP-ase (Wachstein, Meisel 1957); non-specific esterase (Holt 1966); 5'-nucleotidase (Scott 1965); AChE and ChE (Gerebtzoff 1953); aryl sulphatase (Rutenburg et al. — 1952). Deposition of lipids was controlled by staining with oil red O or sudan black B. The actual state of myelination and the glia cytology was studied by routine methods such as the Spielmeyer, Woelcke, Nissl, h + e, Hortega and Cahal techniques.

RESULTS

As it is not our aim to present a thourough demonstration of myelin staining and enzyme histochemistry of the myelination gliosis, which is to be found in our previous papers, we shall only discuss the observed species differences.

Acid phosphatase. As a basic observation the strong acP-activity distributed in neuroglia cells of the white matter during the myelination gliosis in all examined species (mice, rabbits, human infants) should be emphasized. In the mature brain no acP activity was observed in the neuroglia of white matter.

Alkaline phosphatase. The neuroglia of white matter in mice and rabbits lacked any alkP activity, including the myelination period. In neuroglia cells of the cerebral hemispheres in the human infants' brain a weak reaction was present, but no changes appeared in course of myelination gliosis.

Thiaminepyrophosphatase. In mice, rabbits and human infants there is a very characteristic rise in the TPP-ase activity within the cytoplasm of neuroglial cells in course of myelination gliosis (Fig. 1 and 2). The strongest activity in form of distinct lamellae and granules was revealed in the neuroglia of the myelination clusters. In adult rabbits but not in mice a part of the oligodendroglia in the white matter retains a weak intracytoplasmic TPP-ase activity.

Adenosinetriphosphatase. The immature neuroglia of the white matter in mice, rabbits and human infants displays an ATP-ase reaction, with a marked intensification of this activity during myelination (Fig. 3). Yet in the adult rabbits and mice the ATP-ase reaction in the interfascicular oligodendroglia is negative.

Non-specific esterase. The neuroglia of the white matter shows only in the immature brain of human infants a positive reaction for non-

-specific esterase. In rabbits the non-specific esterase activity is visible during the early periods of the extrauterine development in form of small spindles and granules inside the myelinating nerve fibres, but without any conspicious localization in the neuroglia cells.

5'-nucleotidase. This enzyme activity was demonstrated in the neuroglia of the cerebral white matter in course of myelination but only in rabbits. The reaction in the mature oligodendroglia was negative.

Acetylcholinesterase. The studies of AChE activity revealed distinct species differences. The mouse brain is characterized by a well defined AChE activity in the developing neuroglia during the period of myelination and by a complete absence of this activity in the neuroglia of the mature cerebral white matter (Fig. 4). In rabbits there is no AChE activity demonstrable in the neuroglia of the myelination gliosis, whereas some mature oligodendroglia cells at the late period of extrauterine development (30 and 48 day-old animals) exhibit AChE activity. In human infants only singular developing neuroglia cells of the cerebral white matter demonstrate the presence of small intracytoplasmic granules.

Cholinesterase. A positive reaction for ChE was visualized in the mouse brain in many neuroglia cells during the early period of extrauterine development (5th and 10th day of life) (Fig. 5), which than disappeared in the adult white matter. The ChE activity in the neuroglia of the rabbit's cerebral white matter instead increases during brain maturation reaching top intensification in the adult interfascicular oligodendroglia, without any preferential sharp increase during the period of myelination (Fig. 6).

In the brain of human infants only in some neuroglial cells a weakly positive reaction for ChE in the form of small granules is visible.

Aryl sulphatase. In all three examined species a clear ASS activity was evident in bundles of myelinated nervous fibres of the brain. In the rabbits and human infants this reaction was found even prior to the histologically established myelination of nerve fibres. The reaction for ASS was found neither inside the immature nor in the mature neuroglia cells.

DISCUSSION

The development of the membrane system enveloping the nervous fibres, known as the myelination process is preceded by a numerical increase of the neuroglia cells, by their maturation and differentiation. (Roback, Scherer 1935; Fleischhauer 1968).

According to our histoenzymatic studies regardless of some species differences, the general pattern of myelination gliosis is designated also by increased enzyme activities along with the morphological differentiation of immature neuroglia cells, which points to its general biological significance. In the mouse brain the process of myelination is distinguished by the raised activity of acP, ATP-ase, TPP-ase, AChE and ChE in the neuroglia and in the rabbit by the increased activity of acP, ATP-ase, TPP-ase and 5'-nucleotidase. In the neuroglia of human foetuses, in course of myelination gliosis, only the ATP-ase and TPP-ase activities were markedly elevated, and that of acP appeared moderately increased.

The obvious correlation between the order of histologically detectable myelination and the increase of enzyme activities in the neuroglia should be pointed too. It should also be emphasized that it is the clusters of neuroglia cells, which display the apparently highest enzymes activities. This is a most characteristic picture for the early period of myelination, indicating a special significance of the enhancement of enzymic processes for myelinogenesis.

In course of further development the activity of the said enzymes in the interfascicular oligodendroglia diminishes or even disappeares completely.

The exact significance of the observed increase of enzymic activities in the neuroglia during the process of myelination is difficult to explain. The activity of TPP-ase serves as a histochemical marker for the Golgi apparatus. Hence the observed enhanced activity of the enzyme in the neuroglia taking part in the myelination of the nerve fibres might indicate that the Golgi apparatus is relevant for metabolic processes involved in laying down of the myelin sheath substances.

The role of ATP-ase in the metabolism of the brain neuroglia is not yet fully understood, but its very probable connection with the ATP — ADP system suggests a significance of that enzyme in generating energy for the increased lipid and protein turnover during myelinogenesis.

The rise of acP activity, one of the lysosomic enzymes, seen also in hypertrophy and proliferation of the glia in pathological processes, seems to be joint with the increased nucleic acids' metabolism.

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HISTOCHEMIA ENZYMÓW W GLIOZIE MIELINIZACYJNEJ

Streszczenie

W pracy porównano wyniki badań histoenzymatycznych neurogleju u białych myszy, szczurów oraz w materiale ludzkim.

Badano aktywność następujących enzymów: acP, alkP, TPP-azy, ATP-azy, nieswoistej esterazy, 5'-nukleotydazy, AChE, ChE i ASS.

Jak stwierdzono, niezależnie od pewnych różnic gatunkowych, glioza mielinizacyjna charakteryzuje się wzrostem aktywności enzymatycznej (fosfataz i esteraz), równolegle z różnicowaniem niedojrzałych komórek neurogleju. Stwierdzono istnienie wyraźnej korelacji pomiędzy kolejnością mielinizacji poszczególnych układów włókien nerwowych a wzrostem aktywności enzymatycznej neurogleju. W dalszym okresie rozwoju mózgu aktywność enzymatyczna w oligodendrogleju obniża się lub nawet znika całkowicie.

Wzrost aktywności TPP-azy w neurogleju w czasie procesu mielinizacji zdaje się świadczyć o znaczeniu aparatu Golgi'ego w procesach metabolicznych związanych z odkładaniem składników osłonek mielinowych, a ATP-azy o znaczeniu tego enzymu w procesach energetycznych powiązanych ze wzrostem przemiany lipidów białek w czasie mielinogenezy.

Zwiększenie aktywności acP, zjawisko występujące również często w procesach patologicznych, powiązanych z rozrostem i rozplemem gleju, wydaje się być powiązane ze wzrostem metabolizmu kwasów nukleinowych komórek neurogleju.

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ГИСТОХИМИЯ ФЕРМЕНТОВ В МИЭЛИНИЗИРУЮЩЕМ ГЛИОЗЕ

Содержание

В работе сравняются результаты гистохимических исследований нейроглии у белых мышей, крыс и человека. Исследовали активности следующих ферментов: кислой и налкаличной фосфатаз, тиаминовой пирофосфатазы, АТФ-азы, неспецифичной эстеразы, 5-нуклеотидазы, ацетилхолинэстеразы, холинэстеразы и арил-сульфатазы.

Констатировали, что независимо от некоторых видовых различий, миэлинизирующий глиоз характеризован увеличением энзиматической активности фосфатаз и эстераз параллельно к дифференцировке незрелых клеток нейроглии. Констатировали также существование четкого согласования между последствием миэлинизации отдельных систем нервных волокон и увеличением энзиматической активности нейроглии. В очередном периоде развития мозга ферментативная активность олигодендроглии малеет или совершенно исчезает. Увеличение активности тиаминовой пирофосфатазы в нейроглии во время процесса миэлинизации, свидетельствует кажется, о значении аппарата Гольджи в обменных процессах связанных с формировкой компонентов миэлиновых оболочек, а АТФ-азы о значении этого фермента в энергетических процессах связанных с увеличением обмена липопротеидов во время миэлогенеза.

Увеличение активности кислой фосфатазы, наблюдаемое также патологических процессах связанных с разрастанием глии, кажется связанным с увеличением обмена нуклеиновых кислот клеток нейроглии.

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Fig. 1. Rabbit — 12 days old. TPP-ase activity is visible in the clusters of neuroglial cells in the corpus callosum. × 50.

Fig. 2. Infant — 31st week of menstrual age (weight 1280 g). Neuroglia in the cerebral white matter shows the intracellular TPP-ase activity. × 200.

Fig. 3. Mouse — 10 days old. The clusters of neuroglial cells in the corpus callosum demonstrate the distinct ATP-ase activity. × 16.

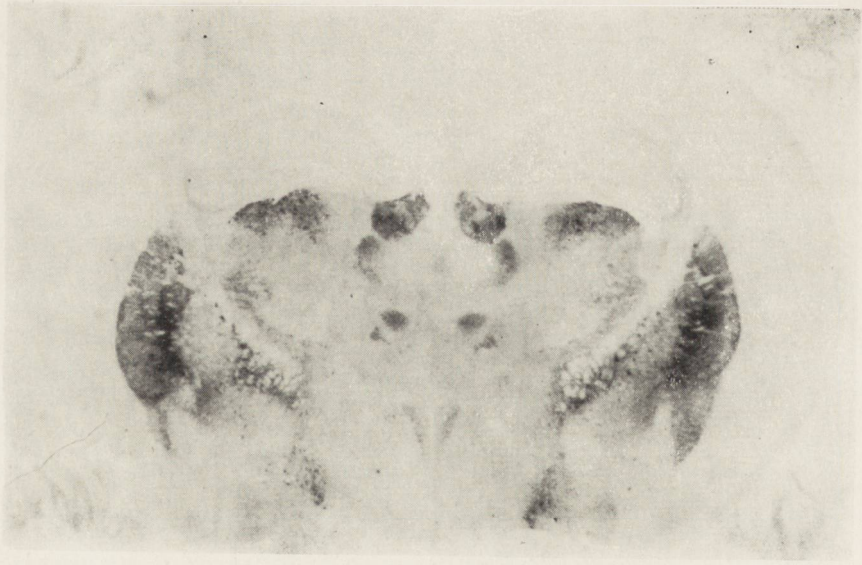

Fig. 4. Mouse — 14 days old. The negative reaction of AChE in the neuroglial cells and in the nerve fibres. The marked AChE activity in the basal ganglia and in other grey structures. × 10.

Fig. 5. Mouse — 10 days old. The ChE activity in neuroglia cells in the corpus callosum. × 180.

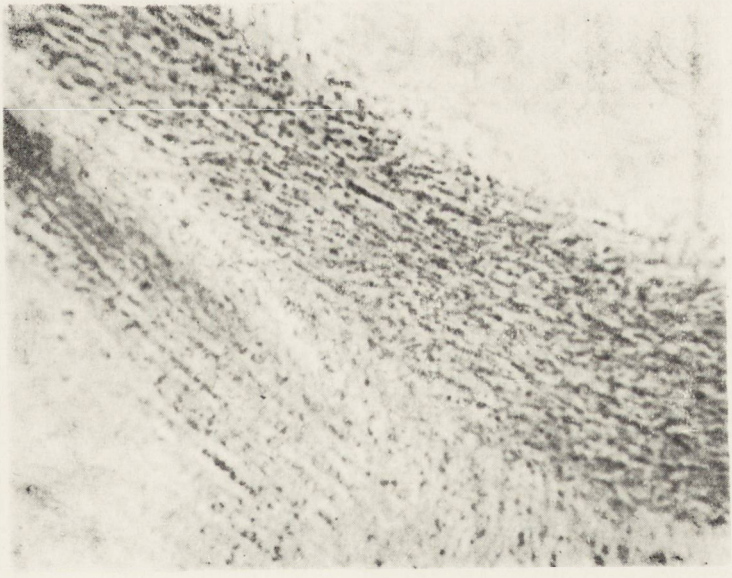

Fig. 6. Rabbit — 120 days old. The distinct ChE activity in the interfascicular oligodendroglia. × 70.

A. N. DAVISON

THE BIOCHEMISTRY OF MYELINOGENESIS

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Up to a few years ago ideas about the chemical composition of myelin had necessarily to be deductive. However, it is now possible to isolate a relatively pure myelin fraction from homogenate of nervous tissue and directly determine its composition (August et al. 1961, Autilio et al. 1964). Analysis shows that isolated myelin contains cholesterol, phospholipids and galactolipids (cerebrosides + sulphatides) in the molar ratio of approx. 2 : 2 : 1 and this composition is similar in all vertebrate species (Cuzner et al. 1965). Myelin accounts for a higher proportion of lipid than any other brain subcellular structure and its lipid content is greater than that of other types of biological membrane. At least 44% of the total brain cholesterol and 68% of the cerebroside is present in myelin.

Myelin contains about 25—30% of its dry weight as protein. Besides containing about 60% of a chloroform-methanol (2 : 1 v/v) soluble protein (proteolipid protein), a basic encephalogenic protein and a third insoluble protein are also present in myelin of the central nervous system. Compared to other membranes myelin is remarkable in its relative enzyme inactivity (Adams et al. 1963); small amounts of leucine aminopeptidase and proteolytic activity, and rather more 2, 3-cyclic nucleotide 3′-phosphohydrolase activity (Kurihara, Tsukada 1967) have been detected in c r u d e myelin fractions but the exact localisation and the possible role of these enzymes is as yet unknown.

Earlier work with a variety of radioactive precursors and with [4-^{14}C] cholesterol had suggested that the myelin constituents were metabolically rather stable (Davison 1968). In 1965, Torvik and Sidman reported radioautographic studies showing persistance of incorporated labelled precursors in the myelin-rich white matter of developing mouse brain and relatively slight uptake into that of adults. The hypothesis of relative metabolic inertness of myelin (Davison, Dobbing 1961) was confirmed when persisting radioactivity in the central

nervous system was shown to be predominantly localised in myelin separated by differential centrifugation (Davison, Gregson 1962, Cuzner et al. 1966).

Although myelin has a constant composition in the adult brain during the early stages of CNS development there are marked differences in its composition. The "early" myelin has a chemical composition similar to that of cytomembranes: no cerebroside, more short chain fatty acid esters than long chain esters and relatively more phospholipid than is present in mature myelin.

It was suggested by Davison, Cuzner, Banik and Oxberry (1966) that the so called "early" myelin was a mixture of both myelin and an intermediate precursor, originating from oligodendroglial plasma membrane. In order to further determine the chemical characteristics and metabolism of these membrane fractions we have examined the effects of blocking synthesis of cholesterol in the developing rat brain. Desmosterol has been shown to be only present in freshly synthesised myelin but the amount of this cholesterol precursor can be markedly increased by treatment of young rats with Triparanol (Banik, Davison 1967). After treatment, brain desmosterol is slowly replaced by cholesterol. Following injection of the drug AY-9944 (Trans-1,4-bis-[2-chlorobenzylaminomethyl]-cyclohexane dihydrochloride) into 5-day old rats there is an accumulation of 7-dehydrocholesterol in the brain. The cholesterol precursor is present in the crude myelin fraction of 15-day old rats where it accounts for about half the total sterol. When early myelin was separated (Norton et al., unpublished) into adult typemyelin and a second mebrane fraction — both were found to contain similar proportions of sterol and precursor. It was also noted that brain microsomes has the same sterol distribution. Even though substantial amounts of 7-dehydrocholesterol are incorporated into early myelin this sterol cannot be detected in the brain 21 days after discontinuing drug treatment. It is, therefore proposed that during the early stages of myelination the newly synthesised glial membrane can readily undergo metabolism. During myelin formation cerebroside may be added to and lecithin be eliminated from the membrane so that one type of structure (e.g. subunit type) may be converted to another (e.g. the unit membrane type). The principles of such transformation reactions have been discussed by Luzzati and Husson (1962), Benson (1966) and Lucy (1968).

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A. N. Davison

BIOCHEMIA MIELINOGENEZY

Streszczenie

Biochemiczne badania mieliny wykazują, że jest to substancja lipoproteinowa zawierająca 75% lipidów. Cholesterol, fosfolipidy i galaktolipidy są obecne w proporcjach molarnych mających się do siebie jak 2 : 2 : 1. U zwierząt dorosłych mielina jest metabolicznie stabilna i nie zawiera żadnych prawie enzymów poza 2′, 3′-cykliczną nukleotydo 3′-fosfohydrolazą oraz aminopeptydazą leucynową. Niniejsza praca zakłada istnienie pośredniego stadium w syntezie mieliny, przy czym postuluje się, że ta „wczesna" mielina jest niezróżnicowaną błoną glejową. Podawanie szczurom różnych środków pozwala na wbudowanie 50% prekursorów cholesterolu do mieliny rozwijającego się mózgu. Nasze badania wykazują, że 7-dehydrocholesterol i desmosterol w mielinie podlegają stosunkowo szybkiej redukcji do cholesterolu. Sugeruje to metaboliczną aktywność „wczesnej" mieliny w przeciwieństwie do jej stabilności w dojrzałym mózgu.

А. Н. Дэвисон

БИОХИМИЯ МИЭЛИНОГЕНЕЗА

Содержание

Биохимическое исследование миэлина указывает, что являтся он липопротеидовым веществом содержащим 75% липида. Холестерин, фосфолипиды и галактолипиды присутствуют в отношениях молярных выраженных отношением 2 : 2 : 1. У взрослых животных миэлин метаболически стабильный и не содержит почти никаких ферментов кроме 2′, 3′-цикло нуклеотид 3′-фосфогидролазой и лейциновой амин-пептидазой.

Нынешняя работа принимает существование посредственной стадии в синтезе миэлина при чем предположено, что этот „ранний" миэлин является недифференцированной глиальной мембраной. Введение крысам разных средств разрешает инкорпорировать 50% прекурсоров холестерина в состав миэлина развивающегося головного мозга.

Наши исследования показывают, что 7-дегидрохолестерин и десмостерол подвергаются в миэлине относительно быстрому восстановлению в холестерин. Свидетельствует это о метаболической активности „раннего" миэлина в сравнении с его стабильностью в зрелом мозге.

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J. N. CUMINGS

THE LIPID COMPOSITION OF PURE MYELIN IN SOME DEMYELINATING DISORDERS

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This paper describes some of the experiments carried out during the past year or so with particular reference to the composition of myelin in the white matter from normal subjects and these with various diseases. This enables comparison to be made with descriptions given of findings in the whole white matter in lipid diseases as described previously (Cuming 1967).

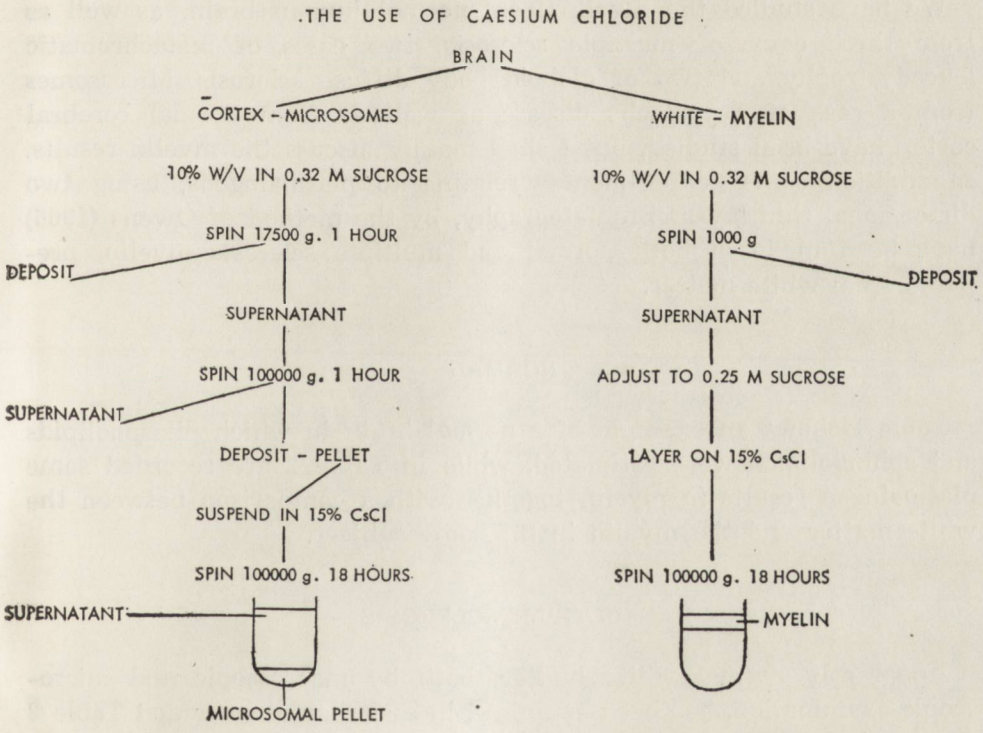

Fig. 1. The use of Caesium chloride.

For the separation of myelin and microsomes we have made use of caesium chloride rather than sucrose in differential centrifugation (Fig. 1). The strength of caesium chloride required, together with the time and speed of centrifugation, had to be determined experimentally and this took same time, but a strength of 15% caesium chloride finally gave us reliable results. Although we initially used an 18 hour spin at the final stage this can be shortened to about four hours, yet 18 hours was convenient, as then the centrifuge could be left on overnight and our working day devoted to processing (Thompson et al. 1967). The electron microscopic appearances demonstrated that both myelin and microsomal preparations were relatively pure.

Lipid extracts of white matter myelin as well as of cortical microsomes have been studied, using thin layer chromatography in a manner described by Müldner, Wherret and Cumings (1962). Phospholipids were determined by their phosphorus content from each individual lipid spot, while sphingolipids were estimated from their sphingosine content by a method recently described (Cumings et al. 1968). Total phosphorus and sphingosine were estimated in the original lipid extracts as also were cerebroside and cholesterol.

We have studied the myelin from normal human brain, as well as from three cases of multiple sclerosis, two cases of metachromatic leucodystrophy and two of globoid body diffuse sclerosis. Microsomes from a case of Tay Sachs disease as well as from normal cerebral cortex have been studied, but I shall mainly discuss the myelin results. In addition some experiments relating to plasmalogens, using two dimensional thin layer chromatography, by the method of Owens (1966) have been made in both normal and multiple sclerosis myelin, prepared from white matter.

NORMAL

Table I shows an example of a normal brain in which phospholipids and sphingolipids were estimated, while in Table 2 are recorded some plasmalogen results in myelin together with a comparison between the white matter and the myelin in the same subject.

MULTIPLE SCLEROSIS

Apparently normal white matter, both by macroscopic and microscopic examination, has been examined in a similar manner and Table 3 shows phospholipid and sphingolipid values for the myelin prepared.

It is seen that ethanolamine levels are slightly but significantly reduced, while other phospholipids do not differ appreciably from the normal. When the figures in Table 4 are examined it is found that ethanolamine plasmalogen is definitely reduced whether calculated against total phospholipids or against the non-plasmalogen ethanolamine.

Table 1. Normal myelin

Phospholipids %		Sphingolipids %	
Ethanolamine	39·9	Ganglioside	0
Choline	24·1	Sphingomyelin	7·1
Serine	16·4	Trihexoside	trace
Sphingomyelin	19·6	Sulphatide	16·1
		Dihexoside	2·8
		Cerebroside	69·6
		Ceramide	4·3

Cerebroside/Sulphatide ratio 4 : 1

Table 2. Plasmalogens in normal brain

	In myelin	In white matter
Ethanolamine plasmalogen	77·3	72
Choline plasmalogen	4·0	4—5
Serine plasmalogen	2·8	2—3

as % of corresponding phospholipid

Table 3. Multiple sclerosis myelin

Phospholipids %		Sphingolipids %	
Ethanolamine	36·22	Ganglioside	1·72
Choline	27·58	Sphingomyelin	3·44
Serine	17·37	Trihexoside	1·72
Sphingomyelin	18·74	Sulphatide	29·73
		Dihexoside	4·74
		Cerebroside	53·44
		Ceramide	5·17

Cerebroside/Sulphatide ratio 1·86 : 1

The sphingolipid analyses (Table 3) show some striking abnormalities. Whereas normally by this technique, and using a standard amount of myelin from a known amount of white matter, no ganglioside is

Table 4. Plasmalogens in multiple sclerosis myelin

	% corresponding phospholipid	% total phospholipid
Ethanolamine plasmalogen	70·5	29·0
Choline plasmalogen	3·6	0·9
Serine plasmalogen	4·0	0·6

seen, in multiple sclerosis ganglioside is definitely present. The normal cerebroside/sulphatide ratio is as 4 is to 1 but in the example given which was characteristic of all three examined, there is a well marked alteration, the result of a definite reduction in cerebroside, as well as a relative increase in sulphatide.

It is possible to calculate the molar proportions of cerebroside, sulphatide and cholesterol when it is found that whereas in the normal myelin the figures are 1·0, 0·2, 1·7 respectively, in multiple sclerosis they are 1·0, 0·31, 1·5 (Cumings, Goodwin 1968).

It is clear from these findings that the „apparently normal" white matter myelin shows the following variations from the normal: a reduced ethanolamine and ethanolamine plasmalogen, increased ganglioside and an altered cerebroside/sulphatide ratio. These all indicate a biochemically disturbed myelin in the „apparently normal" white matter, but whether because it was originally abnormal or whether, as is possible, it indicates the first signs of a new lesion, one cannot as yet say.

METACHROMATIC LEUCODYSTROPHY

The electron microscopic appearance of the myelin prepared from each of the three cases was abnormal (Cumings et al. 1968). A phospholipid analysis shows only relatively minor variation from the normal but the sphingolipid composition is definitely abnormal (Table 5).

Table 5. Sphingolipids of myelin

	In metachromatic leucodystrophy %	In Krabbe's disease %
Ganglioside	1·9	5·99
Sphingomyelin	2·4	14·52
Trihexoside	1·5	2·49
Sulphatide	85·2	8·09
Dihexoside	1·8	3·29
Cerebroside	4·4	58·94
Ceramide	2·8	6·65
Cerebroside/Sulphatide ratio	1 : 19·3	7·3 : 1

The most remarkable finding is the change in cerebroside/sulphatide ratio which is 1 : 20 as compared to the normal 4 : 1, due to gross reduction in cerebroside and a considerable increase in sulphatide. It is worth noting that ganglioside is present.

GLOBOID BODY DIFFUSE SCLEROSIS (KRABBE'S DISEASE)

The myelin is seen to be damaged under the electron microscope but while phospholipid levels are not greatly altered those of the sphingolipid compounds are abnormal (Table 5). Cerebrosides are considerably increased and sulphatides decreased, resulting in a ratio of 7 to 1, or nearly double the normal.

It is now well known that in the ganglioside pattern of the cortical lipids two abnormal bands G_{M3} and G_{M4} are present. These same abnormalities are to be found in the microsomal preparations from white matter as well as from microsomes of cortex.

Further work is in progress relative both to the findings in multiple sclerosis, as well as to myelin from white matter in other diseases.

J. N. Cumings

SKŁAD LIPIDÓW CZYSTEJ MIELINY W PEWNYCH ZABURZENIACH DEMIELINIZACYJNYCH

Streszczenie

Przedstawia się krótki opis dotyczący oddzielenia mieliny i mikrosomów od tkanki mózgowej, w wyniku czego sfingolipidy w tych frakcjach subkomórkowych zostały rozdzielone i określone ilościowo za pomocą metody chromatografii cienkowarstwowej. Za materiał do badań służyła mielina z prawidłowej białej istoty mózgu, jak również ze stwardnienia rozsianego, leukodystrofii metachromatycznej i choroby Krabbego.

Wyniki badań wykazują pewne różnice między materiałem patologicznym w stosunku do prawidłowego, zwłaszcza zmianie ulega stosunek cerebrozydy: sulfatydy, jak też w obecności gangliozydów w ilościach większych niż w stanie prawidłowym.

„Pozornie prawidłowa" istota biała mózgu w stwardnieniu rozsianym wykazuje także zmniejszoną zawartość etanolaminy i plazmalogenu etanolaminowego.

Д. Н. Кэмингс

СОСТАВ ЛИПИДОВ ЧИСТОГО МИЭЛИНА В НЕКОТОРЫХ ДЕМИЭЛИНИЗИРУЮЩИХ РАССТРОЙСТВАХ

Содержание

Представляется краткое описание отделения миэлина и микросомов от мозговых тканей, следствием чего сфинголипиды из этих субклеточных фракций

разделили методом тонкослойной хроматографии и каждый липид измерили количественно. Исследованным материалом был миэлин полученный из нормального белого вещества, из расеянного склероза, метахроматической лейкодистрофии и из болезни Краббэ.

Результаты исследований показывают некие изменения в патологическом материале в сравнении с нормальным особенно в изменении отношения цереброзиды/сульфатиды и в наличии ганглиозидов в больших чем нормально количествах.

„Внешне правильное" белое вещество в рассеянном склерозе также содержало обниженный уровень этанлоамина и этаноламин-плазмалогена.

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F. CHEVALLIER

ETUDE DES TRANSFERTS DE CHOLESTEROL D'ORIGINE PLASMATIQUE DANS LE SYSTEM NERVEUX DU RAT ADULTE ET EN CROISSANCE

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Environ 70 pour 100 du cholestérol d'un rat sont mobiles. Entre un organe quelconque et le plasma, il existe, en effet, des transferts bidirectionnels de cholestérol. Ces transferts constituent le mode essentiel de renouvellement du cholestérol des organes. On s'est attaché à préciser les caractéristiques fonctionnelles de ce type de renouvellement par échange ou remplacement moléculaire (Chevallier 1956, Chevallier 1961, Chevallier, Giraud 1966, Chevallier 1967). On va examiner celles qui concernent le système nerveux du rat.

A — RATS ADULTES

Les rats utilisés sont des mâles de souche Wistar pesant environ 350 g au début de l'expérience.

I. *Détermination de la fraction du cholestérol renouvelable par des transfers de cholestérol plasmatique*

On a utilisé dans ce but la méthode d'équilibre isotopique. Chez des rats ingérant un régime dont la concentration et la radioactivité spécifique du cholestérol sont constantes, la radioactivité spécifique du cholestérol plasmatique atteint une valeur d'équilibre (Chevalier 1956, 1960, 1966). Au bout d'un temps variable suivant la nature de l'organe considéré, la radioactivité spécifique du cholestérol de chaque organe atteindra, elle aussi, une valeur d'équilibre. Le rapport de la radioactivité spécifique du cholestérol d'un organe à celle du cholestérol plasmatique définit, à l'équilibre isotopique, la fraction du cholestérol renouvelé par transfert. Contrairement à de nombreux organes, le temps néces-

saire pour observer un rapport constant est très long pour les différents éléments du système nerveux. L'expérience doit durer au minimum une année. Par ailleurs, on constate que seuls 24 pour 100, en moyenne, du cholestérol du cerveau sont renouvelés par le cholestérol plasmatique, 15 pour 100 pour le nerf sciatique et 12 pour 100 pour la moelle épinière.

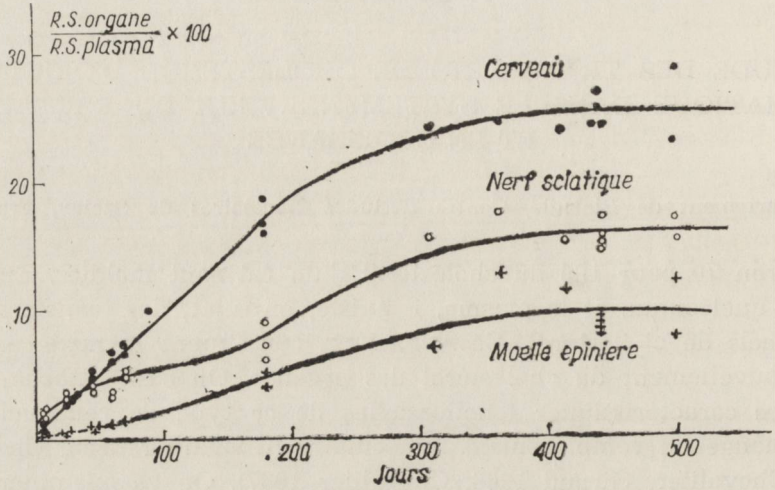

Fig. 1. Pourcentages, par rapport à la radioactivité spécifique du cholestérol plasmatique, de celles du cholestérol du cerveau, de la moelle épinière et du nerf sciatique prélevés chez de rats ayant ingéré, depuis l'âge adulte, du cholestérol-4-^{14}C pendant des durées allant de 7 à 500 jours.

Un travail récent nous permet de compléter et préciser les données reportées ci-dessus. Tout d'abord, six nouveaux rats ont été sacrifiés après 440 et 500 jours d'expérience (fig. 1). Si les valeurs moyennes des rapports de remplacement concernant le cerveau et le nerf sciatique demeurent pratiquement inchangées (respectivement 0,25 et 0,16), celles concernant la moelle épinière étaient nettement surestimées (0,09 au lieu de 0,12). Par ailleurs, le cerveau a été décomposé en 3 éléments: les hémisphères cérébelleux, le bulbe, le cerveau restant. De même, moelle épinière et nerf sciatique ont été sectionnés en 2 ou 3 fragments, de taille approximativement égale: supérieur, moyen, et, inférieur. Les rapports des radioactivités spécifiques du cholestérol de ces divers éléments du système nerveux à celle du cholestérol plasmatique sont reportés dans le tableau 1. On constate que les radioactivités spécifiques du cholestérol des segments supérieur et inférieur de la moelle épinière sont pratiquement égales, et nettement plus élevées qu celle du cholestérol du segment moyen. Pour le nerf sciatique, une relation du même type lie les segments supérieur et moyen par rapport

au segment inférieur. Par ailleurs, la radioactivité spécifique du cholestérol du bulbe est nettement inférieure à celles, égales entre elles, du cholestérol des hémisphères cérébelleux et du cerveau restant. Il est à l'heure actuelle difficile d'interpréter de tels résultats. L'étude de la localisation du cholestérol mobile dans le cerveau fournira un élément de réponse à ce problème.

2. *Preuve de l'existence de transferts cerveau-plasma (Chevallier, Giraud 1966)*

A la suite d'une expérience qui concernait le cholestérol total d'animaux entièrs, on a conclu que la quasi totalité du cholestérol d'origine plasmatique contenu dans les organes du rat, retournait dans son milieu d'origine. La lenteur des transferts de cholestérol au niveau du cerveau nous a engagés à vérifier si cette conclusion s'appliquait effectivement à ce cas particulier.

On a utilisé des rats ayant ingéré du cholestérol -4-^{14}C pendant 24 jours. Ils ont été sacrifiés durant les semains qui ont suivi, puis au bout d'un an. Après un tel délai, on constate que la radioactivité totale du cholestérol, comme sa radioactivité spécifique, ne sont plus que le tiers de leurs valeurs observées au bout du premier mois après la cessation du régime marqué (fig. 2). La cinétique de la radioactivité

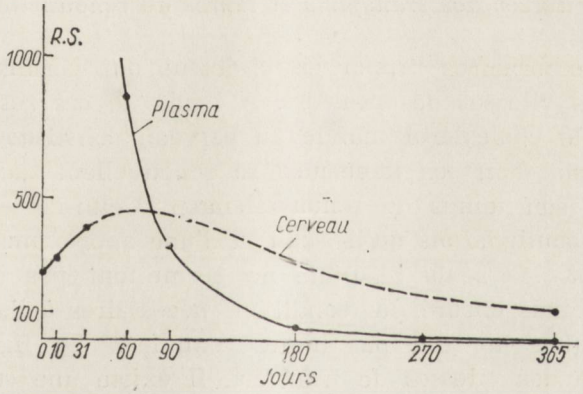

Fig. 2. Radioactivités spécifiques du cholestérol (ipm/mg) du cerveau et du plasma, prélevés chez des rats ayant ingéré, à l'âge adulte, du cholestérol-4-^{14}C pendant 24 jours, et ayant été sacrifiés au cours des mois suivants.

spécifique suggère, du reste, que cette disparition du cholestérol marqué est encore plus importante. En effet, la valeur de la radioactivité spécifique du cholestérol du cerveau au premier mois ne représente probablement pas sa valeur maximale. Quoiqu'il en soit, on peut admettre que le cholestérol d'origine plasmatique contenu dans

le cerveau retourne dans la circulation sanguine. En d'autres termes, 24 pour 100 du cholestérol du cerveau sont réellement mobiles.

Tableau 1

Durée de l'expérience (jours)		Cerveau			Moelle épinière			Nerf Sciatique		
		C.R.	H.C.	B.	S.	M.	I.	S.	M.	I.
60	1		6,1		1,1	0,7	1,2	2,8	2,8	4,3
	2		6,5		1,2	1,0	1,3	2,6	3,1	4,2
180	3		15,5		3,5		3,3	6,0	6,0	8,8
	4		17,5		3,9	2,9	3,9		9	11
440	5	27,5	27	19	9,1	7,5	10,9	15		19,3
	6	27	27,5	19,2	9,8	7,8	9,3	13,3		18,2
	7	28,2	26,6	18,8	9,2	6,5	9,2	14,5		17,8
	8	27,5	27,5	18,5	9,2	7,5	9,2	12,8		12,5
500	9	25,1	26,6	19,5	8,8	6,9	9,0	14,6	13,3	20
	10	39,6	31,6	24,7	13	10	12,6	16,9	16,9	19,4

Radioactivité spécifique du cholestérol de différents éléments du système nerveux, exprimée en pour-cent de celle du cholestérol plasmatique (H.C. : hémisphères cérébelleux, B : bulble, C.R. : cerveau restant, S. : supérieur, M. : moyen, I. : inférieur).

3. *Vitesses des transferts et temps de renouvellement*

Les deux expériences rapportées ci-dessus ont permis de souligner la lenteur des vitesses de transfert. A l'aide de ces résultats, en admettant que le cholestérol mobile du cerveau constitue un compartiment homogène dont les molécules se renouvellent „au hasard", on peut calculer son temps de renouvellement. Celui-ci serait d'environ 4 mois. Nous soulignerons qu'il s'agit là d'une approximation. En effet, non seulement, les seuls résultats ne permettent pas d'effectuer un calcul précis, mais encore, les conditions nécessaires à l'application des calculs classiques ne sont pas toutes remplies. La figure I montre l'hétérogénéité des vitesses de transfert. Il existe une composante rapide assurant le renouvellement d'une petite fraction du cholestérol mobile, tout au moins en ce qui concerne le cerveau et le nerf sciatique (fig. 3). La cinétique suggère aussi que la composante lente principale n'a, peut-être pas, la valeur d'une constante. Cette observation touche essentiellement le nerf sciatique et le moelle épinière. On note à leur propos une légère augmentation de pente entre le 150 et le 300è jour d'expérience. On s'est demandé si elle ne pouvait pas être expliquée par une migration axonale de cholestérol marqué entre, d'une

part, le cerveau et la moelle, et, d'autre part, la moelle et le nerf sciatique. Or, les résultats reportés dans le tableau 1 montrent que les différences entre les radioactivités spécifiques du cholestérol des différents segments demeurent dans des rapports constants. En conclusion, si une migration axonale existe, elle est de faible amplitude et n'est pas détectable par notre méthode.

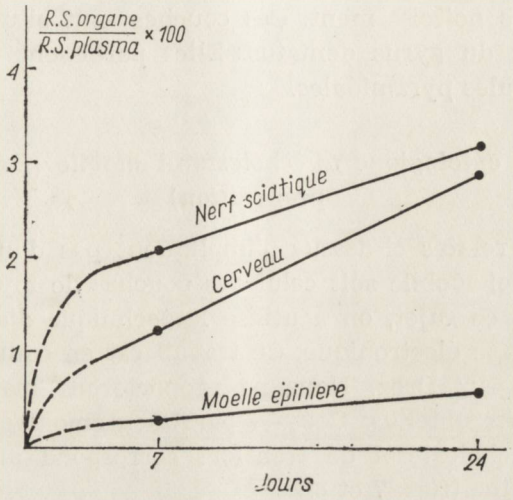

Fig. 3. Agrandissement de la figure 1 pour les courtes durées d'ingestion de cholestérol-4-^{14}C.

4. *Localisation anatomique des zones de cholestérol mobile (Chevallier, Petit 1966)*

Ce problème a été traité par la technique d'autoradiographie. Les cerveaux ont été prélevés sur des rats ayant ingéré un régime contenant du cholestérol — ^{14}C pendant 8 ou 60 jours. Les autoradiogrammes dont les figures 5 et 6 fournissent des exemples, montrent que le noircissement est très intense dans certaines régions anatomiques. Il y a, en fait, une correspondance rigoureuse entre la densité du noircissement d'un autoradiogramme et celle de la coloration de la même coupe obtenue par la technique de Weigert. Pour établir cette comparaison, on s'est servi des reproductions figurant dans le livre: „Craigie's Neuroanatomy of the Rat" (Zeman, Innes 1963). Ainsi, le cholestérol mobile d'une zone anatomique est d'autant plus abondant, que la densité des fibres myélinisées de cette zone est elle-même élevée.

Indépendamment de cette relation, les autoradiogrammes montrent, à côté des zones à fibres myélinisées, un noircissement quasi homogène,

de faible intensité. Ce marquage est rapidement obtenu, et n'évolue pas d'une façon appréciable avec le temps. Ainsi, une faible fraction du cholestérol des régions anatomiques pauvres ou dépourvues de fibres myélinisées, est rapidement renouvelée par transfert. Il y a probalement une correspondance entre cette observation et celle relative à l'existence d'une composante rapide des vitesses de transfert. Enfin, dans ces zones de noircissement de faible intensité, on observe des couches de moindre noircissement. Ces couches sont situées au niveau de l'hippocampe et du gyrus dentatus. Elles paraissent correspondre aux couches des cellules pyramidales.

5. *Localisation cytologique du cholestérol mobile (Droz, Chevallier en préparation)*

Les résultats relatés ci-dessus n'impliquent pas d'une façon certaine que le cholestérol mobile soit celui des couches de myéline. Pour avoir des précisions à ce sujet, on a utilisé la technique d'autohistoradiographie en microscope électronique. Ce travail est en cours dans le laboratoire du Professeur Droz. Nous ne rapporterons maintenant que les premiers résultats obtenus. Ceux-ci paraissent montrer qu'une proportion élevée de cholestérol de transfert correspond au cholestérol des couches de myéline (figs. 7 et 8).

B — RATS EN CROISSANCE

L'existence d'une transfert de cholestérol du plasma dans le cerveau de jeunes animaux (rat ,lapin, poulet) a été prouvée, en particulier par

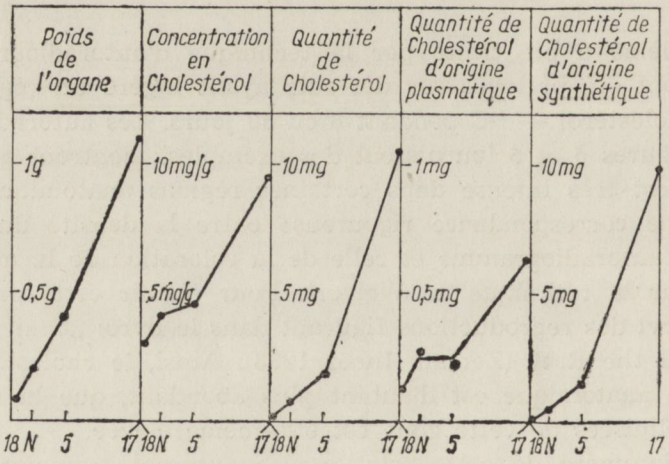

Fig. 4: Données quantitatives concernant le cholestérol du cerveau durant la fin de la vie foetale et les 17 premiers jour de vie (N : naissance).

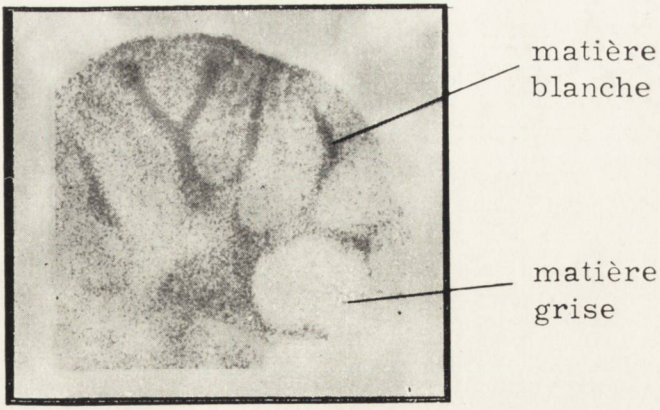

Fig. 5: Autoradiographie d'une section sagittale d'un hémisphère cérébelleux d'un rat ayant ingéré du cholestérol-4-^{14}C.

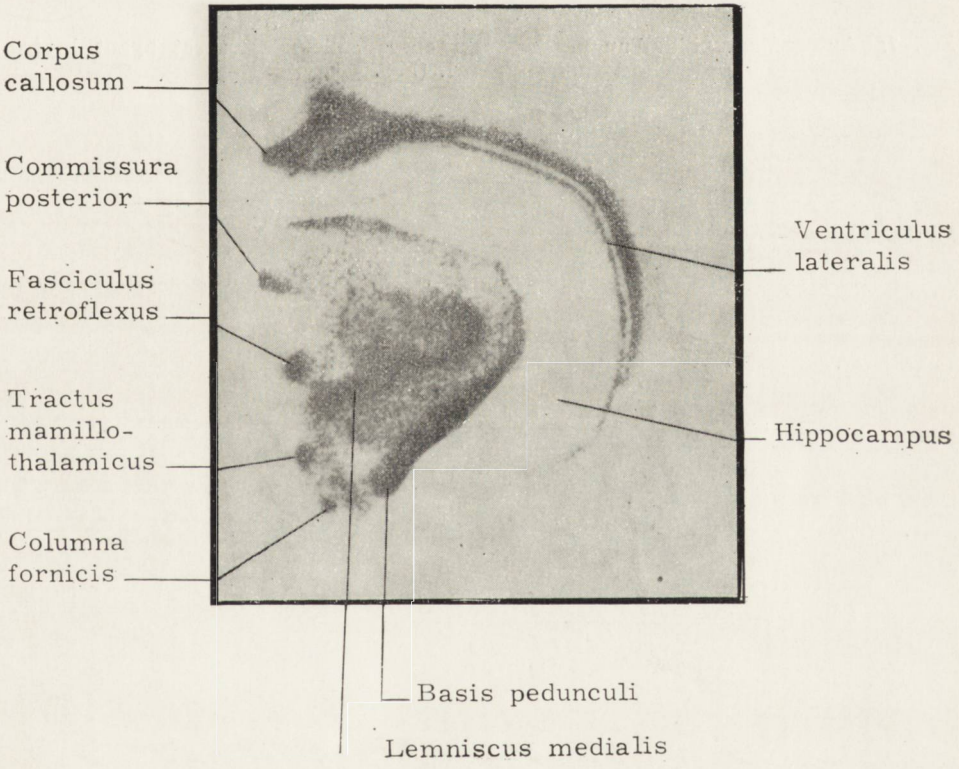

Fig. 6. Autoradiographie d'une section transversale du cerveau d'un rat, ayant ingéré du cholestérol-4-^{14}C.

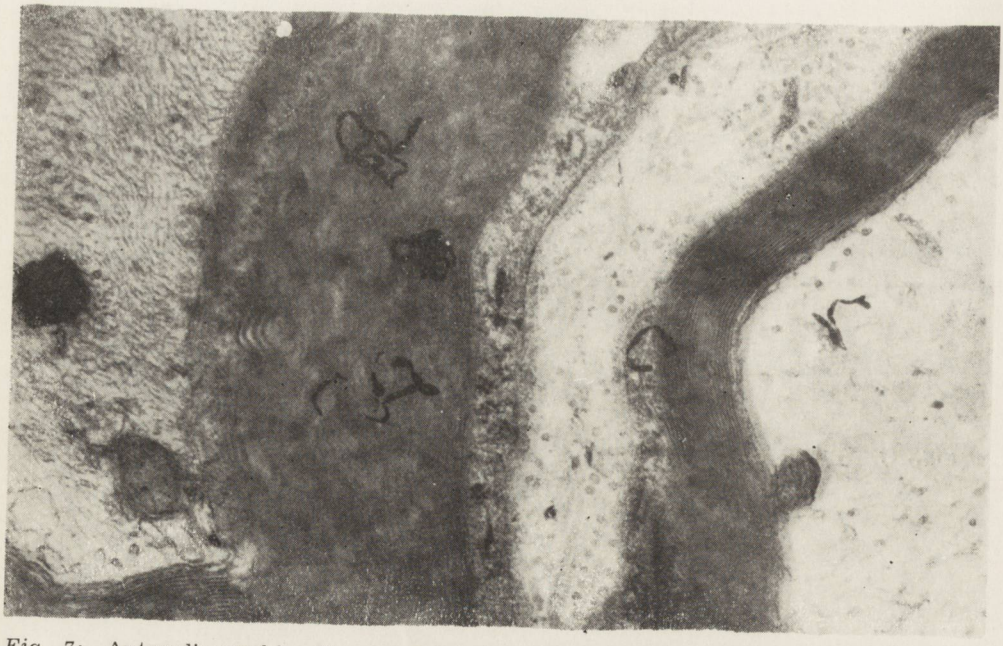

Fig. 7: Autoradiographie d'un segment du corps calleux d'un rat ayant ingéré du cholestérol-^{3}H. (× 51000).

Fig. 8: Autoradiographie d'un segment de ganglion spinal d'un rat ayant ingéré du cholestérol-^{3}H. (× 42000).

Davison et Coll. (1958, 1959), mais aussi par Dobbing (1963) et par Clarenburg et Coll. (1963). Par ailleurs, Davison a souligné la persistance, dans le cerveau d'animaux adultes, du cholestérol marqué, d'origine plasmatique, déposé dans le cerveau des animaux jeunes. De notre côté, on a étudié ce processus de transfert d'un point de vue quantitatif (Chevallier 1964). Par la méthode d'équilibre isotopique, on a précisé les quantités du cholestérol d'origine plasmatique contenu dans le cerveau des rats depuis le 2è jour avant la naissance jusqu'au 17è jour après la naissance. Sur la figure 4 sont reportés les résultats. On constate que, deux jours avant la naissance, le cholestérol de transfert représente 50 pour 100 du cholestérol du cerveau. L'enrichissement en cholestérol de cet organe ne commence réellement que quelques jours après la naissance. La synthèse du cholestérol „in situ" représente l'élément majeur de l'accroissement. Néanmoins, les transferts augmetent aussi. Deux jours avant la naissance, le cerveau contient 0,12 mg de cholestérol d'origine plasmatique; 17 jours après la naissance, il en contient 0,6 mg. A cet âge le pourcentage de cholestérol de transfert par rapport au cholestérol de toutes origines n'est plus que de 6.

Signalons, enfin, que chez le poussin, Kritchevsky et Defendi (1962) ont montré, par autoradiographie, la présence de cholestérol tritié dans la substance blanche du cervelet.

F. Chevallier

BADANIA NAD PRZECHODZENIEM CHOLESTEROLU Z SUROWICY KRWI DO CENTRALNEGO UKŁADU NERWOWEGO U SZCZURA DOROSŁEGO I ZNAJDUJĄCEGO SIĘ W OKRESIE WZROSTU

Streszczenie

U dorosłego szczura wymiana cholesterolu pomiędzy narządem a surowicą krwi jest zasadniczym sposobem odnowy komórkowego cholesterolu. Cholesterol centralnego układu nerwowego nie stanowi wyjątku od tej reguły. Jednakże na tym poziomie, proces ten charakteryzują dwie cechy: Z jednej strony, gdy jedna z frakcji cholesterolu odnawia się następująco: 23, 12, 15% odpowiednio dla mózgu, rdzenia kręgowego i nerwu kulszowego, to z drugiej strony szybkości wymian są niewielkie. Szybkości te odpowiadają podanym wyżej odsetkom i nie mogą być określane przed upływem roku codziennego podawania cholesterolu znakowanego ^{14}C. Wykazano też, że transport plazma-mózg jest sprzężony z transportem w kierunku odwrotnym mózg-plazma. Wyjaśnia to dobrze mechanizm odnowy poprzez wymianę lub zastąpienie. Spostrzeżenia te potwierdzają także względną nieprzenikliwość bariery mózgowej.

Otrzymane wyniki pozwalają na określenie, czy frakcja cholesterolu mózgowego podlegająca wymianie przez przemieszczenie została zlokalizowana w jakiejś szczególnej okolicy anatomicznej. Próby rozwiązania tego problemu dokonano za pomocą autoradiografii z ^{14}C. Stwierdzono, że zaczernienie klisz było ściśle proporcjonalne do gęstości włókien mielinowych. Dla uzyskania większej dokładności lokalizacji cholesterolu podlegającego wymianie na poziomie stref włókien mielinowych rozpoczęto doświadczenia z zastosowaniem autoradiografii w mikroskopii elektronowej z użyciem cholesterolu znakowanego trytem. Praca ta jest w toku przy współpracy z B. Droz.

Шевалье Ф.

ИССЛЕДОВАНИЯ НАД ПЕРЕХДОМ ХОЛЕСТЕРИНА ИЗ КРОВЯНОЙ ПЛАЗМЫ В ЦЕНТРАЛЬНУЮ НЕРВНУЮ СИСТЕМУ ВЗРОСЛОЙ КРЫСЫ И ВО ВРЕМЯ ВОЗРАСТА

Содержание

У взрослой крысы обмен холестерина между органом и кровяной плазмой является основным способом обновления клеточного холестерина. Холестерин центральной нервной системы не исключение от этого правила. Однако на этом уровне процесс характеризуется двумя факторами. С одной стороны одна из фракций холестерина обновляется следующим путе: 23, 12, 15% соответственно для головного мозга, спинного мозга и седалищного нерва. С другой стороны скорости обменов невелики. Скорости эти соответствуют приведенным выше процентам и не могут быть определены раньше чем спустя года ежедневного применения холестерина меченного ^{14}C.

Показано также что транспорт веществ в направлении кровяная плазма--головной мозг сопряжен с транспортом в обратном направлении. Это хорошо объясняет механизм обмена путем замещения или обновления. Настоящие наблюдения потверждают также относительную непроникновенность гемато-мозгового барьера. Полученные результаты позволяют определить в какой степени фракция холестерина подвергающегося обновлению путем перемещения локализирована в какой-то определенной области головного мозга. Попытки решения этой проблемы проведены с помощью авторадиографии с ^{14}C. Констатировали что зачернение плёнок было строго пропорционально к густоте миелиновых волокон.

Начаты опыты методом авторадиографии в электронной микроскопии с применением холестерина меченного тритием. Работа ведется в сотрудничестве с Б. Дроз.

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COMPARATIVE STUDIES ON PHOSPHOLIPIDS IN MORPHOLOGICALLY DIFFERENT AREAS OF THE BRAIN

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It has been shown in the last few years that one of the main factors responsible for the specificity of phospholipid composition in various formations of the central nervous system (CNS) is the relative content of myelin structures. Thus, E. M. Kreps and his coworkers (1963) have found that different regions of the rat brain at the earlier ontogenetic stages are rather similar with respect to their phospholipid content, it is only during myelinization that the differences between the brain regions appear peculiar to the adult animal, concerning both the content and turnover rate of individual phospholipids.

This work is an attempt to characterize the content and the turnover rate of several representatives of phospholipids in the CNS formations which markedly differ from each other as regards the relative content of myelin structures. These formations were as follows: a) brain cortex in which the number of cell element is relatively high and nerve trunks consist mainly of nonmyelinated nerve fibers; b) medulla oblongata in which the main mass consists of myelinated fibers. Besides, both these regions of CNS differ from each other in the character of glial elements. Whereas in the brain cortex the astroglia predominates, in the medulla the glial cells are represented mainly by oligodendrocytes, which according to modern conceptions play an important role in myelin formation and according to some authors (O'Brien, Sampson 1965, Davison et al. 1960) have the same lipid composition as myelin structures.

The work was carried out on adult male rats of Wistar strain, body weight 180—200 g. The animals were injected subcutaneously with radioactive sodium phosphate in a 5 μC dose per 1 g of body weight. Two hours after isotope injection the animals were decapitated, the brain was taken out, thoroughly cleaned from the meninges and blood

vessels and washed out from blood with saline, thereafter the brain cortex and the medulla were separated.

Lipids were extracted according to Folch with chloroform-methanol (2 : 1). The fractionation of phospholipids was done by the method described earlier (Dvorkin et al. 1963). The following phospholipid fractions were separated: phosphatidic acids + polyglycerophosphatides, aminophospholipids (a mixture of diacyl and plasmalogen forms of ethanol-amin- and serine-phosphatides), phosphatidylinositols and choline containing phospholipids. By mild alkaline hydrolysis according to Davson (1960) the aminophospholipid fraction was separated into diacyl and plasmalogen forms, while the choline-containing phospholipid fraction — into lecithins (phosphatidylcholines) and sphingomyelins. Moreover, the phospatidopeptides were extracted by acidic chloroform-methanol. In all these fractions the radioactivity of the phosphorus was determined. The content of lipid phosphorus was calculated in μg per g of fresh tissue. The intensity of phosphate group turnover of each fraction was estimated on the basis of the value of the relative spectific activity (RSA) of phospholipid phosphorus, this value being the ratio of specific activity of the phosphorus of each fraction to the specific activity of the inorganic phosphate of the brain tissue.

Table 1. Phospholipid fractions content (μg) of lipid phosphorus per gram of fresh tissue in the cerebral cortex and the medulla of rats (mean of 12—16 exp.)

	Cerebral cortex	Medulla
Total phospholipids	1843 ±24	2644 ±84
Phosphatidic acids + polyglycerophosphatides	63 ± 3	118 ± 5
Phosphatidylcholines	696 ±13	696 ±18
Diacyl forms of aminophospholipids	520 ±11	589 ±18
Plasmalogen forms of aminophospholipids	326 ± 6	823 ±21
Sphingomyelins	111 ± 3	244 ± 6
Phosphatidylinositols	144 ± 8	255 ± 3
Phosphatidopeptides	18·8± 1·0	137·5± 5

The total phospholipid content in rat medulla is approximately 1·5 that in the brain hemisphere cortex. As seen from Table 1, about 70% of this difference is accounted for by aminophospholipids, mainly due to their plasmalogen forms; the content of the diacyl forms of aminophospholipids in the medulla is only slightly higher than in the brain cortex. The rest of this difference (about 30%) is accounted for by a higher than in the cortex content of phospholipid fractions such

as phosphatidic acids + polyglycerophosphatides, phosphoinositides, phosphatidopeptides and sphingomyelins in the medulla. There is no difference in the absolute phosphatidylcholine content between the regions compared.

Table 2. Relative specific activity of phospholipid fractions in the cerebral cortex and the medulla of rats (mean of 12—16 exp.)

	Cerebral cortex	Medulla	P*)
Total phospholipids	2·86±0·16	2·25±0·13	<0·01
Phosphatidic acids + polyglycerophoshatides	5·30±0·60	7·14±0·74	0·05<P<0·1
Phosphatidylcholines	1·72±0·09	2·05±0·11	<0·05
Diacyl forms of aminophospholipids	1·40±0·12	1·55±0·13	>0·1
Plasmalogen forms of aminophospholipids	0·75±0·07	0·53±0·05	<0·05
Sphingomyelins	0·38±0·04	0·32±0·02	>0·1
Phosphatidylinositols	18·4 ±1·2	7·05±0·51	<0·001
Phosphatidopeptides	29·9 ±2·0	25·0 ±0·5	>0·1

*) P — statistical criterium of difference of RSA values of each fraction between the cortex and the medulla.

As to the turnover rate of the phospholipid fractions studied (Table 2), metabolically the most active, both in the brain cortex and in the medulla, are phosphatidylinositols, phosphatidopeptides and phosphatidic acid + polyglycerophosphatides, the RSA values of all other fractions being much lower. The order of turnover rate values of the phospholipid fraction obtained was the same in both the regions studied,

phosphatidylinositols > phosphatidic acids + polyglycerophosphatides > phosphatidylcholine > diacyl aminophospholipids > > plasmalogen aminophospholipids > sphingomyelins.

When comparing the RSA values of every phospholipid fraction in the brain cortex and in the medulla it is seen that the most marked difference is in the phosphatidylinositol fraction, the turnover rate of which in the brain cortex is about 2·5 times that in the medulla. The RSA of aminoplasmalogens and phosphatidopeptides in the brain cortex is somewhat higher while that of phosphatidic acids + polyglycerophosphatides and phosphatidylcholines somewhat lower than in the medulla. The intensity of turnover of diacyl forms of aminophospholipids and sphingomyelins is equal in both regions of the brain.

This study has shown both the brain cortex and the medulla to possess a similar collection of phospholipids, however, the content and the turnover rate of the same phospholipid fractions is rather different in those two regions.

The most marked differences are observed with respect to the aminoplasmalogens and phosphatidopeptides, the content of which in the medulla is correspondingly 2·5 and 7·2 times as high as in the brain cortex. It is known that the aminoplasmalogen forms in the brain consist of as much as 95% of ethanolamine plasmalogens (Webster 1960), we can therefore consider that the latter are responsible for the difference in the phospholipids content in the brain regions which markedly differ from each other in the number of myelin structures. Indeed, it has been shown by Norton and Autilio (1966) that the ethanolamine plasmalogens predominate in the myelin among all other individual phospholipids and account for about one third of their total amount.

Another phospholipid the content of which in the medulla is more than twice that in the brain cortex is sphingomyelin. It is of interest that sphingomyelin, like etanolamine plasmalogen, is characterized by quite a low metabolic activity considered up till now to be a peculiar feature of the myelin lipids in general. However it would be wrong, basing only on the increased content of these two lipids in the CNS regions rich in myelin formations, to connect their functions only with myelin. Such a point of view is in disagreement with the results of the present work which show both phospholipids to be sufficiently represented in the brain cortex. The concept of these phospholipids playing a role in nonmyelinated formations of the nervous tissue is supported by the data obtained recently (Freysz et al. 1963, Ivanova et al. 1967) as to the presence of ethanolamine plasmalogens and sphingomyelins in the mitochondrial and microsomal fractions of nervous tissue as well as on the presence of ethanolamine plasmalogens revealed by Johnston and Roots (1966) in isolated neurons of the bovine brain vestibular nucleus.

Attention should be drawn to the fact that the medulla as compared with the brain cortex is characterized not only by an increased content of metabolically low active phospholipids such as aminoplasmalogens and sphingomyelins but also of phospholipids with a high turnover rate, namely phosphatidic acids + polyglycerophosphatides, phosphoinositides and phosphatidopeptides. Moreover, even higher values of RSA in the medulla than in the brain cortex are revealed for several phospholipids, such as phosphotidic acids + polyglycerophosphatides,

phosphatidylcholines. There are data in the literature (Mandel, Nussbaum 1966) indicating the turnover rate of polyglycerophosphatides in the myelin sheath fraction to be twice that in the mitochondrial and microsomal fractions of the rat brain tissue. Besides, it is known that polyphosphoinositides the turnover rate of which is much higher than of other known phospholipids are localized mainly in the myelin structures of the nervous tissue (Eichberg, Davson 1965). All these facts indicate that the concept of the metabolic inertness of myelin lipids at present can hardly be considered correct.

The question arises why the same phospholipids possess different metabolic activity depending on the morphological structuress of the CNS they are localized in. These differences can be accounted for by the difference in the chemical composition of these phospholipids and particularly the different pattern of fatty acids in the phospholipids of white and gray matter (O'Brien 1965). It is also quite probable that the same phospholipids localized in different structures differ in the character and the degree of stability of the bonds with the protein and polypeptide components of these tissue structures which, in turn, definitely influence the metabolic activity of these phospholipids.

At present we are still rather far from a complete understanding of the factors responsible for the different level of metabolic activity of the brain tissue phospholipids in dependence on their localization in some formations of the central nervous system. However, further studies in this field are quite promising because the peculiarites of phospholipid metabolism in the morphologically different structures of the CNS can be imagined to reflect the function of the phospholipids in these structures.

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PORÓWNAWCZE BADANIA LIPIDÓW W RÓŻNYCH MORFOLOGICZNIE STRUKTURACH MÓZGU

Streszczenie

Badano zawartość i intensywność metabolizmu niektórych rodzajów fosfolipidów w korze półkul mózgowych i rdzeniu przedłużonym szczurów. Wybrano do badań struktury istotnie różniące się od siebie zawartością komórek nerwowych i włókien a także stosunkiem istoty szarej do białej, jak i charakterem elementów glejowych. Wykazano, że zawartość wolnych fosfolipidów w rdzeniu przedłużonym jest około 1,5 raza większa niż w korze półkul mózgu, przy czym 70% tej różnicy odnosi się do plazmalowych form aminofosfolipidów, podczas gdy zawartość form dwuacylowych aminofosfolipidów w rdzeniu przedłużonym była niewiele tylko wyższa od zawartości ich w korze półkul.

Zawartość frakcji kwasów fosfatydowych, poliglicerofosfatydów, fosfoinozytoli, fosfatydopeptydów oraz sfingomielin w rdzeniu przedłużonym jest średnio dwukrotnie większa od zawartości w korze; zawartość lecytyn jest jednakowa w obu badanych strukturach.

Porównując wartości względnej radioaktywności właściwej (WRW) największe różnice pomiędzy korą a rdzeniem przedłużonym stwierdzono dla frakcji fosfoinozytoli, której WRW w korze była 2,5 raza większa od WRW rdzenia przedłużonego. Wielkość WRW dla plazmalowych aminofosfolipidów i fosfopeptydów w korze mózgowej jest nieznacznie większa, a dla kwasów fosfatydowych i lecytyn mniejsza niż w rdzeniu przedłużonym.

Intensywność metabolizmu grup fosforowych dwuacylowych fosfolipidów i sfingomielin jest jednakowa w obu badanych strukturach.

W pracy przedyskutowano osobliwości zawartości i metabolizmu poszczególnych frakcji fosfolipidów w różnych morfologicznie strukturach centralnego układu nerwowego.

Д. А. Четвериков, В. Я. Дворкин и С. В. Гастева

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ФОСФОЛИПИДОВ В МОРФОЛОГИЧЕСКИ РАЗЛИЧНЫХ УЧАСТКАХ ГОЛОВНОГО МОЗГА

Содержание

Было изучено содержание и интенсивность обмена отдельных представителей фосфолипидов (ФЛ) в коре больших полушарий и продолговатом мозгу крыс-отделах центральной нервной системы, существенно отличающихся друг от друга по содержанию нервных клеток и проводников, соотношению серого и белого вещества и характеру глиальных элементов. Показано, что содержание суммарных ФЛ в продолговатом мозгу примерно в 1,5 раза выше, чем в коре больших полушарий, причем около 70% этой разницы относится за счет плазмалогенных форм аминофосфолипидов, тогда как содержание диацильных фомр аминофосфолипидов в продолговатом мозгу было лишь незначительно выше, чем в коре больших полушарий.

Содержание фракций фосфатидных кислот + полиглицерофосфатидов, фосфоинозитидов, фосфатидопептидов и сфингомиелинов в продолговатом мозгу в среднем в 2 раза выше, чем в коре; содержание лецитинов одинаково в обоих исследованных отделах.

При сравнении величин относительной удельной радиоактивности (ОУР) наибольшие различия между корой и продолговатым мозгом обнаружены для фракции фосфоинозитидов, величины ОУР которой в коре больших полушарий были в 2,5 раза выше, чем в продолговатом мозгу. Величины ОУР для плазмалогенных аминофосфолипидов и фосфатидопептидов в коре больших полушарий незначительно выше, а для фосфатидных кислот и лецитинов — ниже, чем в продолговатом мозгу.

Интенсивность обмена фосфатных групп диацильных аминофосфолипидов и сфингомиелинов одинакова в обоих отделах мозга. Обсуждаются особенности содержания и обмена отдельных фракций ФЛ в морфологически различных участках центральной нервной системы.

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H. JATZKEWITZ

INBORN ERRORS OF METABOLISM AS A CAUSE OF DEMYELINATING DISEASE

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In examinating inborn errors of metabolism as a cause of demyelinating diseases only metachromatic leukodystrophy and Krabb's disease (globoid cell or globoid leukodystrophy) will be considered.

Both demyelinating diseases are characterized by an abnormal amount of brain white-matter lipids. These lipids belong to the class of sphingolipids and are specific components of the myelin sheath. They are cerebrosides and cerebroside sulphates (= sulphatides). Cerebrosides and cerebroside sulphates, however, are not only constituents of brain white matter but also of other tissues e.g. kidney and liver.

Their level in different tissues is regulated by two different enzymes, an anabolic and a catabolic one (Fig. 1). The activity of the two enzymes determines the turnover of a substance in cellular and subcellular structures such as myelin, oligodendroglia, mitochondria, etc.

Inborn errors of metabolism usually follow the recessive mode of inheritance. Garrod developed the concept that such diseases arise because an enzyme governing a single metabolic step is reduced in activity or missing alltogether.

With this one gene — one enzyme principle in mind, a defect of the structural gene (similar to that verified in the hemoglobin variants) could only produce an accumulation of a metabolic endproduct by causing a defect in its catabolic enzyme (Fig. 1, Fig. 2).

On the other hand, a defect of the structural gene which induces the anabolic enzyme should cause a deficiency of the metabolic endproduct (Fig. 2). One of the two possibilities mentioned (deficiency of the catabolic enzyme) is to be seen in metachromatic leukodystrophy, the other one (deficiency of the anabolic enzyme) possibly in Krabbe's disease (Fig. 2).

In our laboratory it could be shown (Mehl, Jatzkewitz 1965, Jatzkewitz, Mehl 1969) that in metachromatic leukodystrophy a deficiency

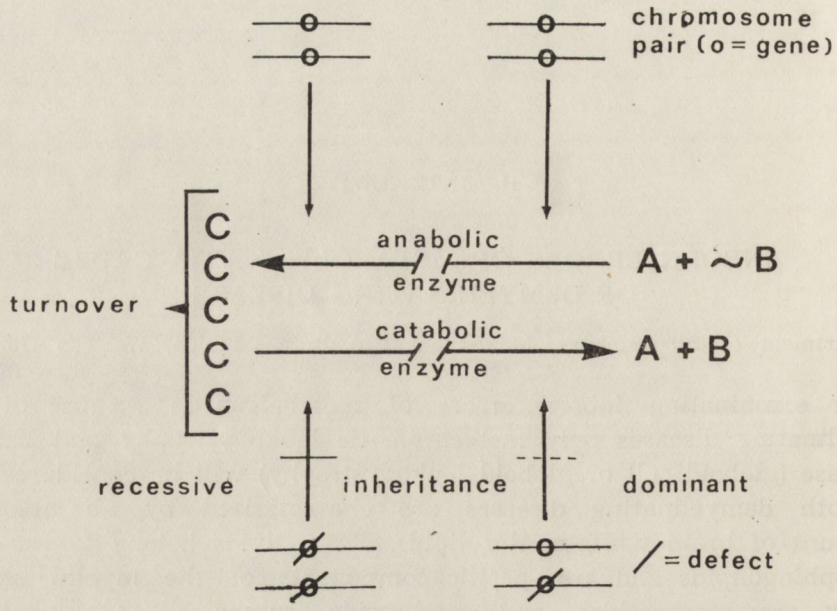


Fig. 1. Metabolic-endoproduct (C) accumulation in diseases caused by deficient structural genes, inducing the catabolic enzyme (left: recessive; right: dominant inheritance).

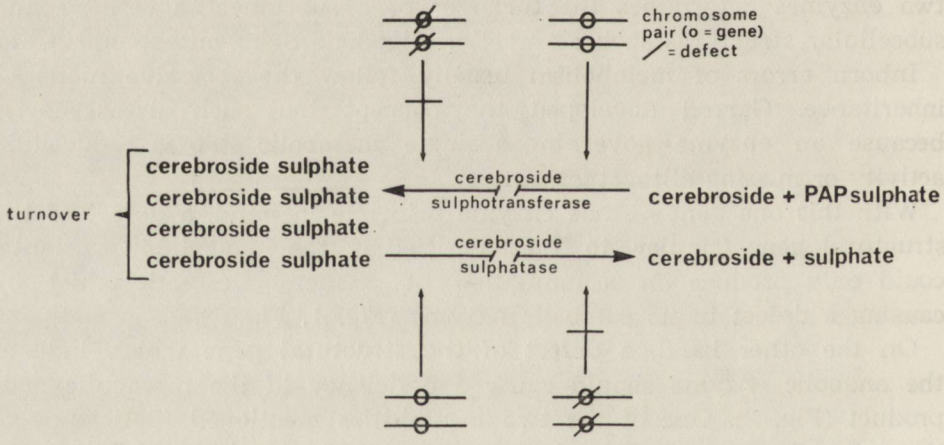
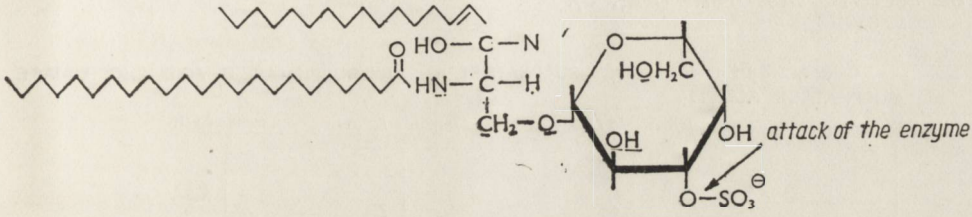
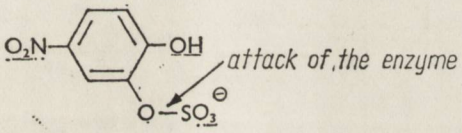


Fig. 2. Diseases caused by deficient structural genes inducing either the anabolic (left) or the catabolic (right) enzyme. Left: Deficient anabolic enzyme (cerebroside sulpho-transferase activity) leading to metabolic-endproduct (cerebroside sulphate) deficiency in patients with Krabbe's (= globoid) leukodystrophy. Right: Deficient catabolic enzyme (cerebroside sulphatase activity) leading to a metabolic-endproduct (cerebroside sulphate) accumulation in patients with metachromatic leukodystrophy. PAPsulphate = phosphoadenosine — phosphosulphate.

in the enzyme cerebroside sulphatase which splits cerebroside sulphate (= sulphatide) into cerebroside and sulphate exists. Accordingly, an accumulation of sulphatides is to be found in brain, kidney and liver, i.e. in all the tissues where sulphatides play a role and a relative deficiency of cerebrosides exists in the brain. In disintegrated cerebral white matter the amount of sulphatides is 2 to 5 times that of normal white matter. On the other hand, in Krabbe's disease sulphatides in disintegrated white matter were nearly absent, due to markedly reduced cerebroside sulphotransferase activity in brain (Bachhawat et al. 1967). This latter enzyme transforms cerebrosides into sulphatides by attaching the activited sulphate grouup (PAPsulphate)to the cerebrosides (Balasubramanian, Bachhawat 1965, McKhann et al. 1965).



Ceramide galactose 3 sulphate
cerebroside sulphate (I)



2-hydroxy-5-nitrophenyl sulphate (II)

Fig. 3. Substrate (I) of cerebroside sulphatase and (II) of its heat-labile component, arylsulphatase A.

In Fig. 3 the chemical structure of the aforementioned substances is shown: cerebroside sulphate, which is accumulated in metachromatic leukodystrophy (the negatively charged SO_3-group is the cause of metachromasia and is responsible for the name) and is deficient in Krabbe's disease, and an unphysiological substance, an arylsulphate (2-hydroxy--5-nitrophenyl sulphate).

These chemical structures are presented for the following reason: In apparent contradiction to the assumption of a single enzyme block in metachromatic leukodystrohpy, Austin et al. (1963) found that an

enzyme, arylsulphatase A, which splits 2-hydroxy-5-nitrophenyl sulphate was also markedly diminished in brain and kidney in this disease. The discrepancy has since been resolved. It has been shown that this arylsulphatase A is a component of the cerebroside sulphatase (Mehl, Jatzkewitz 1968).

The deficiency of cerebroside sulphatase and arysulphatase A was demonstrated in kidney using tissues from 6 patients and 6 controls (Fig. 4). Arylsulphatase B and acid phosphatase activity were determined in order to demonstrate the extent to which the tissues were enzymatically intact. This was necessary since the samples were all obtained by autopsy and stored in a refrigerator, sometimes for years. It was difficult to obtain them, as the usual storage of organs in formalin destroys their enzymatic activity. The same deficiency is to be seen in the brain (Fig. 5).

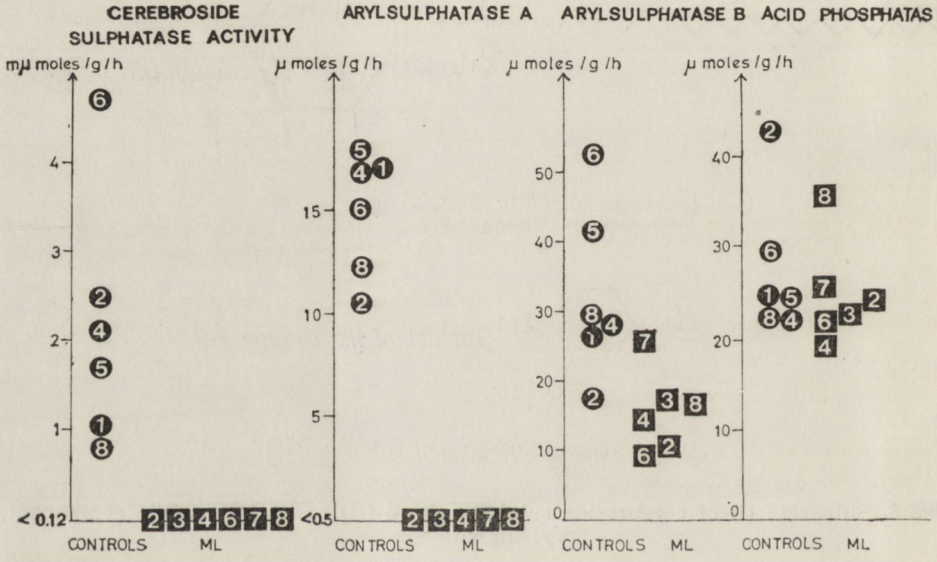


Fig. 4. Deficiency of cerebroside sulphatase activity and arylsulphatase A activity in renal cortex in cases of metachromatic leukodystrophy (ML). Numbers in circles (control patients) and those in squares (ML-patients) refer to the case numbers (see: Jatzkewitz and Mehl, 1969). The enzymic activities are related to fresh tissue weight.

The disease is a generalized one with regard to enzyme deficiency and accordingly to the accumulation product. Therefore, its diagnosis can be made with samples of venous blood by determining the arylsulphatase A activity in leukocyte preparations (Percy, Brady 1968). However, as just mentioned, the disease is a generalized one morpho-

logical alterations (these including disintegration of myelin) being only observable in the white matter of the brain.

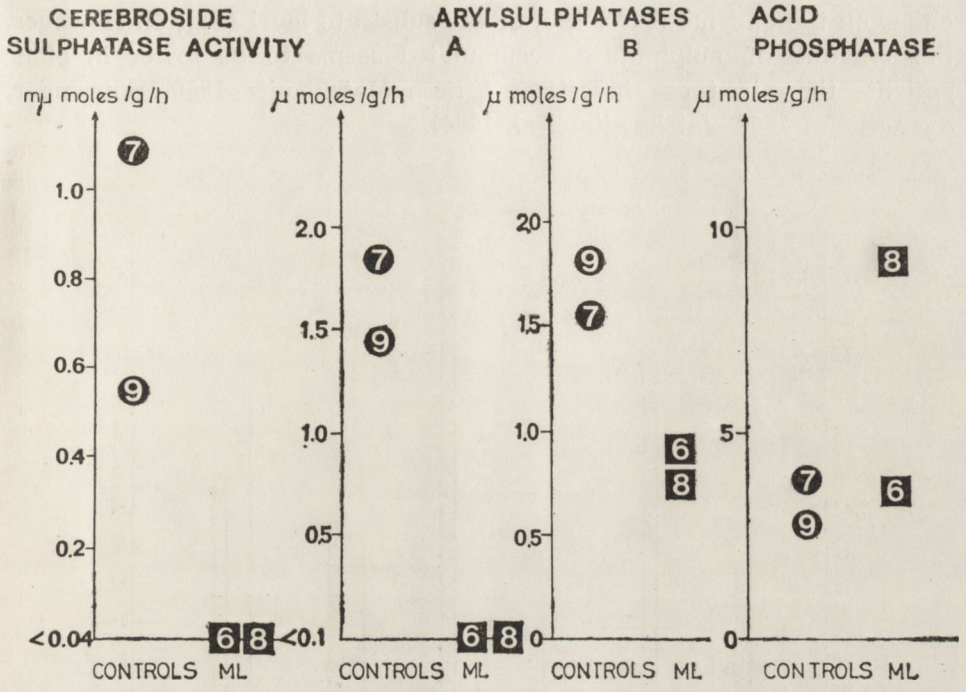


Fig. 5. Defficiency of cerebroside sulphatase activity and arylsulphatase A activity in cerebral white matter in cases of metachromatic leukodystrophy (ML). For further details compare text of Fig. 4.

Morphological examinations indicated that the lysosomes of the oligodendrocytes might be primarily affected in the disease (Résibois 1969). Accordingly, the impairment of the oligodendrocytes directly and/or the induction by the impaired oligodendrocytes of a "false" composition of the myelin could predetermine its later breakdown. For example, an abnormal lipid composition was found in myelin fractions (O'Brien, Sampson 1965, Cumings et al. 1968) which were isolated from autopsy material of patients. The same, namely a "false" composition of the myelin sheath (lack of sulphatides as building blocks), could be the cause for the demyelination in Krabbe's disease.

A final point remains to be clarified. That is the clinical onset and duration of metachromatic leukodystrophy and their relation to the residual catabolic enzyme activity and the amount of the stored sulphatides. Since the disease affects children, young people and adults, it is to be expected that residual cerebroside sulphatase activity

should be higher with increasing age at clinical onset and consequently the accumulation of sulphatides lower. Contrary to these expectations, no such relationships have been found in our cases up to the age of 20, either on the enzyme (Figs. 4, 5) or the substrate level (Fig. 6). However, the amount of sulphatides accumulated seems to be lower in older patients than in those investigated here (Jatzkewitz 1960, Sourander, Svenerholm 1962, Jatzkewitz et al. 1964).

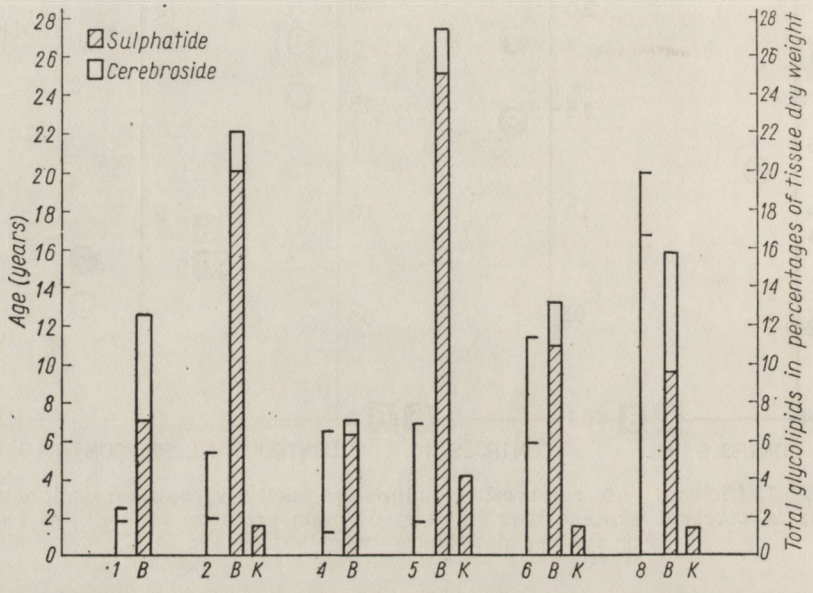


Fig. 6. Total glucolipid content in formalin-fixed brain white matter and kidney of ML-patients, expressed in percentages of dry weight in relation to onset of clinical symptoms and age at death F (years).

Moreover, the disease points to a change from the recessive mode of inheritance to the dominant one (see: Austin et al. 1968). In the aforementioned paper the authors suggest that the lower accumulation of the sulphatides in these cases might be due to a change in the pattern of different multiple forms of the catabolic enzyme (cerebroside sulphatase). Another reason might be that the older patients are heterozygotes (Fig. 1, right) and accordingly only half of the amount of the catabolic enzyme might be defective.

Acknowledgement: I gratefully acknowledge the permission by Pergamon Press to reproduce Figs 4, 5 and 6 from the Journal Neurochemistry.

H. Jatzkewitz

WRODZONE NIEPRAWIDŁOWOŚCI METABOLICZNE JAKO PRZYCZYNA CHOROBY DEMIELINIZACYJNEJ

Streszczenie

Leukodystrofie: metachromatyczna i globoidalna zostały wybrane jako przykłady wrodzonych nieprawidłowości metabolicznych powodujących demielinizację. Defekty enzymatyczne powodujące spichrzanie lub prawdopodobny brak sulfatydów w tych dwu jednostkach chorobowych stanowią podstawę rozważań. Wykazano w leukodystrofii metachromatycznej (nerka i mózg), że enzym katabolizujący odpowiedzialny za rozkład sulfatydów był zredukowany do granic wykrywalności (1—6%) wartości kontrolnych. Ilościowe badania podwyższonego poziomu sulfatydów, spowodowanego brakiem tego enzymu w zdemielinizowanej istocie białej mózgu i w nerkach, nie wykazało związku pomiędzy ilością nagromadzonych sulfatydów a przebiegiem klinicznym oraz czasem trwania choroby lub też wiekiem zejścia śmiertelnego (aż do wieku lat 20). W pracy przedyskutowano rozmaite aspekty genetyczne tego problemu oraz czynniki powodujące wzmożoną chorobę demielinizacyjną.

Г. Яцкевиц

ВРОЖДЕННЫЕ МЕТАБОЛИЧЕСКИЕ ДЕФФЕКТЫ КАК ПРИЧИНА ДЕМИЭЛИЗАЦИОННОЙ БОЛЕЗНИ

Содержание

Метахроматическая и круглоклеточная лейкодистрофии выбраны как примеры врожденных метаболических пороков вызывающих демиэлинизацию. Энзиматические пороки вызывающие нагромождение или же вероятный нехватоκ сульфатидов в обеих болезнях явились основой рассуждений. Показали, что в метахроматической лейкодистрофии (мозг и почки) катаболизирующий фермент ответственный за разложение сульфатидов был редуцирован до границы определяемости (1—6%) контрольных величин. Количественное исследование повышенного уровня сульфатидов вызванного недостатком упомянутого фермента в демиэлинизированном белом мозговом веществе не потвердило существования связи между количеством накопленных сульфатидов а клиническим течением и временем протекания болезни или возрастом смертного исхода (до возраста 20 лет). В работе обсуждаются разные генетические аспекты этого вопроса как и факторы вызывающие усилённую демиэлинизационную болезнь.

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NEUROPAT. POL. 1969, VII, 3

P. BORRI, G. MACCHI, M. TARAMELLI

FATTY ACID COMPOSITION OF BRAIN CHOLESTEROL ESTERS IN SOME NEUROLOGICAL DISORDERS WITH MYELIN INVOLVEMENT

(Preliminary communication)

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It is known that in demyelinating diseases cholesterol esters are present in various amounts, while in normal tissue they are practically absent (Cumings 1953, 1955, Rodnight 1959, Davison, Wajda 1962).

Also in Wallerian degeneration the formation of cholesterol esters is one of the most characteristic features. Cholesterol esters have been reported in what is known as primary demyelinating diseases, such as multiple sclerosis, leucodystrophies and in neurolipidoses.

On the other hand changes have been found in the amount of individual lipids, and of total fatty acids, and in the fatty acid composition. Summing up the results reported by several authors in multiple sclerosis it appears that the main chemical abnormalities concern a decrease of plasmalogens, cholesterol and sphingolipids (mainly sphingomyelin).

Moreover, as far as fatty acids are concerned, the content of long-chain saturated fatty acids (both unsubstituted and hydroxy) and unsaturated fatty acids (polyunsaurated and monoenoic, mainly oleic) is decreased (Davison, Wajda 1962, Gerstl et al. 1963, 1965, Cumings et al. 1965, Amaducci et al. 1965, Kishimoto et al. 1967).

The present research was undertaken to get an insight into the phenomenon of cholesterol esters formation in brain white matter during demyelination.

Our data concern quantitative cholesterol esters determination and their fatty acid composition in white matter specimens from cases of multiple sclerosis. A few data are also reported on cholesterol esters fatty acid composition in noninflammatory diseases, such as meta-

chromatic leucodystrophy, gargoylism, sudanophilic leucodystrophy and infantile amaurotic idiocy.

MATERIALS AND METHODS

Specimens of white matter from 5 normal adult brains, from 4 brains of patients who died of multiple sclerosis, and from one case of metachromatic leucodystrophy and one of infantile amaurotic idiocy were taken for analysis, all from deep-freeze stored brains, preferentially from the same regions of the centrum semiovale.

Three specimens were taken from each multiple sclerosis brain.

Lipids were extracted twice with chloroform-methanol (2 : 1 v/v). Part of the lipid extract was used for total cholesterol, phosphorus and hexoses determinations and proteolipid evaluation. About 20 mg of lipid were applied on preparative silica gel G. Merck plates and developed with petroleum ether-ethyl ether (80 : 20 v/v). The plates were then sprayed with bromthymol blue in water-ammonia solution.

Cholesterol and cholesterol esters bands were scraped off and extracted by threefold washings with chloroform.

The cholesterol esters/cholesterol ratio was evaluated by the Liebermann-Burchard test in known amounts of both eluates. Cholesterol esters were partly applied on silica gel F 254 Merck plates, developed 3 times with heptane-toluene (60 : 25 v/v) according to Alling et al. (1968) to visualize the individual cholesteryl esters fractions, partly concentrated and again applied on preparative silica gel plates developed with the same solvent, sprayed with bromthymol blue, and scraped off. The cholesterol esters were extracted as described above, transmethylated with half-saturated methanolic-hydrochloric acid, taken up in hexane and injected into a Carlo Erba type AID fractovap gas-chromatograph using 20% butanediol succinate on chromosorb W columns at working temperature of 183°C and N_2 pressure of 0·80 atm.

Some silica gel plates without any lipid applied were treated in the same way and the chloroform-extractable substances from silica gel surfaces comparable to that scraped off in the case of cholesterol esters were injected into the gas-chromatograph as well.

This was done in order to reduce background interference which seemed to be of some importance when the cholesterol esters amount was found to be very low, such as in normal white matter and in some pathological specimens. Unknown substances gave peaks with carbon number corresponding to C14:0, C15:0, C16:0, C16:2, C18:0, C18:1, C20:1 fatty acids. The relative proportion was found by repeated observa-

tions to be rather constant, and since an accurate determination of the amounts of both C16:0 and C18:1 fatty acids of cholesterol esters was of basic importance, it seemed necessary to substract in each chromatogram the part of the C16:1 and C18:1 peak due to the background.

Correction was done by evaluating in each cholesterol esters chromatogram the peak with carbon number C16:2 which was found to be the highest peak of the background chromatogram, and which did not correspond to any fatty acid known to occur in a significant amount in cholesterol esters. The height of this peak was taken as the index of contamination and, when present in a chromatogram of cholesterol esters in amounts exceeding 1/15 of the height of the corresponding peak in the sillica gel-solvent background chromatogram, a correction was done, because the C16:0 peak of contaminants exceed that of the C18:1. The C14:0 and C15:0 peaks of cholesterol esters were neglected owing to the large amounts of contaminants.

RESULTS

Table 1 reports the cholesterol esters/cholesterol ratio and the fatty acid composition of brain white matter cholesterol esters from 5 normal adults, 7 out of the 12 multiple sclerosis specimens examined, 2 samples of infantile amaurotic idiocy white matter, and one each from metachromatic leucodystrophy, gargoylism and sudanophilic leucodystrophy cases.

Cholesterol esters in normal adult brain have been found to be present in amounts which, although detectable on TLC after concentration, are very low: about 0·2% of total cholesterol.

Identification of fatty acid higher than C20:4 was only tentative. Fatty acids were identified by calculating their carbon number and using references of main saturated, linolic and arachidonic fatty acid.

Data for normal values in adults are reported as mean values for each fatty acid, together with the standard deviations. Cholesterol esters fatty acid composition of 7 out of 12 multiple sclerosis specimens are also reported, arranged in a sequence based on the degree of esterification of cholesterol found in each specimen, regardless of factors such as brain source and amounts of plaques and of apparently normal white matter.

However, white matter specimens from the same brain showed similar cholesterol esters amounts in all 3 experiments. Data referring to remaining 5 multiple sclerosis specimens are omitted in Table 1 because they are strictly similar to some of those reported, both as cholesterol esters content and fatty acid composition.

Table 1

% Cholesterol esters	5 Normal adults (18—43 years old)		MS specimens (4 brains, from 25—54 y. old patients)							M.L. 7 y.	Garg. 15 y.	Ort.L. 25 y.	IAI 3·5 y.	
Free cholesterol	0·2		1·2	1·4	7·1	10·2	14·7	31·2	38·1	1·0	1·9	2·7	5·0	4·0
Fatty acid	mean ± S. D.													
C16 : 0	33·6	5·4	30·4	30·8	21·9	21·5	21·0	20·7	20·0	50·1	42·2	—	24·4	25·9
C16 : 1	9·0	4·1	9·8	5·9	4·9	3·3	11·0	2·4	2·7	6·4	4·2	13·0	6·0	5·3
C18 : 0	6·1	3·6	3·6	6·7	6·9	4·9	4·2	2·5	7·0	5·3	9·5	5·1	4·4	4·5
C18 : 1	29·2	5·1	36·4	41·4	41·2	46·5	48·0	52·1	48·9	22·0	33·8	28·3	57·6	55·2
C20 : 1	1·0	0·6	—	0·7	2·2	2·0	1·1	2·1	3·0	7·7	1·5	2·8	0·7	1·0
C20 : 2	tr.		—	—	1·8	0·5	1·2	2·3	0·7	—	—	—	—	—
C20 : 3	tr.		2·4	—	0·8	—	2·5	3·1	0·3	—	—	—	—	—
C20 : 4	12·2	4·6	8·5	3·7	6·1	6·9	5·0	4·1	4·1	0·9	2·8	1·4	1·8	2·2
C22 : 1	1·3	1·0	0·6	1·5	0·9	0·7	—	—	0·7	1·9	1·1	1·0	0·5	0·5
C22 : 3	1·2	1·2	tr.	—	1·9	0·8	0·7	0·9	1·0	tr.	0·7	1·3	tr.	tr.
C22 : 4	1·8	1·3	6·7	—	3·9	3·3	0·6	1·0	3·8	1·9	4·1	1·7	3·6	4·2
C22 : 5 (n − 3)	2·8	1·3	1·3	6·1	4·5	6·0	3·5	5·4	4·7	2·9	—	2·2	1·0	1·2
C22 : 6	2·0	1·5	0·4	3·1	3·0	3·5	1·0	3·5	3·0	3·0	—	9·4	tr.	tr.

Fatty acid composition of cholesterol esters in white matter

Also two determinations of cholesterol esters fatty acid from an infantile amaurotic idiocy brain are reported, together with one determination from a case of gargoylism, of metachromatic leucodystrophy and of sudanophilic leucodystrophy. The latter results should therefore be considered as preliminary. Figure 1 shows the cholesterol ester fractions on TLC according to Alling et al. (1968). 1 refers to human plasma cholesterol esters, in which the main band has been demonstrated (Alling et al. 1968) to correspond to cholesterol linoleate. 2 and 4 refer to cholesterol esters from MS and infantile amaurotic idiocy respectively: in both the main band corresponds to that of monoenoic fatty acid in accordance with GLC fatty acid determination.

The normal cholesterol esters pattern (3) shows main bands corresponding to saturated and monounsaturated fatty acids, confirming once more GLC analyses where C16 : 0 and C18 : 1 have been found to be the main fatty acid.

DISCUSSION

Data concerning white matter cholesterol esters fatty acid are rather scanty in the literature: Cumings et al. (1965) report in MS white matter a cholesterol esters fatty acid composition which qualitatively corresponds to that reported by us. However it seems that the percentual content of individual fatty acids in MS cholesterol esters corresponds to our normal values, where the main fatty acids are C16:1, while in our MS specimens the C18:1 is, by fair the dominating fatty acid.

Gerstl et al. (1966) in a case of sudanophilic leucodystrophy although tentatively identifying fatty acids higher than C20:4 as long-chain saturated and monounsaturated up to C25:0, give a qualitative and quantitative pattern of the main white matter cholesterol esters fatty acids corresponding to that observed by us in MS and infantile amaurotic idiocy, where the main fatty component is oleic acid. These authors emphasize the high amount of this fatty acid in brain cholesterol esters as compared with the typical higher content of linolic acid of human plasma.

As far as our normal values are concerned, several determinations give evidence of a higher and almost similar content of C16:0 and C18:1 fatty acids, arachidonic acid giving values around 12%. It should however be pointed out that a rather high degree of variability of values has been observed, as indicated by the standard deviations, probably in connection with the difficulty in obtaining preparations free of contaminants. Further work should be done in this field. The fatty acid

pattern of normal white matter cholesterol esters sharply differing from that of plasma cholesterol esters excludes the possibility that the pattern of normal brain cholesterol esters fatty acid might be contamined by blood remnants in white matter; moreover, cholesterol esters in erythrocytes are known to be absent.

From specimens with high cholesterol esters/cholesterol ratio where the effect of impurities is excluded, the pattern of fatty acids esterified with cholesterol in pathological conditions may be definitively established. However, three groups of cholesterol esters fatty acid patterns may be seen: those of normal adult brain, with 3 main fatty acids, palmitic, oleic and arachidonic, those of samples with high cholesterol esters content showing oleic acid content around 50%, and a third group of pathological specimens with lower cholesterol esters content, where an intermediate pattern is seen.

In the second group both "inflammatory" diseases such as multiple sclerosis and degenerative diseases, such as infantile amaurotic idiocies, are included, indicating that cholesterol esters formations in nervous tissue is an aspecific reaction to demyelination regardless of the agent. It might be pointed out that in infantile amaurotic idiocy, with only about 5% cholesterol esters, as compared with about 40% in some MS specimens, the amount of C18:1, which is the fatty acid preferentially esterified by cholesterol in pathological specimens, is higher than in all MS specimens. Whether this might be simply due to the different age of the patients and to the different brain lipid composition or to some difference between inflammatory and degenerative demyelination, as far as cholesterol esters formation is concerned, is not possible to ascertain. Further work seems necessary also in this field for instance the determination of brain cholesterol esters fatty acid in infants.

Similar conclusions may be drawn from the third group of cholesterol esters fatty acid patterns, where both MS specimens and degenerative diseases are included.

All specimens of this group had a lower content of cholesterol esters, about 1—3% although they differed less as far as age is concerned starting from 7 years in the case of metachromatic leucodystrophy. As already pointed out, data concerning some of the cases reported have to be considered as preliminary owing to the fact that only one cholesterol esters fatty acid determination was done.

As far as the determination of cholesterol esters content and of their fatty acid pattern in 4 brains from multiple sclerosis is concerned, it is worthwhile to point out that fatty acid composition, when a small amount of esterified cholesterol was present in white matter, did not

differ much from normal values, but starting from 7% concentration up to about 40% a similar pattern was observed, showing that oleic acid concentration remained stable arounud 50%, even when cholesterol esters amounts increased in the tissue.

It seems that when a certain amount of cholesterol esters is present in the tissue, a different type of cholesterol esters synthesis or a different source of fatty acid available for esterification with cholesterol is involved.

A brief discussion on two points raised by our results may be of some importance: 1) It is possible that the fatty acid pattern with very low amounts of normal brain cholesterol esters, as seen by gas-chromatographic analysis might be in some way affected by nonfatty acid contaminants.

However, the separation of cholesterol esters components in TLC (Fig. 1) shows that in normal white matter, cholesterol esterified with saturated fatty acids is present in higher amounts, in accordance with

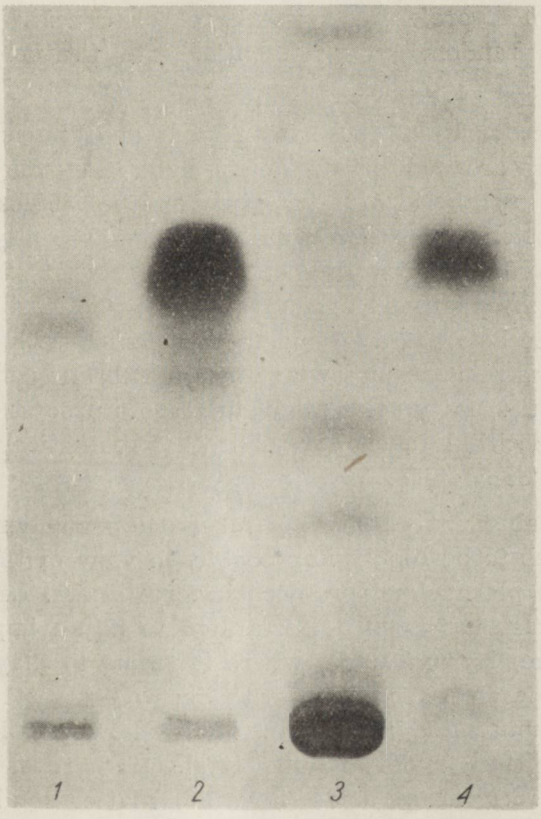

Fig. 1. TLC of cholesterol esters components of human plasma *(1)*; MS white matter *(2)*; normal adult white matter *(3)*; infantile amaurotic idiocy *(4)*. See text.

GLC, where C16:0 is the main fatty acid. 2) The highest cholesterol esters content was found in a case of what is known as "acute" MS, a girl 25 years old, where mostly plaques of recent type were seen in histological examination. Other cases showed mostly "old" plaques. It seems therefore, that the highest esterified cholesterol content may be linked with the clinical form of the disease and with a more recent type of plaque and recent demyelination. However, the fatty acid pattern was found to be almost identical in all cases. If we now take in account that the total white matter cholesterol was drastically decreased in all specimens analyzed, reaching a maximum of 25% depletion, and that in the extreme case of cholesterol esters formation there is about 200 times larger cholesterol esters amount than in normal, it results, from gas-chromatographic analysis, that not only oleic acid, but also several other fatty acids are esterified in greater amounts with cholesterol in pathological specimens. Fatty acids seem to be taken up, probably from glycerol-phospholipids mostly randomly, with the exception of a preferential uptake of oleic acid. Both processes seem to occur random and preferential esterification of cholesterol oleate.

As far as the source of these fatty acids and the new synthesis of cholesterol esters are concerned, several hypotheses may be suggested: 1) an ex-novo synthesis of fatty acids on the cholesterol substrate, 2) cholesterol esters migration from blood, as recently demonstrated for free cholesterol by Chevallier et Giraud (1966), 3) fatty acid might be freed from phospholipids by activated phospholipase A (acting preferentially on the beta position) and then esterified with free cholesterol which has lost its relationships with other membrane components owing to demyelination, 4) fatty acids might undergo a process of direct transacylation from lecithin to cholesterol by a hypothetical brain lecíthin--cholesterol acyltransferase.

The first metabolic mechanism is far from being demonstrated, the second one seems to be highly improbable, in view of the clear-cut difference between plasma and brain cholesterol esters. Lecithin-cholesterol acyl-transferase has been demonstrated to be an important enzyme in plasma (Gjone, Norum 1968), where it seems to play a role in the transport of cholesterol from tissues to liver (Glomset 1968) but it has never ben demonstrated in brain. It is, however, interesting that Cumings et al. (1965) and Amaducci et al. (1965) report a preferential depletion of C18:1 fatty acids in white matter total phospholipids and lecithins in multiple sclerosis; this supplementing our report on the C18:1 increase in white matter cholesterol esters in the same

disease. The mechanism postulating phospholipase A activation might therefore be involved both in the C18:1 depletion of phospholipids reported by these authors and the preferential C18:1 uptake in newly formed cholesterol esters in demyelination.

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SKŁAD KWASÓW TŁUSZCZOWYCH MÓZGOWYCH ESTRÓW CHOLESTEROLU W NIEKTÓRYCH ZABURZENIACH NEUROLOGICZNYCH Z USZKODZENIEM MIELINY

Streszczenie

Przeprowadzono analizę estrów cholesterolowych kwasów tłuszczowych na materiale autopsyjnym istoty białej mózgu z przypadków stwardnienia rozsianego, dziecięcej choroby Tay-Sachsa, leukodystrofii metachromatycznej i maszkaronizmu i porównano wyniki ze składem kwasów tłuszczowych estrów cholesterolu z prawidłowej istoty białej. Główne różnice dotyczą kwasów monoenowych i nienasyconych.

Podjęto też próbę określenia bezwzględnych ilości estrów cholesterolu i innych lipidów mózgu celem upewnienia się o istnieniu współzależności pomiędzy spadkiem zawartości niektórych kwasów tłuszczowych w fosfolipidach i wzrostem ich zawartości w grupie estrów cholesterolu.

П. Борри, Г. Макки, М. Тарамэлли

СОСТАВ ЖИРНЫХ КИСЛОТ ЭФИРОВ ХОЛЕСТЕРИНА В НЕКОТОРЫХ НЕЙРОЛОГИЧЕСКИХ РАССТРОЙСТВАХ ПРОТЕКАЮЩИХ С МИЭЛИНОВЫМ КОМПОНЕНТОМ

Содержание

Проведен анализ жирных кислот эфиров холестерина на секционном материале было вещества головного мозга со случаев рассеянного склероза, детской амауротической идиотии, метароматической лейкодистрофии и гаргоилизма. Результаты сравнены с составом жирных кислот эфиров холестерина нормального белого вещества мозга. Главные различия касаются алифатических и ненасыщённых жирных кислот.

Предпринята попытка определения абсолютных количеств эфиров холестерина и других мозговых липидов целью потверждения существования взаимосвязи между снижением содержания некоторых жирныж кислот фосфолипидов и увеличением их содержания в эфирах холестерина.

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STUDIES ON THE SPECTRUM OF GLOBULINS IN ENCEPHALOMYELITIS DISSEMINATA

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Spectrum of γ-globulins from the cerebrospinal fluid studied by paper-electrophoresis shows in multiple sclerosis an increase in 55——65% of all cases. In cases of MS with all classical symptoms this γ-globulin increase reaches even 75% of them. The results of electrophoretic investigation of serum are different. In literature the data concerning this subject are controversial. Many authors have found a small γ-globulin increase, others report normal values.

In evaluation of our agar-electrophoretic results in 79 cases of multiple sclerosis we tried to distinguish in the course of the disease an acute, subacute and chronic form. Later statistical evaluation of the results of the fluid and serum agar electropherograms was carried out by the U or *T*-test.

T = 1,98 corresponds to a 5% probability. Calculation of the normal values was done on the basis of 23 cerebrospinal fluids and 40 sera from patients without any neurological and psychiatric symptoms. The results concerning cerebrospinal fluid were normal. Table 1 shows the results of c.s.f. agar pherograms of 16 acute multiple sclerosis patients. They show a statistically significant increase of the γ_2- to γ_5-globulin fraction with a peak of γ_3.

Table 2 shows in 28 subacute cases a significant increase of γ-globulin fraction including the γ_1-globulin band. Here also the peak lies at the γ_3-globulin fraction.

Table 3 demonstrates in 35 chronic cases the same rise of γ-globulin content with a γ_3-globulin peak.

Pathological mobilities are not present in these three groups. A graphical demonstration of the increased γ-globulin fractions (Fig. 1) in the acute, subacute and chronic course shows only slight differences in the position of γ-globulin peaks.

Table 1. Statistical evaluation of the spinal fluid agar pherograms in acute multiple sclerosis

	M n = 23	M n = 16	T
V	4·81	4·34	0·83
Alb.	69·12	61·93	1·61
α_1	4·85	**3·79**↙	2·11
α_2	4·68	4·24	0·29
β	7·61	6·66	1·53
δ	3·88	3·92	0·29
γ_1	1·23	1·48	0·62
$\gamma_{1,}$	0·92	1·13	1·31
γ_2	0·92	**2·63**↗	**3·04**
γ_3	1·33	**5·37**↗	**4·30**
γ_4	0·46	**2·62**↗	**3·59**
γ_5	0·17	**0·78**↗	**3·17**

n = Number of cases; M = Mean value in relation %.

Table 2. Statistical evaluation of the spinal fluid agar pherograms in chronic multiple sclerosis

	M n = 23	M n = 28	T
V	4·81	5·54	0·97
Alb.	69·12	**59·60**↙	**3·90**
α_1	4·85	4·83	0·70
α_2	4·68	5·18	0·89
β	7·61	**6·50**↙	**2·44**
δ	3·88	4·23	0·66
γ_1	1·23	1·36	0·76
$\gamma_{1,}$	0·92	**1·22**↗	**3·70**
γ_2	0·92	**2·17**↗	**3·09**
γ_3	1·33	**5·82**↗	**5·00**
γ_4	0·46	**2·62**↗	**4·02**
γ_5	0·17	**0·91**↗	**3·63**

n = Number of cases;
M = Mean value in relation %

Table 3. Statistical evaluation of the spinal fluid agar pherograms in chronic multiple sclerosis

	M n = 23	M n = 35	T
V	4·81	5·62	1·01
Alb.	69·12	**61·14**↙	**4·01**
α_1	4·85	4·37	0·65
α_2	4·68	5·51	1·85
β	7·61	6·91	1·82
δ	3·88	4·16	0·24
γ_1	1·23	1·74	0·06
$\gamma_{1,}$	0·92	**1·41**↗	**2·90**
γ_2	0·92	**1·80**↗	**2·29**
γ_3	1·33	**4·18**↗	**4·66**
γ_4	0·46	**2·28**↗	**3·09**
γ_5	0·17	**0·54**↗	**2·48**

n = Number of cases;
M = Mean value in relation %

Statistical comparison of the significantly increased fractions, is not significant except for a α_1-globulin diminution in acute cases (Table 4).

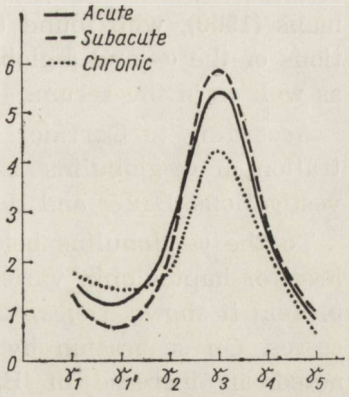

Fig. 1. Correlation of γ-globulin fractions in acute, subacute and chronic multiple sclerosis forms.

The globulin spectrum of the c.s.f. fractions shows in all courses a maximum in the γ_3-part inclusively with an increase of γ_1- to γ_5-globulin bands. The single case of γ_3-globulin augmentation reported in the literature, is difficult to interpret. The three forms show largely conform behaviour of the globulin spectrum so that the antibody production seems in all stages to be the same with slight differences.

Table 4. Statistical comparison of the significant fractions in the different disease types

	M acut	M subacut	T		M acut	M chron	T		M subacut	M chron	T
Alb.	61·93	59·60	0·33	Alb.	61·93	61·14	0·48	Alb.	59·60	61·14	0·31
α_1	3·79	4·83	2·56	α_1	3·79	4·37	2·64	α_1	—	—	—
β	6·66	6·50	0·90	β	—	—	—	β	6·50	6·91	1·12
γ_1	1·13	1·22	0·77	γ_1	1·13	1·41	1·26	γ_1	1·22	1·41	0·91
γ_2	2·63	2·17	0·79	γ_2	2·63	1·80	1·37	γ_2	2·17	1·80	1·03
γ_3	5·37	5·82	0·33	γ_3	5·37	4·18	0·90	γ_3	5·82	4·18	1·17
γ_4	2·62	2·62	0·18	γ_4	4·28	2·62	0·87	γ_4	2·62	2·28	0·98
γ_5	0·78	0·91	0·45	γ_5	0·78	0·54	0·91	γ_5	0·91	0·54	1·16

M = Mean value in relation %

In view of the normal γ-globulin values in the serum it is probable, that the pathophysiological action of multiple sclerosis is predominantly limited to the pia-arachnoidal space and the brain. The participation of the other part of the reticulo-endothelial system as for instance in panencephalitis and syphilitic disease is not relevant.

Studies with labeled globulins show an autologous γ-globulin building in within the liquor spaces, which can increase up to 90% of the total liquor γ-globulin. γ-Globulin bands could only be visualized in single cases in contrast to the findings of Lowenthal (1964) and Here-

mans (1960), who found this fraction very often increased. The alterations of the α- and β-globulins in cerebrospinal fluid are not significant as well as in the serum.

According to Corridori and Pellegrini (1960) an increase in concentration of α_2-globulins is demonstrated in paper electrophoretic investigations. Bauer and Heitmann (1958) could not confirm this is finding.

To the α_2-globulins belong α_2-macro-globulin and haptoglobins. The level of haptoglobin varies in relatively wide limits. As an acute-phase protein it shows a clear increase of concentration in inflammatory processes. On separation by means of polyacrylamide electrophoresis we noted an increase of Hp 1—1 content in the sera of 1—1 and 2—1 multiple sclerosis patients. In our investigations we found all three main types 1—1, 1—2, and 2—2— so that no sure relation could be established between the course of disease and Hp-type. For information Hp-bands in the acrylamid pherogram are shown schematically in Fig. 2. Beside the normal spectrum, in patients with multiple sclerosis, we found in the 1—2 type from the cerebrospinal fluid an additional band.

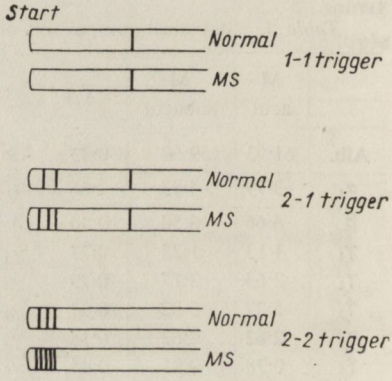

Fig. 2. Scheme of polyacrylamid electrophoretic separation of haptoglobin bands in cerebrospinal fluid of multiple sclerosis patients.

In the 2—2 type there exist even five bands. Our material is at the moment relatively small, so that there was no possibility for statistical analysis. In contrast to this fact, the findings may be important in view of a strengthened function of the barrier. Cendrowski and Sniguowicz (1964) have found in the serum of MS-patients a significant increase of total haptoglobins what stands in agreement with the few increased α_2-fractions in serum electropherograms. The disturbance of the barries is in this illness acceptable as studies by means of immunoelectrophoresis show in c.s.f. an increase of the anodic part of the γ G-line and the existence of a γ-A-band. We state an agreement of

our results with the findings of Schrader and Weise and also Rowlan and Randt.

The results of our electrophoretic studies of the cerebrospinal fluid and blood serum in MS cases may be summarized as follows:

1. The characteristic kinetics (sharp peak) of γ-globulin increase, with a maximum corresponding to γ-3 globulins seems to be of diagnostic importance.

2. No special patterns of γ-globulin increase, associated with various courses of the disease have been detected; it seems therefore likely that there is a continuity of antibody synthesis through the acute to the chronic type of disease.

3. No significant increase of blood serum γ-globulins could be demonstrated.

4. A slight increase of α-globulins in blood serum may depend, among other things, upon the increase of haptoglobin content.

5. In the cerebrospinal fluid of both Hp 1—2 and Hp 2—2 patients, additional Hp bands occur. This may indicate that the barrier function is disturbed.

R. M. Schmidt, H. Diessner

BADANIA GLOBULIN W ROZSIANYM ZAPALENIU MÓZGU I RDZENIA

Streszczenie

1. Charakterystyczna kinetyka w formie ostrego szczytu wzrostu γ-globulin z najwyższym punktem odpowiadającym γ-3 globulinom wydaje się mieć znaczenie diagnostyczne.

2. Nie zaobserwowano szczególnych prawidłowości wzrostu γ-globulin w skojarzeniu z różnymi typami przebiegu choroby; wydaje się przeto prawdopodobne, że istnieje ciągłość syntezy przeciwciał w czasie ostrego i przewlekłego typu choroby.

3. Nie udało się wykazać znamiennego wzrostu γ-globulin w surowicy krwi.

4. Niewielki wzrost γ-globulin w surowicy krwi może zależeć m. in. od zwiększenia ilości haptoglobin.

5. W płynie mózgowo-rdzeniowym zarówno u nosicieli Hp 1—2 jak i Hp 2—2 pojawia się dodatkowe pasmo Hp. Może to wskazywać na uszkodzenie czynności bariery.

Р. М. Шмидт, Г. Дисснер

ИССЛЕДОВАНИЕ ГЛОБУЛИНОВОГО СПЕКТРА В РАССЕЯННОМ ЭНЦЕФАЛОМИЭЛИТЕ

Содержание

1. Характеристическая кинетика в форме острого пика увеличения γ-глобулинов с верхушкой ответствующей γ-3-глобулинам имеет, кажется, диагностическое значение.

2. Не наблюдали особенных правил увеличения γ-глобулинов в сочетании с разными типами течения болезни; вероятно затем, существует непрерывный синтез антител во время острого и хронического типа болезни.
3. Не удалось выявить существенного увеличения γ-глобулинов в кровяной плазме.
4. Небольшое увеличение содержания γ-глобулинов в плазме крови может быть зависимо м.пр. от увеличения количества гаптоглобинов.
5. В спинномозговой жидкости так у носителей Hp 1—2 как и Hp 2—2 появляется добавочная полоса Hp. Это может указывать на повреждение функции барьера.

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CEREBRAL VASCULAR PERMEABILITY IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS *)

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease in which circulating antibody and sensitised cells are produced against a basic protein component of myelin. Most of the evidence suggests that the lesions are due to specifically sensitised lymphocytes, although the role of circulating antibody is still disputed. Delayed hypersensitivity skin reactions to the encephalitogenic protein correlate with the onset and severity of the disease (Shaw et al. 1965), while circulating antibody titres do not (Caspary 1966). EAE has also been transferred to normal animals with suspensions of lymph node and spleen cells (Paterson 1960, Stone 1961, 1968). Serum on the other hand, is ineffective (see Chase 1959) and may even exert a protective effect when injected into rats developing the disease (Paterson et al. 1965).

However, there is some evidence that circulating antibody may play a role in the production of the lesion. Bornstein has shown that serum from animals with EAE will damage myelin and gial cells in tissue culture (Bornstein, Appel 1961, Lamoureux et al. 1966) and Peterson found this "myelinolytic antibody" in the serum of recipient animals after passive cellular transfer (Peterson 1963).

There are also a number of reports suggesting that the increase in cerebral vascular permeability occurring in this disease precedes the appearance of inflammatory cells in the central nervous system. They include autoradiographic studies with ^{131}I and ^{125}I-labelled albumin and — more recently — immunofluorescent localisation of serum proteins in the brain parenchyma (Vulpe et al. 1960, Cutler et al. 1967, Oldstone, Dixon 1968). The demonstration of a vascular leak before the entry of inflammatory cells into the brain and cord would consti-

*) Supported by the Multiple Sclerosis Society (U.K.).

tute important evidence that the lesion of EAE was initiated by antibody rather than by sensitised cells. Since most of the information on this point is derived from qualitative observations made on tissue sections, we have re-examined the problem with a quantitative method using protein labelled with two different radioisotopes.

MEASUREMENT OF PERMEABILITY

The cerebral vessels are relatively impermeable to protein and changes in permeability can be detected by measuring the rate of accumulation of such protein in the extravascular compartment. If a circulating serum protein is labelled with a suitable isotope, the site and severity of the leak can be recognised by the rise in the radioactivity of the affected part that is caused by the extravasation of the labelled compound. A refinement of this technique is to make an allowance for the amount of intravascular isotope by measuring the cerebral blood volume. This is done by injecting protein, labelled with a second isotope immediately before the animal is killed.

In the present investigation female albino guinea pigs were sensitised by the intradermal injection of an emulsion of Freund's complete adjuvant and a suspension of guinea pig whole brain. Each animal received 1 mg of guinea pig brain "dry weight" and 2 mg of M. butyricum. Cerebral vascular permeability was measured in groups of animals sacrificed at intervals after sensitisation and the brain and cord examined for histological evidence of EAE.

Permeability was estimated by injecting ^{131}I-labelled human serum albumin intravenously followed 24 hours later by an injection of ^{125}I-labelled albumin. Immediately after the second injection a blood sample was taken, the animal killed and the brain and cord fixed in formol saline. The radioactivity due to each isotope was estimated in the blood, and in blocks taken from the fore-, mid- and hind-brain and the cerebellum, as well as the cervical, lumbar and thoracic regions of the spinal cord.

The results in the brain and cord were expressed as a ratio of the count in the blood ("Blood equivalents"). Under the conditions of the experiment the ^{125}I blood equivalent represented the blood volume of the specimen. On the other hand, the ^{131}I blood equivalent reflected both intravascular and extravascular ^{131}I-labelled protein. The difference between the two (^{131}I.B.E. — ^{125}I.B.E.) was therefore a measure of extravascular leakage over 24 hours. This is referred to as the "extravascular blood equivalent" (EVBE).

There was a marked increase in cerebral vascular permeability in the brain and cord of animals with EAE compared with normal controls (Table 1).

Table 1. Vascular permeability in allergic encephalomyelitis

	Mean EVBE*)	
	Allergic Encephalomyelitis (days 11—15)	Normal Controls**)
Forebrain	2·16	0·12±0·02
Mid-hindbrain	2·54	0·13±0·03
Cerebellum	1·52	0·18±0·03
Cervical cord	1·52	0·43±0·06
Thoracic cord	6·13	0·55±0·05
Lumbar cord	4·59	0·49±0·09

*) „Extravascular blood equivalent"
**) Mean ± S.E.

The earliest stage at which this increase was detected was 11—12 days after sensitisation. Figure 1 shows the onset of the permeability changes in the forebrain. Similar results were obtained in other parts of the brain and the spinal cord.

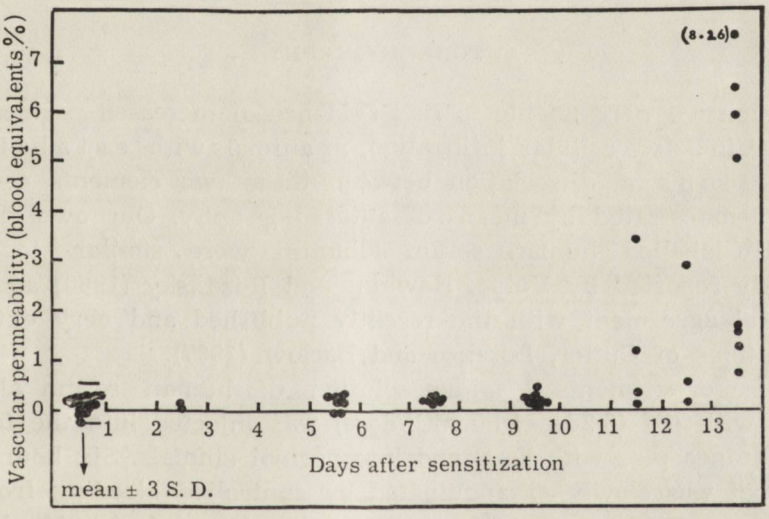

Fig. 1. Vascular permeability (EVBE) in the forebrain of guinea-pigs developing EAE. First appearance of the vascular leak 11—12 days after sensitisation.

On the other hand, the first definite histological lesions were found in animals killed 9—10 days after the sensitising injection and occasional small accumulations of inflammatory cells were found some

days before this. In no case was an increase in vascular permeability found to precede infiltration by inflammatory cells. These results were, therefore, compatible with the view that the EAE lesion is due to sensitised cells and not to circulating antibody.

However, it is to be noted that there have been a number of recent reports suggesting that changes in the brain parenchyma can be detected several days before inflammatory cells appear in the CNS. A widespread activation of proteolytic activity has been claimed by Benetato, Gabrielescu and Boros (1965). They reported that proteolysis was diffusely increased throughout the brain and preceded the cellular infiltration by over a week. There are also the extremely interesting observations of Oldstone and Dixon (1968) on EAE in Levis rats. Using a fluorescent antibody method they observed a leak of serum fibrinogen, β_{1c}globulin and IgG from the cerebral vessels several days before the appearance of inflammatory cells in the central nervous system.

The discrepancy between our own findings and those of Oldstone and Dixon may be due to a species difference. If not, the question resolves itself into a comparison of the methods used. The present investigation has clearly failed to quantitatively confirm results obtained using an immunochemical procedure on tissue sections.

AUTORADIOGRAPHY

Although we were unable to find evidence of increased permeabality in the brain before cellular infiltration, in animals with established EAE lesions a dissociation between these two elements could be clearly demonstrated by means of autoradiography. Our own findings using ^{125}I-labelled human serum albumin were similar to those previously reported by Vulpe, Hawkins and Rozdilsky (1960) and were in general agreement with the recently published and very extensive investigations of Cutler, Lorenzo and Barlow (1967).

In these experiments a saline solution of human serum albumin labelled with ^{125}I (0·25 — 1·50 mCi/dose) was injected into the femoral vein of guinea pigs with EAE and into normal animals. Six hours later the animal was slowly exsanguinated by controlled bleeding from the jugular vein: fluid was simultaneously replaced by infusing 5% dextran into the superior vena cava (method modified from Rozdilsky and Olszewski 1957). After perfusion with formol saline the brain was removed and embedded in paraffin blocks. Autoradiographs were prepared produced by a modification of Curran and Clark's (1961) stripping film technique using Kodak AR10.

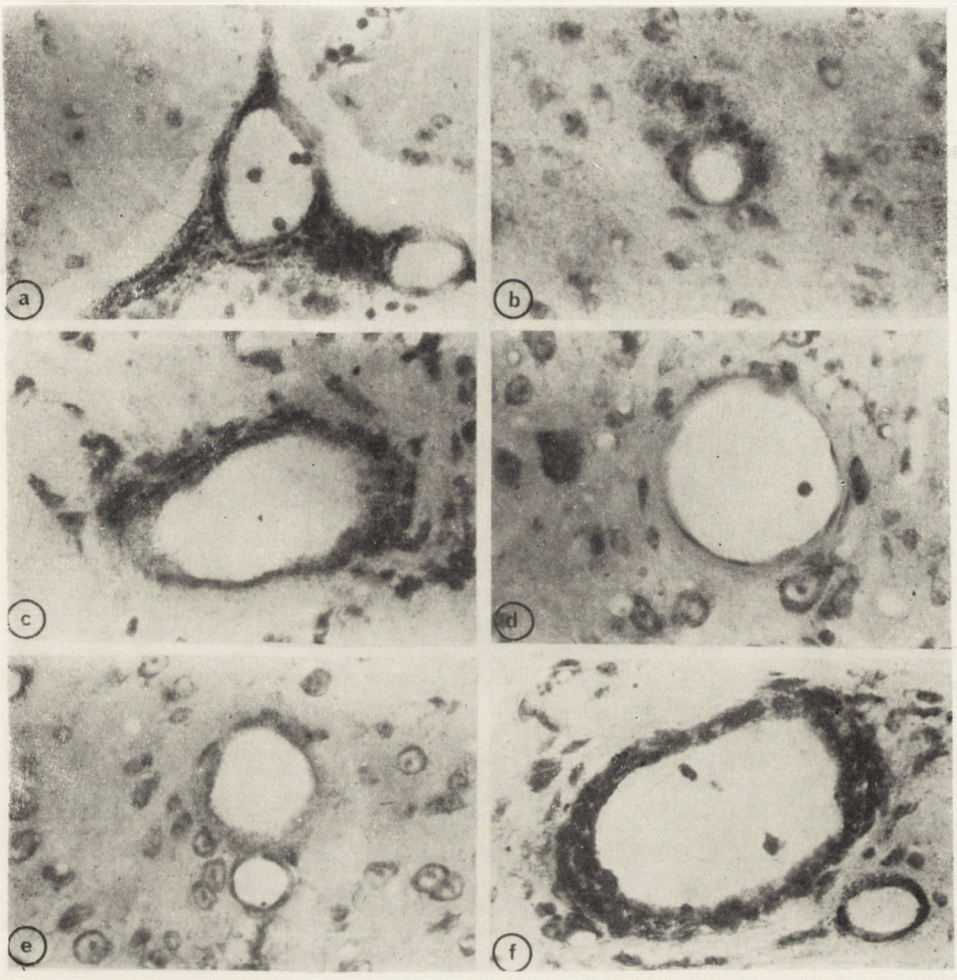

Fig. 2: Meningeal vessel from a guinea pig with EAE showing inflammatory cells and extravasation of labelled protein. Autoradiograph stained with methylene blue. × 450 (a). Cerebral vessel from guinea pig with EAE. Vascular leak with minimal cellular infiltration. Autoradiograph, methylene blue. × 450 (b). Perivascular accumulation of inflammatory cells accompanied by a protein leak in a guinea pig with EAE. Autoradiograph, methylene blue. × 450 (c). Control. Normal cerebral vessel. Autoradiograph, methylene blue. × 450 (d). Vascular leak of labelled protein from a histologically normal vessel. Guinea pig with EAE. Autoradiograph, methylene blue. × 450 (e). Cerebral vessels from a guinea pig with EAE. The larger vessel shows a perivascular cuff of inflammatory cells and extravasation of labelled protein. The smaller one (?branch) shows a similar vascular leak but without inflammatory cells. Autoradiograph, methylene blue. × 450 (f).

Extravascular penetration of ^{125}I-labelled albumin was not found in normal animals but was observed in guinea pigs at an early stage of EAE shortly after the onset of paralysis. Intense areas of radioactivity were found around many of the vessels in the meninges and in the substance of the brain (Fig. 2 a, b, c, d, f). In some cases a more diffuse low-grade radioactivity was also noted over wide areas of adjacent parenchyma.

Increased vascular permeability was demonstrated in many vessels surrounded by inflammatory cells. However, the most interesting finding was the demonstration of histologically normal vessels apparently leaking protein. This remarkable dissociation between the vascular and cellular changes has often been observed and commented upon (Barlow 1956, Vulpe et al. 1960, Cutler et al. 1967). It has generally been held to prove that the permeability changes in EAE precede the arrival of inflammatory cells to provide support for the view that the lesion is due to circulating antibody and not delayed-type hypersensitivity.

However, there is now evidence that such a separation of vascular and cellular elements may be a feature of all manifestations of delayed hypersensitivity and indeed of inflammatory reactions in general (Spector, Willoughby 1968). This phenomenon has been observed in tuberculin reaction in the skin by Wiener, Lattes and Spiro (1967), using intravenous carbon in conjunction with electron microscopy. These authors showed that intramural carbon was to be found in association with emigrating leucocytes as well as in unaffected vessels. Conversely, perivascular cellular infiltration occurred both with and without vascular leakage. The explanation is to be found in the nature of the delayed reaction itself. The initial lesion is due to specifically sensitised lymphocytes, but the more widespread vascular changes are caused by diffusible "permeability factors" that are analogous to the kinins and other pharmacologically active substances liberated at the site of acute inflammatory reactions (Spector, Willoughby 1968, Schild, Willoughby 1967).

Since vascular and cellular changes can be dissociated in all forms of delayed hypersensitivity the autoradiographic findings in EAE are perfectly compatible with the view that the lesion is due to sensitised cells and not to circulating antibody.

Acknowledgements. These experiments were done with the assistance of Miss L. A. Kennedy who also prepared the histological sections and autoradiographs. I should also like to thank Dr. Norman Veal for the preparation of the labelled protein; the illustration and photographic department of Guy's Hospital for the figures; and Mr. R. Morgan for help and advice.

S. Leibovitz

PRZEPUSZCZALNOŚĆ NACZYŃ MÓZGOWYCH W DOŚWIADCZALNYM, ALERGICZNYM ZAPALENIU MÓZGU I RDZENIA (EAEM)

Streszczenie

Badano przepuszczalność naczyń mózgowych u świnek morskich z EAEM. Zastosowano metodę ilościową z podwójnym izotopem promieniotwórczym pozwalającą mierzyć zmiany przepuszczalności niezależnie od zmian objętości krwi w mózgu. Nie potwierdzono istnienia „przecieków" mózgowych powstających przed pojawieniem się komórek zapalnych w centralnym układzie nerwowym.

Jednakże badania autoradiograficzne nad ustalonymi zmianami w EAEM przy pomocy albuminy znakowanej ^{125}J potwierdzają, że przecieki naczyniowe i powstawanie nacieków komórkowych mogą być rozdzielone w czasie.

Obraz zmian przepuszczalności jest zgodny z poglądem twierdzącym że EAEM jest objawem późnej nadwrażliwości i zależy raczej od uczulonych komórek niż krążących przeciwciał.

С. Лейбовиц

ПРОНИЦАЕМОСТЬ СОСУДОВ ГОЛОВНОГО МОЗГА В ЭКСПЕРИМЕНТАЛЬНОМ АЛЛЕРГИЧЕСКОМ ЭНЦЕФАЛОМИЭЛИТЕ (ЭАЭМ)

Содержание

Исслдования велись над проницаемостью мозговых сосудов у морских свинок с ЭАЭМ. Применили количественный метод с двойным радиоизотопом разрешающий измерять изменения проницаемости независимо от изменений объема крови в головном мозге. Не потвердили существования мозговых „течей" возникающих перед появлением воспалительных клеток в центральной нервной системе. Однако авторадиографические исследования обоснованных изменений в ЭАЭМ при помощи альбумина меченного I^{125} потверждают, что сосудистые „течи" и клеточные инфильтраты из воспалительных клеток могут появлятся в разном времени.

Картина изменений проницаемости согласна со взглядами утверждающими, что ЭАЭМ является симптомом замедленной аллергии и зависит в большей степени от сенсибилизированных клеток чем циркулирующих антител.

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BIOCHEMICAL STUDIES IN EAE

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There is some evidence that the pathological process in EAE is associated with an abnormal metabolism in the nervous tissue itself, thus being not entirely a result and reflection of the cell infiltrations and myelin destruction. The abnormalities in the nervous tissue were found by Smith (1965) who stated that the paralysis resulting from demyelination is associated with an abnormal metabolism both inside and outside the myelin sheath. The problem of the presumably primary changes in the cerebral nitrogen metabolism was raised by the investigations of Gershenowicz et al. (1968).

The question, to what an extent, the damage in the myelin can be regarded as a primary or secondary effect of the inflammatory process may be answered by biochemical studies. That is why we have undertaken a systematic study of the biochemical changes occurring in the brain in course of EAE. Following this way we are investigating the chemistry of structural components of the nervous tissue and its metabolism. The present report deals with following subjects: tissue respiration, anaerobic glycolysis, enzymatic mechanism of protein biosynthesis and lipid composition.

MATERIAL AND METHODS

Our experiments were performed using adult outbred guinea-pigs and rabbits of either sexes. Experimental allergic encephalomyelitis was induced by injections of an emulsion of homologous lyophilized total brain with an addition of adjuvant according to Kies et others (1957). The injecting material consisted of 40 mg homogenized brain, 10 mg heat-killed tbc bacilli, 2 ml liquid paraffin, 1 ml 0·9 per cent saline, and 1 ml melted lanoline. After incubation for one hour at 56°C,

0·1 ml of the emulsion was injected intracutaneously into the neck region for three consecutive days.

The brain samples for histological examination were fixed in 10% (w/v) formaline and stained with haematoxyline-eosine and other routine procedures such as the Woelcke and Nissl methods.

For examination of the tissue respiration and anaerobic glycolysis a 10% homogenate of cerebral white and grey matter was prepared. The tissue respiration was studied by the direct Warburg method, at 38°C in an oxygen atmosphere, using the Krebs-Ringer medium with a phosphate buffer of pH 7·4 and glucose at a concentration of 2 mg per ml. The anaerobic glycolysis was examined according to Negelein (1925) in a atmosphere of nitrogen. The composition of the gas mixture was as follows (95% of nitrogen and 5% of carbon dioxide). The activation of amino acids was studied by the hydroxamic method of Hoagland (1955) in extracts of acetone powders of the nervous tissue. The individual activation of 20 amino acids was studied. The protein content in the acetone powder was estimated by the biuret method (Kingsley, 1942).

Brain lipids were estimated after chromatographic separation on thin-layer plates. The phospholipids and cerebrosides were separated according to Svennerholm (1964) and estimated according to Broda (1967) and Rodin et al. (1955) respectively. Cholesterol was separated according to Svennerholm (1964) and estimated using the method of Sperry and Webb (1950). The gangliosides were separated according to Jatzkewitz et al. (1965) and estimated as described by Suzuki (1964).

RESULTS

In animals inoculated with the encephalitogenic mixture the clinical, chiefly neurological, symptoms appeared at the earliest on the 11th and 15th day after the first injection in rabbits and guinea pigs respectively. In the majority they became manifest about the 20th day. The objective signs of illness: loss of weight, loss of motor activity, paralysis of legs as well as other neurological symptoms corresponded with the well — known picture of experimental encephalomyelitis in guinea pigs and rabbits.

The cytological and myelin stains both revealed a typical pathological pattern of meningoencephalomyelitis with inflammatory reactions localized chiefly in perivascular and subependymal regions, predominantly within the cerebral white matter (Fig. 1). Strong inflammatory changes were present in the choroid plexus. Perivascular demyelination was only occasionally observed.

The observed pathological picture is in conformity with descriptions of EAE in guinea pigs and rabbits, and therefore does not require a more detailed account.

Table 1. Respiration and glycolysis in the brain of rabbits in course of EAE

	White matter		Grey matter	
	normal	EAE (paralytic period)	normal	EAE (paralytic period)
Tissue respiration ($-Qo_2 = \mu l\ O_2$/mg of dry tissue/h)	3·05±0·22	2·66±0·10	7·38±0·20	**4·61±0·40**
Anaerobic glycolysis ($Qm = \mu l\ CO_2$/mg of dry tissue/h)	3·20±0·22	2·47±0·61	8·14±0·37	**4·48±0·92**

The results are given as mean ± standard error (significant differences underlined). Number of animals in each group was 6.

The results of our studies on respiration and glycolysis in the brain of rabbits in course of EAE are presented in the Table 1. It is shown that the tissue respiration in the grey matter is markedly diminished in course of the paralytic period of EAE. In the white matter of animals with EAE the tissue respiration is apparently also decreased but the differences are statistically insignificant.

From our studies it appears that also the anaerobic glycolysis is declined in the grey matter of all encephalitic rabbits. However it should be mentioned that there exist great differences between individual animals. The small decrease found in the white matter is insignificant.

The next table (Table 2) presents the rates of amino acids activation in the brain of rabbits in course of EAE. The data presented there indicate that the activation of several amino acids by the sRNA-aminoacyl synthetases so called pH 5 enzymes is increased in the brain of rabbits with EAE both in the white and in the grey matter. The activation of asparagine and phenylalanine appeared to be altered at most. In normal animals the two latter amino acids are activated only in traces, whereas animals with EAE demonstrate high rates of activation. Also the marked increase of glutamic acid activation in the cerebral white matter, should be underlined, while no differences were found with respect to aspartic acid and glutamine activation.

Results concerning the lipid composition of the brain tissue in EAE are presented in Table 3.

Table 2. Rate of amino acid activation in the brain of rabbits in course of EAE

L-amino acids	White matter		Grey matter	
	normal	EAE (paralytic period)	normal	EAE (paralytic period)
Alanine	0·00	0·00	0·00	0·00
Asparagine	0·01±0·001	**0·45±0·08**	0·01±0·002	**0·39±0·02**
Aspartic acid	0·01±0·002	0·00	0·01±0·002	0·00
Glycine	0·01±0·004	0·02±0·07	0·01±0·001	0·02±0·01
Phenylalanine	0·01±0·002	**0·22±0·07**	0·01±0·003	**0·19±0·07**
Glutamine	0·39±0·023	0·48±0·05	0·44±0·036	0·55±0·05
Glutamic acid	0·49±0·027	**1·20±0·19**	0·50±0·019	1·10±0·22
Histidine	0·00	**0·06±0·01**	0·00	0·05±0·02
Cystine	0·00	0·00	0·00	0·00
Leucine	0·00	0·00	0·00	0·00
Isoleucine	0·00	0·00	0·00	0·00
Norleucine	0·00	0·00	0·00	0·00
Lysine	0·00	0·00	0·00	0·00
Methionine	0·00	0·00	0·00	0·00
Proline	0·01±0·003	0·00	0·01±0·002	0·00
Serine	0·00	0·00	0·00	0·00
Threonine	0·00	0·00	0·00	0·00
Valine	0·00	0·00	0·00	0·00
Norvaline	0·00	0·00	0·00	0·00
Tryptophane	0·00	0·00	0·00	0·00

The results are given as mean in μmole of hydroxamates (mg of protein) h ± standard error (significant differences underlined). Number of animals in each group was 6.

It occurs, that a decrease of the free cholesterol content, accompanied by the appearance of cholesterol esters is the most characteristic and regular feature of that experimental disease. It should be emphasized that cholesterol esters are found both in the white and grey cerebral matter.

There was not a single analyzed lipid class, which would remain unchanged in course of this experimental disease. However — no regularity of changes both with respect to their localisation and to the period of disease could be found. With only two exceptions in the grey matter as for phosphatidylcholine and the sphingomyelins, the observed changes, both in the white and grey matter, indicate an increase of the particular lipids in course of EAE.

DISCUSSION AND CONCLUSIONS

The results obtained in the performed investigations indicate that the pathological process of EAE induces metabolic changes in several of the structural components of the nervous tissue.

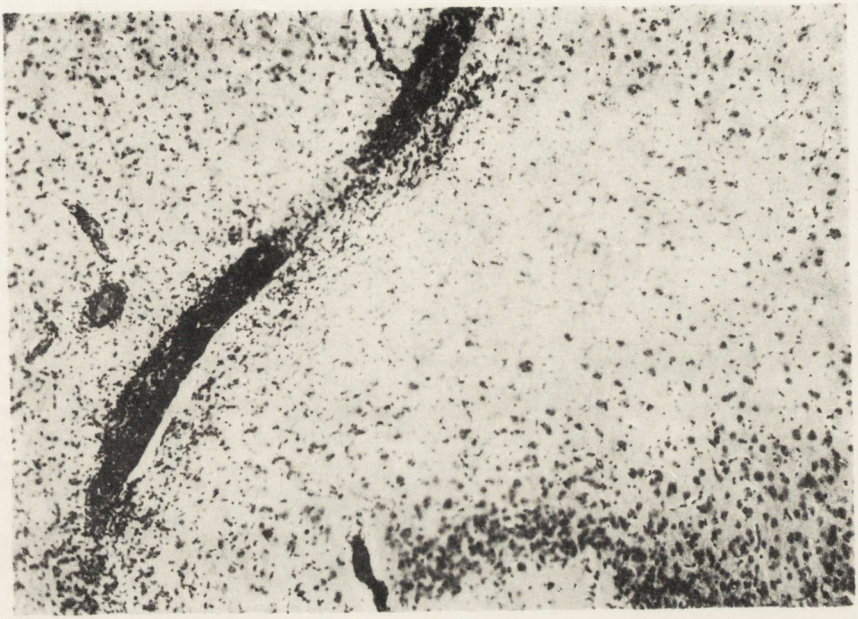

Fig. 1. Perivascular inflammatory reaction in the brain of a guinea pig with EAE. Stained by haematoxylin-eosine. × 80.

Table 3. Brain lipids in guinea pigs in course of EAE

	White matter				Grey matter	
	Normal	EAE (early period)	EAE (paralytic period)	Normal	EAE (early period)	EAE (paralytic period)
Phosphatidyl-choline	5·06±0·15	5·18±0·04	4·90±0·08	5·20±0·05	**3·93±0·16**	**3·46±0·11**
Phosphatidyl-ethanolamine	9·58±0·14	9·45±0·07	9·42±0·27	5·78±0·06	6·66±0·14	**6·84±0·13**
Phosphatidyl-serine	3·20±0·06	**3·90±0·09**	**4·05±0·12**	2·18±0·01	**3·25±0·02**	**3·06±0·03**
Sphingomye-line	2·45±0·05	2·52±0·10	**2·95±0·03**	1·61±0·04	1·51±0·01	**1·29±0·02**
Cerebroside	7·97±0·09	**9·31±0·09**	**9·43±0·21**	4·51±0·06	4·87±0·23	4·69±0·09
Sulphatide	3·60±0·07	**4·22±0·10**	4·05±0·13	1·42±0·03	**2·47±0·08**	**2·40±0·01**
Free choleste-rol	11·22±0·05	**9·60±0·30**	**9·90±0·17**	5·66±0·04	5·56±0·16	5·93±0·22
Cholesterol esters	0·00.	**1·89±0·24**	**1·82±0·29**	0·00	**2·58±0·28**	**1·98±0·39**
Gangliosides	0·56±0·01	0·52±0·01	0·56±0·02	1·09±0·01	1·03±0·04	0·91±0·04

The results are given as mean in g/100 g of dry tissue ± standard error (significant differences underlined). Number of animals in each group was 10

The observed decrease of tissue respiration and anaerobic glycolysis in both the cerebral grey and white matter, the enhanced activation of some of amino acids by the sRNA-aminoacyl synthetases found also here and there, and finally the appearance of cholesterol esters in both the grey and white matter — point to a severe metabolic impairment confined to these two cerebral structures, which both seem to be involved in the pathological process.

The most probable explanation for the observed increase of activation of asparagine and phenylalanine and of some other amino acids is the presence of invading cells which is a prominent feature of this process. We cannot exlude the possibility that we are measuring enzyme activities of these cells which are normally absent in the central nervous system. Although the lesion comprises only a small part of the total of the nervous tissue, cells which have relatively high enzyme activities, involved in the first steps of protein biosynthesis, could affect considerably the overall activity in the normal adult brain, the background level of which is known to be very low. The same explanation may be valid for the rise in the content of some of

phospholipids and galactolipids in the brain of animals with EAE in a sense, that the cellular infiltrations have a different lipid pattern than the normal brain.

The metabolic experiments performed by Smith (1965) with the in vitro uptake of glucose-C^{14} into lipid fractions of brains from animals with EAE have shown that there is an increase in labelling rate of the phospholipid and galactolipid fractions, whereas cholesterol synthesis remained unchanged or slightly depressed. The highest rises in labbeling were seen in the lysolecithin, lecithin, phosphatidyl serine and galactolipids fractions. Further observations made by the same author showed that the glucose-C^{14} incorporation into brain lipids remained high even during the recovery period at a time when pathological changes had largely disappeared and he concluded that at least a part of the observed metabolic changes can be ascribed to the central nervous system tissue itself.

The findings of the metabolic studies presented by Smith (1965) correlate well with our results of lipid determination, and may to some extent explain the observed increase of phospholipids and galactolipids, and the decrease of the free cholesterol content.

Basing on the above presented data, we seem to be justified in concluding that in course of EAE both the white and grey cerebral matter are severely affected at the molecular level, in spite of the obvious preponderence of microscopic changes to the cerebral white matter.

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BADANIA BIOCHEMICZNE W DOŚWIADCZALNYM ALERGICZNYM ZAPALENIU MÓZGU

Streszczenie

Doświadczalne alergiczne zapalenie mózgu wywoływano u świnek morskich i królików przez wstrzykiwanie homologicznego antygenu z dodatkiem adjuwantu Freunda.

W mózgu badano oddychanie tkankowe metodą Warburga, beztlenową glikolizę metodą Negeleina, aktywację 20 aminokwasów metodą Hoaglanda, oraz skład lipidów za pomocą chromatografii cienko-warstwowej.

W wyniku przeprowadzonych badań stwierdzono, że proces patologiczny doświadczalnego alergicznego zapalenia mózgu wywołuje spadek oddychania tkankowego i beztlenowej glikolizy, wzrost aktywacji aminokwasów, szczególnie asparaginy i fenylalaniny oraz pojawienie się estrów cholesterolu zarówno w istocie białej jak i szarej mózgu.

Wzrost aktywacji aminokwasów w przebiegu alergicznego zapalenia mózgu wydaje się być następstwem obecności nacieków komórkowych o dużej aktywności enzymatycznej. Podobnie odmienny skład lipidowy komórek nacieków może częściowo tłumaczyć obserwowany wzrost zawartości niektórych frakcji fosfolipidowych i galaktolipidów.

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БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ В ЭКСПЕРИМЕНТАЛЬНОМ АЛЛЕРГИЧЕСКОМ ЭНЦЕФАЛИТЕ

Содержание

Экспериментальный аллергический энцефалит вызывали у морских свинок и кроликов инъицируя им гомологичный антиген с адъювантом Фрейнда. В головном мозге исследовали тканевое дыхание методом Варбурга, анаэробный гликолиз методом Негелейна, активацию 20 аминокислот методом Хоагланда, состав липидов методом тонкослойной хроматографии. В результате проведенных исследований констатировали что патологический процесс экспериментального аллергического энцефалита вызывает падение уровня тканевого дыхания и анаэробного гликолиза, повышение активации аминокислот, особенно аспарагина и фенилаланина и появление эфиров холестерина так в белом как и в сером веществе головного мозга. Повышение активации аминокислот в течении аллергического энцефалита кажется быть следствием присутствия клеточных инфильтратов обладающих сывокой ферментативной активностью. Также различный состав липидов клеток инфильтратов может частично объяснить наблюдаемое повышение содержания некоторых фракций фосфолипидов и галактолипидов.

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LICHT- UND FLUORESCENZOPTISCHE UNTERSUCHUNGEN BEI DER EXPERIMENTELLEN ALLERGISCHEN ENCEPHALOMYELITIS

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Das Ziel einer Frühdiagnostik der Encephalomyelitis disseminata — entsprechend der Konzeption unserer Arbeitsgruppe — fordert Nachweismethoden, die früher als die Krankheitssymptome einen Hinweis auf den Beginn der Erkrankung geben können. Für die Klinik hat somit die Pathogenese des initialen Frühstadiums der Encephalomyelitis entscheidene Bedeutung.

Im 1. Komplex unserer Untersuchungen bei der experimentellen allergischen Encephalomyelitis (EAEM) baschäftigten wir uns mit dem Nachweis der Störung der Schrankenpermeabilität. Evans blue, Trypaflavin, FITC (Fluoresceinisothiocyanat), TTC (Triphenyltetrazoliumchlorid) wurde Kaninchen und Meerschweinchen kurz vor der Tötung injiziert. Die Substanzen waren im extracerebralen Retikuloendothelium und Nieren nachweisbar, nicht im Bereich der cerebrospinalen Läsionen. Akridinorange sahen wir jedoch perivasal in cerebralen Arealen mit und ohne cellulär entzündliche Reaktionen (Abb. 2, 3). Das Ausmass der perivaskulären Transsudationen wurde durch die Vitalversuche nicht erfasst. Die Ursache kann sein:

1. Die unterschiedliche Permeation der Farbstoffe auf Grund ihres differenten Wertes als Schrankenindikator (Quadbeck 1967).
2. Die bereits erfolgte Rücklaufigkeit der initialen Permeation.

Wie die Beobachtungen erbrachten, laufen mehrere Stadien offensichtlich nebeneinander ab. Es ist anzunehmen, dass die spezifische Sensibilisierung (Seitelberger 1967) zu einem noch unvollständig erfassten Zeitpunkt der initialen Schrankenstörung stattfindet.

Ebenfalls im Hinblick auf die Ereignisse des Frühstadiums berücksichtigten wir im 2. Komplex immunologische Probleme.

I. Der Nachweis von Autoantikörpern mit zytolytischer Aktivität gegen Myelin und Gliazellen ist seit 1961 durch Bornstein und Appel (1961), sowohl bei der EAEM als auch der Encephalomyelitis des Men-

schen bekannt. Zuverlässige Zellkulturen sind für derartige Untersuchungen Voraussetzung. Quantitative Aussagen sind kaum möglich, da dieser zur IgG-Klasse gehörende Antikörper nur in sehr geringer Konzentration im Serum vorhanden ist.

II. Leichter nachweisbar sind zierkulierende Antikörper mit Hilfe der OUCHTERLONY-Technik. Es handelt sich nach den Untersuchungen von Ross und Mitarb. (1965) um einen Serum-Faktor, der gegen Hirnextrakt präzipitiert und zur IgM-Klasse gehört.

In Verlaufskontrollen beobachteten wir das Auftreten von Antikörpern gegen allogene Antigene (Hirn und Rückenmark) mit und ohne Trypsin-Vorbehandlung. Den semiquantitativen Antikörperverlauf zeigt die Abb. 1. Es fand sich, dass die Antikörper-Beobachtungen 2 Typen bei insgesamt 2 Verlaufsformen erbrachten:

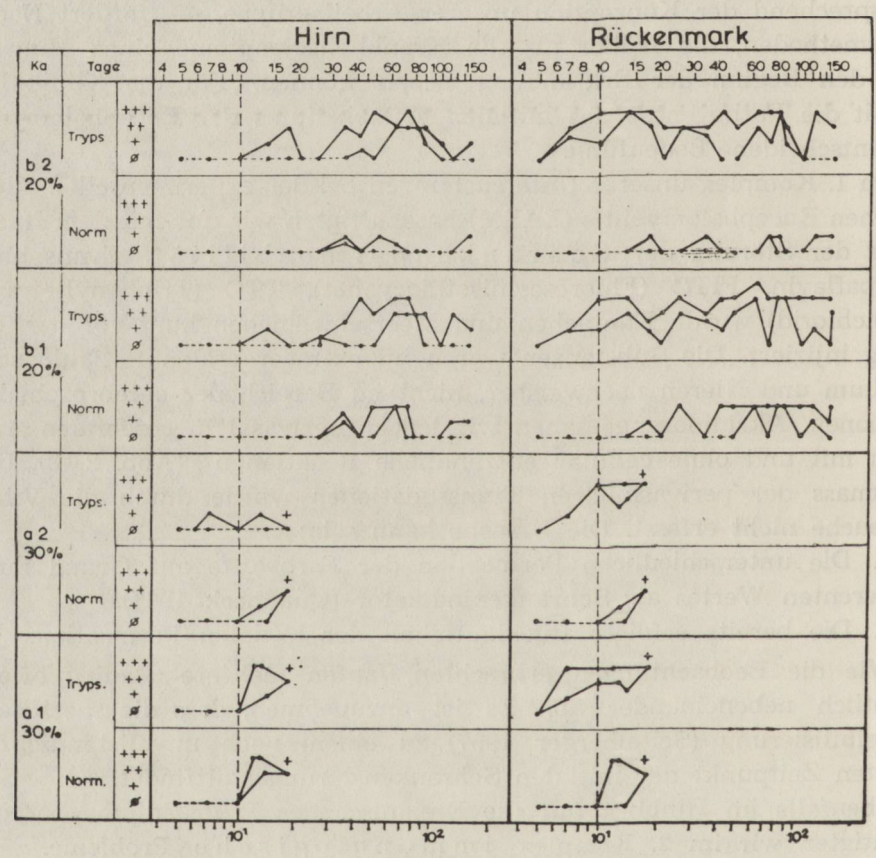

Abb. 1. Antikörper-Verlaufsbeobachtungen bei der EAEM des Kaninchens mit semiquantitativer modif. OUCHTERLONY-Technik: 2 Typen des Antikörperverlaufes (1, 2) gegen normales und trypsinbehandeltes allogens Hirn und Rückenmark bei 2 Verlaufsformen (a, b); (Logarithm. Zeiteinteilung).

1. Einen Typ mit frühem Antikörper-Anstieg gegen alle Antigene, ohne wesentlichen Unterschied, besonders früh, schon zwischen dem 6—7 Tag gegen trypsiniertes Rückenmark, sonst um den 10—11 Tag.
2. Einen Typ mit deutlichem Antikörper-Anstieg vor allem gegenüber trypsin-behandeltem Rückenmark, geringer gegenüber anderen Antigenen.

Die Tiere der Verlaufsform a zeigten früh und ausgeprägt das akute klinische Bild einer EAEM, starben zwischen dem 14 und 18 Tag. Die Tiere der Verlaufsform b boten klinisch ein mehr subakutes Krankheitsbild, teilweise mit intermittierenden Verlauf. Die fermentative Spaltung der Homogenate mit Trypsin ist für das Ausmass, also die Stärke der Antigenität von Bedeutung. Wesentlich für das Auftreten von Präzipitaten ist jedoch, ob eine Übereinstimmung mit dem den pathogenen Mechanismus auslösenden Antigen besteht.

Der Nachweis von Autoantikörpern im Serum von Tieren mit EAEM zeigt, dass ein Prozess der Autoimmunisation besteht (Frick 1967, Seitelberger 1967, Steffen 1968). Er bietet, wie unsere Verlaufsbeobachtungen zeigen, eine partielle Übereinstimmung mit dem klinischen Verlauf im Anfangsstadium. Je früher und komplexer er auftritt, desto akuter ist das Erkrankungsbild. Kuwert (1964) beobachtete analog das Abfallen von Serumkomplement am 6—7 Tag der Erkrankung.

Im 3. Komplex, den immunhistologischen Vergleichsbeobachtungen, wandten wir uns der Frage der Korrelation zum Gewebsprozess zu, was für die Wertigkeit des frühen Nachweises der Antikörper entscheidend ist. Fluorescenzoptisch liessen sich mit FITC-markiertem Anti-Gammaglobulin — wie auch Frick (1967) und Ridley (1963) — in den perivasculären Lymphocyten- und Plasmazellansammlungen des Hirns, ebenso wie in Lymphocyten und Milz Gammaglobuline nachweisen. Bei Bewertung der eigenen positiven Ergebnisse (Abb. 4) stehen diese in keiner Relation zur Ausdehnung der entzündlichen Reaktionen. Die Beobachtungen sprechen lediglich für eine Permeabilitätsstörung und das Vorhandensein immunkompetenter Zellen.

Da die Entmarkung bei der EAEM des kleinen Laboratoriumstieres überwiegend infiltratgebunden auftritt, ist es naheliegend, dass die Markscheide durch einen mehrgliedrigen Ablauf — entsprechend Seitelberger (1967) — alteriert wird. Bedeutsam für den Prozess dürfte sein:
1) Die Störung der Barrierenfunktion der Blut-Hirnschranke. 2) Das Eindringen oder die ortständige Produktion von Mediatorsubstanzen; 3) Das Auftreten immunkompetenter mesenchymaler Zellen, einschliesslich der Phagocyten, wobei auch die Funktion der noch nich eingeord-

neten Gliazellen im Infiltratgebiet von Bedeutung sein dürfte; 4) Die Reaktionskette beginnt mit dem Auftreten spezieller Immunglobuline, besonderen Komplementvorstufen und der Bildung von Lysokephalin (Pette et al. 1965, Seitelberger 1967).

Im Zentrum der Problematik der initialen Vorgänge steht die Frage nach der Auslösung der Antigenmobilisation, inwieweit es sich um eine Toleranzstörung handelt, welche exogenen und endogenen Faktoren auf ein genetisch labiles Gleichgewicht treffen. Spezielle Immunseren differenzierter Faktoren könnten unter Verwendung herkömmlicher fluoreszenzoptischer Verfahren und besonderer Markierungen für elektronenoptische Untersuchungen die initialen Vorgänge im Entzündungsbereich weiter beleuchten.

H. Meyer-Rienecker et al.

BADANIA NAD DOŚWIADCZALNYM ALERGICZNYM ZAPALENIEM MÓZGU METODAMI MIKROSKOPII KONWENCJONALNEJ I FLUORESCENCYJNEJ

Streszczenie

Odkrycia patogenetyczne we wczesnym stadium doświadczalnego alergicznego zapalenia mózgu i rdzenia mają znaczenie dla danych klinicznych we wczesnej diagnostyce rozsianego zapalenia mózgu i rdzenia. Zwrócono uwagę na wartość stosowania wskaźników bariery w trakcie badań nad przeciwciałami i w przebiegu badań immunohistologicznych. W pracy omówiono przebieg wczesnego stadium doświadczalnego alergicznego zapalenia mózgu i rdzenia z podziałem na kilka faz.

Г. Мейер-Ринэкэр и др.

ИССЛЕДОВАНИЯ НАД ЭКСПЕРИМЕНТАЛЬНЫМ АЛЛЕРГИЧЕСКИМ ЭНЦЕФАЛОМИЭЛИТОМ (ЭАЭМ) МЕТОДАМИ СВЕТОВОЙ И ФЛУОРЕСЦЕНТНОЙ МИКРОСКОПИИ

Содержание

Патогенетические открытия в начальном периоде ЭАЭМ имеют значение для клинических данных в ранней диагностике рассеянного энцефаломиэлита. Обращено внимание на стоимость применения индикаторов барьера во время исследований над антителами и в течении иммуногистологических наблюдений. В работе обсуждается течение раннего периода ЭАЭМ с его подразделением на несколько стадий.

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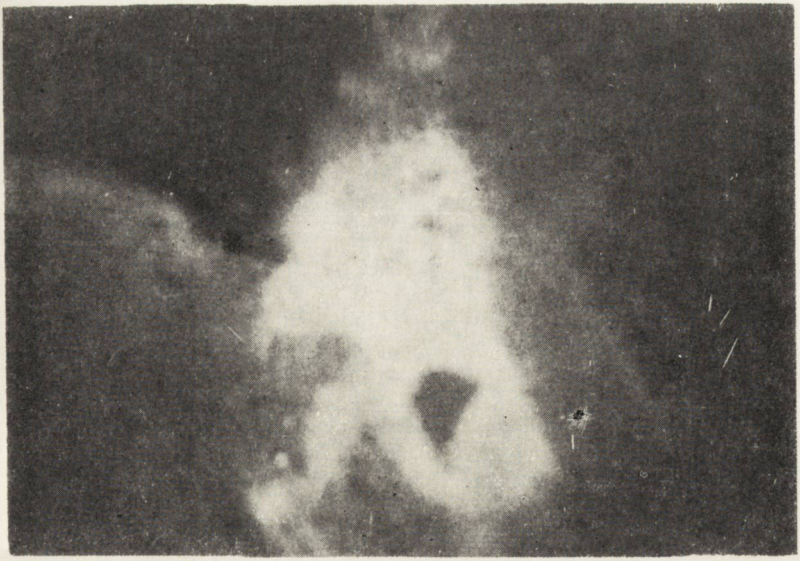

Abb. 2. Gefrierschnitt des Hirns eines Meerschweinchens bei EAEM, dem 4 Minuten ante finem Akridinorange (1 : 1000) intracardial injiziert wurde: diffuse perivaskuläre Fluorochromdarstellung; keine zellulären Infiltrationen im perivasalen Gebiet. (HBO 50, BG 12/OGL-GG 9).

Abb. 3. Akridinorangefluorescenz in einem Gebiet mit zellulären Infiltrationen; gleiche Bedingungen wie unter Abb. 2.

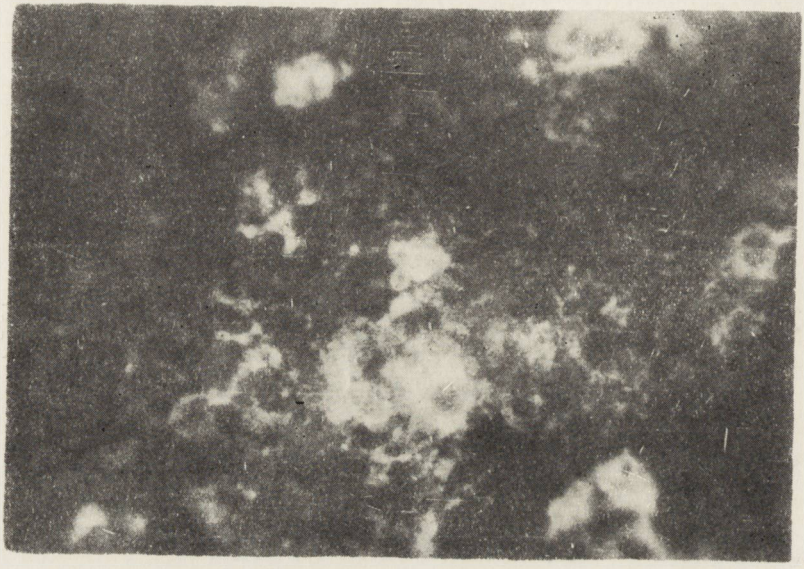

Abb. 4. Gefriernativschnitt des Hirns eines Kaninchens mit EAEM: fluorescierende Infiltratzellen nach Behandlung mit FITC-markiertem Anti-Kaninchen-Gammaglobulin (GAR g — FITC); (HBO 50, BG 12/OGL-GG 9).

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ZUR THEORIE DES ALTERUNGSPROZESSES IM NERVENSYSTEM DES MENSCHEN

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Alterungsprozesse im Nervensystem sind den Medizinern seit sehr langer Zeit bekannt, soweit sie zu schweren funktionellen Ausfällen führen. Erst seit einigen Jahrzehnten rückten auch feinere Veränderungen in den Blickwinkel, zuerst erregten sie wohl das Interesse der Neurohistologen (Hodge 1894/95, Pilcz 1895, Bühler 1898, Mühlmann 1901 ff, Marinesco 1906, Nageotte 1907 ff, Rossi 1906). Besondere Verdienste hat sich der Grossmeister der klassischen Neurohistologie in der Frühzeit der neurologischen Gerontologie erworben, Ramon y Cajal (1905 ff.), von dem die heute schon längst in alle Lehr- und Handbüher eingegangenen Definitionen der Degenerationsstadien des Neurons im wesentlichen gegeben worden sind. Die neuere Neurohistologie hat die Problematik der Altersveränderungen niemals aus den Augen verloren (Andrew 1941, 1952, Bruchmüller und Scharft 1965, de Castro 1921/22, Döring 1948, 1955, Harms 1924, Hermann 1951, Kiss 1933, Levi 1925, Scharf 1958, Stöhr 1949/50, Truex 1939, 1940, 1942, Watzka und Scharf 1951, Zeglio 1936). Das Alterungsgeschehen als eindeutig statistisches, quantitativ-morphologisches Problem erkannten vor allem Corbin und Gardner (1937) sowie Prestige (1965). Eine der interessantesten neurophysiologischen Beiträge zum Alterungsproblem steuerte Pearson (1928) bei, den histochemischen Aspekt betonten Sulkin (1955) sowie Sulkin und Kuntz (1948, 1952). Die kürzlich viel zu früh verstorbene Neurohistologin Emmi Hagen (1951) prägte den markanten Begriff „physiologische Degeneration", der sich zunehmend durchsetzt. Selbstverständlich ist die hier vorgelegte Literaturübersicht nicht vollständig. Einige interessante Aspekte enthält z. B. der Handbuchbeitrag von Bourlière (1966).

*) Mit dankenswerter Unterstützung durch einen Forschungsauftrag des Ministeriums für Hochschulwesen der DDR. Graphik: Akad. Bildhauer Hellmut Helwin.

Stellt man in der Biologie die gleichen Ansprüche, wie sie in den physikalischen Wissenschaften seit Jahrhunderten selbstverständlich sind, dann haben alle bisherigen Untersuchungen über den Alterungsprozess des Nervensystems zwar zu einer umfangreichen Faktensammlung geführt, aber nicht zu Ansätzen für eine Theorie. Im Sinne der Theoretischen Physik kann von einer wirklichen Theorie nur dann gesprochen werden, wenn diese in mathematisch abstrahierter Form darstellbar ist.

Wollte man nun versuchen, den altersbedingten Involutionsprozess der Nervenzelle mit seinen schon cytologisch, cytochemisch und cytophysikalisch noch nicht voll überschaubaren Einzelheiten in eine formelmässig darstellbare Theorie zu fassen, dann würde dies kaum in näherer Zukunft zum Ziele führen. Allein für die Darstellung der Wechselbeziehungen eines einzigen Multienzymsystems in einem noch biochemisch kontrollierbaren Zeitraum von wenigen Minuten brauchte Hess (1968) ein System von etwa 100 nichtlinearen Differentialgleichungen.

Der Versuch, eine Theorie der Alterung im Nervensystem aufzustellen, muss also bei einem einfachen Beispiel beginnen. Untersuchungen von Ronge (1943, 1944), Bolton, Winkelmann und Dyck (1966), Scharf und Blumenthal (1967) sowie Scharf (1967) über die Abhängigkeit der Anzahl der Meissnerschen Tastkörperchen in der Fingerhaut vom Lebensalter können ein genügend einfaches Material liefern, das für den Versuch, eine Theorie aufzustellen, benutzbar ist.

Abb. 1 zeigt eine Schichprobe, die von Bolton, Winkelmann und Dyck (1966) an der Zehenhaut von 49 Personen verschiedenen Alters erhoben wurde. Die Zehenhaut wird hier für die Abbildung gewählt, weil sie keinen Geschlechtsunterschied erkennen lässt, während für die Fingerhaut zwischen weiblichen und männlichen Individuen unterschieden werden müsste. Die weiteren Betrachtungen haben aber die Fingerhaut zum Objekt, wobei der Geschlechtsunterschied zunächst unberücksichtigt bleibt, das heisst alle Berechnungen stellen „den Menschen" gewissermassen als Mittelwert aus Frau und Mann dar. Während beim Neonatus auf 1 mm² Hautfläche etwa 75 Tastköperchen fallen, findet man beim 90 jährigen nur noch etwa 3 Meissner-Körperchen. Der Abklingprozess kann durch eine lineare Differentialgleichung 4. Ordnung mit konstanten Koeffizienten beschrieben werden. Bezeichnet man die Anzahl der Tastkörperchen pro mm² Hautfläche mit M und das Lebensalter in Jahren mit t, dann hat die Differentialgleichung die Gestalt

$$\frac{d^4M}{dt^4} + a_2 \frac{d^3M}{dt^3} + a_1 \frac{d^2M}{dt^2} + a_0 \frac{dM}{dt} = 0 \qquad (1)$$

Die Terme der Gleichung sind biophysikalisch interpretierbar:

$\frac{dM}{dt}$ = Involutionsgeschwindigkeit

$\frac{d^2M}{dt^2}$ = Involuntionsbeschleunigung

$\frac{d^3M}{dt^3}$ = Involutionsbeschleunigungsänderung

$\frac{d^4M}{dt^4}$ = Änderung der Involutionsbeschleunigungsänderung

Das allgemeine Integral von (1) lautet:

$$M(t) = A_0 + A_1 e^{-\gamma_1 t} + A_2 e^{-\gamma_2 t} + A_3 e^{-\gamma_3 t} \qquad (2)\ *)$$

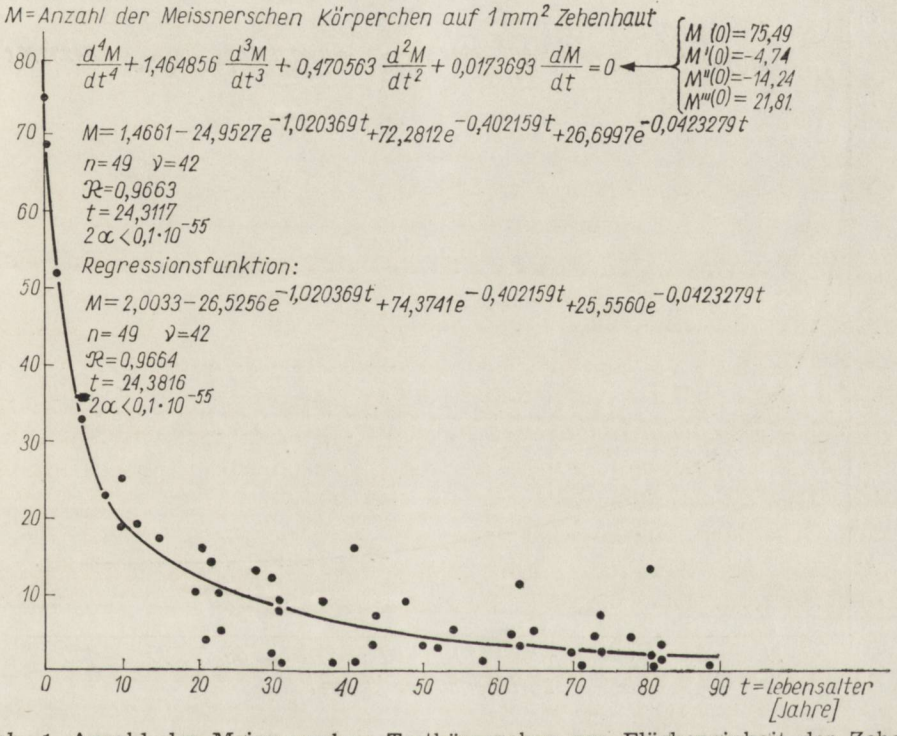


Abb. 1. Anzahl der Meissnerschen Tastkörperchen pro Flächeneinheit der Zehenhaut bei 49 Personen beiderlei Geschlechts in Abhängigkeit vom Lebensalter. Nach den Angaben von Bolton et al. (1966) sind die n=49 Messpunkte aufgetragen. Die Koeffizienten der Differentialgleichung (1) wurden durch Differenzenrechnung und Innere Regression ermittelt. Das Integral (2) wurde mit dem angegeben Anfangsbedingungen exakt berechnet, zum Vergleich wurden die Intergrationskonstanten zusätzlich durch lineare Regressionsrechnung ermittelt. Die beiden Kurven fallen bei Zeichengenauigkeit zusammen und lassen sich erst in der vierten Dezimalstelle des Korrelationskoeffizienten unterscheiden. Kein Geschlechtsunterschied.

*) In dieser und den folgenden Gleichungen sind die exponentiellen Zeitkonstanten mit einem Minuszeichen versehen, um anzudeuten, dass sie alle kleiner als Null sind.

Ein hier passendes partikuläres Integral ist in Abb. 1 angeschrieben, wo sich auch die Koeffizienten von (1) finden. Die 3 verschiedenen exponentiellen Zeitkonstanten in (2) sind grob gerundet:

$$\gamma_1 = -1; \qquad \gamma_2 = -0{,}4; \qquad \gamma_3 = -00{,}4;$$

Wie Abb. 1 zeigt, fällt die Kurve der Anzahl der Tastorganellen pro Flächeneinheit in der Kindheit äusserst rasch, im mittleren Lebensalter weniger drastisch und strebt im Senium asymptotisch einem konstanten Restzustand zu. Abb. 2 zeigt das Verhalten der Meissnerschen Tastkörperchen in der Fingerhaut, wobei — wie schon erwähnt — Ge-

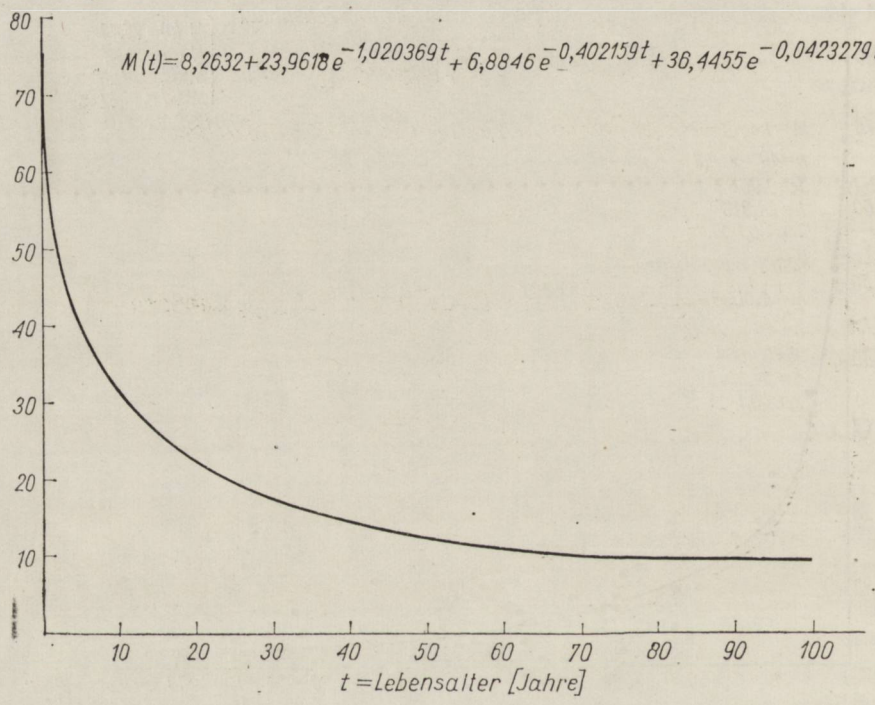


Abb. 2. Wegen des Geschlechtsunterschiedes ergeben sich für die zeitabhängige Anzahl der Meissner-Körperchen in der Fingerhaut (bezogen auf die Hautflächeneinheit) eigentlich getrennte Kurven für Männer und Frauen. Der hier gezeigte Kurvenzug gilt unter Nichtbeachtung des Geschechtsunterschiedes für Homo sapiens, der analytische Ausdruck wurde für 63 Personen durch Regressionsrechnung ermittelt. Messungen von Ronge (1943) sowie Bolton et al. (1966).

schlechtsunterschiede unbeachtet bleiben. Wollte man dieses Verhalten kritiklos akzeptieren, dann müsste man resignieren, denn man hätte zu schliessen, dass der Mensch vom Tage der Geburt an degeneriert. So sinnlos kann aber die menschliche Existenz nicht sein!

Die Lösung des Problems ist im menschlichen Wachstum zu suchen. Die Differentialgleichung für das Wachstum der Fingertasthaut ist.

$$\frac{d^4F}{dt^4} + b_2 \frac{d^3F}{dt^3} + b_1 \frac{d^2F}{dt^2} + b_0 \frac{dF}{dt} = 0 \qquad (3)$$

mit F = Fingertastfläche (mm^2) und t = Lebensalter (Jahre). Das allgemeine Integral ist

$$F(t) = B_0 + B_1 e^{-\beta_1 t} + B_2 e^{-\beta_2 t} + B_3 e^{-\beta_3 t} \qquad (4)$$

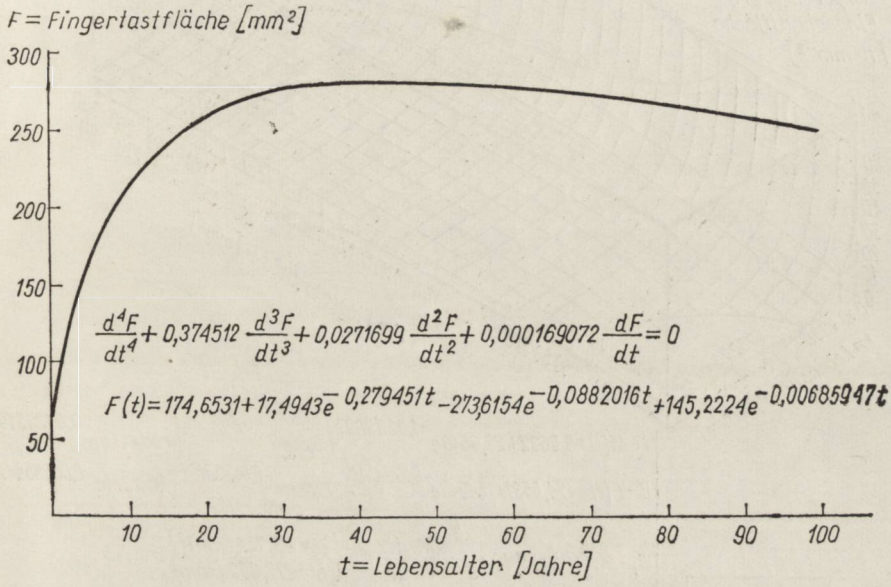


Abb. 3. Abhängigkeit der mittleren absoluten Fingerbeerenfläche vom Lebensalter. Die Wachstumskurve steigt in der Kindheit steil an, bei Adolescenten verlangsamt sich der Zuwachs allmählich. Nach einem Maximum im mittleren Lebensalter fällt die Kurve, weil subcutanes Fettpolster, Wassereinlagerung und elastische Fasern allmählich reduziert werden. Die Konstanten der Differentialgleichung (3) wurden durch Differenzenrechnung und Innere Regression, des Integrals (4) durch lineare Regressionsrechnung ermittelt, nachdem die Exponenten durch Lösen der Differentialgleichung bekannt waren. Unter Verwendung von Messwerten Ronges (1943) und Angaben aus der anatomischen Handbuchliteratur.

Numerisch sind (3) und (4) in Abb. 3 angeschrieben, für (4) steht dort der analytische Ausdruck des passenden speziellen Integrals. Der Kurvenzug der Abb. 3 zeigt ein kräftiges Wachstum der Fingerhaut in der Kindheit, das bis ins mittlere Lebensalter verlangsamt anhält, um schliesslich einer langsamen Flächenabnahme zu weichen. Bekanntlich degenerieren in der Cutis etwa vom 40. Lebensjahr an allmählich die elastischen Fasern, in höherem Alter kommt dann neben dem Turgorverlust noch der allmähliche Schwund des Fettpolsters hinzu.

Betrachtet man nun die Anzahl der Meisner-Körperchen als Funktion zweier Argumente

$$M = f(t, F) \tag{5}$$

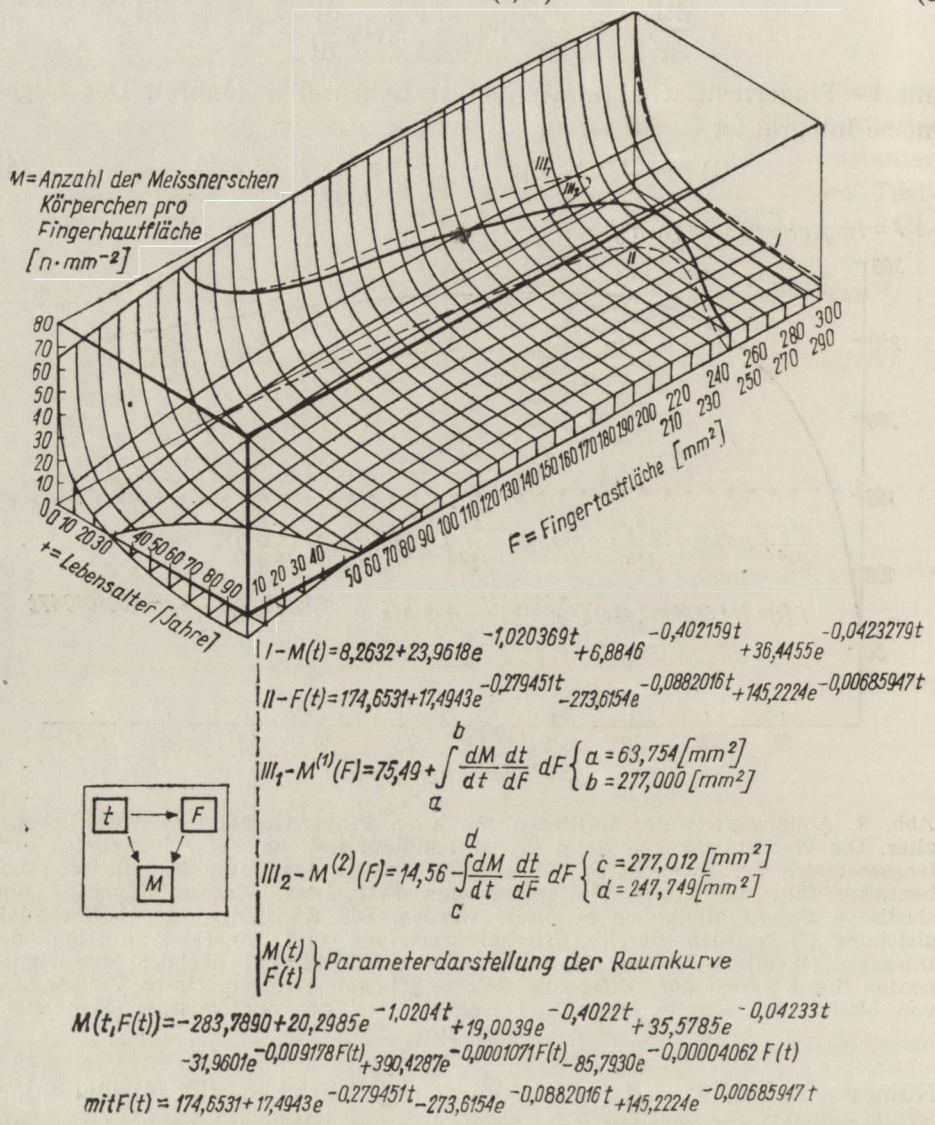


Abb. 4. Abhängigkeit der relativen Anzahl der Meissnerschen Tastkörperchen vom Lebensalter und von der aktuellen Grösse der Fingertastfläche, also M = f (t, F). Die transzendente Fläche (8) ist Lösung der Pfaffschen Differentialgleichung für 2 Argumente (7), sie enthält die Raumkurve (dick ausgezogen), um die alle Messwerte streuen. In die tM-Ebene ist die Kurve (2) aus Abb. 2 projiziert, in die tF-ebene die Kurve (4) aus Abb. 3. Diese Projektionen sind Parameterdarstellungen der Raumkurve. Die 3 mögliche Projektion in die MF-Ebene ist ebenfals eingezeichnet (siehe auch Abb. 5). Unten links das logische Schema der Abhängigkeiten.

dann sind die Gleichungen (2) und (4) die Parameterdarstellungen einer Raumkurve, die in der Fläche (5) enthalten ist (Abb. 4). Das logische Schema der Abhängigkeiten (5) ist ebenfalls in Abb. 4 dargestellt. Wollte man die direkte Abhängigkeit

$$M = f(F) \tag{6}$$

in einem geschlossenen analytichen Ausdruck darstellen, dann wäre dies nur im Komplexen möglich. (6) kann aber im Reellen zumindest

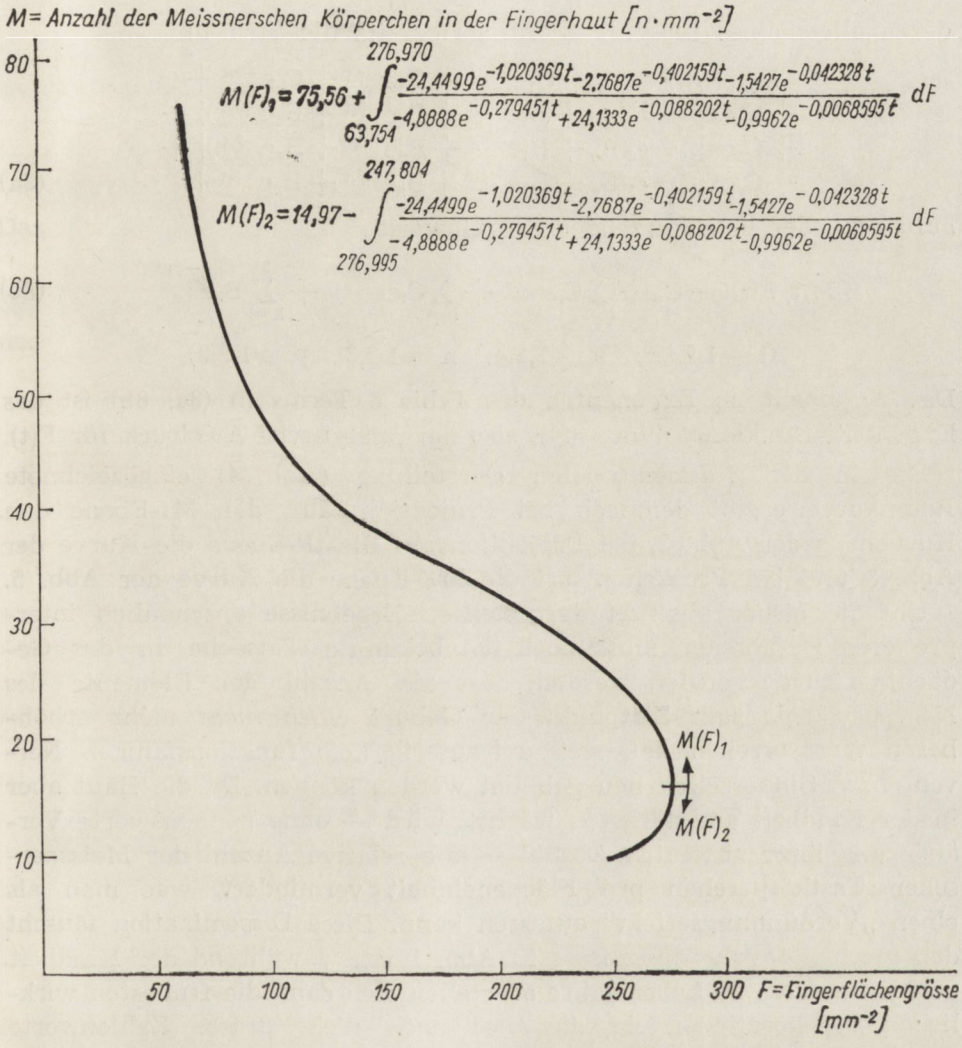


Abb. 5. Abhängigkeit der relativen Anzahl der Meissnerschen Tastkörperchen von der aktuellen Fingerflächengrösse. Die Darstellung im Reellen ist nur in 2 Stücken möglich, die Auswertung der Integrale nur numerisch. Die Kurve stellt die Projektion der Raumkurve (8) aus Abb. 4 in die FM-Fläche gesondert dar. Der untere Kurvenabschnitt gilt für alte Menschen.

numerisch aus zwei Kurvenstücken erhalten werden, wenn man von den beiden in Abb. 5 angegeben Integraldarstellungen ausgeht. Die transzendente Fläche (5) ergibt sich durch Integration der partiellen linearen Differentialgleichung 4.
Ordnung

$$\frac{\partial^4 M}{\partial t^4} + a_2 \frac{\partial^3 M}{\partial t^3} + a_1 \frac{\partial^2 M}{\partial t^2} + a_0 \frac{\partial M}{\partial t} + \frac{\partial^3 M}{\partial F^4} + \nu_2 \frac{\partial^3 M}{\partial F^3} + \nu_1 \frac{\partial^2 M}{\partial F^2} + \nu_0 \frac{\partial M}{\partial F} \qquad (7)$$

deren allgemeines Integral allerdings bereits die Struktur

$$\begin{aligned} M/t, F/t// = {} & C_0 + C_1 e^{-\gamma_1 t} + C_2 e^{-\gamma_2 t} + C_3 e^{-\gamma_3 t} + \\ & + C_4 \exp\{-\lambda_1 (B_0 + B_1 e^{-\beta_1 t} + B_2 e^{-\beta_2 t} + B_3 e^{-\beta_3 t})\} + \\ & + C_5 \exp\{-\lambda_2 (B_0 + B_1 e^{-\beta_1 t} + B_2 e^{-\beta_2 t} + B_3 e^{-\beta_3 t})\} + \\ & + C_6 \exp\{-\lambda_3 (B_0 + B_1 e^{-\beta_1 t} + B_2 e^{-\beta_2 t} + B_3 e^{-\beta_3 t})\} \end{aligned} \qquad (8a)$$

aufweist, oder in abkürzender Schreibweise

$$M/t, F(t)/ = C_0 + \sum_{i=1}^{3} C_i e^{-\gamma_n t} + \sum_{i=4}^{6} C_i e^{-\lambda_p / B_0} + \sum_{k=1}^{3} B_k e^{-\beta_k t} \qquad (8b)$$

$$(i = 1,2,...6; \quad k = 1,2,3; \quad n = 1,2,3; \quad p = 1,2,3)$$

Das Argument im Exponenten des 4 bis 6 Terms in (8a, 8b) ist das Exponentialfunkionspolynom (4), also der analytische Ausdruck für F(t).

Die in der 3-dimensionalen Darstellung (Abb. 4) eingezeichnete Raumkurve ergibt demnach bei Projektion auf die Mt-Ebene den Kurvenzug der Abb. 2, bei Projektion auf die tF-Ebene die Kurve der Abb. 3 und bei Projektion auf die MF-Ebene die Kurve der Abb. 5.

Um die bisher abstrakt dargestellten Ergebnisse anschaulich interpretieren zu können, muss noch die bekannte Tatsache in das Gedächtnis zurückgerufen werden, dass die Anzahl der Elemente des Nervensystems zum Zeitpunkt der Geburt einen nicht mehr erhöhbaren Wert erreicht hat, weil extrauterin kein funktionsfähigen Nerven- bzw. Sinneszellen neu gebildet werden können. Da die Haut aber in der Kindheit am stärksten wächst, wird — ohne nennenswerte Veränderung ihrer absoluten Anzahl — die relative Anzahl der Meissnerschen Tastkörperchen pro Flächeneinheit vermindert, was man als einen „Verdünnungseffekt" aufassen kann. Diese Dissemination täuscht den raschen Involutionsprozess in Abb. 1 und 2 während der Kindheit vor. Etwas vom 20 Lebensjahre an stellen sich dann die frühesten wirklichen Degenerationen ein, die aber noch nicht grosse Zahlenwerte annehmen, was erst vom 35 Lebensjahre and der Fall ist. Die rasche Degeneration in höherem Alter wird dann teilweise wieder dadurch kompensiert, dass die Hautfläche infolge des Altersumbaues etwas verkleinert wird.

Aus der Sicht der Neurophysiologie und der funktionellen Neuromorphologie interessiert nun, wie sich die geschilderten Vorgänge auf die Reizausnutzung auswirken. Bezeichnet man den punktförming wirkenden mechanischen Reizdruck mit P (g.mm^{-2}), den Reizerfolg mit E (%), dann können an Hand von Messungen die Koeffizienten der Differentialgleichung

$$\frac{d^4E}{dP^4} + c_2\frac{d^3E}{dP^3} + c_1\frac{d^2E}{dP^2} + c_0\frac{dE}{dP} = 0 \qquad (9)$$

bestimmt werden. In dieser Differentialgleichung sind die Koeffizienten anschaulich interpretierbar:

c_0 = Rückstellkonstante
c_1 = Dämpfungskonstante
c_2 = Trägheitskonstante

Das Integral dieser Gleich ist

$$E(P) = D_0 + D_1e^{-\mu_1 P} + D_2e^{-\mu_2 P} + D_3e^{-\mu_3 P} \qquad (10)$$

ein passendes partikuläres Integral zeigt Abb. 6. Der dem Augenschein nach näherungsweise lineare Kurvenzug ist nach statistischer Prüfung

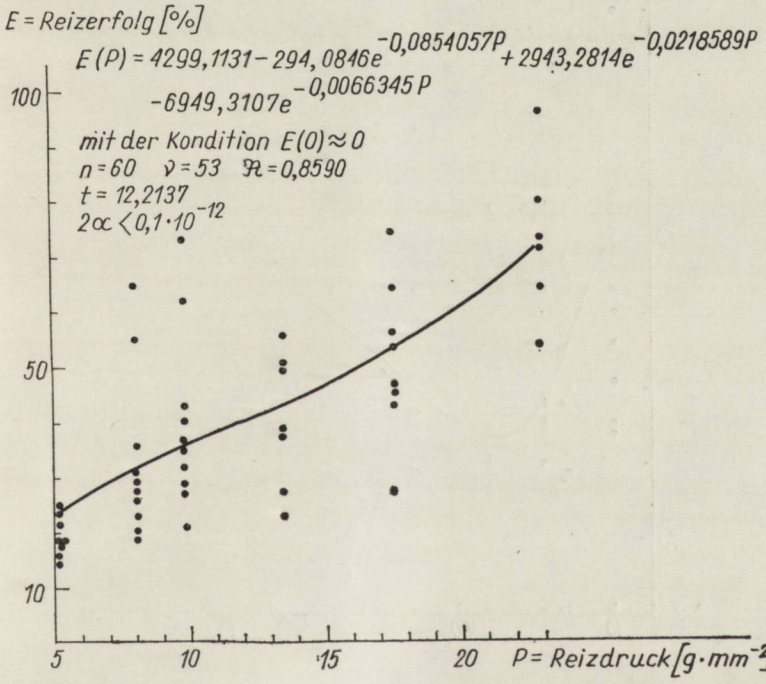


Abb. 6. Abhängigkeit des Reizerfolges vom Reizdruck pro mm² Fingertastfläche. Sehr starke Streuung der Punkte um die Kurve, weil das Lebensalter nicht berücksichtigt ist. Messungen von Ronge (1944). Der analytische Ausdruck (10) — durch Regressionsrechnung gewonnen — befriedigt die Differentialgleichung (9). Vergleiche mit Abb. 7.

mit der Annahme der Linearität nicht verträglich, so dass der Ansatz (9) gerechtfertigt erscheint. Das Gesetz ist einfach und plausibel: Wenn der Reizdruck erhöht wird, steigt auch der Reizerfolg. Die starke Streuung der Punkte um die Kurve ist darauf zurückzuführen, dass die Messungen bei Versuchspersonen unterschiedlichen Alters angestellt worden sind. Betrachtet man nämlich den Reizerfolg E(%) in Abhängigkeit vom Lebensalter (t), dann gilt die Differentialgleichung

$$\frac{d^4E}{dt^4} + s_2\frac{d^3E}{dt^3} + s_1\frac{d^2E}{dt^2} + s_0\frac{dE}{dt} = 0 \tag{11}$$

mit dem allgemeinen Integral

$$E(t) = G_0e^{-\varphi_1 t} + G_2e^{-\varphi_2 t} + G_3e^{-\varphi_3 t} \tag{12}$$

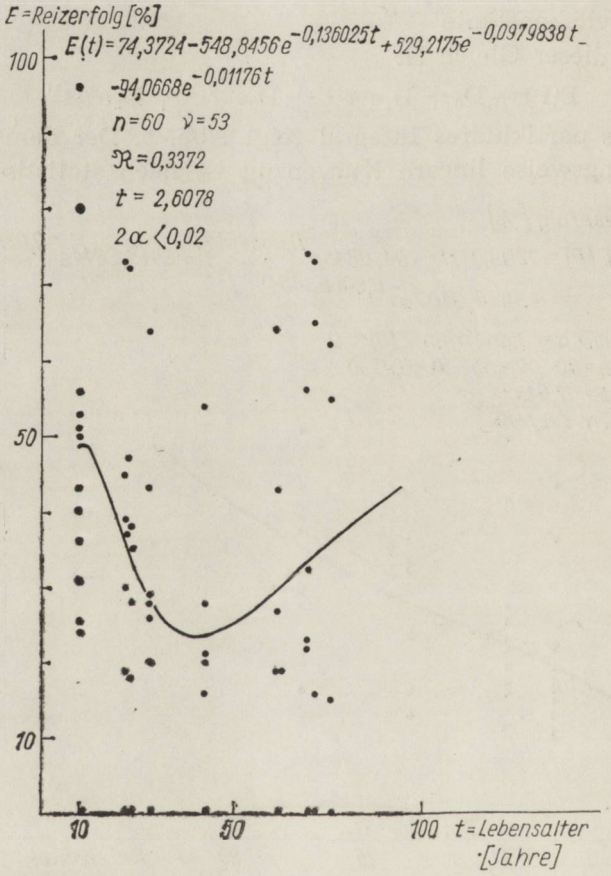


Abb. 7. Abhängigkeit des Reizerfolges in Abhängigkeit vom Lebensalter gemäss Differentialgleichung (11) und deren Lösung (12) Berechnung des analytischen Ausdruckes durch Innere und anschliessende lineare Regression. Die starke Streuung der Punkte ist dadurch bedingt, dass hier der Reizdruck unbeachtet bleibt. Vergleiche mit Abb. 6.

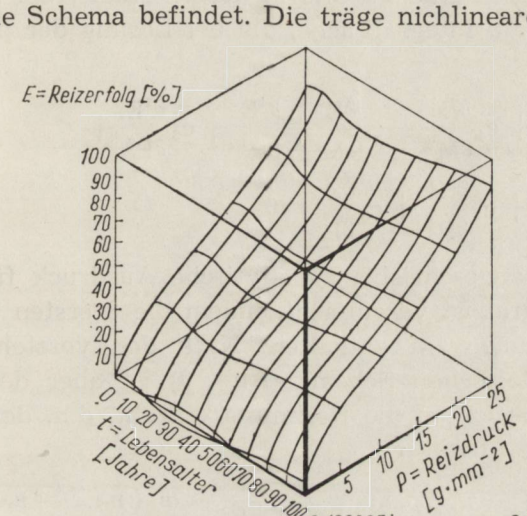
Ein für die Messwerte passendes partikuläres Integral ist in Abb. 7 gezeichnet. Hier ist die Nichtlinearität sofort deutlich, ebenso aber die starke Streuung, die sich ohne weiters erklären lässt, weil hier der aufgewandte Reizdruck ausgeschaltet ist. Die vorstehenden Bemerkungen machen bereits deutlich, dass es sinnwoll ist, anstelle der getrennten Differentialgleichungen (9) und (11) die Summe aus beiden zu verwenden, also die lineare partielle Differentialgleichung vom Pfaffschen Typ

$$\frac{\partial^4 F}{\partial p^4} + c_2 \frac{\partial^3 E}{\partial p^3} + c_1 \frac{\partial^2 E}{\partial p^2} + c_0 \frac{\partial E}{\partial P} + \frac{\partial^4 E}{\partial t^4} + s_2 \frac{\partial^3 E}{\partial t^3} + s_1 \frac{\partial^2 E}{\partial t^2} + s_0 \frac{\partial E}{\partial t} = 0 \qquad (13)$$

deren allgemeines Integral

$$E(t,P) = K_0 + K_1 e^{-\varphi_1 t} + K_2 e^{-\varphi_2 t} + K_3 e^{-\varphi_3 t} + K_4 e^{-\mu_1 P} + K_5 e^{-\mu_2 P} + K_6 e^{-\mu_3 P} \qquad (14)$$

eine transzendente Fläche 4 Ordnung ist. Das die Messwerte befriedigende partikuläre Integral ist in Abb. 8 gezeigt, wo sich auch das logische Schema befindet. Die träge nichlineare Oscillation der Integral-



$$E(t,P) = 4052{,}8878 - 80{,}0584e^{-0{,}136025t} + 95{,}7825e^{-0{,}097984t} - 30{,}4474e^{-0{,}011760t} - 269{,}9429e^{-0{,}085406P} + 2729{,}6624e^{-0{,}021859P} - 6498{,}0430e^{-0{,}006635P}$$

$n = 67 \quad \nu = 54$
$\mathfrak{R} = 0{,}9082$
$t = 15{,}9430$
$2\alpha < 0{,}1 \cdot 10^{-33}$

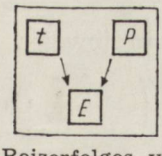


Abb. 8. Abhängigkeit des Reizerfolges vom Reizdruck und vom Lebensalter zugleich gemäss dem logischen Schema unten links. Sehr gute Anpassung der Integralfläche (14) an die Messpunkte, die nicht eingezeichnet sind. Dies ist aber daran zu erkennen, dass trotz Abnahme der Freiheitsgrade (in Abb. 6 und 7 ist jeweils $\nu = 53$) der Korrelationskoeffizient ($\varkappa$) und der Wert von t zugenommen haben. Die Integralfläche befriedigt die Pfaffsche Differentialgleichung (13).

fläche in t-Richtung ist besonders interessant, zeigt sie doch deutlich, dass Kinder überempffindlich sind, Erwachsene eine relative Unterempfindlichkeit aufweisen, während alte Menschen gegenüber akuten Reizen eine an Kinder erinnerndte relative Hypersensibilität erkennen lassen. Histologisch ist dieses merkwürdige Greisenverhalten erklärbar: Die Altersveränderungen der Haut bedingen eine leichte Deformierbarkeit, so dass ein Reiz nicht nur auf direkt betroffene Tastkörperchen wirkt, sondern auch auf solche in der weiteren Umgebung. Diese Tatsache steht nicht im Widerspruch zu der klinisch lange bekannten Greisenindolenz, die für chronisch wirkende Reize gilt: Die absolut niedrige Zahl der sensiblen Organellen lässt alte Menschen „abstumpfen".

Der vorerst letzte Schritt einer analytischen Betrachtung dieses Wechselspiels zwischen Wachstums- und Involutionsprozessen im Zusammenhang mit der Tastsinnesfunktion wirft die Frage auf, ob die Abhängigkeiten vollständig in eine Theorie zu fassen sind. Abb. 9 zeigt das logische Schema und die Integralfläche, die bei Lösung der partiellen Differentialgleichung

$$\frac{\partial^4 E}{\partial M^4} + c_5 \frac{\partial^3 E}{\partial M^3} + c_4 \frac{\partial^2 E}{\partial M^2} + c_3 \frac{\partial E}{\partial M} + \frac{\partial^4 E}{\partial P^4} + c_2 \frac{\partial^3 E}{\partial P^3} + \\ + c_1 \frac{\partial^2 E}{\partial P^2} + c_0 \frac{\partial E}{\partial P} = 0 \qquad (15)$$

entsteht. Der bei Abb. 9 angeschriebene analytische Ausdruck für das passende partikuläre Integral ist vereinfacht, indem die 3 ersten Terme im Exponenten das Argument M enthalten. Nach den vorstehenden Ausführungen und dem logischen Schema (Abb. 9) ist aber deutlich, dass M wiederum als Funktion in die Rechnung eingeht, d.h. der analytische Ausdruck hat die Struktur

$$E(M,P) = J_0 + J_1 e^{-\gamma_1} \Big(C_0 + C_1 e^{-\alpha_1 t} + C_2 e^{-\alpha_2 t} + C_3 e^{-\alpha_3 t} + C_4 e^{-\lambda_1 (B_0 + B_1 e^{-\beta_1 t} + B_2 e^{-\beta_2 t} + \\ + B_3 e^{-\beta_3 t})} + C_5 e^{-\lambda_2 (\ldots)} + C_6 e^{-\lambda_3 (\ldots)} + J_2 e^{-\lambda_2 (\ldots)} + J_3 e^{-\gamma_3 (\ldots)} + J_4 e^{-\mu_1 P} + J_5 e^{-\mu_2 P} + \\ + J_6 e^{-\mu_3 P} \qquad (16a)$$

oder vollständig in abgekürzter Schreibweise

$$E(M,P) = J_0 + \sum_{j=1}^{3} \left\{ J_j \exp \left[-\gamma_j \left(C_0 + \sum_{i=1}^{3} C_i e^{-\alpha_n t} + \sum_{i=4}^{6} C_i \exp - \right. \right. \right. \\ \left. \left. \left. - \lambda_p \left(B_0 + \sum_{k=1}^{3} B_k e^{-\beta_k t} \right) \right) \right] \right\} + \sum_{j=4}^{6} J_j e^{-\mu_m P} \qquad (16b)$$

i=1,2,..., 6; j=1,2,..., 6; k=1, 2, 3; m=1, 2, 3; n=1, 2, 3; p=1, 2, 3;

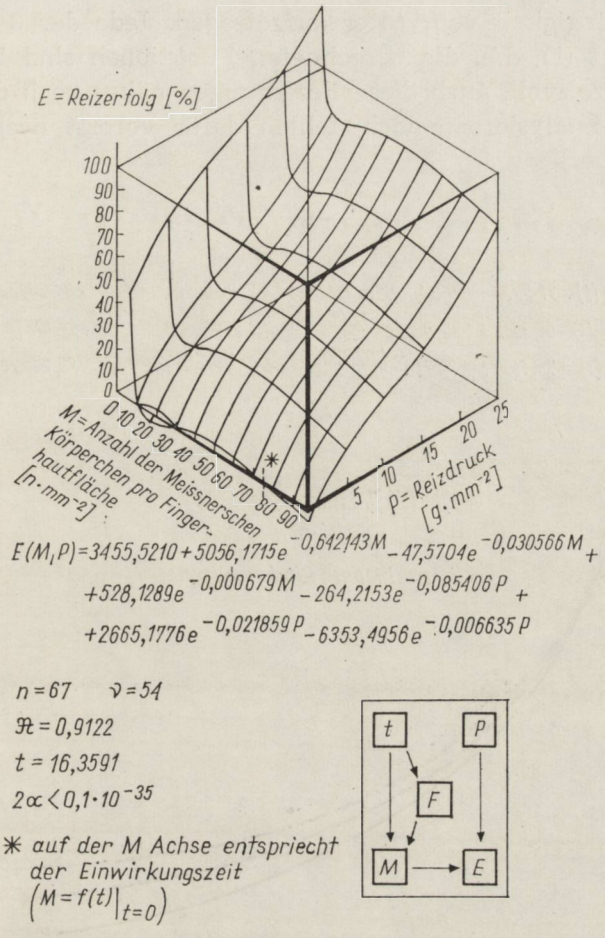


Abb. 9. Abhängigkeit des Reizerfolges vom Reizdruck und von der relativen Anzahl der Meissnerschen Tastkörperchen zugleich. Der vereinfachte analytische Ausdruck hat die Struktur (16), er ist Lösung von (15). Den logischen Aufbau demonstriert das rechts unten eingesetzte Schema. Biologisch sinnvoll ist nur der Flächenabschnitt, der vom Intervall $8 \leqslant M \leqslant 75$ begrenzt wird.

Wie man sieht geht das Exponentialfunktionespolynom (8a) bzw. (8b) als Argument in die Exponenten der drei ersten variablen Terme des Integrals (16b) ein. Damit ist die Abhängigkeit des Reizerfolges

$$E(M/t,\ F/t),\ P)$$

strukturell und kausal nur auf Zeit- und Druckabhängigkeit reduziert. Prüft man die Gültigkeit der Differentialgleichung (1) auf andere Teile des Nervensystems, wobei die abhängige Variable M gegen die jeweils betrachtete auszutauschen wäre, dann lässt sich feststellen, dass (1) weite Gültigkeit hat. In Abb. 10 sind drei andere zeitabhängige Varia-

blen in Abhängigkeit vom Alter aufgetragen. Jede der 3 Intergralkurven befriedigt (1), d.h. die berechneten Funktionen sind Lösungen von (1). Es wäre reizvoll, auch diese Beziehungen näher nach dem gezeigten Schema zu analysieren, doch fehlen dafür vorerst noch verlässliche Messungen als Basis.

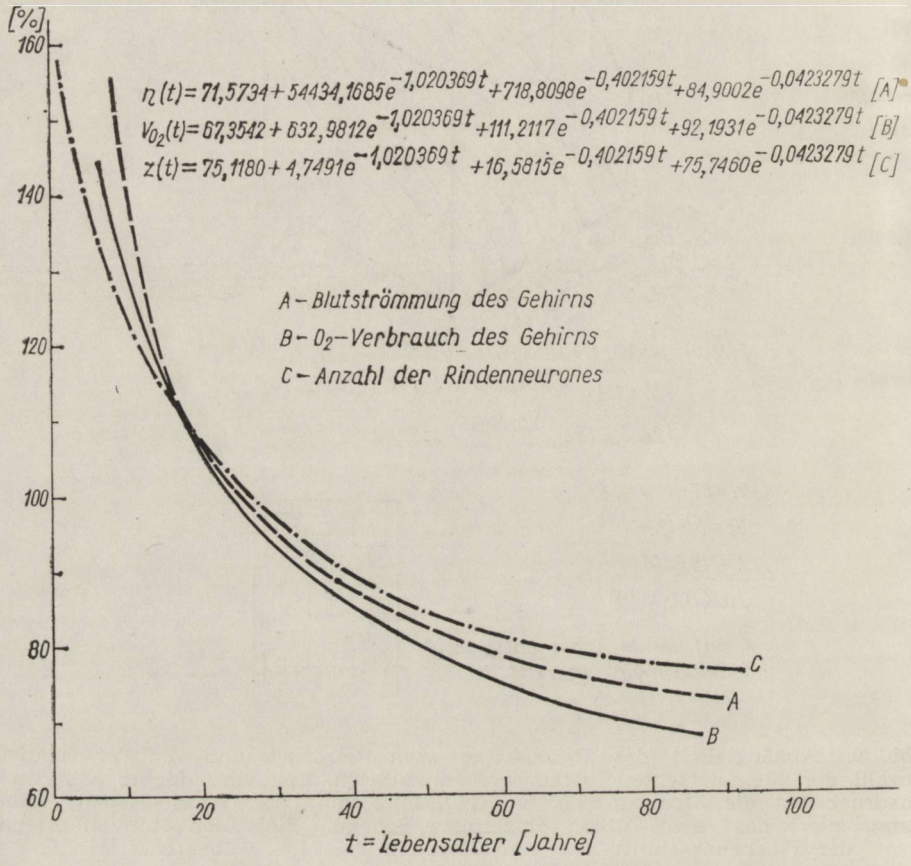


Abb. 10. Die Differentialgleichung (1) beschreibt aus andere altersabhängige Prozesse im Nervensystem, wenn man M als Ordinatenbezeichnung entsprechend auswechselt. Die drei hier gezeigten Kurven sind Lösungen der Gleichung (1) Daten aus Bourlière 1966.

Jedenfalls zeigen die vorstehenden Ausführungen dass die Anwendung der Analysis über die einfache statistische Auswertung hinaus durchaus zu brauchbaren und sinnvollen Modellen führt und damit erlaubt, den Versuch der Aufstellung einer Theorie des Alterns nervöser Strukturen nicht mehr als von vornherein absurdes Unterfangen beiseite schieben zu müssen.

J. H. Scharf

TEORIA PROCESU STARZENIA W UKŁADZIE NERWOWYM CZŁOWIEKA

Streszczenie

Gdy przyjmie się za wzorzec ciałka Meissnera, wyłania się możliwość przedstawienia matematycznej teorii procesu starzenia. Involucja liczby względnej meissnerowskich narządów dotykowych na jednostkę powierzchni tkanek miąższu palca nie jest wyrazem czystego procesu starzenia się, lecz wypadkową pomiędzy wzrostem a starzeniem się. Przedstawia się dwa modele matematyczne, również dla wykorzystania podrażnień.

Г. Шарф

К ТЕОРИИ ПРОЦЕССА СТАРЕНИЯ В НЕРВНОЙ СИСТЕМЕ ЧЕЛОБЕКА

Содержание

На примере осязательных телец Мейсснера пытается установить математическую теорию возраста. Инволюция относительного числа органелл Мейсснера по единице поверхности кожи не является чистым процессом старения, но представляет собой взаимодействие между ростом и старением. Для использования раздражения также предлагаются математические модели.

LITERATUR

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EFFECT OF PRENATAL X-IRRADIATION ON RESPIRATION AND GLYCOLYSIS OF THE BRAIN IN ONTOGENIC DEVELOPMENT

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Among the factors disturbing the regular development of the central nervous system, ionizing radiation plays an important role. In searching for mechanisms responsible for developmental brain alterations as the effect of irradiation of the foetus, biochemical changes during ontogenesis are the subject of the greatest interest. A lot of these changes were noticed during experiments made lately in our laboratory (Mularek, 1968; Wender, 1964; Wender et al., 1964; Wender, 1965).

The purpose of the present experiments was to establish the influence of irradiation of animals during their final foetal life on the respiration process and the glycolysis of the brain, in the different stages of their ontogenic development.

MATERIAL AND METHODS

Experimental animals — rabbits (Chinchilla) — were irradiated with a single dose 150 r of X-ray radiation on the 18th day of foetal life. The day of fecondation was considered the first day of pregnancy. For irradiation Picker's therapeutic Roentgen apparatus was utilized, using the following settings: 18 KV, 15 mA current and 0·5 mm Cu and 1·0 mm Al filters. The distance of the lamp focus to the detector was exactly 72 cm, the dose-rate was 26 r/min. To obtain 150 r dose — the animals were irradiated for 5 min. 48 sec. During irradiation the pregnant females were placed in a special box, made of plexiglass and paraffin, where the quantity of the rate of radiation had been previously determined.

Measurements of the tissue respiration and the anaerobic glycolysis rate in the brain were carried out during the following periods of ontogenic development: the 20th day of foetal life and the 1st, 3rd, 12th, 30th, 48th and 120th day after delivery.

In choosing the age of experimental animals the occurence of the phenomena, which contribute greatly to the development of the nervous system in rabbits were taken into account. According to Palladin (1955) the following stages are especially important in the brain development of the rabbit: the period of extensive differentiation of the nervous tissue (16th - 20th day of foetal life), beginning of the extrauterine life (1st day after delivery), period of appearance of new functions i.e. the sight (9th — 10th day of life) and the period, in which many biochemical processes approach the level specific for adult animals (30th day of life).

The myelination process was also taken into account; this process begins between the 9th — 20th day of extrauterine life and is completed between the 30th — 56th day of life (Edgar, 1955).

The animals were killed by decapitation and the samples of white and grey matter of the brain hemispheres were taken immediately for the experiment. In older animals the white matter and grey matter could be distinguished. In younger animals the tissue was taken from the sites, in which later the grey and white matter appears; the hemispheres of the foetuses were investigated in toto. For biochemical determination 10% homogenate was prepared. The tissue respiration and glycolysis were measured manometrically, using the Warburg apparatus, at 38°C. The oxygen consumption was measured using direct method of Warburg in an atmosphere of oxygen (Umbreit et al., 1959), as a medium the Krebs-Ringer solution with phosphat buffer (pH 7,4) was used. Immediately before performance of measurement, 2 mg/ml glucose was added. The determination of the anaerobic glycolysis was carried out according to Negelein (1956) in an atmosphere of gas mixture: 95% N_2 + 5% CO_2; as a medium the Krebs-Ringer solution with carbonate buffer (pH 7·4) was used. The retention of lactate was taken into consideration. Gasometric data were expressed (Q_{C_2}; Q_M) as quantities of absorbed or liberated gas per mg of dry tissue in one hour.

In the same developmental periods as the irradiated animals — normal rabbits, not irradiated — were taken for experiments as a control group. Fisher's test was used for variancy analysis; the statistical significance of the data was determined using Student's test. Confidence coefficient $P \leqslant 0{\cdot}05$ was taken to determine the significance.

In all examined periods of both experimental groups — the brain was taken for histological studies. The brain was fixed in a solution of 10% formaline. The frozen slices, 20 μ thick, were coloured according to the Spielmeyer's method (Romeis, 1948). On the basis of microscopic

examination the degree of myelination of the nervous fibres in the brain of the irradiated rabbits and the control group was compared.

RESULTS

The results of the investigation of the normal rabbits indicate, that tissue respiration and anaerobic glycolysis rates in the grey and white matter of the brain are related to their maturation and change considerably as the development of the animals proceeds (Tab. 2, 3). During the period which directly follows delivery — the tissue respiration shows similar values for the grey and white matter of the brain. These values diverge during further ontogenic development: as the central nervous system of the rabbits matures — the oxygen consumption in the grey matter of the brain — increases (Fig. 3). In the white matter —

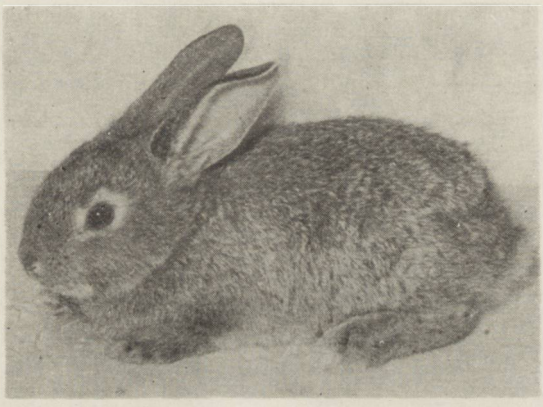

Fig. 1a. The normal rabbit. Age: 30 days.

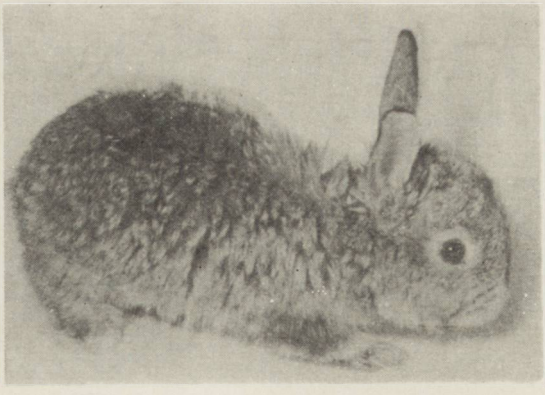

Fig. 1b. The rabbit irradiated on the 18th day of foetal life with a dose 150 r. Age: 30 days.

the tissue respiration assumes the highest values during the period, which directly follows delivery, when the myelination process is beginning. During the next developmental periods — the tissue respiration in the white matter — decreases (Fig. 3). The glycolytic activity in the grey and white matter of the brain — is similar to the tissue respiration (Fig. 4). Very detailed results of investigations of the tissue respiration process and anaerobic glycolysis of the brain during normal ontogenic development of the rabbits were published earlier (Owsianowski, 1968).

The irradiation of the pregnant females has no influence on the delivery term or on the number, appearance, behaviour and weight of the newborn animals.

The first general irradiation effects were seen the third or the fourth week after delivery. The mobility, the gloss of the hair, the weight of the body and the brain of the irradiated animals were much smaller than those of the control group (Tab. 1, Fig. 1a, b).

In the further developmental periods — the irradiated animals differed more distinctly from those of the control group of the same age (Tab. 1, Fig. 2). The results of the biochemical investigations indicate, that the ionizing radiation distrubed also the dynamics of the brain developmental changes, of oxygen consumption and the glycolytic activity of the brain. (Fig. 3, 4). The decrease of oxygen consumption in the brain cortex of the irradiated animals was seen just the first day after

Fig. 2. Rabbits aged 120 days. On the right — rabbit irradiated on the 18th day of foetal life with a dose 150 r: on the left — normal rabbit (control group).

Table 1. The weight of the body and the brain (in grams) of normal and irradiated rabbits

Age		Weight of the body				Weight of the brain			
		normal rabbits	irradiated rabbits	difference of the mean values (%)	confidence coefficient	normal rabbits	irradiated rabbits	difference of the mean values (%)	confidence coefficient
		mean values	mean values			mean values	mean values		
20th day of foetal life		2·25± 0·36	2·20± 0·21	2	$P > 0{\cdot}05$	0·33±0·03	0·27±0·04	18	$P > 0{\cdot}05$
Days after delivery	1	35·83± 2·64	34·30± 2·58	4	$P > 0{\cdot}05$	1·06±0·09	1·07±0·12	1	$P > 0{\cdot}05$
	3	58·00± 1·18	57·17± 7·20	2	$P > 0{\cdot}05$	1·63±0·06	1·61±0·07	1	$P > 0{\cdot}05$
	12	165·00± 20	163·00± 21	1	$P > 0{\cdot}05$	2·91±0·20	2·59±0·23	11	$P > 0{\cdot}05$
	30	535·00± 24	431·00± 23	18	$P < 0{\cdot}001$	6·08±0·06	5·11±0·79	16	$P < 0{\cdot}05$
	48	1253·00±180	614·00± 56	51	$P < 0{\cdot}001$	6·43 0·40	5·00±0·64	22	$P < 0{\cdot}001$
	120	1751·00±159	1306·00±114	25	$P < 0{\cdot}001$	6·75±0·29	6·07±0·19	10	$P < 0{\cdot}001$

Mean values were counted on the base of the weight of 6 animals of each group;
± standard error

the delivery and than the 12th day. The greatest difference of the respiration — in comparison with the control group — was found the 30th day, after delivery. A slighly smaller difference was noticed during the next examined periods: the 48th and the 120th day of life (Tab. 2, Fig. 5). In the white matter — the decrease of tissue respiration was seen the 1st and the 30th day after delivery (Tab. 2, Fig. 6). The great-

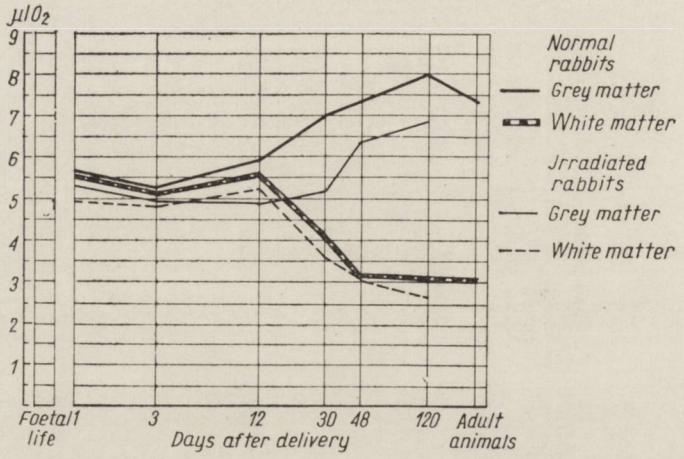

Fig. 3. Tissue respiration of the grey and white matter of the brain of irradiated and normal rabbits — during different periods of ontogenic development. The quantity of µl O_2 corresponds to the mean values of Qo_2 as shown in Table 2.

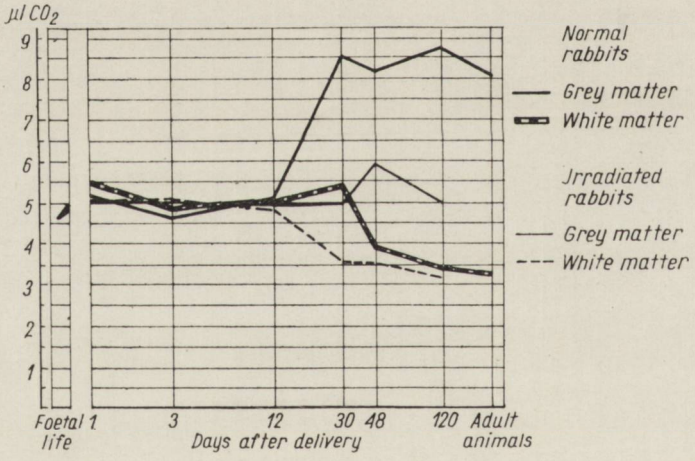

Fig. 4. Anaerobic glycolysis of the grey and white matter of the brain of irradiated and normal rabbits — during different periods of ontogenic development. The qunatity of µl CO_2 corresponds to the mean values of Q_MN_2 as shown in Table 3.

Table 2. The oxygen consumption of the grey and white matter of the brain of irradiated and normal rabbits — during different developmental periods

Age		Grey matter				White matter			
		normal rabbits	irradiated rabitsb	difference of the mean values (%)	confidence coefficient	normal rabbits	irradiated rabbits	difference of the mean values (%)	confidence coefficient
		mean values	mean values			mean values	mean values		
20th day of foetal life		4·64±0·14	4·58±0·29	1	$P > 0·05$	4·64±0·14	4·58±0·29	1	$P > 0·05$
Days after delivery	1	5·69±0·15	5·31±0·29	7	$P < 0·02$	5·64±0·18	4·47±0·43	20	$P < 0·001$
	3	5·29±0·14	4·96±0·41	6	$P > 0·05$	5·17±0·67	4·78±0·17	7	$P > 0·05$
	12	5·96±0·11	4·99±0·46	16	$P < 0·001$	5·55±0·28	5·31±0·24	4	$P > 0·05$
	30	7·03±0·23	5·25±0·64	25	$P < 0·001$	4·06±0·18	3·60±0·30	11	$P < 0·01$
	48	7·39±0·23	6·34±0·33	14	$P < 0·001$	3·23±0·22	3·02±0·25	6	$P > 0·05$
	120	8·03±0·34	6·86±0·47	15	$P < 0·001$	3·08±0·17	2·74±0·37	11	$P > 0·05$

Mean values indicate the number of μl O_2/mg of dry weight of tissue/hour;
± standard error

est decreases of the oxygen consumption, induced by ionizing radiation – was seen — mainly in the brain cortex and partially also in the white matter — during these developmental periods, in which it assumes the greatest rate in normal animals.

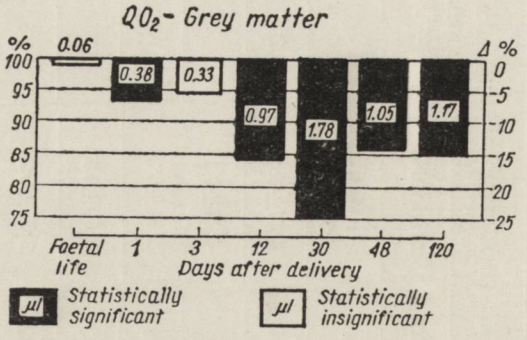

Fig. 5. Difference between the oxygen consumption of the grey matter of the brain of irradiated and normal rabbits — during different periods of development. The differences are indicated in % and in μl O_2.

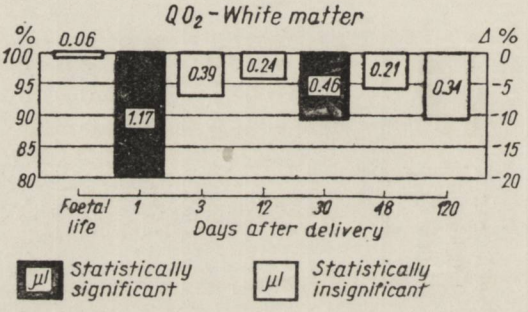

Fig. 6. Differences between the oxygen consumption of the white matter of the brain of irradiated and normal rabbits — during different periods of development. The differences are indicated in % and μl O_2.

The anaerobic glycolysis in the brain of the irradiated animals decreases mainly in the grey matter, but in the next periods it decreases, parallel with the appearence of the growth perturbations (Tab. 3, Fig. 7). In the white matter the significant decrease of glyolytic activity was noticed the 30th day after delivery (Tab. 3, Fig. 8); in the other developmental periods — the anaerobic glycolysis in the white matter of the brain of irradiated animals and the control group is similar. Histological studies of myelination of the nervous fibres of the brain of the irradiated animals showed no visible effect, except a slight retardation of this process.

Table 3. The anaerobic glycolysis of the grey and white matter of the brain of irradiated and normal rabbits — during different developmental periods

Age		Grey matter				White matter			
		normal rabbits	irradiated rabbits	difference of the mean values (%)	confidence coefficient	normal rabbits	irradiated rabbits	difference of the mean values (%)	confidence coefficient
		mean values	mean values			mean values	mean values		
20th day of foetal life		4·66±0·23	4·63±0·37	1	$P > 0·05$	4·66±0·23	4·63±0·37	1	$P > 0·05$
Days after delivery	1	5·22±0·07	5·07±0·24	3	$P > 0·05$	5·48±0·40	5·12±0·25	7	$P > 0·05$
	3	4·66±0·21	4·85±0·40	4	$P > 0·05$	4·80±0·21	5·04±0·24	5	$P > 0·05$
	12	5·11±0·44	4·98±0·34	3	$P > 0·05$	5·07±0·31	4·89±0·49	3	$P > 0·05$
	30	8·56±0·55	4·97±0·70	42	$P < 0·001$	5·38±0·57	3·68±0·84	32	$P < 0·01$
	48	8·24±0·32	5·98±0·24	27	$P < 0·001$	3·83±0·24	3·63±0·30	5	$P > 0·05$
	120	8·72±0·26	4·93±0·34	43	$P < 0·001$	3·40±0·10	3·21±0·50	6	$P > 0·05$

Mean values indicate the number of μl CO_2/mg of dry weight of tissue/hour;
± standard error

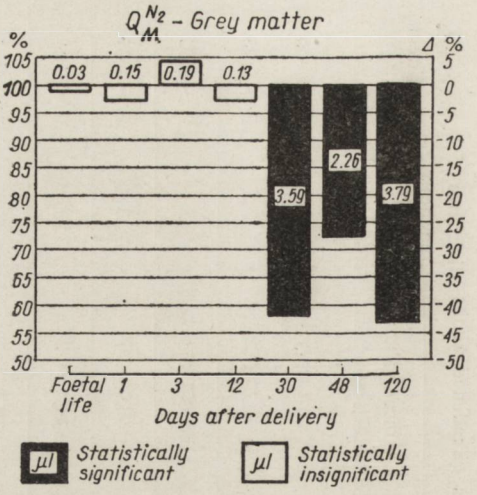

Fig. 7. Differences between the anaerobic glycolysis of the grey matter of the brain of irradiated and normal rabbits — during different periods of development. The differences are indicated in % and in μl O_2.

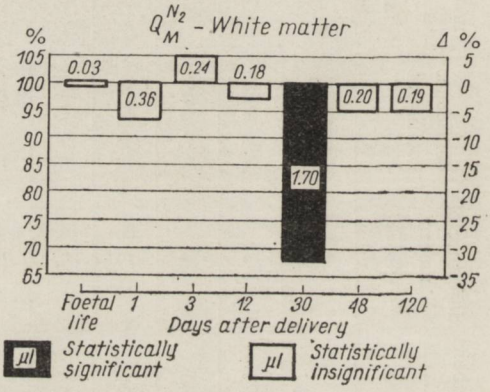

Fig. 8. Differences between the anaerobic glycolysis of the white matter of the brain of irradiated and normal rabbits — during different periods of development. The differences are indicated in % and μl O_2.

DISCUSSION

Beside the morphological consequences of ionizing radiation on the nervous system which has been examined very carefully and in details, the most interesting problems are the biochemical perturbations caused by this factor. Among numerous works on changes of this kind an important place is given to experiments of the metabolism of nucleic acids, the synthesis of which being joined with the mitotic process of brain development (Fitz-Niggli, 1955; Gärtner, 1963; Holmes, 1947; Hovard et al., 1953; Mularek, 1968).

Great attention was also paid to the fact that irradiation of the foetus in the last period of foetal life causes perturbations of the normal developmental changes in the brain electrolytes (Wender, 1964), disturbances of amino acids differentiation in the brain proteins during maturation of the central nervous system (Wender et al., 1964), the reduction of activity of aryl sulphatase (Wender et al., 1964) and the temporary perturbations of brain lipids (Wender, 1963).

The tissue respiration processes and the activity of the enzymes in the irradiated tissues have also been the subjects of examination for the influence of ionizing radiation on the organism, but the results of these experiments are often very diverse.

Florscheim et al. (1952) did not notice-after ionizing radiation of mice with a dose of 500 r-any changes of oxygen consumption in the brain, neither directly after the irradiation, nor 190 hours later. The irradiation of the cell suspensions of mice did not cause — as Schmidlin-Meszaros (1954) have shown — any decrease of the activity of the enzymes of the citric acid cycle. Some authors noticed, that the decrease of tissue respiration can be observed while examining the isolated mitochondries (Fritz-Niggli, 1955; Ryser et al., 1954).

In contradiction to these observations there are some reports indicating the increase of oxygen consumption in the tissue homogenates after the irradiation of rats with a dose 200 — 1400 r (Kunckel et al., 1952). The results of experiments of the influence of irradiation on anaerobic glycolysis are more in accordance. Inhibition of the glycolytic processes was observed in the retina of the rat (Crabtree, 1939) and in the spleen of the mouse (Hickman et al., 1953).

Very interesting investigations of the influence of ionizing radiation on the respiration of the retina were made by Cohen and Noel (1960). They found that irradiation with doses which do not change the bioelectrical function of the retina, have no influence on the oxygen consumption in the tissue whereas stronger doses or radiation, producing evident changes in the electroretinogram, also cause an important inhibition of the respiration and glycolytic processes. These data show a parallelism of the metabolic and functional consequences of ionizing radiation.

The divergent results of investigations of the influence of irradiation on the tissue respiration processes seem to depend on unequal conditions of the experiments, on the time of observation after the action of ionizing radiation and especially on the degree of maturity of the irradiated animals.

Knowing the close connection between tissue respiration and glycolysis with the maturation of the central nervous system (Flexner et al., 1941; Flexner, 1955; Himwich, 1939; Himwich et al., 1941; Owsianowski, 1968), our own experiments concerned the influence of irradiation of the animals in their final foetal life on the dynamic of these processes in the brain during the ontogenic development.

The general radiobiological observations concerning the appearence and the behaviour of the irradiated animals are all in accordance with the results of those of a lot of authors, indicating, that ionizing radiation acting on the organism in the final period of foetal life causes smaller and often more distant morphological consequences than those used in the first phase of the organogenesis (Hicks, 1953; Job et al., 1935; Lamerton et al., 1953; Mularek, 1968; Wilson et al., 1950; Wilson, 1954). The lack of essential perturbations of the myelination process in the nerve fibres in the brain of the irradiated rabbits on the 18th day of the foetal life is in agreement with the results of the examinations of Wender (1965), who noticed only a very slight retardation of this process after having irradiated guinea-pigs in their final period of foetal life. These observations show a relatively slight sensitivity of the myelination process on ionizing radiation acting in the last period of foetal development, in contradiction to the metabolic phenomena, the perturbations of which were presented in this work. The biochemical experiments showed, that ionizing irradiation acting during foetal life — caused a metabolic error distinctly seen, at the time of the ontogenic brain development.

CONCLUSIONS

1. Irradiation of the rabbits on the 18th day of foetal life with a dose 150 r of X-Ray disturbed the normal developmental changes of the tissue respiration and the anaerobic glycolysis of the brain during ontogenesis.

2. The decrease of oxygen consumption induced by ionizing radiation is seen mainly in the grey matter of the brain and partially in the white matter. The greatest decrease of this process occurs during those developmental periods, in which it assumes the greatest rate in normal animals.

3. The anaerobic glycolysis of the brain of irradiated rabbits decreases mainly in the grey matter in later periods of extrauterine development, coinciding with growth perturbation caused by ionizing radiation.

4. The myelination process of the nervous fibres of the brain of irradiated animals is only slightly retarded.

M. Owsianowski

WPŁYW DZIAŁANIA PROMIENI X W ŻYCIU PŁODOWYM NA ODDYCHANIE TKANKOWE I GLIKOLIZĘ MÓZGU W CZASIE ROZWOJU ONTOGENETYCZNEGO

Streszczenie

U królików napromienionych w 18 dniu życia wewnątrzmacicznego pojedynczą dawką 150 r — badano oddychanie tkankowe i glikolizę beztlenową w mózgu w różnych okresach rozwoju ontogenetycznego. Zużycie tlenu przez mózg i aktywność glikolityczną oznaczono metodami manometrycznymi, posługując się aparatem Warburga.

Porównując wyniki badań u królików napromienionych i u zwierząt prawidłowo rozwijających się stwierdzono, że promieniowanie jonizujące, zastosowane w końcowym okresie życia płodowego zaburza prawidłową dynamikę zmian rozwojowych oddychania tkankowego i glikolizy mózgu. Największe obniżenie zużycia tlenu u zwierząt stwierdzono w istocie szarej mózgu; w mniejszym stopniu w istocie białej. Zjawisko to występuje głównie w tych okresach rozwojowych, w których u zwierząt normalnych rozpoczyna się wzrost oddychania tkankowego mózgu.

Glikoliza beztlenowa w mózgu królików napromienionych ulega obniżeniu głównie w istocie szarej; następuje to jednak w późniejszych okresach dojrzewania, kiedy obserwuje się wywołane działaniem promieni jonizujących zmiany w wyglądzie i zachowaniu się zwierząt oraz spadek wagi ich ciała i mózgu.

W przeciwieństwie do stwierdzonych zaburzeń metabolicznych, stanowiących następstwo napromienienia królików w życiu płodowym, nie obserwowano istotnego wpływu napromienienia na proces mielinizacji włókien nerwowych w mózgu w czasie jego dojrzewania.

W pracy omówiono niektóre aspekty działania promieniowania jonizującego na ośrodkowy układ nerwowy.

M. Овсяновски

ВЛИЯНИЕ РЕНТГЕНОВЫХ ЛУЧЕЙ ВО ВНУТРЕУТРОБНОМ ПЕРИОДЕ НА ТКАНЕВОЕ ДЫХАНИЕ И МОЗГОВОЙ ГЛИКОЛИЗ ВО ВРЕМЯ ОНТОГЕНЕТИЧЕСКОГО РАЗВИТИЯ

Содержание

У кроликов облучённых в 18 дне внутриутробной жизни одиночной дозой 150 р. — в различных периодах онтогенетического развития исследовали тканевое дыхание и анаэробный гликолиз головного мозга. Потребление мозгом кислорода и гликолитическую активность определяли манометрическими методами при помощи аппарата Варбурга.

Сравнив результаты этих исследований над облученными и правильно развивающимися животными констатировали что ионизирующее облучение нарушает правильную динамику возрастных изменений тканевого дыхания и гликолиза в мозге. Наибольшее падение потребления кислорода у облученных жи-

вотных констатировали в сером, а частично и в белом мозговом веществе. Явление это наблюдается главным образом в тех периодах развития, когда у нормальных животных начинается увеличение тканевого дыхания.

Анаэробный гликолиз в мозге облученных кроликов снижается главным образом в сером веществе, однако в более поздных периодах созревания, когда наблюдаются изменения в выгляде и поведении животных, снижение их веса тела и мозга вызванные ионизирующим облучением. В противоположности к констатированным метаболическим расстройствам являющимся последствием облучения кроликов во внутреутробном периоде дозой 150 р., доза эта примененная во время созревания мозга не имела никакого влияния на процесс миэлинизации.

В работе обсуждаются некоторые аспекты влияния ионизирующего облучения на центральную нервную систему.

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INFORMACJA

Wydział Nauk Medycznych PAN ogłosił w „Służbie Zdrowia" z dn. 2.II.69 r.

konkurs

na wykonanie prac badawczych z zakresu następujących problemów:

1. PODSTAWOWE I KLINICZNE BADANIA REAKCJI USTROJOWYCH I KOMÓRKOWYCH, ISTOTNYCH W ZWIĄZKU Z PRZESZCZEPIANIEM NARZĄDÓW, TKANEK I KOMÓREK.
2. BADANIA Z DZIEDZINY GENETYKI CZŁOWIEKA.
3. BADANIA NAD NOWYMI LEKAMI, A W SZCZEGÓLNOŚCI PSYCHOTROPOWYMI, PRZECIWNOWOTWOROWYMI I PRZECIWWIRUSOWYMI.
4. KONSTRUKCJA I ZASTOSOWANIE NOWOCZESNYCH PROTEZ I URZĄDZEŃ REJESTRUJĄCYCH, WZMACNIAJĄCYCH LUB ZASTĘPUJĄCYCH FUNKCJĘ NARZĄDÓW.
5. OCENA JAKOŚCI ŻYWIENIA I WYŻYWIENIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM BADANIA STANU ODŻYWIENIA I WARTOSCI ODŻYWCZYCH PRODUKTÓW SPOŻYWCZYCH.

Szczegółowe warunki konkursu zostały podane w komunikacie w „Służbie Zdrowia".

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A. RADOMIŃSKA-PYREK, T. CHOJNACKI, T. KORZYBSKI

ON THE BIOSYNTHESIS OF PHOSPHOLIPIDS AND OTHER PHOSPHODIESTERS VIA CYTIDINE MECHANISM

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The pathways of the biosynthesis of the most common animal phospholipids have been elucidated by Kennedy and his group in the late 1950-ies. Two mechanism of this biosynthesis, both involving cytidine derivatives as intermediates are know. In the formation of phosphatidylcholine cytidine triphosphate reacts with phosphorycholine to form cytidine diphosphate choline. The latter compound passes its phosphorylcholine moiety onto a diglyceride. In the formation of phosphatidylethanolamine the reaction sequence is similar except that cytidine diphosphate ethanolamine instead of the choline derivative functions here as intermediate. The other mechanism represents a different type of biosynthesis, where the lipid component forms the nucleotide intermediate. Thus in the formation phosphatidylinositol, cytidine triphosphate reacts with phosphatidic acid and the resulting cytidine diphosphate diglyceride undergoes the so called inositololysis. The cytidylyltransferases involved in the formation of lecithin and cephalin, are widely distributed in nature. The one responsible for phosphorylcholine activation is easily separable from the other. This can be achieved by gel filtration of cytidylyltransferases on Sephadex G-200. The molecular weights calculated from the elution volumes of phosphorylcholine cytidylyltransferase and phosphorylethanolamine cytidylyltransferase are 130.000 and 40.000 respectively. (Chojnacki, Radomińska-Pyrek, Korzybski, 1967). In our laboratory the enzyme synthetizing cytidine diphosphate ethanolamine has been studied more extensively. In more elaborate separations on Sephadex G-200 two discrete peaks of enzyme activity were observed in all studied tissues. (Radomińska-Pyrek, 1969). There were found peculiar differences in the pH optimum of the enzyme synthetizing cytidine diphosphate ethanolamine with respect of the tissue; thus pH optimum of the brain enzyme is about 7·8, the other tissues exhibit two pH optima one at pH 7·8 and the second one at 6·2.

Besides its function in the formation of ethanolamine phospholipids cytidine diphosphate ethanolamine is involved in the biosynthesis of non-lipid phosphodiesters in tissue of some species. In this process cytidine diphosphate ethanolamine passes its phosphorylethanolamine moiety onto L-serine to form serine ethanolamine phosphate. This type of diesters is known to be present only in fishes, reptiles, amphibians and birds. Its biosynthesis was first elucidated in chicken tissues. (Rosenberg H., Ennor A., Sugai S., 1964). This step closely ressembles the last stage in the formation of some phospholipids.

The specificity of the two stages of the cytidine pathways of the formation of phospholipids was studied some years ago mainly in our Institute and in the Department of Experimental Neuropharmacology, Medical School, Birmingham, (Chojnacki, Ansell, 1967). From these studies a general conclusion was drawn, that only cytidine diphosphate choline and cytidine diphosphate ethanolamine could be formed in animal tissues. No other phosphorylated base could react with cytidine triphosphate. However the second step of the formation of phospholipids, i.e. the transfer of phosphorylated base from the cytidine coenzymes into the lipid acceptor when studied with the use of chemically synthetized analogues of cytidine diphosphates ethanolamine exhibited an almost complete lack of specificity. Thus only the first step of phospholipid biosynthesis is specific, and the second one is unspecific. We have tried to obtain some insight into the biosynthesis of other phosphodiesters whether this was a general rule in the pathways involving cytidine intermediates. The experiments were made in co-operation with Dr Allen of the Medical School in Canberra. (Table 1). It clearly showed that the analogues of cytidine diphosphate

Table 1. Incorporation of ^{32}P from CMP-^{32}P-bases into phosphodiesters and phospholipids in the chicken gut microsomes

Substrate used	Water soluble phosphodiesters		Phospholipids	
	counts/min.	mμmoles mg/prot/hr	counts/min.	mμmoles mg/prot/hr
CMP — ^{32}PE	8·650	2·62	11·092	3·32
CMP — ^{32}PGE	36	0·01	17·515	3·6
CMP — ^{32}P-APr	160	0·06	1·044	0·40
CMP — ^{32}PAiPr	690	0·24	4·280	1·31

ethanolamine are well acceptable in the phospholipid synthetizing system. While the yield from cytidine diphosphate ethanolamine was 3·23 mμmoles per mg of protein per hour, the one from cytidine disphosphate guinidinoethanol was 3·60 mμmoles, from one of the cytidine

diphosphate propanolamine — 1·31 mμmoles. The same analogues of cytidine diphosphate ethanolamine did not function in the synthesis of water soluble diesters. While the transfer of phosphorylethanolamine was 2·62 mμmoles per mg of protein per hour the transfer of others was only negligible. It seems therefore that in the bisynthesis of serine ethanolamine phosphate in chicken tissues both enzymic steps are specific.

In the second step of the biosynthesis of phospholipids the lack of specificity was observed in case of about twenty of analogues of cytidine diphosphate ethanolamine containing other related aminoalcohols instead of ethanolamine. We have tried recently to obtain a completely different phosphatidyl derivative containing sugar instead of ethanolamine using cytidine diphosphate glucose and the phospholipid synthetizing system from rat liver. Cytidine diphosphate glucose is a physiological compound in several microorganisms and it was obtained in the ^{32}P-labelled form for our studies by using the enzyme from S. typhimurium. (Chojnacki, Sawicka, Korzybski, 1968). The enzyme synthetizing cytidine diphosphate glucose was isolated from bacteria on Sephadex G-200 column. The compound was then prepared starting from glucose-1-^{32}P-phosphate and cytidine trophosphate. It behaved as expected on mild acid hydrolysis. The test performed with the phospholipid synthetizing system of rat liver did not show the formation of labelled phospholipid from ^{32}P-cytidine disphosphate glucose. It should therefore be concluded that the previously described unspecificity of the system is restricted to phosphorylaminoalcohols.

A. Radomińska-Pyrek, T. Chojnacki, T. Korzybski

BADANIA NAD BIOSYNTEZĄ FOSFOLIPIDÓW I INNYCH FOSFODWUESTRÓW DROGĄ MECHANIZMU CYTYDYNOWEGO

Streszczenie

Uprzednie badania nad swoistością mechanizmu cytydynowego w tworzeniu głównych fosfolipidów tkankowych wykazały jego bardzo niski stopień dyskryminacji dla szeregu analogów cytydynodwufosfocholiny oraz cytydynodwufosfoetanolaminy. Jedynym analogiem tego typu, nie będącym źródłem estrów fosforanowych była cytydynodwufosfoglikoza. Czynność analogów koenzymów cytydynowych w tworzeniu dwuestrów fosforanowych porównano w systemie syntetyzującym fosfolipidy i L-serynoetanoloaminofosforany w mikrosomach jelita kurczęcia. System syntetyzujący L-serynoetanoloaminofosforany uważamy za bardziej swoisty.

А. Радоминьска-Пырек, Т. Хойнацки, Т. Коржибски

БИОСИНТЕЗ ЦИТИДИНОВЫМ МЕХАНИЗМОМ ФОСФОЛИПИДОВ И ДРУГИХ ФОСФОДИЭСТРОВ

Содержание

Прежние исследования над специфичностью цитидинового механизма в синтезе главных тканевых фосфолипидов показали низкую степень дискриминации этого механизма в отношении к некоторым аналогам цитидин-дифосфохолина и этанолоамина.

Единственным аналогом этого типа не явившимся донором фосфодиэстов был цитидин-дифосфоглюкоз. Роль аналогов цитидиновых коферментов в синтезе диэстров фосфатов проведена на системе синтезирующей фосфолипиды и L-серин-этилоаминофосфаты в микросомах кишечника цыплёнка. Система синтезирующая L-серин-этаноламинофосфаты найдена авторвми более специфичной.

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L. S. CHERKÁSOVA, A. T. PYKULEV, L. L. DOVGALEVICH,
L. S. YAKUBOVICH

GLUTAMIC ACID METABOLISM IN THE WHITE AND GREY MATTER OF THE BRAIN UNDER NORMAL CONDITIONS AND UNDER THE INFLUENCE OF CERTAIN FACTORS

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As a result of research carried out in recent years a high metabolic activity of glutamic acid (GA) in the brain tissue and also its close relation to the metabolism of aspartic acid (AA) and gamma-amino--butiric acid (GABA) have been established, so have the inclusion of GA through the processes of transamination and oxidative desamination in the citric acid cycle (CAC) and in the processes of binding of ammonium, and other substances (1, 2, 3, 4, 5, 6, 7) *. It is well known that the metabolic reaction of the organism to various factors of the environment is determined by the functional state of the neural-endocrine system and particularly by the hypothalamo-hypophyseo-adrenal system.

In our previous investigations it was shown that the levels of free and bound GA, AA and GABA in homogenates and subcellular fractions of the brain are altered by whole body X-ray or neutron irradiation with relatively small doses. This is accompanied by changes in the activity of such key enzymes of amino-acid metabolism as alanine- and aspartate-aminonitransferase (AlT, AsT; 2.6.11, and 2.6.1.2) (8, 9).

Taking into consideration all the data obtained in this Laboratory which point to the fact of an increase in the corticosterone content in the blood outflowing from the adrenals of rat irradiated with the same dose (10), and bearing in mind all that is known about the role of the hypophyseo-adrenal system as an intermediate of various influences, we undertook investigations on the dependence of the metabolism of the above mentioned amino acids and the activity of

* Exceptionally in this paper the references are quoted in the numerical order.

AlT and AsT in various parts of the brain tissue at the corticosteroid level in the organism.

Experiments were carried out with adult Wistar rats of a heterozygotic strain which were kept on a standard diet. With the aid of differential centrifugation we obtained a subfraction of mitochondria, containing myelin and a supernatant fraction (hyaloplasm + microsomes) (from 0·25 M sucrose prepared on Tris-bufer pH 7·4). Experiments were made with intact animals subjected to surgical adrenalectomy and adrenalectomized rats which after the surgery received during a period of 4 days cortisol in doses of 2 mg per 100 gr body weight or adrenalin in doses of 2 mgr per 100 gr of body weight and cortisol and adrenalin simultaneously. Moreover experiments were carried out to study the dependence of glutamic acid metabolism on various stress factors, in animals subjected to false adrenalectomy, or intact and also adrenalectomized animals, 24 hrs after X-rays irradiation in a dose of 40 R.

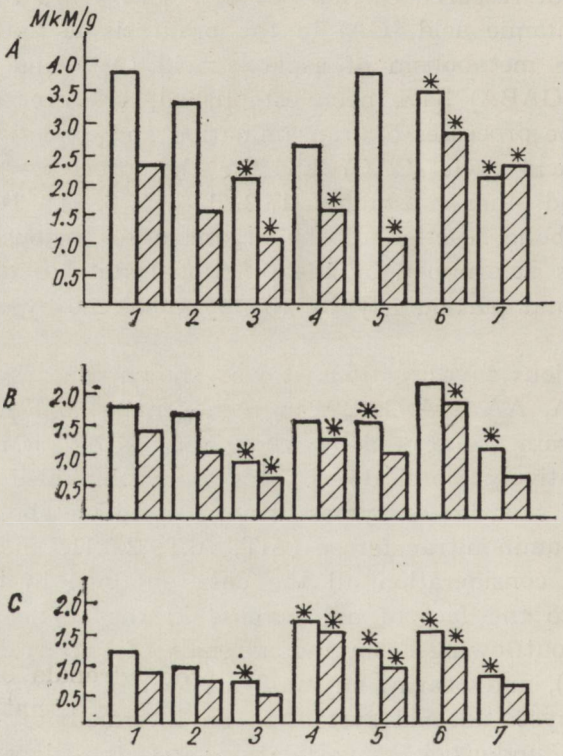

Fig. 1. Content of free (empty column) and bound (striped column) GA (A), AA (B) and GABA (C) in the grey matter; *1* — intact, *2* — false adrenalectomy, *3* — adrenalectomy, *4* — adrenalectomy + glucocorticoid, *5* — adrenalectomy + + adrenalin, *6* — X-ray irradiation, ✱ — statistically proved.

The results obtained in studying the changes in the content of free and bound GA, AA, and GABA in the grey and white matter expressed as MkM/g of tissue are presented in Figs 1, 2. As seen the

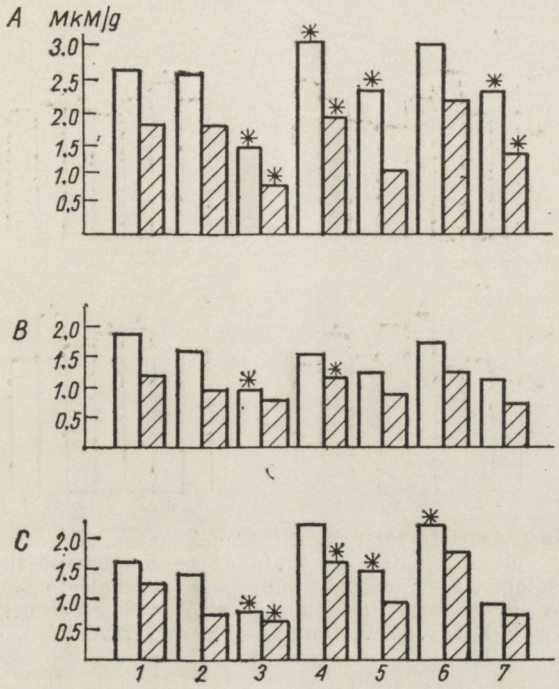

Fig. 2. Content of free and bound GA, AA, GABA in the white matter. Legend as in *Fig. 1.*

amounts of free and bound glutamic and aspartic acids, and GABA are practically unchanged in the grey and white matter in cases with false adrenalectomy, whereas there was substantial decrease on the sixth day after adrenalectomy when the corticosteroid level in the organism was at its minimum. In these figures statistically superrated data are represented by dots. Compensatory administration of cortisol to adrenalectomized animals reestablishes the levels of free and bound forms of aspartic acid in the grey and white matter of the brain, at the same time the glutamic acid level and that of GABA is altered. There is an increase of free and bound GABA in the white and grey matter, and a decrease of GA in the grey matter. Introduction of adrenalin alone cannot compensate the resulting changes. Exposure of intact animals to X-rays produces in the grey matter increase of bound forms of all the three acids whereas their level in the white matter remains unaltered. Irradiation of adrenolectomized animals causes

various changes in the grey matter and has no effect on the AA and GABA content in the white matter.

The results of the changes in AlT and AsT activity in homogenates and subcellular organelles are presented in Fig. 3—6. For the sake

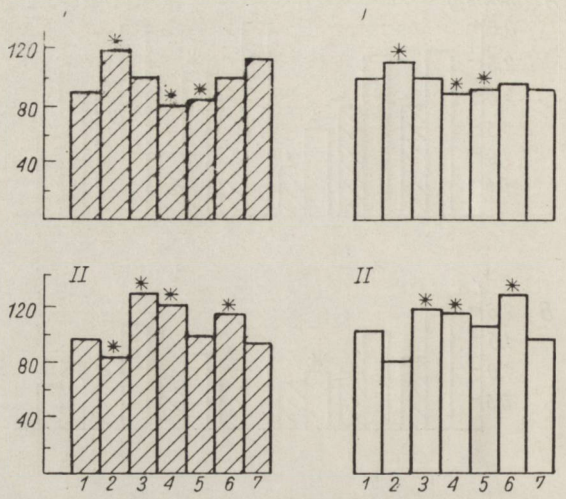

Fig. 3. AlT (*I*) and AsT (*II*) activity in homogenates of grey (striped column) and white (empty column) matter of brain as percentage of the control values. *1* — false adrenalectomy, *2* — adrenalectomy, *3* — adrenalectomy + glucocorticoid, *4* — adrenalectomy + adrenalin, *5* — adrenalectomy + glucocorticoid + adrenalin, *6* — adrenalectomy + X-ray irradiation, *7* — X-ray irradiation, ✱ — statistically proved.

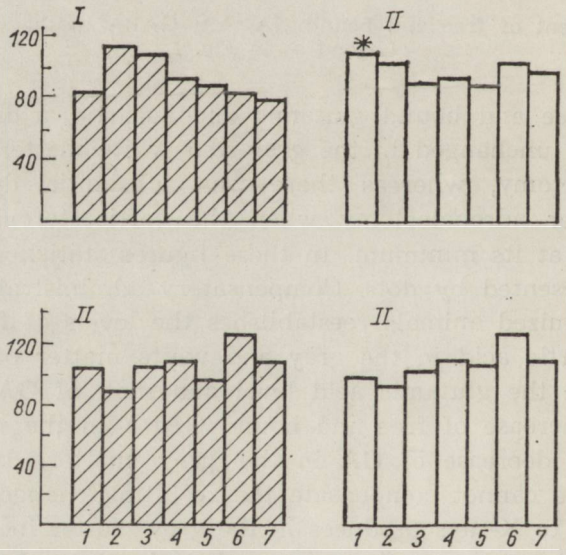

Fig. 4. AlT and AsT activity in mitochondria of grey and white matter of brain as percentage of the control values. Legend as in *Fig. 3.*

of a more convenient comparison of the results of different series, the data in this figure are given as percentage of the control values.

The activity of AlT and AsT is considerably changed under the given experimental conditions. In homogenates from the grey matter of adrenalectomized animals (Fig. 3) there is a statistically proved increase in AlT activity, whereas in mitochondria (Fig. 4) and supernatant (Fig. 6) these changes are not supported statistically. Introduction of cortisol into adrenalectomized animals compensates the resulting changes, at the same time administration of adrenalin with cortisol

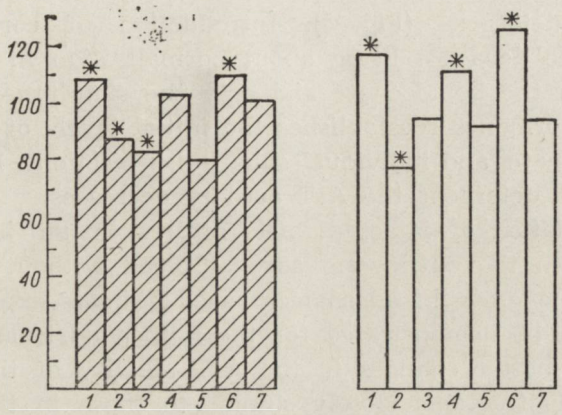

Fig. 5. AlT and AsT activity in mitochondria of the grey and white matter of brain exposed to detergent as percentage of the control values. Legend as in *Fig. 3.*

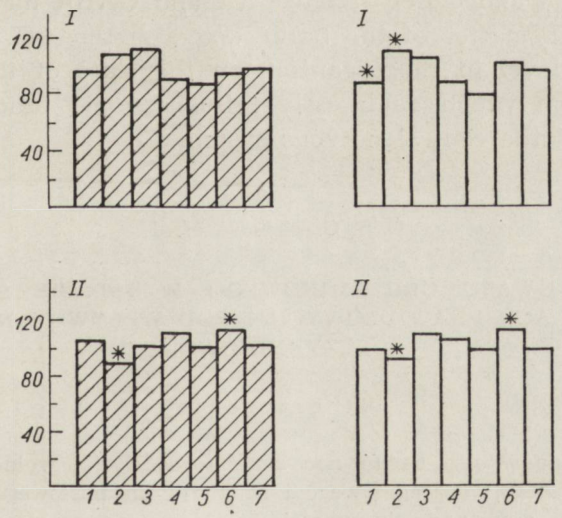

Fig. 6. AlT and AsT activity in supernatant (hyaloplasma + microsomes) of grey and white matter of brain as percentage of the control values. Legends as in *Fig. 3.*

does not bring normalisation and there is a sharp decrease of AlT activity in the homogenates from the grey matter. On investigation of the activity of this enzyme in mitochondria and supernatant w found no such effects. The AlT activity in homogenates and mitochondria from the white matter is unstable, but statistically supported changes are found only for homogenate and supernatant.

The activity of AsT depends to a somewhat greater extent on th glucocorticosteroid level in the organism than the activity of AlT Adrenalectomy results in a decrease of AsT activity in the homogenate from the grey matter, in supernatant and in mitochondria exposed t digitonin as detergent (Fig. 5). Introduction of cortisol alone o adrenalin alone does not bring about normalisation in the activity o the enzyme in the homogenate and only the simultaneous administration of both hormones reestablishes the initial levels of these activitie in the homogenate, and individual subcellular fractions. In mitochondri exposed to the detergent the AsT activity decreases.

X-ray irradiation of intact animals results in an increase of th cortisol level in the outflowing adrenal blood without change of th AsT activity, whereas in adrenalectomized animals exposed to X-ray this effect can be demonstrated for the mitochondria and supernatant In the white matter changes in the AsT activity of the homogenate and subcellular fractions closely similar to those in the grey matte should be noted.

The foregoing considerations point to the dependence of glutami acid metabolism and closely related AA and GABA metabolism in th grey and white matter of the brain, and also the AlT activity at th corticosteroid level in the organism, on the state of the hypophyseo -adrenal system which doubtlessly influences the functional level o the activity of the central nervous system.

L. S. Czerkasova et al.

METABOLIZM KWASU GLUTAMINOWEGO W ISTOCIE SZAREJ MÓZG W WARUNKACH PRAWIDŁOWYCH I POD WPŁYWEM NIEKTÓRYCH CZYNNIKÓW

Streszczenie

Przedstawia się wyniki badań nad zmianą zawartości wolnego i związaneg kwasu glutaminowego, asparaginowego i gamma-aminomasłowego w szarej i bia łej istocie mózgu oraz nad aktywnością aminotransferaz alaminy i asparagin w homogenatach i strukturach subkomórkowych białej i szarej istoty mózg u białych szczurów.

Badano zwierzęta poddane chirurgicznej adrenalektomii, którym od drugiego dnia po zabiegu w ciągu 4 dni podawano kortizon (2 mg/100 g wagi ciała), adrenalinę (2 mikrogramy/100 g wagi ciała) oraz adrenalinę i kortizon równocześnie, następnie w celu wykrycia wpływu czynników stresowych badano zwierzęta poddane adrenalektomii w 24 godziny po napromienieniu rentgenowskim dawką 40 r oraz poddane pseudo-adrenalektomii i nieoperowane.

Na 6 dzień po adrenalektomii następuje istotny spadek zawartości wspomnianych aminokwasów w szarej i białej istocie mózgu (ryc. 1, 2). Kompensacyjne podanie kortizonu zwierzętom po adrenalektomii przywraca poziom wolnego i związanego kwasu asparaginowego. Rentgenowskie napromienienie nieoperowanych zwierząt prowadzi do nagromadzenia w istocie szarej mózgu form związanych wszystkich trzech aminokwasów, przy ich niezmienionym poziomie w istocie białej. Napromienienie zwierząt poddanych adrenalektomii prowadzi do zmian o innym charakterze. W homogenatach istoty szarej mózgu zwierząt poddanych adrenalektomii wzrasta aktywność amino-transferazy alaninowej oraz spada aktywność amino-transferazy asparaginowej. Spadek aktywności amino-transferazy asparaginowej spotrzegamy w nadsączu i w mitochondriach (ryc. 3, 4, 6).

Podanie kortizonu lub adrenaliny nie prowadzi do normalizacji aktywności enzymów w homogenacie istoty szarej mózgu i tylko równoczesne podanie obu hormonów przywraca wyjściowy poziom aktywności amino-transferazy asparaginowej w homogenacie i poszczególnych frakcjach subkomórkowych (ryc. 3, 4, 5, 6).

Zmiana aktywności amino-transferaz alaninowej i asparaginowej następuje zawsze po napromienieniu zwierząt nieoperowanych jak i adrenalektomizowanych.

Przytoczone dane świadczą o zależności metabolizmu kwasu glutaminowego, asparaginowego oraz gamma-amino masłowego w szarej i białej istocie mózgu, a także aktywności amino-transferaz alaninowej i asparaginowej od poziomu kortykosterydów w organizmie, a przez to i od stanu układu przysadkowo-nadnerczowego, co znajduje bezwzględnie swój wyraz na poziomie aktywności czynnościowej centralnego układu nerwowego.

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ОБМЕН ГЛУТАМИНОВОЙ КИСЛОТЫ В БЕЛОМ И СЕРОМ ВЕЩЕСТВЕ ГОЛОВНОГО МОЗГА ПРИ НОРМАЛЬНЫХ УСЛОВИЯХ И ВОЗДЕЙСТВИИ НЕКОТОРЫХ ФАКТОРОВ

Содержание

Приводятся результаты исследований по изучению изменений содержания свободных и связанных глютаминовой (ГК), аспарагиновой (АК) и гамма-аминомасляной кислоты (ГАМК) в сером и белом веществе головного мозга и активности аланин и аспартат-аминотрансфераз (АЛТ; 2.6.1.2. КФ) в гомогенатах и субклеточных органеллах белого и серого вещества мозга белых крыс подвергнутых хирургической адреналэктомии, адреналэктомированных крыс, которым со второго дня после операции в течении 4-х дней вводили кортизол (2 мг/100 г веса), адреналин (2 мкг/100 г) и адреналин и кортизол одновременно, и для выявления воздействия стрессорных факторов у животных подвергнутых ложной адреналэктомии, интактных и адреналэктомированных животных через сутки после рентгеновского облучения в дозе 40 р.

На 6 сутки после адреналэктомии наступает существенное снижение содержания указанных аминокислот в сером и белом веществе мозга (рис. 1, 2). Компенсаторное введение кортизола адреналэктомированным животным восстанавливает уровень свободной и связанной АК.

Рентгеновое облучение интактных животных приводит к накоплению в сером веществе мозга связанных форм всех трех кислот, при неизменном уровне их в белом. Облучение адреналэктомированных животных вызывает иной характер изменений.

В гомогенатах серого вещества мозга адреналэктомированных животных увеличивается активность АЛТ и снижается активность АСТ, активность АСТ снижается в надосадочной жидкости и митохондриях (рис. 3, 4, 6).

Введение кортизола или адреналина не приводит к нормализации активности ферментов в гомогенате серого вещества мозга и только одновременное введение двух гормонов востанавливает исходный уровень активности АСТ в гомогенате и отдельных субклеточных фракциях (рис. 3, 4, 5, 6). Закономерное изменение активности АЛТ и АСТ наступает после облучения интактных и адреналэктомированных животных.

Указанное свидетельствует о зависимости обмена ГК и связанных с ней АК и ГАМК в сердом и белом веществе мозга, а также активности АЛТ и АСТ от уровня кортикостероидов в организме а следовательно, и состояния гипофиз-адреналовой системы, что безусловно находит свое отражение на уровне функциональной активности центральной нервной системы.

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THE GAMMA-AMINOBUTYRIC ACID (GABA) SYSTEM OF THE CEREBRAL WHITE MATTER AND OF BRAIN TUMOURS

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The discovery that GABA has an inhibitory effect on the nervous system of mammals has given rise to numerous experiments aiming at determination of its functional role. It is assumed that GABA is an inhibitory transmitter affecting the activity of nervous and synaptic transmission (Krnjevic, Schwartz 1967, Obata et al. 1967, Otsuka et al. 1967, Sytinsky 1968). The role of the neuroglia in the regulation of physiological activity in the brain and its participation in the metabolism of GABA is little known. Data on the distribution of GABA and the enzymes of its metabolism (glutamate-decarboxylase (GAD) and GABA-transaminase-gamaketoglutaric acid (GABA-T) in the human brain matter and brain tumours are insufficient (Awapara et al. 1950, Okumura et al. 1958, Fukai 1959, Iunoue 1959, Hirosuke 1960. Waksman, Faienza 1960, Jinnai, Mori 1960, Muller, Langeman 1962, Wollemann, Devenyl 1963, Sytinsky et al. 1965, 1968, Berezov 1966, Promyslov, Andreeva 1966, Sheridan et al. 1967).

The purpose of the present investigation was to study the content of components of the GABA system of the white matter of human brain, of gliomas consisting only of glial cells and of human brain tumours of other types.

METHODS

GABA was quantitatively determined by paper chromatographic analysis in the butanol-acetic acid-water (4:1:5, v/v) system. All spectrophotometric readings were made with model SF-4A spectrophotometr at 512 mμ. GAD and GABA-T activity were determined according to the amount of the final products of enzymatic reaction. Samples were incubated in closed tubes for 30 min. at 37° in anaerobic conditions (N_2) (Sytinsky, Priyatkina 1966). A part of each tumour or each sample of

brain tissue was examined histologically (Tchaika, Laboratory of Pathoanatomy, A. L. Polenov Institute of Neurosurgery, Leningrad).

RESULTS AND DISCUSSION

The data of the components of the GABA system in the human brain are shown in Table 1. It was found earlier that postmortem changes in the brain tissue are followed by an increase of GABA content (Avenirova et al. 1966) only during the first 2—3 minutes.

Table 1. Gamma-aminobutyric acid system of the cerebral grey and white matter of human brain

	GABA mg/100 g fresh wt	GAD activity M GABA/g/hr	GABA-T activity M GA/g/hr
Cortex:			
Frontal white	7·3±0·4	2·0±0·9	8·3±2·4
Frontal grey	19·5±1·2	6·1±2·8	25·0±3·0
Temporal white	3·2±0·2	1·7±0·6	7·5±1·5
Temporal grey	20·0±0·75	5·7±1·7	24·1±2·7
Occipital white	7·0±1·0	2·4±0·6	10·2±0·9
Occipital grey	12·3±0·7	8·8±3·0	26·0±1·7
Pons	10·0±1·8	0·9±0·4	—
Corpus callosum	7·0±0·7	—	6·8±1·3
Dura matter	2·2±0·1	—	—
Pia matter with arachnoidea	traces	—	—

Mean ± S.E.M. for 5 samples; GA = glutamic. acid.

Thus analysis of autopsy material with some errors and standardization of the preparation time of the brain samples may supply comparable data with those concerning GABA content in the brain in pathological processes of the central nervous system. In the grey matter the GABA content from all parts of human brain examined and the activity of the enzymes involved in its metabolism are 2—3 times higher than in white matter.

The low GABA content in the pia of the human brain should be stressed. Analysis of the data of the GABA system components in tumour tissue of human brain shows that their content is very low (Table 2). It is possible, however, that the GABA level and the activity of enzymes involved in its metabolism are approximately two times higher in neuroectodermal tumours than in meningovascular growths. The GABA content decreases in both kinds of human brain tumorus in conformity with the degree of tumour tissue cataplasia. GAD activities are not found in malignant tumour samples.

Table 2. Gamma-aminobutyric acid system of human brain tumours.

	GABA mg/100 g fresh wt	GAD activity M GABA/g/hr	GABA-T activity M GA/g/hr
Arachnoendothelioma (non-malignant tumors)	traces —2·0 (10)	0·1—0·25 (10)	0·2—0·42 (7)
Arachnoendotheliosarcoma	traces (7)	no detectable activity (5)	traces —0·3 (5)
Glioma-astrocytoma (non-malignant tumors)	traces —4·2 (8)	0·1—0·4 (8)	1·7—2·9 (6)
Glioma-astrocytoma (malignant--tumors)	traces (5)	no detectable activity (6)	traces —0·4 (4)

Number of investigations in parenthesis

The GABA-T activity in the tissue of multiform spongioblastoma however, was approximately 3 μMGA per g per hour. The anaplasia and differentiation process influence no doubt the metabolism of glial cells. The complete disappearance of even GABA traces can be explained by being used up in the processes of the energy consumption in tumour cells metabolism. Exact data concerning the distribution of the GABA system components between neurons and glial cells are not available as yet.

In neuron degeneration and glial expansion of the cat's medial geniculate body GABA content was changed slightly (Utley 1963). In conformity with the foregoing statement GABA is apparently distributed equally between nerouns and glia. However, in the studies of Ruscak et al. (1967) the drop of GABA level in rat brain cortex during necrobiotic processes in neurons and their substitution with reactive astroglia was observed. The morphological variety of glial cells corresponds to their neurochemical character.

The protoplasmatic actrocytes poor in mitochondria may be expected to have a high GAD activity and higher GABA level. Other glial cells (oligodendroglia) immediately surrounding the neurons bodies and nerve endings are relatively rich in mitochondria; the fractions of which are characterized by GABA-T activity.

Apparently as a result of this the neuroglial cells are able to remove and inactivate the products of GABA metabolism in neurons, thus forming a nervous system functional unit with neurons and not taking part directly in the synaptic transmission.

I. Sytinsky

SYSTEM KWASU GAMMA-AMINOMASŁOWEGO (GABA) ISTOTY BIAŁEJ MÓZGU I GUZÓW MÓZGOWYCH

Streszczenie

Niniejsze badania podjęto w celu określenia zawartości składników systemu GABA w istocie białej mózgu człowieka, jak również glejaków składających się wyłącznie z komórek glejowych oraz w innych guzach mózgu człowieka.

GABA badano ilościowo za pomocą metody chromatografii bibułowej. Aktywności dekarboksylazy glutaminowej oraz transaminazy alfaketoglutarowej GABA określono w odniesieniu do produktu końcowego reakcji enzymatycznej.

И. Ситинский

СИСТЕМА ГАММА - АМИНОМАСЛЯННОЙ КИСЛОТЫ (ГАБА) БЕЛОГО ВЕЩЕСТВА ГОЛОВНОГО МОЗГА И МОЗГОВЫХ ОПУХОЛЕЙ

Содержание

Настоящие исследования предприняты целью определения состава компонентов системы ГАБА в белом мозговом веществе человека как и глиом состоящих исключительно из глиальных клеток и в других опухолях головного мозга.

ГАБА определеляли количественно при помощи метода хроматографии на фильтровальной бумаге. Активности ферментов: глютамат-декарбоксилазы и альфа-кетоглютаровой трансаминазы ГАБА определяли в отношении к окончательному продукту ензиматической реакции.

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INVESTIGATION ON THE MACROMOLECULAR STRUCTURE OF BRAIN DNA IN IRRADIATED ANIMALS

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In radiobiological literature the opinion is generally held that exposure to ionizing radiation of active mitotic and sensitive tissues results in degradation of DNA not directly through the action on its molecule but by means of DNA-ase liberated from the injured cells. The enzyme is liberated from subcellular structures already within three hours. The process is particularly rapid during the first 28—48 hrs after irradiation.

In regard to the differences in radioresistance of tissues, one of the current problems of modern radiobiology seems to be the question why these tissues, though injured by radiation, regenerate from local ionizing effects, and what distinguishes them from radiosensitive tissues.

Studies confirming the destructive effect of radiation on brain DNA of adult animals are scarse and samples of DNA in the experiments carried out were usually collected very late, several hours after irradiation (Caster, 1958).

We investigated the structural changes in macromolecular DNA obtained from the brain of adult animals immediately after irradiation.

Ionizing radiation can cause damage of the polynucleotide chain of DNA and makes it more liable to enzymatic degradation. To check this hypothesis we examined the kinetics of enzymatic splitting of brain DNA by acid DNA-ase.

Rabbits were once irradiated with X-rays in 700 R. dose. In order to exclude the possibility of an increase in DNA-ase activity, decapitation was carried out not later than 10 min. after irradiation.

Splitting of DNA by means of acid DNA-ase was followed spectrophotometrically in ultraviolet light by determination of the byproducts of enzymatic degradation (Schneider W. C., Hogeboom G. H., 1955). As substrate highly polymerized DNA was used, prepared by the method of Gulland in Schapot's modification (Schapot W. S., 1950).

As a source of enzyme 5% spleen homogenate or 10% brain homogenate was used. To obtain a relatively high quantity of DNA, it was extracted from the whole brain. In order to obtain the possibly best model of the processes taking place in the living organism DNA and DNA-ase from the same organ were used.

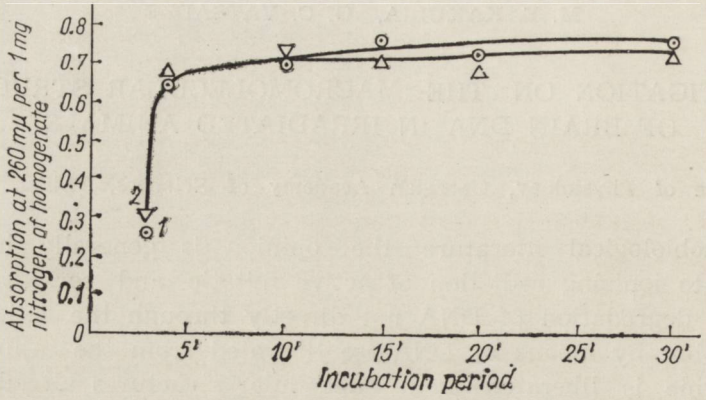

Fig. 1. Splitting of the brain DNA with the brain acid DNA-ase. *1* — DNA of the control animal, *2* — DNA of the irradiated animal. Each point represents the mean value of a series of experiments.

The curves of enzymatic splitting of DNA collected from irradiated brains and of those from control animals did not differ from each other.

It is possible that radiation damage of the brain DNA in these experiments could not be demonstrated because brain DNA-se as known from literature, is active only against single-stranded DNA (Marmur J., Rownd R., Schild Kraut C., 1963), therefore we performed experiments in which against brain DNA much more active DNA-ase from the spleeen was used.

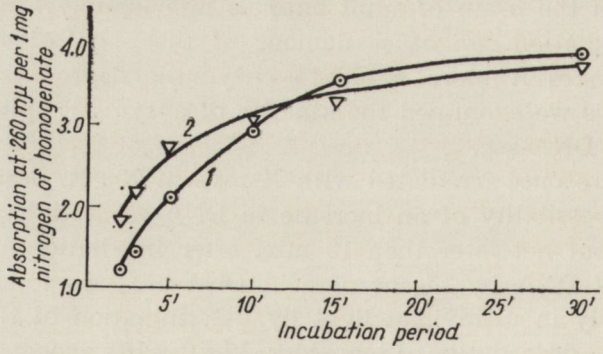

Fig. 2. Splitting of the brain DNA of the irradiated animal with acid DNA-ase of spleen. *1* — DNA of the control animal, *2* — DNA of the irradiated animal. Each point represents the mean value of a series of experiments. Deviations are statistically reliable.

Enzymatic hydrolysis of brain DNA from irradiated animals by means of spleen DNA-ase runs faster in the initial phase of the reaction as compared with that in controls. This can be explained by the fact that there is summation of the damage to the phosphoester bonds produced by the irradiation and the action of enzyme.

On the basis of the above mentioned data it may be presumed, that brain DNA is also damaged by irradiation, but in radioresistant brain tissue there are conditions for recovery of the DNA structure. In radiolabile organs, under the influence of activated DNA-ases, the process of decay of DNA is intensified.

The changes of the macromolecular structure of DNA in irradiated animals were established spectrophotometrically on the basis of studies of heat denaturation.

In one part of the experiments the head of the animal was irradiated with an X-ray dose of 9000 R, in the other part — wholebody irradiation was applied in the range from 50 to 9000 R. The brain was collected at different time intervals after irradiation: 2, 10, 30, 90 min. from 3 to 19 hrs and up to 1·5 month.

It is known that brain is a very heterogeneous organ in its cellular structure. Glial elements are for instance 10 times more common in it than neurons. While capillary vessels are more frequent in grey matter than in white matter; the glia is distributed rather proportionally in both substances.

The study of relative radiosensitivity of such cells, as neuron, several types of glial cells, the endothelium of capillary vessels within organs of the animal organism is extremely difficult. We therefore limited our work to separate samples of grey and white matter. It is known that the interneuronal space in grey matter is filled with all types of glia, while in white matter there are only oligodendrocytes and fibrillary astrocytes. Oligodendroglia does not contact vessels and has the closest connections with nerve fibers. It was ascertained by means of the electron microscope that the oligodendroglia is the place of myelin formation. Astrocytes are involved in maintaining ion concentration and osmotic pressure in the CNS and especially in the active transport of potassium and sodium ions.

In investigations on heat denaturation of DNA and DNP of white and grey matter of the brain it was shown that besides the splitting of phosphoester bonds in DNA, taken from the brain of irradiated animals — the profile of molecular melting is changed even upon irradiation with small doses (100 R).

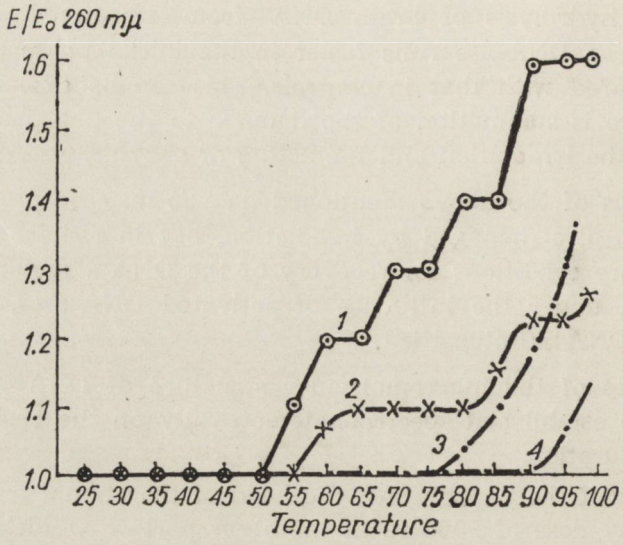

Fig. 3. Thermal denaturation of DNP 10 minutes after total irradiation at the dose of 150 R. *1* — DNP of grey matter, *2* — DNP of white matter, *3* — DNA of the control animal, *4* —DNP of the control animal.

Melting of DNA starts in irradiated animals at 45°—50°C instead of 77°C as in the control materials. It is known that, normal nonirradiated DNP begins to disintegrate at a temperature by 14°C higher than does nonirradiated DNA. Irradiated DNP and DNA start to melt at the same temperature. In irradiated animals the differences in melting of DNA of white and grey matter are not significant, however the changes in the melting profile of DNP are much more distinct — DNP of grey matter suffers more from radiation as compared with that of white matter.

This may perhaps be explained by the fact that the density of cells and thus DNA concentration is higher in grey than in white matter. The higher degree of damage of DNP in grey matter may be also result of the fact that the neuron in the waking state is constantly stimulated there. Lesion of neurons due to ionizing radiation may be not only primary, but also secondary, owing to damage to the endothelium of capillaries and glial satellites.

The well known fact of relative radioresistance of brain tissue may be also explained by the special conditions favouring rapid postradiation recovery of cells from damage. It is known that in yeast suspensions through which oxygen is passed recovery from postradiation damage is very rapid, reaching 60%.

As the oxygen requirement of tissues is not influenced by radiation, the reparative systems are also radioresistant as confirmed by our experiments — reparation was observed in the range of doses of 100—9000 R.

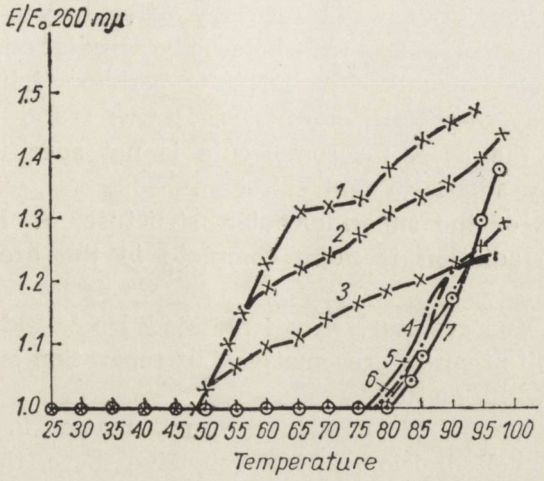

Fig. 4. Thermal denaturation of the brain DNA of rabbit during the irradiation of head at the dose of 9000 R. *1* — 10 minutes after the irradiation, *2* — 3 hours after the irradiation, *3* — 24 hours after the irradiation, *4* — 1·5 hours after the irradiation, *5* — 3 hours after the irradiation, *6* — 5 hours after the irradiation, *7* — DNA of the control animal.

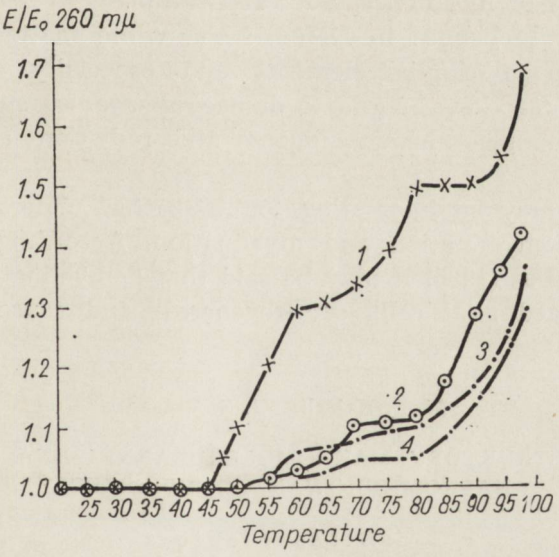

Fig. 5. Thermal denaturation of DNP and DNA 14 days after the total irradiation at the dose of 150 R. *1* — DNP of white matter, *2* — DNP of grey matter, *3* — DNA of white matter, *4* — DNA of grey matter.

As criterium of recovery we took molecular melting profile of DNA and DNP. In irradiation of the head only, with a dose of 9000 R, after 1·5 — 5 hrs in 30% of cases almost complete and in the others only partial, reparation was noted.

In wholebody irradiation even with small doses we did not observe complete reparation, only weak recovery after longer time intervals was observed in most cases. In whole-body irradiation, reparation of the macromolecular structure of DNA is perhaps inhibited by the action of hypophyseo-adrenal hormones. It was found that the effect of radiation on the DNA macromolecule is not specific and may be produced also by adrenalin and estrogen.

Reconstruction of the supermolecular structure of DNA and DNP in wholebody irradiation is more complete in the grey than in the white matter.

As shown by our results X-ray irradiated DNA and DNP are less influenced in white than in grey matter, but reparation is more complete in grey than in white matter.

Better reconstruction of DNA in the grey matter is also supported by the fact that cell division, is less intensive there, this giving more time for reparation.

In the grey matter of the brain the volume of the capillary network is 3—4 that in the white matter. Accordingly the grey matter uses 5 times more oxygen (Thews 1960) than the white one, therefore the former disposes of more energy for reparation.

The structural changes in DNA of the white matter may well be related to damage of glial elements and especially oligodendroglia. Of course the reactions of glia to damaging agents, such as trauma, temperature changes, chemical agents and radiation are mostly the same.

Our results may be interesting for a better understanding of the processes of demyelination and myelination. According to the statement of Galambos (1965) it may be said that demyelination is a disease of glia. Damaged glial cells can regenerate and cause remyelination of the axon.

M. E. Kakulia, G. S. Vatsadze

ZMIANA STRUKTURY MAKROMOLEKULARNEJ DNA W MÓZGU NAPROMIENIOWANEGO ZWIERZĘCIA

Streszczenie

Praca przedstawia wyniki badań nad makromolekularną strukturą DNA mózgu zwierząt napromieniowanych promieniami Roentgena. Enzymatyczna hy-

droliza DNA z mózgu zwierzęcia napromieniowanego prowadzona kwaśną DNA-zą ze śledziony przebiega szybciej w początkowym etapie reakcji w porównaniu z DNA z nienapromieniowanego mózgu zwierzęcia. Fakt ten jest prawdopodobnie uwarunkowany sumowaniem uszkodzeń fosfoestrowych połączeń wywołanych napromieniowaniem i działaniem enzymu.

Poza rozerwaniem połączeń fosfoestrowych w DNA ekstrahowanym z mózgu napromienionych zwierząt, zmieniony jest także wykres topliwości molekularnej. Topnienie to rozpoczyna się od 45—50°C, zamiast 77°C w kontroli. Bardziej poglądowo zmiany te są wyrażone w wykresie topnienia DNP. DNP istoty szarej mózgu są silniej uszkadzane promieniowaniem jonizującym niż DNP istoty białej.

Popromienna regeneracja uszkodzeń następuje w odwrotnej kolejności: Lepiej regenerują DNA i DNP istoty szarej niż białej. W popromiennej regeneracji komórki zasadnicze znaczenie ma zużycie tlenu i jest ono promienioodporne. W naszych doświadczeniach obserwowaliśmy regenerację w zakresie dawek 150—9000 r. Procesy regeneracyjne są wyraźniejsze przy lokalnym napromienieniu głowy. Po napromienieniu ogólnym procesowi regeneracji makromolekularnej DNA prawdopodobnie przeszkadza podwyższenie aktywności układu przysadkowo-nadnerczowego.

Zmianę budowy DNA istoty białej należy odnieść prawdopodobnie do elementów glejowych w szczególności oligodendrogleju. Ponieważ reakcja gleju na rozliczne czynniki szkodliwe jest jednakowa, otrzymane dane mogą przyczynić się do zrozumienia procesów demielinizacji i mielinizacji.

М. Е. Какулия, Г. С. Вацадзе

ИЗМЕНЕНИЕ МАКРОМОЛЕКУЛЯРНОЙ СТРУКТУРЫ ДНК ГОЛОВНОГО МОЗГА ОБЛУЧЕННОГО ЖИВОТНОГО

Содержание

В работе исследовалась макромолекулярная структура ДНК головного мозга при облучении животного лучами Рентгена. Ферментативный гидролиз ДНК головного мозга облученного животного кислой ДНК-азой селезенки по сравнению с ДНК головного мозга необлученного животного, в начальной стадии реакции происходит быстрее. Этот факт повидимому обусловлен суммированием повреждений фосфорноэстерных связей, вызванных облучением и действием фермента.

Кроме разрыва фосфорноэстерных связей у ДНК, извлеченной из мозга облученого животного изменен профиль молекулярного плавления. Плавление начинается с 45—50° С, вместо 77° С в контроле. Более наглядно выражены изменения в профиле плавления ДНП: ДНП серого вещества больше повреждается ионизирующей радиацией, чем ДНП белого вещества, а пострадиационное восстановление повреждений происходит в обратном порядке: лучше восстанавливается ДНК и ДНП серого вещества, чем белого. В пострадиационном восстановлении клетки основное значение имеет потребление кислорода, а оно радиорезистентно. В наших опытах в диапазоне доз от 150 до 9000 р наблюдалось восстановление.

Процессы восстановления лучше выражены при локальном облучении головы. При общем облучении восстановлению макромолекулярной структуры ДНК, надо полагать, препятствует повышение активности гипофизарно-адреналовой системы.

Изменение структуры ДНК белого вещества, повидимому, следует отнест зяа счет глиальных элементов, в частности, олигодендроглии. Так как реакци глии на различные вредоносные агенты одинакова, полученные данные могу быть привлечены к пониманию процессов демиэлинизации и миэлинизаци

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S. H. HAIDARLIU

FUNCTIONAL CHANGES OF THE RNA CONTENT IN CELLULAR COMPONENTS OF THE SPINAL CORD

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The biochemistry of the nervous system both theoretically and practically is one of the most complicated and at the same time extremely difficult fields in human and animal biochemistry owing to a number of morphological and functional peculiarities of the nervous system. First of all the extreme morphological complication of texture, and the multiplicity and high lability of physiological functions and metabolism of the nervous system should be noted.

The morphological and biochemical heterogenicity of the nervous tissue is conditioned in particular by the presence of two main types of cells — the nerve and glial cells which compose the joint system of neurons-neuroglia. Until recently main attention was devoted to examination of the neurons. But the results of recent morphological (De Robertis, Gerschenfeld, 1961; Luse, 1962; Nakai, 1963; Hagaborn et al., 1963; Alexandrovskaja et. al., 1965) and electrophysiological investigations (Galambos, 1961; Roitbak, 1963; Svaetichin et al., 1965) show that neuroglia take part in the specific function of neurons. The confrontation of the results of separate studies on the changes in the metabolism of neurons and of neuroglial cells during changes in the functional state of the nervous system was of great interest.

Recent data show that these changes concern mainly nucleic acid metabolism and can be demonstrated quantitatively by means of modern research equipment. For this reason neurobiologists are greatly interested in the problems of nucleic acids.

Th role of glial cells in the metabolism and functional activity of the neuron is at present intensively studied (Hydén and Pigon, 1960; Egyházi and Hydén, 1961; De Robertis and Gerschenfeld, 1961; Galambos, 1961; Hydén, 1961 a, b; Gomirato, 1963; Giacobini, 1964; Svaeti-

chin et al., 1965; Pevzner, 1965, 1966; Hydén and Lange, 1966; Pevzner, Haidarliu, 1967).

It is now evident that RNA is present in nerve fibers (Edström, Eichner and Edström, 1962; Edström, 1964, 1964a). The concentration of this compound in nerve fibers is, however, relatively low. Owing to the morphological heterogeneity it is difficult to evaluate the changes in RNA content in various structures of the nervous system by the methods widely used in biochemistry. It should be expected that confrontation of the results obtained by different methods and characterizing changes in the metabolism of various tissue elements of a definite region of the nervous system can in some degree solve this problem.

In the present investigation we used a quantitative cytochemical method — ultraviolet cytospectrophotometry — to determine the nucleic acid content in the individual nerve and surrounding glial cell satellites and tried to establish the relation between the nucleic acid content in these cells and the functional state of the nervous system. The investigation was also undertaken for determining the functional changes in RNA content in the "process component" (axons and dendrites, the processes of glial cells) of rat spinal cord by an indirect way — by confrontation of the results obtained by spectrophotometric determination of the RNA content in individual nerve and glial cells and those of chemical analysis of small samples from the same tissue region during changes in the functional state of the nervous system.

METHODS

Rats of the Wistar strain weighing between 180 and 220 gm., 5—6 months old, i.e. after completion of the myelination process (Kuhlman and Lowry, 1956) and stabilization of brain weight (Brizzee, et al., 1964), were used as experimental animals in all the experiments.

The skin of rat paws was electrically stimulated through a metal-barred floor of a special chamber inducing a strong excitement of the nervous system and intensive motor activity in the experimental animals most pronounced during the first 5—10 min. of stimulation. After 55—60 min. the animals were in a state of exhaustion, the active movements almost disappeared. The ability of the animals for making active mowements usually returned only after a rest of 5—10 min.

After 5, 10, 20 and 60 min. of stimulation and after 1, 2, 4 and 18 hrs of rest after cessation of the 60-minute stimulation the rats were decapitated and the spinal cord was extracted in the region of lumbar enlargement. The sample was divided into two parts, one of which (2—4 mg. of gray matter from the anterior horns of the spinal cord)

was used for chemical analysis to determine the nucleic acid content in the whole tissue by the modified (Hairdarliu, 1967) method of Tsanev and Markov (1960), another part was fixed in cold Brodsky solution: formalin-ethanol-acetic acid (3 : 1 : 0·3, by vol.) (Brodsky, 1960, Pevzner, 1965), embedded in paraffin and cut into 5—7 μ slices.

The nucleic acid concentration in individual nerve and glial cells was determined by the two-wavelength method of ultraviolet cytospectrophotometry (Ornstein, 1952, Patau, 1952, Mendelsohn, 1958, Agroskin et al., 1960) with a cytospectrophotometer, the scheme of which, the measuring procedure and calculations have been published elsewhere (Pevzner, 1963, 1966).

Determination of the cell volume by means of the previously described methods (Hydéen, 1955, Pevzner, 1960, 1965) allowed to calculate the amount of nucleic acids per cell.

Each mean value was calculated after determination of the nucleic acid amount in 60—120 cells from each of 5—10 animals, kept under the same conditions. The results were elaborated statistically by the t test.

RESULTS

Analysis of the homogenate of the anterior horns of rat spinal cord after the first 5 min. of stimulation (Fig. 1, A) revealed a decrease of the RNA content. Later, however, the RNA content increased and during the following period of stimulation and rest after cessation of the 60-min. stimulation, there was no difference as compared with the control values.

Cytospectrophotometric analysis revealed more significant changes (Fig. 1, B). Five minutes after the beginning of stimulation the RNA content increased in the perikaryons of motor neurons and simultaneously significantly decreased in the surrounding satellite glial cells. In the next 5 min. the RNA content decreased also in the cytoplasm of motor neurons. But 20 min. after the beginning of stimulation the nucleic acid content increased both in nerve and glial cells and practically did not differ from control values. Only prolonged stimulation in the course of 60 min. resulted in a significant decrease of nucleic acid content both in the motor neurons and in the surrounding satellite glial cells.

Return to the normal nucleic acid level (Fig. 2, B) began only after a 1-hr period of rest and was completed in 4 hrs in the neuroglia and in 18 hrs in the motor neurons.

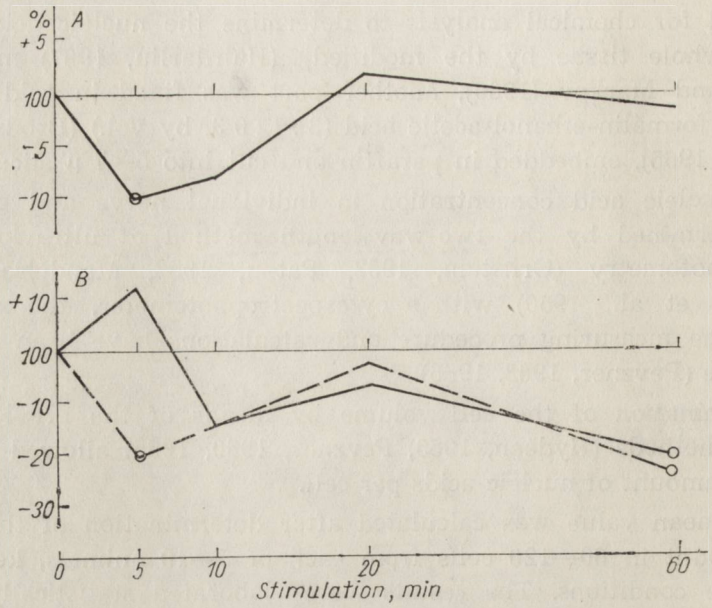

Fig. 1. Relative changes in nucleic acid content in rat spinal cord during electric stimulation of skin (percentage to control value).
A — RNA in homogenates of the spinal cord gray matter of anterior horns; *B* — continous line — cytoplasmic RNA in motor neurons of the same region; *B*, — dashed line — total nucleic acid content in surrounding satellite glial cells; *O* — statistically significant changes.

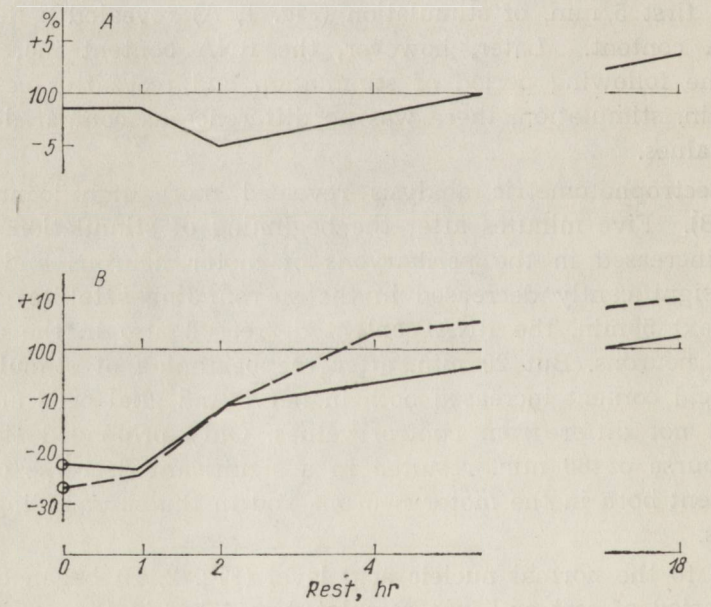

Fig. 2. Relative changes in nucleic acid content in rat spinal cord during the period of rest after cessation of 60-minute stimulation. Legend as in Fig. 1.

DISCUSSION

Cytospectrophotometric analysis of individual cells in the nervous tissue makes possible the detection of significant changes of nucleic acid concentration in nerve and glial cells, whereas chemical analysis of whole tissue homogenates may reveal only slight changes.

The determination of the RNA content in the cells of excited and intensively moving animals can reveal an increase of the RNA content in the cytoplasm of motor neurons in the anterior horns of the spinal cord and a simultaneous decrease of nucleic acid content in the surrounding satellite glial cells. (In glial cells the total of the RNA and DNA was determined. We believed the changes of nucleic acid amount in glial cells occurring in such short time periods as in our experiments to be caused by chanses of the RNA content). Such dynamics of RNA content changes in the mentioned components of the neuron-neuroglia system confirms the previously advanced hypothesis concerning the supporting function of the neuroglia metabolism for maintaing the metabolic processes in excited neurons. The experiments were performed on nerve and glial cells of Deiters' nucleus (Hydén, 1962) and on the cells of the superior cervical sympathetic ganglion of cat (Pevzner, 1965, 1966).

We gave particular attention to the fact of increase of nucleic acid content both in nerve and glial cells after 20 min. of stimulation taking place after the decrease of nucleic acid content in these two components of the neuron-neuroglia system as the result of 10-minute stimulation. Taking into account the simultaneous decrease of motor activity of the animals it may be supposed that in this case some physiological changes of adaptive character could occur accompanied by metabolic changes, so that the disturbed correlation of anabolic and catabolic processes would be re-established. But a prolonged stimulation, probably, results ultimately in a new substantial disturbance of this correlation. Such an explanation, naturally, must be subjected to experimental (in particular to physiological) verification.

The rest of the changes in nucleic acid content in nerve and glial cells revealed in exhausted animals and during the period of restitution corresponded on the whole to previously received data characterizing the metabolism of nucleic acids in the neuron-neuroglia system (Hydén, 1962, Pevzner, 1965).

The decrease of the RNA content revealed in homogenates after 5 min. of intensive motor activity of the animals (Fig. 1, A), could occur owing to a decrease of the RNA content in glial cells (Fig. 1, B). On the other hand, the insignificant increase in RNA amount in motor

neurons, owing to their small quantity, probably had only a slight influence on this decrease. In the remaining elements of nervous tissue no changes took place or a certain drop of the RNA level was noted.

The most pronounced differences between the results obtained by means of chemical and cytospectrophotometric analysis were revealed by examination of exhausted animals which had been electrically stimulated for 60 min. In the cytoplasm of neurons and the surrounding satellite glial cells there occurred a decrease of nucleic acid content, whereas homogenate analysis did not reveal any significant changes. In this case the RNA amount in the process components could increase in some degree. Probably therefore, in spite of the decrease of the RNA content in the bodies of nerve and glial cells, the total analysis did not reveal any significant changes.

Such conclusions, however should be advanced with circumspection, since the different tissue treatment in the course of examination of brain homogenates and sections could also lead to the differences in composition of the RNA fractions and changes in its physical and chemical properties, which could be reflected in the results of quantitative RNA determination.

Thus, the results of cytospectrophotometric analysis allowed to characterize the changes in RNA metabolism in the bodies of nerve and glial cells. But these changes did not exert a decisive influence upon the results of whole tissue analysis, mostly in view of the comparatively small volume of bodies in the total mass of nervous tissue. Comparison of these data with those of chemical analysis of tissue from the same regions of the nervous system afforded indirect data on RNA metabolism as a whole in the processes of nerve and glial cells, i.e. in the "process component".

These findings seem to indicate that with pronounced changes in the functional state of the nervous system, there also occur definite changes in the RNA content in the processes of nerve and glial cells, which can influence the results of whole tissue analysis. Therefore in biological investigations carried out on whole nervous tissue homogenate the possibility of such changes should also be taken into account.

I am very grateful to Dr. Leonid Pevzner for his interest and helpful comments.

S. Ch. Haidarliu

CZYNNOŚCIOWE ZMIANY ZAWARTOŚCI RNA W ELEMENTACH KOMÓRKOWYCH RDZENIA KRĘGOWEGO

Streszczenie

Heterogeniczność morfologiczna tkanki nerwowej jest przyczyną istotnych trudności w ocenie zmian zawartości RNA w różnych strukturach tej tkanki.

W niniejszej pracy przy pomocy metody cytospektrofotometrii w ultrafiolecie o dwóch długościach fali określono zawartość RNA w komórkach nerwowych i otaczających je glejowych komórkach satelitarnych, a także zbadano zależność między poziomem zawartości kwasów nukleinowych w tych komórkach i stanem czynnościowym układu nerwowego. Oprócz tego podjęto próbę określenia drogą pośrednią zmian zawartości RNA w „komponencie wypustkowej" (neuryty, i dendryty komórek nerwowych, wypustki komórek glejowych) istoty szarej rdzenia na podstawie porównania danych otrzymanych po spektrofotometrycznej analizie RNA w pojedynczych komórkach nerwowych i glejowych z chemiczną analizą małych naważek tych samych okolic rdzenia po zmianie stanu czynnościowego układu nerwowego.

W warunkach elektrycznego drażnienia skóry, zastosowanego przez nas do niniejszych doświadczeń, zmiany zawartości RNA w ciałach komórek nerwowych i glejowych nie wykazały decydującego wpływu na wyniki analizy całkowitej (pełnej) tkanki, prawdopodobnie na skutek stosunkowo niewielkiej objętości ciał tych komórek w ogólnej masie tkanki nerwowej. Otrzymane wyniki pozwalają przypuszczać, że przy gwałtownych zmianach stanu czynnościowego układu nerwowego zachodzi również określona zmiana zawartości RNA w wypustkach komórek nerwowych i glejowych, mogąca wpływać na wyniki analizy pełnej tkanki.

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ФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ СОДЕРЖАНИЯ РНК В КЛЕТОЧНЫХ КОМПОНЕНТАХ СПИННОГО МОЗГА

Содержание

Морфологическая гетерогенность нервной ткани приводит к значительным трудностям при оценке изменений содержания РНК в различных ее структурах.

В настоящей работе методом двухволновой УФ-цитоспектрофотометрии было определено количество РНК в нервных и окружающих их глиальных клетках--сателлитах, а также изучена зависимость между уровнем содержания нуклеиновых кислот в этих клетках и функциональным состоянием нервной системы. Кроме того, была предпринята попытка косвенным путем определить изменения содержания РНК в „отростковом компоненте" (аксоны и дендриты нервных клеток, отростки глиальных клеток) серого вещества спинного мозга на основе сравнения данных, полученных при спектрофотометрическом определении РНК в отдельных нервных и глиальных клетках и химическом анализе малых навесок тех же участков мозга при изменении функционального состояния нервной системы.

В условиях электрокожного раздражения, которые мы применили в наших экспериментах, изменения количества РНК в телах нервных и глиальных клеток не оказали решающего влияния на результаты анализа цельной ткани, по-видимому, главным образом вследствие сравнительно небольшого объема тел клеток в общей массе нервной ткани. Полученные данные позволяют думать, что при резких изменениях функционального состояния нервной системы также происходят определенные изменения содержания РНК в отростках нервных и глиальных клеток, которые могут влиять на результаты анализа цельной ткани.

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SPIS TREŚCI

HISTOCHEMIA MIELINY I NEUROGLEJU

BIOCHEMIA MIELINY I DEMIELINIZACJI

BIOCHEMIA W DOŚWIADCZALNYM ALERGICZNYM ZAPALENIU MÓZGU

BIOCHEMIA TKANKI NERWOWEJ