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BIOCHEMISTRY OF CEREBRAL TUMOURS

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NEUROPAT. POL. 1972, X, 2

E. GROSSI-PAOLETTI, S. PEZZOTTA, P. PAOLETTI

METABOLIC CHARACTERISTICS OF ETHYLNITROSOUREA — INDUCED BRAIN TUMOURS

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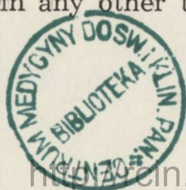
Sterols show the most remarkable characteristics among the lipid components of brain tumours. Evidences were given that both human (Azarnoff et al. 1958) and experimental brain tumours (Paoletti et al. 1966, Fumagalli et al. 1969) incorporate simple precursors into cholesterol at a much faster rate than normal adult brain of the same species.

Our group demonstrated that, very efficient sterol synthesis in brain tumours is accompanied by a defect in the last step of cholesterol formation; that is transformation of desmosterol to cholesterol. Desmosterol differs from cholesterol only by the presence in the molecule of a double bond in position 24—25 of the lateral chain, which is saturated to form cholesterol by means of desmosterol reductase (Avigan, Steinberg 1961). This enzyme activity is very high in the liver, since desmosterol is saturated very quickly (Dempsey 1965), while it must be deficient in immature brain and in brain tumours since both tissues accumulate desmosterol (Fumagalli, Paoletti 1963, Fumagalli et al. 1964, Paoletti et al. 1965).

In fact biosynthetic studies have shown that both in developing brain (Grossi-Paoletti 1971) and brain tumours (Fumagalli et al. 1969) desmosterol formation is much faster than cholesterol synthesis. Desmosterol has been detected in human gliomas (Fumagalli et al. 1964) in spontaneous animal tumours (Fumagalli et al. 1966), and in experimental ependymoblastomas (Fumagalli et al. 1966).

Very recently we have produced tumours of the nervous system in mature rats treated with methylnitrosourea (Schiffer et al. 1970) or in rats born from mothers treated with ethylnitrosourea during pregnancy (Grossi-Paoletti et al. 1970) according to Druckrey et al. (1971). Desmosterol concentration has been measured in these tumours and it has been shown to be higher than in any other tumour type tested (Weiss et al. 1970).



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The present report deals with the chemical analysis of neurinomas both Gasserian and of the spinal cord induced in Long Evans rats by administering ethylnitrosourea within 24 hrs. after birth either subcutaneously or intracerebrally. The total sterols and desmosterol levels have been measured. Protein and DNA concentration and H^3-Thymidine incorporation into DNA have also been evaluated in brain tumours as well as in liver, and in nervous tissue of tumour bearing rats and of non-treated controls.

EXPERIMENTAL DATA

Animals: Litters of Long Evans rats have been divided into three groups at the day of birth:

Group I: control rats injected with saline at the day of birth.

Group II: newborn rats injected subcutaneously with 50 mg/Kg ENU within 24 hrs after birth.

Group III: newborn rats injected intracerebrally (in parietal lobe) with 50 mg/Kg ENU within 24 hrs after birth.

The experimental conditions referring to drug solution and animal care have been reported previously (Grossi-Paoletti et al. 1970).

Thymidine incorporation into DNA: Control rats and rats bearing a tumour of the central nervous system were injected subcutaneously with 6-^{3}H-Thymidine 50 μCi/Kg (specific activity: 10 μCi/μmole) after 4 hour fasting. The rats were sacrificed 4 hrs. after the administration of the labelled compound and carefully dissected. Aliquots of tissues were taken for chemical analysis and aliquots were spared for histological examination performed by prof. Schiffer and Fabiani of the Neurological Clinic, University of Turin.

The tissues were weighed and homogenized in distilled water (2 ml/100 mg wt.). Aliquots of the homogenate (0.4 ml) were transferred to discs of filter paper and dried carefully with a fan. Afterwards they were immersed in cold 10% trichloracetic acid (TCA) and allowed to stand for 2 hours. After transferring into cold 5% TCA (1 hr), in ethanol: ether 75 : 25 (30 min.) and in ethyl ether (30 min), the discs, completely dried, were then dropped into counting vials and measured for radioactivity with a Packard 3000 Scintillation counter. It was assumed that all the radioactivity found was present as DNA (Bucher et al. 1964). The counting efficiency was determined by redissolving some of the samples in NCS (Nuclear Chicago) and counting.

Chemical determinations: Separate aliquots of the tissue homogenates were precipitated with cold TCA and the lipid material

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was extracted with ethanol and ether by repeated washings. DNA and RNA were obtained from the residual precipitate by redissolving in TCA and heating. DNA content was measured according to Burton (1956). The residue, free of nucleic acids, was dissolved by heating in N NaOH and the proteins were measured according to Lowry et al. (1951). The total sterols, purified through saponification of the lipid material and extraction with ether, were measured according to Abell et al. (1952). After acetylation of an aliquot of the sterol fraction, desmosterol content was evaluated by gas-liquid chromatography (Galli, Grossi-Paoletti 1967).

RESULTS AND DISCUSSION

The incidence of tumours of the nervous system was very high both when ethylnitrosourea was injected subcutaneously as already observed by Wechsler et al. (1969), and when the drug was injected directly into the brain.

The results obtained using this last route of administration are in contrast with those obtained by Kelley et al. (1968) using rats and mice. These authors did not obtain brain tumours in both species after intracerebral injection of MNU (methylnitrosourea) in newborn animals, which instead died of leukemia, lung or kidney tumours. The high incidence of brain tumours in our rats may be explained by drug difference: MNU is usually effective on mature rats, while ENU and other related ethyl derivatives are very effective only when given to fetal or newborn rats, as pointel out by Druckrey et al. (1971).

The tumour induction time and the tumour types obtained in the rats treated at the day of birth are reported elsewhere (Grossi-Paoletti et al. in publication). However, it should be noticed that more than 80% of the treated rats of both Group II and III had tumours of the nervous system and that the tumour types obtained were very similar to those of the transplancentally treated rats (Grossi-Paoletti et al. 1970). Neurinomas were the most frequent tumours and the most suitable for estimation of total sterol and desmosterol content, DNA concentration and synthesis.

The total sterol content was determined in tissues both from control and tumour bearing rats (Fig. 1). The liver sterols averaging 2 mg per gram tissue in control rats were higher only in Group III rats. In the brain, the total sterol content of gray matter was slightly higher in tumour-bearing rats than in control ones, while in the white matter significantly lower amounts were present in tumour-bearing animals; there was also a tendency in the spinal cord toward a decrease of the total sterols.

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As observed for other tumours of the nervous system (Brante 1949, Gopal et al. 1963) the neurinomas both of the Gasser ganglion and of the spinal cord have a low sterol content, ranging from 2 to 5.5 mg per gram of wet tissue.

Desmosterol content has been determined. The liver and nervous tissue of control rats did not show the presence of this sterol, while in some of the samples of gray and white matter and of spinal cord of treated rats appreciable amounts of desmosterol were detected (1—2% of the total sterols). The desmosterol content of the examined neurinomas (Fig. 2) was larger in Gasser tumours (7—7.5% of total sterols) than

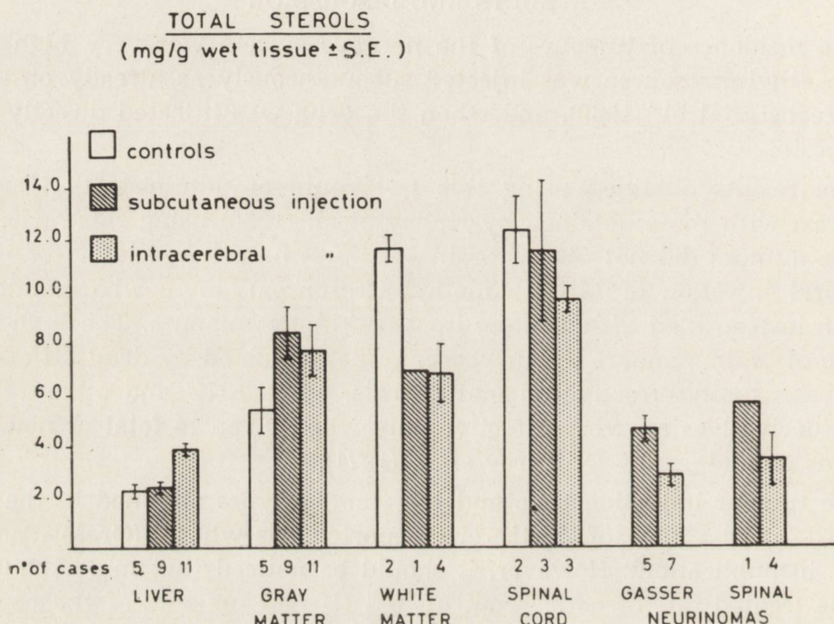

Fig. 1. Total sterol content measured in tissues of control rats (Group I) and of rats injected at birth with ENU either subcutaneously (Group II) or intracerebrally (Group III) and sacrificed when clear tumour symptoms occurred.

in spinal cord tumours (4.5—5.5%) of both group II and III rats. This result agrees well with that found in neurinomas induced by ENU transplacental administration, which have high desmosterol levels in contrast with MNU induced neurinomas, in which desmosterol was not detectable (Weiss et al. 1970).

The present data are in agreement also with the finding of desmosterol in other brain tumours, that is in human (Fumagalli et al. 1964), animal spontaneous (Fumagalli et al. 1966) and mice transplantable gliomas (Paoletti et al. 1965). It should be noticed however that the Gasser neurinomas induced by ENU administration have a desmosterol content

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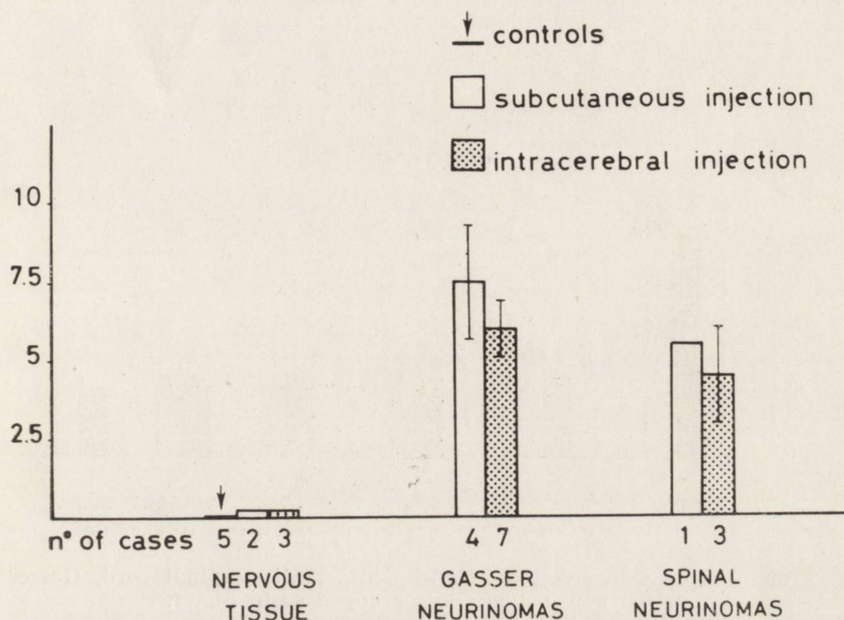

Fig. 2. Desmosterol content (as percent of the total sterols) in brain and tumours. Rats grouped as in *Fig. 1.*

superior to any other tumour except for MNU or ENU-induced oligodendrogliomas (Weiss et al. 1970).

Protein content has been measured in all the above mentioned tissues (Fig. 3). Liver proteins do not differ substantially in control and treated rats and range between 160 and 180 mg per gram of wet tissue. The protein content of gray and white matter is also similar in control and treated rats (65—80 mg per gram tissue). Spinal cord samples show a slightly lower protein content as well as the neurinomas (60—70 mg per gram tissue). Literature data on brain tumour proteins indicate an increase of the soluble proteins in the tumours (Corridori et al. 1960, Gerhardt et al. 1963), while in experimental neurinomas the total protein content is lower than in normal brain tissue. A workup of the other analytical data on a protein basis did not change considerably the results, which for this reason are reported on a wet weight basis.

A remarkably high DNA content is present in neurinomas both of the Gasser ganglion (4.5 mg/g tissue) and of the spinal cord (3—5 mg/g tissue), with a large variation in some of the cases (Fig. 4) and a low DNA content in normal nervous tissue. The present data correspond well

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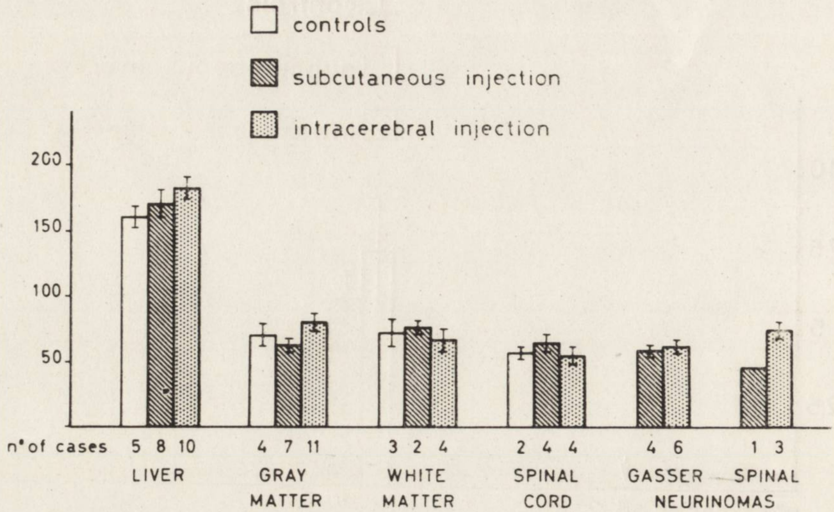

Fig. 3. Protein content in liver, gray and white matter, spinal cord, Gasser and spinal neurinomas. Rats grouped as in *Fig. 1.*

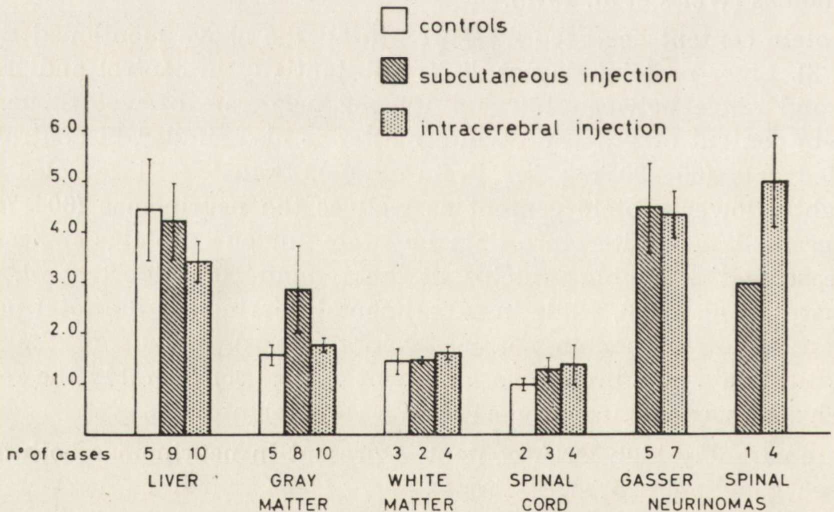

Fig. 4. DNA content in liver, gray and white matter, spinal cord, Gasser and spinal neurinomas. Rats grouped as in *Fig. 1.*

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with the data reported in the literature for other brain tumours (Heller, Elliott 1955, Nayyar 1963, Yonemasu 1968).

The incorporation of thymidine into nucleic acids, which expresses DNA synthesis, has been measured with the aim of establishing a parallelism between tumour proliferation rate and desmosterol content. The results, expressed as dpm per gram of tissue of ^{3}H-thymidine incorporated into DNA are reported (Fig. 5). It may be noticed that a very small amount

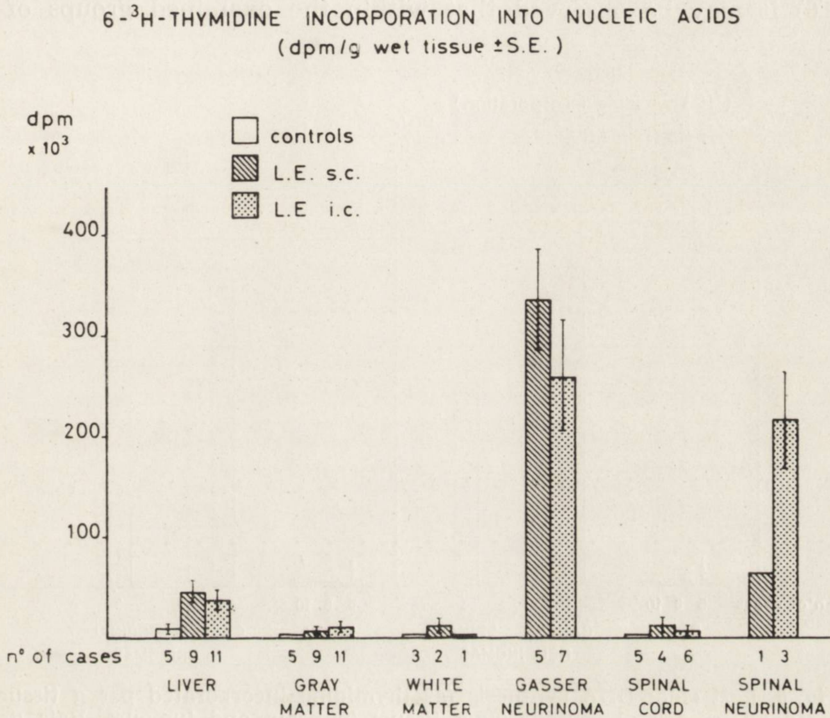

Fig. 5. Thymidine incorporation into nucleic acids (DNA) as dpm incorporated/g wet tissue. Rats grouped as in *Fig. 1.*

of incorporation was detected in the liver of control rats; while a significantly higher incorporation was present in the liver of Group II and III rats. Gray and white matter as well as spinal cord, as expected, did not incorporate thymidine into DNA to an appreciable extent, while the same tissues of treated rats in some cases showed DNA synthesis. A remarkable thymidine incorporation was detected in the tumours and the Gasser neurinomas were particularly efficient in this respect.

It has been shown that a correlation can be made between growth rate and thymidine incorporation into DNA of hepatomas (Lea et al. 1966)

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and also into kidney tumours (Lea et al. 1968), and it has been observed that brain tumours concentrate labelled thymidine presumably in DNA, as shown by autoradiography (Johnson et al. 1960, Kissel et al. 1967, Wechsler et al. 1969). The present data are indicative of a high DNA synthesis in ENU induced neurinomas, which agrees very well with the high proliferation rate and malignancy of these tumours (Grossi--Paoletti et al. 1970, Wechsler et al. 1969).

When comparing DNA synthesis and desmosterol content of tumours (Fig. 6) it may be observed that within the examined groups of rats

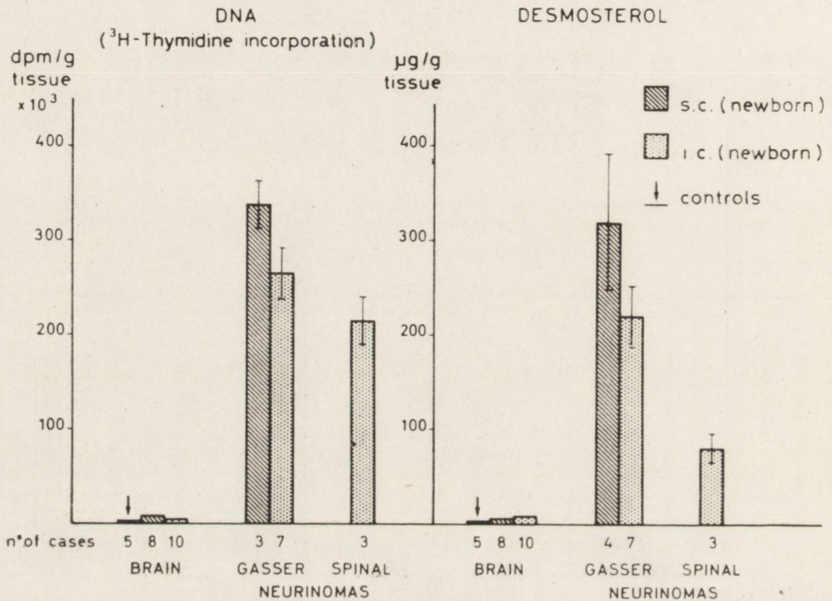

Fig. 6. Left side: DNA synthesis (as thymidine incorporated per g tissue). Right side: Desmosterol content (µg/g tissue) in brain and tumours. Rats grouped as in *Fig. 1.*

a larger incorporation of thymidine in Gasser neurinomas (dpm/g tissue) is accompanied by a larger desmosterol content (µg/g tissue) in the same tumours. It is therefore concluded that there may be a correlation between these two parameters which represent respectively cell proliferation and metabolic activity of the tumours.

The presence within the brain or the spinal cord of rats, sacrificed either for a Gasser or spinal neurinoma, of areas showing thymidine incorporation and desmosterol in detectable amounts, may be correlated with the oligodendroglial foci which are observed in the brain of these rats (Grossi-Paoletti et al. 1970). The fact that in the animals surviving

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longer oligodendriogliomas, gliosarcomas and other tumours appear, together with the biochemical findings, entitles us to interpret these as pretumoral areas (Schiffer et al. 1970, Grossi-Paoletti et al. in publication).

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E. Grossi-Paoletti, S. Pezzotta, P. Paoletti

METABOLICZNE WŁAŚCIWOŚCI GUZÓW MÓZGU WZBUDZONYCH ETYLONITROZOMOCZNIKIEM

Streszczenie

Badano zawartość i strukturę steroli w nerwiakach zwoju Gassera i rdzenia kręgowego szczura, wzbudzonych jednorazowym, podskórnym lub domózgowym wstrzyknięciem etylonitrozomocznika w dniu urodzenia. W guzach tych stwierdzono wysoki poziom desmosterolu.

Zawartość DNA w guzach była wysoka w porównaniu z zawartością w prawidłowej tkance, a jego synteza ze znakowanej tymidyny bardzo wydajna. Guzy wykazujące najwyższe włączanie tymidyny do DNA charakteryzowały się również największą zawartością desmosterolu.

Е. Гросси-Паолетти, С. Пеццотта, П. Паолетти

МЕТАБОЛИЧЕСКИЕ СВОЙСТВА ОПУХОЛЕЙ МОЗГА, ВЫЗВАННЫХ ЭТИЛНИТРОЗОМОЧЕВИНОЙ

Резюме

Исследовали содержание и структуру стеролей невриноm Гассерова узла и спинного мозга крысы, вызванных однократной, подкожной или внутримозговой инъекцией этилнитрозомочевины в дне рождения. В этих опухолях был обнаружен высокий уровень десмостерола.

Содержание ДНК в опухолях было высокое в сравнении с содержанием в нормальной ткани а его синтез из меченного тимидина давал очень большой выход. Опухоли обнаруживающие самую высокую степень включения тимидина в ДНК характеризовались также самым высоким содержанием десмостерола.

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THE IIIrd NATIONAL SYMPOSIUM
OF NEUROPATHOLOGY

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NEUROPAT. POL. 1972, X, 2

M. I. Prockorova, S. Y. Tumanova, T. V. Tchaika

LIPID METABOLISM OF HUMAN BRAIN TUMOURS

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A common feature of neoplastic degeneration of the cells is the alteration of their membranes. An unitary membrane defect can be postulated to account for the variability characteristic of tumour behaviour (Wallach 1968). It changes the structure of lipoprotein complex which forms a functional unit of any membrane (Curtis 1967). Since lipids constitute more than 50% of the whole mass of this complex, neoplastic degeneration of the brain tissue could result directly in the change of lipid metabolism. The phospholipids and glycolipids play a particular role in the membrane structure. From the latter, especially the gangliosides are specific for neuronal, dendritic and nervous endings membranes (Landolf et al. 1966, Lapetina et al. 1967). The phospholipid composition of tumour tissue has been extensively investigated and a detailed comparison of these compounds with those occuring in the normal tissue has given much information on the general metabolic behaviour of tumour tissue (Figard, Greenberg 1962, Gopal et al. 1963, Gray 1963, Kawanami 1967, Snyder et al. 1969, Djatlovizka et al., 1969a, 1969b, 1970, 1971, Wood, Healy 1970). The glycolipids in tumour tissue have received very little attention and the information about their metabolism in the human brain tumours is very scanty (Seifert 1966, Brady et al. 1969, Kóstic et al. 1970).

This paper presents our data concerning the total lipids of some neuroectodermal and meningovascular tumours with particular emphasis on the gangliosides and cerebrosides.

MATERIAL AND METHODS

All tumours specimens vere obtained from the surgical material from Polenov's Neurosurgical Clinic, Leningrad. In certain cases the adjacent cortex was removed to serve as control material. The histological examination of the control portions of the brain revealed no pathological changes. The histological diagnosis of neoplastic tissue was performed in the Anatomopathological Department of Polenov's Neurosurgical

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Clinic. The following intracranial tumours were analysed: brain astrocytomas (typical, continued growth, dedifferentiated) neurinoma n. VIII, malignant ependymoma; meningiomas (typical, dedifferentiated, malignant), angioreticuloma.

All neoplastic tissues as well as the adjacent normal brain tissue were washed with an isotonic saline solution to remove traces of blood. The homogenized tissue was extracted with 20 times the tissue weight of chloroform-methanol (2 : 1 v/v). The total lipids of neoplastic tissues were extracted and washed by the method of Folch et al. (1957). The total lipids were fractionated on silica gel KCK. Approximately equal amounts of total lipids were taken on each plate. The solvent system used to develop the chromatograms was hexane-ether-acetic acid (70 : 30 : 2 v/v). The chromatograms were developed with iodine vapour. Components were identified by comparison with commercial standards run on the same plate. Phospholipids remained at the origin. The neutral lipid content was analyzed by thin-layer chromatography in the above system.

Gangliosides were extracted by the method of Svennerholm (1956) as modified by Folch-Pi (1959). After dialysis and lyophilization, the gangliosides were dissolved in chlorophorm-methanol (1 : 1 v/v) and analysed for the total N-acetylneuraminic acid by the resorcinol method described by Svennerholm (1957) and modified by Miettinen and Takki-Lunkkainen (1959). The ganglioside mixture was separated by thin-layer chromatography on silica gel KCK in chloroform-methanol-water (60 : 35 : 8 v/v). Gangliosides were localized by impregnation of the plates with the resorcinol solution. The individual gangliosides were identified by comparison with a standard mixture of brain gangliosides chromatographed under the some conditions.

Cerebrosides were extracted by the method of Uzman (1953). The content of cerebrosides was estimated by galactose using the orcin method of Svennerholm (1955). After crystallization from acetone, chromatography of cerebrosides on silica gel KCK was done in chloroform--methanol-water (60 : 35 : 8 v/v). Cerebrosides were localized by exposure of the plates to iodine vapours.

RESULTS AND DISCUSSION

All types of the examined tumours contained scanty amounts of lipids (Table 1). The normal human brain tissue contains average 11.3% total lipids per wet weight. Their level in the examined tumours was considerably decreased. The lowest content of the total lipids was found in the case of the meningioma III° (malignant meningioma) — 0.64%. At

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the same time dedifferentiated astrocytoma contained 6% of total lipids. The total lipids of the angioreticuloma constituted 5.7% and in neurinoma — 1.7%. In the meningovascular tumours the decrease of total lipids was manifested more sharply than in the neuroectodermal tumours. If in the line of meningovascular tumours one could observe the decrease of total lipids depending on the grade of anaplasia so in the line of neuroectodermal tumours this relationship was evidently disturbed (typical astrocytoma — 3.5%; continued growth astrocytoma — 3.0%; dedifffe-rentiated astrocytoma — 6.0%; malignant ependymoma — 2.0%). At present it is impossible to explain this phenomenon in a suitable way.

Table 1. Total lipids, gangliosides and cerebrosides of human brain tumours

Samples	Total lipids % w.w.	Gangliosides μm NANA	Cerebrosides % w.w.
Controls	11.3	1.20	1.5
Neuroectodermal tumours			
typical astrocytoma	3.5	0.37	0.40
cont. growth astrocytoma	3.0	0.39	0.31
dedifferen. astrocytoma	6.0	0.11	0.25
malignant ependymoma	2.0	0.34	0.04
Neurinoma VIII	1.7	0.45	0.43
Meningovascular tumours			
typical meningioma	3.0	0.25	0.40
dedifferen. meningioma	2.4	0.25	0.43
malignant meningioma	0.6	0.11	0.94
angioreticuloma	5.7	0.58	0.31

It had been shown before that the phospholipid compositions of the tumour cells did not markedly differ from those of a normal brain tissue. It was of some interest to define if there were any variations in composition of neutral lipids in human brain tumours. As may be concluded from the results obtained by thin layer chromatography, numerous fractions of neutral lipids are present in the neoplastic tissue but their number is lower than that found in the control material. We succeded in detecting the following fractions in normal tissue of human brain: phospholipids which remained at the origin, cholesterol (Rf-0.23), monoglycerides (Rf-0.37), free fatty acids (RF-0.42), diglycerides (Rf-0.55), triglycerides (Rf-0.62) a nonidentified fraction (Rf-0.73) and cholesterol esters (Rf-0.81). On the chromatographic plates, almost all of the examined tumours showed the following distinct fractions: free fatty acids, cholesterol esters, triglycerides and nonidentified fraction, which we like to attributed to the fatty acid esters. The enhanced occurence of the ester linkages in neoplasms is not due to an increased biosynthesis of

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ester bonds but could occur owing to the absence of enzymes that cleave the ester bond (Snyder et al. 1969).

We have not succeeded in detecting cholesterol in the cases of dedifferentiated astrocytoma, angioreticuloma, malignant ependymoma. In the literature, the loss of feedback control in cholesterol biosynthesis in tumours had already been reported (Siperstein et al. 1966).

A slowly moving fraction with Rf-0.09 appeared close to the phospholipids in dedifferentiated astrocytoma and angioreticuloma.

The absence of the mono- and diglycerides was a common feature of all the examined tumours with the exception of dedifferentiated astrocytoma, angioreticuloma and neurinoma where the diglycerides have been detected (Table 2). Such a difference in a character of the neutral lipids component may result from the break in the metabolism of different lipids in every specific case.

Table 2. Components of total lipids of human brain tumours

	Phosphol.	Nonident.	Cholester.	Monoglycer.	Fatty acid	Diglycer.	Triglycer.	Nonident	Ester chol.
Controls	+	−	+	+	+	+	+	+	+
Neuroectodermal tumours									
typical astrocytoma	+	−	+	−	+	−	−	+	+
cont. growth astrocytoma	+	−	+	−	+	−	−	+	+
dedifferen. astrocytoma	+	+	−	−	−	+	+	+	+
malignant ependymoma	+	−	+	+	+	−	−	+	+
Neurinoma VIII	+	−	+	−	−	+	+	+	+
Meningovascular tumours									
typical meningioma	+	−	+	+	+	−	−	+	+
dedifferen. meningioma	+	−	+	+	+	−	−	+	+
malignant meningioma	+	−	−	−	−	−	+	+	+
angioreticuloma	+	+	−	−	−	+	+	+	+

The content of gangliosides in the tumours sharply decreased (Table 1). The level of the gangliosides in dedifferentiated astrocytoma and meningioma III° was ten times lower than that in the normal tissue.

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A recent quantitative investigation by Slagel et al. (1967) showed that in some human glial brain tumours, particularly those with a high degree of malignancy, a lower concentration of total ganglioside NANA was also found. The concentration of gangliosides in the tumours with a low degree of malignancy (astrocytoma, neurinoma, meningioma, angioreticuloma) was only two to three times less than that found in normal surrounding brain tissue.

The distribution of different gangliosides in brain tumours was changed (Figs. 1 and 2). In normal human brain, 9 individual fractions

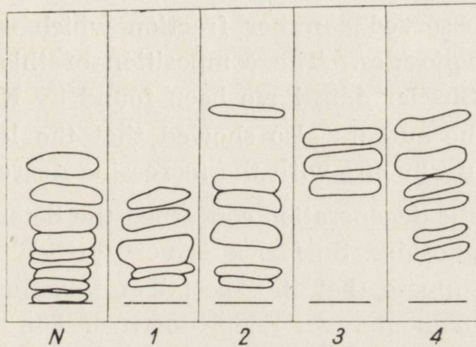

Fig. 1. Ganglioside patterns of human brain tumours. *N* — control, *1* — typical meningioma, *2* — dedifferentiated meningioma, *3* — malignant meningioma, *4* — angioreticuloma

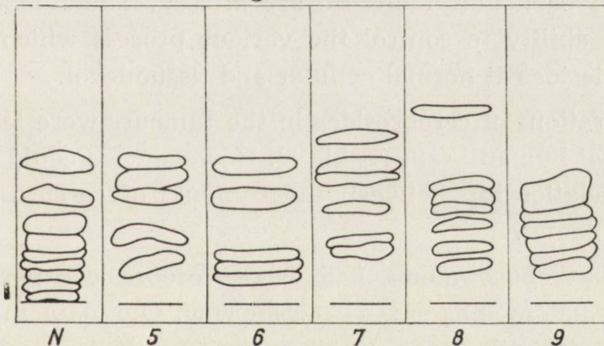

Fig. 2. Ganglioside patterns of human brain tumours. *N* — control, *5* — typical astrocytoma, *6* — continued growth astrocytoma, *7* — dedifferentiated astrocytoma, *8* — neurinoma VIII, *9* — malignant ependymoma

of gangliosides were detected. Every examined brain tumour exhibited its own character of distribution of gangliosides; this considerably differed from the control. The alterations were due to the general decrease in number of slowly moving fractions and increase in number of the fast moving ones.

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The malignant meningioma had only three polar fractions of monosialogangliosides and no di- and trisialogangliosides at all. In all examined tumours, the slowest two fractions of trisialogangliosides were absent. These data differed somewhat from the results obtained by Kóstic et al. (1970). These authors demonstrated that the amount of less polar gangliosides was increased in malignant tumours while that of the more polar gangliosides relatively decreased. Nevertheless, these authors showed that astrocytoma had a lower di- and trisialogangliosides content than normal human brain tissue. In five examined tumours (dedifferentiated and malignant meningioma, angioreticuloma, neurinoma, dedifferentiated astrocytoma), we observed a minor fraction which moved faster than other monosialogangliosides. The composition of this fraction remains to be determined. Similar data have been found by Kóstic et al. (1970) in meningiomas. The authors also showed that the level of the minor fraction increased in tumours with the increasing degree of malignancy.

Thus, the neoplastic degeneration concerned the heterogenity of mono-, di- and trisialogangliosides. So far, it is very difficult to explain these results. One may suppose that the special enzymes catalysing the biosynthesis of these compounds are deeply perturbed in different tumours. Numerous enzymes are involved in the synthesis of gangliosides and it is likely that the activities of certain enzymes participating in the formation of a particular molecule are altered. The tumour cells may be loosing the ability to control the various process which permit synthesis of gangliosides at normal cellular and tissue level.

The concentrations of cerebrosides in the tumours were less than those found in normal human brain (Table 1). However, we could not establish any straight relationship between the contents of cerebrosides and the degree of malignancy.

The malignant ependymoma had 0.04% cerebrosides per wet weight and the malignant meningioma — 0.94% w.w. Our data in that respect are in agreement with those of other authors. It is possible that the decrease in cerebroside content is connected with the cerebrosidase activity of tumour cells. Popova and Promyslova (1971) reported that astrocytomas and oligodendriomas possess such an activity. The increase in level of cholesterol esters and the drop in level of cerebrosides may result from the demyelination process occuring in brain tissue involved by the tumour.

The heterogeneity of cerebrosides in tumours varied considerably (Figs. 3 and 4), from one tumour to the other. Samples of normal human

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brain revealed five individual cerebroside fractions. The first two fractions were sulphatides and the others were neutral ceramide hexosides. Thus beside the number of fractions, their mobility was changed too. In meningioma and angioreticuloma we detected 3 fractions, in astrocytoma — 6 individual fractions, whereas in all other tumours only 4 fractions were found. The alteration in the mobility of those fractions is a result of the change in cerebroside structure of tumours. Perhaps it is related to the variation of mono-, di-, tri- and tetrahexoside ceramides.

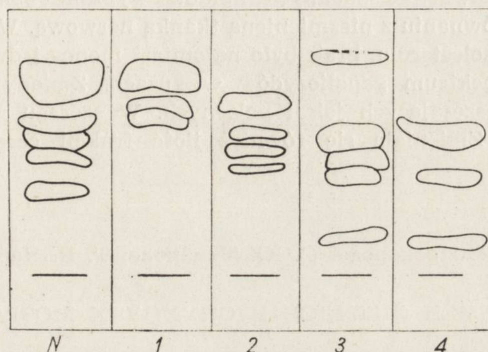

Fig. 3. Cerebrosides of human brain tumours. *N* — control, *1* — typical meningioma, *2* — malignant meningioma, *3* — dedifferentiated meningioma, *4* — angioreticuloma

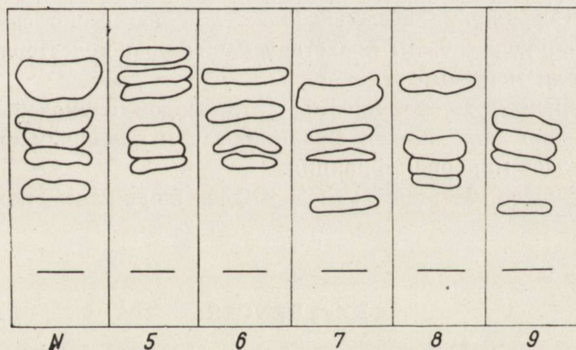

Fig. 4. Cerebrosides of human brain tumours. *N* — control, *5* — astrocytoma, *6* — continued growth astrocytoma, *7* — dedifferentiated astrocytoma, *8* — malignant ependymoma, *9* — neurinoma VIII

The lack of glycolipid standards as well as the lack of detailed analyses of composition of those compounds does not allow to come to definite conclusions. In the future, however, such an approach can provide useful information concerning the relationships of different lipids in various types of brain tumours.

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M. I. Prockorowa, S. J. Tumanowa, T. W. Czajka

METABOLIZM LIPIDÓW W GUZACH MÓZGU

Streszczenie

Przedmiotem badań autorów był metabolizm lipidów w wybranych guzach mózgu pochodzenia neuroektodermalnego i oponowo-naczyniowego. Zawartość i charakter tłuszczów obojętnych, gangliozydów i cerebrozydów określano przy pomocy chromatografii cienkowarstwowej.

Stwierdzono, że zawartość badanych lipidów wybitnie obniżała się w tkance nowotworowej w porównaniu z niezmienioną tkanką nerwową. We wszystkich guzach występowały estry cholesterolu, brak było natomiast mono- i dwuglicerydów. Zmieniało się również spektrum gangliozydów w guzach. Zmiany dotyczyły zarówno frakcji wolnoprzemieszczających jak i polarnych. W szeregu guzów pojawiała się frakcja dodatkowa. Zmieniała się również ilość frakcji cerebrozydów oraz ich ruchliwość.

М. И. Прохорова, С. Ю. Туманова, Т. В. Чайка

ОБМЕН ЛИПИДОВ В ОПУХОЛЯХ МОЗГА

Резюме

Нейтральные липиды, ганглиозиды и цереброзиды ряда нейроэктодермальных и менинговаскулярных опухолей исследовались методом тонкослойной хроматографии. Содержание исследованных липидов резко снижалось в опухолях. Во всех опухолях были представлены эфиры холестерина и, как правило, отсутствовали моно- и диглицериды.

Изменялся характер гетерогенности ганглиозидов опухолей, изменения касались как медленно движущихся фракций, так и полярных фракций. В ряде опухолей появлялась минорная фракция.

Уменьшалось число фракций цереброзидов, изменялась подвижность этих фракций.

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DNA ESTIMATIONS IN CEREBRAL TUMOURS OF MAN

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In the course of the last 20 years the DNA amount in cerebral tumours was investigated with biochemical analyses (Heller, Elliot 1954, Broun et al. 1962, Lutsenko, Promyslova 1962) and with microscopical methods without (Gros et al. 1955, Roth, Gyergyay 1962, Schiffer et al. 1964) and with cytophotometric evaluation (Lapham 1959, Scarlato, Müller 1959, Pevzner et al. 1964, Kortmann 1968, Patzer 1969). The up to now cytophotometric investigations were performed on sections of specimens embedded in paraffin. Therefore we can suppose, that with regard to the complicated calculation from the slice to a whole nucleus, especially in the cases of polymorphic forms like in the glioblastomas, these "plug" — methods give no correct values.

We report on cytophotometric scanning measurements of DNA amounts in complete nuclei of gliomas (9 oligodendrogliomas, 3 astrocytomas, 5 glioblastomas, 8 medulloblastomas). The gliomas were classified according to a modified grading-system of Ringertz (Müller, Schröder 1968) into three grades. The measurements were performed with a Barr and Stroud Integrating Microdensitometer of the type described by Deeley (1955). Air dried smear preparations of fresh operated tumour tissue were fixed in ice-cold formalin- ethanol and stained by a standardized Feulgen procedure. Monochromatic light at wave lenght 500 µm was used to determine the light absorption. For every nucleus, three absorption measurements were made, and the averages, expressed in arbitrary units, were used for pooling the results. As a rule corresponding to the statistical distribution for every tumour 50 or 100 nuclei were measured. In our figures the ordinate represents the number, on the abscissa are indicated the steps of ploidy. The arbitrary units and the ploidy steps were calculated on a logarithmic basis. The symbol N notifies the total of measured cells.

The diploid level was obtained by measurements of a great collection of segmented granulocytes of normal blood of healthy persons. In accordance with numerous investigations (Lit. s. Müller, Sandritter 1961)

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based on cytophotometric and chemical techniques the values of granulocytes appear as a useful reference for the standardization of the diploidy. In the tumour preparations as a control granulocytes likewise were checked.

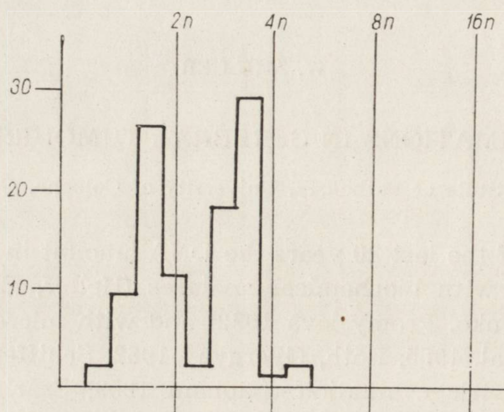

Fig. 1 Oligodendroglioma I (No. 11727-22y's-male) N = 100

In the oligodendroglioma-group we find in the grade I DNA amounts assembled with peaks between the 2n and 4n line or as for instance in the first figure with a maximum between the diploid and tetraploid range and a second maximum between the tetra- and octoploid step (Fig. 1).

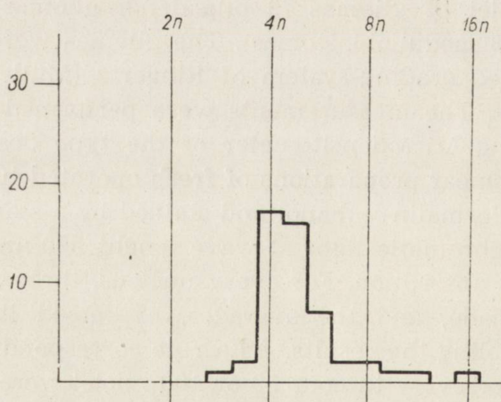

Fig. 2. Oligodendroglioma II (No. 13069-40y's-female) N = 50

With increased malignity as in the Fig. 2 a distribution of the values with shifting of the maximum to the 4n line appears, or we can find (Fig. 3) a peak in the hyperdiploid level and a second hypertetraploid maximum. If we take into consideration the stem-line concept, we can regard this case as an oligodendroglioma with a hypertetraploid stem-line.

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Figure 4 shows the data obtained in an astrocytoma of grade I with a very large distribution of the Feulgen values up to the hypooctoploid area without development of a definite peak. The second example of

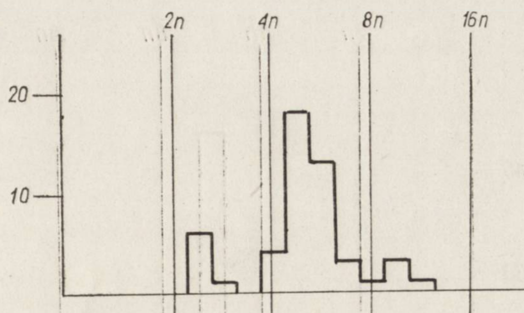

Fig. 3. Oligodendroglioma II (No. 13237-33y's-male) N = 50

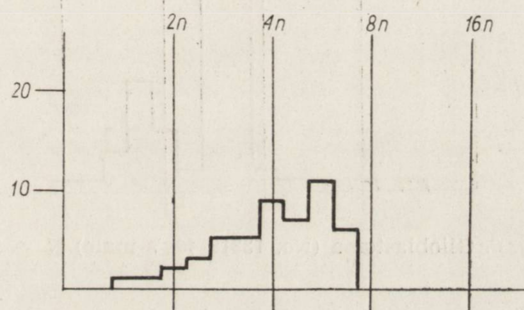

Fig. 4. Astrocytoma I (No. 11670-23y's-male) N = 50

astrocytoma (Fig. 5) is a more malignant tumour with a very expanded distribution of the values and without distinct maxima or ploidy steps.

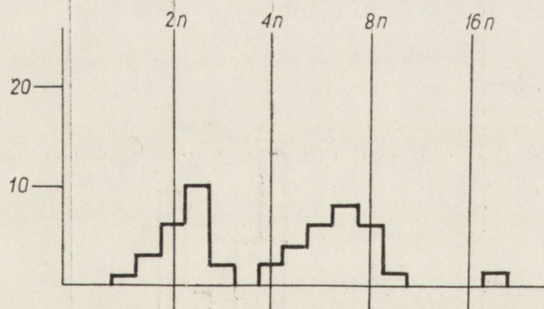

Fig. 5. Astrocytoma II (Nr. 13175-44y's-male) N = 50

The following two diagrams (Figs. 6 and 7) concern the group of gliomas grade III or glioblastomas. Both of them exhibit a very large

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distribution of the Feulgen values and the latter shows two definite maxima exactly in the tetraploid and octoploid range, with the stem line in the 4n step.

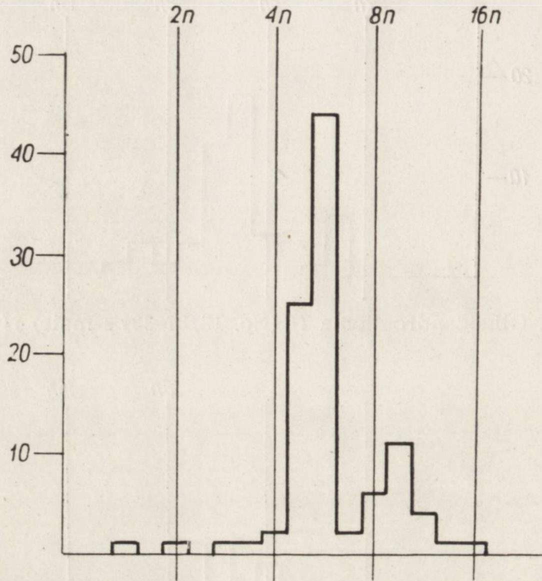

Fig. 6. Glioblastoma (No. 13318-46y's-male) N = 100

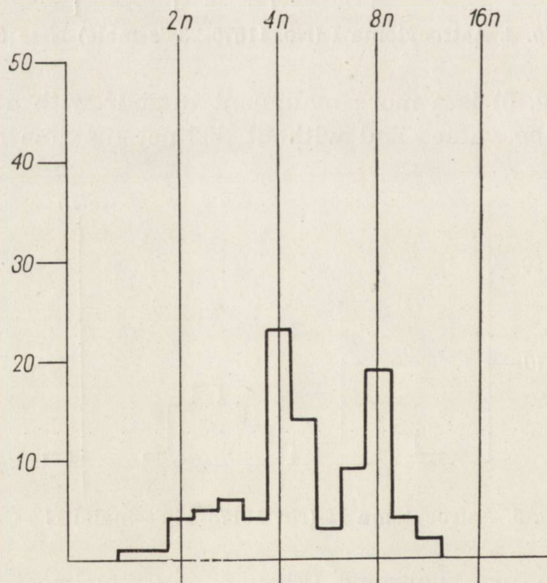

Fig. 7. Glioblastoma (No. 13060-38y's-male) N = 100

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In regard to the relatively small number of cases, these examples of this part of the glioma group seem to allow us to drow the following conclusions:

1) with increasing malignity the amount of DNA in the single nuclei increases, so that the spectrum of the values becomes wider than in normal diploid cells;

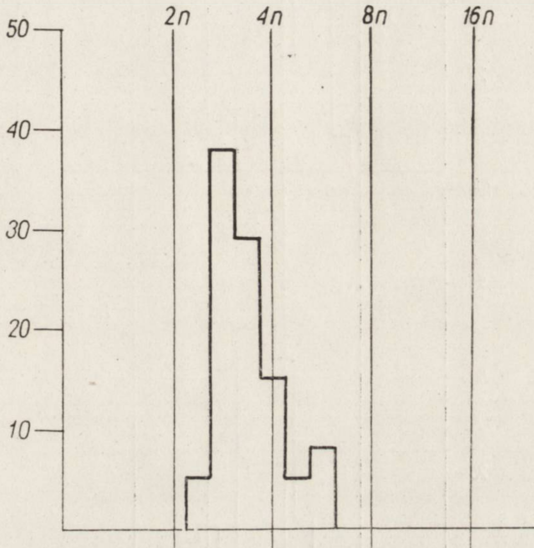

Fig. 8. Medulloblastoma (No. 12802-33y's-female) N = 100

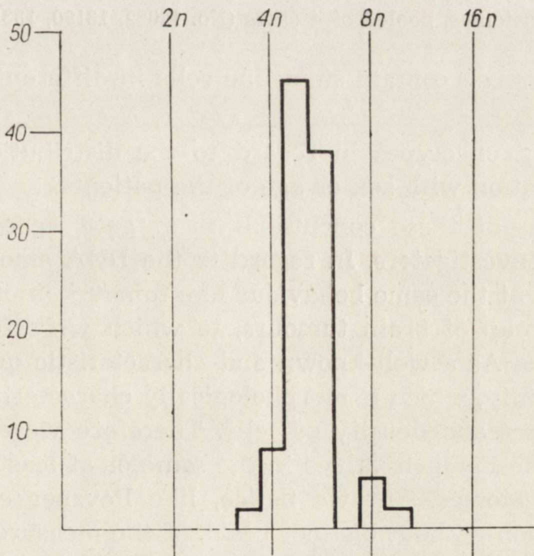

Fig. 9. Medulloblastoma (No. 12022-8y's-male) N = 100

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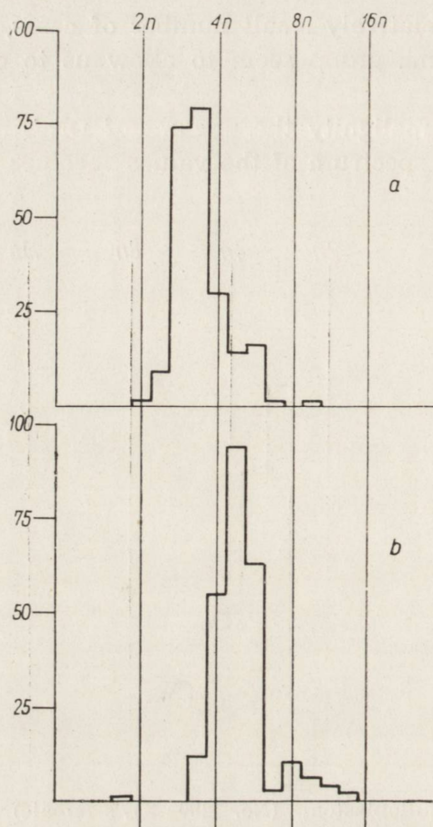

Fig. 10a. Medulloblastomas, pooled of 4 cases (No. 11802, 12847, 13150, 13053) N = 225
Fig. 10b. Medulloblastomas, pooled of 4 cases (No. 12022, 13190, 13311, 13374) N = 250

2) the tumours can contain stem-line cells in different and significant ploidy steps;

3) the different oncotypes in regard to the distribution of the DNA are not in correlation with sex or age of the patients.

The first point of these conclusions is in good agreement with the results of other investigators. In regard to the DNA amount the gliomas obviously represent the same behaviour like tumours in other organs.

The second group of brain tumours, to which we refer now, are the medulloblastomas. As a well-known and characteristic quality we notice their high malignity, which is morphologically characterized by the large number of mitoses and density of cells. Therefore we could expect the distribution of the Feulgen values in the same or at least similar manner as in the glioblastomas. But we notice, like Pevzner et al. (1964) not a great distribution without distinct peaks of the measured values, rather we find distributions with a slight tendency to the great values and

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a remarkable concentration of the most values at a definite maximum. The peaks of the maxima are either in the area between the 2n and 4n (Fig. 8) or the 4n and 8n (Fig. 9) line. Compiling the values of these two types of maxima of the explored medulloblastomas we get two well defined kinds of hypo-respectively hypertetraploidic tumours (Fig. 10).

We think that both of these oncotypes possibly are correlated with the small or large cell medulloblastomas. This conception is in good agreement with the results of Stolpmann (1968), who detected that with increasing the number of the great nuclei in medulloblastomas the amount of DNA rises like-wise.

With reference of the conception that the enzyme-equipment of a cell is genetically determined, the observed variety of the different distribution-patterns of the Feulgen values in the investigated gliomas altogether and the lack of well defined ploidy levels in the particular kinds of these tumours seems to be one possible reason for the nonuniforme enzyme-spectrum in the same kinds of gliomas and therefore explains the discrepancies in the informations of several investigators referring to this.

W. Müller

OZNACZANIE DNA W GUZACH MÓZGU U LUDZI

Streszczenie

Autorzy oznaczali DNA w glejakach ludzkich metodą Feulgena. Badania przeprowadzono na skrawkach materiału operacyjnego przy użyciu mikrodensytometru integrującego. Stwierdzono, że gwiaździaki oraz skąpodrzewiaki charakteryzują się rozmieszczeniem DNA, odchylającym się od wartości diploidalnych, przy wykształceniu się linii rodzajowych.

Glejaki wielopostaciowe wykazują rozdziały wartości odczynu Feulgena charakterystyczne dla guzów złośliwych, z szerokim rozrzutem i poliploidalnym maksimum.

Wśród rdzeniaków stwierdzono dwie grupy — ze szczytami gęstości odpowiednio w zakresach hipo- i hipertetraploidalnym.

В. Мюллер

ОПРЕДЕЛЕНИЕ ДНК В ОПУХОЛЯХ МОЗГА У ЛЮДЕЙ

Резюме

Авторы определяли в человеческих глиомах ДНК методом Фойлгена. Исследования проводились на срезах операционного материала при использовании интегрирующего микродензитометра.

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Было обнаружено, что астроцитомы и олигодендроцитомы характеризуются распределением ДНК, откланяющимся от диплоидных величин при формировании видовых линий.

Полиморфные глиомы обнаруживают разделы реакции Фойлгена характерные для злокачественных опухолей с широким разбросом и полиплоидным максимумом.

Среди медуллобластом были обнаружены 2 группы — с максимумами соответственно плотности в гипо- и гипертетраплоидном диапазонах.

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NEUROPAT. POL. 1972, X, 2

G. Viale, H. Kroh

TRANSFER AND RIBOSOMAL RIBONUCLEIC ACIDS IN HUMAN GLIOMAS

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Modifications in the molecular structure and in biological activity of t-RNA occur during differentiation and neoplastic processes (Baliga et al. 1965, Srinivasan, Borek 1966).

The changes are mainly related to the absolute increase of methylated nucleosides (Bergquist, Matthews 1959, 1962). In all types of brain tumours methyllated nucleosides occur in the t-RNA in greater amount than in normal brain tissue (Table 1). It seems that the increase of methylated nucleosides in particular types of gliomas is proportional to the degree of malignancy (Viale et al. 1968).

Table 1. Methylated nucleosides in t-RNA from human brain and brain tumours (expres sed in M%M)

Normal brain (frontal lobe)	2.08
Medulloblastoma	3.86
Spongioblastoma	5.40
Astrocytoma I	4.76
Astrocytoma II—III	7.17
Oligodendroglioma I	4.54
Oligodendroglioma I—III	5.23
Glioblastoma	10.36
Neurinoma	3.84
Sarcoma (fibrocellular)	3.46

The nucleoside composition of tumoral t-RNA differs in various tumours mainly as concerns the degree of methylation of particular bases (Viale et al. 1967). The increase of 2-o-methylated riboses is observed in all types of gliomas (Table 2).

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Table 2. Nucleoside composition of t-RNAs from human brain and undifferentiated human gliomas

Nucleosides (M%M)	Normal brain	Medullo-blastoma	Spongio-blastoma	Astrocy-toma I	Astrocyto-ma II—III	Oligoden-droglioma I	Oligoden-droglioma II—III	Glioblas-toma
Cytidine	29.48	29.44	28.92	29.44	29.20	30.12	29.06	27.03
5-Methylcytidine	0.12	0.26	0.72	0.24	0.64	0.33	0.30	0.72
2'-0-Methylcytidine	0.12	0.26	0.20	0.14	0.48	0.18	0.37	0.58
Adenosine	19.36	17.86	17.64	19.38	17.65	17.87	18.15	17.96
N^6-Methyladenosine	0.18	0.45	0.54	0.54	0.86	0.38	0.62	1.14
N^6-Dimethyladenosine	0.12	0.36	0.32	0.32	0.56	0.32	0.45	0.68
2'-0-Methyladenosine	0.04	0.06	0.08	0.08	0.35	0.12	0.16	0.42
Guanosine	30.26	29.14	28.48	28.25	27.41	29.16	29.22	25.84
1-Methylguanosine	0.02	0.46	0.88	0.48	0.84	0.57	0.52	1.58
N^2-Methylguanosine	0.32	0.74	0.72	0.90	0.62	0.72	0.64	1.88
N^2-Dimethylguanosine	0.54	0.52	1.04	0.76	0.98	0.66	0.73	1.13
2'-0-Methylguanosine	0.25	0.30	0.38	0.26	0.84	0.44	0.60	0.90
Uridine	13.56	15.18	16.22	14.44	15.24	14.52	15.18	14.42
Ψ-Uridine	5.05	4.16	3.84	3.75	3.60	3.84	3.12	3.86
5-Methyluridine	0.32	0.08	0.36	0.64	0.42	0.51	0.36	0.62
2'-0-Uridine	0.03	0.05	0.12	0.04	0.40	0.16	0.32	0.46
Inosine	0.20	0.34	0.22	0.36	0.14	0.24	0.12	0.32
1-Methylinosine	0.02	0.32	0.04	0.36	0.18	0.15	0.16	0.27

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The rate of methylated purines to methylated pyrimidines differs markedly in tumours of various types (Viale et al. 1967). Accordingly, t-RNA methylase activity *) is increased (Viale et al. 1970). The phenomenon is demonstrated by Figures 1, 2, 3, where: methylases from mali-

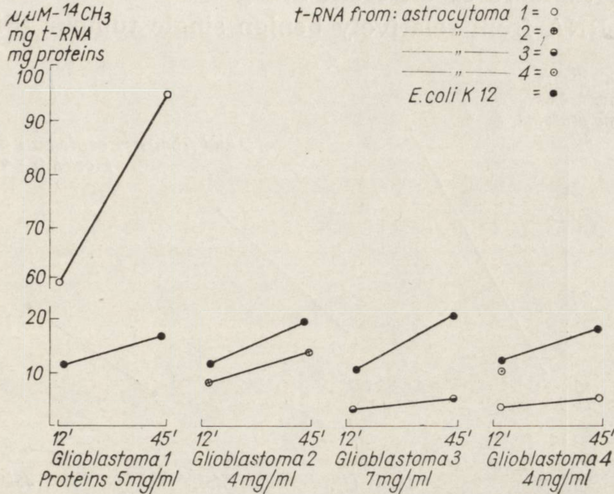

Fig. 1. The activity of the methylases originating from glioblastomas (1, 2, 3, 4) acting on the t-RNA (2.5 mg/ml) from different astrocytomas (1, 2, 3, 4) and on t-RNA from methyl-deficient E. coli K 12, in condition of rate.

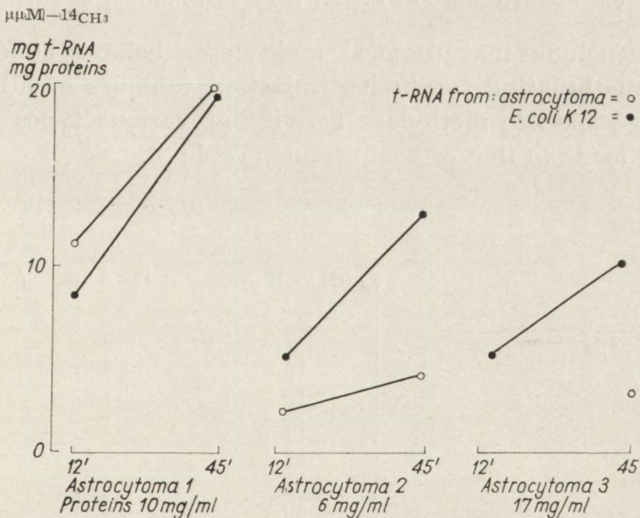

Fig. 2. The t-RNA methylase activities of various astrocytomas (1, 2, 3) acting on the substrate (2.5 mg/ml t-RNA) from other astrocytoma and on t-RNA from methyl-deficient E. coli K-12, in condition of rate.

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*) t-RNA methylase activity (rate) means a measure of the number of methylase molecules present.

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gnant tumours methylate t-RNAs derived from E. coli and t-RNAs from less malignant tumours from various individuals (Fig. 1); methylases from relatively bening tumour from different individual act upon t-RNA from E. coli and t-RNA from similar tumour (Fig. 2); methylases from different types of tumours of different malignancy act on the t-RNA from E. coli and t-RNA from relatively benign single tumour (Fig. 3).

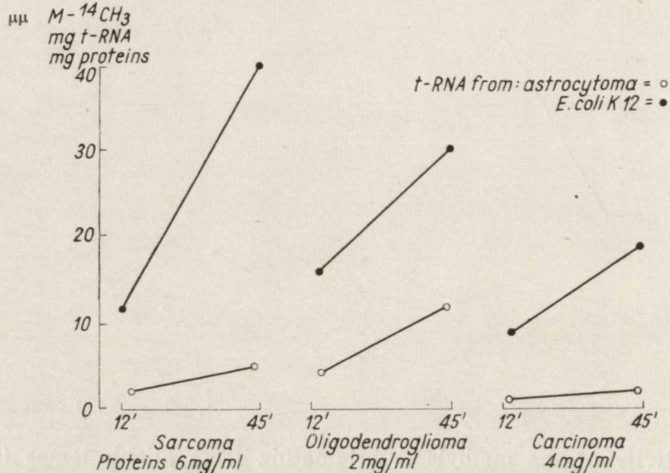

Fig. 3. The t-RNA methylase activities of different brain tumours (sarcoma, oligodendroglioma, carcinoma) acting on astrocytoma t-RNA (2.5 mg/ml), and on t-RNA from methyl-deficient E. coli K 12, in condition of rate.

One can conclude that tumoral methylases behave abberantly and their tissue methylating specificity is lost in tumours of all types.

The average specific methylase activity of various types of tumours is always higher than that of brain tissue (Table 3).

Table 3. The determination of average activity of t-RNA methylases from human brain tumours

	μμM $^{14}CH_3$/mg /	t-RNA/mg	/ Proteins/45'
Normal brain (frontal lobe)	7.7	±0.5	(8)
Astrocytoma	16.3	±3.5	(12)
Oligodendroglioma	22.7	±1.2	(13)
Malignant oligodendroglioma	13.2	±1.7	(14)
Glioblastoma	18.7	±2.4	(24)
Meningioma	11.5	±2.0	(8)
Sarcoma	29.2	±6.4	(10)
Carcinoma (brain metastasis)	10.6	±3.5	(13)

Average values ± standard deviation (number of determinations)
2.5 mg/ml E. coli K 12 t-RNA; 3—7 mg proteins/ml;
30 mμM/ml $^{14}CH_3$-S-adenosyl-methionine

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In accord to increased methylase activity also the methylation capacity *) of t-RNA is increased.

Observed phenomena point out, that tumour enzymes, in conditions of extent of methylation, methylate t-RNA extracted from tumours of the same or different type (Figs. 4, 5, 6, 7).

Transfer RNA can be still methylated more by sequential methylation in conditions of capacity measurments (Figs. 8, 9). Sequential methylation points again to the increased t-RNA capacity and lost tissue specificity.

No marked modifications have been found in the composition of classical nucleosides and their reciprocal ratios in both 18 S and 28 S fractions of the r-RNAs from tumours, when compared with normal brain (Table 4).

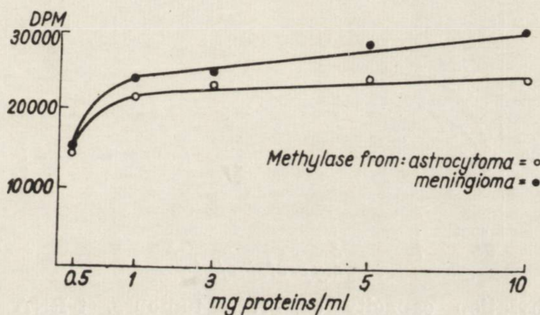

Fig. 4. The methylation capacity of brain tumours. t-RNA from astrocytoma 1 (0.1 mg/ml) methylated by methylase from astrocytoma 2 and meningioma, in condition of excess amount of proteins. Incubation time 45 min.

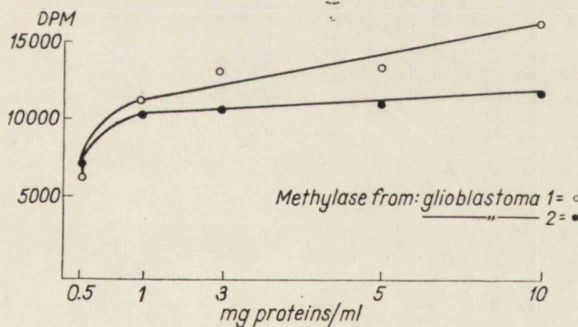

Fig. 5. The methylation capacity of brain tumours. t-RNA from oligodendroglioma (0.1 mg/ml) is acted upon by methylases from two different glioblastomas, in condition of excess amount of proteins. Incubation time 45 min.

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*) The capacity means the measure of the total number of sites on t-RNA which a given group of t-RNA methylases can recognize. The capacity, called also the extent reflects qualitative differences between t-RNA methylases.

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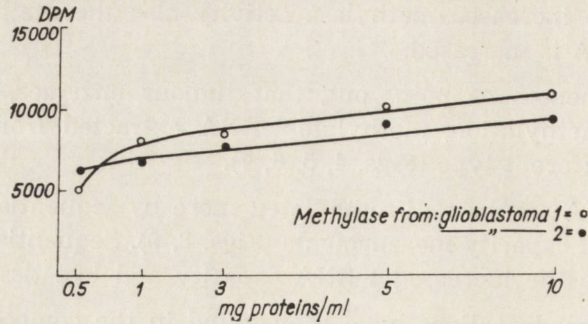

Fig. 6. The methylation capacity of brain tumours. t-RNA derived from meningioma (0.1 mg/ml) is methylated by methylases from two different glioblastomas, in condition of excess amount of proteins. Incubation time 45 min.

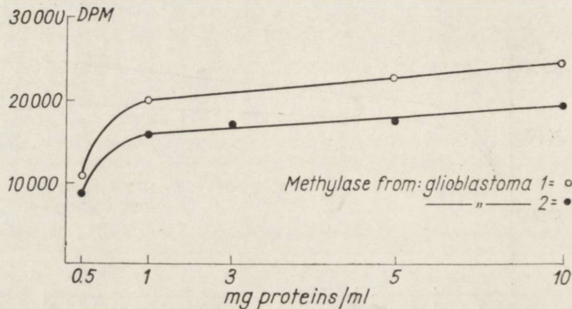

Fig. 7. The methylation capacity of brain tumours. t-RNA from astrocytoma (0.1 mg/ml) is methylated by two different glioblastomas, in conditions of excess amount of proteins. Incubation time 45 min.

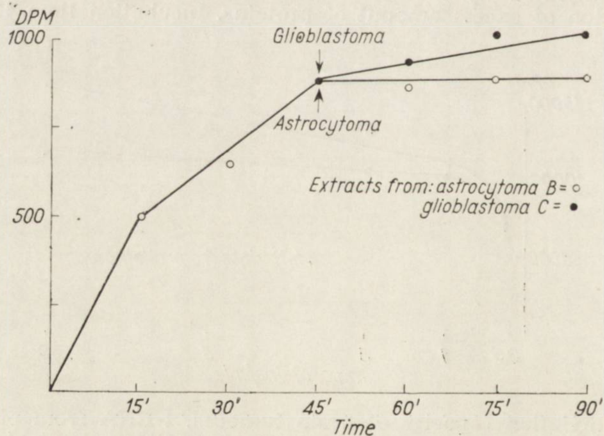

Fig. 8. The effect of sequential incubations in a system in which t-RNA (0.1 mg/ml) from an astrocytoma (Tumour A) is first incubated with extracts (10 mg/ml proteins) from another astrocytoma (Tumour B). Aliquots corresponding to 0,01 mg t-RNA were taken at selected intervals. At the point of saturation of the system, the incubation mixture was divided in two parts and extracts (10 mg/ml proteins) from tumour B and from a glioblastoma (Tumour C) were added separately. Glioblastoma extracts still methylated the already hypermethylated substrate.

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Table 4. Methylated nucleosides in the ribosomal RNAs from human brain tumours

	Human brain		Medullo-blastoma		Spongio-blastoma		Astro-cytoma I		Astro-cytoma II—III		Oligoden-droglioma I		Oligoden-droglioma II—III		Glioblasto-ma	
	18 S	28 S	18 S	28 S	18 S	28 S	18 S	28 S	18 S	28 S	18 S	28 S	18 S	28 S	18 S	28 S
Methylcytidine (N_4-?)	0.15	—	0.22	<0.05	0.10	—	0.26	0.05	0.18	0.12	0.15	—	0.15	0.07	0.10	0.12
N^6-Methyladenosine	—	—	<0.05	0.08	—	—	<0.05	0.14	0.09	0.14	—	0.08	0.12	0.10	0.15	0.08
N^6-Dimethyladenosine	0.16	0.12	0.15	0.10	0.24	<0.05	0.20	—	0.23	<0.05	0.16	0.20	0.22	0.08	0.26	0.14
N^2-Dimethylguanosine	0.28	0.08	0.44	0.15	0.25	0.18	0.36	0.12	0.42	0.16	0.22	0.14	0.41	0.22	0.32	0.10
2'-0-Methylnucleosides	0.32	0.24	0.45	0.22	0.33	0.28	0.15	0.22	0.35	0.26	0.24	0.12	0.36	0.24	0.38	0.13
Total (M%M)	0.91	0.44	1.31	0.60	0.92	0.51	1.02	0.53	1.27	0.73	0.77	0.54	1.26	0.71	1.21	0.57

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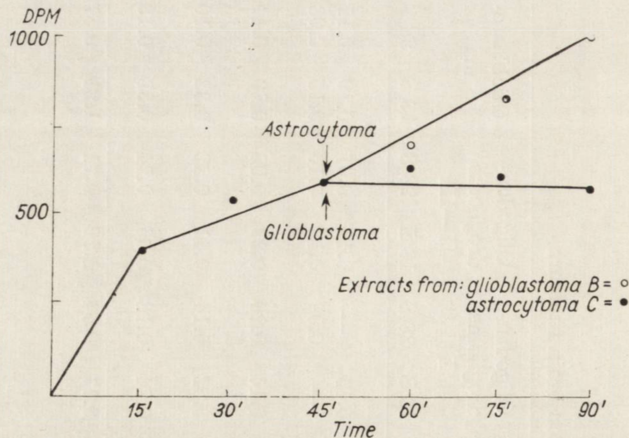

Fig. 9. The effect of sequential incubations in a system in which t-RNA (0.1 mg/ml) from an astrocytoma (Tumour A) is first incubated with extracts (10 mg/ml proteins) from glioblastoma (Tumour B). Aliquots corresponding to 0.01 mg t-RNA were taken at selected intervals. At the point of saturation of the system, the incubation mixture was divided in two parts and extracts (10 mg/ml proteins) from tumour B and from another astrocytoma (Tumour C) were added separately. Astrocytoma extracts still methylated the already hypermethylated substrate.

The increase of methylated nucleosides is more marked in 18 S fraction than the 28 S fraction. The degree of methylation of both fractions from tumours is higher than that from normal brain.

Presented findings demonstrate following:

Transfer RNA of the brain tumour tissues exhibits increased rate of methylation.

The pattern of base specific methylation is different in various brain tumours.

Transfer RNA methylase activity and t-RNA capacity in brain tumours are increased; the tissue specifity of t-RNA methylases is lost in all types of tumours.

The increase of methylated nucleosides of r-RNA is more discrete than in t-RNA.

G. Viale, H. Kroh

KWAS RYBONUKLEINOWY PRZENOSZĄCY I RYBOSOMALNY W GLEJAKACH LUDZKICH

Streszczenie

Badania przeprowadzone na materiale glejaków ludzkich wykazały wzrost zawartości metylowanych nukleozydów w porównaniu z niezmienioną tkanką mózgu. Wzrost ten jest zależny od stopnia złośliwości nowotworu.

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Obserwowano wzrost aktywności metylaz kwasu rybonukleinowego przenoszącego (t-RNA methylases). Na podstawie przeprowadzonych badań stwierdzono utratę właściwości specyficznych tego enzymu w odniesieniu do wszystkich typów guzów.

Wzmożonej aktywności metylaz towarzyszy wzrost pojemności metylacyjnej kwasu rybonukleinowego przenoszącego (t-RNA capacity), która również zmienia się niespecyficznie.

Kilkakrotnie powtarzana metylacja również wskazuje na zwiększenie pojemności metylacyjnej t-RNA i utratę własności specyficznych.

Zmiany w składzie nukleozydów we frakcjach 18 S i 28 S kwasu rybonukleinowego rybozomowego nie są tak wybitnie zaznaczone, aczkolwiek stopień metylacji obu frakcji w guzach mózgu jest znacznie wyższy niż w prawidłowym mózgu.

Г. Виале, Г. Крох

ТРАНСФЕР-РИБОНУКЛЕИНОВАЯ КИСЛОТА И РИБОСОМЫ В ЧЕЛОВЕЧЕСКИХ ГЛИОМАХ

Резюме

Исследования, проведенные на человеческих глиомах, обнаружили увеличение метилированных нуклеотидов по сравнению с неизмененной мозговой тканью. Это увеличение зависит от степени злокачественности опухоли.

Наблюдается также рост активности метилаз трансфер-рибонуклеиновой кислоты (t-RNA methylase). Проведенные исследования обнаружили потеру специфических свойств этого энзима по отношению к всем типам опухолей.

Вместе с усиленной активностью метилаз наблюдается увеличение метиляционной емкости трансфер-рибонуклеиновой кислоты (t-RNA capacity), которая также изменяется неспецифически.

Несколько раз повтораемая метиляция указывает также на увеличение метиляционной емкости т-РНК и потеру специфический свойств.

Изменения в составе нуклеотидов во фракциях 18 S и 28 S рибозомной рибонуклеиновой кислоты выделяются не так заметно, хотя степень метиляции обеих фракций в опухолях мозга значительно выше чем в нормальном мозгу.

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NEUROPAT. POL. 1972, X, 2

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CHARACTERIZATION OF NUCLEIC ACIDS ISOLATED FROM HUMAN BRAIN TUMOURS

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Extensive histochemical investigations by Hyden (1962) suggest a direct involvement of RNA in the function of the central nervous system. The biochemical studies provided evidence for the incorporation of RNA precursors into RNA of anatomically and functionally different brain regions (Borkowski 1962).

Nevertheless the entire interpretation of the biochemical studies is complicated by considerable morphological heterogeneity of cerebrum. In the cortex itself, where various types of neuronal cells are responsible for creation and transmission of impulses, numerous neuroglial cells exist which are not directly involved in the specific function of the central nervous system.

Attempts have been made for several years (Rose 1967) to obtain a morphologically homogenous material from cerebral tissue by separating the neuronal and the glial cells by means of differential centrifugation.

Neoplastic cerebral tumours of glial origin undoubtedly show certain morphological homogenity, they exhibit the ability of division and neoplastic growth, in contrast to neuronal cells. On the basis of material gathered from the Neurosurgical Clinic, and fully aware of the basic biological differences between normal and neoplastic glial cells, we have undertaken the studies to determine the RNA and DNA content in glial tumour tissue as well as in normal cerebrum and to characterize comparatively the nucleic acids extracted from neoplastic and normal tissue.

MATERIAL AND METHODS

The experiments were carried out on different regions of the human brain and various types of human brain tumours. Quantitative determinations of total RNA and DNA were carried out on the fresh tissue by

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means of the methods described previously (Borkowski et al. 1967). The quantities of RNA and DNA were expressed as μg of P per 1 g of fresh tissue.

Extraction of nucleic acids: The tissues were homogenized in a Potter-Elvehjem type homogenizer in 0.14 M NaCl in 0.01 M tris-HCl buffer pH 7.2 at 0°. After homogenization, SDS *in substantia* at 2% was added and extraction was carried out with 1 vol of neutral phenol for 1 hr at 0°, with constant stirring. Aqueous phase obtained by centrifugation was separated and the remaining phenol phase and interphase were re-suspended in 0.5 M NaCl in the same buffer containing 0.5% SDS and extracted for 30 min. in room temperature. After centrifugation, the aqueous phase was separated and both the phenol phase and the interphase were resuspended in 0.14 M NaCl in the same buffer and reextracted for 10 min at 65°. After centrifugation, 1 vol of 95% ethanol was added to the combined aqueous phases. The fibres formed were collected from the solution with a glass rod. The fibres constituted fraction I of nucleic acids. To the remain another volume of ethanol was added. The obtained sediment constituted fraction II. The phenol phase and the interphase were precipitated with 2 vol of ethanol and the nucleic acids determined quantitatively were regarded as fraction III.

Fractionation of nucleic acids was carried out on methylated albumin kieselguhr column- MAK-column (Mandell, Hershey 1960). Enzymatic hydrolysis of RNA was performed according to Marmur (1961) using crystalline pancreatic ribonuclease in citrate buffer. The ribonuclease was purified before use by heating at 80° for 10 min (Marmur 1961). Nucleotide composition of RNA and DNA was determined according to the method described previously by Borkowski et al. (1969, 1970).

RESULTS

The concentrations of both nucleic acids in various anatomical regions of normal human brain are presented in Table 1.

Table 1. Concentration of nucleic acids in various regions of normal human brain tissue

Anatomical region of brain	μgP/1 g of fresh tissue		RNA/DNA
	RNA	DNA	
Region parietal	45.4±9.2	30.6±5.1	1.47
Region occipital	44.5±7.4	30.8±3.0	1.44
Region temporal	39.1±7.0	28.5±7.0	1.37
Region frontal	64.6±5.2	38.4±3.0	1.68
White matter, parietal region	38.7±4.2	14.5±4.5	2.66
Gray matter, parietal region	38.0±7.4	82.5±3.0	0.46

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Both the RNA and the DNA content are equal in the parietal, occipital and temporal regions. Higher concentrations of both RNA and DNA were found in frontal region. The RNA concentrations in the white and gray matter were similar, whereas the DNA concentration in the gray matter was five times higher than that in the white matter.

The nucleic acid content in tumours depended on their cytological structure. The concentrations of RNA and DNA determined in various types of tumours are presented in Table 2.

Table 2. Concentration of nucleic acids in various types of human brain tumours

Type of tumour	μgP/1 g of fresh tissue		RNA/DNA
	RNA	DNA	
Ependymoblastoma	119.0±7.8	103.3±6.3	1.1
Astrocytoma	72.0±5.1	70.9±4.5	1.1
Meningioma	96.0±4.1	87.0±5.1	1.1
Oligodendroglioma	96.5±3.2	72.4±3.0	1.3
Neurinoma	54.2±3.7	124.0±6.3	0.44
Astrocytoma in glioblastoma vertens	26.7±6.2	94.5±3.7	0.3

In ependymoblastoma, astrocytoma and meningioma the concentration of RNA was only slightly higher than that of DNA, that means the RNA /DNA ratio was 1.1. In oligodendroglioma, the RNA/DNA ratio was 1.3 respectively, which points to the higher RNA content. In neurinoma and astrocytoma in glioblastoma vertens, the DNA concentration was higher and the RNA/DNA ratio amounted to 0.3 and 0.44 respectively.

Three-fold phenol extraction in tris-HCl buffer with different NaCl and SDS concentration and carried out at different temperatures gave different recoveries of nucleic acids, depending on the tissue type.

Table 3. Repartition of nucleic acids between aqueous and phenol phases

Tissue	Per cent of nucleic acids	
	in combined aqueous phase	in phenol phase and interphase
Normal brain	82.0±5.0	18.0±4.5
Oligodendroglioma	89.0	11.0
Ependymoblastoma	98.5	1.5
Glioma	91.0	9.0
Astrocytoma in glioblastoma vertens	86.0	14.0

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Under these conditions, the amount of the unextracted nucleic acids in the neoplastic tissue made 1.5 — 15% of the total nucleic acid content, whereas the value found for the normal tissue was about 18%.

The fibres of DNA (fraction I) were contaminated by RNA. The concentration of RNA in this fraction depended on the type of tissue, the DNA level being always higher. After digestion with crystalline pancreatic ribonuclease the fibres of DNA were purified on a MAK-column. Under these conditions, the RNA degradation products were eluted at a low NaCl concentration, whereas one peak of pure DNA was obtained at 0.6 M NaCl. Clear franctions of DNA were precipitated with 1 vol of ethanol and the concentration of ribonuclease-resistant RNA was determined (Borkowski et al. 1967, Mandel, Borkowska 1964). For the neoplastic tissue it appeared to be 2.8 — 4.6% of the total DNA in fraction I, whereas for the normal tissue it was only 2.0%.

Table 4. Concentration of RNA-ribonuclease-resistent in DNA fractions obtained from normal brain tissue and various types of tumours

Type of tissue	μgP-DNA	μgP-RNA ribonuclease-resistent	Per cent of RNA ribonuclease-resistent
Normal brain region occipital	131.0	2.75	2.06
Oligodendroglioma	42.4	1.25	2.86
Ependymoblastoma	75.5	3.06	3.90
Astrocytoma	40.3	1.25	3.10
Glioma	27.1	1.07	3.80
Astrocytoma in glioblastoma vertens	60.2	2.90	4.59

For the closer characterization of the types of RNA in fraction II the nucleic acids were separated on a MAK-column. Elution was carried out with a continous NaCl gradient and finally with 1.5 M NaCl in 0.2 M NH_4OH (Mahler et al. 1966). We have found five peaks of absorption at 260 nm peak I at 0.2 M, peak II at 0.4 M, peak III at 0.6 M, peak IV at 0.7 — 0.8 M and peak V at 1.5 M NaCl in ammonia solution. According to Mandell and Hershey (1960) peak I was formed by degradation products of nucleic acids, peak II corresponds to soluble RNA (s-RNA), peak III to DNA, peak IV to ribosomal RNA (r-RNA), whereas peak V consists of both types of nucleic acids. The first peak was always higher in the normal than in the neoplastic tissue. The r-RNA concentration was much higher in the neoplastic tissue than in the normal ones.

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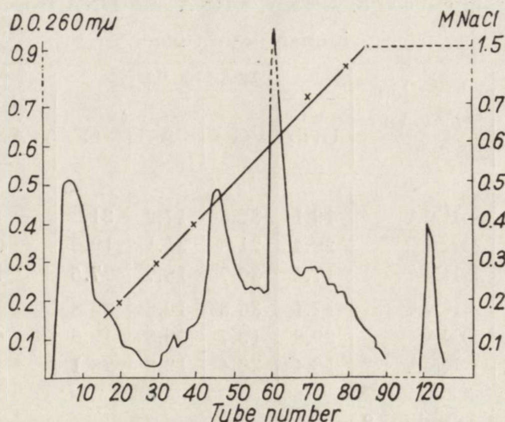

Fig. 1. Chromatogram of nucleic acids (fraction II) extracted from the normal human brain on a MAK (methylated albuminekieselguhr) column. From 100 — 150 OD of nucleic acids were loaded on the column in 0.01 M Tris-HCl buffer, pH 7.1 in 0.14 M NaCl. Elution was carried out with a linear gradient from 0.14 M to 1.5 M NaCl in Tris buffer and finally with 1.5 M NaCl in 0.2 M NH_4OH.

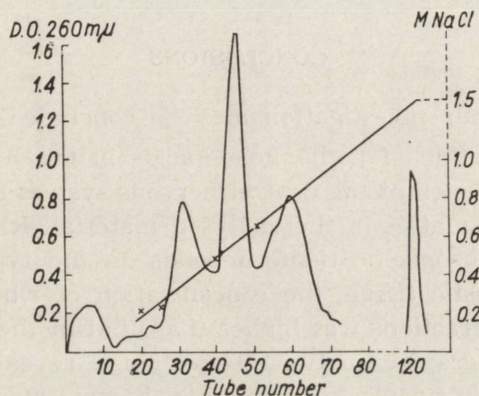

Fig. 2. Chromatogram of nucleic acids (fraction II) extracted from neoplastic human brain tissue on a MAK column. From 100 — 150 OD of nucleic acids were loaded on the column in 0.01 M Tris-HCl buffer, pH 7.1 in 0.14 M NaCl. Elution was carried out with a linear gradient from 0.14 M to 1.5 M NaCl in Tris buffer and finally with 1.5 M NaCl in 0.2 M NH_4OH.

This phenomenon may be explained by a higher activity of ribonuclease in the normal brain tissue than in the neoplastic one (Roth 1959). The s-RNA, DNA and r-RNA fractions were precipitated with 95% ethanol and their base composition was determined.

The GMP content in s-RNA of the neoplastic tissue was higher, and UMP content lower in comparison with s-RNA from the normal tissue. DNA base composition in both tissues was identical. Nucleotide composition of r-RNA in both tissues was very similar, as established on the basis of the G+C/A+U and purine/pyrimidine ratio. It is interesting

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Table 5. Nucleotide composition of s-RNA, r-RNA and DNA from normal and tumours human brain tissue

Tissue	Fraction of nucleic acids	Mole % U(T)	Mole % G	Mole % A	Mole % C	$\frac{G + C}{A + U(T)}$	$\frac{Pur}{Pyr}$
Normal brain	s-RNA	19.1	32.5	17.2	31.3	1.76	0.99
tissue	DNA	28.4	21.5	31.1	19.0	0.68	1.11
Region occipital	r-RNA	17.1	36.7	18.7	27.5	1.79	1.24
Neoplastic	s-RNA	17.1	36.3	16.8	29.8	1.95	1.14
tissue	DNA	29.8	19.7	30.8	19.6	0.65	1.02
	r-RNA	16.4	36.4	19.2	28.1	1.82	1.25

The results are means values 8 experiments.

that the G+C/A+U ratio in s-RNA obtained from both tissues is very high. The nucleotide composition of nucleic acids obtained from various types of tumours was similar.

CONCLUSIONS

The results of our investigation may be concluded as following:

1. The concentration of both nucleic acids in the normal brain tissue depended on the region of the central nervous system and on the content of white and gray matter in the analysed material. RNA and DNA concentration in the neoplastic tissue depends on the type of tumour.

2. In the neoplastic tissue, the concentration of ribonuclease-resistent RNA in the DNA fractions was higher than that in the normal tissue.

3. The chromatographic analysis of fraction II revealed higher concentration of r-RNA in neoplastic tissue, which may suggest that the ribonuclease activity was lower in the analysed tumour tissues.

4. The nucleotide composition of analysed nucleic acids obtained from various types of neoplastic tissue was similar.

S. Kulesza, T. Borkowski

CHARAKTERYSTYKA KWASÓW NUKLEINOWYCH IZOLOWANYCH Z GUZÓW MÓZGU U LUDZI

Streszczenie

W różnych obszarach ośrodkowego układu nerwowego człowieka oraz kilku typach nowotworów mózgu przeprowadzono ilościowe oznaczenia stężenia RNA i DNA.

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Stężenie RNA i DNA zależne było od struktury morfologicznej badanej tkanki. Opracowano schemat ekstrakcji, zapewniający maksymalne wydobycie kwasów nukleinowych z obu typów badanych tkanek. Otrzymane preparatywnie kwasy nukleinowe z tkanki nowotworowej i prawidłowej rozdzielono na dwie frakcje: frakcję I — bogatą w DNA i frakcję II — zawierającą głównie RNA. Po trawieniu rybonukleazą frakcji I i oczyszczeniu na kolumnie MAK oznaczono zawartość RNA związanego z DNA. Ilość RNA rybonukleazoopornego związanego z DNA była wyższa w tkance nowotworowej. Frakcję II poddano chromatografii na kolumnie MAK i w otrzymanych t-RNA i r-RNA oznaczano składy nukleotydowe, a w DNA skład zasad.

С. Кулеша, Т. Борковски

ХАРАКТЕРИСТИКА НУКЛЕИНОВЫХ КИСЛОТ ИЗОЛИРОВАННЫХ ИЗ ОПУХОЛЕЙ ЧЕЛОВЕЧЕСКОГО МОЗГА

Резюме

Проведены количественные определения концентрации РНК и ДНК в разных районах центральной нервной системы человека и в нескольких типах опухолей человеческого мозга.

Концентрация РНК и ДНК зависела от морфологической структуры исследованной ткани. Разработана схема экстрагирования нуклеиновых кислот из обоих типов исследованных тканей. Полученные препаративным путем нуклеиновые кислоты из опухолевой и нормальной ткани разделялись на две фракции: фракцию I — богатую ДНК, и фракцию II — содержащую главным образом РНК. После переваривания фракции I рибонуклеазой и очистке на колонке МАК определялось содержание РНК, связанной с ДНК. Количество рибонуклеазорезистентной РНК, связанное с ДНК было выше в опухолевой ткани. Фракция II подвергалась хроматографии на колонке МАК и в полученных т-РНК и р-РНК определялся нуклеотидный состав а в ДНК — состав оснований.

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BRAIN NITROGEN METABOLISM IN RATS WITH INOCULATED EXTRACEREBRAL SARCOMAS

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The interaction between the organism and a tumour arises from early stages of development of the latter. It is manifested in a modification of the metabolism in organs and tissues distant from the tumour.

The nervous system reaction to the development of the tumour is of phasic character. At the initial stages of the process, excitation of the nervous system is observed; this is considered to be a compensatory and protective reaction. Inhibition and exhaustion of the nervous system follows (Latmanizova 1956, Samundgan 1956, Ukoleva et al. 1966). It is known, that the influence of functional deviation and exhaustion of the nervous system or impairment of connections between the damaged organ and the nervous system create favourable conditions for the growth of a tumour.

The changes of the functional state of the central nervous system are accompanied by modification of brain metabolism. However, this aspect of tumour biochemistry has been unsufficiently investigated sofar. It is very important to find the connection between the level and trend of brain metabolism and the properties of the tumour activity.

The special features of protein and nucleic acid metabolism of tumours result in mobilisation of all energetic and plastic resources of the organism. One of the most important research directions should be the study of protein synthesis regulation and of the state of nitrogen metabolism in the brain of animals. Regulation of protein synthesis becomes of utmost importance in tissue regeneration, embryogenesis and tumour growth. The intensity of synthesis of ribosomes is known to depend on a number of factors. One of them is the concentration of potassium and ammonium ions. They cannot be substituted by other cations. Ammonium ions have some advantages as compared with those of potassium (Schoffeniels 1969).

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Ammonia concentration in the brain increases upon excitation, under the action of extreme factors, in the first stages of postembryonal development, regeneration and in other states causing intensification of protein synthesis.

The stimulation of protein synthesis with ammonium ions has been demonstrated in normal liver tissue cells and in the cells of ascites hepatoma of Novikoff (Griffin et al. 1968). In the model system it was shown that the protein synthesis can be stimulated by increasing the ammonium concentration from 1 mM to 10 mM (Maden, Monro 1968).

The ammonia level in the brain is maintained by the systems of its binding and liberation. The most active system is that of glutamic acid--glutamine. Glutamine synthetase and glutaminase take part in transformation within the system. The role of amino acids and urea in brain metabolism is important and specific (Gershenovich et al. 1970). One of the specific properties of brain proteins is their ability to form complexes with low-molecular-weight ligands. It is suggested that such complexes have some specific functions or may play an accumulative role in relation to the reserves of low molecular compounds. In this connection the necessity arose to characterize the state of nitrogen brain metabolism in the development of extracerebral tumours.

MATERIALS AND METHODS

The investigations were carried out on white rats with inoculated sarcomas (C — 45, M — 1, novocaine-synestrol sarcoma) (Konoplev 1960, Ukolova 1960). The inoculation was done hypodermically with 50 per cent tumour cells suspension in physiological saline; 1 ml of suspended matter was injected.

Inoculated sarcomas develop during 28 — 30 days and by that time they reach a weight of 30 — 40 g. The biochemical studies were carried out on the 7th, 14th, 21th, 30th day after transplantation.

Ammonia and glutamine were determined by Selingthon's method (1951) in Silakova's modification (1968). Amino acids determination was carried out by descending paper chromatography (Paschina 1961) in combination with electrophoresis after Dose (1951). Urea was determined by the urease method with ammonium.

In the brain of animals with sarcoma C-45, besides the above mentioned parameters, the activity of enzymes of synthesis and degradation of glutamine was studied. These were: glutamine synthetase, determined according to Elliot (1964), glutaminase I according to Krebs (1935), glutamic-acidic aminotransferase according to Richterich (1958), in Fry's (1945) and Speck's (1955) modification.

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Protein and nonprotein nitrogen was determined according to the micromethod of Kjeldal. Brain protein binding with urea and amino--acids was also studied (Elliot 1964). To characterize brain protein properties, the degree of their amidation was determined (Gershenovich et al. 1960) as well as the content of guanidine radicals. The latter was determined after protein hydrolysis in 2N HCl and 85% formic acid for 11 hours at 100°. The amount of arginine was determined after Sakaguchi (1925).

Ammonia, glutamic acid and glutamine in the brain of rats with tumours

Ammonia content in the brain of control rats is 0.34 ± 0.04, of glutamine- 3,4 ± 0.24, of glutamic acid- 10.1 ± 1.0 μmoles per 1 g of tissue.

The growth of inoculated extracerebral sarcomas is accompanied by changes in ammonia content in the brain. During the first week of sarcoma C-45 growth, the amount of ammonia in the brain decreases. From the beginning of the second up to the fourth week of its growth, the amount of ammonia increases (Table 1).

It should be noted that the content of ammonia in the brain during sarcoma growth does not correspond, to our knowledge, with the correlation of excitation and inhibition of the nervous system and ammonia level. Brain ammonia increases at the stages of maximal development of the tumour growth process (3 — 4 weeks of growth) in conditions of observable exhaustion of the nervous system (Latmanizova 1956). It may be considered that brain ammonia concentration is correlated with the level of metabolic activity more than with the functional state of tissue.

Glutamine concentration in the brain decreases in the first two weeks of sarcoma C-45 growth, while during the third and fourth week it increases considerably.

A similar picture of ammonia and glutamine dynamics in the brain is observed in the growth of inoculated sarcoma M-1. In our experiments this tumour grew for three weeks and in the periods of its maximal growth (second and third weeks) the amounts of ammonia and glutamine increased simultaneously.

The dynamics of ammonia and glutamine in the rat brain manifests less definitely in the growth of inoculated novocaine-synestrol sarcoma. The first weeks of growth were accompanied by a considerable increase of the ammonia content in the brain. The peak of ammonia concentration fell to the third week of growth of the sarcoma. The amount of glutamine decreased in the third week. Towards the end of tumour growth the glutamine content increases. In the development of novocaine-synestrol

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Table 1. Brain ammonia-glutamine system in the process of growth of inoculated extracerebral sarcomas (in μmoles per 1 g of tissue M ± m)

Period of tumour growth	Sarcoma — 45			Sarcoma M-1			Novocaine-synestrol sarcoma		
	ammonia	glutamine	glutamic acid	ammonia	glutamine	glutamic acid	ammonia	glutamine	glutamic acid
1 week	0.2±0.03 (10)	3.26±0.32	9.08±0.7	0.41±0.1 (10)	4.77±0.5	9.2±1.0	0.47±0.05 (10)	5.7±0.46	12.0±0.8
2 weeks	0.8±0.14 (10)	2.49±0.3	9.4±0.2	0.43±0.27 (10)	5.5 ±0.47	10.3±0.75	0.4 ±0.08 (10)	3.6±0.44	3.4±0.3
3 weeks	0.7±0.04 (10)	5.7 ±0.5	11.8±1.0	0.8 ±0.06 (13)	6.1 ±0.6	8.9±0.8	0.77±0.06 (10)	3.0±0.3	11.8±1.4
4 weeks	0.9±0.07 (11)	4.4 ±0.3	10.3±0.5	—	—	—	0.48±0.08 (10)	4.6±0.3	10.9±0.3

In parantheses — number of animals examined.

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sarcoma, the decrease of glutamic acid concentration takes place only during the second week of growth. In other periods the amount of glutamic acid in the ammonia-glutamic system of the brain differed only slightly from normal. In certain periods of the growth of sarcoma C-45 and M-1 the tendency to a decrease of the amount of glutamic acid in the brain was observed.

The ammonia level in the brain is maintained in a very complex way and manifested by the glutamine level and by the activity of enzymes taking part in its binding and liberation. Evidently, the process of glutamine synthesis, not decomposition, predominates in the brain when a tumour is growing. It was necessary to confirm this supposition experimentally; therefore we studied the activity of glutaminase I and II and glutamine-synthetase.

Glutamine-metabolizing enzymes activity (Table 2)

Table 2. Activity of glutamine metabolizing enzymes in the brain of rats with tumours M ± m

Period of growth	Glutaminase 1 (in μmoles of nitrogen)	Glutaminase 2 (in μmoles of nitrogen)	Glutamine synthetase (in mg of glutamylhydroxamic acid)
Control	22.8±1.2 (11)	10.0±0.8 (7)	21.3±0.8 (10)
1 week	24.3±1.36 (12)	11.1±1.4 (8)	20.4±1.3 (10)
2 weeks	25.6±1.9 (7)	11.1±1.25 (8)	23.4±1.1 (10)
3 weeks	25.8±1.2 (14)	12.1±0.9 (10)	25.1±0.9 (10)
4 weeks	27.4±2.38 (12)	18.1±2.9 (9)	26.6±0.9 (8)

In parantheses — number of animals examined

Tumour development does not lead to a noticeable activation of glutaminase I ($P<0.5$), what suggests, that the role of this enzyme in ammonia formation during the tumour growth is quite insignificant. Glutaminase II activity in the brain increases with the growth of sarcoma reaching its maximum by the fourth week (36% increase).

Glutamine-synthetase activity in rat brain during the growth of sarcoma C-45 increases, and by the fourth week reaches 22%.

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Thus, the peculiar pathogenetic participation of the enzymes under investigation in the brain metabolism of rats with tumours has been established. Their activity in the brain concerns ammonia binding, which may explain the relatively low level of ammonia and the higher level of glutamine in the brain of rats with tumours.

Protein and non-protein brain nitrogen in rats with sarcoma C-45

Starting from the suggestion that ammonia may play a regulatory role in the process of protein biosynthesis, we studied the characteristics of protein metabolism in the brain of an organism with an extracerebral malignant tumour. For this reason protein and nonprotein nitrogen and acid proteinase activity were investigated (Table 3).

Table 3. Protein nitrogen and non-protein nitrogen and acid proteinase activity in the brain of rats with tumours M ± m

Period of tumour growth	Protein nitrogen (in mg of nitrogen/100 mg wet tissue)	Nonprotein nitrogen (in mg of nitrogen/ 100 mg wet tissue)	Proteolytic activity (in µg of tyrosine/ 100 mg wet tissue)
Control	1300±119 (15)	187± 7.6 (15)	2.25±0.04 (15)
1 week	900± 77 (12)	70.5± 3.4 (12)	1.18±0.11 (13)
2 weeks	813± 68 (15)	197± 4.6 (15)	1.24±0.1 (15)
3 weeks	880±108 (14)	238± 6 (14)	2.25±0.16 (14)
4 weeks	837± 39 (7)	259±13.6 (7)	2.9 ±0.2 (9)

In parantheses — number of animals examined

When the inoculated sarcoma develops in an organism, the amount of protein nitrogen in the brain decreases progressively.

Within the first week of tumour growth the amount of non-protein nitrogen decreases sharply. However, its amount increases during further development.

The data obtained reveal discoordination of the processes of protein synthesis and degradation in the brain of rats with tumours.

The intensity of proteolysis in the brain is a marker of the trend of the process. In the brain of normal animals the intensity is 2.25 mg of tyrosine per 100 grams of wet tissue. Although, initially tumour growth in an organism depresses the proteolytic activity in the brain, in the further course of tumour growth the proteolytic activity increases. Products of degradation amount to 2.9 µg of tyrosine per 100 mg wet tissue.

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When investigating the liver and spleen proteinases activity, intensification of proteolysis was also revealed (Shvedova, Firsova 1967).

Activation of proteinases may cause this intensification. Our data concerning the increase of brain cystine concentration serve as base for such an assertion (Table 6). An increase of substrate accessibility, i.e. the change of brain proteins properties, is also possible.

We have determined some functional groups which may provide information about the state of brain proteins of rats with tumours.

Amide and guanidine groups of brain proteins in rats with inoculated extracerebral sarcomas (Table 4)

Table 4. Amide groups in brain proteins of rats with tumours (μ moles nitrogen/g dry protein M ± m)

Periods of tumour growth	Total	Amide groups		
		fast-bound	easily hydrolyzable	strongly bound hydrolyzable
Control	1141.9±30.0	593.0±23.9	548.8±20.1	0.93
1 week	978.0±16.5	521.2±14.4	456.9± 8.0	0.88
2 weeks	860.0±17.4	554.2±22.3	311.9±16.1	0.56
3 weeks	725.5±22.8	470.5±20.0	254.9±11.9	0.54
4 weeks	953.1±11.5	671.3±13.8	286.8± 9.0	0.43

Growth of sarcoma in the rat organism causes changes in the correlation of labile and strongly bound amide groups in brain proteins. The number of total amide groups in proteins becomes less. At the same time the number of fast-bound amide groups increases while the number of easily hydrolyzable amide groups decreases.

Changes in amidation are caused either by deamidation of proteins or by modification of their amino acid content. The latter is shown by the increase of the arginine amount in proteins (Table 5).

Table 5. Arginine level in rat brain proteins during the period of tumour growth

Period of growth	Arginine in water soluble proteins (mg/g protein, M ± m)	Arginine in total protein (mg/g protein, M ± m
Control	62.3±0.99	68.3±1.96
1 week	67.1±1.6	71.4±2.3
2 weeks	72.2±2.0	75.5±1.2
3 weeks	77.3±2.4	78.2±1.7
4 weeks	81.3±3.0	79.5±4.8

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The changes in the properties and in amino-acid content of brain proteins are followed by changes of the degree of binding of proteins with urea and amino acids.

Free and bound amino acids and urea in the brain in the process of sarcoma C-45 growth

Among the amino acids under investigation, the level of glutamic acid, aspertic acid and of gamma aminobutyric acid appeared to undergo the most evident changes (Table 6).

Table 6. Free amino acids and urea in brains of rats with tumours M ± m

Indexes	Control (13)	Periods of tumour growth			
		1 week (16)	2 weeks (21)	3 weeks (10)	4 weeks (10)
Glutamic acid	8.1 ±0.15	6.4 ±0.4	6.0 ±0.15	6.1 ±0.6	7.2 ±0.2
Aspartic acid	3.3 ±0.2	3.3 ±0.14	3.4 ±0.15	2.9 ±0.2	2.7 ±0.15
GABA	2.1 ±0.06	2.1 ±0.08	1.05±0.04	1.2 ±0.06	1.3 ±0.09
Basic amino acids	0.21±0.02	0.16±0.01	0.14±0.01	0.13±0.01	0.14±0.02
Glutamine	4.0 ±0.2	4.7 ±0.3	5.2 ±0.06	5.8 ±0.17	6.1 ±0.2
Cystine + cysteine	0.81±0.07	0.95±0.07	0.91±0.08	0.9 ±0.09	1.06±0.05
Serine	1.86±0.1	2.5 ±0.1	1.78±0.1	2.1 ±0.17	1.77±0.16
Glycine	2.23±0.2	2.6 ±0.14	2.5 ±0.2	1.9 ±0.2	2.0 ±0.09
Methionine + valine	0.13±0.01	0.13±0.01	0.14±0.01	0.15±0.01	0.13±0.04
Leucine + isoleucine	0.14±0.01	0.14±0.01	0.13±0.02	0.15±0.01	0.09±0.01
Urea	4.98±0.29	5.7 ±0.25	5.1 ±0.39	6.03±0.3	6.7 ±0.4

In parantheses — number of animals examined

Already at the initial stages of tumour growth in rat brain the amount of glutamic acid becomes less. This low level remains low during the whole period of sarcoma growth. Glutamic acid is involved in glutamine synthesis and owing to this it takes part in the partial removal of ammonia. On the other hand, it is possible that glutamic acid subjected to oxidative deamination (Cometiani 1954, Takagaki et al. 1957) is the source of ammonia in the conditions of tumour growth and is involved in energetic cycles in the brain. Active periods of tumour growth in an organism are accompanied by a decrease of aspertic acid, GABA, as well as of glycine and basic amino acids in the brain.

The dynamics of the investigated amino acids in the brain of rats with tumours suggest their very complicated and deep disturbances and modifications, which influence the functional brain activity in the pathological state.

We have ascertained that the concentration of free urea in the brain increases with tumour growth.

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The depth of brain proteins damage can be determined by the degree of complexation of amino acids and urea with brain proteins. The amount of brain bound urea and amino acids decreases as the tumour progresses (Table 7).

Table 7. Bound amino acids and urea in the brain of rats with tumours (in mg/g, M ± m

Index	Control	Periods of growth			
		1 week	2 weeks	3 weeks	4 weeks
Glutamic acid	1.6 ±0.27	1.4 ±0.15	1.5 ±0.14	1.2 ±0.1	1.2 ±0.09
Aspartic acid	0.57±0.07	0.62±0.06	0.6 ±0.03	0.44±0.07	0.39±0.06
GABA	0.46±0.03	0.44±0.04	0.66±0.07	0.34±0.04	0.32±0.04
Urea	0.58±0.06	0.62±0.04	0.63±0.06	0.46±0.02	0.43±0.04

Tumour growth in an organism influences the functional brain activity by modifing the protein structure and properties.

Thus, the development of inoculated sarcoma leads to deep disturbances of the most important aspects of brain metabolism. The data suggest, that in this case the brain develops a complex of protective and accomodative reactions with considerable signs of damage.

The low molecular weight metabolites of nitrogen metabolism seem to act in the direction of the protection of brain functional activity, this being manifested in particular by the intensification of ammonia-binding processes.

The state of the protein structure of animals with tumours, the total decrease of energetic processes show the prevalence of catabolic processes in the nerve tissue and damage of the brain protein structure, which correspond to exhaustion of brain functional activity in terminal periods of tumour growth in the organism.

The discovered impairment of nitrogen metabolism and changes in the functional structure of brain proteins and their biological properties are pathogenetic mechanisms of brain participation in the development of tumour processes in the organism.

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METABOLIZM AZOTOWY W MÓZGACH SZCZURÓW Z WSZCZEPIONYMI MIĘSAKAMI POZAMÓZGOWYMI

Streszczenie

W mózgach szczurów z podskórnymi mięsakami C-45, M-1, względnie mięsakami indukowanymi nowokaino-synestrolem, oznaczano poziom azotu białkowego i niebiałkowego oraz niektórych związków azotowych, a ponadto aktywność niektórych enzymów biorących udział w syntezie i degradacji glutaminy.

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We wszystkich przypadkach stwierdzano znaczne zmiany w poziomie amoniaku, glutaminy i kwasu glutaminowego, którym towarzyszył stały spadek zawartości azotu białkowego i grup amidowych w białkach oraz wzrost aktywności glutaminazy II, syntetazy glutaminowej oraz enzymów proteolitycznych. Powyższe wyniki świadczą o ogólnym zaburzeniu metabolizmu białkowego mózgu szczurów z nowotworami pozamózgowymi.

А. В. Архангельская, | З. С. Гершенович | А. Крычевская

АЗОТНЫЙ МЕТАБОЛИЗМ В МОЗГАХ КРЫС С ТРАНСПЛАНТИРОВАННЫМИ ВНЕМОЗГОВЫМИ САРКОМАМИ

Резюме

В мозгах крыс с подкожными саркомами С-45, М-I или саркома индуцированной новокаин-синестролом, определялся уровень белкового и не-белкового азота и некоторых соединений азота, и кроме того некоторых энзимов, участвующих в синтезе и разложении глютамина. Во всех случаях были обнаружены значительные изменения в уровне аммиака, глютамина и глютаминовой кислоты, которые сопровождались постоянным снижением содержания белкового азота и амидных групп в белках и увеличением активности глютаминазы II, глютаминовой синтезы и протолитических энзимов. Выше указанные результаты свидетельствуют об общем нарушении белкового метаболизма у крыс с внемозговыми опухолями мозга.

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EXPERIMENTAL STUDIES ON THE TIME COURSE OF NECROBIOSIS IN HUMAN GLIOMAS

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As well known, the ischemia tolerance time differs from one organ to the other. In relation thereto, the malignant tumours are able to survive for a very long time under full lack of oxygen and of externally introduced feeding substances (Warburg et al. 1958).

In a series of publications Maker et al. (1967) and especially Kirsch (1965, 1967), Kirsch and Leitner (1967a, 1967b) and Kirsch et al. (1967, 1969) have reported upon results of their studies on normal brain and glial tumours under ischemic conditions. It was shown with microchemical methods that while the brain is reacting with an extraordinarily strong anaerobic glycolysis, leading within 30 minutes to the exhaustion of all energy-rich metabolites, the tumours show a moderate production of lactate at 37° continuing for at least 4 hours. In this respect, the experimental and human gliomas exhibit no essential differences.

On this basis the possibility of the successful in vitro cultivation of fresh autopsy materials of several human brain tumours (Manuelidis 1965) is finding its explanation. Moreover it is intelligible, that the histochemically demonstrable activity of some dehydrogenase enzymes and diaphorases does not show differences between biopsy and fresh autopsy material (Chason et al. 1963). Of course, this may be essentially influenced by the outer temperature.

While the metabolism of gliomas under „warm" ischemia is already well examined up to 4 hours, data on the moment of the exhaustion of the energy supplying processes are absent. The aim of the following study was to define this moment more approximately, using the enzyme-histochemical methods. As to the selection of the enzymes to be tested, it is to consider, that in several experimental models of infarction, among these also in that of the brain, the loss of activity of cytochrome oxidase, of different dehydrogenases, or diaphorases and of β-glucuronidase in general occurs a long time before that of the phosphate-splitting enzymes

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and of esterases (Gössner 1955, Wachstein, Meisel 1957, Rudolph, Scholl 1958, MacDonald, Spector 1963, Seifert 1967 and others). Therefore it seems more reasonable to favour those enzymes, which loose their activity very quickly. We decided for lactate dehydrogenase (LDH method after Hess et al. 1958), which is present in gliomas in high concentration in connection with the intensive lactate formation (Viale 1969). The other enzymes chosen were: mitochondrium-bound succinic dehydrogenase (SDH method after Nachlass et al. 1957), the β-glucuronidase (β-GU) which is localized preferentially at the lysosomes (method after Seligman et al. 1954) and the acid phosphatase (method after Barka and Anderson 1962).

We proceeded in the following manner: From fresh biopsy material *) of 4 astrocytomas and 4 glioblastomas, solid tissue pieces weighing about 250 mg were taken with sterile instruments and placed in a prewarmed medium. For each time interwal, 2 samples were deposited: one for kryostat-sections to accomplish the enzyme-histochemical reactions, and one for paraffin-embedding, so that there were up to 14 tissue blocks per case. In addition to that, another sample was immediately frozen in carbon dioxide. Two tissue pieces were incubated in 20 ml of medium at 37° in closed 100 ml-Erlenmeyer-flasks for different time intervals up to 8 days. The medium applied was that which has been used for tissue culture**) (lactalbumin and yeast containing Hanks-solution with 30% heat inactivated cow serum and 10% cow amnion; pH — indicator phenol-red; addition of penicillin and streptomycin). Since Erlenmeyer-flasks were not moved up to the end of the experiment, tissue samples did not suffer from perfusion. In addition, samples of tumors for routine diagnosis were taken.

The application of a feeding medium gives a more thorough moistening, better sterilization and preservation of shape as compared with the „moist chamber". Moreover, this gives the adventage, that a peripheral zone of the tissue block is held under approximately the same conditions as in tissue culture. So in addition we receive data on the vitality and width of this border zone, which is important for autoradiographic studies *in vitro*, and those of a beneath arising extended necrosis and a gradual decrease in supply of nutritive substances under these unfavourable conditions. Moreover, we see the advantage to compare the expected loss of enzymes in the centre with that in the periphery, which facilitates the judgment of the sections.

*) We are very grateful to Prof. Dr R. A. Frowein (Neurochirurgische Universitätsklinik Köln) for making biopsy tissue available to us.

**) We thank Dr. H. J. Sehrbundt (at present Hals-Nasen-Ohren-Klinik der Universität Köln) for his kindness, that we are alowed to make use of the laboratory of tissue culture.

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In the first figure we see an example. On the left side after an incubation time of 2.5 hours, we find an activity of LDH spread over the whole section. On the right side the reaction after 20 hours is shown. A narrow edge of about 500 μ thickness shows nearly the same enzyme activity as the fresh tumour. Abruptly it passes over into an area with entire loss of the enzyme. Here only a few deeply lying vessels are discernable by their still clear enzymic activity.

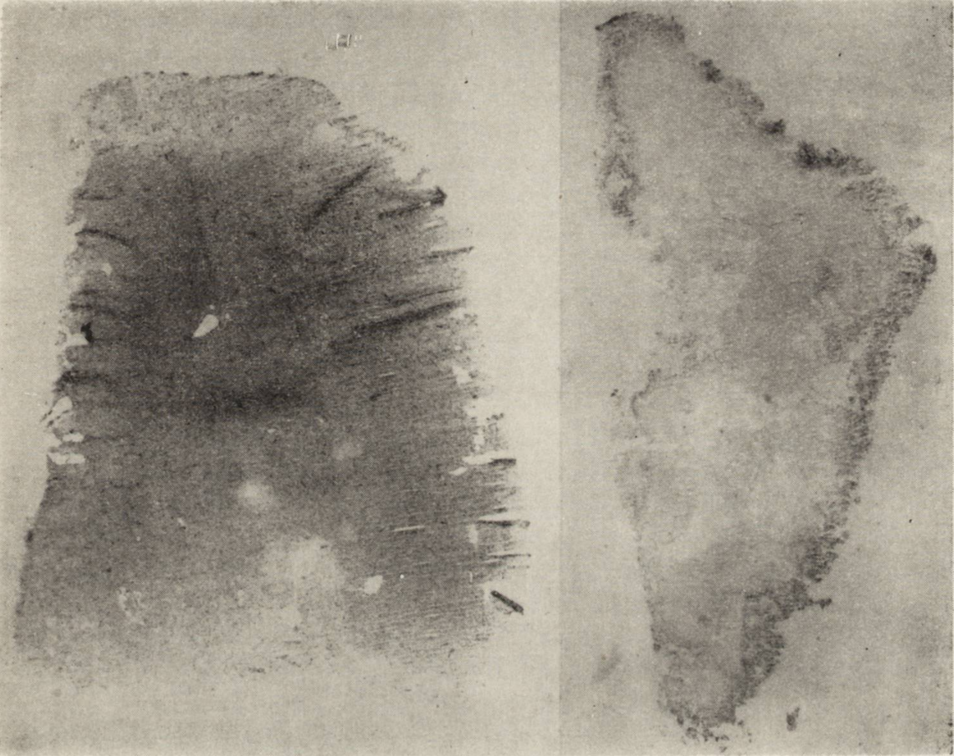

Fig. 1. Lactate dehydrogenase reaction after 2.5 (left) and 20 hours (right) of incubation.

In an attempt to arrange the respective enzyme activity in a half quantitative scheme (Fig. 2) the following time course may be established for the LDH. On the abscissa there is a logarithmic time scale, the ordinate gives the valuation. In the periphery (the filled symbols) the enzyme activity is preserved for a long time and is markedly diminished not earlier than at a time, at which it changes also in the tissue culture not fed with a fresh medium for a long time. As this enzyme is water soluble (Gahan, Kalina 1965, Kalina, Gahan 1965, Osske, Jänisch 1967) and passes over into the culture medium already in the first day (Gahl, Hartmann 1967), one must assume, that in this case protein synthesis

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takes place, which stationarily replaces this enzyme. In the centre of the autolysing block (the open symbols) the first loss of activity occurs already after 5 hours. After 20 hours we can not demonstrate the enzyme activity any more. The behaviour of astrocytomas and glioblastomas is identical.

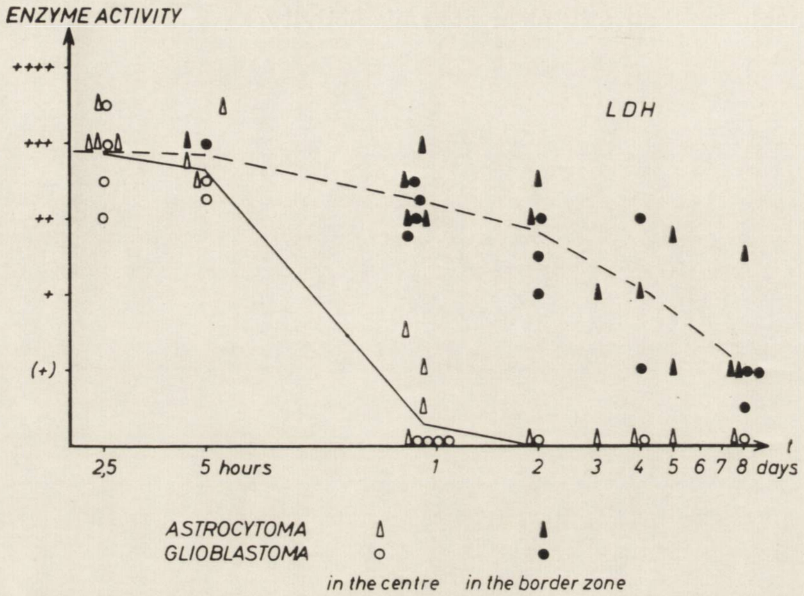

Fig. 2. Lactate dehydrogenase, activity in relation to the incubation time in the peripheral and central parts of the tissue block.

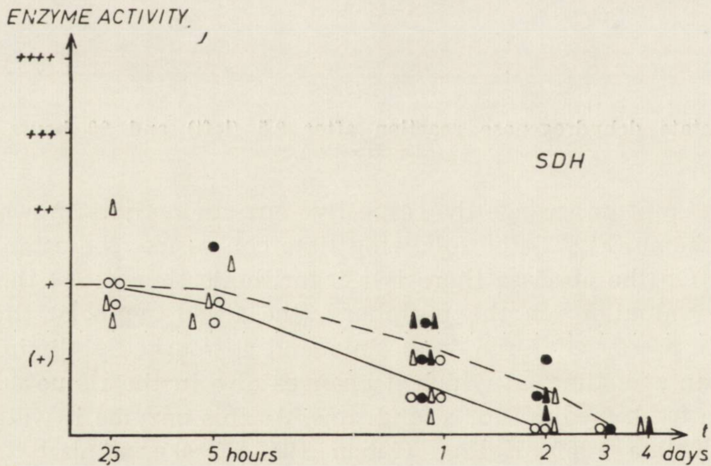

Fig. 3. Succinic dehydrogenase activity in relation to the incubation time in the pheripheral and central parts of the tissue block. Symbole as in Fig. 2.

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Also the SDH (Fig. 3) shows a similarly decreasing activity in the central parts. But its reaction in the border zone diminishes quicker than that of the LDH. This may indicate, that in this case, the energy requirements are satisfied more by anaerobic performances of the metabolism — in contrast to the situation in tissue culture, where the decrease of SDH is observed for the first time after a long cultivation (Zimmermann et al. 1965, Kreutzberg et al. 1966).

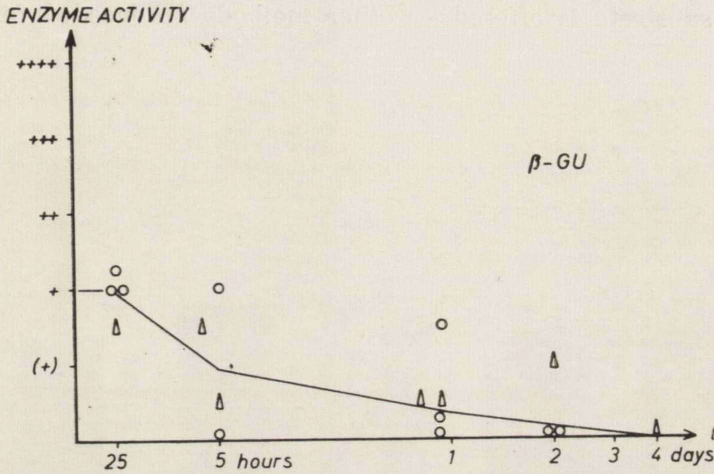

Fig. 4. β-glucuronidase activity in relation to the incubation time in the peripheral and central parts of the tissue block. The same symbols for these two zones, because they show equal density of the reaction product. Symbols as in Fig. 2.

By contrast, the β-glucuronidase (Fig. 4) offers no distinct reactions in the peripheral and central parts. Its activity becomes declined considerably after 5 hours of incubation. The likewise prevailing lysosomal acid phosphatase remains unchanged for 8 days of incubation.

Further it is to mention that after 2 days, a storage of neutral lipids (by colouring with Sudan black B) is appearing in the deep layers at the border zone. The depth of this still preserved zone diminished from 500 to 300 μ after 4 days. Of course we can not find pseudopalisading of the tumour cells. Also the increased activity of the dehydrogenases, hydrolases and acid phosphatase (Głuszcz 1963, Hanefeld 1965, 1967, Schiffer et al. 1965, 1969: Iwanowski 1967, and others), which is very characteristic of a linear necrosis of fresh tumour material in the solid tumour tissue, is absent. Probably for the rise of this typical tissue formation, a better oxygen supply is needed.

The first morphological signs of autolysis in the centre of the pieces were discernable after 4 days in the form of increased frequency of pycnotic pictures of nuclei.

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Under ischemic conditions, LDH and SDH show after 5 hours a small and β-glucuronidase an even stronger decrease of their effect, whereas after 20 hours an almost complete loss of activity of all three enzymes was noted. So we had to search in this time interval for the end of the arranged function of these tumour cells.

As we do not know exactly for how long time the single enzyme may be active after the stop of the regulated cell metabolism — when the adequate substrate is offered — other methods are needed for confirmation.

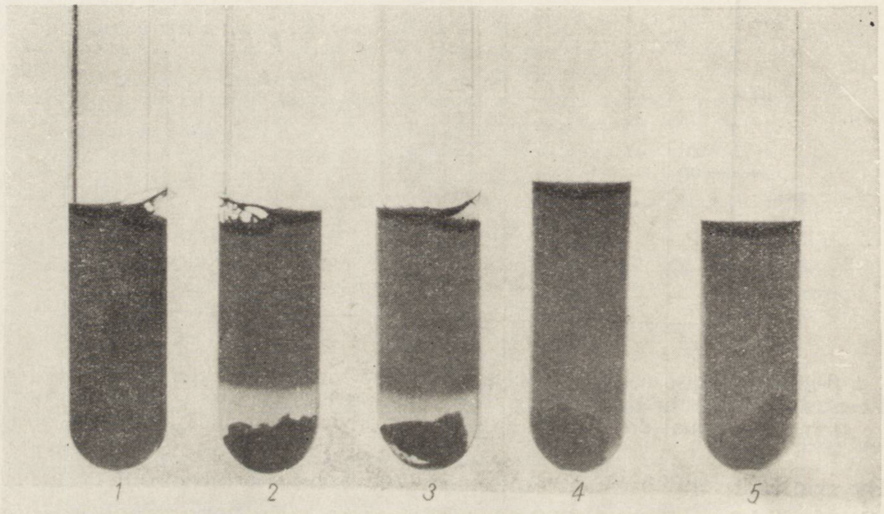

Fig. 5. Colour change of the pH indicator in the neighbourhood of the tumour pieces (tubes 2 and 3) in comparison with its absence in the tubes 4 and 5 with necrotic tumour material. Tube 1 as a control.

The lactate, which is produced in large quantities in the growing tissue under ischemic conditions, causes in the surrounding medium (Fig. 5), which contains phenol-red as pH-indicator, in tubes 2 and 3 at pH of 6.8 a colour change from red to yellow with a well defined boundary layer, providing that the tubes are not moved.

The first tube, where no tumour tissue is present, serves for the colour comparison. The tubes 4 and 5 contain necrotic material from the same case of a glioblastoma. It exhibits no lactate production (see also Kirsch et al. 1967). In the tumour-containing tubes, the colour change layer becomes higher gradually. The sharpness of this layer is preserved, which means that the concentration gradient remains equal. Therefore

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this displacement may not exclusively be reduced to a simple equal distribution by diffusion of a definite quantity of acid valences, but may express their continuous production by the tumour piece.

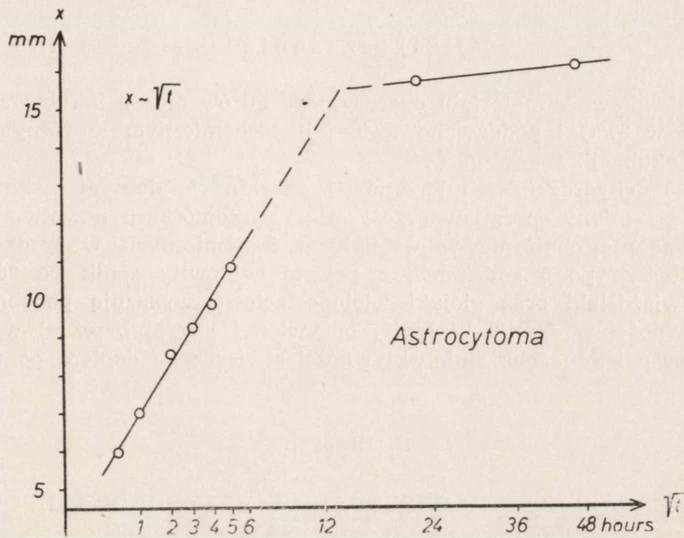

Fig. 6. The diffusion length of the acid valences, marked by the colour change boundary of phenol-red, in relation to the square root of incubation time.

Figure 6 presents dependence of the height of this colour change boundary (the ordinate) or the diffusion length on the time, which is plotted as square root value corresponding to the diffusion laws. So by this representation we could expect a rectilinear relationship. This figure is an example for a number of experiments, all passing the same way without difference for astrocytomas and glioblastomas. A quick rise in the first 6 hours is relieved by a small or sometimes absent progress after a certain period of time. Thereby it is striking, that now the colour change takes place in a wider growing zone. We like to assume, that in this phase the lactate production is succumbed. The cut of the 2 lines in the figure yields for the time at nearly 12 hours.

As here a certain time delay may play a role we can estimate the duration of ischemia at 37°, which leads to the decay of the function in these glioma cells and to the beginning of the autolysis, by 5—10 hours. The drop in activity of LDH, SDH and β-glucuronidase may reflect the early changes in a good temporary agreement, whereby especially the LDH facilitates the judgment of the functional situation because of the high initial intensity.

We wish to thank especially Miss Renate Kott and Miss Marianne Lehnen for their accurate technical assistance.

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R. Schröder

BADANIA DOŚWIADCZALNE NAD CZASOWYM PRZEBIEGIEM NEKROBIOZY W GLEJAKACH U LUDZI

Streszczenie

Wycinki tkankowe z materiału operacyjnego guzów mózgu inkubowano w płynnej pożywce w 37°C i poddawano testom histochemicznym w różnym czasie od rozpoczęcia inkubacji.

Aktywność dehydrogenazy mleczanowej okazała się dobrym wskaźnikiem intensywności przemiany energii w przeżywających komórkach nowotworowych, wykazując nieznaczne obniżenie w warunkach ischemicznych w środkowej części wycinków dopiero po 5 godzinach, a prawie całkowity zanik po 20 godzinach inkubacji. Gwiaździaki oraz glejaki wielopostaciowe wykazują podobny przebieg zaniku aktywności w czasie. W lepiej odżywionej strefie brzeżnej wycinków do głębokości 500 μ istotne obniżenie aktywności stwierdzano dopiero po 4—8 dniach.

Р. Шредер

ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ ВРЕМЕННОГО ПРОТЕКАНИЯ НЕКРОБИОЗА В ГЛИОМАХ У ЛЮДЕЙ

Резюме

Тканевые срезы из операционного материала инкубировали на жидкой среде в 37°Ц и подвергали гистохимическим тестом в разное время после начала инкубации.

Активность лактатдегидрогеназы оказалась хорошим показателем интенсивности обмена энергии в нативных опухолевых клетках, обнаруживая незначительное снижение в исхемических условиях в средней части срезов только спустя 5 часов и почти полное исчезновение после 20 часов инкубирования. Астроцитомы и полиморфные глиомы обнаруживают аналогичный ход исчезновения активности во времени. В лучше питаемой краевой зоне срезов на глубине до 500 мк существенное снижение активности обнаруживалось только спустя 4—8 дней.

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NEUROPAT. POL. 1972, X, 2

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CHANGES OF LACTIC DEHYDROGENASES ISOENZYMES IN TISSUE CULTURES OF HUMAN BRAIN TUMOURS

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The transformation of the LDH isoenzymes in various tissues, tumours and their tissue cultures has been investigated by Philip et al. (1962), Nitowski et al. (1964), who concluded, that during tissue cultivation a progressive loss of the H isoenzymes and an increase of the M isoenzymes occur. The alterations seemed to occur irrespective of whether the cells originated from adult or embryonic, normal or malignant, human or animal tissue. The influence of higher oxygen tension on the increase of H isoenzymes has been also established (Dawson et al. 1964, Lindy et al. 1966, Güttler et al. 1969). The isoenzymatic changes are considered mostly as a sign of dedifferentiation. The in vitro environment fails to maintain the controlling factors which are present in the intact organism and the complex structural and biochemical interactions are lost (Nitowski et al. 1964, Langvad 1970).

The purpose of the present work was to establish the transformation of LDH isoenzymes of brain tumours of different malignity in short term cell cultures. It was known from previous investigations (Gerhardt et al. 1963, Wollemann et al. 1965, 1971) that LDH isoenzymes changed parallely on the malignancy of gliomas: loss of H isoenzymes, characteristic for the normal human brain was observed in all tumours. The tumours of mesodermal origin such as meningiomas and neurinomas showed also a predominance of M-izoenzymes. The isoenzyme changes were localized not only in the supernatant but also in the mitochondrial and nuclear cell fractions, so was the increase the total LDH activity (Nagy et al. 1971).

MATERIAL AND METHODS

The fresh brain and tumour samples were obtained at the time of surgical intervention. One half was kept for histological and biochemical studies, the other part was dissected into 1—2 mm pieces aseptically and

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cultured in Falcon plastic bottles, the bottoms of which were coated with a mixture of hen plasma and chicken embryo extract in equal parts. After the fixation of tumour explants on the bottom of culture flasks, 20 ml cultivation medium was added to each bottle; this consisted of 80% TC-199 medium and 20% calf serum. Cell migration started after 1—3 days of explantation. The tumours were kept in the bottles until the cells were grown over the whole bottom of the flask, this lasted from 14 to 32 days depending on the character of the tumour. Medium was changed three times a week. The cells were removed with 0.25% trypsin solution from the bottom of the bottles, and after several times washing with Tyrode solution they were spinned and dissolved in distilled water for the disc electrophoretic separation of LDH isoenzymes. Parallel to the above tumour cell cultures, on cover glasses placed in Leighton tubes, were grown for histochemical purposes. Histochemical LDH activity measurements according to Hess et al. (1958) were performed in the cells of the growing zone developed around the explants. Fifteen tumour and 1 normal brain samples were examined. The following brain tumours were studied: glioblastoma multiforme (8); malignant astrocytoma (2); protoplasmic astrocytoma (1); metastatic carcinoma (1); endothelial meningioma (3). The normal brain sample was taken from the left temporal lobe, resected from a patient with an aneurysm.

Tissue samples for biochemical investigation were immediately cooled at 0°C, homogenized in 4.5 vol. 0.25 M sucrose, and cellular fractionation was performed according to Brody and Bain (1952). The cell fractions were submitted to disc electrophoresis. Two tenth of ml was applied in 20% sucrose or Sephadex G 200 and run at 5°C for one hour in parallels, and one of each samples was stained for protein according to the method of Davis (1964). In order to solubilize the enzymes of the mitochondrial fractions, these were treated with Triton-X 100 in 1% end concentration. The LDH isoenzymes were stained according to the method of Dewey et al. (1960). Protein was stained with Naphthalene Black 10 B in gels. Scanning of the isoenzymes was performed on a Joyce Chromoscan.

RESULTS

Three of eight investigated glioblastomas did not show any change in the number of LDH isoenzymes after cell cultivation (No 83, 148, 149). In three cases the number of M isoenzymes increased (No 79, 175, 197) and in two tumours the number of bands decreased (No 212, 258). In four of the glioblastomas the most intensively stained isoenzymes were the same in the original tumour homogenates as in the cultivated samples. In five cases band 3 (H_2M_2), and in three tumours band 4 (HM_3) were

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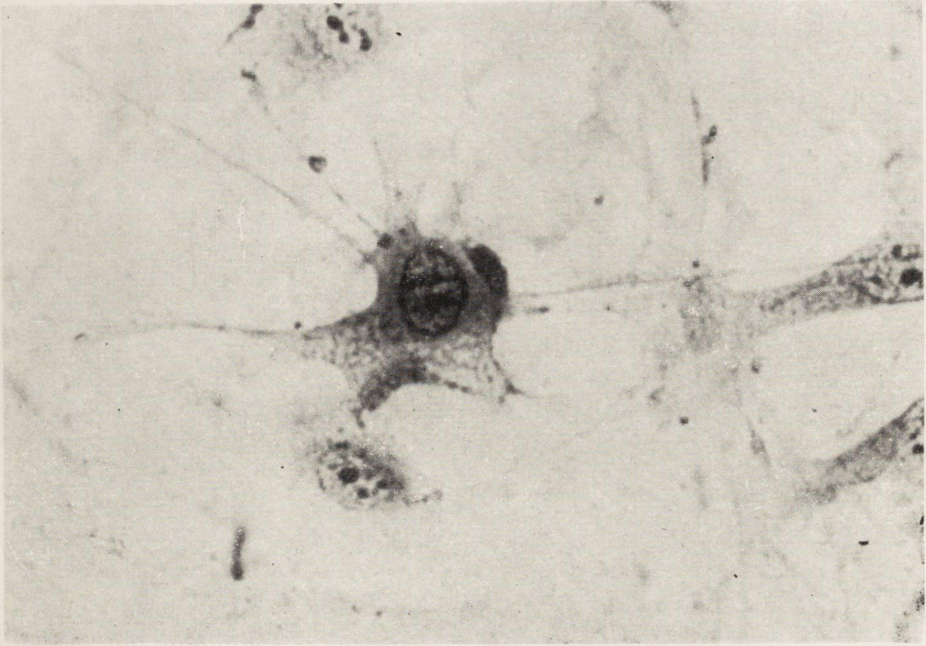

Fig. 5. Histochemical staining for LDH of cell culture of endothelial meningioma No. 144. Note positive reaction in arachnoideal cell and negative staining in the epithelial cells.

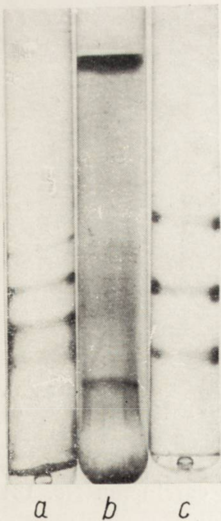

Fig. 6. Glioblastoma multiforme No. 149 before (a) and after 15 days of cell cultivation (b, c) stained for LDH isoenzymes (a, c) and for protein fractions (b). Note unchanged LDH pattern and relatively high gamma-globulin fraction.

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Fig. 7. Histochemical staining for LDH of cell culture of glioblastoma multiforme No. 149. Note positive reaction in a piloid astrocyte and negative staining in the other cells.

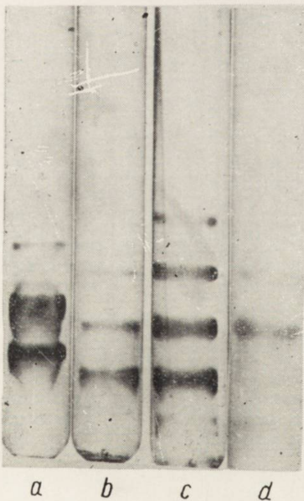

Fig. 8

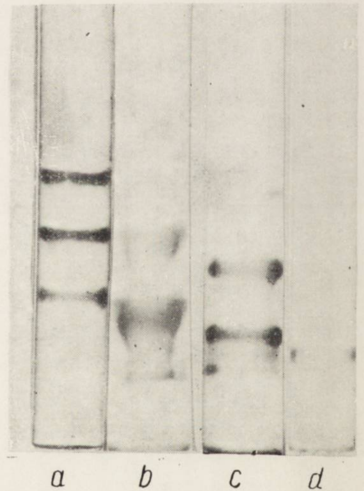

Fig. 9

Fig. 8. Glioblastoma multiforme No. 175. LDH isoenzyme pattern from nuclear (a), mitochondrial (b) and supernatant (c) cell fractions before and after 32 days of cell cultivation (d).

Fig. 9. LDH isoenzyme patterns from astrocytoma protoplasmicum No. 161, before (a) and after (b) 27 days of cell cultivation and from metastatic carcinoma No. 184 before (c) and after (d) 14 days of cell cultivation.

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Table 1. Distribution of LDH isoenzymes in human brain and brain tumours before and after cell cultivation

Sample	Before					After					Time of cultivation
	H_4	H_3M	H_2M_2	HM_3	M_4	H_4	H_3M	H_2M_2	HM_3	M_4	
Temporal lobe No. 104	30	32	28	10	—	100	—	—	—	—	32 days
Glioblastoma multiforme No. 79	11	20	32	37	—	9	18	22	43	8	21 days
Glioblastoma mf. No. 83	30	32	32	6	—	8	29	36	27	—	32 days
Glioblastoma mf. No. 148	8	10	38	44	—	10	12	36	42	—	20 days
Glioblastoma mf. No. 149	28	35	37	—	—	30	36	34	—	—	25 days
Glioblastoma mf. No. 175	18	22	30	36	—	10	36	26	23	5	32 days
Glioblastoma mf. No. 197	22	32	46	—	—	—	18	38	42	22	21 days
Glioblastoma mf. No. 212	12	24	28	26	10	—	25	75	—	—	23 days
Glioblastoma mf. No. 258	20	28	34	18	—	—	4	32	64	—	19 days
Astrocytoma mal. No. 54	36	30	30	4	—	28	32	32	8	—	29 days
Astrocytoma mal. No. 81	21	27	31	20	—	6	20	28	34	12	23 days
Astrocytoma prot. No. 161	38	38	24	—	—	8	34	42	16	—	27 days
Meningioma end. No. 80	13	16	25	34	12	8	12	26	41	13	23 days
Meningioma end. mal. No. 144	14	26	38	22	—	—	10	90	—	—	20 days
Meningioma end. No. 261	18	32	50	—	—	26	44	26	4	—	22 days
Carcinoma met. No. 184	—	8	42	50	—	—	6	12	82	—	14 days

Figures represent percentage contribution of each band to the total activity.

1964). The LDH pattern in cell cultures is changed by several factors, such as elevation of oxygen or CO_2 tension (Goodfriend et al. 1966), or addition of substrate, inhibitors and hormones (Cahn 1963, 1964). Infection of tissue cultures with the oncogenic adenovirus 12, causes the culture to develop the malignant type of pattern long before control uninfected cultures (Latner et al. 1965). Polyovirus infected cultures did not differ from the controls. We attribute also a possible role to the presence of oncogenic viruses in induction the M subunits in our malignant tumours and cell cultures. This view is strengthened by the appearance of other new multiple forms of enzymes in brain tumours and their cell cultures. This concern such enzymes as creatine-phosphokinase and malate dehydrogenase, among them, the M type creatine-phosphokinase which is not present in normal adult and embryonic brain (Róna et al. 1971, Wollemann et al. 1971).

Schapira et al. (1968), showed in rat hepatomas, that normal liver M LDH changes toward H LDH, this being similar to the embryonic liver tissue. They concluded therefore that cancerous isoenzymic modi-

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fications reflect tissue dedifferentiation rather that a shift to a glycolytic metabolism.

Our experiments prove a tumour dependent metabolism, which is different from the adult, as well as from the embryonic type.

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ZMIANY W IZOENZYMACH DEHYDROGENAZY MLECZANOWEJ LUDZKICH GUZÓW MÓZGU W WARUNKACH HODOWLI TKANEK

Streszczenie

W krótko trwających hodowlach tkanki guzów mózgu obserwuje się, zależnie od rodzaju guza, przesunięcie wzorca dehydrogenazy mleczanowej w kierunku izoenzymów M. Prawidłowy mózg i guzy łagodne, w materiale krótko hodowanym, wykazują inny wzorzec.

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ИЗМЕНЕНИЯ В ИЗОЭНЗИМАХ ЛАКТАТДЕГИДРОГЕНАЗЫ ИЗ ЧЕЛОВЕЧЕСКИХ ОПУХОЛЕЙ МОЗГА В УСЛОВИЯХ ТКАНЕВОЙ КУЛЬТУРЫ

Резюме

В короткосрочных культурах ткани опухолей мозга наблюдается, в зависимости от вида опухоли, сдвиг образца лактатдегидрогеназы в направлении изоэнзимов М. Нормальный мозг и доброкачественные опухоли обнаруживают другой образец в коротко культивированном материале.

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NEUROPAT. POL. 1972, X, 2

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GAMMA-AMINOBUTYRATE-ALPHA-OXOGLUTARATE TRANSAMINASE (GABA-T) ACTIVITY OF HUMAN BRAIN TUMOURS OF A VARIOUS DEGREE OF ANAPLASIA

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The gamma-aminobutyric acid (GABA) is considered as a natural inhibitory transmitter of the nervous system. The subcellular localization of GABA system components in the synaptic endings proves GABA to act as a transmitter. The activity of GABA was shown to mimic the inhibitory transmitter both in producing the membrane hyperpolarization and in increasing the membrane conductance for Cl^-ions (Eccles 1965, Florey 1967, Krawitz 1968, Krnjevic 1970, Roberts et al. 1970, Sytinsky 1970).

The intimate contact between glial cells and synapses supports the concept that the neuroglial tissue may modulate the control of the central synaptic transmission. But the role of the neuroglia in the metabolism of GABA remains poorly clarified.

We have also relatively scarse data concerning the content and distribution of GABA and enzymes of its metabolism in the human brain tumours both of neuroectodermal and mesodermal origine. It was shown that GABA content was apparently decreased in various types of brain tumours. The gliomas have the GABA content much lower then the neuroglia from which they are deriving. It was also found that the activity of glutamate decarboxylase (GAD) which catalyzes the formation of GABA from L-glutamic acid was not found in the malignant tumours (Müller, Langeman 1962, Wollemann, Dévényl 1963, Promyslov, Andreeva 1969, Promyslov et al. 1970, Sytinsky et al. 1965, 1968, Sytinsky 1969). The information about a new type of GAD occuring in the glial cells and human brain gliomas has been obtained in recent investigation carried out in the laboratory of Roberts (Haber et al. 1970).

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The data on the GABA-T activity (E.C.2.6.1.19), the enzyme catalyzing the reversible transamination of GABA with alpha-oxoglutarate which is the first step in the main pathway of the utilization of GABA in the Krebs cycle showed a considerable variation of values in the human brain tumour tissue (Waksman, Faienza 1960, Sytinsky 1969). However, the exact data concerning the GABA-T activity of human brain neuro-ectodermal and meningovascular tumours at variable degree of the anaplasia are not available so far.

The main aim of the present study was to obtain information concerning the GABA-T activity in human brain tumours (astrocytomas and meningiomas) at a variable degree of anaplasia.

MATERIAL AND METHODS

Samples of each brain tumour were examined histologically immediately after the surgical intervention. Tumours were classified depending on the degree of the tissue anaplasia as typical, dedifferentiated and malignant ones after Chominsky's classification (1969). Samples of the normal brain tissue derived from the patients killed in accidents without the cranium damage were taken as controls. In these cases GABA-T activity of the normal human brain tissue was measured 12 hours after the death. Sometimes the brain tissue adjoining to the tumour, removed at the time of operation was also examined for the sake of a control comparison. The tissue samples (0.5 g) were homogenized in the ice-cold 0.25 M tris-buffer (2.8 ml) of pH 8.6. The colorimetric method of determination of the GABA-T activity is based on the interaction of 3-methyl-2-benzthiazolone-2-hydrazone (MBTH) with succinic semialdehyde (SSA) which appears during the enzymatic reaction (Vasiljev, Eremin 1968). The incubation mixture consisted of 0.5 ml of 0.25 M tris-buffer of pH 8.6 and 0.5 ml of each of the solutions containing 40 μmoles of alpha-oxoglutaric and gamma-aminobutyric acids and the brain homogenate. The final volume was adjusted to 1.5 ml. After the incubation which was performed for 1 hr at 37°C the enzymatic reaction was stopped by adding 0.5 ml of 20% trichloroacetic acid which was also added to the control samples before the incubation. After the precipitation of proteins the samples were centrifuged. Fifty μl of the deproteinized incubation medium containing 0.02 — 0.05 μmoles of SSA were then added to 0.5 ml of the 1% MBTH solution. The samples were heated for 3 min. in a boiling-water bath and cooled down to 18—20°C. Thereafter 1 ml of the 0.25% ferric chloride ($FeCl_3$ of pH 2.0) was added to each sample followed in 5 min. by the addition of 4 ml of acetone. After shaking the concentration of

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SSA in the samples was determined with the photoelectrocolorimeter, model FEK-M 56. The activity of GABA-T was expressed as micromoles of SSA formed per g of the fresh tissue per hour. The data obtained were examined statistically by the t-test (Fisher 1958).

RESULTS AND DISCUSSION

The data concerning the GABA-T activity in normal human brain tissue are shown in Table 1. The comparison of the GABA-T activity

Table 1. GABA-T activity in normal human brain tissue

μmoles SSA/g/hr	
postmortem brain tissue	biopsy brain tissue
36.0	22.8
32.0	26.0
37.9	25.5
38.0	37.1
	32.0
	23.4
Mean ± S.E.M. 31.1±1.95	(22.8—38.0)

found in the samples of human and rabbit brain tissue determined by the same method revealed in both cases similar values (27.0 μmoles SSA/g/hr the rabbit brain and 31.1 μmoles SSA/g/hr — human brain).

It was also found that the postmortem changes in the brain tissue exerted influence on the GABA-T activity. On the other hand, it has been stated that the brain tissue adjoing to the tumour and removed during the operation can not serve as to an exact control, sample for the normal brain tissue. However, it is rather difficult to explain the difference between the GABA-T activity of the postmortem and biopsy brain tissue.

The data on the GABA-T activity in the human brain tumours are shown in Table 2 and Figure 1. The analysis of the data of the GABA-T activity in the tumour tissue of the human brain is rather difficult. The activity of the enzyme in a typical astrocytoma was increased by 23%. These changes in the GABA-T activity as compared with the normal ones are statistically significant ($p < 0.01$). The activity of this enzyme was in some samples of the typical tumours 1.5 times higher than that in the adjoining normal brain tissue. However, the GABA-T activity of the dedifferential astrocytoma was at the same level as in normal

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tissue. The degree of the tumour tissue anaplasia without any doubt influences the GABA-T activity, which was lower in the malignant tumours (malignant ependymoma and meningeal sarcoma) than in the normal brain tissue. Thus it is possible to come to the conclusion that the GABA-T activity is decreased in accordance with the degree of the anaplasia and the defferentiation process. The figures shown in the

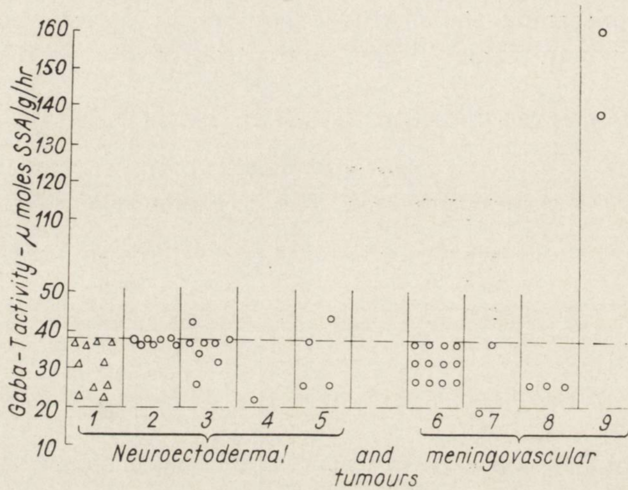

Fig. 1. The GABA-T activity of human brain tumours. *1* — control, *2* — typical astrocytoma, *3* — dedifferentiated astrocytoma, *4* — malignant ependymoma, *5* — neurinoma, *6* — typical meningioma, *7* — dedifferentiated meningioma, *8* — malignant meningioma, *9* — angioreticuloma.

Table 2. GABA-T activity of human brain tumours

Type of tumour	Number of investigations	μmoles SSA/g/hr	
		mean ± S.E.M.	range
neuroectodermal tumours:			
typical astrocytoma	7	38.3±0.02 p<0.01	37.1—38.7
dedifferentiated astrocytoma	8	31.4±2.3	26.0—43.0
malignant ependymoma	1	21.8	
neurinoma	4	33.3±4.26	26.0—44.2
meningovascular tumours:			
typical meningioma	11	32.1±1.4	25.5—37.1
dedifferentiated meningioma	2	27.7	18.4—37.1
malignant meningioma (meningeal sarcoma)	3	25.5±0.14 p<0.05	25.5—26.0
angioreticuloma	2	149.0	115.0 ; 160.0

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Table 2 illustrates the great variety of values of the GABA-T activity. The two cases of the vascular tumour (angioreticuloma) show the highest activity of GABA-T.

Our attempt to demonstrate quantitative differences between the GABA-T activity of the normal and neoplastic brain tissues doesn't allow us to come to definite conclusions. The data of Promyslov and Andreeva (1969) indicated that the process of transamination of GABA to alpha-oxoglutarate occurs approximately 4 times slowlier in all types of the brain tumours than in the normal brain tissue. However, in their opinion, the GABA-T activity doesn't depend on the degree of the tumour tissue anaplasia. On the other hand the GAD activity was not found in malignant tumour samples (Promyslov, Andreeva 1969, Sytinsky 1969). Only recently a new type of GAD has been obtained from the human brain gliomas (Haber et al. 1970). It is obvious that the morphological variety of glial cells corresponds to their neurochemical character. Apparently the GABA-T activity reflects only to a considerable extent the existence of the oxidative metabolism in the brain tumours.

Obviously, the peculiarities of the brain tumours and thereby the loss of the specific metabolic functions of the neuroglial cells explain the disappearance of GABA with its functional role in the activity of the central nervous system. In this case GABA is only the substrate used up in the processes of energy consumption in tumour cells with metabolic acceleration of the intermediate products of glycolysis occuring under the influence of GABA.

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AKTYWNOŚĆ GAMMA-AMINOMAŚLAN-ALFA-OKSOGLUTARAN-AMINO-TRANSFERAZY (GABA-T) W GUZACH MÓZGU O RÓŻNYM STOPNIU ANAPLAZJI

Streszczenie

Celem badań tej pracy było ustalenie aktywności GABA-T w różnego stopnia anaplazji guzach mózgu człowieka. Aktywność enzymu oznaczano przy pomocy kolorymetrycznej metody, opierającej się na reakcji kondensacji powstającego w przebiegu enzymatycznej reakcji półaldehydu bursztynowego z 3-metylo-2-benzotiazyl-2-hydrazonem (metoda własna).

Stopień anaplazji guzów określano według klasyfikacji Chomińskiego, szeregując guzy do trzech grup typowych, odróżnicowanych i złośliwych. Aktywność GABA-T wykazywała znaczne zróżnicowanie nawet w tych samych typach guzów. Autorzy postulują, że stopień anaplazji nowotworu wywiera decydujący wpływ na aktywność enzymatyczną, która obniża się w nowotworach złośliwych. GABA w nowotworowej tkance przestaje odgrywać charakterystyczną dla układu nerwowego rolę mediatora hamowania, a pozostaje tylko substratem w przemianie materii.

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АКТИВНОСТЬ ГАММА-АМИНОБУТИРАТ-АЛЬФА-ОКСОГЛУТАРАТ-АМИНО-ТРАНСФЕРАЗЫ (GABA-T) В ОПУХОЛЯХ МОЗГА РАЗЛИЧНОЙ СТЕПЕНИ АНАПЛАЗИИ

Резюме

Цель настоящего исследования состояла в установлении активности ГАМК-Т в опухолях различной степени анаплазии мозга человека. Активность фермента определяли разработанным в нашей лаборатории колориметрическим методом, который основан на реакции конденсации образующегося в ходе ферментативной реакции янтарного полуальдегида с 3-метил-2-бензотиазолон-2-гидрозоном. Степень анаплазии опухолей определяли согласно классификации Хоминского — типические, дедиффенциальные и злокачественные. Величина активности ГАМК-Т имеет весьма большой размах колебаний даже при определении в одних и тех же типах опухолей. По-видимому, степень катаплазии оказывает влияние на ферментативную активность, которая снижается в злокачественных типах опухолей головного мозга. ГАМК в опухолевой ткани головного мозга теряет свою специфическую роль медиатора торможения в нервной системе и является лишь субстратом в обмене веществ.

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NEUROPAT. POL. 1972, X, 2

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VERGLEICHENDE ASPEKTE BEZÜGLICH AKTIVITÄT UND ISOENZYMMUSTER DER LACTATDEHYDROGENASE BEI SPONTANEN SOWIE EXPERIMENTELLEN HIRNGLIOMEN *)

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Lactatdehydrogenase (LDH, EC 1.1.1.27) stellt als Katalysator der Hin- und Rückreaktion (Oxydation-Reduction) zwischen Lactat und Pyruvat in Gegenwart von Nicotinamidadenindinucleotid ein wichtiges Enzym der Glycolyse dar.

Warburg (1926) konnte nachweisen, dass der Energiebedarf des Tumorgewebes hauptsächlich durch anaerobe Glycolyse gedeckt wird, wodurch sich das neoplastische Gewebe vom normalen unterscheidet. Folgerichtig ist die Untersuchung des Verhaltens der LDH und ihrer Isoenzyme bei Prä- bzw. Neoplasien von besonderer Bedeutung. Dies stellt nämlich eine Möglichkeit dar, die gestörten bzw. vorherrschenden Stoffwechselsysteme während der Tumorentwicklung mit den morphologisch fassbaren Gewebsveränderungen in Beziehung zu setzen.

Unser Interesse gilt der vergleichenden Betrachtung der Verteilung der LDH-Isoenzyme bei Hirngliomen des Menschen (100) einerseits und bei vergleichbaren spontanen (Hund 8) sowie experimentell mittels Methylnitrosoharnstoffs induzierten Hirngliomen verschiedener Tierspecies (Kaninchen, Ratte 250) andererseits. Insbesondere sollte geprüft werden, inwieweit die verschiedenen Tumorgruppen auch durch ein unterschiedliches Isoenzymmuster gekennzeichnet sind und inwiefern innerhalb einer Tumorgruppe Verschiebungen im LDH-Isoenzymmuster auftreten, die sich mit den morphologisch fassbaren Malignitätsmerkmalen korrelieren lassen.

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Hinsichtlich des Vorkommens bzw. der Verteilung der LDH sowie ihrer Isoenzyme bei vergleichbaren spontanen und experimentellen Hirngliomen wurden keine grundsätzlichen Unterschiede festgestellt. Im folgenden wird daher auf die bei den einzelnen Tumorgruppen gemachten Beobachtungen gemeinsam näher eingegangen.

1. Oligodendrogliom I, II: Bei allen untersuchten isomorphen (Grad I) wie pleomorphen (Grad II) Oligodendrogliomen wurde eine positive LDH-Reaktion festgestellt. Die LDH-Aktivität ist bei den isomorphen Oligodendrogliomen gering bis mässig, wobei die gebildeten Formazangranula gleichmässig über das ganze Tumorgewebe verteilt sind (enzymatische Isochromie). Bei pleomorphen Oligodendrogliomen ist eine, zumindest gebietsweise, erhöhte LDH-Aktivität zu beobachten (Abb. 1 a, b, f und 2 a, c).

Hinsichtlich des Verhaltens der LDH-Isoenzyme ist festzustellen, dass sowohl bei den isomorphen als auch bei den pleomorphen Oligodendrogliomen im allgemeinen alle fünf LDH-Banden nachweisbar sind. Zwischen den beiden Dignitätsgraden treten jedoch Unterschiede in der prozentualen Verteilung der einzelnen LDH-Fraktionen auf. Während bei isomorphen Oligodendrogliomen die anodischen Banden die kathodischen überwiegen, wird bei den zur Pleomorphie neigenden Tumoren eine Rechtsverschiebung in den Pherogrammen deutlich (Abb. 1 g und 2 d). Das LDH-Pherogramm isomorpher Oligodendrogliome ist ferner dadurch charakterisiert, dass hier die anodische Fraktion LDH_1 die höchste prozentuale Aktivität aufweist.

2. Astrocytome I, II: Im Vergleich zu den Oligodendrogliomen sind die Astrocytome durch eine intensivere LDH-Reaktion gekennzeichnet. Das Reaktionsprodukt ist nicht nur, wie bei den Oligodendrogliomen in den Perikarya, sondern auch in den Astrocytenfortsätzen lokalisiert (Abb. 1 c, d, e). Länge und Anzahl der LDH-aktiven Astrocytenfortsätzen sind unterschiedlich. Während namlich bei isomorphen Astrocytomen zahlreiche, lange Fortsätze — die manchmal bis zur Capillarinserierung hin verfolgt werden könenn — vorkommen, lassen sich bei den zur Pleomorphie neigenden Astrocytomen (Abb. 2 b) wenige plumpe und kurze Fortsätze erkennen. Zwischen den Zellen isomorpher Astrocytome sind kaum Aktivitätsunterschiede zu beobachten. Bei den pleomorphen Tumorbezirken lassen sich jedoch starke zellindividuelle Aktivitätsschwankungen nachweisen.

Die Verteilung der LDH-Isoenzyme bei den astrocytären Tumoren ist in der Regel ähnlich jener bei den Oligodendrogliomen (Abb. 1 g). Die höchste prozentuale Isoenzymaktivität bei den isomorphen Astrocytomen weisen die anodischen LDH-Fraktionen auf, wobei das Aktivitätsgefälle

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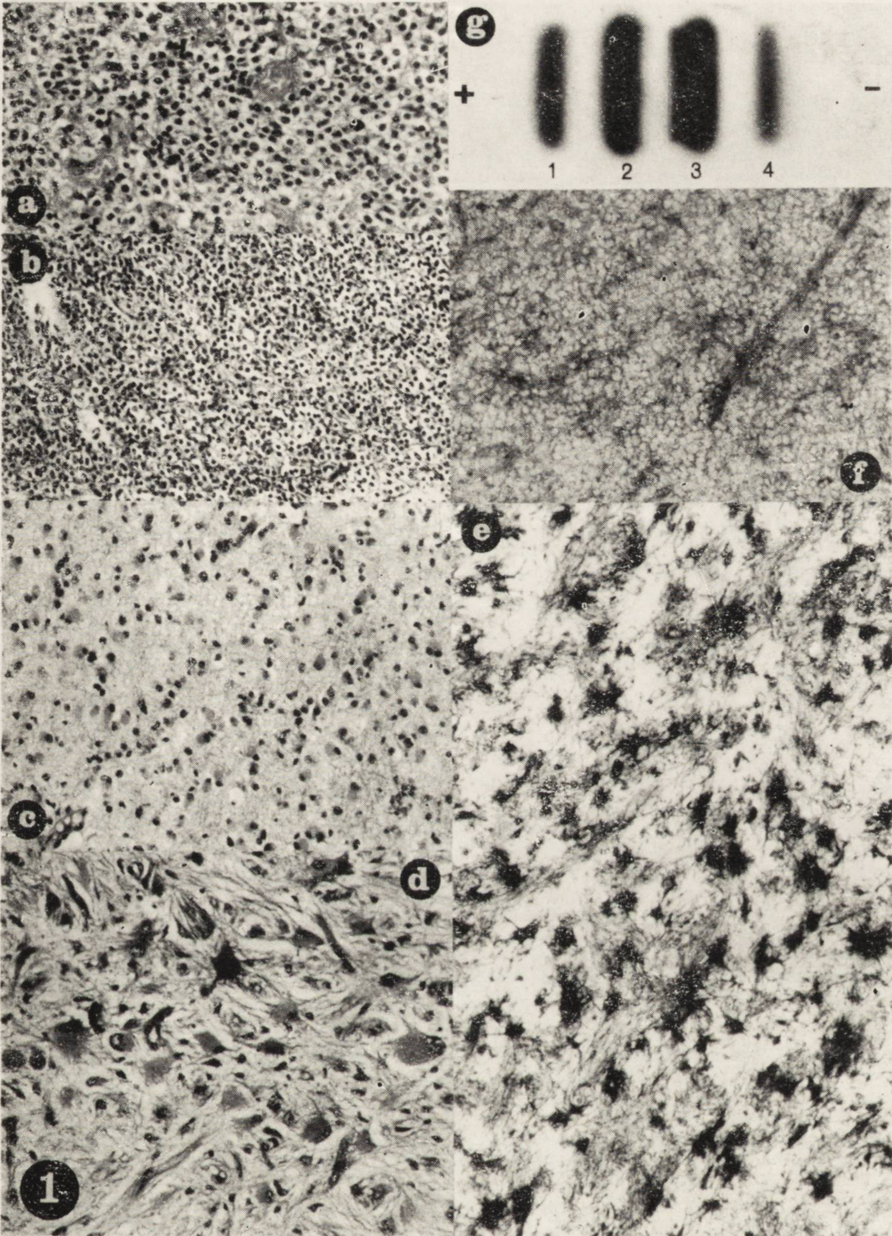

Abb. 1. Spontanes (Mensch, a) bzw. experimentelles (Ratte, b) Oligodendrogliom I mit typischen „Honigwaben"-Architekturen. Spontanes (Mensch) protoplasmatisches (c) bzw. fibrilläres (d) Astrocytom I. Gleichmäßig verteilte LDH-Aktivität (enzymatische Isochromie) sowohl bei Oligodendrogliom I (f) als auch bei Astrocytom I (e). Typische Verteilung der LDH-Isoenzyme (g) bei dem isomorphen Astrocytom (c) mit überwiegend anodischen LDH-Fraktionen. (a, d HE-Färbg. 150x; b, c NISSL-Färbg. 120x; f bzw. e LDH 120x bzw. 150x).

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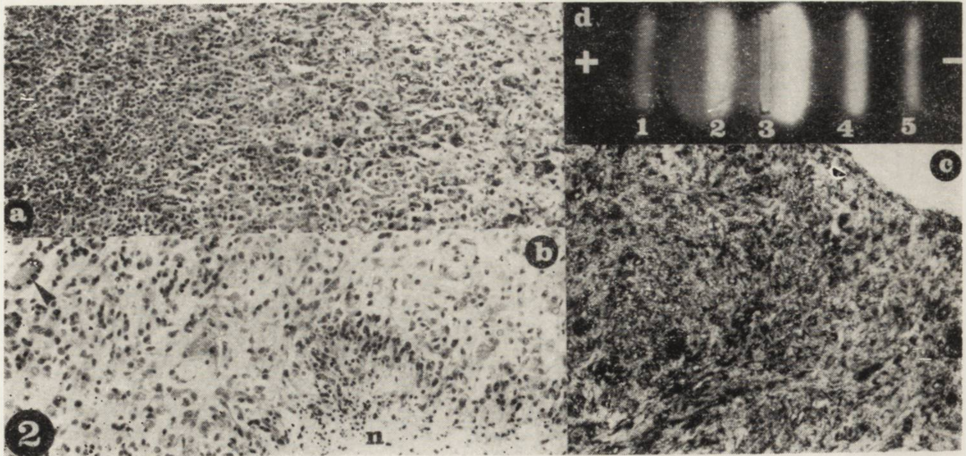

Abb. 2. Experimentelles Oligodendrogliom II (Ratte, a) und Astrocytom II (Ratte, b). Deutliche LDH-Aktivitätssteigerung bei einem experimentellen Oligodendrogliom II (Ratte, c) im Vergleich zu Oligodendrogliom I (1 f). Rechtsverschiebung im LDH-Pherogramm eines spontanen Oligodendroglioms II (Mensch, d). (a, b NISSL-Färbg. 120x; c LDH 120x).

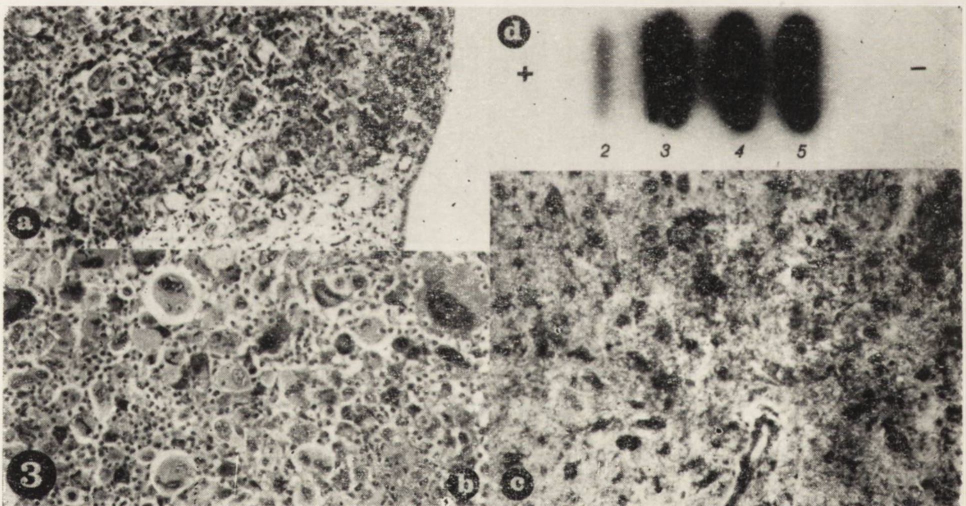

Abb. 3 Experimentelles (Kaninchen, a) bzw. spontanes (Mensch, b) Glioblastom mit Monsterzellen und zahlreichen Mitosen. Ausgeprägte ungleichmäßige Verteilung der LDH-Aktivität (enzymatische Anisochromie) bei einem spontanen Glioblastom (c). Verteilung der LDH-Isoenzyme (d) bei dem Glioblastom der Abb. 3 b mit überwiegend kathodischen LDH-Fraktionen. (a NISSL-Färbg. 120x; b HE-Färbg. 150x; c LDH 120x).

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zwischen der LDH_1 und den übrigen anodischen Banden nicht so stark ist wie bei den Oligodendrogliomen. Bei den Astrocytomen des Malignitätsgrades II ist eine deutliche Abnahme der Aktivität der anodischen zugunsten der kathodischen LDH-Fraktionen festzustellen.

3. Pleomorphe Gliome bzw. Glioblastome: Bei diesem Tumortyp lassen sich bei der LDH-Reaktion die stärksten zellindividuellen Aktivitätsunterschiede nachweisen (enzymatische Anisochromie). Monsterzellen sowie riesenzellähnliche Bildungen weisen die intensivste Reaktion auf (Abb. 3 a, b, c). Im Cytoplasma von Monsterzellen lassen sich oft umschriebene, kernnalhiegende Bezirke nachweisen, die durch eine starke Formazanbildung charakterisiert sind und von einem formazangranulaarmen Gürtel umgeben werden.

Das LDH-Isoenzymmuster der pleomorphen Gliome bzw. Glioblastome unterscheidet sich wesentlich von jenem der Oligodendrogliome und Astrocytome. Für die Glioblastome ist in der Regel die Abnahme der Aktivität der ersten LDH-Fraktionen (LDH_{1-2}), die von einer entsprechenden Aktivitätszunahme der übrigen Banden (LDH_{3-5}) begleitet wird, typisch (Abb. 3 d). Die Aktivität der anodischen LDH-Fraktionen ist oft so gering, dass in den Pherogrammen nicht auswertbare Spuren oder überhaupt keine Aktivität nachgewiesen werden kann (Abb. 3 d). Letzteres gilt besonder für die experimentellen Gliome, bei denen oft nur die kathodischen LDH-Isoenzyme nachgewiesen werden können.

Gleichwohl die anodischen LDH-Fraktionen bei isomorphen Oligodendrogliomen und Astrocytomen die kathodischen überwiegen, scheint dennoch zwischen den beiden Tumortypen ein unterschiedliches Verhalten der anodischen Banden vorzuliegen. Während nämlich bei isomorphen Oligodendrogliomen LDH_1 die höchste Aktivität entfaltet, sind bei isomorphen Astrocytomen LDH_{2-3} am stärksten vertreten. Dieser Befund korreliert mit den von Victor und Wolf (1937) sowie Heller und Elliot (1955) erzielten Ergebnissen über den hohen O_2-Verbrauch der Oligodendrogliome, welchletzerer auch durch das Verhalten der Cytochromoxydase bei spontanen (Hanefeld 1965) wie bei experimentellen (Stavrou 1969b; 1970b) Oligodendrogliomen dokumentiert wird. Diese übereinstimmenden biochemischen und histochemischen Daten deuten auf die wohl unterschiedliche Stoffwechselleistung bzw. Funktion der beim Tumoraufbau beteiligten Gliazellen hin.

Aufgrund der bislang vorliegenden Befunde (Lit. bei Stavrou 1970a) liefert der topochemische Nachweis der LDH, vor allem in Verbindung mit anderen Enzymreaktionen, bei der Klassifikation sowohl spontaner als auch experimenteller Hirntumoren wertvolle Hinweise. Insbesondere stellt die Klärung der Frage des Vorliegens einer enzymatischen Isomorphie bzw. Pleomorphie (Schubert, Kreutzberg 1967) oder einer enzy-

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matischen Isochromie bzw. Anisochromie (Stavrou 1970b) eine wesentliche Voraussetzung für die Beurteilung des Differenzierungsgrades von Hirntumoren dar. Dies ist umso bedeutender, als die enzymtopochemischen Methoden auch in der Schnellschnittdiagnostik optimal eingesetzt werden können.

Die Objektivierung der Dignität von Tumoren des Nervensystems scheint mit Hilfe der quantitativen Erfassung der LDH-Isoenzyme möglich zu sein. Nach den vorliegenden Befunden und unter Berücksichtigung der Literaturangaben (Gerhardt et al. 1963, 1967; Sherwin et al. 1968; Haglid et al. 1970) überwiegen bei gut differenzierten bzw. isomorphen Hirngliomen die anodischen die kathodischen LDH-Fraktionen (vorwiegend aeroble Glycolyse). Mit fortschreitender Entdifferenzierung kommt es in den LDH-Pherogrammen zu einer entsprechenden Rechtsverschiebung. Diese Rechtsverlagerung der Isoenzymaktivität erreicht bei den pleomorphen Gliomen bzw. Glioblastomen ihr Maximum. Maligne Hirntumoren sind also dadurch charakterisiert, dass sie vor allem kathodische LDH-Isoenzyme besitzen (vorwiegend anaerobe Glycolyse).

Ähnliches Verhalten der LDH-Isoenzyme konnte auch bei experimentellen Tumoren des peripheren Nervensystems der Ratte beobachtet werden (Stavrou et al. 1970). Auffallend ist jedoch, dass bei experimentellen pleomorphen Gliomen und malignen Schwannomen oft nur Spuren oder überhaupt keine anodischen LDH-Fraktionen nachweisbar sind. Dieses Verhalten der LDH-Isoenzyme entspricht der ausgeprägten zellindividuellen enzymatischen Variabilität beim topochemischen Nachweis der LDH sowie dem bunten histologischen Bild und deutet auf die starke Entdifferenzierung experimenteller Tumoren des Nervensystems hin.

Zusammenfassend kann aufgrund der bisherigen Ergebnisse gesagt werden, dass der topochemische Nachweis der LDH, verbunden mit der elektrophoretischen Auftrennung bzw. quantitativen Erfassung ihrer Isoenzyme einen wesentlichen Fortschritt bei der „differenzierten" Hirntumordiagnostik darstellt.

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BADANIA PORÓWNAWCZE AKTYWNOŚCI I SKŁADU IZOENZYMÓW DEHYDROGENAZY MLECZANOWEJ W SPONTANICZNYCH I DOŚWIADCZALNYCH GLEJAKACH MÓZGU

Streszczenie

Porównując rozmieszczenie i występowanie dehydrogenazy mleczanowej i jej izoenzymów w ludzkich guzach mózgu oraz w spontanicznych i doświadczalnych guzach mózgu u różnych gatunków zwierząt, nie stwierdzono pomiędzy nimi żad-

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nych istotnych różnic. Glejaki izomorficzne (skąpodrzewiaki i gwiaździaki) charakteryzują się umiarkowaną, względnie wysoką aktywnością dehydrogenazy mleczanowej, rozmieszczoną regularnie w całych skrawkach z kriostatu (izochromia enzymatyczna).

W rozdziale elektroforetycznym dehydrogenazy mleczanowej w obu typach guzów frakcje anodowe przeważają nad katodowymi. W elektroforogramach z guzów o II° zróżnicowania (skąpodrzewiaki II° i gwiaździaki II°) jest widoczne zmniejszenie ilości frakcji anodowych na rzecz katodowych. Glejaki wielopostaciowe charakteryzują się dużym zróżnicowaniem w lokalizacji enzymów, odrębnym dla każdej komórki (anizochromia enzymatyczna). Elektroforogramy dehydrogenazy mleczanowej tej grupy guzów różnią się od elektroforogramów glejaków izomorficznych najwyższą aktywnością frakcji katodowych. Glejaki wielopostaciowe charakteryzują się najwyższymi poziomami aktywności izoenzymów LDH_{3-4}, w których frakcje anodowe są widoczne słabo lub wcale nie występują. Dotyczy to zwłaszcza glejaków wielopostaciowych wywołanych doświadczalnie i wiąże się z silnie zaznaczoną anaplazją tych guzów.

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СРАВНИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ АКТИВНОСТИ И СОДЕРЖАНИЯ ИЗОЭНЗИМОВ ЛАКТАТДЕГИДРАЗЫ СПОНТАННЫХ И ЭКСПЕРИМЕНТАЛЬНЫХ ГЛИОМ МОЗГА

Резюме

Сравнивая распределение и нахождение лактатдегидразы и ее изоэнзимов в человеческих опухолях мозга, а также в спонтанных и экспериментальных опухолях мозга животных не обнаружено между ними никаких существенных разниц. Изоморфные глиомы (олигодендроцитомы и астроцитомы) характеризуются умеренной, относительно высокой активностью лактатдегидразы, распространенной регулярно в целых срезах из криостата (энзиматическая изохромия).

При электрофоретическом разделении лактатдегидразы в обоих типах опухоли анодные фракции преобладают над катодными. На электрофорограммах опухолей II° диференциации (олигодендроцитомы II° и астроцитомы II°) видно уменьшение анодных фракций по сравнению с катодными фракциями. Плейоморфные глиомы и мультиформные глиобластомы характеризуются большой дифференциацией локализации энзимов, отдельной для каждой клетки (энзиматическая анизохромия). Электрофорограммы лактатдегидразы этой группы опухолей отличаются от электрофорограмм изоморфных глиом самой большой активностью катодных фракций. Плейоморфные глиомы характеризуются наивысшими уровнями активности изоэнзимов LDH_{3-4}, анодные фракции которых слабо видны или вовсе не заметны. Это касается особенно мультиформных глиобластов, вызванных экспериментально и это связано с сильно выраженной анаплазией этих опухолей.

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THE OCCURRENCE OF TRACE ELEMENTS IN CEREBRAL TUMOURS

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The structure and mineral elements constitute more than 99 per cent of the elementary composition of a human body. All other elements are found in small concentrations and thus are called trace elements; a distinction is made between essential and non-essential trace elements. Essential trace elements require only small concentrations to perform their biological functions, for example as activators, regulators, co-factors and stabilizers; therefore they are also called anorganic active substances. On the other hand, non-essential trace elements are considered as contaminators or „fellow-traveller elements", which have no physiological function (Bersin 1963; Comar, Bronner 1964; Kasperek et al. 1970; Schicha et al. 1971).

The trace element content of biological tissues may be changed principally by two reasons:

1) as a result of primary disturbed metabolism; in this case secondary effects with respect to essential trace elements may occur;

2) as a result of primary environmental factors; in this case special emphasis has to be placed on the non-essential toxic heavy metals (Hueper, Conway 1964); disturbances of metabolism may occur here as a secondary effect.

This paper reports on the trace element content of human cerebral tumours.

MATERIAL AND METHODS

From patients, who underwent operations for cerebral tumours, a total of 40 tissue samples were examined *. The samples were taken during

*) We are indebted to Prof. Dr. R. A. Frowein (Neurosurgical Clinic of the University of Cologne) for the dissection of biopsy tissue samples.

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operation; they were not formaline-fixed and did not come into contact with other substances with the exception of bi-distilled water, which was used for draining to remove blood. The dry tissue sample weights were between 50 and 400 mg.

The samples consisted of:

1) normal cerebral tissue (n=12): 6 samples of brain cortex and 6 of brain white matter;

2) benign cerebral tumours (n=16): 3 astrocytomas (grade I), 3 oligodendrogliomas (grade I), 2 spongioblastomas, 2 ependymomas and 6 neurinomas;

3) malignant cerebral tumours (n=7): 5 glioblastomas, 1 oligodendroglioma (grade II) and 1 medulloblastoma;

4) separate samples (n=5): 4 tumour necroses and 1 calcified oligodendroglioma (grade I).

The trace element content of the tissue samples was determined by neutron activation analysis (Kasperek 1969). This analytical method is based on the transformation of the naturally stable isotopes into radioactive isotopes by means of neutron irradiation. This is shown schematically in Fig. 1. The radioisotopes, obtained by neutron capture, decay with characteristic half-lifes and usually emit beta and gamma-rays. The energy spectra of the gamma-emission are measured for quantitative analysis. The radioactivities of the single radioisotopes allow computing the corresponding stable isotope contents and thus the respective element concentrations, according to the correlations shown at the bottom of Fig. 1.

The special technique used measures long-lived radioisotopes, but excludes radiochemical separation. By this method the number of elements, which may be analyzed, is limited; on the other hand, the possibility of contamination and analytical failure may practically be eliminated. The dried tissue samples were irradiated for 10 days with an average thermic neutron flux of 5×10^{13} neutrons per cm^2 and second, i.e. with a neutron dosis of 5×10^{19} neutrons per cm^2. The gamma spectra of the activated samples were registered by a germanium-lithium detector coupled to a 1600 multichannel pulse height analyzer after a decay time of 40 days. The gamma energy spectrum of an activated brain tissue sample is given in Fig. 2; the abscissa indicates the gamma energy in KeV, the ordinate the pulse rate. These complex spectra were analyzed according to standard programs using the IBM 360/75 computer.

The elements: silver (Ag), cobalt (Co), chromium (Cr), caesium (Cs), iron (Fe), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se) and zinc (Zn) were determined quantitatively. The concentrations are

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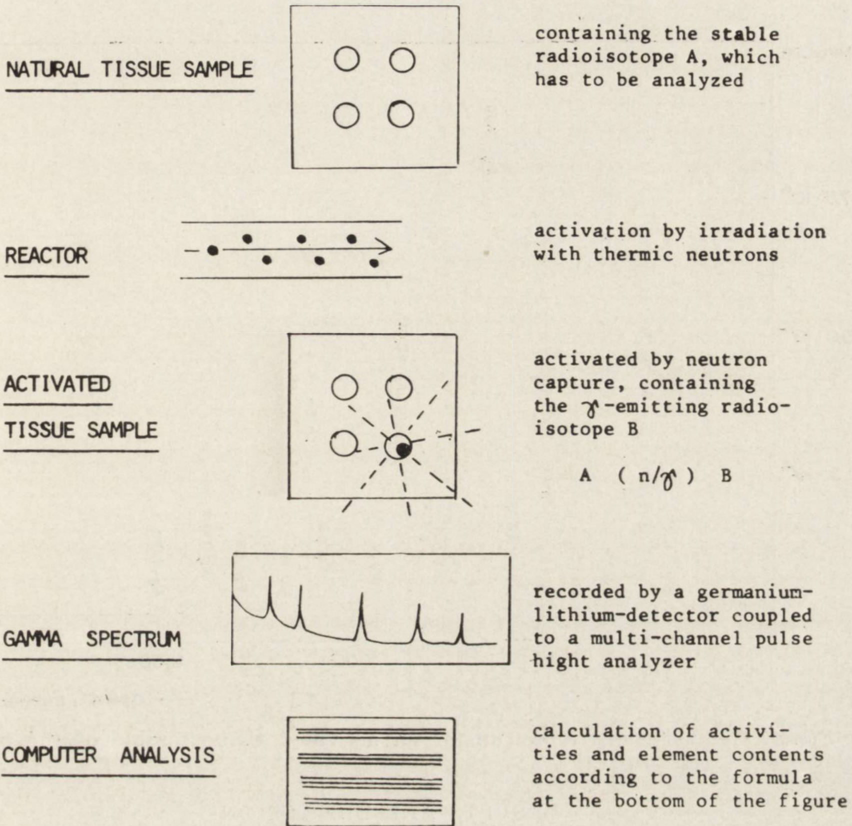
ACTIVATION ANALYSIS



$$\text{radioactivity induced} \sim N \cdot \sigma \cdot \phi \cdot (1-e^{-\lambda t_B}) \cdot (e^{-\lambda t_A})$$

N = number of atoms, which can be activated

σ = cross section of the isotope

ϕ = thermic neutron flux

t_B = irradiation time

t_A = decay time

λ = decay constant of the radioisotope, $\lambda = \ln 2 / T$

T = half-life of the radioisotope

Fig. 1. Pattern of neutron activation analysis

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related to the dry tissue weights. The relative analytical error for cobalt, iron, rubidium, selenium and zinc is on the average less than 5 percent, and for the other elements it ranges between 10 and 20 percent.

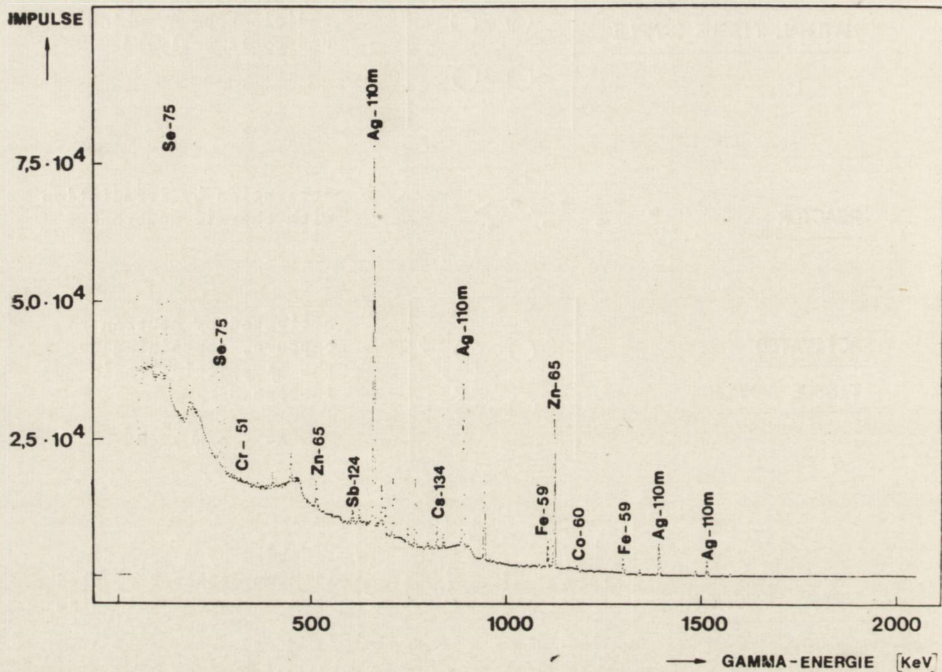

Fig. 2. Gamma-energy-spectrum of an activated tissue sample of the brain.

RESULTS AND DISCUSSION

Neutron activation analysis is at present the most sensitive and exact method for quantitatively and simultaneously determining numerous trace elements in biological tissues (Kasperek 1969). Whereas histochemical examinations do not permit really selective and quantitative statements, the activation analysis allows a qualitatively specific as well as quantitatively sufficient measurements of trace element concentrations.

The mean cerebral element contents measured are in relatively good agreement with the values of other investigators, as far as comparative values may be referred to Bersin (1963); Comar, Bronner (1964); Tipton, Cook (1963); Tipton et al. (1965). As in other biological tissues, also in the brain relatively small variations of essential trace element concentrations are found, whereas the non-essential elements partly show extremely high differences, even in adjacent samples in a single brain (Kasperek

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et al. 1970; Schicha et al. 1969; 1970; 1971; Tipton, Cook 1963; Tipton et al. 1965). Table 1 shows the variation factors V_F, the ratio maximal/minimal element content and the standard deviations in percentage of the mean (S. D.), measured in the normal brain tissue.

Table 1. Variation of essential and non-essential trace elements in normal brain tissue

		S.D. (%)	V_F
Essential trace elements	Co	44	4.1
	Fe	48	2.8
	Rb	42	3.3
	Se	15	1.6
	Zn	13	1.4
Non-essential trace elements	Ag	—	87
	Cr	—	51
	Cs	—	12
	Sb	—	80
	Sc	—	75

S.D. = Standard deviation in % of the mean
V_F = Variation factor, maximal/minimal value

Amongst these elements cobalt, iron, selenium and zinc are essential for the human being, whereas silver, caesium, antimony and scandium are probably not essential (Bersin 1963; Comar, Bronner 1964; Kasperek et al. 1970; Schicha et al. 1969; Schröder 1965). In this Table rubidium was grouped as essential and chromium as non-essential; however, both elements might possibly be essential.

The group of essential elements shows narrow standard ranges: approximately 65 to 80 percent of the single values are measured within the mean ± 30 per cent. The variation factors (V_F) average 2.6. However, as far as the non-essential elements are concerned, the single values differ extremely. The variation factors (V_F) are generally more than 50 and a statistical analysis is here of no use. Therefore, the non-essential trace elements are excluded from the following statistical considerations.

Fig. 3 shows the essential trace element content of the normal brain. The mean content of cerebral cortex is normalized to 1 (open columns) and the ± standard error is given. It is obvious, that in the cerebral white matter (shaded columns) the elements: cobalt, selenium and zinc are significantly lower than in the cortex (student t-test: $P_{Co} < 0.0025$, $P_{Se} < 0.01$, $P_{Zn} < 0.0005$), whereas iron, rubidium and the non-essential

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elements show no significant differences. The reasons for different element contents of cerebral white matter and cortex are unknown.

Since the metabolism in tumours principally is quantitatively and not qualitatively altered (Buchwald, Hudson 1955; Richterich 1958), the concentrations of the essential trace elements are expected to reflect this situation. Therefore the distribution of the trace element contents in the various groups was compared.

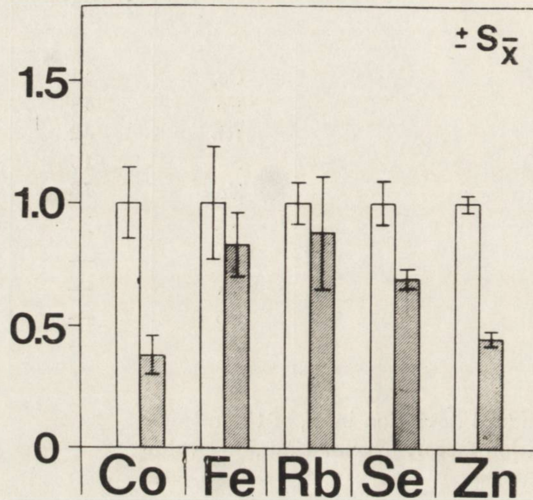

Fig. 3. Element content of normal cerebral tissue. C = cortex, M = white matter open columns — cortex, values normalized to 1 shaded columns — white matter ± standard error is given.

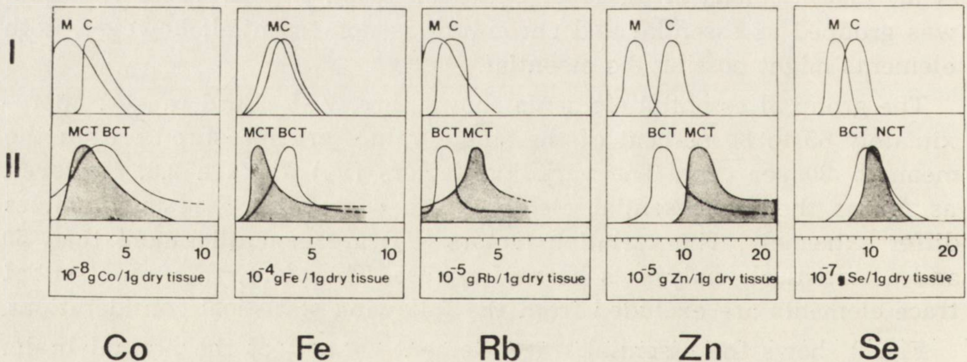

Fig. 4. Trace element distribution in brain. C = normal cerebral cortex; M = normal cerebral white matter; BCT = benign cerebral tumours; MCT = malignant cerebral tumours.

Abscissa: element concentrations in gram element per 1 gram dry tissue

Ordinate: number of cases (relative values, peak heights normalized to a constant height)

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In Fig. 4 the concentrations of cobalt, iron, selenium, rubidium and zinc are given: in line I in the normal brain tissue, separated for cerebral cortex (C) and white matter (M), and in line II in 16 benign cerebral tumours (BCT, open area) and in 7 malignant cerebral tumours (MCT, shaded area). Contrary to the relatively narrow range of values in the normal brain shown in line I, the tumours exhibit greater variations. Moreover, from the distribution curves seen in line II some distinct tendencies of trace element displacements in cerebral tumours may be noted: in the benign cerebral tumours (BCT) cobalt is increased 2-times and selenium 1.5-times the normal values ($p_{Co} < 0.05$, $p_{Se} < 0.001$), whereas the other elements show only slight deviations from the normals given in line I.

Compared with the benign tumours the malignant tumours (MCT, shaded area) exhibit a decrease of cobalt and iron, but an increase of rubidium and zinc and questionably of selenium (significance in the student t-test is given only for zinc, $p < 0.05$). Similar changes of trace element content were observed in other malignant tumours (Addink, Frank 1959; Buchwald, Hudson 1944; Kishi, Nakahara 1937; Pfeilsticker 1965; Schicha et al. 1969; 1970). These deviations of trace element content are likely related to metabolic changes in malignant tissue: the iron decrease indicates a diminution of respiratory enzymes, whereas the elevated rubidium- and zinc-values point to an enhanced metabolic activity and an increase of the nucleic acid content (Butenandt, Dannenberg 1956; Schicha et al. 1969; Schröder 1965).

The concentration of the non-essential elements showed similarly to normal tissue, also in the tumours extremely high individual differences. The silver content of the malignant tumours seemed to be rather diminished.

A detailed correlation of trace element contents with the histological findings was not made, as the number of cases in still too small at present.

Among the tumours examined 4 were partly necrotic: Fig. 5 shows the trace element content in the tumour necroses (shaded columns), compared with the contents in the corresponding solid tumour portions, which are normalized to 1 (open columns). Especially the essential trace elements are diminished on the average in the necrotic tissue, probably as a consequence of the loss of metabolic functions.

In Fig. 6 the data of a calcified oligodendroglioma (grade I) are presented. The element content of the solid tumour portion is normalized to 1 (open columns). The corresponding calcified tumour portion (shaded columns) shows greatly decreased caesium content, whereas zinc is in-

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creased 3.3-times and antinomy 13-times the respective value of the solid portion of the tumour. The reasons for these phenomena are not clear.

In conclusion it can be stated, that trace element displacements in cerebral tumours occur predominantly in malignant ones and here again especially with respect to essential trace elements. The changes are mostly discrete and probably are a secondary effect of disturbed metabolism.

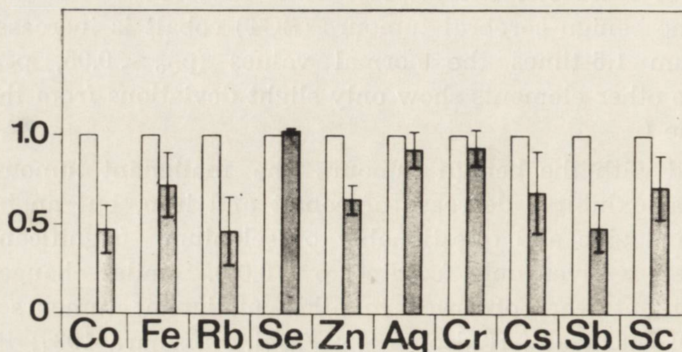

Fig. 5. Relative trace element content in tumour necroses (n=4); open columns — respective solid tumour portion, content normalized to 1; shaded columns — necrosis, ± standard error.

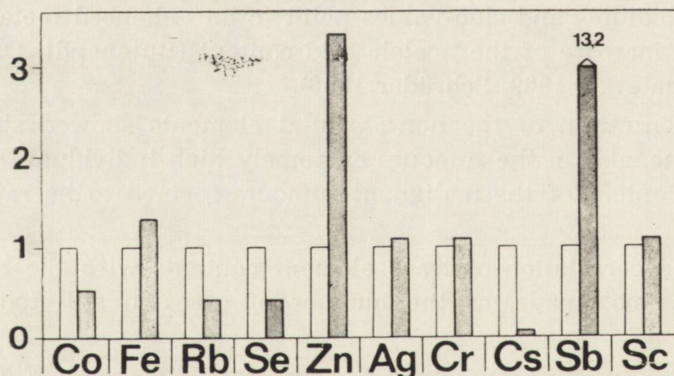

Fig. 6. Trace element content of a calcified oligodendroglioma; open columns — solid tumour portion, normalized to 1 shaded columns — calcified tumour portion.

H. Schicha, W. Müller, K. Kasperek, R. Schröder

WYSTĘPOWANIE PIERWIASTKÓW ŚLADOWYCH W GUZACH MÓZGU

Streszczenie

Zawartość pierwiastków śladowych w guzach mózgu i w prawidłowej tkance mózgu była badana przy pomocy analizy aktywacji neutronów. Wykazano, że zawartość badanych pierwiastków śladowych (Ag, Co, Cr, Fe, Rb, Sc i Zn) w tkance

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mózgu *in vivo* jest zgodna z zawartością w mózgu z materiału autopsyjnego. Materiał stanowiły wycinki 62 guzów otrzymanych podczas operacji.

Podstawowe pierwiastki śladowe wykazywały względnie niski poziom wartości, podczas gdy pierwiastki nie-podstawowe częściowo wykazywały znaczne różnice zawartości.

Ogólnie, zawartość pierwiastków śladowych w niezłośliwych guzach mózgu charakteryzowała się znacznymi odchyleniami od wartości prawidłowych, podczas gdy w guzach złośliwych zawartość żelaza była obniżona, a zawartość rubidu i cynku podwyższona.

W przypadku jednego skąpodrzewiaka napromienianego przed operacją stężenie pierwiastków śladowych było szczególnie wysokie. Inny, zwapniały skąpodrzewiak wykazywał znaczne podwyższenie zawartości cynku. W martwicach w guzach zawartość elementów śladowych była obniżona.

Przedstawione odchylenia w zawartości elementów śladowych są niewątpliwie związane z różnicami metabolicznymi i strukturalnymi tkanki nowotworowej.

Г. Шиха, В. Мюллер, К. Касперек, Р. Шредер

НАЛИЧИЕ СЛЕДОВЫХ ЭЛЕМЕНТОВ В ОПУХОЛЯХ МОЗГА

Резюме

Содержание следовых элементов в опухолях мозга и в нормальной ткани мозга исследовалось при помощи анализа активации неутронов. Обнаружено, что содержание исследованных следовых элементов (Ag, Co, Cr, Fe, Rb, Sc, Zn) в мозговой ткани in vivo согласуется с содержанием этих элементов в мозгу из автопсионного материала. Материал составляли срезы 62 опухолей, полученных во время операции.

Основные следовые элементы обнаруживали относительно низкий уровень, тогда когда не-основные элементы обнаруживали значительные разницы в содержании.

Вообщем, содержание следовых элементов в не-злокачественных опухолях мозга характеризовалось значительными отклонениями от нормальных величин, в то время когда в злокачественных опухолях содержание железа было снижено, а содержание рубидия и цинка — повышено.

В случае одной олигодендроглиомы, облученной перед операцией концентрация следовых элементов была особенно высока. Вторая, кальцифицированная олигодендроглиома обнаруживала значительное повышение содержания цинка. В некрозах опухолей содержание следовых элементов было снижено.

Перечисленные отклонения в содержании следовых элементов частично отчетливо вызваны метаболическими и структурными разницами.

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THE PROPORTION CONSTANCY OF ENZYMES OF THE PHOSPHOTRIOSE-PHOSPHOGLYCERATE GROUP IN BRAIN TUMOURS

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In normal cells and organs the enzyme activity is regulated according to the requirements of metabolism. In this way patterns of enzymes are formed which under normal conditions of metabolism are typical for the organ and the species. Owing to biological variation these patterns are not strictly constant but move between certain boundaries.

In tumours, on the contrary, even in those of the same type, there exists a multiplicity of enzyme patterns. Potter and Watanabe (1968) found that of 41 hepatomas with partially the same morphology there were not two with the same enzyme pattern.

In an earlier investigation we have found similar facts to be true of human brain tumours as well (Fischer 1967).

Fig. 1 demonstrates how great the differences can be in the proportion of the activity of two enzymes of the same metabolic pathway by the example of 11 human brain tumours. The activity of two glycolytic enzymes, lactate dehydrogenase and phosphoglucoisomerase, is represented there. When plotted on a logarithmic scale, equal proportions of enzyme activity show equal distances in the graph, independent of their absolute amounts. A distance, according to the proportion 1 : 5, is shown for comparison. Lactate dehydrogenase is represented by full circles, phosphoglucoisomerase by squares. The figure shows no constant proportion to exist between the two enzyme activities. Extremely low amounts of phosphoglucoisomerase are corresponding partly to high activities of lactate dehydrogenase and vice versa. The proportion between lactate dehydrogenase and phosphoglucoisomerase reaches from 1 : 13 in the meningioma number 120 to 48 : 1 in the glioblastoma number 129; these two proportions are different by a factor of about 620.

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The cause of these great differences is a defect in the control and regulating mechanisms leading to an incongruity between the real requirements of the cells and their enzyme synthesis.

Pette and collaborators (1962) have found the proportions of a group of glycolytic enzymes in various materials, from yeast to rat organs to be characterised by a quite extraordinary stability. These enzymes, triosephosphate isomerase, glyceraldehydephosphate dehydrogenase, glyceratephosphate kinase, glyceratephosphate mutase and enolase are located in a segment of the glycolytic chain from which no essential

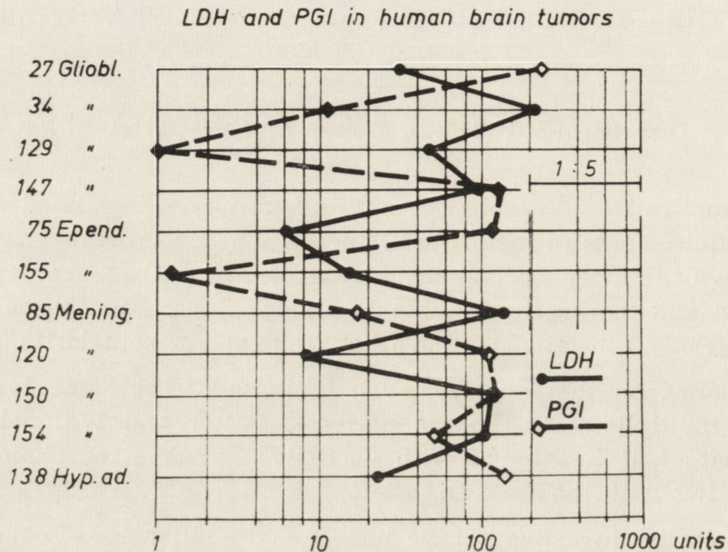

Fig. 1. Activity of LDH and PGI in 11 human brain tumours. 1 unit = 1 μmole/g dry weight/min. Gliobl. = gliblastoma; Epend. = ependymoma; Mening. = meningioma; Hyp. ad. = pituitary adenoma. Logarithmic scale. Equal proportions of enzyme activity show equal distances on this scale. Note the absence of a constant proportion between the two enzyme activities.

by-paths are branching off. Therefore no special regulation of the individual enzymes is necessary. Although the absolute amounts of their activity fluctuate within rather wide limits their proportions are nearly constant. This constancy is to be understood in a biological sense, as there are maximal deviations by a factor of about three.

No systematic investigations on the proportion constancy of these enzymes were made in tumours so far. With regard to the defects of the systems regulating and coordinating the enzyme synthesis, generally established in tumours, it seemed to be of interest here to examine this proportion constancy.

The investigations were made on 17 human brain tumours, composed of 2 glioblastomas, 4 astrocytomas, 1 spongioblastoma, 1 ependymoma,

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1 neurofibroma, 7 meningiomas and 1 pituitary adenoma. Moreover 10 tumours of the brain and of the nerve roots were used which were induced in rats by means of methylnitrosourea and ethylnitrosourea. All of them were sarcoma-like. Seven cell cultures of such experimental tumours, partly after cloning, were investigated too. Brains of six normal Hauben rats were used for comparison. The activity of the enzymes: triosephosphate isomerase, glyceratephosphate kinase, glyceratephosphate mutase and enolase was determined by means of standardized optical tests in the supernatants of homogenates centrifuged at 20 000 g (Bücher et al. 1964).

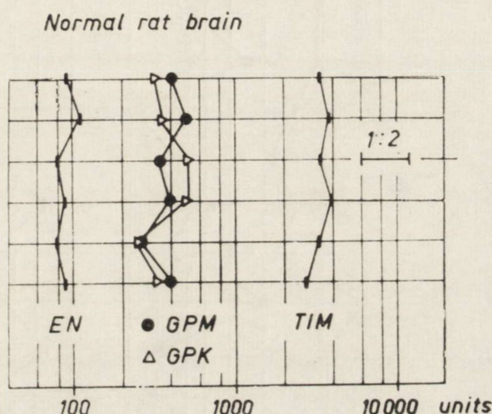

Fig. 2. Enzyme activity in normal rat brain. EN = enolase; GPM = glyceratephosphate mutase; GPK = glyceratephosphate kinase; TIM = triosephosphate isomerase; 1 unit = 1 μmole/g protein/min. Logarithmic scale.

Fig. 2 shows the results obtained with the brains of the normal rats on a logarithmic scale. All six animals display only little variation in the amount of the activity of the 4 enzymes and therefore in their proportions too. This variation is caused biologically as well as by analytical errors and it represents the conditions found in a normal organ.

Fig. 3 shows the activities found in 17 human brain tumours. The amount of activity of each enzyme varies in these tumours within a range of about 1 : 10. The proportions of the 4 enzymes however are fairly constant, as may be seen from the nearly equal distances in the logarithmic scale of the graph. The variations of the proportions are not substantially greater than those of the brains of normal rats.

Fig. 4 shows the activity of the 4 enzymes in 10 tumours of the brain and of the nerve roots of rats induced by means of methylnitrosourea and ethylnitrosourea. Here again the proportion constancy is maintained to an extent similar to that found in the normal rat brain.

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Fig. 5 shows the enzyme activities in cell cultures of experimental tumours of the brain and of the nerve roots. Here the activity of glyceratephosphate mutase was not determined in some cases. A nearly complete constancy of the proportions exists, independent of the differing absolute amounts of enzyme activities.

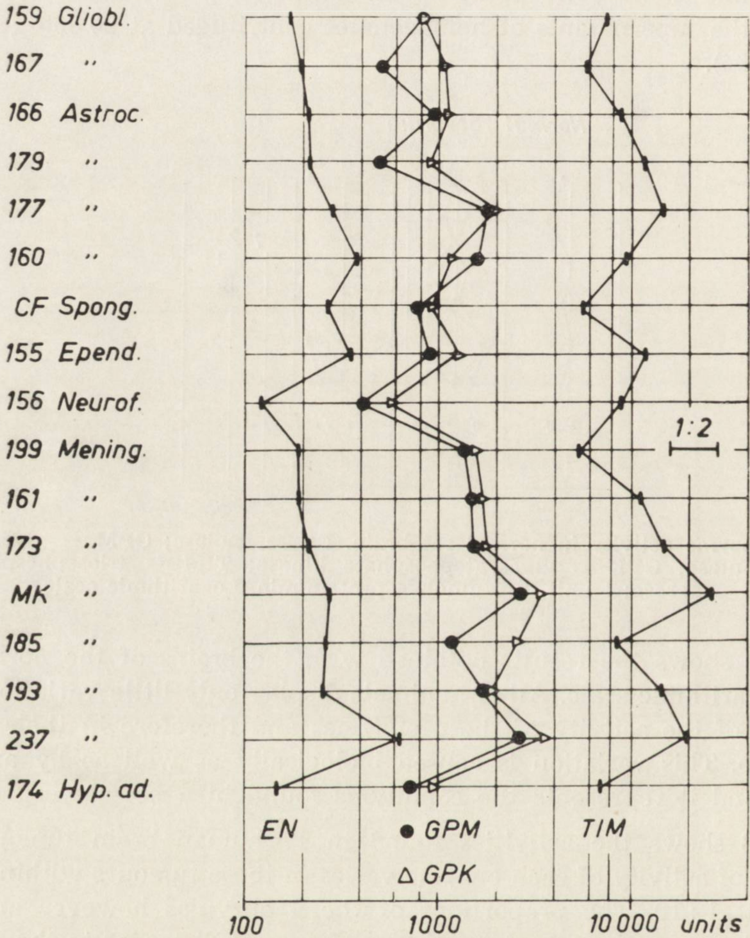

Fig. 3. Enzyme activity in human brain tumours. Enzymes and unit as in *Fig. 2.* Gliobl. = glioblastoma; Astroc. = astrocytoma; Spong. = spongioblastoma; Epend = ependymoma; Neurof. = neurofibroma; Mening. = meningioma; Hyp. ad. = pituitary adenoma.

Consequently, it is established that there is no substantial impairment of the proportion constancy of these enzymes in all the investigated tumours without any exception. This result is very striking with regard

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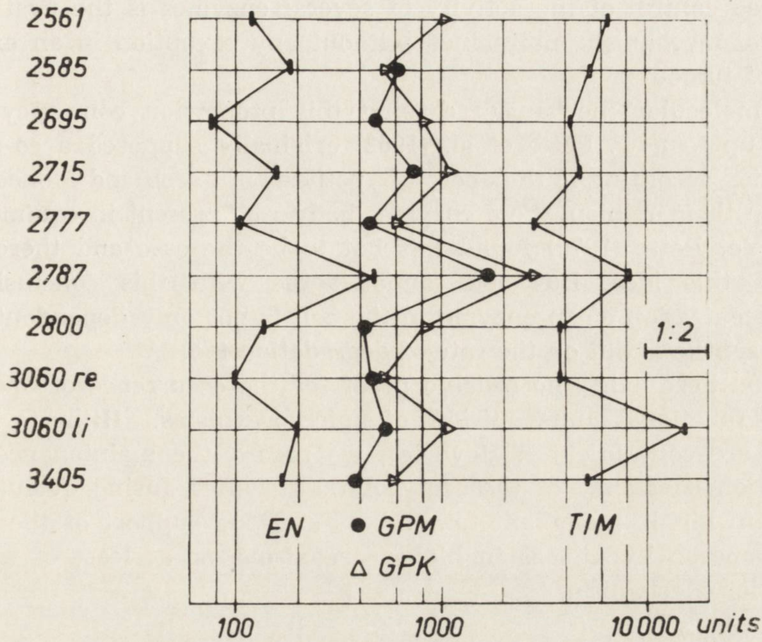

Fig. 4. Enzyme activity in tumours of the brain and of the nerve roots of rats induced by means of methylnitrosourea and ethylnitrosourea. Enzymes and unit as in *Fig. 2.*

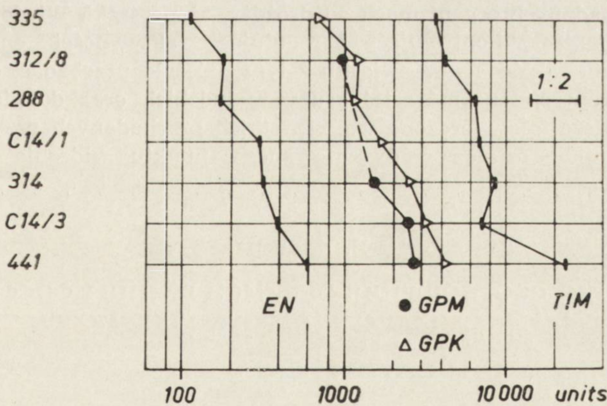

Fig. 5. Enzyme activity in cell cultures of experimental tumours of the brain and of the nerve roots of rats. Enzymes and unit as in *Fig. 2.*

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to the well known disturbances in the systems controlling and regulating enzyme activity generally seen in tumours. To our knowledge this co-ordinated control of the activity of several enzymes is the first case of a control mechanism maintained without any exception in an extensive series of tumours.

The molecular mechanism causing this proportion constancy is unknown up to now. Pette et al. (1962) originally supposed a co-ordinate regulation according to the operon hypothesis of Jacob and Monod (1961). Then, in their opinion, the 5 enzymes had to be present in equimolecular ratios. Yet Pette (1970) found this not to be the case and therefore he rejected this idea. But one cannot agree with this conclusion, for the concentration of an enzyme in the cell is not only dependent on the rate of synthesis but on the rate of degradation too.

So far there exists no reliable proof for the occurrence of operons or similar functional units outside of microorganisms. Hitherto existing results are contradictory. However, we regard the maintenance of the proportion constancy of these glycolytic enzymes during evolution and finally in highly malignant tumours as a strong support of the validity of the operon hypothesis in higher organisms, or at least of a similar regulation mechanism.

W. Fischer

STAŁOŚĆ STOSUNKU ENZYMÓW GRUPY FOSFOTRIOZY — FOSFOGLICERANU W GUZACH MÓZGU

Streszczenie

Aktywność 4 enzymów glikolitycznych: izomerazy fosfotriozy, kinazy fosfoglicerolowej, mutazy fosfoglicerolowej oraz enolazy występuje w stałych proporcjach we wszystkich badanych organizmach od drożdży, aż po saski. Wiadomo również, że w guzach mechanizmy kontrolujące i regulujące aktywności enzymatyczne są w różnym stopniu zakłócone. W związku z tym należało przebadać, czy jest zachowana stałość proporcji enzymów tej grupy w ludzkich oraz doświadczalnych guzach mózgu. Stałość tę stwierdzono we wszystkich przebadanych przypadkach. Znaczenie tego wyniku zostało przedyskutowane z punktu widzenia biologii molekularnej.

В. Фишер

ПОСТОЯНСТВО СООТНОШЕНИЯ ЭНЗИМОВ ГРИППЫ ФОСФОТРИОЗЫ--ФОСФОГЛИЦЕРАТА В ОПУХОЛЯХ МОЗГА

Резюме

Активность 4 гликолитических энзимов: изомеразы фосфотриозы, фосфоглицеральной киназы, фосфоглицеральной мутазы и энолазы находится в постоянных пропорциях во всех исследованных организмах от дрожжей вплоть

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к млекопитающим. Известно также, что в опухолях контрольные и регуляционные энзиматические механизмы активности в разной степени нарушены. В связи с этим следовало исследовать сохраняется ли постоянство пропорции энзимов этой группы в человеческих и экспериментальных опухолях мозга. Это постоянство находили во всех исследованных случаях. Значение этого результата обсуждается с точки зрения молекулярной биологии.

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E. RÓNA, A. NAGY, M. WOLLEMANN, F. SLOWIK

LOCALIZATION OF VARIOUS ISOENZYMES IN DIFFERENT CELL FRACTIONS OF BRAIN TUMOURS

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The detections of various isoenzymes in the pathologically altered brain tissue, cysts, cerebro-spinal fluid and sera of patients with brain tumour have been described by several authors as an important sign of early pathological changes (Gerhardt et al. 1963, Wollemann et al. 1965, Loewenthal et al. 1964, Szliwowski et al. 1961, Sano et al. 1966, Sherwin et al. 1968). The isoenzymes of lactic dehydrogenase (LDH) were most frequently investigated, but aldolase, esterases, phosphatases and malic dehydrogenase isoenzymes were also examined in a few cases (Sugimura et al. 1970, Gerhardt et al. 1963, Wollemann et al. 1965, 1967, 1970, Fischer et al. 1970, Loewenthal et al. 1961).

The alterations in the isoenzyme pattern generally took the form of a diminution of the H (heart) or B (brain) type isoenzymes and of an increase in the M (muscle) type isoenzymes of LDH and aldolase in the malignant brain tumours. Two different concepts exist in the literature on the origin of these changes. According to Goldmann et al. (1964), changes in the glycolytic metabolism from the aerobic to the anaerobic form caused these alterations in the isoenzyme pattern. However, Schapira et al. (1968) pointed to the similarity of embryonic isoenzyme patterns in cases of hepatomas, where the normally present M type isoenzymes in the liver changed to H type ones which were present also only in the embryonic liver. Both conceptions can be traced back to the original theory of Warburg (1930) based on the high anaerobic glycolysis of tumours, which is similar to that of the embryonic tissue.

In order to test the validity of these concepts we investigated the distribution of several isoenzymes of glycolytic (LDH, aldolase, creatine-phosphokinase — CrPK) and oxidative (malic dehydrogenase — MDH, monoaminooxidase — MAO, and glutamic dehydrogenase — GDH) pathways. The distribution within the different cell fractions of some enzymes was

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also investigated. As marker enzymes for cell cytoplasm LDH and glucose-6-phosphate dehydrogenase (G-6-PDH) and for mitochondria, succinic dehydrogenase (SDH) activities were measured.

MATERIAL AND METHODS

Sixty eight brain tumours (22 astrocytomas, 16 glioblastomas, 13 meningiomas, 5 medullobastomas, 4 ependymomas, 3 oligodendrogliomas, 2 spongioblastomas, 2 mixed gliomas and 1 neurinoma) and 8 control brain samples (3 temporal lobes, one removed for epilepsy, two for aneurysm treatment and 5 apparently normal tissue samples surrounding brain tumours) were obtained from surgical interventions. Samples were immediately cooled at 0°C, homogenized in 4.5 vol. 0.25 M sucrose and cellular fractionation was performed according to Brody and Bain (1952). In some cases heavy and light mitochondrial fractions were separated on a ficoll gradient, according to the method of Stahl et al. (1963).

Quantitative LDH activity measurement were performed after Kubowitz and Ott (1943). SDH activity was measured according to the method of Slater (1963) and G-6-PDH activity according to that of Kornberg and Horecker (1955). The cell fractions and fluids were submitted to disc gel electrophoresis. Two-tenth of ml was applied in 20% sucrose or Sephadex G and run at 5°C for one hour in parallels, and one of each samples was stained for protein according to the method of Davis (1964). In order to solubilize the enzymes of the mitochondrial fractions, they were treated with Triton-X in 1% end concentration.

The various isoenzymes were stained according to the following methods: LDH (Dewey, Conklin 1960), aldolase (Penhoet et al. 1966), creatine-phosphokinase (Deul et al. 1964, Rosalki 1966), MDH (Thorne et al. 1963), GDH (Van der Helm 1962), MAO (Glenner et al. 1957), esterase (Nachlas et al. 1949). Quantitative evaluation of the isoenzymes were made on a Joyce Chromoscan.

RESULTS

Within the glioma group the LDH isoenzyme pattern changed parallely to the degree of malignancy in all cell fractions, in the direction from the dominant towards the M LDH isoenzymes. The most obvious changes appeared in the supernatant fractions (Figs. 1 and 2). Meningiomas revealed also similar patterns to those of the malignant tumours, owing to the mesenchymal origin of their cell type (Table 1).

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Table 1. Distribution of lactic dehydrogenase in various areas of normal human brain and in human brain tumours

Brain area or type of tumour	Band number							
	+	1	2	3	0	4	5	—
Corpus striatum		32.3	48.6	18.8	—	—	—	
Cerebellar cortex		84.2	7.7	7.2	—	—		
Cortical gray matter		75.2	15.7	9.2	—	—		
Subcortical white matter		50.7	30.0	19.3	—	—		
Glioblastoma								
No. 174		18	29	30	23	—		
No. 48		22	45	23	9	—		
No. 195		14	23	34	29	—		
No. 411		11	17	37	21	13		
Oligodendroglioma								
No. 257		60	23	17	—	—		
No. 350		40	39	21	—	—		
Astrocytoma								
No. 160		20	24	35	21	—		
No. 126		35	31	34	—	—		
Meningioma (end.)								
No. 193		2	5	20	34	40		
No. 432		17	14	41	28	—		
No. 440		11	20	31	25	12		
No. 197		14	26	36	24	—		
No. 348		7	17	30	46	—		
Meningioma (fibr.)								
No. 166		10	18	42	30	—		
Metastatic carcinoma								
No. 164		26	34	40	—	—		
No. 167		7	11	29	53	—		
No. 90		15	36	28	21	—		
Neuroblastoma								
No. 132		20	32	25	23	—		

Figures represent percentage contribution of each band to the total activity.

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The quantitative LDH activity measurements revealed elevated values mainly in the nuclear and mitochondrial fractions as compared with apparently normal brain samples. The origin of this changed compartmentation is discussed elsewhere (Nagy et al. 1971). LDH pattern similar to that of malignant brain tumours is detectable also in embryonic brain tissue (Bonavita et al. 1962, Gerhardt et al. 1963, Wollemann et al. 1971), and in experimentally induced brain tumours (Katona et al. 1967). Tumour surrounding tissue, cysts, and sometimes CSF also showed changes in the LDH pattern, which were parallel to the LDH pattern in the tumours (Figs. 1, 2, 3, 4).

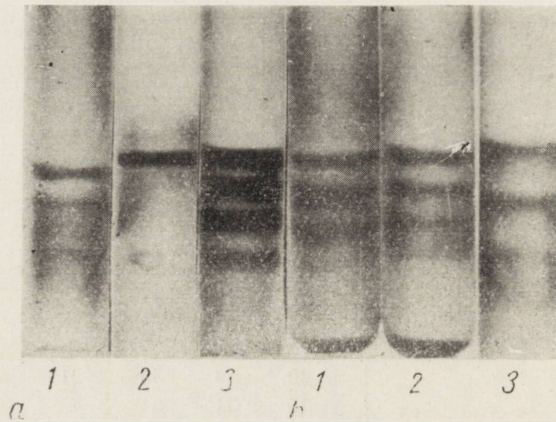

Fig. 1. LDH isoenzymes from: tumour surrounding tissue (a) and tumour (b), astrocytoma No. 33. Nuclear (1), mitochondrial (2) and supernatant cell fractions (3).

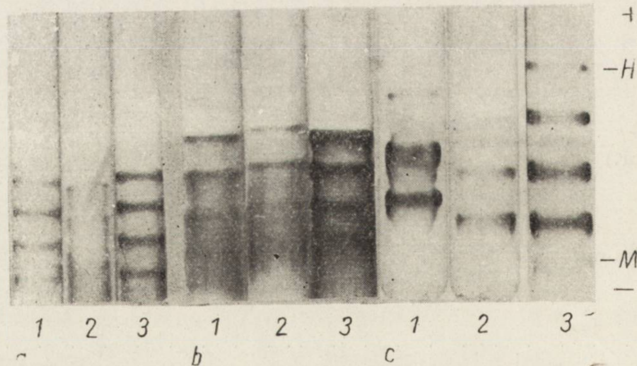

Fig. 2. LDH isoenzymes from: cell fractions of spongioblastoma surrounding tissue (a), spongioblastoma No. 105 (b) and glioblastoma No. 175 (c). Nuclear (1), mitochondrial (2) and supernatant (3) cell fractions. H — heart, M — muscle type.

Principally the same changes were obvious in the aldolase isoenzyme pattern. The brain type was altered in malignant gliomas and embryonic

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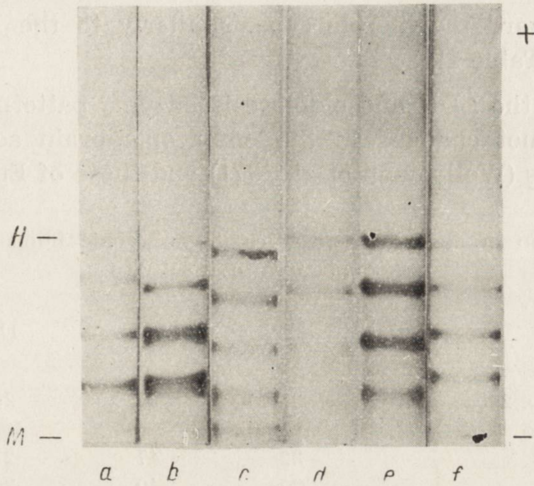

Fig. 3. LDH isoenzymes from: CSF (a) and cyst (b) of glioblastoma multiforme No. 114, tumour (c) and CSF (d) from medulloblastoma No. 279, cystic fluid from mixed glioma No. 319 (e) and from oligodendroglioma No. 265 (f).

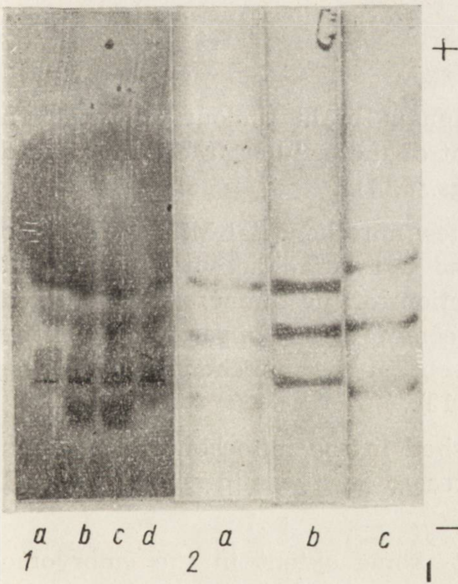

Fig. 4

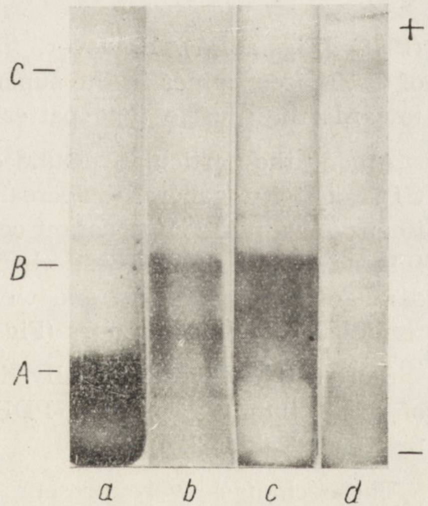

Fig. 5

Fig. 4. LDH isoenzymes from: CSF (a), cystic fluid (b, c) and serum (d) from craniopharyngeoma No. 23 demonstrated by starch gel (1) and disc gel (2) electrophoresis.

Fig. 5. Aldolase isoenzymes crystallized from rabbit muscle (a), from glioblastoma multiforme No. 288 (b), mixed glioma No. 319 (c) and glioblastoma multiforme No. 258 (d). A — muscle type, B — liver type, C — brain type.

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brain tissue toward the muscle type, similarly to the LDH isoenzyme pattern (Fig. 5, Table 2).

In contrast to the LDH and aldolase isoenzyme pattern, the CrPK isoenzymes were not changed in the embryonic brain according to our own observations (Wollemann et al. 1971) and those of Eppenberger et al.

Table 2. Distribution of aldolase isoenzymes in embryonic human brain and brain tumours in % a total activity

	+ I	II	III	IV –
Embryonic brain No. 290,	35	37	28	—
28 weeks old: cortex				
hypothalamus	32	47	21	—
mesencephalon	30	40	30	—
Embryonic brain No. 292,				
20 weeks old: cortex	21	35	29	15
hypothalamus	15	34	31	20
mesencephalon	19	30	28	23
subcortical white matter	12	34	31	23
glioblastoma multiforme				
No. 288	9	31	34	26

(1970) (Figs. 6, 7). However, in malignant brain tumours the pattern of CrPK isoenzymes in the supernatant of the cellular fractions changed towards the muscle type pattern (Figs. 6, 7).

Among the particulate bound enzymes, mitochondrial MDH, MAO and GDH activity generally decreased and a relative activity increase of isoenzymes in the supernatant cell fractions of the tumours was observed (Figs. 8, 9, 10, 11). These changes seemed to be in parallel with the damage of mitochondria and were absent in the mitochondria from the tissue surrounding tumours (Figs. 12, 13).

SDH activity was generally diminished in the mitochondrial fraction of brain tumours and G-6-PDH increased in the supernatant of cell fractions (Tables 3, 4).

These changes were present also to some extent in the embryonic brain tissues where the respiratory activity is diminished in comparison to the glycolytic activity (Wollemann et al. 1971).

Among lysosome - bound enzymes esterase and cholinesterase activities did not parallely develop in brain tumours and embryonic brain tissue. Although acetylcholinesterase activity is diminished in both types of tissue (Wollemann et al. 1961), butyrylcholinesterase activity is increased

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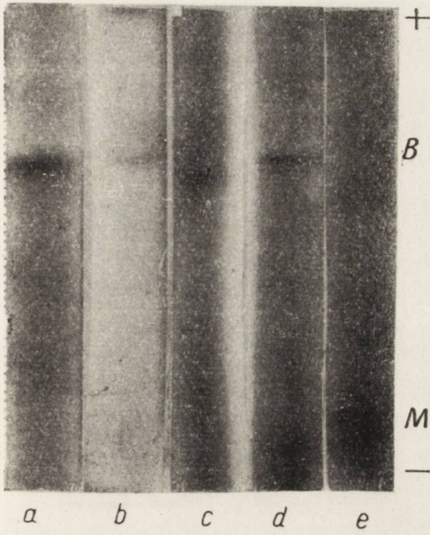

Fig. 6

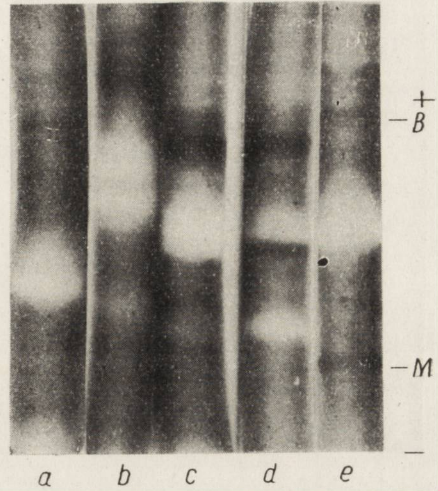

Fig. 7

Fig. 6. Creatinephosphoferase isoenzymes demonstrated according to Deul et al. (1964) from: normal human cortical gray matter supernatant (a), embryonic human cortical gray matter 28 weeks old (b), astrocytoma No. 318-cyst (c) and tumour (d), glioblastoma multiforme No. 288 (e). B — brain type, M — muscle type.

Fig. 7. Creatinephosphoferase isoenzymes demonstrated according to Rosalki (1966) from normal human cortical gray matter supernatant (a), embryonic human cortical gray matter 28 weeks old (b), astrocytoma No. 318 cyst (c) and tumour (d), glioblastoma multiforme No. 288 (e). B — brain type, M — muscle type.

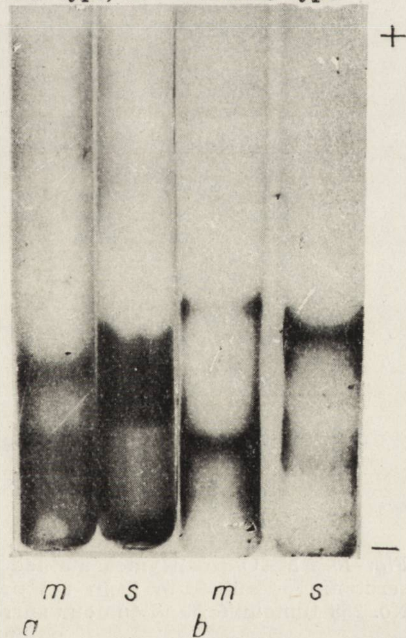

Fig. 8. Malate dehydrogenase isoenzymes from normal human (a) and rat brain (b). Mitochondria (m) and supernatant (s); cell fractionation was performed according to the method of Brody et al. (1952). Mitochondrial enzymes were solublized with Triton-X. MDH activity was stained according to Thorne et al. (1963).

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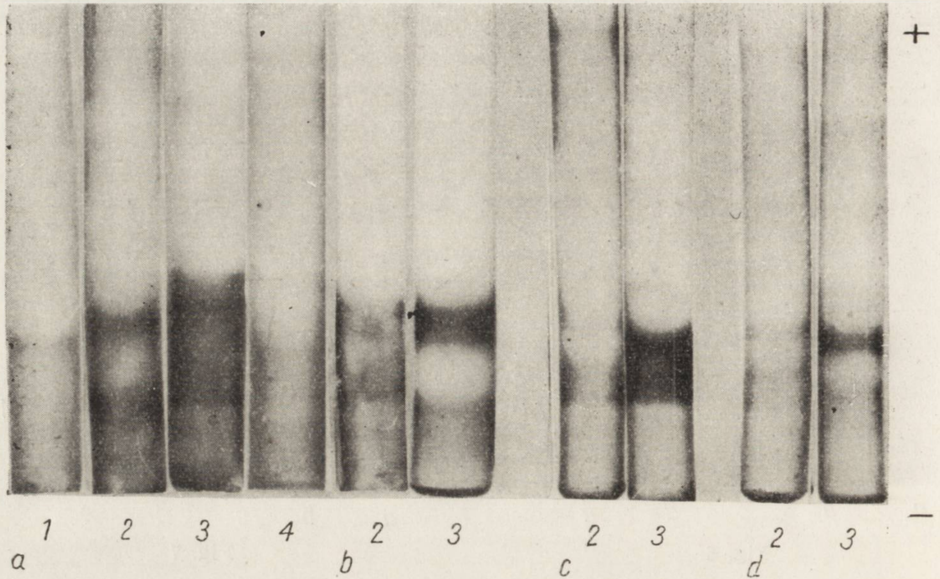

Fig. 9. MDH isoenzymes from nuclear (1) mitochondrial (2) supernatant (3) cell fractions and CSF (4) from a case of astrocytoma No. 282 (a); mitochondrial (2) and supernatant (3) cell fractions from medulloblastoma No. 316 (b); tumour surrounding tissue (c) and tumour (d) of glioblastoma multiforme No 258.

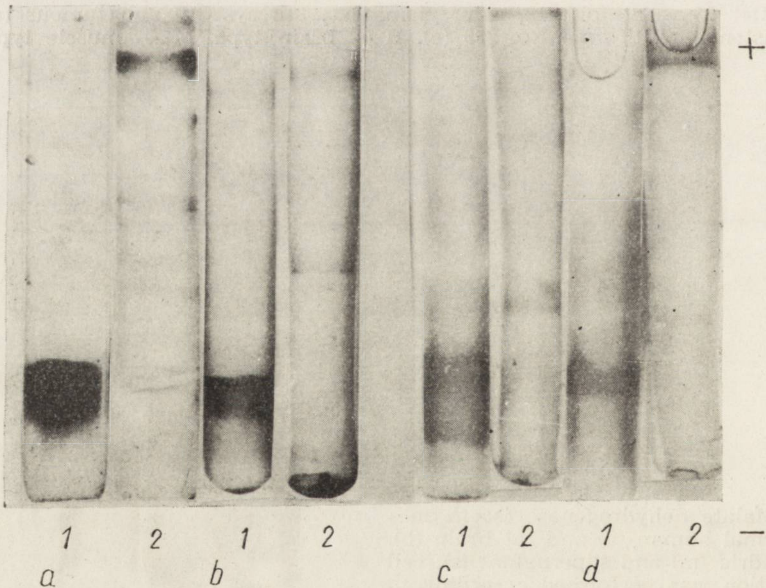

Fig. 10. MAO isoenzymes stained by the method of Glenner et al. (1957) using serotonin as substrate from rat brain (a), cat brain (b), glioblastoma multiforme No. 258 tumour (c) and tumour surrounding tissue (d); mitochondrial (1) and supernatant (2) cell fractions.

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only in brain tumours. High aromatic esterase activities are present in ten isoenzymes in the embryonic brain (Wollemann et al. 1971). In brain tumours only lysosome bound esterase activity is increased as revealed from histochemical investigations. Isoenzymes in the prealbumin, albumin and alpha globulin fractions are diminished in glioblastomas and metastatic carcinomas and present in oligodendrogliomas, astrocytomas and meningiomas (Wollemann et al. 1965).

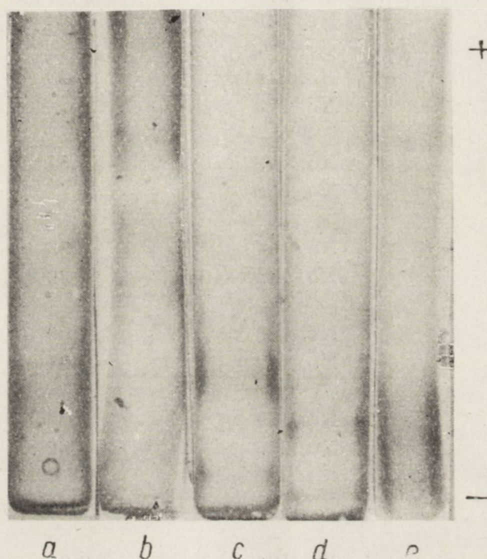

Fig. 11. Multiple forms of GDH from crystalline beef liver enzyme (a) mitochondria of mesencephalon of human embryonic brain (28 weeks old) (b) rat liver mitochondria (c), rat brain mitochondria (d), glioblastoma multiforme No. 288 mitochondria (e). Activity stained according to the method of van der Helm (1962).

DISCUSSION

Our investigations concerning the localization of various isoenzymes in the different cell fractions of brain tumours and normal adult and embryonic brain tissue resulted in the astonishing fact, that brain tumours differ in several aspects not only from normal adult but also from the embryonic brain. Thus tumour tissue induces the synthesis of those isoenzymes, which are best adapted for the metabolism of tumour cells. Latner et al. (1965) stated that the infection of tissue cultures with the oncogenic adenovirus 12 causes cell cultures to develop the malignant type of LDH isoenzyme pattern long before control uninfected cultures. Our results (Wollemann et al. 1971a) from tissue culture of brain tumours showed also that the most malignant tumours revealed rapid change toward the more malignant LDH pattern during the relatively short term

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Table 3. SDH activity of mitochondrial fractions of brain tumours (a) and tumour surrounding tissues (b)

		ΔOD/mg prot.	
		a	b
Astrocytoma	No. 33	1,57	1,97
Astrocytoma	No. 87	1,02	—
Astrocytoma	No. 25	1,02	1,08
Astrocytoma	No. 106	0.81	—
Oligodendroglioma	No. 78	0.73	—
Oligodendroglioma	No. 123		
Glioblastoma	No. 79	0.81	
Glioblastoma	No. 258	0.4	0.9
Ependymoma	No. 84	0.85	
Ependymoma	No. 145	0.29	
Spongioblastoma	No. 105	1.09	1.24
Spongioblastoma	No. 38	1.01	0.64
Plexus papilloma	No. 127	0.75	0.39
Plexus papilloma	No. 249	0.62	
Meningioma		0.56	
Lobus temporalis	No. 104	0.9	
Lobus temporalis	No. 211	0.65	

SDH activity was measured according to Slater et al. (1963).

cell cultivation (13 — 15 days) as compared with the original LDH isoenzyme pattern of the tumours.

These results strengthened our view on the possibility of the oncogenic virus origin of the brain tumours, which was furthered also by the appearance of the M type CrPK isoenzymes in the brain tumours and their absence in embryonic brain tissue. The diminution of mitochondrial enzyme activities and the increase of the soluble form of these enzymes might indicate also a compensatory function in response to mitochondrial damages.

In conclusion the importance of isoenzyme investigations in brain tumours is emphasized also in those cases where quantitative activity measurements did not show any alterations, because diminution in H or B type isoenzymes and increase in M type may result in unchanged total activity.

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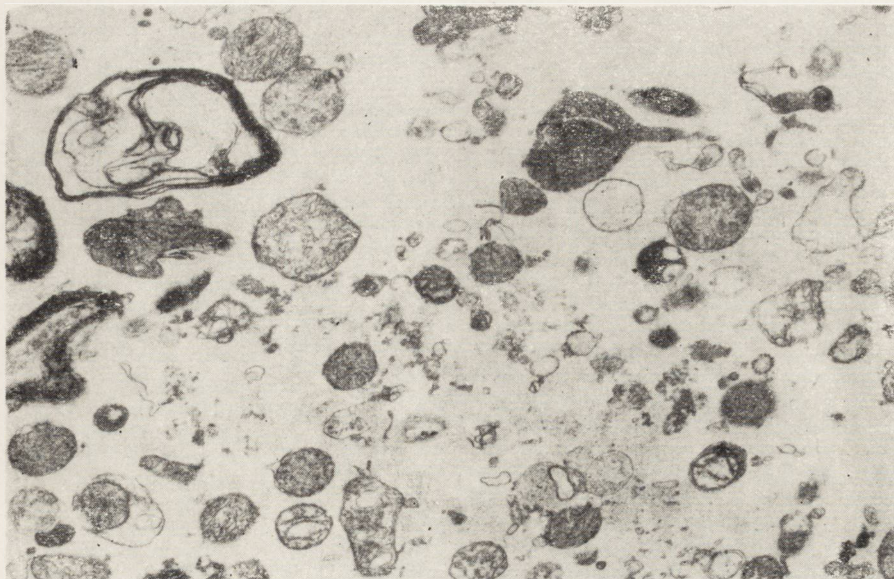

Fig. 12. Electronmicrograph from tumour surrounding mitochondrial fraction of glioblastoma multiforme No. 258. × 18 000.

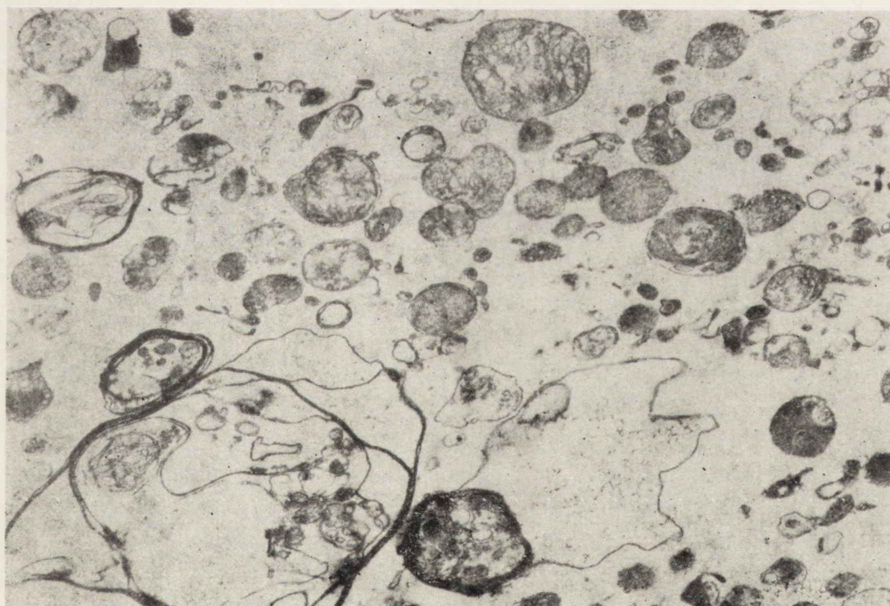

Fig. 13. Electronmicrograph from tumour mitochondrial fraction of glioblastoma multiforme No. 258. × 18 000.

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Table 4. Glucose — 6 phosphate dehydrogenase activity from supernatant of brain tumours (a) and brain tumour surrounding tissues (b)

		ΔOD/mg prot.	
		a	b
Astrocytoma	No. 106	0.41	
Astrocytoma	No. 87	0.2	
Oligodendroglioma	No. 123	0.54	
Sponglioblastoma	No. 38	0.2	0.1
Spongioblastoma	No. 105	0.70	0.46
Ependymoma	No. 84	0.59	
Ependymoma	No. 195	0.61	
Meningioma end.	No. 144	0.78	
Plexus papilloma	No. 127	0.40	0.22
Lobus temporalis	No. 104	0.28	
Lobus temporalis	No. 47	0.2	
Lobus temporalis	No. 211	0.11	

Glucose-6-phosphate dehydrogenase activity was measured according to Kornberg et al. (1953).

E. Róna, A. Nagy, M. Wollemann, F. Slowik

LOKALIZACJA RÓŻNYCH IZOENZYMÓW W POSZCZEGÓLNYCH FRAKCJACH KOMÓRKOWYCH GUZÓW MÓZGU

Streszczenie

Enzymy i izoenzymy cyklu glikolitycznego (dehydrogenaza mleczanowa i aldolaza), oksydacyjne (dehydrogenaza jabłczanowa, glukozo-6-fosforanu, bursztynianowa i glutaminianowa), esteraza i monoaminoksydaza były badane w 68 guzach. Stwierdzono obniżenie aktywności enzymów mitochondrialnych i kompensację enzymów rozpuszczalnych.

Pojawienie się typu M izoenzymów CrPK w guzach mózgu i ich brak w embrionalnej tkance mózgu nasuwa przypuszczenie, że guzy mózgu powstają na skutek działania wirusów rakotwórczych.

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Е. Рона, А. Наги, М. Воллеманн, Ф. Словик

ЛОКАЛИЗАЦИЯ РАЗНЫХ ИЗОЭНЗИМОВ В РАЗНЫХ КЛЕТОЧНЫХ ФРАКЦИЯХ ОПУХОЛЕЙ МОЗГА

Резюме

Энзимы и изоэнзимы гликолитического цикла (лактатдегидрогеназа и алдолаза), окислительные (малатдегидрогеназа, глюкозо-6-фосфатдегидрогеназа, сукцинатдегидрогеназа и глютаматдегидрогеназа), эстераза и моноаминооксидаза исследовались в 68 опухолях. Обнаружено снижение активности митохондриальных энзимов и компенсацию растворимых энзимов.

Появления типа М изоэнзимов CrPK в опухолях мозга и их отсутствие в эмбриональной ткани мозга позволяет полагать, что опухоли мозга возникают вследствие действия канцерогенных вирусов.

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HISTOCHEMISTRY OF CEREBRAL TUMOURS

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ENZYME HISTOCHEMICAL INVESTIGATIONS OF REPEATEDLY TRANSPLANTED BRAIN AND NERVE TUMOURS OF THE RAT

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Transplanted experimental brain tumours originally induced by chemical carcinogens have often been used for histo- and biochemical investigations (Ogawa, Zimmerman 1959, Saiko 1963, Lehrer et al. 1966, Maker et al. 1966). The results obtained from such tumourgrafts after being transplanted in many generations of animals were believed to be characteristic for experimental brain tumours in general. It has not been taken sufficiently into consideration, that the tumourgrafts might have changed their metabolism in the course of the transplantations. Our investigations were carried out to study possible changes in the enzyme patterns of an intracerebral and a nerve tumour of the rat.

MATERIAL AND METHODS

Both tumours were induced by N-methyl-N-nitrosourea. The induction of the original brain tumour in a rat of the inbred strain E was described previously (Warzok et al. 1971). The nerve tumour was obtained in a Wistar rat after 18 intravenous injections of the carcinogen (Güthert et al. 1968, test series VI).

Parts of the tumours were cut small enough, in order to insert the fragments by a trocar into the brain of the rats of the inbred strain E, about 4 weeks old. In this way the grafts of the brain and the nerve tumour were subsequently transplanted up to the 21st and the 18th generation, respectively. The primary tumours as well as the tumourgrafts were examined histologically and histochemically. The following enzymatic activities were studied: 1) alkaline phosphatase, 2) acid phosphatase, 3) non-specific esterase, 4) β-D-glucosidase, 5) β-D-galactosidase, 6) 5'-nucleotidase, 7) $NADH_2$-tetrazolium reductase, 8) succinate dehydrogenase, and 9) lactate dehydrogenase. For enzyme histochemical methods used see Osske et al. (1971).

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RESULTS

Biological observations

Following transplantation of the brain tumour, the survival of the rats decreased from 40 days after the first transplantation to 10 or 12 days after the 10th generation. In the case of the nerve tumour the interwals shortened from 3 to 4 months after the first transplantation to about 3 weeks after the 10th one.

Histological observations

The primary brain tumour, diagnosed as a sarcoma, and its transplants are composed of small densely packed cells with irregular nuclei slightly differing in shape and size. A typical behaviour of the neoplasm and especially of its transplants is the spreading of the tumour cells along the blood vessels and from the lumen of the vetricles into the adjacent brain tissue. The histological feature does not change in the course of 21 generations of grafting.

The nerve tumour examined was a neurosarcoma of the sciatic nerve of rat. The original tumour consisted mainly of long cells regularly arranged. In the third generation of the tumourgrafts we observed some areas containing polymorphous cells. In the following isografts, the polymorphous character prevailed and polynuclear giant cells and frequent mitotic figures occurred.

Histoenzymatic observations

a) B r a i n t u m o u r: Alkaline phosphatase is, like in other experimental brain tumours of the rat (Osske et al. 1969, Stavrou 1969, 1970), a constituent of the blood vessels in the primary tumour as well as in the tumourgrafts. Beside the vessel walls, the enzyme activity is present in polymorphs which can be found around the blood vessels and sometimes in areas of necrosis.

In the primary tumour the acid phosphatase activity is diffusely distributed in the cytoplasm; however, the staining reaction is weak. With continuous grafting the enzyme activity increases and can be observed in areas at one side of the cell nuclei (Fig. 1). The other parts of the cytoplasm show only scarce or no activity at all. Cells at the border of necrotic areas stain also intensively red. On account of their corpuscular and diffuse reaction they can be easily distinguished from living tumour cells.

The changes in the activity and distribution of the non-specific esterase within the first tumour generation were more striking than those

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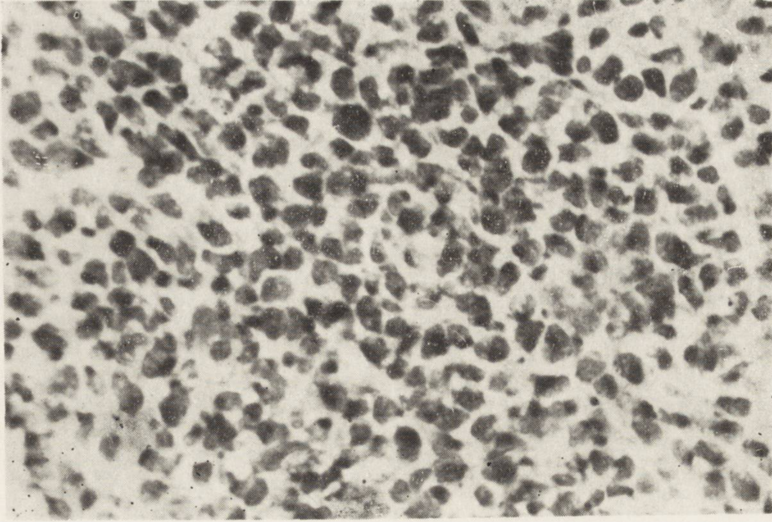

Fig. 1. Rat No. 1498. 20th generation of the brain tumour. High activity of the acid phosphatase at one side of the nuclei. × 550.

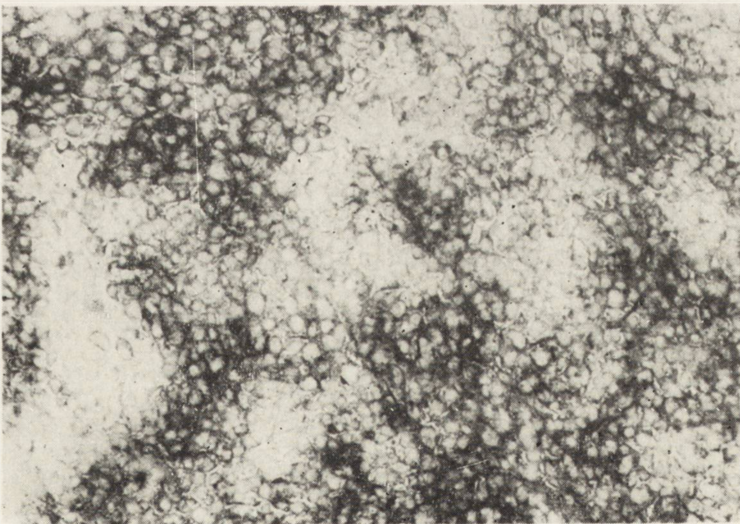

Fig. 2. Rat No 1316. 12th generation of the brain tumour. Non-specific esterase in ill — defined irregular areas. × 220.

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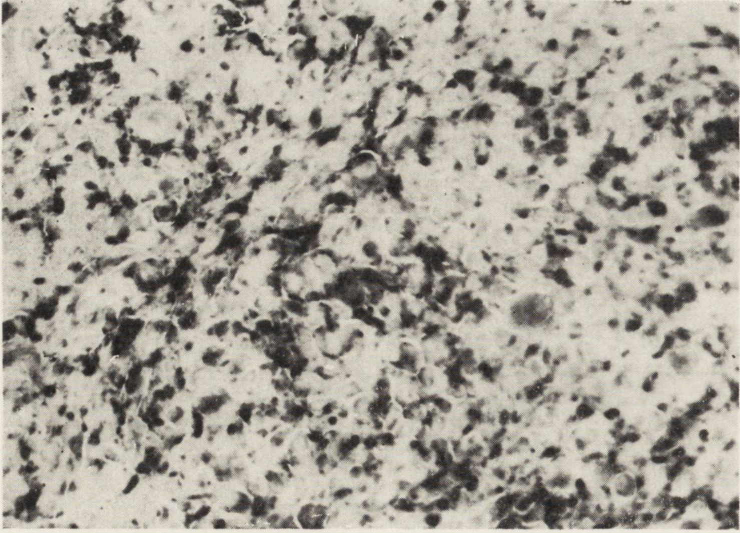

Fig. 3. Rat. No. 1562. 3rd generation of the neurosarcoma. Granular activity of the acid phosphatase in all tumour cells. × 300.

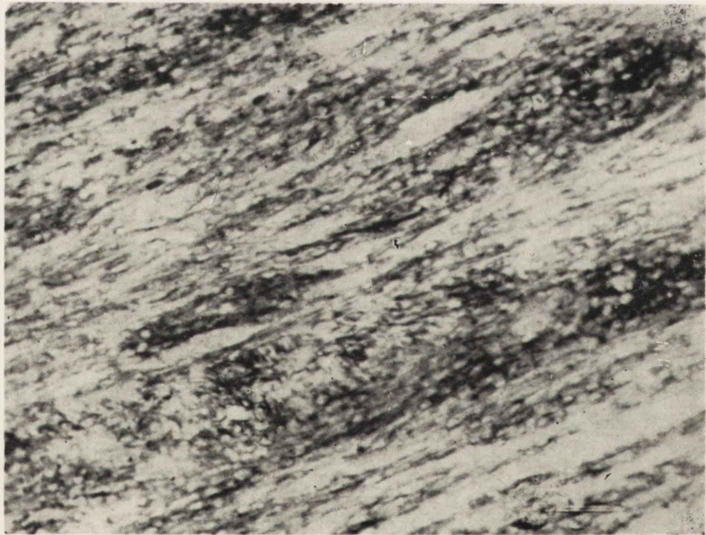

Fig. 4. Rat No 607. Primary nerve tumour. $NADH_2$-tetrazolium reductase in the cytoplasm of the oblong tumour cells. × 190.

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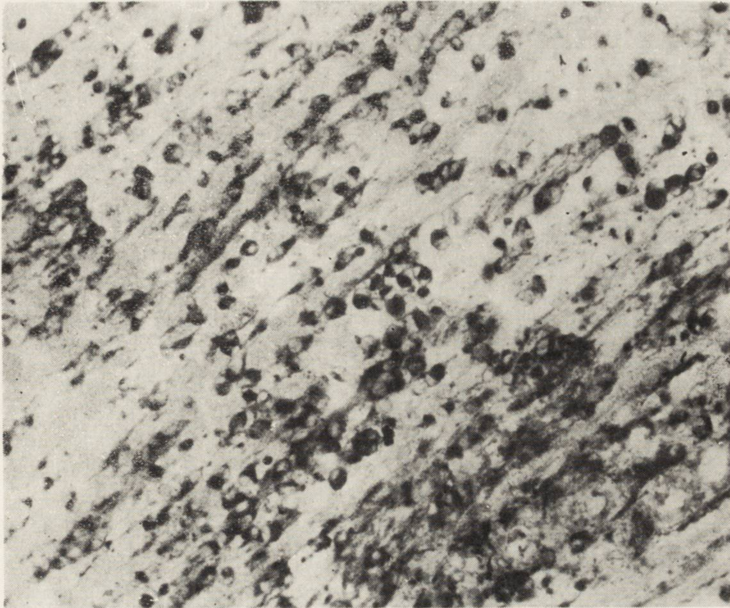

Fig. 5. Rat No 607. Primary nerve tumour. $NADH_2$-tetrazolium reductase localized at one side of the nuclei. × 220.

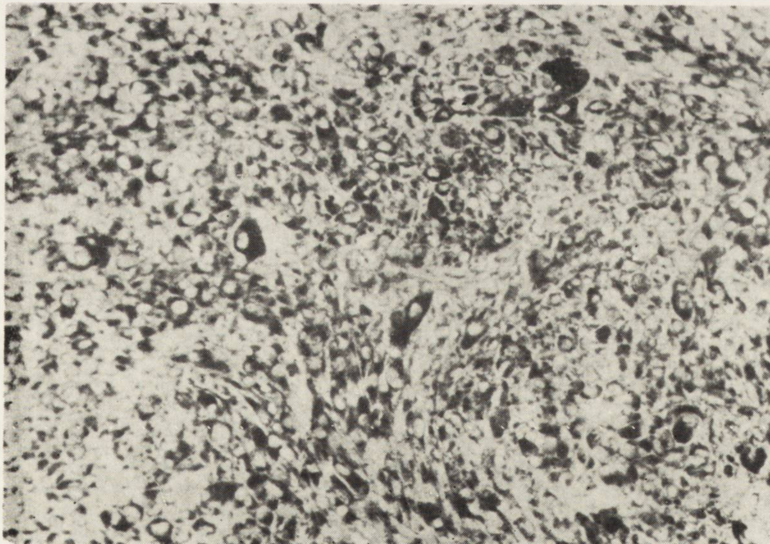

Fig. 6. Rat No 2235. 13th generation of the neurosarcoma. Intense activity of $NADH_2$-tetrazolium reductase especially in the giant cells. × 190.

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of the acid phosphatase. In the original tumour as well as in the grafts of the first and the second generation we found only a low activity in the cytoplasm of a few scattered cells. The following transplants in contradistinction to the first ones are characterized by **illdefined irregular** areas of high activity alternating with places of low or lacking activity (Fig. 2). These contrasts become more pronounced from generation to generation. Usually the highly active areas are situated in the centre of the grafts, whereas the peripheric parts of the transplants stain pale or remain unstained. The non-specific esterase activity is indicated by a fine granular reaction products within the cytoplasm. On the other hand, the perivascularly arranged tumour cells as well as the degenerated areas show a coarser precipitate.

5'-Nucleotidase occurs in some blood vessel walls, but the intensity of the reaction differs from graft to graft. The enzyme activity is also localized in necrobiotic areas, where some of the cells show especially high activity. Attempts to demonstrate other hydrolytic enzymes in the tumours, like glucose-6-phosphatase, β-D-glucosidase and β-D-galactosidase, failed.

The $NADH_2$-tetrazolium reductase, the lactate and the succinate dehydrogenase are present in the primary tumour as well as in the tumourgrafts. Their distribution remains unchanged in the course of the transplantations. But it seems, that the reaction activity of lactate dehydrogenase and the $NADH_2$-tetrazolium reductase is moderate and that of succinate dehydrogenase is very weak. Both enzymes are localized especially in the perinuclear zone and sometimes the formazane granules are more concentrated at one side of the nucleus.

Using the gel incubation method for the demonstration of the lactate dehydrogenase we observed this enzyme by contrast to the $NADH_2$-tetrazolium reductase in the whole cytoplasm of all the tumourgrafts.

b) Nerve tumour: The results obtained for alkaline phosphatase are similar to those of the examined brain tumour. The enzyme distribution does not change during the transplantations, but in the later generations we often found enlarged blood vessels, sometimes with slightly decreased activity.

In the primary tumour, the granular products of the activity of acid phosphatase are limited to single cells or small cell groups scattered between enlarged myeline sheaths undergoing degeneration. In the remaining tumour tissue as well as in that of the first and the second generation only a diffuse and weak reaction is present. After the 8th generation all cells especially, the polymorphous are filled with an intense granular reaction product (Fig. 3).

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The non-specific esterase activity, not detectable in the original neurosarcoma, is very scarce and diffuse in the transplants. An intense granular esterase and acid phosphatase activity is found only in the perinecrotic areas.

As to the other hydrolytic enzymes, such as glucose-6-phosphatase, 5'-nucleotidase, β-D-glucosidase and β-D-galactosidase no changes of their pattern depending upon the transplantation can be observed. It should be mentioned only, that both glycosidases are present within the degenerating myeline sheaths of the original neurosarcoma as well as in regressive areas of the transplants.

The activity of $NADH_2$-tetrazolium reductase and succinate dehydrogenase in the sciatic nerve tumour is moderate and weak, respectively. Activities of both enzymes are distributed in the whole cytoplasm of the spindle-shaped cells (Fig. 4). In a number of other tumour cells, however, the enzymes are concentrated at one side of the nucleus (Fig. 5).

In the course of the transplantation, the succinate dehydrogenase activity decreases. whereas an intense reaction of $NADH_2$-tetrazolium reductase appears, especially in the giant cells (Fig. 6). Contrary to these dehydrogenases, the lactate dehydrogenase displays a steady and an intense activity in the cytoplasm of all tumour cells.

DISCUSSION

The most striking differences between the primary tumours and their subsequent intracerebral transplants concern the distribution pattern of acid phosphatase and in the case of the brain tumour of non-specific esterase too. High activity of acid phosphatase and non-specific esterase found in human brain tumours (Schiffer et al. 1965, 1968) and in experimental brain and nerve tumours of the rat (Fabiani et al. 1970) is interpreted by these authors as a sign of regressive changes of the tumour cells. The decreasing survival of the rats in the course of our transplantation experiments, however, points to an undiminished vitality of the tumour cells. The enzyme histochemical alterations observed during the transplantations probably indicate a dedifferentiation of the tumour cells and their adaptation to the surrounding brain tissue.

In the original nerve tumour two morphological and histochemical different kinds of tumour cells occured (Figs. 4 and 5).

Regarding both these results and the polymorphous character of the transplanted neurosarcoma (Fig. 6) a dedifferentiation of the two cell types is possible, on the other hand a selection and an overgrowth of one type cannot be ruled out.

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BADANIA HISTOENZYMATYCZNE WIELOKROTNIE PRZESZCZEPIANYCH GUZÓW MÓZGU I NERWU U SZCZURA

Streszczenie

Przeprowadzone badania miały na celu ustalenie ewentualnych różnic w obrazie enzymatycznym guzów układu nerwowego po wielokrotnych transplantacjach.

Przy pomocy metod histoenzymatycznych autorzy przebadali mięsak śródmózgowy i nerwiako-mięsak nerwu kulszowego, wzbudzone przy pomocy N-metylo-N-nitrozomocznika i kolejne ich przeszczepy śródmózgowe, aż do 21 i 19 pokolenia.

W linii mięsaka śródmózgowego lokalizacja i aktywność kwaśnej fosfatazy i nieswoistej esterazy zmieniały się w pierwszych pokoleniach przeszczepu guza; począwszy od 5 pokolenia pozostawały niezmienione. W przeszczepach guza nerwu kulszowego kwaśna fosfataza wzrastała po 8 pokoleniu, wykazując potem stałe rozmieszczenie ziarnistego enzymu w komórkach guza.

Uzyskane wyniki sugerują pewne zmiany w metabolizmie guzów neurogennych w przebiegu transplantacji, chociaż w obu seriach guzów zarówno fosfataza zasadowa jak i niektóre dehydrogenazy nie wykazywały żadnych zmian wykrywalnych przy pomocy metod histoenzymatycznych.

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ГИСТОЭНЗИМАТИЧЕСКИЕ ИССЛЕДОВАНИЯ МНОГОКРАТНОПРИВИВАНЫХ ОПУХОЛЕЙ МОЗГА И НЕРВА КРЫСЫ

Резюме

Проведенные исследования заключались в установлении возможных разниц в энзиматической картине опухолей нервной системы после многократных трансплантаций.

При помощи гистоэнзиматических методов авторы исследовали внутримозговую саркому и нейросаркому седалищного нерва, вызванных N-метил-N-нитрозомочевиной, а также их внутримозговые трансплантаты вплоть до 21 и 19 генерации.

В линии внутримозговой саркомы локализация и активность кислой фосфатазы и неспецифичной эстеразы изменялись в первых генерациях трансплантата опухоли и оставались неизменными начиная с 5 генерации. В трансплантатах опухоли седалищного нерва кислая фосфатаза увеличивалась после 8 генерации, обнаруживая потом постоянное распределение зернистого энзима в клетках опухоли.

Полученные результаты говорят о некоторых изменениях метаболизма нейрогенных опухолей в процессе трансплантации, хотя в обеих сериях как щелочная фосфатаза так и некоторые дегидразы не обнаруживали никаких изменений, наблюдаемых при помощи гистоэнзиматических методов.

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HISTOENZYMOLOGY OF HUMAN AND EXPERIMENTAL BRAIN TUMOURS: REMARKS ON THE INTERPRETATION OF HYDROLYTIC ENZYME REACTIONS *)

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The histochemistry of hydrolytic enzymes in human cerebral tumours is relatively well known. Generally, activities of these enzymes increase in connection with regressive and catabolic processes, even if other cell conditions have been recognized as responsible for the enzyme variations. The same pictures as observed in human oncotypes can be found in nitrosourea-induced experimental brain tumours of rats; as examples there may be noted: cyst formation in neurinomas and the transformation of isomorphic oligodendrogliomas to polymorphic gliomas. Also in „in vitro" cultures of experimental nitrosourea neurinomas of rats, cell regression is accomplished through an increase of hydrolytic enzymes content. It is to be remarked that these tumours display a formidable growth rate. In the spindle cells a few lysosomes are revealed by intravital staining with acridin orange and by histoenzymological procedures. On the contrary, in regressive cells such as amoeboid cells or microglia-like cells, there is an intense formation of large cytosomes and the enzyme reaction strongly increases, as demonstrated by the previously mentioned methods.

Generally, the granular reaction is interpreted in terms of lysosomal localization of the enzymes, while the diffuse one, which is manifested with diazo-methods, may be attributed either to enzyme release from lysosomes, being in this case indicative of cell regression, or to a newly synthetized enzyme in the endoplasmic reticulum, being in this case indicative of cell progression. The problem is practically that of establishing the exact significance of the diffuse reaction. It should be noted that frequently the diffuse reaction is considered mainly to be an artifact as compared with the „true" reaction, which is granular. Even if new

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electron microscope techniques have furnished some information on this question, most electron microscopy investigations have been carried out almost exclusively on acid phosphatase and by the lead method of Gomori. On the contrary, we know that the diffuse reaction is characteristic of diazo-methods so that, on the grounds of this difference, hypotheses have been put forward that the two methods reveal different enzymes.

In order to contribute to the elucidation of this problem two different experiments on human cerebral tumours were carried out.

At first, beta-glucuronidase was studied because biochemically it has been found mainly as free or soluble activity in cerebral tumours (Allen et al. 1970) and because there is much evidence of its double intracellular localization. Fishman et al. (1969) demonstrated that the coupler diazotized pararosanilin, used for histochemical evidentiation of the enzyme, has an inhibitory effect on the enzyme reaction. This effect is particularly evident on the diffuse reaction and is proportional to the coupler molarity. We utilized the method of Hayashi et al. (1964) with diazotized pararosanilin of various molarities from 0.6 mM to 4.2 mM (substrate naphthol AS-BI beta-D-glucuronide 0.15 mM) on freezing microtome sections from a small block fixed in buffered formalin pH 7.2 with 0.1 M cacodilate and containing 0.88 M sucrose.

We observed that with the decrease of the coupler molarity, the intensity of the histochemical reaction increases. This is particularly true for the diffuse reaction which, with 0.6 mM pararosanilin, becomes clearly evident in the protoplasmic astrocytes of gliomas, in astrocytes of cerebellar spongioblastoma, in astrocyte-like cells of reticular areas of neurinoma, etc. On the contrary, no reaction can be found in medulloblastoma with the obvious exception of pericytes, phagocytes, etc. In particular, a clear-cut difference in glioblastomas is observed between the cell elements of perinecrotic areas, showing a prominent granular and corpuscular reaction (cytolysosomes) and those of the florid areas, showing a prominent diffuse reaction. Between these extreme pictures, there are intermediate stages in which the diffuse reaction becomes at first confined to the Golgi area of the cell and then associated with a granular reaction. Together with the increasing number and volume of the granules, the diffuse staining disappears. This passage from one to another type of the reaction is very interesting if we take into account the situation of the Golgi apparatus regarded on one hand as a receptacle of enzymes produced by endoplasmic reticulum and on the other as a source of lysosomes.

In the second experiment we examined with the electron microscope the intracellular localization of the final reaction product of the Gomori reaction for acid phosphatase in two glioblastomas. At the optical level

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these tumours presented two cell types as far as the character of the enzyme reaction is concerned: one type with a prevailing granular reaction and the other with such a fine granular reaction as to be the closest possible to the diffuse reaction of naphthol methods. Ultrastructurally, three cell types have been identified on the basis of the situation of the final reaction product: the first type consists of small cells with the final reaction product localized in large cytosomes, associated to large vacuoles which occupy almost the entire cytoplasm; the second type consists of large cells with the FRP still localized in cytosomes; the third type consists of large cells with the FRP localized also in the cisternae of endoplasmic reticulum or in other ultrastructures which could correspond to Golgi apparatus. We believe that the enzyme reaction found in the third cell type might correspond to the diffuse reaction of diazo-methods.

Our results not only confirm the hypothesis that the histochemical diffuse reaction may be the expression of protein synthesis, and therefore of a progressive reaction of the cell but also, and above all, they prove that, even if enzyme release from lysosomes cannot be excluded, it is actually the transformation of a diffuse reaction into a granular one which indicates cell regression.

All our findings on hydrolytic enzymes in human as well as in experimental brain tumours are interpretable on the grounds of the prospected hypothesis. The phenomena of cell differentiation, anaplasia and regression are strictly correlated. Cell regression may also be realized independently of the former two phenomena; as is well known, in this case the preexisting lysosomes are utilized.

The problem discussed concerns directly the fundamental question of the interrelation of the cytotypes in cerebral tumours which recently arose again in connection with the heteromorphism of experimental tumours .In view of its importance we feel that this problem deserves further consideration.

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HISTOENZYMOLOGIA LUDZKICH I DOŚWIADCZALNYCH GUZÓW MÓZGU — ZAGADNIENIE INTERPRETACJI ODCZYNÓW ENZYMÓW HYDROLITYCZNYCH

Streszczenie

Autorzy omawiają zagadnienie różnicowania komórkowych zmian wstecznych i postępowych w guzach mózgu.

Badano aktywność kwaśnej fosfatazy i glukuronidazy, enzymów charakteryzujących się dwoistą lokalizacją śródkomórkową i dwojakiego typu odczynem histochemicznym — ziarnistym związanym z lizosomami i rozlanym (zwłaszcza przy

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użyciu metod dwuazowych) stanowiącym wyraz bądź uwalniania się enzymu z lizosomów, bądź też ich lokalizacji w siatce śródplazmatycznej.

W celu wyjaśnienia dwoistości odczynu enzymatycznego badano aktywność glukuronidazy i jej zmiany w zależności od stężenia substratu. Wykazano wzrost natężenia reakcji, zwłaszcza typu rozlanego, przy obniżeniu molarności roztworu barwnika. Zjawisko to występowało jednak w pewnych tylko typach komórek, takich np. jak astrocyty protoplazmatyczne w glejakach, w astrocytach w spongioblastoma cerebelli, czy gwiaździstych komórkach nerwiaków. Nie obserwowano go natomiast w komórkach rdzeniaków. Różnice w charakterze odczynu wykazywały również związek ze stanem biologicznym tkanki nowotworowej — w otoczeniu martwic przeważał ziarnisty typ reakcji, w okolicach bujnego wzrostu dominował odczyn rozlany. Można było również prześledzić komórki, w których odczyn rozlany zanikał na korzyść odczynu ziarnistego.

Badanie mikroskopowo-elektronowe aktywności fosfatazy kwaśnej w wybranych przypadkach glejaków wielopostaciowych pozwoliło na wyodrębnienie 3 typów odczynu: małe komórki ze strątami w dużych cytozomach, duże komórki ze strątami w cytozomach oraz duże komórki ze strątami w zbiornikach siatki śródplazmatycznej lub w strukturach aparatu Golgiego. W przeświadczeniu autorów ostatni typ odczynu może odpowiadać reakcji rozlanej występującej w technikach dwuazowych.

Uzyskane wyniki potwierdzają przypuszczenie, że rozlana reakcja histochemiczna może być wyrazem syntezy białek, stanowiąc wykładnik zmian postępowych w komórce. Przemiana odczynu rozlanego w ziarnisty wskazuje na zmiany wsteczne w komórce. W konkluzji autorzy stwierdzają, że różnicowanie, anaplazja i zmiany wsteczne są zjawiskami ściśle łączącymi się ze sobą.

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ГИСТОЭНЗИМОЛОГИЯ ЧЕЛОВЕЧЕСКИХ И ЭКСПЕРИМЕНТАЛЬНЫХ ОПУХОЛЕЙ; ЗАМЕЧАНИЯ КАСАЮЩИЕСЯ ГИДРОЛАЗ

Резюме

Работа касается проблеммы дифференцирования клеток и дегенеративных и прогрессивных изменений в опухолях мозга. Активность энзимов усиливается в зависимости от дегенеративных и катаболических изменений. Это касается опухолей мозга у людей, экспериментальных опухолей у крыс, вызванных производными нитрозомочевины, и экспериментальных невром в тканевой культуре.

С целью выяснения двойственности энзиматической реакции исследовалась глюкуронидаза по методу Гаяши с применением разных концентраций субстрата. Было обнаружено, что увеличение интенсивности реакции, особенно разлитого характера, зависит от уменьшения молярности раствора красителя. Эта реакция касается протоплазматических астроцитов в глиомах, астроцитов в спонгиобластомах мозжечка, астроцитов в ретикулярных полях невром, но не обнаруживается в паренхмие медуллобластом. Выдающиеся разницы сводятся на пример к грануляционной, цитолизосомальной реакции в районах вблизи некроза и к разлитой реакции, выдающейся в районах обильного роста. Встречаются также переходные виды. Можно наблюдать клетки, в которых

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разлития реакция исчезает в пользу грануляционной реакции. Предполагается, следовательно, что аппарат Гольджи ведет себя или как вместилище энзимов вырабатываемых эндоплазматическим ретикулом или как источник лизозомов.

Исследования 2 злокачественных глиом в световом микроскопе обнаружили 2 типа клеток, с типичной грануляционной реакцией или преципитацией, сходной с продуктом реакции имеющимся в разлитой реакции в нафтоловом методе. В.М.Е. эти клетки обнаруживали 3 типа реакции: малые клетки с преципитатом в больших цитозомах, большие клетки с преципитатом в цитозомах, большие клетки с преципитатом в цистернах эндоплазматического ретикуллома или в структурах, соответствующих аппарату Гольджи. Этот тип соответствует вероятно разлитой реакции в двуазовом методе. Результаты подтверждают гипотезу, что разлитая гистохимическая реакция может быть проявлением синтеза протеинов, и следовательно прогрессивных изменений в клетке. В этом свете дифференцирование, анаплазия и дегенеративные изменения тесно соприкасаются.

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H. KROH

MORPHOLOGICAL AND ENZYMATIC CHARACTERISTICS OF EXPERIMENTAL CEREBRAL TUMOURS IN MICE*)

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Morphological studies of experimental brain tumours in mice in general point to their similarity with human spontaneous tumours.

The paper presents the results of experiments carried out to investigate some enzymatic properties of chemically induced brain tumours in mice in order to compare them with their human counterparts. The study was carried out on about 100 tumours induced by methylcholantrene implantation into cerebral hemisphere of inbred mice C_3H, R III, and DBA.

Besides routine histological staining, reactions for following hydrolases: alkaline and acid phosphatase, non-specific esterase, for following oxidazing-reducing enzymes: lactic, succinic and glucoso-6-phosphate dehydrogenase and also for aminopeptidase, were performed. The particulars concerning applied methods are described in previous papers (Kroh 1969, 1970a, 1970b, Kroh, Szumańska 1971).

RESULTS

All the tumours were morphologically classified as gliomas, sarcomas and as mixed tumours — gliosarcomas. Three types of gliomas, i.e. oligodendrogliomas, astrocytomas of different types and glioblastomas and few cases of ependymomas were observed.

Among these cerebral tumours there were some of „pure" and some of „composite" character.

Complete lack or marked decrease of alkaline phosphatase activity in vascular walls is observed in all examined types of gliomas except astroblastoma.

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Activity of acid phosphatase varies from hardly visible to prominent in the cytoplasm of neoplastic cells independent on the type of cell or tumour (Fig. 1).

Non-specific esterase activity in general coincides with the latter. Only the ependymomas exhibit rather uniformly distributed low cytoplasmic activity.

Lactic dehydrogenase activity in all classified and unclassified gliomas is markedly increased in the cytoplasm of neoplastic cells as compared with cellular elements of brain tissue (Fig. 2).

Glucose-6-phosphate activity in cytoplasm of investigated astrocytomas and ependymomas of various degrees of differentiation is of lesser intensity (Fig. 3), when succinic dehydrogenase activity is very low or entirely lacking.

The low activity of aminopeptidase is noted only in the cytoplasm of oligodendroglioma cells.

The intermediary gliosarcoma group is divided further according to the proportions of different types of tissues present. There are the gliomas with the neoplastic proliferation of vascular walls, the tumours with equal participation of elements of glial and mesodermal origin and the sarcomas with concomitant neoplastic glial proliferation (Fig. 4).

The activity of alkaline phosphatase in all vessels is absent or markedly decreased in all glial or mesodermal parts of tumours.

The acid phosphatase activity is characterized by marked differences in intensity and localization, in cells of both origins.

The non-specific esterase reveals an almost analogous picture.

On the basis of our experience with extracranial gliosarcomatous parts of intracerebral gliomas, high activity of lactic dehydrogenase is always found, though various cells differ in intensity, almost absent succinic dehydrogenase activity and intermediate glucose-6-phosphate dehydrogenase activity.

Four groups of brain sarcomas were distinguished: fibrosarcomas of the dura mater, endothelial meningosarcomas, osteosarcomas and monstrocellular sarcomas of the brain.

Alkaline phosphatase activity in vascular walls is very much decreased in all sarcomas, as compared to normal brain vessels, especially in monstrocellular sarcomas.

Osteosarcoma shows remarkable high cytoplasmic activity.

Acid phosphatase differs markedly in intensity from cell to cell (Fig. 5). Monster cells do not exhibit any activity of this enzyme.

The distribution of non-specific esterase does not show any distinctive features and differs in intensity in all sarcomas.

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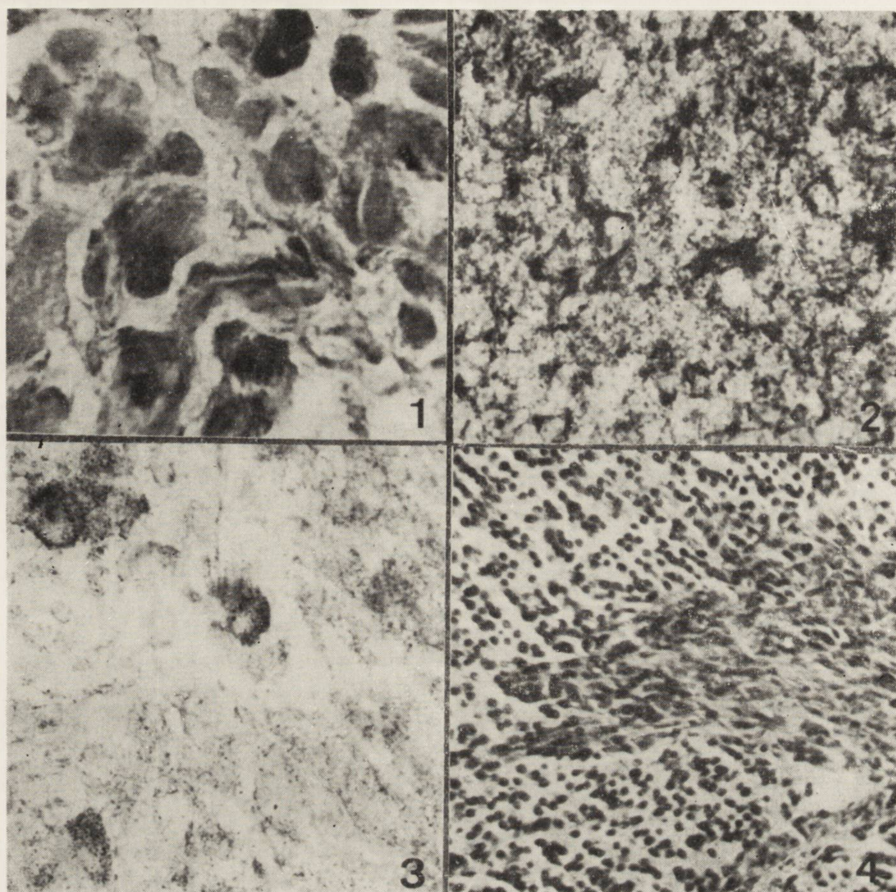

Fig. 1. Astrocytoma III°. Non uniform cytoplasmatic distribution of acid phosphatase activity. × 800.

Fig. 2. Astrocytoma II°. High lactic dehydrogenase activity. × 400.

Fig. 3. Astrocytoma II/III°. Low, non uniform activity of glucose-6-phosphate dehydrogenase. × 400.

Fig. 4. Gliosarcoma. Representation of tissues of glial and mesodermal origin. HE. × 200.

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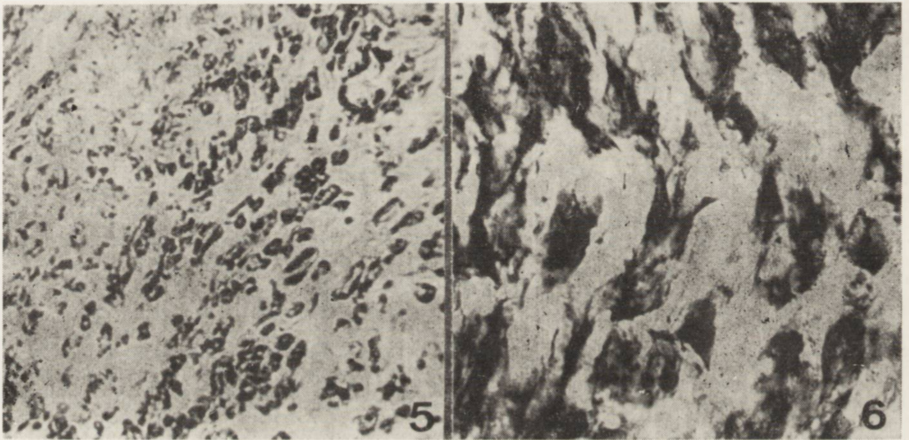

Fig. 5. Fibrosarcoma. Non uniform distribution of acid phosphatase activity. × 65.

Fig. 6. Fibrosarcoma. High activity of lactic dehydrogenase in the cytoplasm and processes of tumor cells. × 400.

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The activity of lactic dehydrogenase is high in fibrosarcomas and monstrocellular sarcomas without preference in particular types of cells (Fig. 6).

Both fibrosarcomas and monstrocellular sarcomas exhibit low activity of glucose-6-phosphate dehydrogenase.

Succinic dehydrogenase activity in both above mentioned types of neoplasms is completely absent in most cells.

Aminopeptidase activity is noted in the cytoplasm of dispersed singular cells or nests of fusiform cells. Some more regular increase in aminopeptidase activity can be observed in the cells of the periphery of tumours.

DISCUSSION

In the material investigated, neoplasms were encountered which resembled those found in human central nervous system. As far as their origin is concerned they are either „pure" or „mixed".

Mixed character of tumours is connected probably with simultaneous action of carcinogen on the tissue of different origin. The more rapid proliferation of one type of neoplastic tissue or the induction of one type of neoplasm by another is to be also considered. The same applies to the „composite" gliomas (Rubinstein 1964, Kroh 1970 a, b).

The morphological structure of induced gliomas and sarcomas shows remarkable similarities to the structure of spontaneous human tumours. This refers both to the development of primary structures and to the secondary structures.

Morphological similarity to the human tumours does not fully reflect on the enzymatic properties, especially in view of different opinions concerning histochemistry of human tumours.

Constant decrease of alkaline phosphatase activity in the vascular walls is perhaps related to deep disturbances in the blood-brain barrier (Meier-Ruge 1966).

The observations of acid phosphatase activity differ from the observations of other authors concerning the intensity of reaction in particular types of tumours. It seems that there is a lack of typical constant pattern of distribution of this enzyme in experimental gliomas and sarcomas.

As far as non specific esterase is concerned the tumors do not show any special patterns.

The activity of aminopeptidase observed mainly in sarcomas is in some cases localized in the periphery of tumours, in others in dispersed cells.

The increase of the lactic dehydrogenase activity in the cells of all types of tumours as well as the decrease or lack of succinic dehydrogenase

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activity probably indicates only the changed metabolism of neoplastic cells in the direction of anaerobic respiration (Kroh, Szumańska 1971). These observations are in agreement with many authors opinions concerning the activity of investigated enzymes in spontaneous human tumours (Nasu, Muller 1964).

H. Kroh

MORFOLOGICZNE I ENZYMATYCZNE CECHY DOŚWIADCZALNYCH GUZÓW MÓZGU MYSZY

Streszczenie

Przedstawiono wyniki badań morfologicznych i enzymatycznych 100 doświadczalnych nowotworów mózgu wywołanych metylocholantrenem.

Poza rutynowym badaniem histologicznym przeprowadzono reakcje enzymatyczne dla następujących enzymów: fosfataza zasadowa i kwaśna, esteraza nieswoista, aminopeptydaza, dehydrogenaza mleczanowa, bursztynianowa i glukozo-6-fosforanu.

Nowotwory zostały sklasyfikowane jako glejaki, mięsaki, i glejako-mięsaki. Wśród glejaków obserwowano skąpodrzewiaki, różnego typu gwiaździaki, glejaki wielopostaciowe i wyściółczaki, a wśród mięsaków — włókniako-mięsaki opony twardej, oponiako-mięsaki, kostniako-mięsaki i mięsaki olbrzymiokomórkowe.

Obserwowane nowotwory wykazywały znaczne podobieństwo morfologiczne do odpowiednich samoistnych nowotworów mózgu człowieka, dotyczące tak struktur pierwszorzędowych jak i drugorzędowych. Zarówno glejaki jak i mięsaki wykazywały podobne cechy enzymatyczne. Fosfataza zasadowa występowała wyłącznie w komórkach kostniako-mięsaka, natomiast stała aktywność tego enzymu w ścianach naczyń w tkance prawidłowej ulegała we wszystkich rodzajach nowotworów zanikowi lub znacznemu obniżeniu, co wiąże się z zaburzeniami przepuszczalności naczyń. Aktywność fosfatazy kwaśnej wykazywała duże wahania nasilenia i lokalizacji i nie posiadała cech charakterystycznych dla żadnego rodzaju guza. Aktywność dehydrogenaz we wszystkich guzach wykazywała bardzo wysokie nasilenie dehydrogenazy mleczanowej, mierne glukozo-6-fosforanu, śladowe dehydrogenazy bursztynianowej. Takie nasilenie aktywności dehydrogenaz prawdopodobnie wskazuje na zmieniony metabolizm komórek nowotworowych w kierunku oddychania beztlenowego.

Г. Крох

МОРФОЛОГИЧЕСКИЕ И ЭНЗИМАТИЧЕСКИЕ СВОЙСТВА ЭКСПЕРИМЕНТАЛЬНЫХ ОПУХОЛЕЙ МОЗГА МЫШИ

Резюме

Представлены результаты морфологических и энзиматических исследований 100 экспериментальных опухолей мозга, вызванных метилхолантреном.

Кроме обыкновенного гистологического исследования проводились энзиматические реакции для следующих энзимов: щелочная и кислая фосфатазы,

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неспецифическая эстераза, аминопептидаза, лактатдегидрогеназа, сукциндегидрогеназа и дегидрогеназа глюкозо-6-фосфата.

Опухоли класифицировались как глиомы, саркомы и глиосаркомы. Среди глиом наблюдали олигодендроглиомы, всякого типа астроцитомы, полиморфные глиобластомы и эпендиомы, а среди сарком — фибросаркомы твердой мозговой оболочки, менингиомы, остеосаркомы и гигантоклеточные саркомы.

Наблюдаемые опухоли обнаруживали значительное морфологическое сходство к соответствующим спонтанным опухолям мозга человека, касающееся как перворазрядных так и второразрядных структур. Как глиомы так и саркомы обнаруживали подобные энзиматические свойства. Щелочная фосфатаза находилась исключительно в клетках остеосаркомы, в то время когда постоянная активность этого энзима в стенках сосудов нормальной ткани подвергалась во всех видах опухолей исчезновению или значительному снижению, что связывается с нарушениями проницаемости сосудов. Активность кислой фосфатазы обнаруживала большие колебания интенсивности и локализации и не имела характерных свойств ни для одного вида опухоли. Активность дегидрогеназ во всех опухолях обнаруживала очень высокую интенсивность лактатдегидрогеназы, умеренное — глюкозо-6-фосфата и следовое — сукциндегидрогеназы. Такая интенсивность активности дегидрогеназ вероятно указывает на измененный метаболизм опухолевых клеток в направлении анаэробного дыхания.

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SOME HISTOCHEMICAL STUDIES ON MEDULLOBLASTOMAS WITH REFERENCE TO THEIR LOCALIZATION

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Medulloblastoma is characterized in classical descriptions as a tumour of childhood, located in the midline of the cerebellum and leading to a rapid lethal end. However, tumours located in the cerebellar hemisphere are reported in adolescents and young adults. They are well circumscribed, abound in reticulin fibers and have a better prognosis when successfully extirpated. „The circumscribed arachnoid sarcoma of the cerebellum" (Foerster, Gagel 1939) gave rise to different opinions among the neuromorphologists with regard to its nosologic differentiation. Some authors (Kernohan, Uihlein 1962) consider it as a cerebellar fibrosarcoma, while others (Rubinstein, Northfield 1964) believe it to be a variety of medulloblastoma and call it „desmoplastic" type.

The purpose of the present paper was to compare the localization of the classic and „desmoplastic" types of medulloblastoma and their histological and histochemical characteristics.

Eighteen tumours were studied; histochemical studies were performed in 10 of them. The methods applied were: 1) routine histological: hematoxylin-eosin stain and stainings according to Gomori, Nissl, Bodian, Holzer, Weil-Davenport; 2) histochemical: for oxidoreductases (succinic-, malic-, isocitric-, lactic-, glutamic-, glycero-phosphate- (NAD and menadion), glucose-6-phosphate dehydrogenases, NAD- and NADP-diaphorases); for hydrolytic enzymes (acid phosphatase, nonspecific esterase, beta-glucuronidase, N-acetyl-beta-glucosaminidase, alcaline, phosphatase, adenosine triphosphatase, phosphorylase, aldolase); in addition histochemical reactions for lipids and glycogen were performed.

The localization of the cerebellar neoplasms is presented according to operative and autopsy findings. Twelve of the tumours were located in

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the vermis and spread towards the IV ventricle and/or the cerebellar hemispheres (2 of them reached the cervical spine while a third one involved the dural sinuses as well). The other 6 tumours were located in the cerebellar hemisphere as a circumscribed node or they infiltrated the brain stem and the meninges.

The hematoxylin-eosin staining and that for reticulin fibres showed in 9 tumours (7 located in the vermis and 2 in the cerebellar hemisphere) the typical picture of medulloblastoma. The other 9 tumours (5 located in the vermis and 4 in the cerebellar hemisphere) corresponded to the „desmoplastic" type of Rubinstein and Northfield. In these tumours bundles of reticulin fibers enclosed islands or strands of cells (Fig. 1) which did not differ from the cells of the classic type of medulloblastoma. No relationship was established between the gross appearance of the tumour and the content of reticulin fibers. The latter were observed both in a well circumscribed tumours located deeply in the cerebellar hemisphere as well as in tumours infiltrating the IV ventricle, the brain stem, the cerebellar hemisphere, the meninges. Histochemical tests for oxidoreductases showed no differences in the intensity, character or distribution of the reactions among the tumoral cells of classic type and the cells constituting islands and strands of „desmoplastic" type. The oxidoreductase reactions were weaker in the bundles of reticulin fibers when compared to those in the islands and were concentrated mainly around the lighter, elongated nuclei among the fibers. Neither was any difference found between the two types of medulloblastomas in the activity of lysosomal enzymes. The reactions for nonspecific esterase and acid phosphatase were weak, diffuse in the tumoral parenchyma of classic type and in the islands of „desmoplastic" type. The reactions for beta-glucuronidase and N-acetyl-beta-glucosaminidase were almost negative. There were scattered cells which showing no difference in size from the rest of the parenchymal cells revealed a more intensive reaction for all four lysosomal hydrolases (Fig. 2) especially for nonspecific esterase. The reactions for glycogen and phosphorylase were negative in the tumoral parenchyma of most medulloblastomas. This was very clear in the „desmoplastic" type where, in contrast to the negative reaction in the tumour cell islands, the bundles of reticulin fibers showed a granular and comparatively intensive diffuse reaction. Quite a reverse picture was observed after staining with Sudan black for lipids. The tumoral cells gave a dust-fine granular reaction while there was none in the bundles of reticulin fibers. A positive reaction for alkaline phosphatase was found in the parenchyma of four medulloblastomas, irrespective of their type.

The total data from the hematoxylin-eosin stain and the staining methods for neuronal and glial elements (Nissl, Bodian, Weil-Davenport,

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Holzer) showed that in 5 of the tumours (3 located in the vermis and 2 in the cerebellar hemisphere) there were features of neuronal and/or glial differentiation. Neurons with unequal differentiation were found, their cytoplasm showed intensive or moderate diffuse basophilic coloration. Intracytoplasmic or axonal neurofibrils were found in one of the two tumours where these cells were present (Fig. 3a). The activity of all enzymes representing oxidizing-reducing group was high and located both in the cellular bodies and at the beginning of the axons (Fig. 3b). These cells stood out among the rest of the parenchyma with moderate, very fine-granular or diffuse reaction in the tests for lysosomal enzymes. We could not identificate them in the reactions for glycogen and phosphorylase, while Sudan black B stained them diffusely blue. Cells with morphological features of glial elements were observed in 3 medulloblastomas (1 located in the vermis and 2 in the cerebellar hemisphere). They were astrocyte-like cells, the size of which and location provided no reasons to doubt that they were not local hypertrophied astrocytes (Fig. 4a). In addition we found in one of the tumours an area with features of oligodendrocytoma. The Weil-Davenport stain proved the glial origin of these cells. They exhibited apparently higher oxidoreductase and hydrolase activity than the rest of the parenchyma (Fig. 4b), even for enzymes which were but little pronounced as a rule.

In 2 medulloblastomas (1 classic and 1 „lesmoplastic" type) on the background of cellular population characteristic for these tumours, patchy areas with apparent polymorphism were found of the first place. This polymorphism concerned cellular nuclei; bi- or multinuclear cells, monstrous nuclei, a great number of normal and abnormal mitotic figures were present therein. The oxidoreductase reactions in these patchy areas were characterized by diversity in the particular cells and thus they contrasted with the isomorphic pattern in the rest of the tumour parenchyma (in the same tumour) and in other medulloblastomas. The intensity of the reactions was not dependent on the size of the cellular nucleus (Fig. 5). This histochemical polymorphism was more evident in the activity of mitochondrial form of glycerophosphate dehydrogenase than in the enzymes of the Krebs cycle. Some of the larger cells having irregular nuclei stood out among the rest and showed a more intensive reaction for acid phosphatase, nonspecific esterase and aldolase. These cells were fully negative when tested for glycogen and phosphorylase.

In 2 classic type medulloblastomas (located in the vermis) scattered or clustered pseudoxanthomatous cells with large diformazan granules in nearly all oxidoreductases, with an intensive coarse granular reaction for the lysosomal enzymes and with high lipid content were observed. In general, the vascular component was slightly marked in both types

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of medulloblastomas but in 2 tumours (1 classic and 1 „desmoplastic") there were some regions with haemangioma appearance. The vessels irrespective of their caliber, showed a rather higher activity than the tumoral cells when tested for glucose-6-phosphate dehydrogenase and the NADP-diaphorase.

DISCUSSION

The results of our study showed that there was no direct correspondence between the localization of the tumours and their histological structure. In reality, the greater part of the classic-type medulloblastomas of our material were located in the vermis while the „desmoplastic" ones were almost evenly distributed in the cerebellar hemispheres and the midline. The lack of correlation between the localization of the medulloblastomas, their histological type and the postoperative survival terms has been pointed out by Miles and Bhandari (1970). We found no difference between the tumoral cells of the classic-type medulloblastoma and the cells constituting islands in the „desmoplastic" type when applying standard histological methods. Neither was any difference observed when histochemical methods were used. Thus, our results fully support the concept of Rubinstein and Northfield (1964) that the „circumscribed arachnoid sarcoma of the cerebellum", described by Foerster and Gagel is in fact a variety of medulloblastoma. Its characteristic difference from the classic type of medulloblastoma — the presence of reticulin fiber bundles — is attributed by Rubinstein and Northfield (1964) to the early invasion of the leptomeninges and the unclarified properties of some tumours, medulloblastoma being one of them, to stimulate the proliferation of connective tissue. Another argument in favour of the concept of Rubinstein and Northfield (1964) is the presence of features of neuronal and glial differentiation in some of the studied medulloblastomas, both classic and „desmoplastic". This finding is related to the histogenesis of medulloblastoma, a point at issue much discussed lately (Gullotta 1966, 1967; Kane, Aronson 1967; Kreuzberg, Gullotta 1967; Matakas et al. 1970; Monticone 1965; Müller 1967; Polak 1967; Viale, Candia 1967; Voigt 1968; Zülch, Wechsler 1968), but we shall not go into details in the present paper. We would like only to draw attention to the rather isomorphic not only histologic but also histochemical characteristics of the cells exhibiting neuronal and glial differentiation, irrespective of the type, localization or gross appearance of the medulloblastomas.

Kernohan and Uihlein (1962) associated the presence of reticulin fibers with their origin from the sarcomatous tumoral cells, and the islands,

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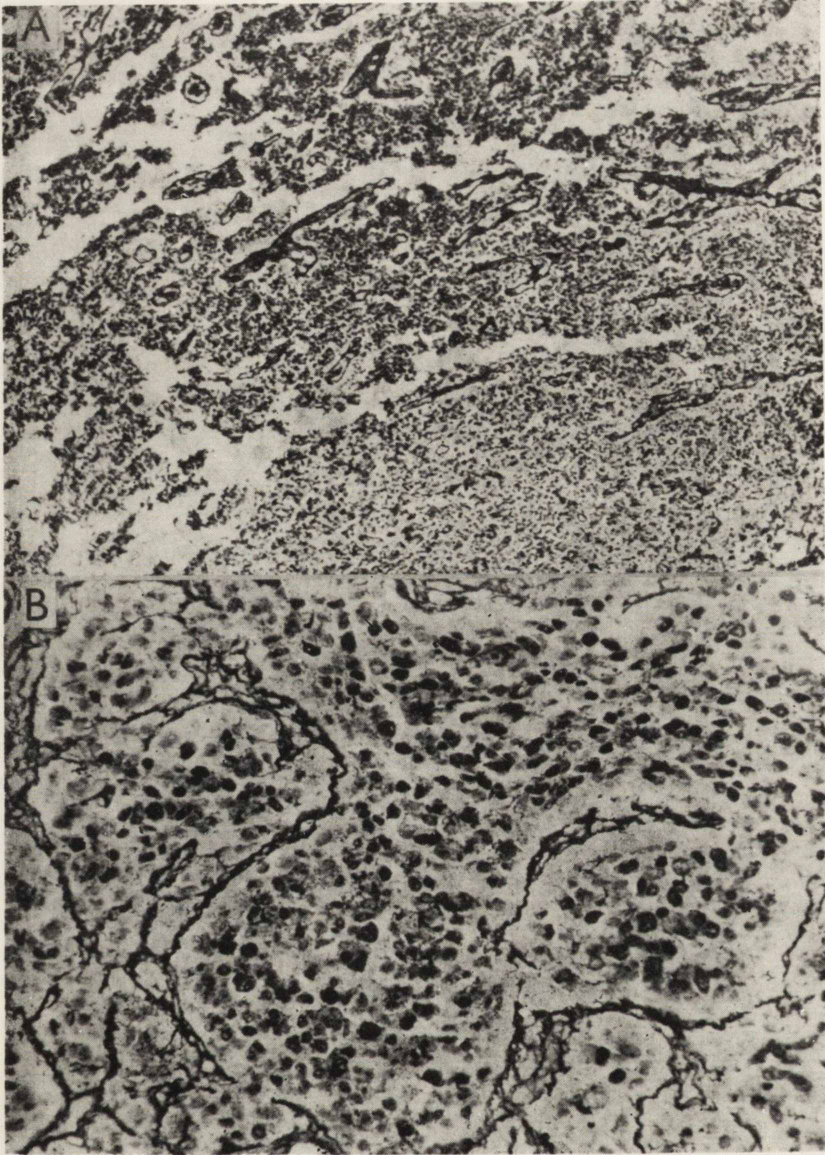

Fig. 1. Reticulin fibres staining according to Gomori. A) Classic type of medulloblastoma: reticulin fibers are seen only in the vascular wall, × 63. B) „Desmoplastic" type of medulloblastoma: the bundles of reticulin fibers enclose islands of cells, × 157.5.

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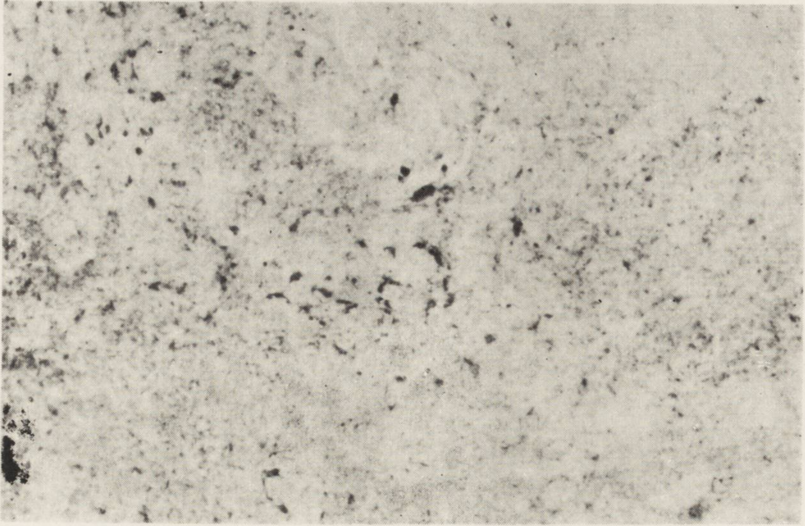

Fig. 2. Acid phosphatase activity: a more intensive reaction is seen in the pericytes, the perivascular and some scattered parenchymal cells. × 63.

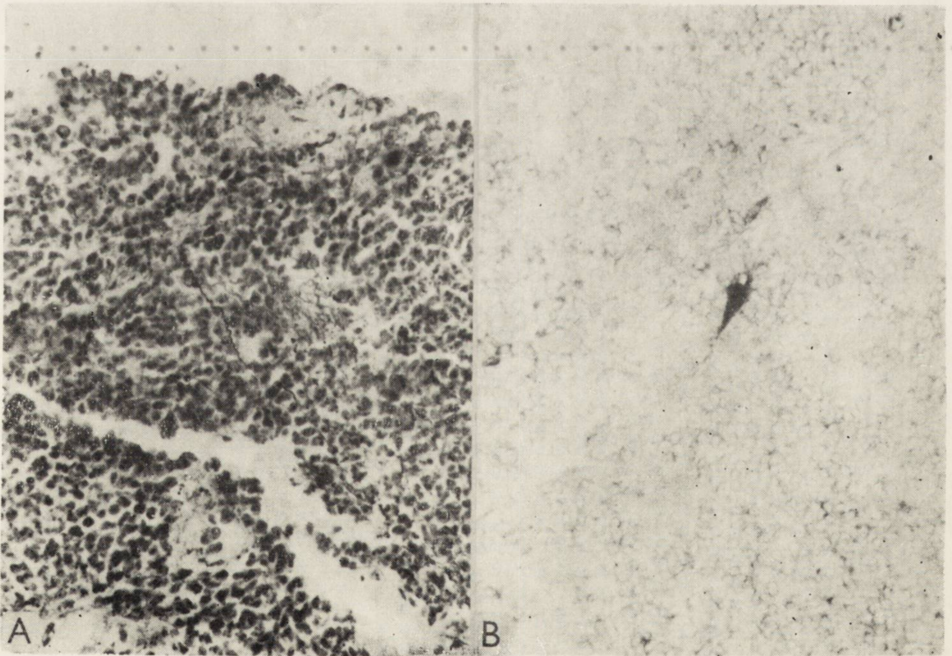

Fig. 3. A) Neurofibrils stained according to Bodian, × 252. B) NAD-diaphorase activity. Cells with neuronal differentiation are prominent. × 157.5.

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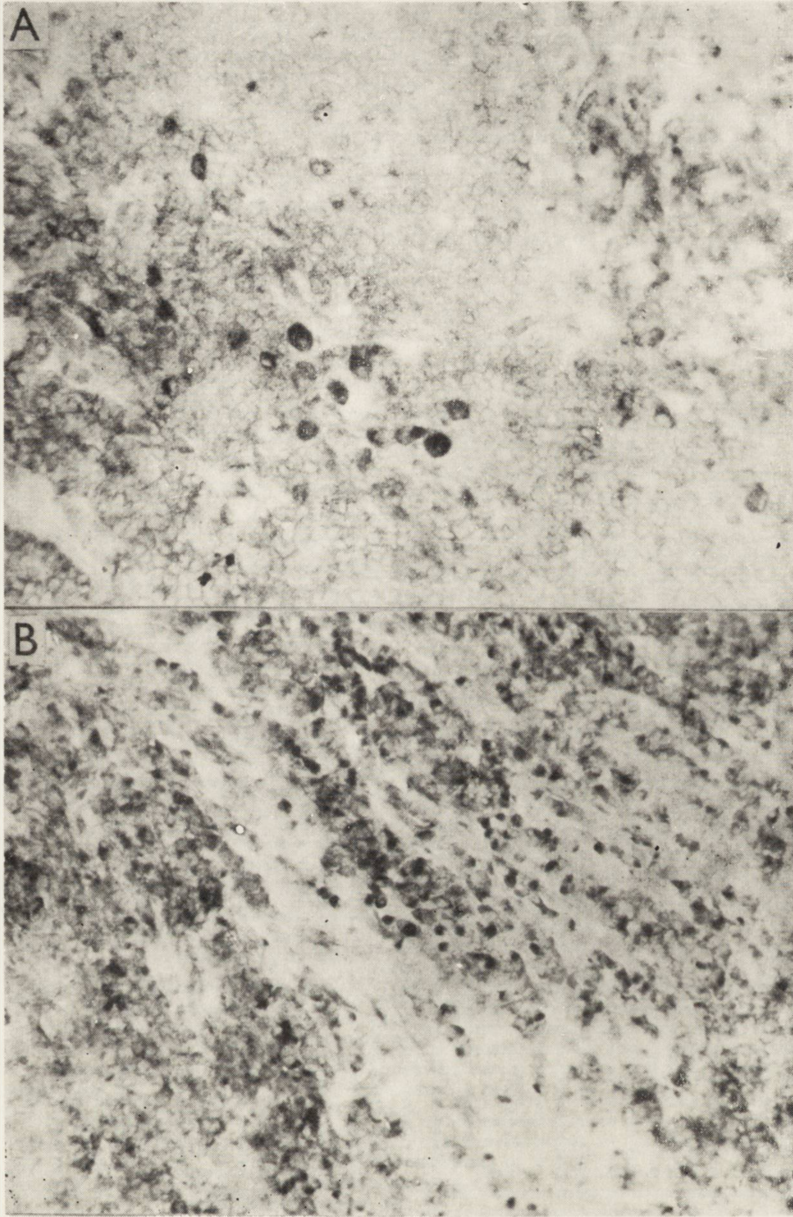

Fig. 4. A) Glutamic dehydrogenase activity is intensive in the cells showing glial differentiation. B) Glutamic dehydrogenase activity, high activity in the oligodendrocytelike cells, 157.5.

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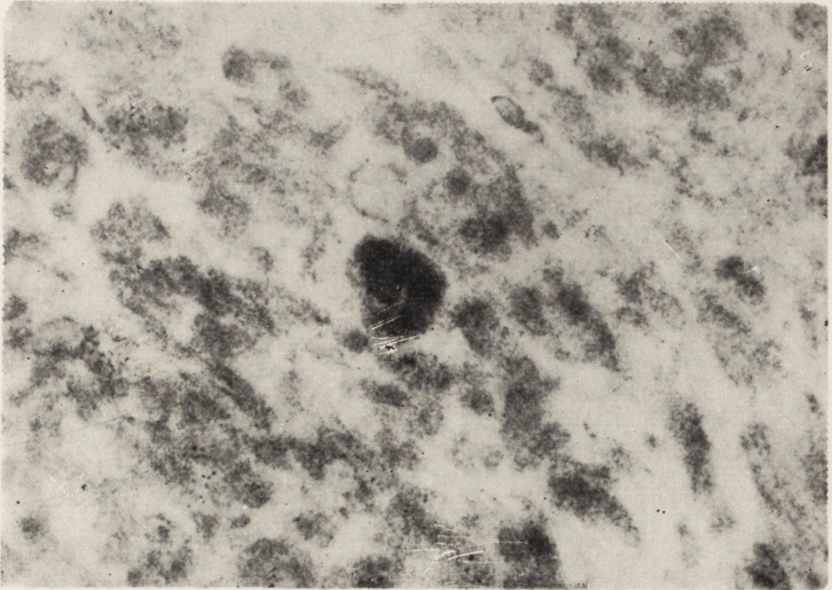

Fig. 5. Lactic dehydrogenase activity. Cells with high activity are prominent in the polymorphous areas.

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according to them, were foci of degeneration where the reticulin fibers were torn. Our histochemical studies showed that the reticulin fiber bundles and the cells among them differ from the cells in the islands. In addition, the intensity and character of the reactions both for oxidative and lysosomal enzymes demonstrated no degenerative changes and differed from those in the edematous parts and foci of microdestruction.

There exists a hypothesis that the „desmoplastic" medulloblastomas are mixed tumours. Schenk (1966) presented a case where (according to him) the prominent cellular and nuclear polymorphism, the atypical fusiform and giant cells, and the abundance of mitoses were indicative of sarcoma. We found 2 medulloblastomas with foci of polymorphism, but one of them was of classical type, with no reticulin fibers present. Similar foci were observed in patients after X-ray therapy (Oppenheimer 1969) in tumors which later showed destruction. Pleomorphism in these tumours was considered as a reaction for the radiation. We are inclined to admit such an explanation of the finding, although no X-ray therapy was applied in our cases.

I. Lolova, A. Ivanova, S. Bojinov, V. Christov

HISTOCHEMICZNE BADANIA RDZENIAKÓW O RÓŻNEJ LOKALIZACJI

Streszczenie

Autorzy przeprowadzają korelację pomiędzy danymi histologicznymi i histochemicznymi, a wiekiem i umiejscowieniem rdzeniaków móżdżku w 18 przypadkach. Do oceny aktywności enzymów oksydo-redukcyjnych i hydrolitycznych, lipidów i glikogenu zastosowano klasyczne techniki histologiczne i metody histochemiczne.

Wyniki badań histologicznych i histochemicznych nowotworów linii środkowej i półkul móżdżku dotyczą ogólnej budowy, elementów komórkowych, typowych rozet, obecności włókien retikulinowych, związku z oponami, obecności atypowych form komórkowych i mitoz.

Dziewięć rdzeniaków wykazywało typową budowę morfologiczną, 9 zaś przedstawiało typ desmoplastyczny, charakteryzujący się dużym udziałem włókien retikulinowych, otaczających wysepki typowych komórek. Większość nowotworów robaka miała klasyczną budowę morfologiczną, natomiast rdzeniaki półkul miały charakter desmoplastyczny.

Aktywność enzymów oksydo-redukcyjnych i hydrolaz nie wykazywała różnic w charakterze, nasileniu, czy lokalizacji w obu typach nowotworów. Prawie we wszystkich przypadkach obu typów guzów odczyny na glikogen i fosforylazy były ujemne w parenchymie, podczas gdy włókna retikulinowe wykazywały reakcję ziarnistą i rozlaną. Barwienie na lipidy Sudanem czarnym wykazywało reakcję dodatnią w komórkach miąższu nowotworów.

Podobieństwo morfologiczne i histochemiczne obu typów nowotworu przemawia za ich wspólnym pochodzeniem wbrew sugestiom innych autorów.

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И. Лолова, А. Иванова, С. Божинов, В. Христов

ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ МЕДУЛЛОБЛАСТОМ С РАЗНОЙ ЛОКАЛИЗАЦИЕЙ

Резюме

Авторы приводят корреляцию между гистологическими и гистохимическими данными а возрастом и локализацией медуллобластом мозжечка в 18 случаях. В оценке активности окислительно-восстановительных и гидролитических энзимов, липидов и гликогена применялись классические гистологические техники и гистохимические методы. Результаты гистологических и гистохимических исследований опухолей срединной линии и полушарий мозжечка касаются общего строения, клеточных элементов, типичных розет, наличия ретикулиновых волокон, связи с оболочками, наличия атипичных клеточных форм и митозов.

Девять медуллобластом обнаруживали типичное морфологическое строение, а 9 представляли собой десмопластический тип, характеризующийся большим участием ретикулиновых волокон окружающих островки типичных клеток. Большинство опухолей червя имела классическое морфологическое строение, в то время когда медуллобластомы полушарий были десмопластического характера. Активность окислительно-восстановительных энзимов и гидролаз не обнаруживали разниц в характере, интенсивности или локализации в обоих типах опухолей. Почти во всех случаях обоих типов опухолей реакции на гликоген и фосфорилазу были отрицательными в паренхиме, тогда как ретикулиновые волокна обнаруживали зернистую и разлитую реакцию. Окрашивание на липиды суданом черным обнаруживало положительную реакцию в клетках паренхимы опухолей.

Морфологическое и гистохимическое сходство обоих типов опухолей говорит за их общим происхождением вопреки предположениям других авторов.

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J. KAŁUŻA, H. SZYDŁOWSKA

A COMPARATIVE HISTOCHEMICAL STUDY ON THE FUNCTIONAL GROUPS IN PROTEINS AND SOME OXIDIZING-REDUCING ENZYMES IN REACTIVE GLIA AND GLIAL TUMOURS

I. GLIAL TUMOURS

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Contrary to the reactive glia, the glial tumours display a significant differentiation of the cytoenzymatic activity, depending on the cellular content and the degree of anaplasia (Kałuża 1970, Schiffer et al. 1965, Smith 1963). Among the enzymes of the Krebs and the Embden — Meyerhoff — Parnas cycle, which exhibit essential differentiation of their activity, the NADH-dependent tetrazole reductase and the lactate dehydrogenase (LDH) occupy a particular position.

The biological studies revealed a significant LDH content in the neoplastic tissue. In the normal tissue, 30 — 95% of ^{14}C-glucose is metabolized into lactic acid within 30 sec. — 8 min. following intravenous injection. However, the transfer of the carbon isotope from lactic acid into other substances occurs in the normal tissue much more extensively than in the neoplastic one. According to Busch et al. (1962), the amount of lactic acid metabolized in the neoplasm makes less than 21% of that in the normal tissue. On the other hand, Zamecnik et al. (1951) proved that the rate coefficient for the incorporation into proteins of the glucose carbon is ten times higher in hepatoma than in the normal liver and the amino acids connected with individual links in the Krebs cycle exhibit a specific activity which is higher in tumours than in the normal tissue.

Studying the differences between the enzymatic activities in various cell groups in glial tumours, we were able to observe a certain correlation between the enzymatic activity and the cytoplasm/nucleus ratio, similar to that found in the studies on functional groups in proteins (Szydłowska 1970a, 1970b). We therefore decided to study comparatively the oxidizing-reducing enzymes and the functional groups in proteins.

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MATERIALS AND METHODS

Materials, methods as well as the techniques applied for histoenzymatic reactions and reactions on functional groups in proteins were as described in the article on reactive glia (Szydłowska, Kałuża 1972). A part of the samples of glial tumours used in histoenzymatic studied were preincubated 3 hrs in a glucose enriched Parker solution (glucose concentration — 0.01 M) since — as could be concluded from studies by other authors — glucose is very quickly metabolized in the normal tissue, in brain by about 80%. This modification, however, remained without effect on the intensity of the *in vitro* cytoenzymatic reaction in the neoplastic tissue.

RESULTS

Among the examined enzymes, the LDH (Figs. 1a, 1b) and the NADH — dependent tetrazolium reductase (Figs. 2a, 2b) exhibited an extremely high activity. NADPH- dependent tetrazolium reductase, although less active than the above two enzymes, turned out to be more active than the remaining ones.

Intensity of the enzymatic activity is in the first instance connected with the change in cytoplasm/nucleus ratio. For that reason tumours with a high degree of anaplasia, and thus with a significant cellular polymorphism, appeared the most suitable for our studies, since there the cytoenzymatic differentiation as well as that of functional groups in proteins was strongly marked (Figs. 3a, 3b, 3c, 4a, 4b, 4c).

The high enzymatic activity is characteristic for cytoplasm rich cells, such as various gemistocyt-like forms and giant cells (Figs. 1b, 2b). These cells distinguish themselves by their high LDH and NADH — dependent tetrazolium reductase activity, although the other enzymes also exhibited a relatively higher activity that in the remaining cells. Majority of these cells displayed significant amounts of functional protein groups. Gemistocytes of gemistocytomas and multiform glioblastomas are rich in all functional groups (Figs. 3a, 3b, 3c, 4a, 4b, 4c). An uniformity of protein group reactions turned out to be a general feature of large gemistocytes and giant cells. The surface layers of the cytoplasm exhibit a strong reaction, whereas the cytoplasm inside the cell reacts very weakly. Sometimes intensification of the reaction occurs at one of the cell poles.

The cells poor in cytoplasm are characterized by a low activity of all enzymes but LDH and NADH — dependent tetrazolium reductase, which are relatively more active than the other enzymes (Figs 1a, 2a). Changes in the content of functional groups take place almost exclusively in

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nuclei and are expressed by an increase in the amount of these groups, especially by that of SH groups.

A variety of intermediate reactions exists between the extremely strong histoenzymatic reaction in the cytoplasm-rich cells and an extremely weak one in the cytoplasm-poor cells. The mutual relationships between the functional groups of proteins in nuclei of these cells also undergo several transformations (Figs. 3a — 5c).

Among the group of intermediate intensity of histoenzymatic reaction, there can be numbered: fibrillary astrocytomas, especially those localized in cerebellum and rarely occuring oligodendrogliomas without astrocytic component or those exhibiting features of anaplastic growth.

Characteristic for all types of examined tumours, however, irrespective of the degree of cellular polymorphism, was the low activity of glutamate dehydrogenase. Only in highly differentiated, diffuse astrocytomas (Fig. 5d), an increase in activity of these enzymes could be noted less significant, however, than that of LDH. The glutamate dehydrogenase activity was relatively higher as compared with that of succinate dehydrogenase.

DISCUSSION

The histochemical correlation between the activity of oxidizing-reducing enzymes and the presence of terminal protein groups in glial tumours was difficult to establish. No correlation was found even between the high activity of LDH or NADH — dependent tetrazolium reductase and the increase in functional groups in proteins of either the cytoplasm — rich cells or cells localized in necrotic regions, where the tissue desintegration is significant and connected with the rise in level of terminal groups. Similarly, no results were obtained as to the correlation between the weak activities of the remaining enzymes like SDH, MDH and GDH, and the changes in the content of terminal groups in proteins.

The amount of SH groups does not depend upon the enzyme activity. The rise in level of these groups was observed paralelly with the development of tumour anaplasia and occured first of all in nuclei, particularly in nuclear membranes, preferentially in malignant tumours, in the cells of the front of tumour growth. No results, however, were obtained indicating any correlation between the number of SH groups and the rate of neoplastic growth (Bahr, Moberger 1958, Müller 1966, Müller et al. 1958).

In most of the cytoplasm-rich cells, however, we were able to detect an increase in N-terminal α-amino groups and a positive reaction for COOH groups, not only in the cell — body, but also in some processes.

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The question as to the way, in which the tumour tissue metabolises considerable amounts of lactic acid, remains unanswered. The rise in level of SH groups in nuclei of individual cytoplasm — rich cells may be indicative for the intensification of synthetic processes, since these groups are connected with DNA and RNA synthesis (Barron 1953, Bell 1958, Gieorgiew et al 1960), with the transport of RNA from the nucleus to the cytoplasm (Sirlin 1960, Srinivason et al. 1964), as well as with the regulation of mutual relationships between the metabolism of lipids and polysaccharides (Krimsky, Racker 1952, Singer, Barron 1945, Srere 1965, Stern et al. 1952). It is thus possible that the tumour very rapidly synthesizes long polypeptide chains and therefore the histochemical methods are not efficient enough for detecting the increasing amounts of proteins. This should confirm the fact, that the compounds blocking the SH groups inhibit the tumour growth (Alow et al. 1967, Anisimow 1967, Friedman et al. 1949). This phenomenon found application in the synthesis of numerous cytostatics.

CONCLUSIONS

1) No correlations exists between the activities of the oxidizing-reducing enzymes in the cells of glial tumours and functional protein groups. This concerns in particular the lactate dehydrogenase and the NADH — dependent tetrazolium reductase.

2) The cytoenzymatic differentiation and the intensity of the reactions on NH_2, COOH and SH groups depend upon the cellular content of the tumour and on the cytoplasm/nucleus ratio.

3) The shift of cytoplasm/nucleus ratio in the direction of the cytoplasm proceeds along with an increase in activity of LDH and NADH — dependent tetrazolium reductase, whereas the intensity of the reactions on functional protein groups does not exhibit this tendency.

J. Kałuża, H. Szydłowska

HISTOCHEMICZNE BADANIA PORÓWNAWCZE GRUP CZYNNOŚCIOWYCH BIAŁEK I NIEKTÓRYCH ENZYMÓW OKSYDACYJNO-REDUKCYJNYCH W GLEJU ODCZYNOWYM I NOWOTWORACH GLEJOPOCHODNYCH

I. Nowotwory glejowopochodne

Streszczenie

Autorzy porównywali zmiany nasilenia reakcji na grupy czynnościowe białek z aktywnością niektórych enzymów oksydacyjno-redukcyjnych, zwłaszcza dehydrogenazy kwasu mlekowego i NADH reduktazy tetrazolowej, których aktywność w nowotworach jest bardzo wysoka.

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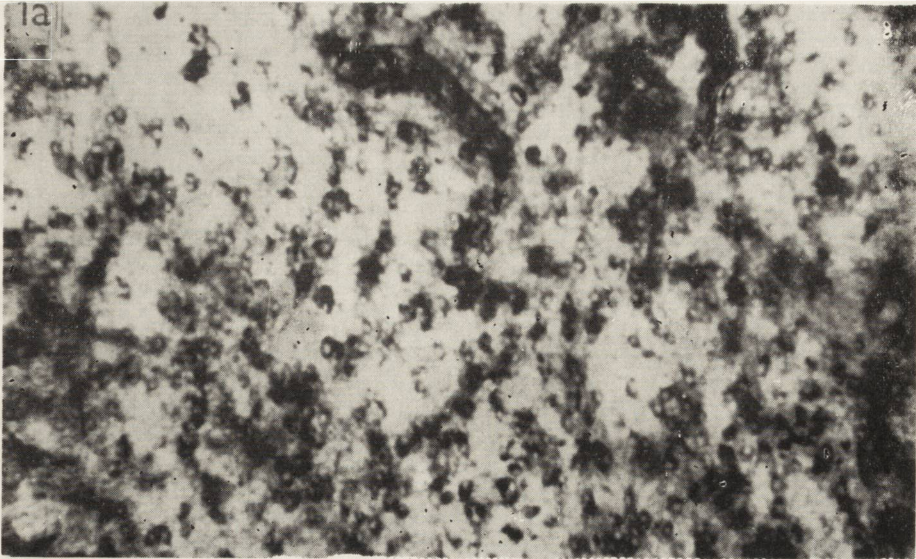

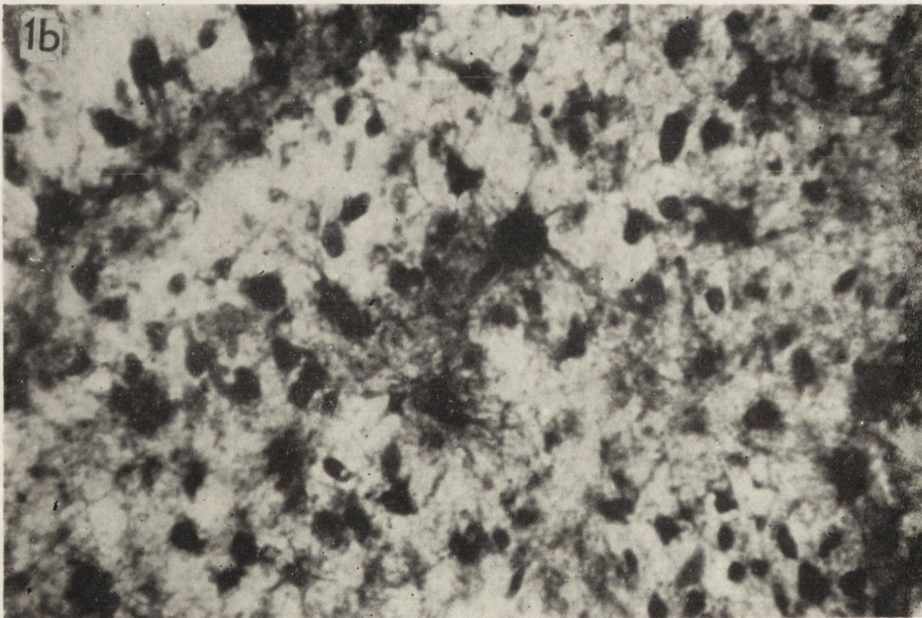

Fig. 1. Lactic acid dehydrogenase activity in neoplastic cells. × 1 170: a — Oligodendroglioma anaplasticum. Cytoplasm-poor cells, b — Glioblastoma multiforme. Cytoplasm-rich cells.

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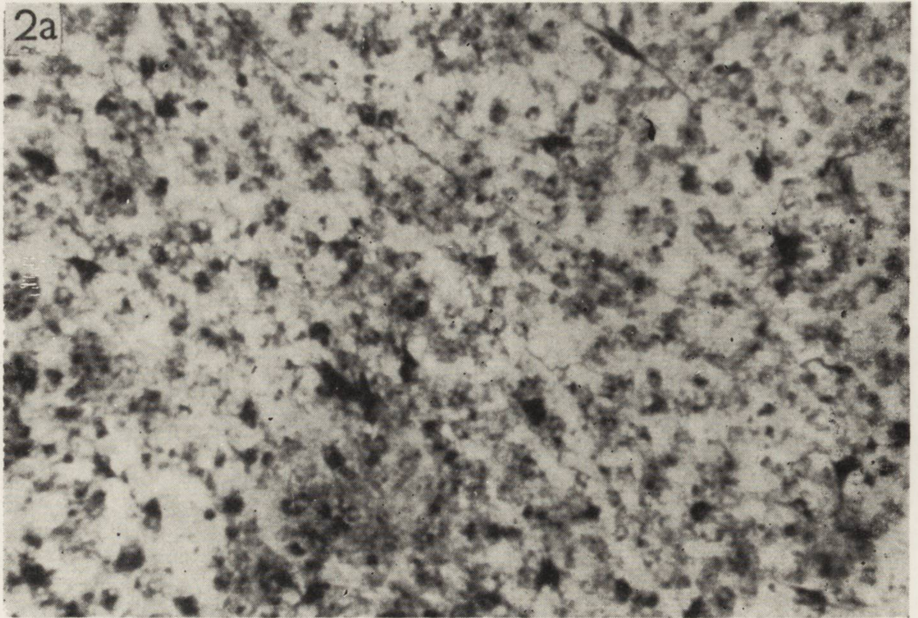

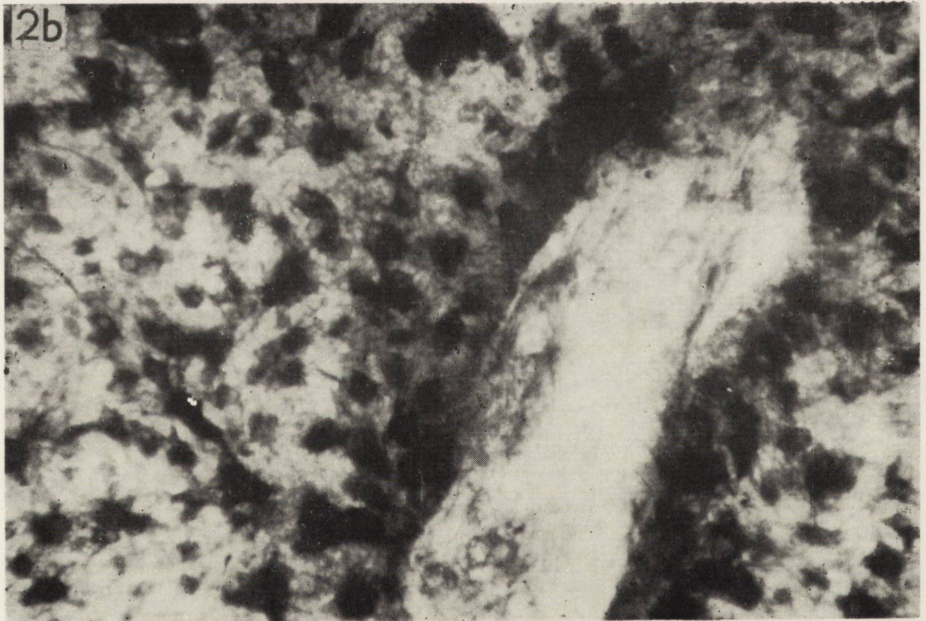

Fig. 2. NADH-dependent tatrazolium reductase activity in neoplastic cells. × 1 170: a — Oligodendroglioma anaplasticum. Cytoplasm-poor cells, b — Glioblastoma multiforme. Cytoplasm rich-cells.

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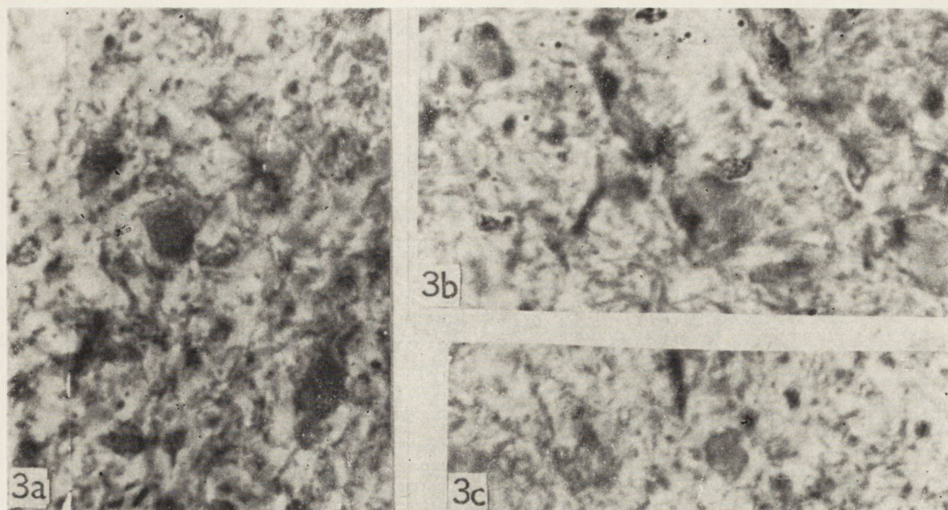

Fig. 3. Astrocytoma gemistocyticum. Reaction for functional groups in proteins. × 1 170: a) Strong NH_2 group reaction with ninhydrin and Schiff reagent in gemistocyte-like cells, b — SH group reaction in gemistocyte-like cells. Method by Bennet, c — Weak Barrnett-Seligman reaction for COOH group in gemistocyte-like cells.

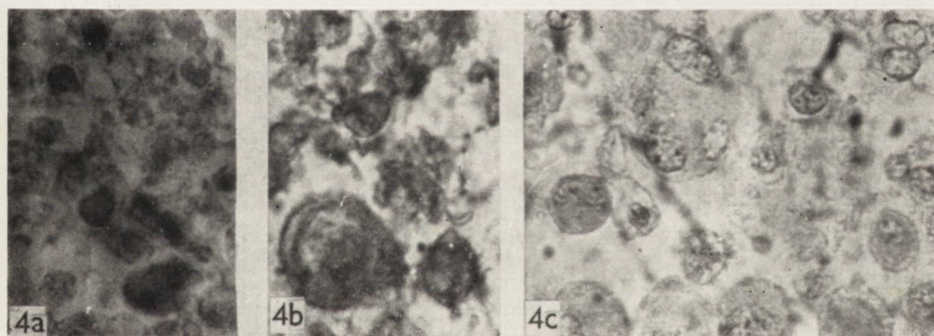

Fig. 4. Glioblastoma multiforme. Reactions for functional groups in proteins. × 1 170: a — Strong NH_2 group reaction with ninhydrin and Schiff reagent in gemistocyte--like cells, b — Strong Barrnett-Seligman reaction for COOH groups in giant cells, c — SH group reaction in gemistocytes-like and giant cells. Method by Bennet. Weak reaction in cytoplasm, strong in nuclei.

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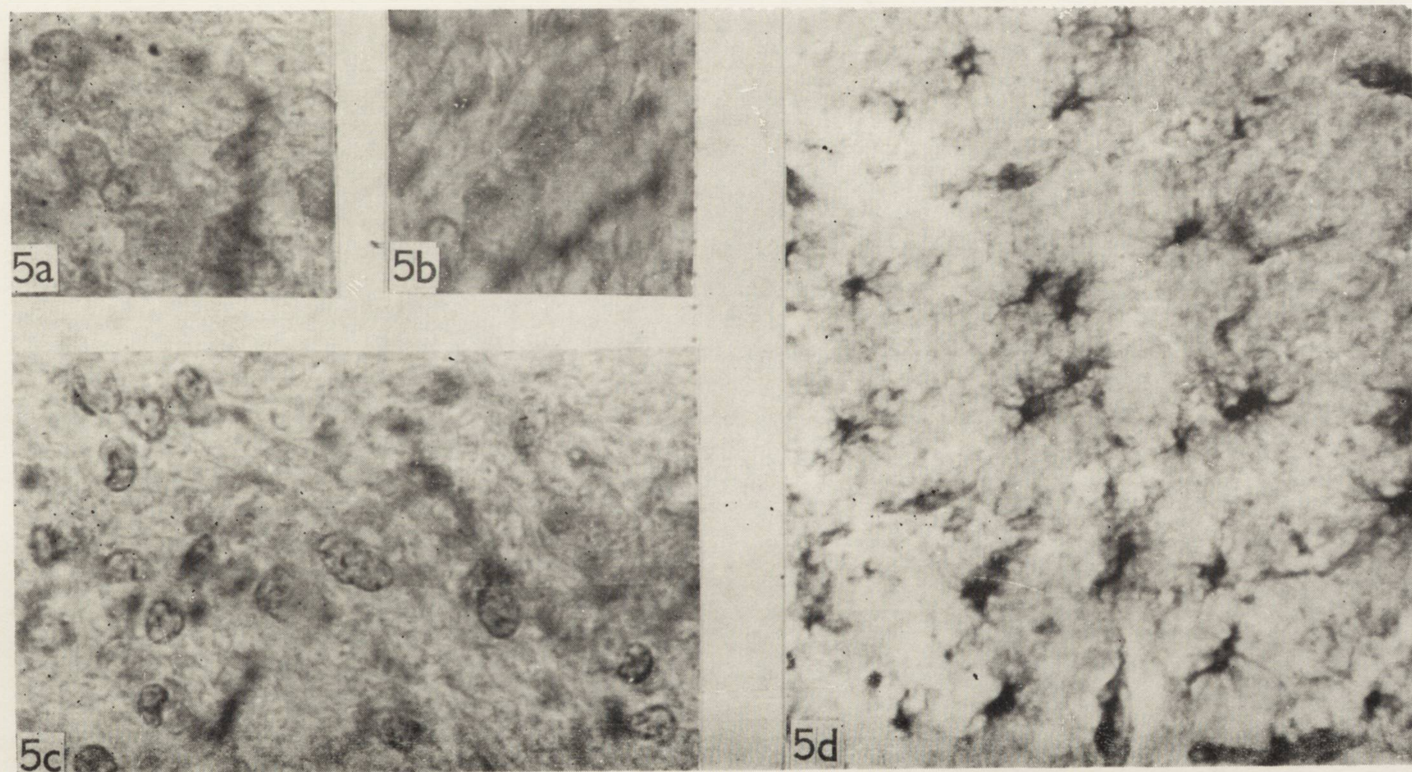

Fig. 5. Astrocytoma diffusum. × 1170: a — Very weak NH_2 group reaction with ninhydrin and Schiff reagent, diffused regularly in the whole texture, b — Very weak Barrnett-Seligman reaction for COOH groups, diffused regularly in the whole texture, c — SH group reaction, well-marked exclusively in cell nuclei Method by Bennet, d — Significant glutamic acid dehydrogenase activity.

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Z badań wynika, że aktywność cytoenzymatyczna komórek nowotworowych wiąże się ze zmianą stosunku plazmo-jądrowego, nie towarzyszą jej natomiast równoległe zmiany przyrostu lub spadku ilości grup czynnościowych białek.

Я. Калужа, Г. Шидловска

ГИСТОХИМИЧЕСКИЕ СРАВНИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ ФУНКЦИОНАЛЬНЫХ ГРУПП БЕЛКОВ И НЕКОТОРЫХ ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНЫХ ЭНЗИМОВ В РЕАКТИВНОЙ ГЛИИ И ГЛИОПРОИЗВОДНЫХ ОПУХОЛЯХ

I. ГЛИОПРОИЗВОДНЫЕ ОПУХОЛИ

Резюме

Авторы сравнивали изменения интенсивности реакции на функциональные группы белков с активностью некоторых окислительно-восстановительных энзимов, в частности лактатдегидразы и тетразольной редуктазы, активность которых в опухолях очень высокая.

Из исследований следует, что цитоэнзиматическая активность опухолевых клеток связана с изменением плазмо-ядерного соотношения и не сопутствуют ней параллельные изменения увеличения или уменьшения количества функциональных групп белков.

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ACID PHOSPHATASE ACTIVITY IN HUMAN BRAIN TUMOURS *)

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Activity of acid hydrolases increases in human and experimental brain tumours, mainly in relation to the frequency and intensity of catabolic processes (Schiffer et al. 1965, 1968, Fabiani et al. 1970). Histochemically they have also been discussed in connection with the processes of cell differentiation and anaplasia.

From a histoenzymological standpoint, the distribution, localization and character of the final reaction product (FRP) are the most important factors for a correct evaluation of the above cellular and tissue events.

As known, the FRP of the histochemical hydrolytic pictures may be granular and/or diffuse. While the granular reaction must be generally associated with the lysosomes, the diffuse one cannot be always regarded as an expression of lysosomal changes, and thus of cellular regression. In fact, in various physiological and pathological cell conditions the diffuse diazo-reactions are linked with the particular substrate employed and the current protein synthesis.

In order to evaluate the significance of the enzyme reactions in tumour cytotypes, histochemical tests for acid phosphatase have been carried out either in fresh or fixed tissue of many cerebral tumours, including: medulloblastoma, cerebellar spongioblastoma, protoplasmic astrocytoma, glioblastoma multiforme, neurinoma, meningioma, craniopharyngioma, metastatic carcinoma. The FRP has been studied with various effectors (10^{-3}M NaF; 10^{-3}M pCMB; $2 \cdot 10^{-3}$M Pb $(NO_3)_2$; $2 \cdot 10^{-3}$M $HgCl_2$: the latter two only by the azo-techniques) and Triton X-100 of various concentrations (0.1%; 0.5%; 1%) with the use of beta-glycerophosphate, naphthyl-phosphate, naphthol AS-BI and naphthol AS-TR phosphate as substrates. Moreover, the fragility test of Bitensky was performed on fresh tissue with the above mentioned substrates.

The results may be summarized as follows:

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a) The distribution pattern of the enzyme activity is generally similar in fresh and in fixed material after the same incubation time, the reaction intensity being low in differentiated or immature tumors, like meningiomas or medulloblastomas, and high in anaplastic tumors, like glioblastoma multiforme. Moreover, independently of the tumor type, the activity either granular and/or diffuse, clearly prevails in regressive areas. These observations confirm the well known relationship between acid phosphatase and regressive events.

b) Although the effector action is comparable in fresh and fixed material, in the former, however, the efficiency is generally higher. Among the inhibitors, NaF and pCMB are the most effective; in all tumors the granular reaction is most sensitive, either with Gomori- or diazo-methods. On the contrary, in differentiated oncotypes, like meningiomas or A areas of neurinomas, the diffuse azo-dye FRP is partially resistant. Heavy metals give similar effects on the two components of the diazo FRP.

c) Triton X-100 treatment gives different results in fresh and fixed tissue, with diazo and Gomori-methods and in relation to the concentration.

In fresh tissue the effect is quite similar to that known in biochemistry: after 15 min. at room temp., as little as 0.1% of the detergent provokes a considerable reduction or complete disappearance of the enzyme activity, both in Gomori or diazo-methods. At higher concentrations the reactions are always negative. In fixed material, parallely with the rise of detergent concentration, the Gomori-reaction progressively subsides and disappears. Conversely, diazo-reactions are not modified or are enhanced, particularly at lower concentrations.

d) The lysosomal fragility test of Bitensky shows an early appearance of the granular FRP with Gomori- and diazo-techniques, e.g. after 5 min. of incubation, in anaplastic cells of several tumors (glioblastoma multiforme, metastases, etc...) and in regressive areas of undifferentiated or immature tumors.

More prolonged incubation results in a diffuse cytoplasmic activity and for even longer periods nuclear staining. If the results are completely similar for the granular FRP, a particular finding regarding the diffuse reaction must be emphasized. This concerns the differentiated tumors where the diffuse FRP appears earlier than the granular one, with azodye procedures.

The significance of the diffuse stainings for acid phosphatases as well as for other hydrolytic enzymes is not always easy to interpret. However, cytological investigations in fresh tissue by the controled temperature freezing-sectioning technique allow us to demonstrate lysosomal structures according to the biochemists' concept.

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If with the Gomori method only the last stage of the lysosomal activation is characterized by a diffuse stain, on the contrary with azo-dye procedures, especially when using naphthol AS-substitutes, we observe a clear diffuse reaction in differentiated tumours earlier than the granular one, so that it cannot be explained by lysosomal diffusion. Moreover, the same reaction is resistant to NaF. These observations agree with those made on the general effects of Triton X-100 and of inhibitors, either biochemically (Tappel et al. 1963, Meany et al. 1967, Allen et al. 1970), histochemically (Novikoff 1963) or zymographically (Bernsohn, Barron 1964, Allen et al. 1965). They indicate that multiple molecular forms of acid phosphatase may exist, and that in some cytotypes of brain tumours the diffuse reaction may have a primary extra-lysosomal situation. In this regard it is to be remarked that ultrastructural data show in several cell-types, including tumoral astrocytes, an ER localization of acid phosphatase (Novikoff 1963, Schiffer et al. 1970, etc.).

In conclusion, our cytochemical findings support the hypothesis of a relation between cell differentiation and diffuse non-lysosomal acid phosphatase activity. This enzyme activity seems to have substrate variations and NaF-resistance, so that it might correspond to that identified by Barron et al. (1964) as 2, 3 and 4 bands in neural zymographic studies.

Further experiments on enzyme release and zymographic separation are in progress.

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AKTYWNOŚĆ KWAŚNYCH HYDROLAZ W GUZACH MÓZGU U LUDZI

Streszczenie

Przedmiotem badań autorów była aktywność kwaśnej fosfatazy w różnych typach guzów ośrodkowego układu nerwowego. Celem badań było ustalenie wpływu różnorodnych związków chemicznych na ziarnisty lub rozlany charakter produktów odczynu histochemicznego w zależności od stosowanej metody, stanu badanej tkanki i rodzaju nowotworu.

Otrzymane wyniki można zreasumować następująco:

1) najskuteczniejszym inhibitorem jest NaF i pCMB, a reakcja typu ziarnistego — najwrażliwsza. W guzach zróżnicowanych, jak oponiaki, rozlany odczyn uzyskiwany przy użyciu metod dwuazowych, był częściowy;

2) Triton X-100 powodował w świeżej tkance całkowite zniesienie reakcji, zarówno przy zastosowaniu metody Gomoriego, jak i metod dwuazowych. W tkance utrwalonej reakcja Gomoriego stopniowo się zmniejszała, aż do zaniku, odpowiednio do wzrastającego stężenia detergentu. Przeciwnie, reakcje dwuazowe były wzmożone, szczególnie przy niskich stężeniach;

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3) test Bitensky'ego wykazuje wczesne pojawienie się reakcji ziarnistej (w metodach Gomoriego i dwuazowych) w guzach anaplastycznych lub w okolicach ze zmianami wstecznymi, podczas gdy w guzach zróżnicowanych, jak w oponiakach, reakcja rozlana pojawia się wcześniej niż ziarnista.

Ostateczne wyniki wykazują, że rozlany lub ziarnisty charakter KPR zależy, w ustalonych warunkach, od użytego substratu.

Wykazanie rozlanej reakcji metodami dwuazowymi, a zwłaszcza w stanie inercji lizosomalnej, można uważać za wynik lokalizacji cytoplazmatycznej, nielizosomalnej, prawdopodobnie w siateczce endoplazmatycznej, a zatem za wyraz zmian postępowych w komórce.

А. Фабиани, Г. Кровери, Л. Форнатто, Д. Шиффер

АКТИВНОСТЬ КИСЛЫХ ГИДРОЛАЗ В ОПУХОЛЯХ МОЗГА ЧЕЛОВЕКА

Резюме

Многочисленные биохимические и гистохимические исследования указывают на усиленную активность кислых гидролаз в опухолях мозга. Усиленная активность этих энзимов вызвана катаболическими процессами, имеющими место внутри опухоли, а также является результатом процессов дифференцирования клеток и анапластических процессов.

Полученные результаты можно резюмировать следующим образом:

1) самым эффективным ингибитром является NaF и pCMB, а реакция грануляционного типа — наиболее чувствительна. В дифференцированных опухолях, как менингиомы, разлитая реакция получаемая при использовании двуазовых методов была частичной;

2) Тритон X-100 вызывал в свежей ткани полное снесение реакции, как при применении метода Гомори так и двуазовых методов. В фиксированной ткани реакция Гомори постепенно уменьшалась вплоть к исчезновению, соответственно с увеличающейся концентрацией детергента. И наоборот, двуазовые реакции были усилены, особенно при низких концентрациях;

3) тест Битенского обнаруживает раннее появление грануляционной реакции (в методах Гомори и двуазовых) в анапластических опухолях или в районах с дегенеративными изменениями, в то время когда в дифференцированных опухолях, как менингиомы, разлитая реакция появляется раньше грануляционной.

Окончательные результаты указывают, что разлитый или грануляционный характер KPR зависит, в установленных условиях, от использованного субстрата.

Обнаружение разлитой реакции двуазовыми методами, и особенно в состоянии лизосомальной инерции, можно считать результатом цитоплазматической локализации, нелизосомальной, вероятно в эндоплазматическим ретикулюм, и стало быть — проявлением прогрессирующих изменений в клетке.

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HISTOCHEMICAL STUDIES ON PITUITARY ADENOMAS WITH REFERENCE TO TYPE AND CLINICAL PICTURE

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The different hormonal functions of the pituitary cells types are associated with definite staining characteristics used as identification criteria in light-microscopy. In addition, there are data concerning a correlation between the enzyme-histochemical and biochemical characteristics, and the hormonal activity of the pituitary cells (Pearse 1956, Bleicher et al. 1961, Balogh 1962, Schreiber 1962, Sobel 1962, Fand, Wattenberg 1963, Smith, Farquhar 1970).

The lack of an absolute staining specificity, on the one hand, and the opinion of Furth and Klifton (1966) that „the pituitary tumours might be monomorphous in type but not monohormonal", on the other hand, made us undertake the task to establish: 1) whether there is any correlation between the histological structure and the enzyme-histochemical characteristics of pituitary tumours; 2) whether there exists a certain correlation between the above mentioned criteria and hormonal activity.

MATERIAL AND METHODS

Biopsy material of 30 pituitary tumours was studied. The paraffin sections, fixed in Zenker, Carnoy and 10% neutral formalin solutions, were stained by hemalum-eosin, Mallory, Azan and PAS reactions. Histochemical methods were applied in 17 of the tumours: on fresh and fixed cryostat sections. They were used, to demonstrate: succinic-, malic-, isocitric-, lactic-, glycerophosphate (NAD and menadion)-, glutamic-, beta-oxybutyric dehydrogenases, NAD- and NADP-diaphorases, acid phosphatase, nonspecific esterase, beta-glucuronidase, N-acetyl-beta-glucosaminidase, alkaline phosphatase, aldolase. Lipids were stained with Sudan III or Sudan black.

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RESULTS

The studied tumours (classified according to histological criteria) were: 23 chromophobe-, 4 mixed type-, 1 acidophilic-, 1 malignant adenoma and 1 infundibuloma.

Chromophobe adenomas, conformly to the monomorphous histological pattern, were characterized also by a marked uniformity of reaction for oxidoreductases. The reaction product was fine-granular, evenly distributed in the cytoplasmic body. Seldom, larger mono- or binuclear cells with more intensive, predominantly perinuclear reaction were observed. Comparing the intensity, we could grade the reactions for the different dehydrogenases and diaphorases as follows: NAD-diaphorase, glycerophosphate (menadion)-, lactic dehydrogenases, NADP-diaphorase, succinic-, isocitric-, malic-, glutamic-, beta-oxybutyric-, glycerophosphate (NAD) dehydrogenases. The tumoral parenchyma displayed moderate uniform reactions for acid phosphatase and nonspecific esterase and relatively weaker reactions for beta-glucuronidase and N-acetyl-beta--glucosaminidase. Only in the acid phosphatase reaction there was a more intensive staining zone round the vessels and near the connective tissue strands. The pericytes and some scattered cells, located mainly around the vessels and close to connective tissue strands showed intensive granular reaction for all 4 enzymes. The tumoral parenchyma was negative when tested for alkaline phosphatase except to two cases: one of them with patches of foetal type.

Histological investigation established in two chromophobe adenomas circumscribed or rather large regions of cells with a relatively large cytoplasmic body, with marked granular cytoplasmic reaction but with no definite affinity for the staining agents (Fig. 1a). The reactions for oxidoreductases were more intensive in these regions when compared to the rest of the tumoral parenchyma (Figs. 1b and 1c). The reaction for acid phosphatase was stronger in the perivascularly arranged cells as compared with those in the small-cell regions (Figs. 2a and 2b). The leading symptoms in the clinical picture of the patients of this group were sight disturbances and/or increased intracranial pressure (with corresponding changes in the *sella turcica)*. In 3 cases there were indications of hypogonadism (amenorrhea, sexual impotence).

Two of the adenomas were classified as chromophobe with a transition towards acidophilic. In one of them, the acidophilic cells were scarce while in the second case, pathologically verified, these cells involved whole regions and had a more typical structure (Figs. 3a and 3b). Giant cells and cellular syncytia manifested themselves usually by a most intensive reaction for oxidoreductases (Fig. 3c) and acid phosphatase

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(Fig. 3d). In addition, the rest of the parenchyma, even in the regions with relatively monomorphous histological structure exhibited a marked histochemical polymorphism both for the oxidoreductases and for the hydrolytic enzymes with the exception of alkaline phosphatase. Clinically, the first tumour caused apparent hormonal disorders (acromegaly, sexual impotence) and minimal sight disturbances while in the second tumour the sight disturbances were the leading symptoms in the absence of any hormonal disorders whatsoever.

Four of the adenomas were classified as mixed type according to the criteria of Tönnis et al. (1953) and Müller (1954). Histochemically, this group of tumours was characterized by a marked diversity of the oxidoreductase activity, regardless of the cell size. On the whole, the reactions for acid phosphatase and nonspecific esterase were more intensive as compared with those in chromophobe adenomas. The reaction for acid phosphatase was centrally located in the larger cells. More pericytes and round cells with intensive reaction for hydrolases were noted in comparison with the chromophobe adenomas. Alkaline phosphatase activity was noticeable only in some of the vessels. Clinically, two mixed type adenomas produced typical acromegalic changes, one was hormonally inactive and one presented a Cushing-like syndrome. The latter tumour had been twice operated upon in 2 years. At the first operation the pattern was of a chromophobe adenoma of diffuse type with microfoci of moderate, predominantly nuclear, polymorphism (Fig. 4a). The removed tumoral part at the second operation displayed fully the pattern of a mixed adenoma (Fig. 4b).

The acidophil adenoma, clinically manifested by acromegaly, corresponded histologically to the classic descriptions. No histochemical investigation was done owing to difficulties in the operation but later the case was pathologically verified.

The peculiar histological structure of one adenoma provided grounds to be classified as malignant. It consisted of oval cells of different size with well lined borders. The nuclei were light, vesicular. They had one or more prominent nucleoli, eccentrically located. The nuclear membrane was sometimes oddly undulated. Mitoses and cells with more than one nucleus were very often observed. The cytoplasm was homogeneous, with well marked acidophilia. Among the cells just described small undifferentiated cells with scarce cytoplasm, and one or more rich in chromatin nuclei, were scattered or clustered (Fig. 5). There were no clinical data for hormonal disorders but only 2 months later the tumour produced full atrophy of the optic nerves followed by loss of sight.

The tumoral parenchyma of the infundibuloma (Fig. 6a) displayed a high oxidoreductase activity except succinic dehydrogenase. The cel-

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lular bodies were uniformly filled with diformazan granules and the processes could be followed for some distance (Fig. 6b). The pituicytes with large cytoplasmic bodies, eccentrically located nuclei and long processes gave the most prominent enzymatic reaction among the rest of tumoral parenchyma, this being true especially for succinic dehydrogenase. The reactions for lysosome enzymes were moderate in intensity and when testing nonspecific esterase, the reticular structure of the tumour could be seen. The large pituicytes were distinguished by a more intensive and predominantly diffuse reaction for hydrolases. The alkaline phosphatase reaction was rather intensive in the vascular walls and moderate, almost diffuse, in the tumoral parenchyma. The tumour has produced clinically sight disturbances but no hormonal disorders.

DISCUSSION

We noticed a number of peculiarities when comparing the results of the present histological and histochemical study of pituitary adenomas. In the first place a lack of any definite correlation between the histological structure and the presence of hormonal disorders. Similar observations are reported by other authors (see Brilmayer et al. 1957, Marks 1959, Russel, Rubinstein 1963). Some tumours with an evident pattern of acidophil adenomas and those of mixed type were hormonally inactive. On the other hand, the tumour producing a Cushing-like syndrome with a primary chromophobe structure proved to be a typical mixed one at reoperation. On the one hand, this testifies to a possible transition or emergence of one histological type of tumour into another, and on the other hand, that histological criteria are insufficient for the evaluation of a tumour as hormonally active or inactive.

Our study showed that the regions of large cells in chromophobe adenomas differed in their enzyme activity from the remaining ones. Provided we admit that an initial phase of differentiation of the chromophobe cells specifically granulated ones occurs (Russel 1957); it might be supposed that in the earliest stage of this process there is an increase of the enzyme activity, especially of oxidoreductases.

Chromophobe tumours with histological signs of transition to acidophilic or mixed types were characterized by distinct histochemical polymorphism. The more intensive reaction distinguished not only the giant mono- or multinuclear cells, including those with eosinophil granules, but also some of the smaller cells which showed characteristics of chromophobe ones. This renders impossible the distinction of the different types of adenoma cells on the basis of their oxidoreductase activity. These

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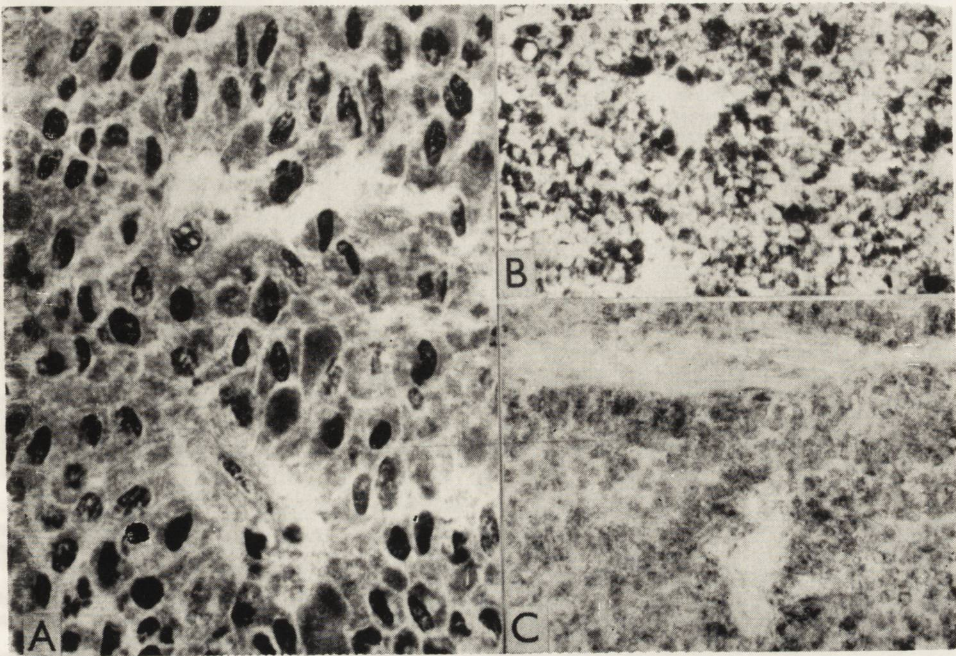

Fig. 1. Chromophobe adenoma. A) Area of cells with large cytoplasmic bodies. Stain according to Mallory. × 396. B) Succinic dehydrogenase activity in a similar area. × 157. C) Succinic dehydrogenase activity in an adjacent area composed of small cells. × 157.

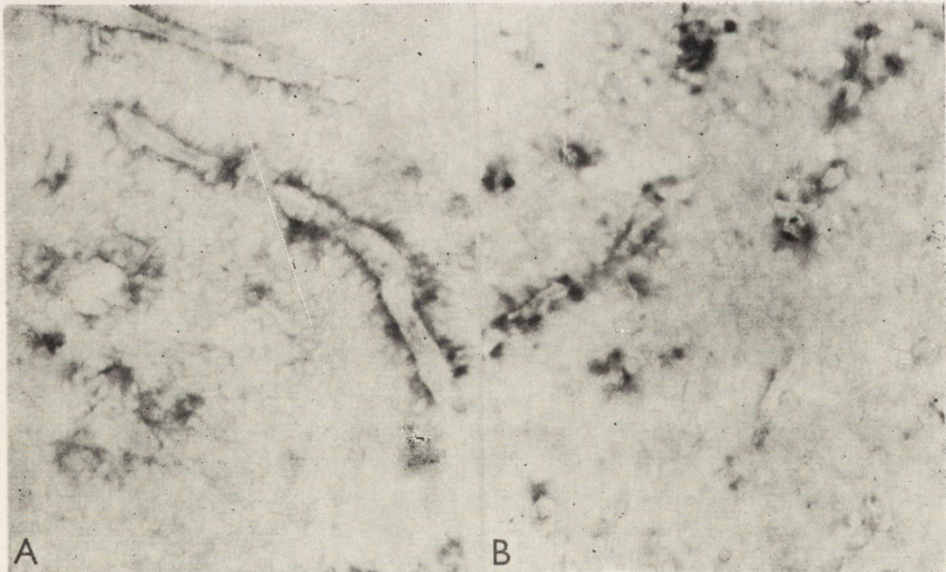

Fig. 2. Chromophobe adenoma: reaction for acid phosphatase. A) Large-cell and B) small-cell regions. × 157.

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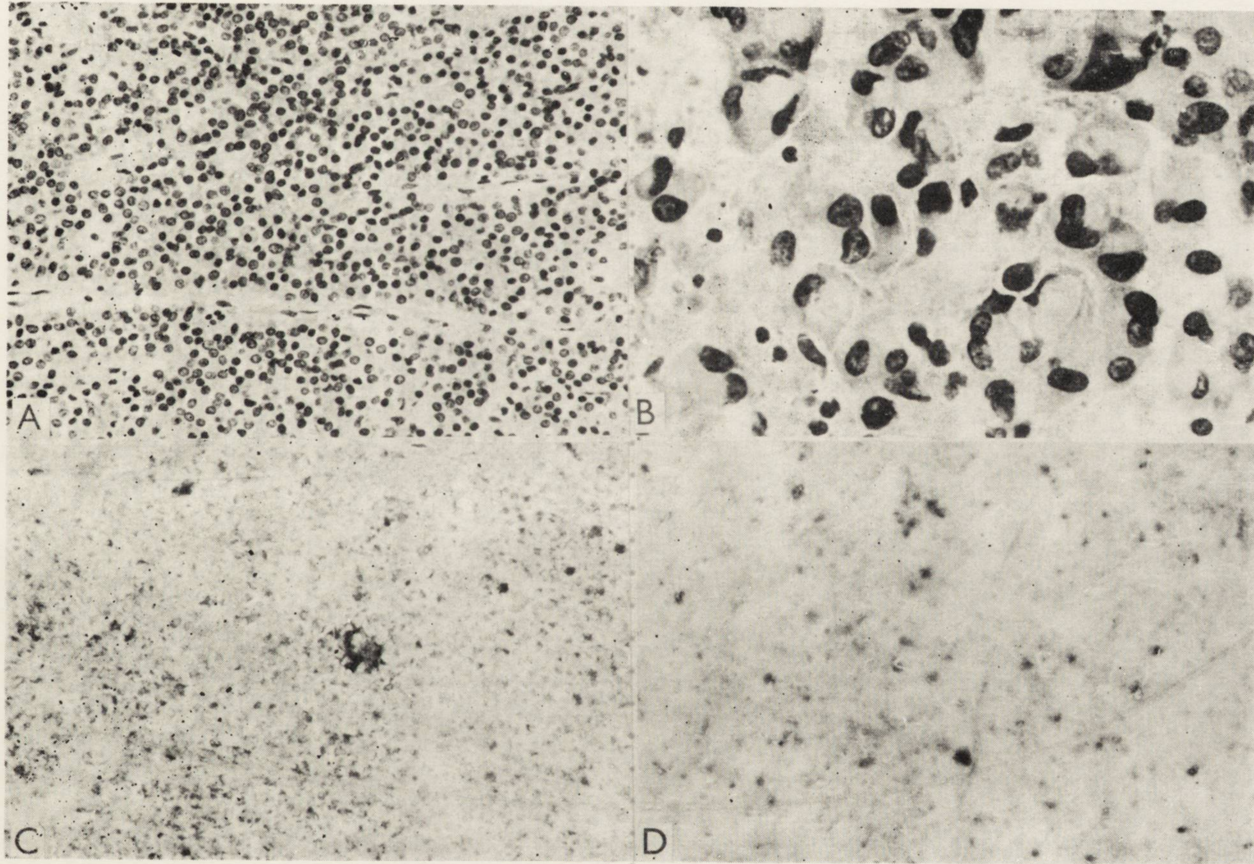

Fig. 3. Chromophobe adenoma with transition towards acidophilic one. A) Structure of chromophobe adenoma. Hemalum-eosin stain. × 157. B) Large cells with acidophilic granules in cytoplasm. Hemalum-eosin stain. × 396. C) Diverse in intensity reaction for NAD-diaphorase. × 63. D) Intensive reaction for acid phosphatase in cells of various size. × 63.

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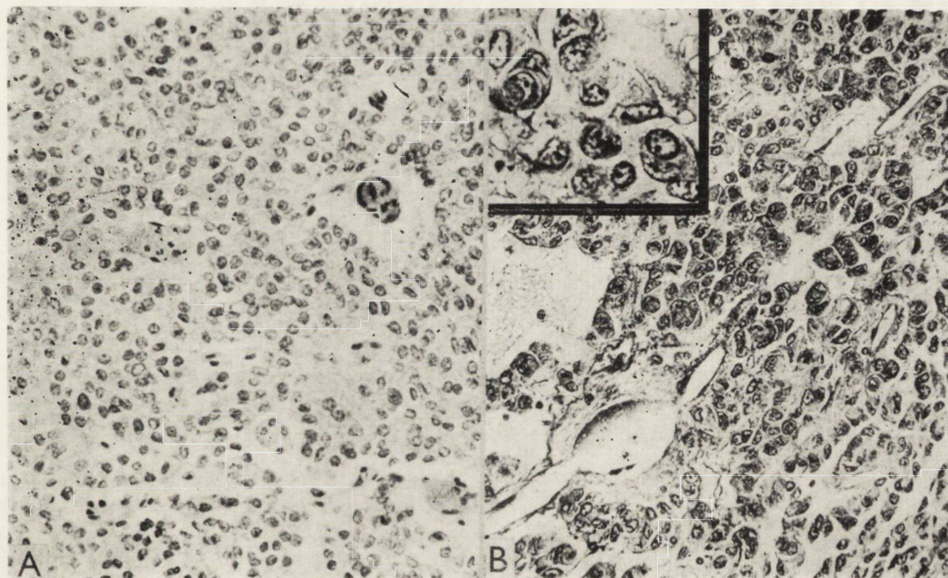

Fig. 4. A) Chromophobe adenoma. Hemalum-eosin. stain. × 157. B) At second operation: pattern of mixed type adenoma. Stain according to Mallory. × 157. Detail: endocytogenesis.

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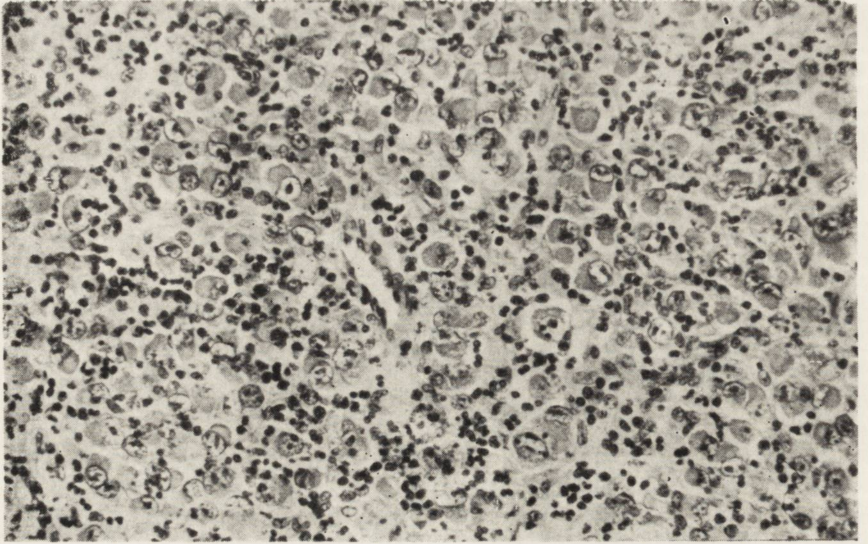

Fig. 5. Malignant adenoma. Hemalum-eosin stain. × 157.

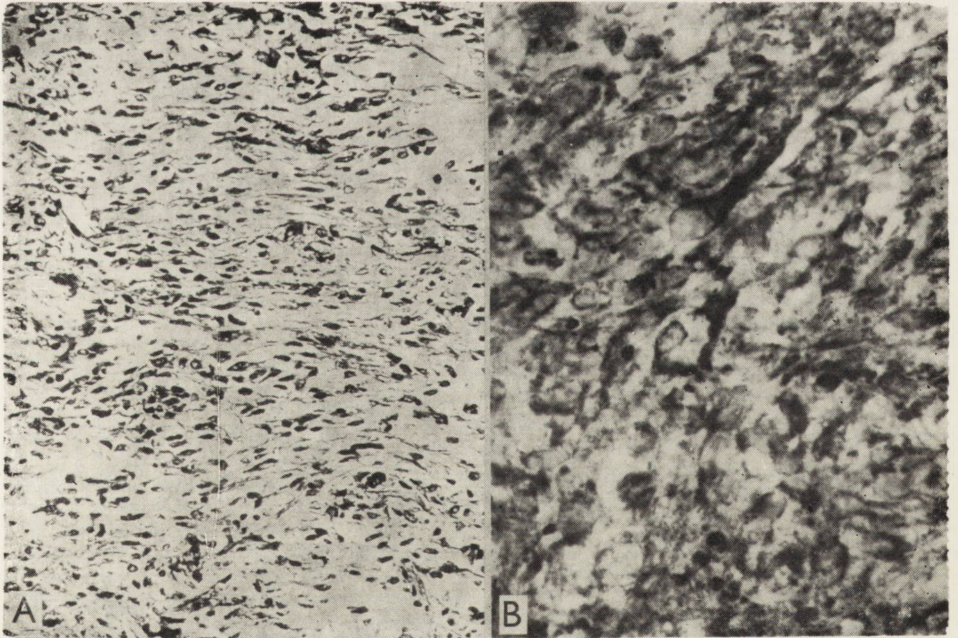

Fig. 6. Infundibuloma. A) Phosphotungstic acid-hematoxylin stain. × 157. B) Intensive NAD-diaphorase activity in tumoral parenchyma. × 396.

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results support the opinion of Pearse (1962) and Nasu (1964) and do not correspond to the observations of Viale (1965) as regards higher oxidative activity in the acidophil cells.

The results regarding the activity of the hydrolases in pituitary adenomas are not uniform (Melchior, Micuta 1956, Büttger 1957, Feigin, Wolf 1959, Nasu 1964, Bingas 1966, Hanefeld 1966, Sil 1970). Most authors are inclined to admit that the hydrolytic enzymes are in closer relation with the secretory activity of the adenoma cells. Our study also showed clearly more intensive and more varied reactions for hydrolytic enzymes, and especially for acid phosphatase in the transitional or mixed types.

However, this was true both for tumours producing hormonal disorders and for the hormonally inactive ones.

A number of structural-functional correlations have been determined, based on extensive detailed studies of the pituitary in man and experimental animals. These correlations are clearly disturbed in pituitary tumours. The structure of the tumour, as a whole, and of the tumoral cells, in particular, is subordinated to qualitively new laws — the laws of blastomatous growth. The structural differentiation is in some cases of a low functional value. On the other hand the presence of a specific function in the absence of an adequate structure as well as the different functions in apparently the same structures probably result from the new subordination. The total data of the present study raise the question how far is the enzyme activity related to each of these processes.

The results at this stage of our study do not provide grounds enough to draw any definite conclusions for establishing additional histochemical criteria for the classification of pituitary tumours.

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BADANIA HISTOCHEMICZNE GRUCZOLAKÓW PRZYSADKI W ODNIESIENIU DO TYPU I OBRAZU KLINICZNEGO

Streszczenie

Na materiale biopsyjnym z 30 nowotworów przysadki podjęto próbę wyjaśnienia, czy istnieje korelacja między budową histologiczną i właściwościami histochemicznymi gruczolaków przysadki oraz czy istnieje korelacja pomiędzy kryteriami histologicznymi i aktywnością enzymatyczną a zaburzeniami hormonalnymi i objawami klinicznymi.

Badany materiał obejmował 23 gruczolaki barwnikooporne, 1 kwasochłonny, 4 typu mieszanego, 1 złośliwy gruczolak, 1 guz lejka.

Miąższ gruczolaków barwnikoopornych wykazywał dość jednorodną aktywność enzymów oksydoredukcyjnych o różnym nasileniu odczynu, zależnie od rodzaju

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enzymu, również umiarkowana była aktywność hydrolaz. U większości pacjentów występowały zaburzenia wzroku, u 3 tylko hipogonadyzm. W dwu przypadkach gruczolaków przejściowych występowały również komórki kwasochłonne, a obraz histochemiczny był urozmaicony. U jednego pacjenta obserwowano akromegalię, impotencję, zaburzenia wzroku, u drugiego występowały jedynie zaburzenia wzroku.

Cztery gruczolaki typu mieszanego odznaczały się wybitnymi różnicami w aktywności enzymów oksydoredukcyjnych i aktywnością hydrolaz wyższą niż w gruczolakach barwnikoopornych. Klinicznie dwa przypadki wykazywały akromegalię, 1 zespół Cushinga, 1 był nieczynny hormonalnie.

Przypadek czystego gruczolaka kwasochłonnego z akromegalią nie był badany enzymatycznie, podobnie jak gruczolak złośliwy. Guz lejka wykazywał wysoką aktywność oksydoreduktaz.

Na podstawie przeprowadzonych badań stwierdzono brak korelacji pomiędzy strukturą histologiczną guzów i obecnością zaburzeń hormonalnych.

Niektóre z guzów były nieczynne hormonalnie, w innych rodzaj zaburzeń hormonalnych nie odpowiadał budowie histologicznej. Wydaje się również niemożliwe odróżnienie typów komórek gruczolaków na podstawie aktywności oksydoreduktaz. Hydrolazy wydają się być aktywniejsze w typach przejściowych i mieszanych zarówno w gruczolakach czynnych hormonalnie, jak i nieczynnych.

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ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ АДЕНОМ ГИПОФИЗА ПО ОТНОШЕНИЮ К ТИПУ И КЛИНИЧЕСКОЙ КАРТИНЕ

Резюме

На биопсионном материале, взятом из 30 опухолей гипофиза, была предпринята попытка выяснить существует ли корреляция между гистологическим строением и гистохимическими свойствами аденом гипофиза, а также — имеется ли корреляция между гистологическими критериями и энзиматической активностью а гуморальными нарушениями и клиническими симптомами.

Исследованный материал охватывал 23 хроморезистентные аденомы, 1 ацидофильную аденому, 4 смешанного типа, 1 злокачественную аденому и 1 опухоль воронки.

Паренхима хроматорезистентных аденом обнаруживала весьма однородную активность окислительно-восстановительных энзимов с разной интенсивностью реакции в зависимости от вида энзима. Умеренной была также активность гидролаз. У большинства пациентов имелись нарушения зрения; у 3 только гипогонадизм. В 2 случаях обнаруживались ацидофильные клетки и гистохимическая картина была разнообразной. У 1 пациента наблюдалась акромегалия, импотенция и нарушения зрения; у второго имелись только нарушения зрения.

Четыре аденомы смешанного типа отличались выдающимися разницами в активности окислительно-восстановительных энзимов и высшей активностью гидролаз чем в хроморезистентных аденомах. Клинически два случая обнаруживали акромегалию, 1 — синдром Кашинга и 1 был гуморально неактивен.

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Случай чистой ацидофильной аденомы с акромегалией не исследовался энзиматически, так как и злокачественная аденома.

Опухоль воронки обнаруживала высокую активность оксидоредуктаз.

На основании проведенных исследований было обнаружено отсутствие корреляции между гистологической структурой опухолей и наличием гуморальных нарушений.

Некоторые из опухолей были гуморально неактивны, в других — вид гуморальных нарушений не соответствовал гистологическому строению. Кажется также невозможным отличение типов клеток аденом на основании активности оксидоредуктаз. Гидролазы кажутся быть более активные в переходных и смешанных типах, как в гуморально активных так и в гуморально неактивных аденомах.

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NEUROPAT. POL. 1972, X, 2

D. FELICETTI, U. BONK, C. COUTELLE, R. COUTELLE, F. W. RATH, F. TRAUB

EXPERIMENTAL INVESTIGATIONS ON INVASIVE TUMOUR GROWTH

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The invasive growth — infiltration and destruction of the surrounding host tissue is a main characteristic of a malignant tumour. Therefore a detailed knowledge of this process is very important for the interpretation of malignancy. At present there are two contradicting conceptions:

1) The infiltrating tumour cells prepare or facilitate the invasion by leakage or secretion of enzymes, which break up the surrounding host tissue (we mention the so called "spreading factor" — Dontenwill 1955; Grossfeld 1961) and proteolytic enzymes (Sylven, Malmgren 1957).

2) The tumour destroys the surrounding tissue only by its growth pressure. The destruction of the surrounding tissue therefore is the result of the tumour spread (Coutelle et al. 1969; Rath et al. 1969).

A team led by C. Coutelle tried to come nearer to the solution of this bias with light- and electron-microscopical, histo- and biochemical methods. The transplant of Ehrlich ascites carcinoma into the mouse brain served as model for invasive growth. It grew without interfering lymphocellular reaction to a solid tumour with immediate contact to surrounding brain tissue. The results obtained have been already published (Coutelle et al. 1969, 1970; Rath et al. 1969; Bonk et al. 1969; Felicetti et al. 1970). Some of the biochemical investigations will be presented here:

With a freezing microtome the mouse brain was cut into 100 μ thick horizontal slices. From these 3 areas of the tissue were separated: tumour, tumour surrounding and normal brain tissue from the contralateral tumour free hemisphere. In these we determined the activity of 4 transferases, 1 oxydoreductase, 3 hydrolases and 10 proteolytic enzymes (Coutelle et al. 1970; Felicetti et al. 1970). Some enzymes have a higher, some a lower and a few the same activity as the normal brain tissue.

To exclude methodical errors we performed the following controls:

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1) Removal of remaining tumour cell-nests in the tumour surrounding by microdissection. The obtained values of this almost tumour cell free preparation did not differ from these obtained with the above described method (Coutelle et al. 1970; Felicetti et al. 1970).

2) With hemoglobine as test substance was shown that no diffusion of macromolecules takes place during the preparation of the tissue slices.

In order to decide, if the elevated enzyme activities in the surrounding brain tissue were not due to the presence of enzymes originating from tumour tissue, we tried to find destinguishing characteristics of some enzymes of cancer cells and normal brain tissue. The amino acid arylamidase and the β-glucuronidase activities which were significantly elevated in the tumour surrounding brain tissue compared with that of normal brain were chosen for examination of their isoenzyme pattern.

For the characterization of the arylamidases (Felicetti 1971) mouse brains (excluding cerebellum) were homogenized in Tris-HCl buffer with adden Triton X 100, centrifuged and filtrated on Sephadex G 200 column. Ascites tumour cells were treated in the same manner. The arylamidases of each of these two tissues came down from the column in two fractions which were then tested for their substrate specificity, their reactivity to inhibitors and activators and the hydrolytic dependency from temperature. The fractions 1 behave differently from fractions 2, but on comparing each of these two fractions from the brain and from the EAC cells no significant differences could be stated. Neither was it possible to find any differences between the isoenzymes pattern of β-glucuronidase from mouse brain and from EAC cells (Coutelle, Rosa 1971). Therefore these experiments were of no use for the assessment of the origin of these enzymes.

Combining the results of our investigations with the different above mentioned methods we conclude that in our model the alteration of the tumour surrounding tissue found with morphological methods is not due to tumour enzymes but caused by pressure of the growing tumour and circulatory disturbances following its perivascular spread.

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BADANIA DOŚWIADCZALNE NAD INWAZYJNYM WZROSTEM GUZÓW

Streszczenie

Inwazyjny charakter wzrostu guzów był badany na przeszczepach Ehrlich ascites carcinoma do mózgu myszy przy użyciu różnych metod.

Badania nie dostarczyły dowodu, że zmiany w tkance gospodarza otaczającej nowotwór były wywołane przez enzymy pochodzące z komórek guza, natomiast były one spowodowane przez ucisk rosnącego guza i zaburzenia naczyniopochodne.

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ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ ИНВАЗИОННОГО РОСТА ОПУХОЛЕЙ

Резюме

Инвазионный характер роста опухолей исследовался на трансплантатах Ehrlich ascites carcinoma в мозг мыши при использовании разных методов.

Исследования не дали доказательства, что изменения в ткани хозяина, окружающей опухоль, были вызваны энзимами, происходящими из клеток опухоли, а позволили заключить, что эти изменения были обусловлены давлением растущей опухоли и сосудистыми нарушениями.

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BIOCHEMISTRY AND HISTOCHEMISTRY OF THE GLIA

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NEUROPAT. POL. 1972, X, 2

E. THOMAS

CONTRIBUTION OF ENZYME HISTOCHEMISTRY AS TO THE NATURE AND DIFFERENCES AMONG MANTLE CELLS (SATELLITE CELLS), AMPHICYTES, AND SCHWANN CELLS IN REMAK FIBER BUNDLES AND OF MYELINATED FIBERS IN DORSAL ROOT GANGLIA, SYMPATHETIC GANGLIA AND IN PERIPHERAL NERVES OF VARIOUS ANIMALS

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Sheath cells around nerve cells in peripheral ganglia are not recognizable as a special type of cell with usual methods of histology. The mantle cells (satellite cells of the English literature) and the Schwann cells are said, according to light and electron microscopic findings, to have the same function and structure (Scharf 1958; Peters et al. 1970).

It is the purpose of this report to draw attention to the differentiation among the various types of sheath cells in the peripheral nervous system, and to their metabolic properties. Glial cells in the central nervous system are different from these cells in many respects, and have to be considered seperately. They are not discussed in this paper except for a special type of active glia around motor neurons in the spinal cord.

The layer of mantle cells around a sensory ganglion cell forms a closed unit separating this cell (with its one pseudounipolar neurite) from the rest of the tissue. The amphicytes *) in the sympathetic ganglia lie in a "plasmodium" between the ganglion cells thus enwrapping these nerve cells and their multipolar processes.

As to Schwann cells, two types can be discerned histochemically, i.e. the Schwann cell of the Remak fiber bundle enwrapping several unmyelinated axons, and the Schwann cell around an internodium of the myelinated fiber.

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*) This name was selected in order to distinguish them from „interstitial cells" as they are called sometimes in the Anglo-American literature. Interstitial cells are cells in the so-called terminal reticulum (vide Stohr 1957, p. 119).

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Enzyme histochemistry has shown that sheath cells are active in many respects and have outstanding metabolic capabilities. This is especially so for alkaline phosphatase. Some other hydrolytic enzymes, which play likewise a role in these sheath cells are also considered. Of the oxydative enzymes we took α-glycerophosphate menadione tetrazolium reductase because this enzyme is localized preferably extraneuronally and is active in the mantle cell (Thomas, Pearse 1961).

MATERIAL AND METHODS

The species examined were: man, rhesus monkey, mandrill, dog, Vietnamese pig, cat, rabbit, lynx, mink, rat, mouse and frog.

The material studied was: dorsal root ganglia from man and all species mentioned; peripheral spinal nerves from most of the species mentioned; and sympathetic trunk with sympathetic ganglia from man and a mandrill.

The material was cut unfixed with a cold microtome. For the hydrolytic enzyme reactions the sections were dried at room temperature for at least 24 hrs.

The enzymes examined as described by Pearse (1960) were: 1) α-glycerophosphate menadione tetrazolium reductase (no coenzyme); tetrazolium salts: MTT or Nitro BT. 2) Adenosine triphosphatase (after Padykula and Herman), incubation time 5 to 45 min. 3) Butyryl-cholinesterase (Koelle method, modified by Gomori); substrate butyrylthiocholine iodide; incubation time 30 min. or longer. 4) Indoxylesterase (after Holt and Withers), substrate 5-bromo-4-chloro indoxylacetate, incubation time 30 min. or longer. 5) alkaline phosphatase, azo-dye coupling method (after Burstone), with naphthol AS-TR phosphate and fast blue VB salt, incubation time 5 min. to 45 min. Sometimes the Gomori calcium cobalt method was applied too.

RESULTS

1. α-glycerophosphate menadione tetrazolium reductase (αGlyPO$_4$D). In the spinal ganglion this enzyme is highly active in mantle cells. The large nerve cells of many animals are negative or less active than are the mantle cells (Fig. 1a). But in the cat these large nerve cells contain more enzyme than the mantle cell units. In the pig mantle cells were negative or only very slightly reacting, but the nerve cells stained well in this animal.

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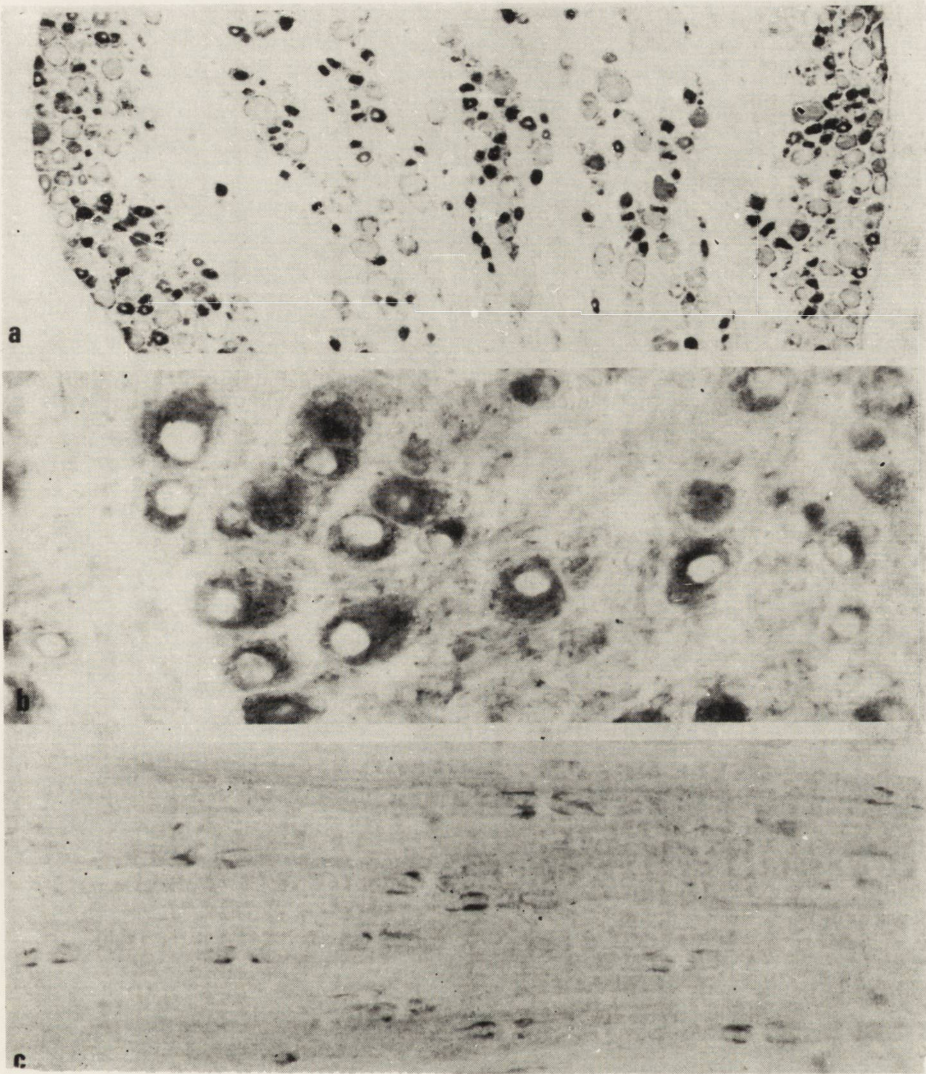

Fig. 1. α-glycerophosphate menadione tetrazolium reductase, a—c — reaction with Nitro BT, d — reaction with MTT, a — dorsal root ganglion, dog; the enzyme reaction is seen in the mantle cell units around the large nerve cells, × 40, b — sympthetic ganglion, mandrill; some reaction lies in the amphicytes, but the nerve cells are by far more active; the nerve at the left is negative, × 250, c — Vietnamese pig, spinal nerve; the enzyme reaction is visible only in the juxtancdal processes of the Schwann cells, × 140.

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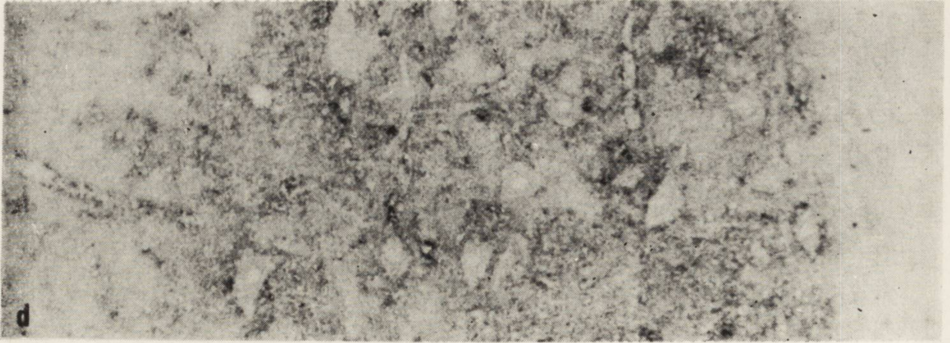

Fig. 1d. — monkey *(pygathrix naemeus)*, spinal cord, longitudinal section; very active areas mostly around motor neurons, probably belonging to glial cells are evident, × 70.

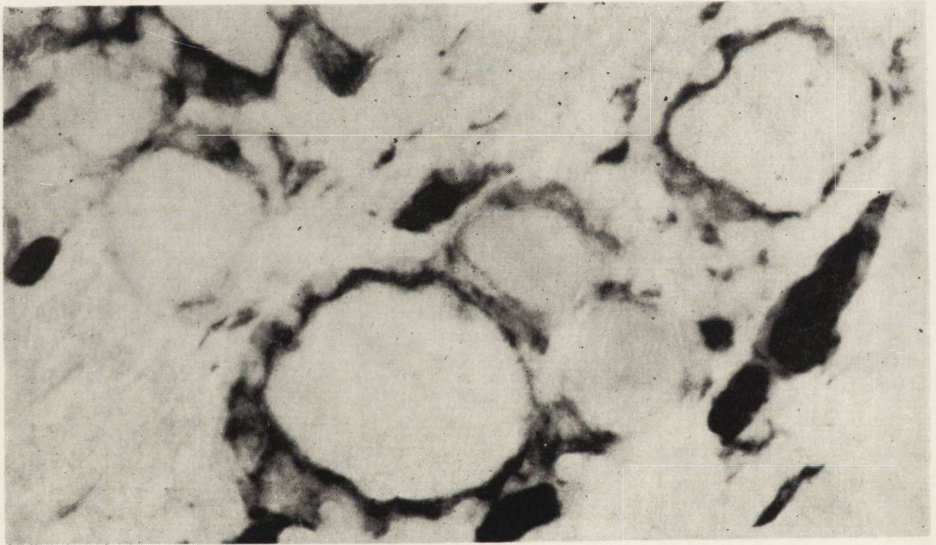

Fig. 2. Adenosine triphosphatase, incubation time 45 min. Dog, dorsal root ganglion. The enzyme reaction lies between the mantle cell units and in the mantle cells. It is higher around the large nerve cells, × 320.

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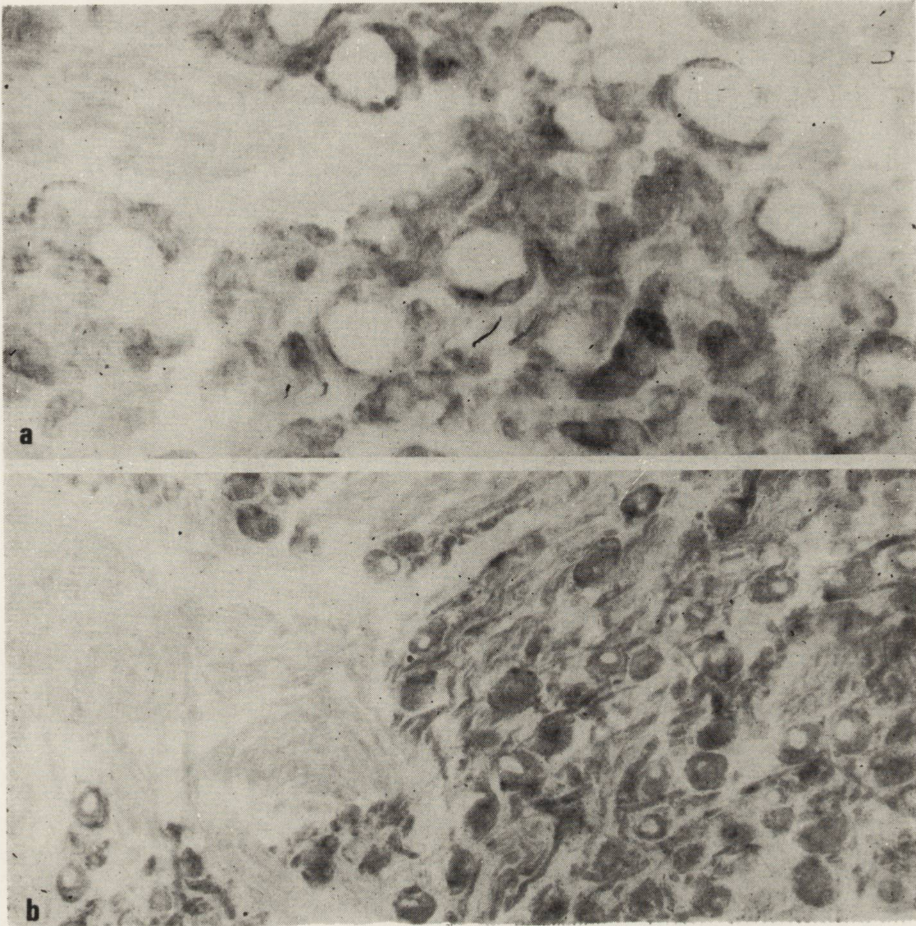

Fig. 3. Sympathetic ganglion, mandrill, a — butyrylcholinesterase, incubation time 45 min; the amphicytes contain the enzyme; a very faint reaction is seen along the nerve on the left, × 250, b — indoxylesterase, incubation time 60 min.; the esterase is located in amphicytes, nerve cells and their processes; the nerve on the left is only very weakly stained, × 125.

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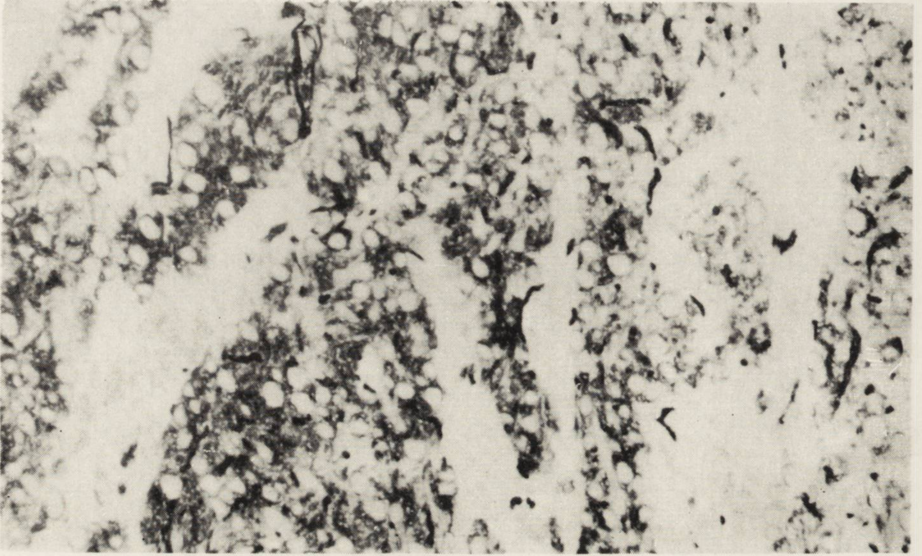

Fig. 4. Alkaline phosphatase, azo-dye coupling method, incubation time 10 min. Sympathetic ganglion, mandrill. Amphicytes contain much aPase, as do the endothelial cells. Nerve fiber bundles with Schwann cells lying in the ganglion are negative, × 60.

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In the sympathetic ganglia amphicytes are less active than are nerve cells, but they show some enzyme activity (Fig. 1b).

In the spinal nerves, especially well in the pig, a juxtanodal activity in the Schwann cells of myelinated fibers is to be seen (Fig. 1c). No activity is seen around the Schwann cell nucleus where other dehydrogenases are present. In the Remark fiber bundles after prolonged incubation time there is a very low overall activity e.g. in the rat. This activity is increased in the proximal stump after a nerve lesion. Finer structural details are not recognizable with the light microscope.

In the central nervous system, namely the spinal cord, enzyme accumulations in the form of relatively large active spots were seen especially in the dog, but also in other animals and man. These are around motor neurons (Fig. 1d). We assume that they belong to a special type of glial cell. Staining is more pronounced with the tetrazolium salt MTT than with Nitro BT. With the latter, the difference to an active neuropil is no longer so striking.

2. Adenosine triphosphatase (ATPase) is active in many places in the peripheral nervous system. Our results are consistent with those of Novikoff et al. (1966).

It may be added here, that in the dog the activity in the mantle cell units differs from one to the other (Fig. 2). Amphicytes contain ATPase in equal amounts. In unmyelinated fiber bundles one cannot decide by light microscopy where the strongly positive reaction product lies in the fine structure. Around some myelinated fibers one sees positive sheaths; these seem to be more often present in primate than in other animals. The same fiber may have a negative sheath in other parts of their length.

3. Butyrylcholinesterase (BChE) is located in mantle cells. The activity is different in the various species, but often is high (Table 1). Amphicytes contain BChE (Fig. 3a). Along the nerve fiber bundles in the sympathetic ganglion one sees a weak overall activity and in the *truncus sympathicus* additionally some more active strands are visible. One cannot decide with the light microscope whether the reaction is due solely to the Schwann cells or to the axons as well.

In the spinal nerves some small positive bundles run along the nerve and one can assume that these are Remak bundles. Stained strands of various length are also seen and they seem to be Schwann cell prolongations enwrapping myelinated fibers. The sheaths of most of the fibers were negative.

There are animals (pig), where only a very weak reaction was detectable after 20 hours of incubation. No activity was seen around Ranvier nodes. Single fibers of the nerve of a mandrill and a rabbit exhibit over a short distance activity in the Schmidt-Lanterman incisures.

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Table 1. Enzyme Activity in Mantle Cells

	aPase	ATPase	BChE	IE	αGlyPO$_4$D
Man	+	+	+	— ; (+)	+
Rhesus monkey	+	+	+		+
Mandrill	+	+	+	+	+
Dog	+	+	+	+	+
Pig	+	+	—	— ; (+)	— ; (+)
Cat	+	+	+	— ; (+)	(+)
Rabbit	+	+	+	+	+
Lynx	+	+	+		
Mink	+	+	+	+	
Rat	(+)	+	+	— ; (+)	+
Mouse	+			— ; some(+)	
Frog	(+)	(+)	—	—	

\+ present
(+) weak, present after prolonged incubation
— negative

4. I n d o x y l e s t e r a s e (IE). In this enzyme activity considerable species differences exist, as is shown for the mantle cells in table 1. These differences seen in the mantel cells also exist in ganglion cells, which are active in the ganglions of the mink and the frog, but not in the dog. The amphicytes contain IE (Fig. 3b). The results in the Schwann cells were the same as for BChE.

5. A l k a l i n e P h o s p h a t a s e (aPase). This enzyme is most striking in respect to the high activity in the mantle cells and in the amphicytes. There was a very pronounced and rapid hydrolytic reaction in the mantle cell units of spinal ganglia of all species examined. Differences in the activity from one mantle cell unit to another were observed in the pig and the dog; higher activity was located around the bigger nerve cells. No Schwann cells were stained in the sensory ganglia.

In the amphicytes a very strong aPase activity is seen (Fig. 4). There are areas in the ganglion with high enzyme concentrations and other areas where amphicytes are not so active. Schwann cells also in sympathetic ganglia were not stained.

It was only in the peripheral spinal nerves of some animals that what was assumed to be Remak fiber bundles reacting positively (Table 2). In most animals studied no reaction was seen. Likewise, only in the Schmidt-Lanterman incisures of some animals aPase was seen. In the rat, our results were inconsistent. But a high activity developed in irregular incisures along single fibers in what was assumed to be a retrograde reaction after a peripheral nerve lesion.

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Table 2. Alkaline Phosphatase Activity in Sheath Cells

	Mantle cells (sensory ganglion)	Amphicytes (sympathetic ganglion)	Schwann cell of Remak bundles	Schwann cell of myelinated fibers	
				Schmidt-Lanterman Incisures	Ranvier nodes
Man	+	+	—	mostly —	—
Rhesus monkey	+		—	+	some +
Mandrill	+	+	—	+	some +
Dog	+		+	—	—
Pig	+		—	—	—
Cat	+		—	—	—
Rabbit	+		—;seldom(+)	—	—
Lynx	+		—	—	—
Mink	+		+	—	—
Rat	(+)		—	+ ; —	some +
Mouse	+		—	—	—
Frog	(+)			—	—

\+ present
(+) weak present after prolonged incubation
— negative

DISCUSSION

Enzyme activity in mantle cells for α-glycerophosphate menadione tetrazolium reductase (Thomas, Pearse 1961), nucleoside phosphatases (Novikoff et al. 1966), butyrylcholinesterase (Koelle 1950), indoxylesterase (Thomas 1963) and alkaline phosphatase (Shimizu 1950) has been repeatedly reported in the literature. The amphicytes are likewise enzymatically very active in their hydrolytic capabilities and they seem to be a comparable kind of sheath cell in the sympathetic ganglia to the mantle cell in the sensory ganglia. With the oxidative enzyme $\alpha GlyPO_4D$, however, they are not comparable. Only the mantle cells (of many species, not all) seem to be more active than the nerve cells, but the amphicytes in the rat are less active than are the autonomic nerve cells (Härkönen 1964) and we observed the same in the mandrill.

Gomori (1941) has shown that there exist species differences in aPase activity in the amphicytes. The mantle cells of all species studied contained this enzyme generally in high concentrations. In some species it was more active in the units round the large nerve cells. The same is the case for ATPase. With the electron microscope Matsuura et al. (1970) described aPASE in some "dark" mantle cells of the trigeminal ganglia of

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the rat in the endoplasmic reticulum, on ribosomes, and on/or between the membranes of both nerve cells and mantle cells and their interdigitations.

In unmyelinated fiber bundles, Schwann cells exhibit relatively low but distinct activities for BChE and IE. The enzymes are found along the whole length of the Remak fiber bundles. In Schwann cells around myelinated fibers activity, if seen at all, is restricted to cytoplasmic areas around the nucleus and to strands of cytoplasm lying along the internode or juxtanodally. Very seldom and inconsistently we saw BChE in Schmidt-Lanterman incisures of single fibers. This localization does not seem to be the rule, or our methods are too crude to detect a small activity. APase on the other hand can be seen very well in the incisures (Pinner et al. 1964). Here we assume thet species differences are responsible for the variety of findings. The inconsistency of a positive reaction in the rat under standardized preparation procedures suggests a different behaviour at these incisures under different circumstances. A dynamic point of view as to their enzyme content seems justified also because under pathologic conditions these incisures can become very active for alkaline phosphatase.

In the Remak fiber bundles aPase was seen in the dog, and the mink, but with the light microscope one is not absolutely sure where the reaction really lies. In the rabbit the finding was sporadic and in the other species examined there was no activity seen in the Schwann cells around unmyelinated fibers. Thus, for aPase we can state that the sheath cells around axons behave different than those around neurons.

As the function of aPase is not known one can only speculate about its role in the mantle cells and the amphicytes. Involvement of aPase in transport processes is discussed in the literature (Cohen 1970). The localization of aPase seems to justify this idea. But for the sheath cells in the peripheral nervous system we have to consider other functional roles also. The activity of non-specific cholinesterase and of indoxylesterase may be interpreted as a measure of protection of the non-neural tissue against products of the neural parenchyma. Here, species differences confront us with difficulties in interpretation.

Other specialized cells bordering the nervous tissue as a whole seem to have partly similar functions to the sheath cells; these are the perineurium cells and the arachnoidal cells which can contain aPase too in high quantities. But here again species differences (Hennig 1972) render interpretations very difficult. Only for the mantle cells there seems to exist a broader morphologic principle from a phylogenetic point of view because these cells were positive for aPase in all the species so far examined. This seems important to state for this kind of cell, because

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here we studied the same animals which can give negative results in the other cells mentioned.

Acknowledgement: We are indebted to Dr. G. Klinghardt and Dr. H. Gräfin Vitzthum for kindly providing the material of some of the animals studied. The technical assistance of Charlotte Beyer and Ilonka Kamperschrör is gratefully acknowledged.

E. Thomas

HISTOCHEMIA ENZYMATYCZNA JAKO CZYNNIK RÓŻNICUJĄCY CHARAKTER KOMÓREK OSŁONKOWYCH (SATELITARNYCH), AMFICYTÓW I KOMÓREK SCHWANNA WŁÓKIEN BEZMIELINOWYCH I MIELINOWYCH W ZWOJACH MIĘDZYKRĘGOWYCH, ZWOJACH WSPÓŁCZULNYCH I NERWACH OBWODOWYCH U RÓŻNYCH GATUNKÓW ZWIERZĄT

Streszczenie

Oznaczono aktywność reduktazy menadion α-glicerofosforanu, adeninotrójfosfatazy, butyrylcholinesterazy, esterazy indoksylowej i monofosfatazy zasadowej w komórkach osłonkowych nerwów obwodowych u różnych gatunków zwierząt.

Komórki osłonkowe zwojów czuciowych i amficyty zwojów współczulnych wykazują nieoczekiwanie wysoką aktywność większości badanych enzymów.

Komórki Schwanna otaczające bezmielinowe włókna również wykazują aktywność enzymatyczną, lecz u większości badanych gatunków zwierząt brak aktywności monofosfatazy zasadowej. W komórkach Schwanna otaczających włókna mielinowe aktywność enzymatyczna niektórych enzymów czasem występuje tylko ogniskowo w miejscach przewężeń Ranviera i wcięć Schmidt-Lantermana.

Zróżnicowanie enzymatyczne pozaneuronalnych elementów tkankowych jest znacznie większe u różnych badanych gatunków zwierzęcych, niż można by oczekiwać na podstawie identycznych obrazów morfologicznych.

Autor wysuwa przypuszczenie, że aktywność enzymatyczna komórek osłonkowych jest związana przynajmniej częściowo z zabezpieczeniem tkanek położonych poza neuronem. Ponieważ z punktu widzenia histochemicznego komórki osłonkowe i amficyty różnią się od siebie czynnościowo, wydaje się uzasadnione utrzymanie odrębności ich nomenklatury. Komórki Schwanna, natomiast, należy traktować jako jeszcze inną postać komórek, pozostających w ścisłej zależności od włókien przez nie otaczanych.

Е. Томас

ЭНЗИМАТИЧЕСКАЯ ГИСТОХИМИЯ КАК ФАКТОР ДИФФЕРЕНЦИРУЮЩИЙ ХАРАКТЕР САТЕЛИТНЫХ КЛЕТОК, АМФИЦИТОВ И КЛЕТОК ШВАННА ВЕЗМИЕЛИНОВЫХ И МИЕЛИНОВЫХ ВОЛОКОН В МЕЖПОЗВОНОЧНЫХ ГАНГЛИЯХ, СИМПАТИЧЕСКИХ УЗЛАХ И ПЕРИФЕРИЧЕСКИХ НЕРВАХ У РАЗНЫХ ВИДОВ ЖИВОТНЫХ

Резюме

Определялась активность редуктазы менадион α глицерофосфата, аденинтрифосфатазы, бутирилхолинэстеразы, индоксиловой эстеразы и основной монофосфатазы в клетках оболочки периферических нервов разных видов животных.

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Клетки оболочки чувствительных ганглиев и амфициты симпатических узлов обнаруживают неожиданно высокую активность большинства исследованных энзимов.

Клетки Шванна, окружающие безмиелиновые волокна также обнаруживают энзиматическую активность, но у большинства исследованных видов животных отсутствует активность основной монофосфатазы. В клетках Шванна, окружающих миелиновые волокна энзиматическая активность некоторых энзимов обнаруживается иногда только очагами в местах перетяжек Ранвье и вырезок Шмидт-Лянтермана.

Энзиматическая дифференциация вненейронных тканевых элементов значительно больше, у разных исследованных видов животных, чем можна бы этого ожидать на основании идентичных морфологических картин.

Автор полагает, что энзиматическая активность клеток нервных оболочек по крайней мере частично связана с обеспечением тканей находящихся вне нейрона. Так как с гистохимической точки зрения клетки нервных оболочек и амфициты отличаются от себя функционально кажется обоснованным сохранение независимости их номенклатуры. Клетки Шванна, зато, следует рассматривать как еще другой вид клеток, остающихся в тесной зависимости от нервных волокон.

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A COMPARATIVE HISTOCHEMICAL STUDY ON FUNCTIONAL PROTEIN GROUPS AND SOME OXIDIZING-REDUCING ENZYMES IN REACTIVE GLIA AND GLIAL TUMOURS

II. REACTIVE GLIA

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Tumour growth in the CNS produces changes in the surroundings of the tumour, resulting from its action upon the tissue. In connection therewith, we examined both the tumour and the surrounding glia, in particular at the line of junction of the tumour infiltration to the normal tissue. A so-called "uncertain region" can be distinguished within the region of infiltration, the intensity and diversity of the former being significant both for estimating the neoplastic growth and for checking its complete removal. This transitory region can form a subject of studies on the changes occuring in the surroundings and on the reaction of the tissue on tumour infiltration.

Since morphologically the tumour-surrounding glia differs only slightly — if at all from the reactive glia contacting the other — non neoplastic — pathologic processes, the examination of the former meets with considerable difficulties. The morphological methods turned out to be insufficient for distinguishing the uncertain region from the changes occuring around the tumour.

The histoenzymatic studies (Kałuża 1970, Rubinstein et al. 1962, Schiffer et al. 1965) revealed a difference between the normal and tumour-surrounding reactive glia as to the activities of some respiratory enzymes. On the other hand, as may be concluded from the studies on functional protein groups (Szydłowska 1970b), the intensity of reactions for NH_2, COOH and SH groups in the reactive glia varies from one pathologic process to the other. Furthermore, both the intensity of the reactions and their localization in the reactive glia differ from those in the neoplastic glia (Szydłowska 1970a, 1970c).

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The connections of the metabolism of amino acids with the Krebs cycle, as well as the high enzymatic activity of reactive astrocytes, prompted us to study comparatively the activities of same oxidizing--reducing enzymes and the functional protein groups.

MATERIAL AND METHODS

For histochemical studies of enzymes and functional protein groups, human material has been used. Samples of tumours and surrounding tissue were taken at surgery. The samples for histoenzymatic studies were immediately frozen in dry ice. Slices of 15 microns were cut in a kryostat, dried at room temperature and stored at —4° — 0°C for not longer than 24 hrs. The histoenzymatic reactions were carried out under standard conditions (incubation time — 45 min., temperature — 25°C) acc. to Nachlas et al. (1958) using Nitro-BT as indicator. Incubation was stopped by transferring the samples into 4% formaldehyde solution. Samples were embedded in glycerol gel.

The following enzymes were investigated: lactate dehydrogenase (LDH), isocitrate dehydrogenase, succinate dehydrogenase, glutamic dehydrogenase, malonic dehydrogenase as well as NADH and NADPH — dependent terazolium reductase.

The material designated for histochemical investigations on functional protein groups was fixed in Carnoy solution (6 : 3 : 1) at —4°C for 6 hrs. Thereafter the samples were transferred into absolute alcohol, cooled to —4°C, for 24 hrs. Following that, they were passed through methyl benzoate with celloidine and rapidly embedded in paraffin. The thickness of the slices was 5—7 microns. The NH_2 groups were detected by reaction with ninhydrine and Schiff reagent, after Yasuma and Itchikawa (1953). The control experiments were performed on samples deaminated with a mixture of 10% acetic acid and 5% aqueous solution of sodium nitrite for 24 or 48 hrs at —4°C (Pearse 1960). For identification of amino acids with free NH_2 groups, reaction with 1% solution of DNFB*) in 90% alcohol was performed for 16 hrs at room temperature. The COOH groups were visualized with the method of Barrnett and Seligman (1956, 1958). Reactions were carried out on two series of samples. One of them was previously passed through a solution of sodium bicarbonate in order to show exclusively the C-terminal carboxylic groups. In the other series, which was not washed at all, the COOH groups of the radicals of dicarboxylic acids could be revealed in addition (Karnowsky, Fasman 1960).

The control samples were methylated before the reaction in 1% solution of HCl in methyl alcohol for 1 hr at 60°C (Lillie 1958).

*) DNFB — Dinitrofluorobenzene

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The SH groups were detected on the basis of the mercaptan reaction with a method of Bennett (1951) in a modification of Szydłowska et al. (1967). The SH groups in the control samples were blocked before the reaction with 0.1 M N-methylmaleimide solution for 4 hrs at 37°C (Lison 1960).

RESULTS

In the neighbourhood of abscesses, cerebro-meningeal scars and metastatic foci, which are accompanied by collateral edema of different intensity, the individual enzymes show changing activities, not always depending on the type of pathology.

In all of the mentioned types of diseases, however, the reactive glia shows an extremely high activity of LDH (Figs. 1a, b, 2), but very low activities of malonate, isocitrate and succinate dehyrogenases.

The NADH — dependent tetrazolium reductase (Fig. 1a) of the reactive glia displays a high activity as compared with the normal tissue, but is less active than in the neoplastic glia. The NADPH — dependent tetrazolium reductase gives a similar, but unequally distributed reaction, with individual cells characterized by a very low activity of the enzyme.

The most intensive reactions of the mentioned enzymes were observed around the abscesses, especially those accompanied by a wide isomorphic gliosis, coinciding with a lack of productive changes. The reactive glia around metastatic tumours exhibited a somewhat lower activity of these enzymes. The relatively lowest activity could be detected in the glia surrounding the cerebro-meningeal scars.

In the neighbourhood of glial tumours, and in particular around the perineoplastic cysts, a region appears which may be described as a region of enzymatic depletion (Figs 1 a, b). On its background, single astrocytes with an enhanced enzymatic activity were observed, neither resembling the neoplastic glia with their shape nor with length and character of their processes. Single astrocytes of a similar morphological and histochemical character were also found in some regions of the neoplastic tissue. The same observations were done previously by other authors (Smith 1963).

Contrary to the changes in activity of the above described enzymes, the glutamate dehydrogenase exhibits a relatively high activity in the astrocytes arround the abscesses and cerebro-meningeal scars (Fig. 3a), and is much less active in the surroundings of glial tumours and metastatic foci. Changes in activity of this enzyme coincide with some changes in intensity of the reactions for functional protein groups. The

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number of the latter increases unequally in the reactive astrocytes, the increase depending on the pathologic process (Szydłowska 1970b).

Cytoplasm of the astrocytes surrounding abscesses and cerebro-meningeal scars is characterized by an exstraordinarily strong NH_2 — group reaction (Fig. 3b) and a very weak reaction of COOH and SH groups (Figs. 3c, d). Around the glial tumours, in particular around the malignant ones, the reaction of NH_2 groups is moderate, and those of COOH and SH groups very strong. In the cytoplasm of reactive astrocytes around the metastatic tumours all three reactions are very weak.

A certain correlation may be concluded between the intensity of the NH_2 group reaction and the activity of glutamic acid dehydrogenase (Fig. 1c).

Numerous reactive astrocytes around the glial tumours found within the region of "enzymatic depletion" exhibit a much lower level of functional protein groups than those which are localized at a significant distance from the front of the neoplastic growth. The former are characterized in the first instance by a drop in level of COOH groups, but also of NH_2 groups. They are morphologically changed and both their shape and the type of processes do not exclude the possibility of their transformation into neoplastic cells (Szydłowska 1970a). A significant part of the cells, however, exhibits morphological properties of reactive astrocytes and a high level of functional groups. Single cells exhibiting a strong functional protein group reaction as well as those with a high enzymatic activity were also present in the neoplastic tissue.

DISCUSSION

The cytoenzymatic reactions are less, and the reactions of functional protein groups more differentiated in the reactive than in the neoplastic glia. This difference between neoplastic and reactive glia may be due to the fact, that the activity of the oxidizing-reducing enzymes is not only connected with protein metabolism, but also with metabolism of lipids and carbohydrates. The coincidence of the intensity of some reactions of functional protein groups with that of glutamate dehydrogenase activity may be explained by the key function fulfilled by this enzyme both in catabolism and synthesis of a majority of amino acids.

The high activity of glutamate dehydrogenase in reactive astrocytes around abscesses and cerebro-meningeal scars as well as a significant rise in level of NADH- and NADPH- dependent tetrazolium reductase therein, indicate either an increased rate of deamination of L-glutamate to α-oxogluterate, or an enhancement of the reversed process (reductive

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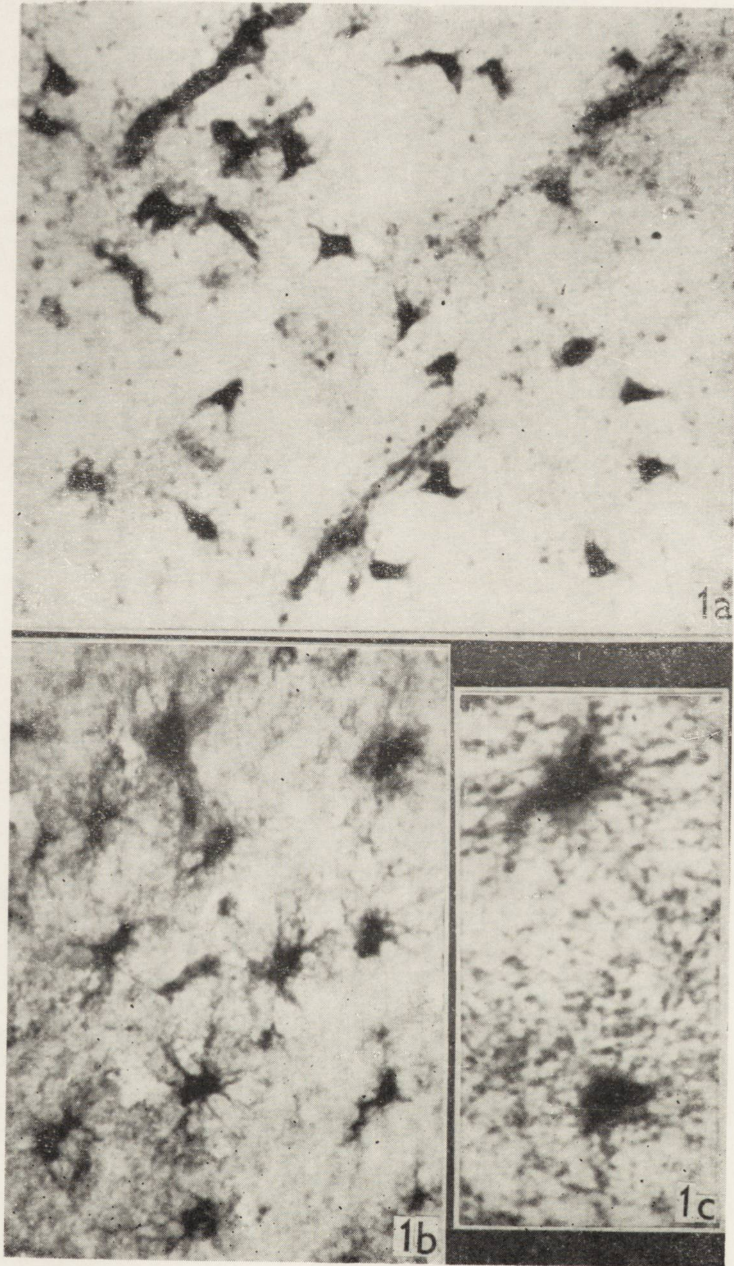

Fig. 1. Reactive astrocytes around the neuroglial tumour. x 1170; a — NADH — dependent tetrazolium reductase, b — Lactic acid dehydrogenase, c — Strong Barnett-Seligman reaction for COOH groups.

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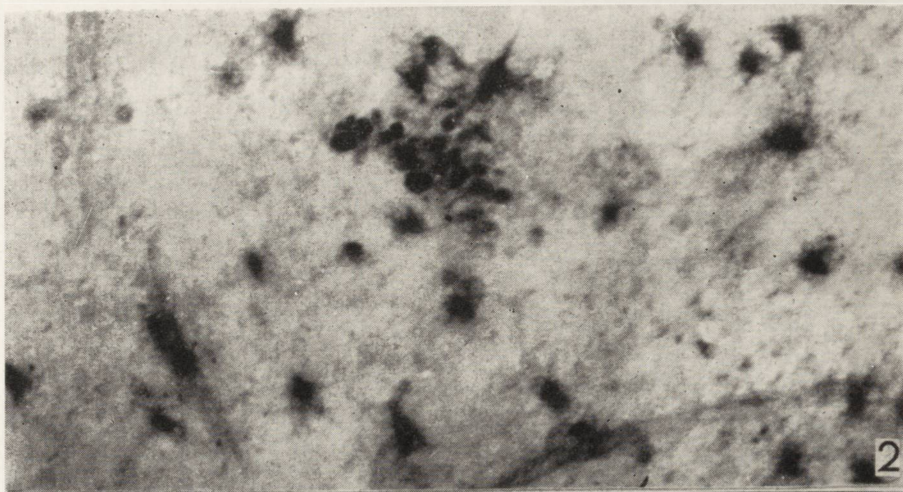

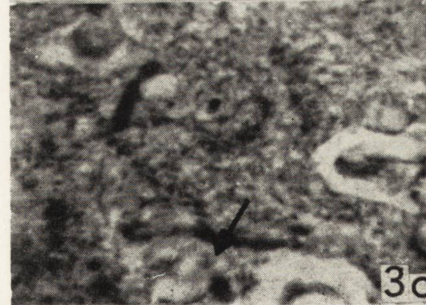

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amination), both catalyzed by this enzyme with participation of NAD and to a lesser extent of NADP (Baldwin 1963, Frunton, Simmonds 1958, Greenberg 1961, Pearse 1960, Kączkowski 1968).

A weak reaction of COOH groups may be indicative for a shift in the direction of deamination of L-glutamate to α-oxoglutarate. Although the Barrnett — Seligman method (1956, 1958) is supposed to display the α-acyloaminocarboxylic groups (R-CO-NH-CH COOH), thus the C-terminal groups (Barrnett, Seligman 1956, 1958, Friede 1962, Lison 1960, Pearse 1960), numerous authors doubt the specifity of this method. To their opinion, this method reveals at least a part of COOH groups of decarboxylic amino acids, in particular those of glutamic acid radicals (Ball, Gersztein 1966, Gersztein 1966, Gersztein, Cwietkova 1960, Pearse 1960, 1968).

The intensity of the reaction on COOH groups performed without washing the samples with sodium bicarbonate, a procedure known to reveal both types of carboxylic groups (Karnkowsky, Fasman 1960) hardly differed from that on washed samples. The latter difference, however, was much higher in the case of normal astrocytes, which contain considerable amounts of glutamic acid (Bairati 1958) and display a much lower activity of glutamate dehydrogenase. This may be indicative of a lower glutamic acid content in astrocytes surrounding non-neoplastic processes than in normal ones.

α-oxoglutarate formed in the reaction could either get metabolized via the Krebs cycle or take part as an intermediate in deamination of other amino acids. Incorporation of α-oxoglutarate into the Krebs cycle would cause an increase in activity of the enzymes of this cycle, like isocitric and succinate dehydrogenases. In this case, however, the activity of the mentioned enzymes is low. Moreower, the reaction on SH groups, which activate these enzymes, was weak. It may be assumed, therefore, that α-oxoglutarate, formed in the cytoplasm of reactive astrocytes around abscesses and cerebromeningeal scars, is an intermediate in the process of deamination of amino acids. A possibility that it participates in the synthesis of the heme-ring without entering Krebs cycle, seems very unlikely.

Fig. 2. Lactic acid dehydrogenase in reactive astrocytes around the metastatic tumour. Metastatic tumour cells in the centre around the blood vessel. x 1170.

Fig. 3. Reactive astrocytes around the abscess. x 1170; a — High glutamic acid dehydrogenase activity, b — Strong NH_2 group reaction with ninhydrin and Schiff reagent, c — Arrows indicate places characterized by a weak Barrnett-Seligman reaction for COOH groups, d — Arrows indicate places characterized by a weak SH group reaction. Method by Bennet.

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The strong reaction of NH_2 groups in cytoplasm of these astrocytes, coinciding with a weak reaction on COOH groups, seems to confirm the above observations. It provides evidence for a presence, along with small amounts of N-terminal α-NH_2 groups, of other amino groups of the type R-CH_2 — NH_2, like e.g. the ε-NH_2 group of lysine an δ-NH_2 group of ornithine which as side chain groups do not undergo deamination. The fact that these amino acids, usually present in núclei occur in high concentration in the cytoplasm, may be due to their resistance towards deamination (Baldwin 1963, Baranowski 1963, Barrnett, Seligman 1958, Bell 1958). Although ornithine has never been found in protein hydrolysates and its occurrence in the animal tissues is of a transitory character, its presence in reactive astrocytes may be concluded from the positive reaction with ninhydrine and Schiff reagent in numerous cells of DNFB — treated samples. The presence of ornithine, which was absent both in the normal tissue and around the glial tumours, may be indicative for the incorporation into the ornithine cycle of the NH_2 group released in the process of deamination.

In the so-called „region of enzymatic depletion" around the glial tumours, a high enzymatic activity and a strong reaction of SH groups were observed in the same astrocytes indicating the enhancement of the Krebs cycle processes. Considering in addition the low activity of glutamate dehydrogenase, one may assume that the processes taking place in astrocytes around gliomas differ from those in reactive astrocytes around non-neoplastic pathological foci, the difference in NH_2/COOH ratio remaining in connection therewith. It seems, however, that the positive reaction on COOH coinciding with a moderate reaction on NH_2 cannot be ascribed to the presence of glutamic acid despite the high glutamate transaminase activity detected around the glial tumours (Berezow, Lutsenko 1963). The latter conclusion could be drawn from the comparison of the results of COOH tests performed in the presence and absence of bicarbonate, respectively. Even assuming a certain aspecificity of the reaction, the main proportion of these groups have to be considered as C-terminal COOH groups. Since the reaction on NH_2 groups was relatively weak, it seems correct to attribute a part of COOH groups to L-phenylacetylglutamine, not containing NH_2 groups and formed in the nervous tissue in connection with removal of toxic metabolites.

The low activity of glutamic acid dehydrogenase and weak reactions on functional protein groups in astrocytes around the metastatic tumours, coinciding with a high activity of the other enzymes tested therein indicate, that contrary to the cases of gliomas and non-neoplastic diseases, the metabolic disorders of the Krebs cycle in the cells around metastases are not very much related to the protein metabolism. Two factors are

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supposed to have caused such a result, one of them be a particular character of metastasis in relation to the nervous tissue, another — a short growth time of metastasis and a rapid desintegration of a tissue in this type of a tumour. This could explain a certain functional insufficiency of reactive glia around the metastatic foci.

CONCLUSIONS

1) The cytoenzymatic activity and the intensity of the reactions of the functional protein groups (NH_2, COOH, SH) vary from one pathologic process to the other.

2) A parallelity exists between the changes in glutamate dehydrogenase activity and the intensity of the NH_2 group reaction.

3) In single astrocytes around the glial tumours, the high activity of such enzymes like lactate, malonate, isocitrate and succinate dehydrogenases as well as of NADH- and NADPH-dependent tetrazolium reductase, is accompanied by an increased number of COOH and SH groups.

4) The low glutamate dehydrogenase activity and the high activity of remaining oxidizing-reducing enzymes around the metastatic tumours coincide with very weak reactions for functional protein groups in reactive astrocytes.

H. Szydłowska, J. Kałuża

HISTOCHEMICZNE BADANIA PORÓWNAWCZE GRUP CZYNNOŚCIOWYCH BIAŁEK I NIEKTÓRYCH ENZYMÓW OKSYDACYJNO-REDUKCYJNYCH W GLEJU ODCZYNOWYM I NOWOTWORACH GLEJOPOCHODNYCH

II. Glej odczynowy

Streszczenie

Autorzy porównywali nasilenie reakcji na grupy NH_2, COOH i SH w astrocytach odczynowych w otoczeniu różnych nienowotworowych procesów chorobowych, nowotworów glejopochodnych i nowotworów przerzutowych, z aktywnością wybranych enzymów oksydacyjno-redukcyjnych w tych komórkach (dehydrogenaza kwasu glutaminowego, mlekowego, malanowego, izocytrynowego i bursztynowego oraz NADH- i NADPH- reduktaza tetrazolowa).

Z badań wynika, że jedynie zmiany aktywności dehydrogenazy kwasu glutaminowego odpowiadają zmianom nasilenia reakcji na grupy NH_2. Zmianom aktywności pozostałych enzymów nie odpowiadają równoległe zmiany w nasileniu reakcji na grupy czynnościowe białek.

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Г. Шидловска, Е. Калужа

ГИСТОХИМИЧЕСКИЕ СРАВНИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ АКТИВНЫХ ГРУПП БЕЛКОВ И НЕКОТОРЫХ ОКСИДАЦИОННО-РЕДУКЦИОННЫХ ЭНЗИМОВ В РЕАКТИВНОЙ ГЛИИ И ГЛИОПРОИЗВОДНЫХ ОПУХОЛЯХ

II. Реактивная глия

Резюме

Авторы сравнивали выраженность реакции на группы NH_2, COOH и SH в реактивных астроцитах разных болезненных неопухолевых процессов, глиопроизводных опухолей и метастазных опухолей с активностью в этих клетках дегидрогеназы глютаминовой, молочной, малоновой, изолимонной и янтарной кислоты, а также NADP и NADPH тетразольной редуктазы.

Из исследований следует, что только изменения активности дегидрогеназы глютаминовой кислоты соответствуют изменениям интенсивности реакции на группы NH_2. Изменениям активности остальных энзимов не соответствуют параллельные изменения интенсивности реакции на активные группы белков.

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OXIDOREDUCTASES IN DEVELOPING NEUROGLIA

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Consideration of developmental and differentiation problems of the neuroglia in terms of enzyme cytochemistry seems to present an important step on the way leading to elucidation of these processes (for a review of this subject see Meyer 1963, and Adams 1965). The results of our up to date investigations concerning the development of neuroglia (Wender, Kozik 1969; Wender et al. 1969, 1970a) have revealed that, regardless of some species differences the early developmental events (beginning with matrix cells up to appearance of spongioblasts and other immature forms of glia cells) comprise not only morphological differentiation but also significant alterations in the enzymic activities of phosphatases, mainly of acid phosphatase, ATP-ase and TPP-ase.

In the late foetal stage, the activities of acid and alkaline phosphatases, of 5'-nucleotidase, of ATP-ase and of TPP-ase as well as of acetylcholinesterase and of cholinesterase are localized in the ependymal cells. Furthermore there is a visible biological distinction between the multilayered plate of nondifferentiated matrix cells and the ependymal cells — the phosphatase activity appearing relatively weaker in the matrix cells. In the mantle layer the activities of some phosphatases i.e. of acid phosphatase, ATP-ase and TPP-ase and to some extent also of one of the esterases (ChE) increase significantly in the course of cell differentiation, but no marked differences between spongioblasts and neuroblasts were noted.

During postnatal development the activities of phosphatases (acid phosphatase, TPP-ase and ATP-ase) and also in some species of some esterases (AChE and ChE) are substantially elevated during the period of enhanced biological activity of the neuroglial cells i.e. during myelination gliosis. The later period of brain development is also accompanied by changes in the enzyme activity of the neuroglia. The fact should be stressed that in mice it is ATP-ase activity in the vascular feet of the neuro-

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glia which seems to be enhanced, whereas in rabbits butyrylcholinesterase and acetylcholinesterase show increased activity in the course of maturation of the oligodendroglia.

As a further step in these studies, investigations of the activity of oxidoreductases in the developing neuroglia were performed. The results were compared with those for various phosphatases and esterases.

MATERIAL AND METHODS

Our investigations were performed on white Wistar rats of either sex. The experimental material was divided into 6 age groups, each consisting of 8 animals; the respective age groups were as follows: 1, 8, 17, 40 and 60 days. Rat foetuses from the 17th day of intrauterine life were also included in the study. The animals were killed by decapitation, and the brains immediately removed and subjected to histoenzymatic studies.

For the assay of oxidoreductases, 12 μ thick sections were cut in a cryostat. The material for determination of esterases and phosphatases was fixed at 4°C for 18 hours in Baker solution. A part of the fixed brains was embedded in paraffin and stained by the method of Klüver-Barrera and H+E.

The activities of the following oxidoreductases were studied: glycerol-3-phosphate dehydrogenase (1-glycerol-3-phosphate: NAD oxidoreductase — E.C. 1.1.1.8) — incubation time 20 min.; lactate dehydrogenase — LDH (1-lactate: NAD oxidoreductase — E.C. 1.1.1.27) — incubation time 50 min.; 3-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate: NAD — oxidoreductase — E.C. 1.1.1.30) — incubation time 60 min.; glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NAD-P-oxidoreductase — E.C. 1.1.1.49) — incubation time 30 min.; succinate dehydrogenase (succinate: acceptor-oxidoreductase — E.C. 1.3.99.1) — incubation time 30 min.; threo-D-5-isocitrate: NAD — E.C. 1.1.1.41 (decarboxylating-iso-citric-dehydrogenase) — incubation time 35 min.; reduced NADP: tetrazolium dehydrogenase (reduced NADP: acceptor-oxidoreductase — E.C. 1.6.99.1) — incubation time 30 min.; reduced NAD: tetrazolium dehydrogenase (reduced NAD: acceptor-oxidoreductase — E.C. 1.6.99.3) — incubation time 35 min.

For the assay of the individual dehydrogenase activities, the basal incubation medium prepared according to Pearse (1960), and containing Nitro BT tetrazolium salt, together with the appropriate substrate as recommended by Niweliński (1963) was used.

For details concerning the methodology used in phosphatase and sterase assays, the readers are referred to the following references: acid and al-

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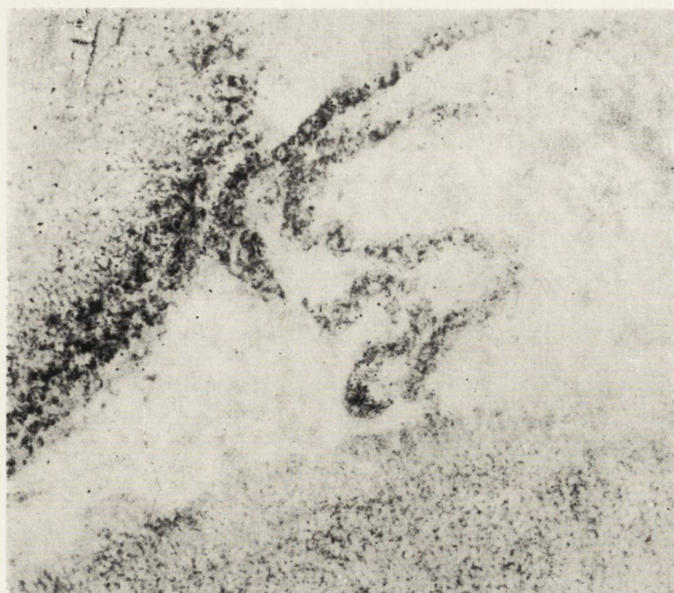

Fig. 1. Succinate dehydrogenase activity in epithelial cells of the chorioid plexus, in ependyma and in matrix cells. Rat-1-day-old. × 110.

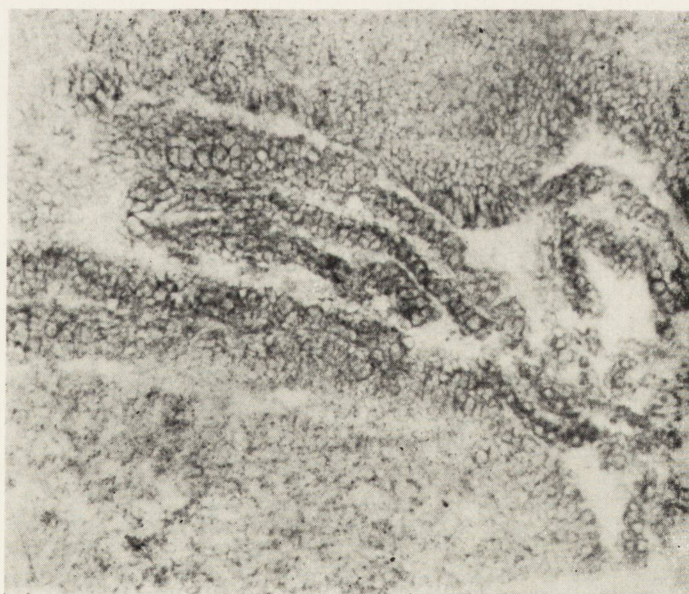

Fig. 2. Low activity of threo-D-5-isocitrate dehydrogenase in matrix cells. Rat — 17 days old. × 110.

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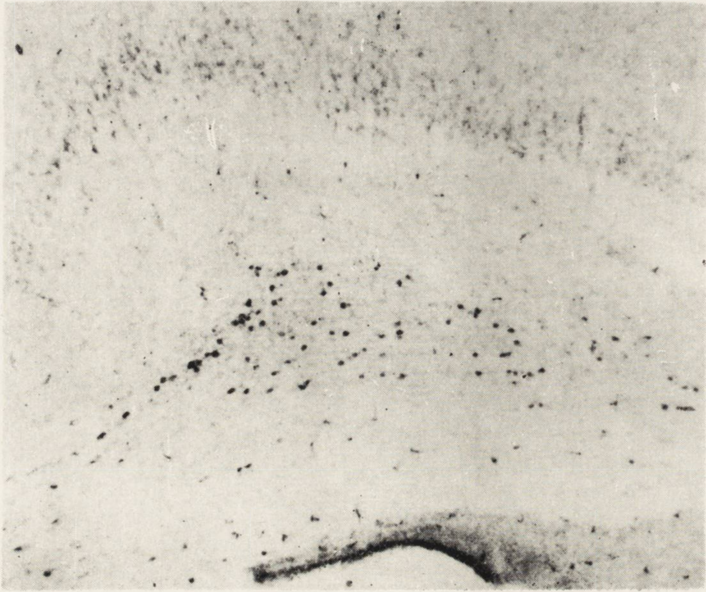

Fig. 3. AcP activity in matrix layer and neuroglial cells of the corpus callosum at the period preceding myelinogenesis. Rat — 8 days old. × 50.

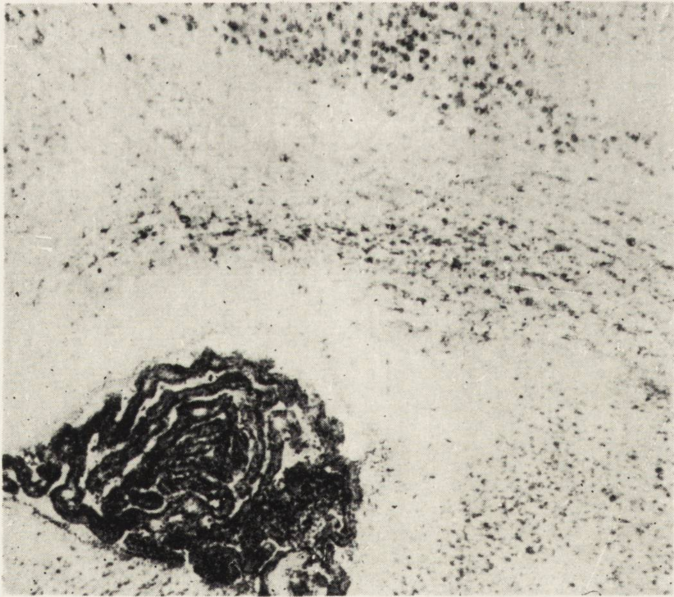

Fig. 4. Myelination gliosis with intensive acP activity. Rat — 17 days old. × 100

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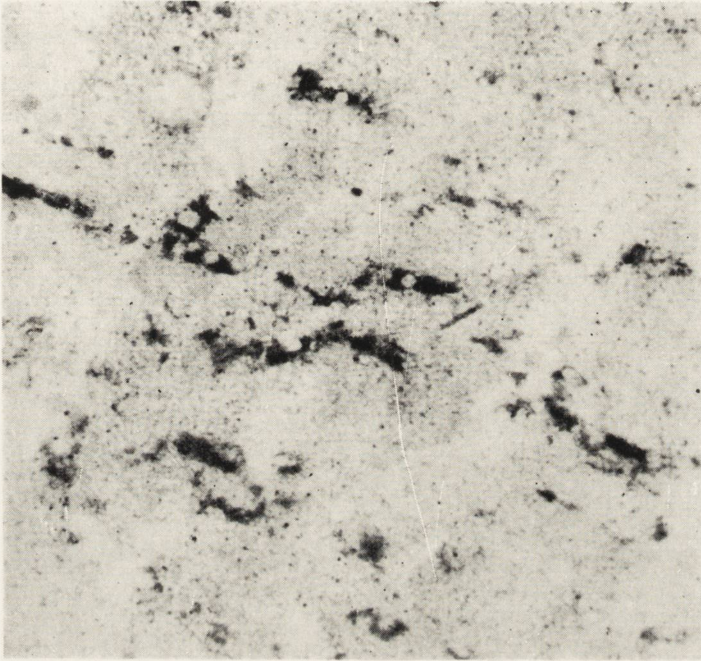

Fig. 5. Activity of lactate dehydrogenase in oligodendroglial cells of the corpus callosum in the late period of extrauterine development. Rat — 40 days old. × 450

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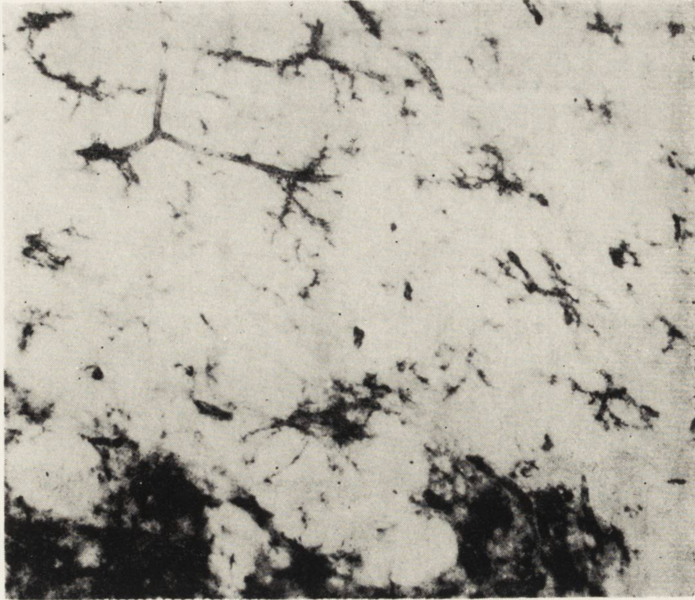

Fig. 6. Rat — 40 days old showing ATP-ase activity emerging in astrocytes and their perivascular feet. × 120.

Fig. 7. TPP-ase activity in astroglia of the white matter. Rat — 60 days old. × 120.

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kaline phosphatases (Gomori 1953); TPP-ase (Novikoff, Goldfischer 1961); ATP-ase (Wachstein, Meisel 1957); non-specific esterase (Holt 1966); AChE and ChE (Gerebtzoff 1953).

RESULTS

As the differences between individual oxidoreductase activities were rather insignificant, the results of these assays will be discussed jointly.

The performed studies have shown that ependymal cells exhibit in the late stage of foetal and in the early period of extrauterine life strong oxidoreductase activity. The cells of the chorioid plexus react similarly (Fig. 1).

The multilayer lamina of nondifferentiated matrix cells displays somewhat different biological properties as compared with ependymal cells: the activity of the investigated oxidoreductases is lower in the matrix and in the differentiating cells of the mantle layer (Fig. 2).

Contrary to the former enzymes these cells exhibit high activities of phosphatases (acP, alkP, TPP-ase, and ATP-ase) and low nonspecific esterase activity without detectable differences between spongioblasts and neuroblasts (Fig. 3).

In the period preceding myelinogenesis (8-day-old rats) as well as during myelination gliosis (17-day-old animals), neuroglial cells do not display increased activities of the investigated oxidoreductases acting within the glycolytic and pentose shunts. On the contrary at the time of myelination gliosis and parallel to differentiation of the immature neuroglia — the glial cells exhibit increased acP, ATP-ase and TPP-ase activities (Fig. 4). The said activity disappears during the later period of brain maturation. Nevertheless in the course of this period of extrauterine development (40-day-old animals), neuroglial cells of the white matter, especially oligodendroglial ones show an enhanced oxidoreductase activity, persisting until adult stage (Fig. 5).

At this late period of development ATP-ase and TPP-ase activities appear in astrocytes and their vascular feet (Fig. 6 and 7).

Acetylcholinesterase and non-specific cholinesterase activities could not be demonstrated in neuroglial cells throughout the whole period of investigation, i.e. from the last period of foetal life up to adult age.

DISCUSSION

The above presented results as well as those obtained in our previous investigations indicate that the activities of phosphatases (acid phosphatase, ATP-ase and TPP-ase) increase indeed in the period of intensified

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biological activity of the neuroglial cells, i.e. during their differentiation and myelinogenesis (Wender et al. 1970, Wender et al. 1970b) as well as during neuroglial hypertrophy and hyperplasia in pathological conditions (Wender et al. 1966, Wender, Kozik 1969). This is not the case with the oxidoreductases acting within the glycolytic and pentose shunts.

According to the observations of Friede (1961) and Yonezawa et al. (1962) during myelinogenesis, the oligodendroglia exhibited increased $NADH_2$ — tetrazolium reductase, succinate dehydrogenase, and $NADPH_2$ — tetrazolium reductase activities. The above results, however, cannot be regarded as entirely opposed to in view to the differences in experimental conditions under which these studies were conducted.

Relatively low activity of oxidative enzymes is displayed by normal astrocytes, but after becoming hypertrophic in pathological conditions, these cells exhibit high activities of a wide range of dehydrogenases. The problem is thoroughly discussed in another paper of this issue by Mossakowski and Penar (1972). Dehydrogenases involved in the pentose shunt and in glycolysis are detectable in mature oligodendrocytes. In contrast to this, enhancement of biological activity of the neuroglia during differentiation and during hyperplasia in the myelination clusters in the course of myelinogenesis in white rats is not accompanied by a concomitant increase of oxidoreductase activities in the maturating neuroglia. The otherwise high activity exhibited by the ependymal epithelium and by the choroid plexus may probably be referred to their secretory and absorptive function (Vigh 1967).

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OKSYDOREDUKTAZY W ROZWIJAJĄCYM SIĘ NEUROGLEJU

Streszczenie

Przeprowadzono badania histoenzymatyczne aktywności następujących oksydoreduktaz w neurogleju rozwijającego się mózgu białego szczura: dehydrogenazy glycerolo-3-fosforanowej, mleczanowej, 3-hydroksymaślanowej, glukozo-6-fosforanowej, bursztynianowej, treo-D-5-izocytrynianowej, dehydrogenazy tetrazolowej $NADPH_2$ i dehydrogenazy tetrazolowej $NADH_2$. Wyniki zestawiono z morfogenezą neurogleju oraz porównano z aktywnością całego szeregu fosfataz i esteraz.

Wyniki badań doprowadziły do następujących wniosków: Komórki ependymy wykazują silną aktywność oksydoreduktaz już w końcowym okresie życia płodowego i we wczesnym okresie po urodzeniu zwierzęcia. Podobną reakcję stwierdza się w komórkach nabłonkowych splotu naczyniówkowego. Wielowarstwowa blaszka niezróżnicowanych komórek macierzy odznacza się nieco odmiennymi właściwościami biologicznymi, w porównaniu z komórkami wyściółki. I tak aktywność wielu spośród badanych oksydoreduktaz jest niska w komórkach macierzy i w różnicujących się

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komórkach warstwy płaszczowej. Komórki te natomiast wykazują silną aktywność wielu fosfataz bez uchwytnych różnic pomiędzy spongioblastami a neuroblastami.

W okresie poprzedzającym mielinogenezę, a także podczas gliozy mielinizacyjnej, komórki neurogleju nie wykazują wzrostu aktywności badanych oksydoreduktaz. W dalszym okresie rozwoju pozapłodowego komórki neurogleju istoty białej, zwłaszcza oligodendroglej wykazują wzrost aktywności oksydoreduktaz, który utrzymuje się w mózgu dojrzałym.

М. Вендер, М. Козик, О. Мулярек

ОКСИДОРЕДУКТАЗЫ В РАЗВИВАЮЩЕЙСЯ НЕЙРОГЛИИ

Резюме

Были проведены гистоэнзиматические исследования активности следующих оксидоредуктаз в нейроглии развивающегося мозга белой крысы: глицеро-3--фосфатной дегидразы, лактатдегидразы, 3-гидроксимасланной дегидразы, глюкозо-6-фосфатной дегидразы, сукциндегидразы, трео-D-5-изолимонной дегидразы, тетразольной $NADPH_2$ дегидразы и тетразольной $NADH_2$ дегидразы. Результаты сопоставлялись с морфогенезом нейроглии и сравнивались с активностью целого ряда фосфатаз и эстераз.

Результаты исследований привели к следующим заключениям: клетки эпендимы обнаруживают сильную активность оксидоредуктаз уже в конечном периоде плодовой жизни и в раннем периоде после рождения животного. Аналогичная реакция обнаруживается в эпителиальных клетках сосудистого сплетения. Многослойная пластинка недифференцированных клеток матрикса отличается немного другими биологическими свойствами, по сравнению с клетками эппендимы. Таким образом активность многих, среди исследованных оксидоредуктаз, низка в клетках матрикса и в клетках дифференцирующихся палиума. Зато клетки эти обнаруживают сильную активность многих фосфатаз без заметных разниц между спонгиобластами и нейробластами.

В периоде предшествующим миелиногенезу, а также во время миелинизационного глиоза, клетки нейроглии не обнаруживают роста активности исследованых оксидоредуктаз. В дальнейшим периоде внеплодового развития клетки нейроглии белого вещества, и в частности олигодендроглия — обнаруживают рост активности оксидоредуктаз, который удерживается в зрелом мозгу.

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NEUROPAT. POL. 1972, X, 2

L. VENKOV

ISOENZYMATIC CHARACTERISTICS OF SOME NONSPECIFIC ESTERASES IN THE RABBIT SPINAL CORD

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Nonspecific esterases belong to the group of enzymes, coded as E.C.3.1.1.1, E.C.3.1.1.2, E.C.3.1.1.6 etc. Depending on their sensitivity to E 600 (diethyl-p-nitrophenyl phosphate), p-chlormercury benzoate and insolubility in water, they are often referred to as A, B, C esterases (Aldrige 1953, Bergman et al. 1957, Bernsohn et al. 1963, 1964).

Their heterogenicity has been emphasized by Eranko et al. (1964), Harkönen (1964), Kokko (1965). It has been already demonstrated (Venkov et al. 1967) that nonspecific esterases can be separated into five isoenzymic fractions by agar gel electrophoresis. One of the fractions has proved to be resistant to a wide variety of organophosphorus compounds. These results were obtained with aqueous protein extracts, derived from rabbit spinal cord. It seemed interesting to ascertain the intracellular localization of the organophosphorus resistant isoenzymes. Primarily, the resistant fraction was detected only in the microsomal and the soluble fractions. We assumed that the other cellular fractions did not manifest it, presumably it was firmly bound to the membrane structures. To clarify this, we treated the material with the detergent Triton X 100.

MATERIAL AND METHODS

1. Preparation of extracts from total homogenate

Extracts from water — soluble proteins. Rabbit spinal cord was homogenized in distilled water to obtain a 40% homogenate. It was frozen at —25°C and thawed, repeating the procedure two times more. The homogenate was centrifuged at 50 000 ev.g. The resulting clear, strongly opalescent supernatant was subjected to electrophoresis.

Preparation of extracts, following Triton X100 treatment. Following freezing and thawing, the homogenate was

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incubated with 0.1% Triton X 100 at 0°C for 30 min. The homogenate was centrifuged and handled as above.

2. Preparation of extracts from subcellular fractions

The subcellular fractions were isolated as described previously (Venkov 1968). All the procedures employed were the same as above.

3. Electrophoresis

Electrophoretic separation was carried out on 7 × 2.5 slides, at 12 V/cm and 0.5 mA/cm for 45 min. One hundred micrograms of protein was the amount employed.

4. Staining of isoenzyme bands

Staining of isoenzyme bands was performed according to Uriel (1961), using alpha- and beta-naphthylacetate as substrates.

RESULTS AND DISCUSSION

The aqueous extracts yielded 5 bands, denoted by a_1, a_2, a_3, s and k_1 (Fig. 1). The a_1 fraction was organophosphorus-resistant (Fig. 2). The subcellular extracts revealed different isoenzymatic patterns. The mitochondrial extracts were negative (Fig. 3). The microsomal ones displayed fractions a_1, a_2, a_3, s and k_1 (Fig. 4). The nuclear extracts had strong a_3 and s. While a_1 and a_2 were but slightly manifested (Fig. 5). The soluble fraction exhibited strong a_1 activity (Fig. 6).

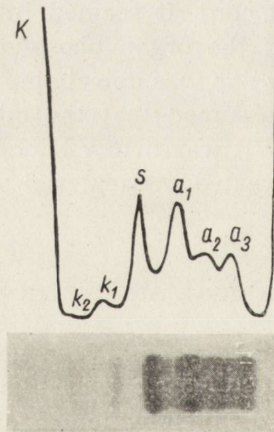

Fig. 1. Isoenzymes of nonspecific esterases. Aqueous extracts.

Following Triton X 100 treatment, the mitochondrial extracts manifested bands a_1, a_2, a_3 and k_1, the latter (Fig. 3) being intense. Band a_1 and a_2 became more intense in the nuclear fraction (Fig. 5).

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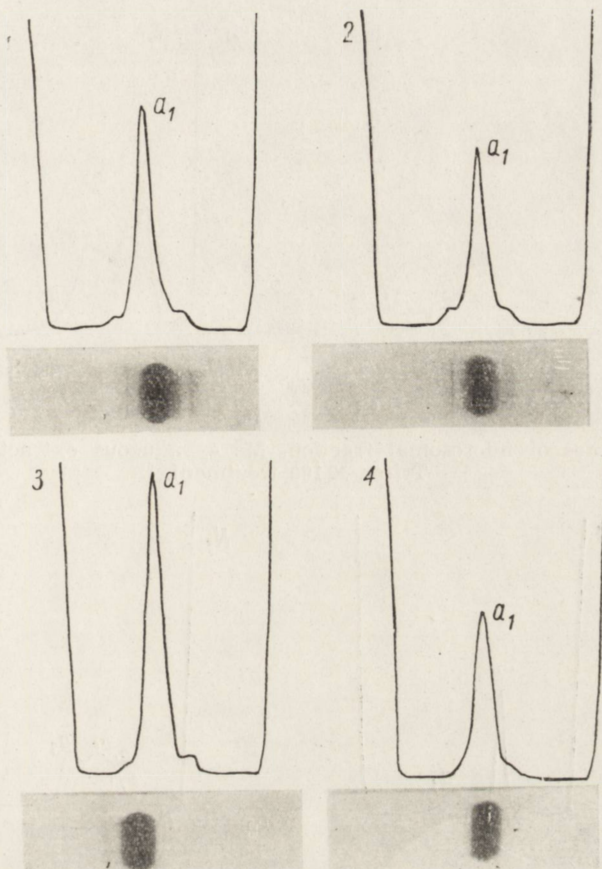

Fig. 2. Organophosphorus-resistant fraction. After treatment with: 1 — Mipafox 10^{-3}M; 2 — Phosdrin 10^{-4}M; 3 — Parathion 10^{-3}M; 4 — Chlorthion 10^{-4}M.

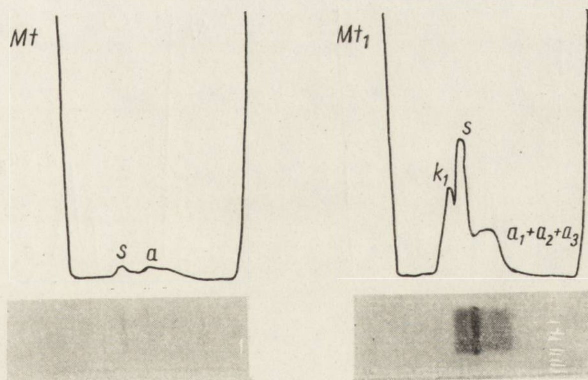

Fig. 3. Isoenzymes of mitochondrial fraction. Mt — aqueous extracts. Mt_1 — after Triton X 100 treatment.

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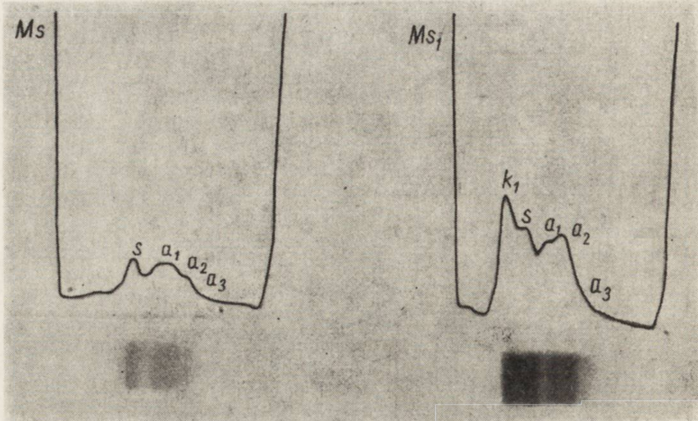

Fig. 4. Isoenzymes of microsomal fraction. Ms — aqueous extracts. Ms_1 — after Triton X 100 treatment.

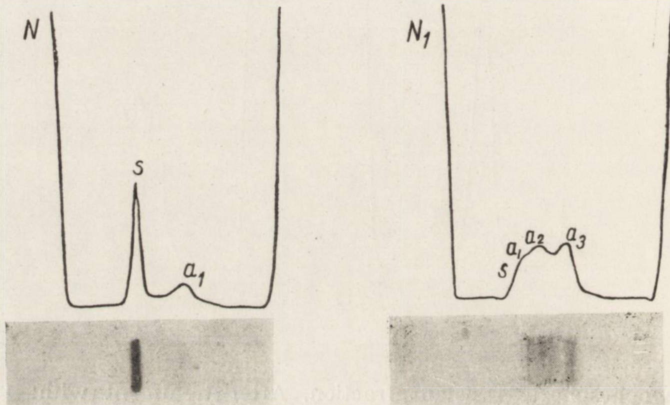

Fig. 5. Isoenzymes of nuclear fraction. N — aqueous extracts. N_1 — after Triton X 100 treatment.

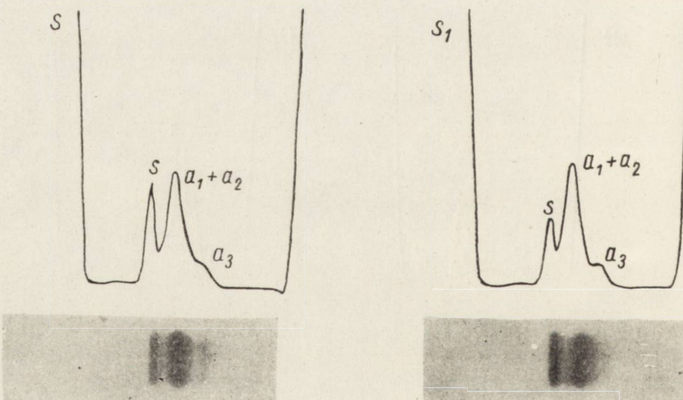

Fig. 6. Isoenzymes of soluble fraction. S — aqueous extracts. S_1 — after Triton X 100 treatment.

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These findings suggest that type B nonspecific esterases consist of several isoenzymic bands (Fig. 7). The high activity of k_1 fraction is characteristic of the membrane structures. This isoenzyme fraction is probably lipoprotein bound.

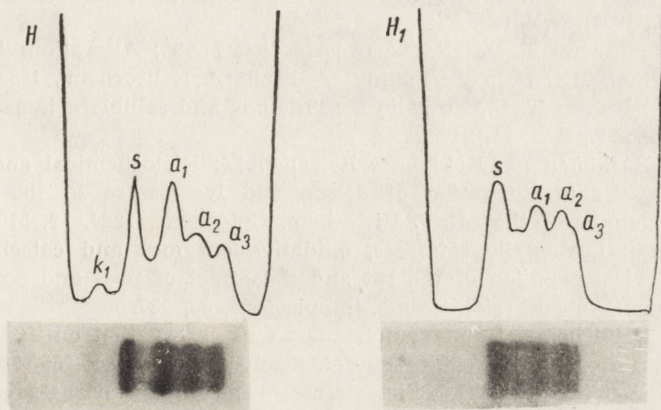

Fig. 7. Isoenzymes of nonspecific esterases. H — aqueous extracts. H_1 — after Triton X 100 treatment.

L. Venkov

WŁAŚCIWOŚCI IZOENZYMÓW NIEKTÓRYCH NIESWOISTYCH ESTERAZ W RDZENIU KRĘGOWYM KRÓLIKA

Streszczenie

Otrzymane z rdzenia kręgowego królika izoenzymy nieswoistych esteraz charakteryzują się ruchliwością elektroforetyczną, opornością przeciwko silnym inhibitorom, rozmieszczeniem w różnych frakcjach komórkowych i wrażliwością na działanie detergentów.

Л. Венков

ХАРАКТЕРИСТИКА ИЗОЭНЗИМОВ НЕСПЕЦИФИЧЕСКИХ ЭСТЕРАЗ СПИННОГО МОЗГА КРОЛИКА

Резюме

Изоэнзимы неспецифических эстераз, полученных из спинного мозга кролика характеризуются разницами в электрофоретической подвижности, сопротивляемостью к сильным ингибиторам, распределением в разных клеточных фракциях и чувствительностью к детергентам.

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NEUROPAT. POL. 1972, X, 2

K. RENKAWEK

ON THE HISTOCHEMICAL AND ENZYME-HISTOCHEMICAL DIFFERENCES BETWEEN VARIOUS TYPES OF GLIAL CELLS CULTURED *IN VITRO**)

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Head of Department: Prof. Dr. M. J. Mossakowski

Identification of particular glial cells in a material stained with routine histological methods is often connected with serious difficulties. These difficulties become even more pronounced in case of glial tissue cultures, especially in young ones with ill-differentiated cellular population (Kraśnicka, Mossakowski 1965; Kraśnicka, Borowicz 1971). In addition these authors stress that even in highly-differentiated cultures, a higher or lower percentage of cells remains, which is not to be classified properly within a definite neuroglial type. The authors define these cells as non--differentiated glial cells, the definition finding its support in the ultrastructural picture (Borowicz et al. 1972).

It seems thus, that examination of cytochemical and cytoenzymatic properties, which are representative for the biological and metabolic differences of cells, may become an essential factor in enabling the morphological identification of glial cells of various types. It also may with certain probability — shed some light on the direction of development of non-differentiated glial cells.

A possibility of distinguishing the glial cells in this way is of a particular importance for experimental studies on tissue cultures, since it is a requisit of estimating the way in which the particular types of glia react on defined impairing factors. Observations derived from human neuropathology namely are indicative of a different participation of various types of glia in a number of pathological processes involving the central nervous system and of their differentiated reactions on various types of noxious factors.

The aim of the present work was to point out the differences between the histochemical and enzyme-histochemical properties of glial cells, representing astrocytic and oligodendroglial lines. The differences facilitate the identification of individual types of glial cells in tissue cultures.

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MATERIAL AND METHODS

Histochemical and enzyme-histochemical studies were carried out on the glial cells of newborn rat cerebellum, cultured *in vitro* according to the method, used routinely in our Laboratory and described by Kraśnicka and Mossakowski (1965).

Cultures aged from 2 to 58 days were studied. Out of a wide range of histochemical and enzyme-histochemical reactions performed, only those were chosen, which revealed evident defferences between two types of neuroglial cells. Histochemical properties of microglial cells, were described elsewhere (Kraśnicka, Renkawek 1969). The following histochemical reactions were studied: periodic acid Schiff reaction, according to McManus-Lowry, with diastase digestion and dimedon blocking (Bulmer 1959), dialysed iron staining after Hale and Sudan black B staining.

Enzyme histochemical reactions concerned activity of both oxidizing-reducing and hydrolytic enzymes.

Oxidizing-reducing enzymes: tetrazolium reductaze of coenzyme I and II, glucose-6-phosphate-, α-glycerophosphate-, lactic-, malic-, glutamic-, isocitric-, succinic- and ethanol dehydrogenases and cytochrome oxidase.

Hydrolytic enzymes: acid monophosphatase, non-specific esterase, β-glucuronidase and butyrylcholinesterase.

Activity of the majority of enzymes were tested according to methods described in previous papers (Mossakowski et al. 1965, Renkawek, Mossakowski 1966, Renkawek 1967, Hoppe 1971). Activity of β-glucuronidase was studied according to method of Hayaschi et al. (1964), and that of butyrylcholinesterase after Gerebtzoff (1959) as modified by Koelle (Roessmann, Friede 1966).

RESULTS

Oxidizing-reducing enzymes

The activity of dehydrogenases appears as early as at the first day of culture, in cells growing from the explant, both in bipolar spongioblasts and in cells resembling astrocytes. In this period of growth, the enzymatic activity is generally low and final products of it are uniformly distributed in the cell cytoplasm. Any enzymatic activity is hard to be seen in cellular processes. G-6P-DH and GLP-DH activity appear as the earliest, LDH activity is also pronounced, even in poorly differentiated cells.

Starting from the 5th day of culture the activity of dehydrogenases in astrocytes becomes more intensive both in cellular pericaria and in numerous, branched cell processes. Formazan granules are arranged in a regu-

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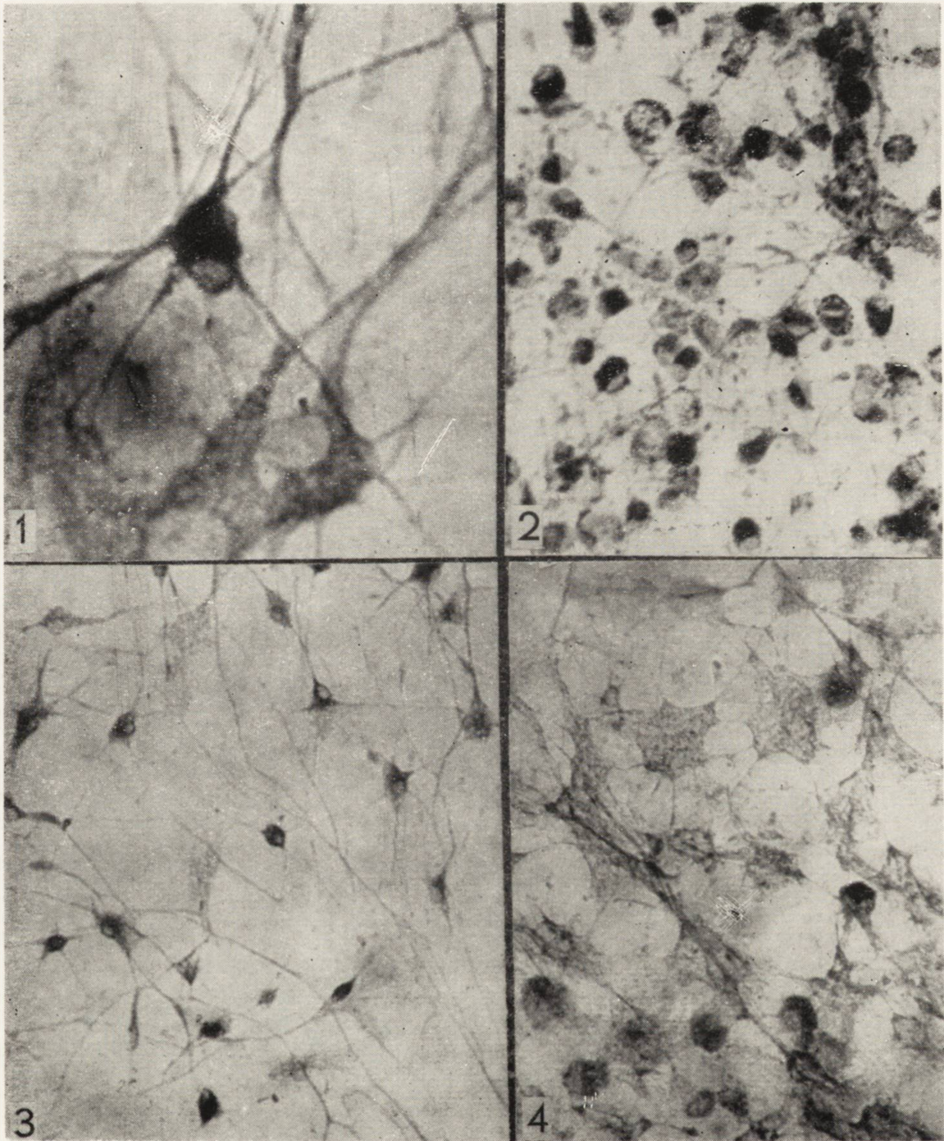

Fig. 1. Ten-day culture. Glutamic dehydrogenase. Marked prevalence of activity of oligodendroglia over astroglia cells. × 400.

Fig. 2. Twenty-day culture. Glucose-6-phosphate dehydrogenase. Distinct predominance of enzymatic activity in oligodendroglia and accumulation of formazan grains in the form of perinuclear caps. × 400.

Fig. 3. Thirthy-five-day culture. Succinic dehydrogenase. Oligodendroglia shows high activity whereas in astroglia activity marked diminishes. × 200.

Fig. 4. Five-day culture. Cytochrome oxidase. Marked differences in intensity of enzymatic activity in astroglia and oligodendroglia cells. × 400.

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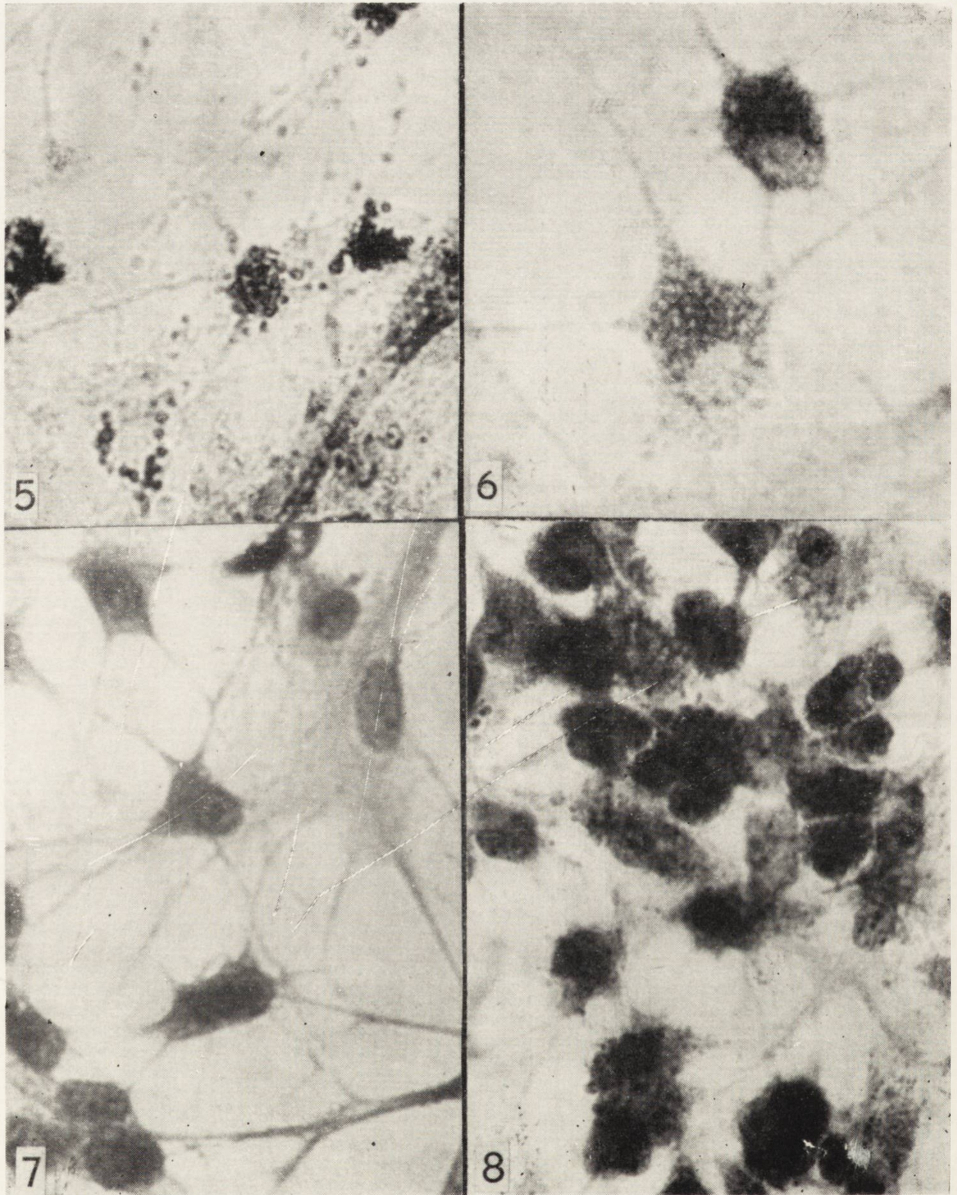

Fig. 5. Fourteen-day culture. Acid phosphatase. Marked differences in the distribution of enzymatic activity; in astroglia the products of enzymatic reaction are situated around the nuclei, in oligodendroglia in the cell pole. × 400.

Fig. 6. Ten-day culture. Non-specific esterase. Marked differences in intensity of activity in astroglia and oligodendroglia. × 400.

Fig. 7. Fifteen-day culture. PAS reaction. Great accumulation of PAS positive granules in oligodendroglia cells. × 400.

Fig. 8. Twenty one-day culture. Dialysed iron method. Distinct accumulation of positive granules more packed in oligodendroglia than in astroglia. × 400.

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lar fashion around cell nuclei or at origin of cellular processes. The highest enzymatic activity is noted in the second and third weeks of culturing. In the subsequent periods the activity of all dehydrogenases studied in astrocytes decreases to a considerable extend.

The activity of the dehydrogenases in oligodendroglia appears after one week of culture. At this period of growth it is manifested histochemically in the form of single formazan grains, spread in the cell cytoplasm. Than the dehydrogenase activity in oligodendrocytes increases rapidly, so that between 7th and 10th day of culture it becomes higher in the cytoplasm and processes of these cells than in astrocytes (Fig. 1). The most intensive activity of dehydrogenases in oligodendrocytes has been noted between 14th and 21st days of culture. Formazan grains, indicating the sites of enzymatic activity are typically distributed in the cytoplasm of oligodendrocytes, taking the form of "perinuclear caps" situated on one site of nuclei (Fig. 2). Activity of dehydrogenases in oligodendroglia remains high up till the 4th week of culture growth, exceeding greatly that of astrocytes (Fig. 3). The high enzymatic activity of oligodendroglia concerns all the dehydrogenases under study, G-6P-DH and GLP DH activities being the highest particularly during the second week of culturing. LDH and tetrazolium reductase of coenzyme I activities are also high. In older cultures, G6P-DH and GLP DH activities decrease only slightly, whereas that of the remaining dehydrogenases is markedly reduced.

Cytochrome oxidase activity during first and second weeks. of culture growth is moderate both in astrocytes and in oligodendroglia, although from the earliest period of culturing the evident and characteristic differences in the intracellular distribution of enzyme activity in both types of cells are present (Fig. 4). They consist in uniform and equal spread of final product grains in the cytoplasm of astrocytes and in cap-like perinuclear condensation of them in oligodendrocytes. In both types of cells the enzyme activity in cellular processes is very weak. During the third week of culturing the considerable enhancement of cytochrome oxidase activity occurs, this involving mainly oligodendrocytes. Even in old cultures the enzyme activity remains high in oligodendroglial cells, exceeding to great extend that of astrocytes.

Hydrolytic enzymes

Acid monophosphatase activity. The activity of acid phosphatase in young cultures, after 2 or 3 days of growth can be demonstrated in the form of single grains distributed rather irregularly throughthout of cell

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cytoplasm. In this period of growth the highest activity is exhibited by the cells with few processes and in bipolar spongioblasts. In 5 — 7 day old cultures, acid phosphatase activity in astrocytes is low, and rather uniformly distributed within cell cytoplasm. Enzyme activity in these cells becomes more intensive with maturation of culture being the highest between 14th and 21st day of culturing. In this period of growth the final products of enzyme activity have a tendency to be accumulated around cellular nuclei. After 4th week of growth in vitro a rapid fall of acid phosphatase activity in astrocytes is observed. In oligodendrocytes cultured in vitro this enzyme activity appears between 5th and 7th days. It is already high and continues to increase gradually. Between 14th and 28th day of culture growth the whole cytoplasm of oligodendrocytes is densly filled with final products of enzymatic reaction. However, quite often they tend to accumulate in only one pole of the cell (Fig. 5). In older cultures acid phosphatase activity in oligodendrocytes decreases slightly.

Non-specific esterase activity. Low enzyme activity is present in all cells outgrowing from the explant already in the earliest periods of culturing, being most evident in cells with few processes. From the 7th day of culture growth the non-specific esterase activity in astrocytes gradually increases reaching its highest level in 14 — 21 day old cultures. The grains of final product of enzymatic reaction are filling the cellular cytoplasm in an uniform fashion. In older cultures, when general activity of this enzymes in astrocytes is reduced to a great extend, the remains of it are observed in perinuclear situation.

High enzyme activity in oligodendrocytes appears rather rapidly at 7th day of culture growth. The highest level of it is present between 14th and 21st day of culturing (Fig. 6). In the contrary to astrocytes, the oligodendroglial non-specific esterase activity remains high even in older cultures. Perinuclear localization of enzyme activity is the most common feature.

β-glucuronidase activity. In young cultures the activity of this enzyme is low in all cells, regardless their types, shape and size. It increases remarkably after second week of culture growth being much more intensive in astrocytes than in oligodendroglial cells. Great variances in the intensity of enzyme activity among the cells of the same type and identical morphological picture form the most striking and typical feature of this enzyme.

Butyrylcholinesterase activity. No activity of this enzyme is observed in glia in the outgrowth zone, either in young or in older cultures. On the contrary a high activity of enzyme is present in all neurons and glial

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cells persisting in culture explant. The greatest intensity of enzyme activity is noted in oligodendrocytes arranged along the myelinating nerve fibres.

Other histochemical reactions

Periodic acid Schiff reaction. In the early periods of culture growth the granular PAS-positive deposits are present only in a slight number of small, processless glial cells. In 5 — 7 day old cultures rather single PAS-positive granules are disseminated in the cytoplasm of astrocytes. The number of these deposits greatly increases in 2 week cultures both in astrocytes and oligodendrocytes. In astrocytes the number and size of PAS-positive granules vary to a great extend from cell to cell. In some cells they are fine single, and localized mostly in pericaria, whereas in others they are coarse, numerous and filled entire cell cytoplasm and processes. In oligodendrocytes PAS-positive deposits are more uniform, as a rule they fill entirelly the cell cytoplasm, forming there in many instances compact aglomerations of PAS-positive material (Fig. 7). In older cultures, after 4th week of growth the amount of PAS-positive granules diminished in both types of glial cells. PAS-dimedon control of the reaction permits to exclude glycogen nature of deposits.

Dialysed iron staining after Hale. Oligodendrocytes contain in their cytoplasm a great amount of densly packed positively stained granules (Fig. 8). Similar granules, somewhat coarser occure in astrocytic cytoplasm, wherein they are more loosely distributed. The amount of dialized-iron positive granules in glial cells cytoplasm is increasing with the age of culture. The most pronounced granular deposits are present in hypertrophied astrocytes, with a large, voluminous cytoplasm.

Sudan black B staining. In healthy cultures glial cells of both types do not contain lipid deposits as revealed by staining with this method. In old degenerating cultures fatty degeneration of astrocytes and oligodendrocytes appears showing no specific type differences.

DISCUSSION

Our histochemical observations are indicative that it is possible to determine characteristic differences between the two types of neuroglial cells cultured in vitro on the ground of variances in the activity of particular enzymes and other histochemical properties. These differences concern mostly the intensity of enzyme-histochemical activity, its intracellular localization and the time sequences in its appearance, increase, and eventual decrease during the course of cell differentiation and ma-

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turation. The usefulness of particular histochemical and enzyme-histochemical reactions varies to a great extend from one to another.

The most characteristic feature, making possible to distinguish the particular types of glia cultured *in vitro* is the prevalence of the activity of all oxidizing-reducing enzymes in oligodendroglia as compared with that in astrocytes. The prevalence of the activity of both dehydrogenases and cytochrome oxidase, persisting during the entire observation period is in general agreement with Friede's (1965) observations, made on glial tissue in situ.

In tissue culture material a sharp increase of the activity of oxidizing--reducing enzyme in second and third weeks of growth, corresponding to myelination period, is very typical for oligodendrocytes, while the changes in the activity of the above mentioned enzymes in astrocytes occur more gradually and are less profound. The most pronounced differences between astro- and oligodendroglia concern the activity of G6P-DH and GLP DH. Another difference between two types of neuroglia is characteristic distribution of enzyme activity within cell cytoplasm. In oligodendrocytes formazan grains, representing the final products of enzyme activity, are as a rule agglomerated on one site of cell nuclei in a form of so-called „perinuclear caps", whereas in astrocytes these products are rather uniformly distributed throughout the whole cytoplasm, with only a slight tendency for more dense aggregation around cell nuclei. It seems interesting to notice that characteristic differences in the activity of cytochrome oxidase are present already in the first week of culture growth, while almost entire cellular population is represented by undifferentiated neuroglial cells.

Acid monophosphatase activity is much less useful for differentiation between astrocytes and oligodendrocytes, since variances in its intensity and distribution are less pronounced than these in the activity of oxidizing-reducing enzymes. This seems to be quite understandable, as investigations on the ultrastructure of neuroglia have revealed a similar lysosomal content and pattern in both types of glial cells (Farquhar, Hartmann 1957). On the other hand, non-specific esterase activity shows characteristics analogous to those of dehydrogenase. Its activity is much higher in oligodendrocytes, and it is arranged in typical perinuclear agglomerations, whereas in astrocytes it is lower and uniformly distributed in cell cytoplasm. These differentiating features in particular types of glial cells are most pronounced in older cultures, after third week of growth. The studies on the activity of β-glucuronidase are less useful in differentiation of glial types. Despite of an evident increase of this enzyme activity in astrocytes after second week of growth, our experi-

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ments showed significant differences in its activity between individual cells, representing the same type of glia.

Butyrylcholinesterase activity is absent in all glial cells, situated in a zone of cell growths in glial cultures, but it is considerably high in neurons, nerve fibres and glial cells located along them within tissue explants. The role of this enzyme in nervous tissue is not exactly known. Activity of this enzyme in nervous tissue in situ has been demonstrated in oligodendrocytes in the period of myelination (Koelle 1954, Roessmann, Friede 1966) and in normal and reactive astrocytes (Cavanagh et al. 1954, Koelle 1954, Gerebtzoff 1959). However, the butyrylcholinesterase activity varies to a great extend in dependence on the animal species (Brightman, Albers 1959, Friede et al. 1964). Our negative results concerning this enzyme activity in glial cells in tissue culture may be indicative differences between glial cells in vitro and in situ conditions. Technical inaccuracy of the method applied for in vitro condition, which has to be taken into consideration, might be ruled out, since under the same condition neurons and glia located within tissue explant reveal enzymatic activity.

Periodic acid Schiff reaction, revealing characteristic accumulation of mucopolysaccharide substances in the cytoplasm of both types of glial cells in tissue culture is of a great value in their differentiation on the ground of differences in the amount, size and distribution of PAS-positive granules. So is dialysed iron staining after Hale.

The above mentioned histochemical and enzyme-histochemical properties of glial cells, resulting from their metabolic and biological functions make possible to differentiate two types of neuroglial cells cultured in vitro. On the other hand they might be of an importance in evaluation of pathological changes in glial cells under various experimental conditions carried out on tissue culture.

K. Renkawek

RÓŻNICE HISTOCHEMICZNE I HISTOENZYMATYCZNE POMIĘDZY RÓŻNYMI TYPAMI KOMÓREK GLEJOWYCH W HODOWLI IN VITRO

Streszczenie

Badano właściwości histochemiczne i aktywność enzymatyczną tkanki glejowej hodowanej *in vitro*. Hodowle glejowe pochodziły z komórek móżdżku noworodków szczurzych przetrzymywanych we flaszkach Carrela. Hodowle w wieku 2—58 dni barwiono metodami rutynowymi, oraz wykonywano reakcję histochemiczną PAS, barwienie żelazem dializowanym i Sudanem czarnym B. Badano aktywności enzymów oksydoredukcyjnych i hydrolitycznych.

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Znaczne różnice występujące we właściwościach histoenzymatycznych komórek gleju ułatwiają rozpoznanie poszczególnych typów komórek glejowych. Cechą najbardziej różnicującą astroglej i oligodendroglej jest przewaga aktywności enzymów oksydoredukcyjnych i esterazy nieswoistej oraz charakterystyczne nagromadzenie produktów reakcji enzymatycznej w okołojądrowej części plazmy w oligodendrogleju. Aktywność butyrylcholinesterazy nie występowała w strefie wzrostu hodowli, jedynie słabą aktywność obserwowano w komórkach glejowych zachowanych w eksplantacie. Aktywność β glukuronidazy nie była charakterystyczna dla typu komórki glejowej. Aktywność enzymatyczna gleju w hodowli znacznie różni się od aktywności tych enzymów w tkance, natomiast badania w hodowli tkankowej dają wyniki istotnie porównywalne, jeśli materiał hodowany pochodzi od tego samego zwierzęcia, hodowany jest według tej samej techniki oraz barwiony przy użyciu tych samych metod histochemicznych i enzymatycznych.

К. Ренкавек

ГИСТОХИМИЧЕСКИЕ И ГИСТОЭНЗИМАТИЧЕСКИЕ РАЗНИЦЫ МЕЖДУ РАЗНЫМИ ТИПАМИ ГЛИАЛЬНЫХ КЛЕТОК В КУЛЬТУРЕ ІН ВИТРО

Резюме

Исследовались гистохимические свойства и энзиматическая активность глиальной ткани, выращиваемой ин витро. Глиальные культуры происходили из клеток можджечка новорожденных крыс выдерживаемых в сосудах Карреля. Культуры возраста 2—58 дней окрашивались обычными методами, а также подвергались гистохимической ПАС реакции, окрашиванию диализированным железом и черным Суданом В. Исследовалась активность окислительно-восстановительных и гидролитических энзимов.

Значительные разницы, имеющиеся в гистохимических свойствах клеток глии, облегчают установление отдельных типов глиальных клеток. Свойством которое самым сильным образом отличает астроглию от олигодендроглии является преобладание активности окислительно-восстановительных энзимов и неспецифической эстеразы и характерное скопление продуктов энзиматической реакции в приядерной части плазмы в олигодендроглии. Активность бутирилхолинэстеразы не обнаруживалась в районе роста культуры, лишь слабая активность наблюдалась в глиальных клетках, сохраненных в эксплантате. Активность β глюкуронидазы не была характерна для типа глиальной клетки. Энзиматическая активность глии в культуре значительно отличается от активности этих энзимов в ткани, зато исследования в тканевой культуре дают результаты действительно сравнимаемые, если выращиваемый материал происходит от одного и того же животного, культивируется той же самой техникой и окрашивается с использованием тех же самых гистохимических и энзиматических методов.

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SOME ASPECTS OF THE HISTOCHEMISTRY OF THE REACTIVE GLIA *)

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Glial reaction is one of the most common features in the pathology of the central nervous system. This accompanies a great number of the pathological processes of the nervous tissue and as a rule may involve all types of glia. However, the richest literature concerns astroglial reactions. This type of glial reaction forms also the subject of the present report.

Increase in cell diameter and cytoplasm volume, enlargement of nuclei and their displacement towards the cell periphery, hypertrophy of cellular processes are the most typical morphological characteristics of reactive astrocytes. In chronic pathological processes they take form of typical plumb cells (Fig. 1).

The vast section of neuropathological literature concerns changes in the histochemical properties of the reactive astrocytes under various pathological and experimental conditions. However, it has to be pointed out that histochemical understanding of reactive astrocytes is not univocal with the morphological one. Histochemical reactive changes often appear earlier than the above mentioned morphological transformations (Rubinstein et al. 1962, Mossakowski 1963, Domańska 1970, Petrescu 1972) and in many instances they are reversible in nature and not accompanied by any modifications in morphological structure of the cells (Mossakowski et al. 1968, Mossakowski, Zelman 1971).

The most typical feature characterizing reactive astrocytes is a remarkable increase of the activity of oxidizing-reducing enzymes (Figs. 2 and 3), which is particularly striking, since in normal astrocytes their activity is very low. In human pathology, an increase of oxidizing-redu-

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cing enzymes was observed in various pathological processes. This was described in the surroundings of brain tumours (Schiffer, Vesco 1962, Rubinstein, Sutton 1963, Mossakowski 1962) and atheromatous cerebral blood vessels (Śmiałek, Wiśniewska 1966, Nareantiu, Tantu 1969), in demyelinated plaques (Ibrahim, Adams 1963, Friede 1961) and in some lipid storage diseases (Wallace et al. 1963). Similarly the same types of changes were seen in the majority of experimentally induced pathological processes, such as edema (Rubinstein et al. 1962) anoxic-ischemic encephalopathy (Becker 1961, Spector 1963) and many other (Friede 1966).

The time of appearance of changes in enzyme histochemical reactios and variations in the activity of different enzymes deserves a special attention. Rubinstein et al. (1962) in their studies on experimental edema noted that the earliest increase of enzyme-histochemical reaction, appearing as early as 12 hrs following injury concerned mostly glutamate dehydrogenase, while the following 12 hrs yielded an enhancement of the activity of NAD-diaphorase and dehydrogenases linked with coenzyme I. Friede (1966) considered that the earliest enzymic changes occuring already 6 hrs after injury concerned at first the enzymes of glycolytic pathway and hexosemonophosphate shunt and later those of the citric acid cycle. Domańska (1970) in our laboratory observed an increase of glucose-6-phosphate dehydrogenase activity as early as 3 hrs after hypoxia in rats.

Besides the increase of oxidizing-reducing enzyme activity, the reactive astrocytes exhibit also a markedly intensified activity of other enzymes such as acid monophosphatase (Fig. 4) (Koenig, Barron 1962, Schiffer et al. 1967), beta-glucuronidase (Schiffer, Cognazzo 1968), butyryl-cholinesterase (Roessmann, Friede 1966), ATP-ase (Ibrahim, Adams 1963) and others.

This increase of the activity of various enzymes is considered univocally to be an universal feature, occuring in all cases of astrocytic reaction regardless the nature of the pathological processes which are the cause of tissue reaction. On the other hand, however, there is a number of observations by different authors and those by our own, which point at the histochemical variances of the reactive astrocytes, despite of morphologically identical substrate involved.

In that respect the problem of glycogen accumulation in the reactive astrocytes deserves a special attention, as this feature constitutes the most common non-specific glial reaction resulting from a great number of pathological processes. The glycogen deposits were reported in astrocytes in the vicinity of brain tumours (Oksche 1961), stab wounds (Friede 1954, Shimizu, Hamuro 1958, Guth, Watson 1968) and particularly as an effect of radiation (Klatzo et al. 1961, Miquel, Haymaker 1965).

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In our studies carried out on various models of experimental hypoxia (Mossakowski et al. 1968 Mossakowski, Zelman 1971, Pronaszko et al. 1971, Long et al. 1972) we have also stated the occurrence of glycogen deposits within astrocytes (Fig. 5). These changes indicating disturbances in metabolism, transport and utilization of glucose in the central nervous system (Mossakowski et al. 1968, Klatzo et al. 1970) were fully reversible. Astrocytic glycogen deposits appeared and disappeared in characteristic time sequences, depending upon the type of experimentally induced hypoxia. Usually they appeared at 6th — 10th hour after experiment and disappeared completely at time intervals varying from few to several days. However, in striking contrast to that there were areas, mostly those surrounding necrotic foci, where astrocytic glycogen deposits persisted for several weeks. It has to be noticed, that none of the glycogen bearing astrocytes, except those within necrotic foci and their vicinity, had manifested any features of cellular hypertrophy and after the disappearance of polysacharide deposits, they were morphologically identical with those in the undamaged tissue. On the other hand, in astrocytes undergoing gemistocytic transformation granular glycogen deposits also disappeared in the course of several days; however, their cytoplasm remained PAS-dimedon positive during many weeks following hypoxia (Fig. 6). This morphological alteration of glycogen might, in turn, indicate variation in its biochemical or physical state and/or its binding with cellular proteins.

In young animals with immature central nervous system the astrocytic accumulation of glycogen deposits occured both in the gray and white matter, which was in a striking contrast with previous observations of Miquel et al. (1965) dealing with irradiation and those of ours concerning the effect of hypoxia on the mature nervous system.

Miquel et al. (1965) considered that glycogen storage was a specific property of the protoplasmatic astroglia. The limitation of astrocytic accumulation of glycogen in the white matter to the areas undergoing an active myelination, might indicate that glycogen accumulation depended more on the functional state of astrocytes that upon their histological type.

In the some series of experiments we noticed parallel changes in the activity of glycogen-metabolizing enzymes (Mossakowski et al. 1968, Mossakowski, Zelman 1971). These consisted in the appearance of histochemically detectable UDPG-transferase and phosphorylase a activities already within 1 hr following hypoxia (Figs. 7, 8) and disappeared complFirst within 3 days for UDPG-transferase and within 7 days for phosphorylase a. However, the reversibility of the enzyme changes depended on several factors. The phosphorylase activity, found to disappear from

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astrocytes within 7 days in the case of experimental hypoxia, persisted several months in hypertrophied astrocytes within degenerating spinal cord tracts (see below).

In our recent studies on the histochemical properties of the reactive astrocytes in hemisectomized spinal cord in cats we turned our attention to the differences between the astrocytic reaction in areas of the glial scar surrounding necrotic foci, and that within degenerating spinal tracts. The differences reflected already on the morphological picture of cells. Within perinecrotic areas the reactive astrocytes took the form of typical gemistocytes already in the course of several weeks after cord injury whereas in degenerating spinal pathways, the glial proliferation was accompanied by considerable cellular hypertrophy, but without formation of typical plumb cells. Gemistocytes within glial scars revealed strong intracytoplasmatic, granular glycogen aggregations which persisted in here considerably longer than in the surroundings of the experimental stab wounds, as reported by Friede (1954) and Shimizu and Hamuro (1958). Glycogen deposits within gemistocytes cytoplasm in this group of experiments differed in their granular nature from those in gemistocytes in hypoxic experiments. At the same time hypertrophied astrocytes in degenerating spinal tracts showed no glycogen deposits.

The variations in histochemical glycogen reactions were accompanied by differences in the activity of glycogen metabolizing enzymes, first of all in that of the phosphorylases. These differences between gemistocytes from the glial scars arround necrotic foci and hypertrophied astrocytes from the degenerating spinal tracts consisted in the intensity and cellular localization of phosphorylase activity (Figs. 9, 10), which was found to be much more intensive and compactly distributed in the latter localization. The persistence of increased phosphorylase activity in this case has been already mentioned. Similar differences concerned the activity of other enzymes, such as aldolase (Fig. 11) and succinate, lactate (Fig. 12) and glucose-6 P-dehydrogenases.

Despite of all well known reservations, concerning interpretation of section histochemical findings, it seems justified to consider, that differences in histochemically detectable activity of various enzymes and in the intracellular contents of some chemical substances, like e.g. glycogen, between normal and reactive astrocytes are indicating changes in the metabolism of these cellular components in the central nervous system occuring under the influence of various pathological processes. Therefore, the above presented variations of histochemical properties of the reactive astroglia itself, might, in turn, indicate the differences in these metabolic disturbances, depending upon the type of reacting glia, its functional state, localization and on the kind, nature and duration of the noxious

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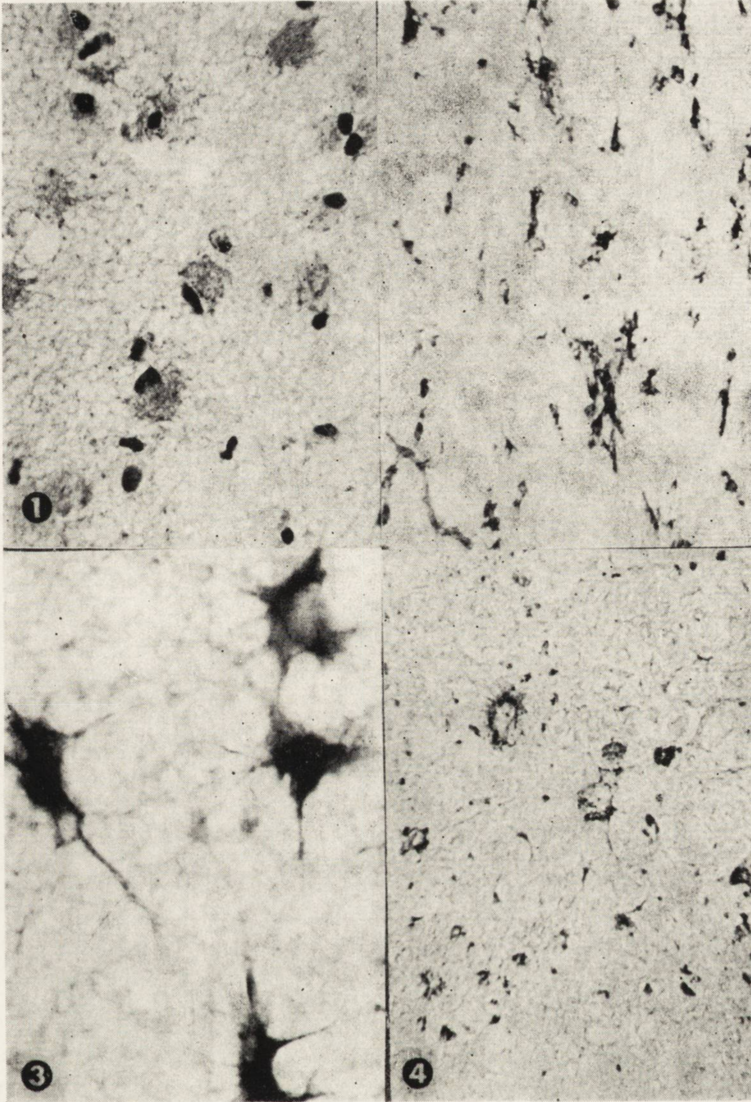

Fig. 1. Plump-astrocytes in the white matter of cerebral hemisphere in a case of diffuse sclerosis. H-E. × 400.

Fig. 2. Glucose-6-phosphate dehydrogenase activity in astrocytes of the cerebral white matter in a case of experimental hypoxic-ischemic encephalopathy (rat), 24 hrs following hypoxia. × 160.

Fig. 3. Succinate dehydrogenase activity in the hypertrophied astrocytes, surrounding a glial brain tumour in man. × 800.

Fig. 4. Acid phosphatase activity in hypertrophied astrocytes within a degenerating tract of the hemisectomized spinal cord in cat. × 400.

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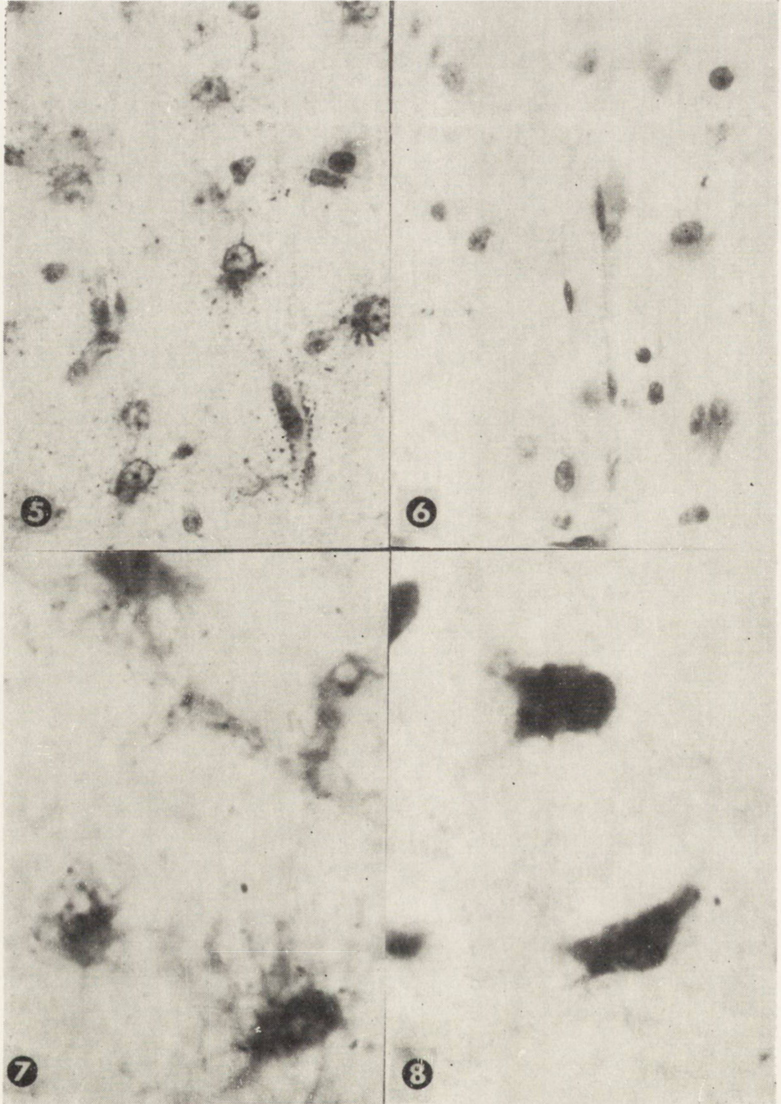

Fig. 5. Glycogen accumulation within the cytoplasm and cellular processes of the white matter astrocytes in an asphyxiated newborn monkey, 24 hrs following asphyxiation. × 780.

Fig. 6. PAS-dimedon positive cytoplasm of gemistocytic astrocytes within 7 day old necrotic focus of the brain in an asphyxiated newborn monkey. × 600.

Fig. 7. UDPG-transferase activity within the white matter astrocytes in an asphyxiated newborn monkey, 1 hr following asphyxiation. PAS staining, × 760.

Fig. 8. Glycogen phosphorylase a activity within the white matter astrocytes in an asphyxiated newborn monkey 1 hr following asphyxiation. PAS staining. × 760.

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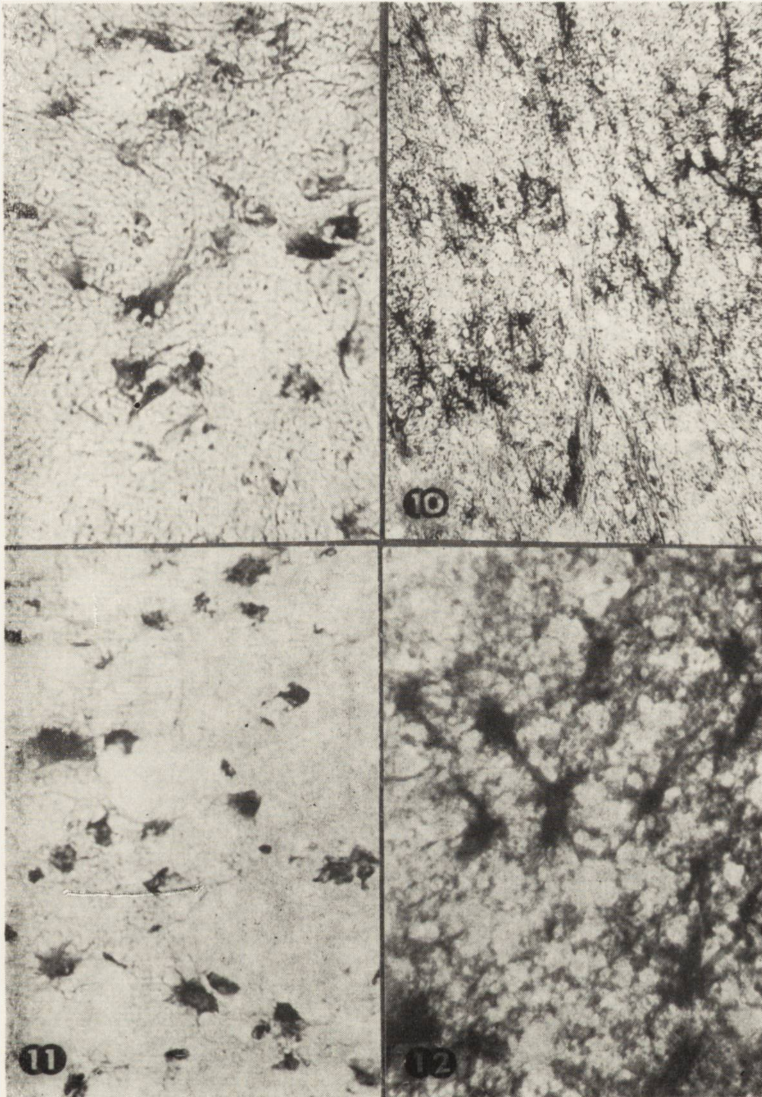

Fig. 9. Glycogen phosphorylase activity in reactive astrocytes from the vicinity of necrotic focus within hemisectomized spinal cord in cat. Iodine staining. × 600.

Fig. 10. Glycogen phosphorylase activity in hypertrophied astrocytes from the degenerating tract of the hemisectomized spinal cord in cat. Iodine staining. × 600.

Fig. 11. Aldolase activity in the reactive astrocytes surrounding necrotic focus within hemisectomized spinal cord in cat. × 400.

Fig. 12. Lactate dehydrogenase activity in the reactive astrocytes from the glial scar in the hemisectomized spinal cord in cat. × 600.

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factors, provoking glial response. The data, presented at this Symposium by Szydłowska and Kałuża (1972), concerning the histochemistry of protein functional (terminal) groups in reactive glia in the areas surrounding various pathological foci in the brain are strongly supporting this view. This would point to the possibility that the morphologically homogenous pathological feature, known under the name of reactive astrocytes, corresponds to various, changeable biological states.

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WYBRANE ZAGADNIENIA HISTOCHEMII GLEJU ODCZYNOWEGO

Streszczenie

Na podstawie przeglądu piśmiennictwa i własnych obserwacji prowadzonych na różnych modelach doświadczalnych autorzy wykazują zróżnicowanie właściwości histochemicznych odczynowych astrocytów.

Autorzy wykazują, że gromadzenie się glikogenu w astrocytach, stanowiące jedną z najpospolitszych nieswoistych reakcji tkanki nerwowej na działanie różnorodnych czynników uszkadzających, wykazuje zróżnicowanie uwarunkowane z jednej strony rodzajem gleju, jego stanem czynnościowym, położeniem, a rodzajem, czasem trwania i charakterem czynnika uszkadzającego z drugiej. Takie same różnice dotyczą aktywności enzymów metabolizujących glikogen i niektórych innych enzymów takich jak aldolaza, dehydrogenaza bursztynianowa, mleczanowa i glukozo-6-fosforanowa.

Autorzy postulują, że zróżnicowanie cech histochemicznych, stanowiących wykładniki metabolicznych właściwości tkanki, wskazuje na niejednorodność pojęcia „glej odczynowy", mimo niejednokrotnej identyczności obrazów morfologicznych.

М. Е. Моссаковски, Б. Пенар

ИЗБРАННЫЕ ВОПРОСЫ ГИСТОХИМИИ РЕАКТИВНОЙ ГЛИИ

Резюме

На основании литературного обзора и собственных наблюдений, проводимых на разных экспериментальных моделях, авторы доказывают дифференциацию гистохимических свойств реактивных астроцитов.

Авторы доказывают, что скопление гликогена в астроцитах, составляющее одну из самых распространенных неспецифических реакций нервной ткани на воздействие разных повреждающих агентов, обнаруживает дифференциацию, обусловленную — с одной стороны — видом глии, ее функциональным состоянием, положением и видом, временем воздействия и характером повреждающего агента — с другой стороны. Такие же разницы касаются активности энзимов метаболизирующих гликоген и некоторых других факторов, таких как альдолаза, сукцинатдегидрогеназа, лактатдегидрогеназа и глюкозо-6-фосфатдегидрогеназа.

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Авторы полагают, что дифференцирование гистохимических свойств, составляющих показатели метаболических свойств ткани, указывает на неоднородность понятия „реактивная глия" несмотря на неоднократную идентичность морфологической картины.

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NEUROPAT. POL. 1972, X, 2

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SOME ASPECTS OF HISTOCHEMISTRY OF GLIA AND GANGLION CELLS OF HYPOTHALAMUS IN THE AGE ASPECTS

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As known, hypothalamus is the main subcortical centre, which ensures the unity of the nervous and endocrine regulation.

Some morphological and cytochemical changes occur in the secreting neurons of the hypothalamus during the process of neurosecreta production. These morphological changes take the form of hypertrophy concerning not only perikarya of the secreting neurons but also their processes and even nerve endings. Cytochemical changes consist in the synthesis of simple and complex proteins as components of neurosecreta; this being performed with the active participation of ribonucleic acid (RNA). It was demonstrated by Levinson and Utkin (1949) and by Polenov (1964) that during the synthesis of neurosecreta the quantity of RNA undergoes alteration. According to the data of Brotsky (1960) and Levinson and Isakov (1961) an increase of the quantity of RNA in neurosecreting cells takes place in the first phase of influence of specific agents, whereafter a functional overstrain of cells occurs resulting in a decrease of RNA.

Some of authors showed that Gomori-positive neurosecretion products contained not only proteins but also lipids and sometimes polysaccharides, formed not only with the participation of RNA but also in connection with the Golgi apparatus.

During recent years the enzymes of the neurosecreting cells have been studied extensively. Moreover, attemps were made to study not only their localization but also changes in their activity during fulfilling the vital function of the secreting neurons. Schiebler (1951) and Tolanti et al. (1958) revealed a high activity of acid and alkaline phosphatases in these cells. Schiebler (1952) showed the activity of succinic dehydrogenase and Yono (1955) and Eichner (1958) that of cholinesterase within the neurosecretory ganglion cells. Kivalo et al. (1958) detected acetylcholinesterase and succinic dehydrogenase activity there.

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Morphological and histochemical changes of the neurosecreting cells, the histotopography of the neurosecreting granules as well as their stainnig properties may be assumed as features characterizing their functional state. However, the histochemical properties of glial tissue in this area have not been sufficiently investigated in this respect.

The above mentioned investigations were performed on animals. Studies concerning histochemical properties of human neurosecreting cells and neuroglia of neurosecretory hypothalamic nuclei are still scarce. We therefore attempted to study the character of morphological and histochemical changes of glia and neurosecreting cells in the supraoptic and paraventricular nuclei of the human hypothalamus in the aspect of age.

We examined the hypothalamus of thirty eight, practically healthy people, who perished in accidents at the age of 20 to 85 years. The autopsy material for the investigation was taken 6 to 12 hours after death. The following methods were applied: Schiff-reaction (after Shabadash) to localize glycogen and neutral mucopolysaccharides; reactions for localizing acid mucopolysaccharides with alcyan-blue after Steedman, with dialyzed colloid iron after Hale and with toluidin blue after Lison, Hess and Hollander; staining of DNA after Feulgen, staining of RNA after Brachet, cytochrome oxidase activity after Winkler-Schulze and Gräff, iron after Perls, lipids after Lison and ascorbic acid after Stiran and Leblond.

Additionally digestion tests with amylase, saliva ptyaline and with bacterial hyaluronidase were performed.

Staining with hematoxylin eosin, picrofuchsin after van Gieson and after Gomori in modification of Mayorova for localization of neurosecretion products were also used.

Our investigations showed that in all age groups the astroglial cells revealed a homogenous, slightly positive Schiff-reaction. This staining was more intensive in neurosecreting ganglion cells, containing Gomori-positive neurosecreta. The Schiff positive reaction of the cytoplasm of both neuroglial and neurosecreting cells did not change after amylase digestion, this being indicative for the presence of small amounts of neutral mucopolysaccharides in their cytoplasm.

The Steedman, Hale and Hess — Holander reactions were slightly positive in astroglia in all age groups. The intensity of these reactions was a little higher in astroglia near the neurosecreting nuclei and was particularly significant in the cytoplasm of neurosecreting cells, decreasing or disappearing completely in the older age groups parallely with the accumulation of lipofuscin in their cytoplasm. These reactions

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demonstrate the presence of acid mucopolysaccharides in cytoplasm of astroglia and particularly in neurosecreting cells. Their content decrease with age.

The Perls'es reaction was negative in all age groups indicating the lack of iron in the cytoplasm of both secreting neurons and astroglia.

Reaction for cytochrome oxidase activity was negative in astroglia in all age groups. This was slightly positive in the cytoplasm of neurosecreting cells in young people and negative in old ones, when the accumulation of lipofuscin in their cytoplasm was high.

Investigation of DNA showed that regardless of age it was present in the nuclei of secreting cells in a low concentration, taking the form of small weakly stained granules.

In younger age groups RNA was localized in the cytoplasm of ganglion cells in the form of dustfine diffuse granules. In old age when lipofuscin was accumulated in the cytoplasm of ganglion cells in a high concentration, RNA was not revealed therein.

Investigations on ascorbic acid showed that it was contained in the neurosecreting cells in the form of fine dustlike formation, staining black and spreading to the cells periphery. There were also such granules accumulated around cell nuclei. Ascorbic acid was also accumulated along the processes, in particular along the axones of hyperthrophied neurosecreting cells. Cells in state of hypertrophy were generally rich in vitamin C. Ascorbic acid was distributed uniformly in the cytoplasm of glial elements. A high concentration of ascorbic acid was also noted along the nerve fibres.

Lipids were found in astroglia and cytoplasm of ganglion cells in the form of small sudanophilic granules, their intracellular concentration increasing with the age.

Neurosecretion products, determined after Gomori had the form of small granules. Their cytotopography and the quantity were aproximately equal in all age groups. Tigroid was revealed in the form of small and large granules, their contents being inversely proportional to the quantity and size of neurosecretion granules.

The increasing concentration of Schiff-positive substances and ascorbic acid as well as activity of the cytochrome oxidase in neurosecreting cells in the state of hypertrophy were a very constant and striking feature.

In the state of „rest" of the neurosecreting cells the contents of Schiff positive substances, and ascorbic acid was slightly decreased. So was the activity of cytochrome oxidase.

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S. Kasabyan, J. Romanchikov

NIEKTÓRE ZAGADNIENIA HISTOCHEMII GLEJU I NEURONÓW PODWZGÓRZA W ASPEKCIE WIEKU

Streszczenie

Autorzy przebadali zmiany morfologiczne i histochemiczne w gleju i neuronach jądra nadwzrokowego i przykomorowego podwzgórza u ludzi w wieku 20—85 lat.

Materiał pobierano 6 — 12 godzin po zgonie. Wykonano reakcje na neurosekret, glikogen, kwaśne i obojętne mukopolisacharydy, DNA i RNA, oksydazę cytochromową, kwas askorbinowy, żelazo i lipidy.

Autorzy stwierdzili obniżenie zawartości kwaśnych mukopolisacharydów i RNA oraz wzrost zawartości lipidów w neuronach w starszych grupach wieku i wzrost substancji PAS-dodatnich, aktywności oksydazy cytochromowej i zawartości kwasu askorbinowego związany ze stanem pobudzenia komórek wydzielniczych.

С. Касабян, Е. Романчиков

НЕКОТОРЫЕ ВОПРОСЫ ГИСТОХИМИИ ГЛИИ И НЕЙРОНОВ ГИПОТАЛАМУСА В АСПЕКТЕ ВОЗРАСТА

Резюме

Авторы исследовали морфологические и гистохимические изменения в глии и нейронах супраоптического и перивентрикулярного ядра гипоталамуса у людей возраста 20—85 лет.

Материал брали 6—12 часов после смерти. Провели реакции на нейросекрет, гликоген, кислые и нейтральные мукополисахариды, ДНК и РНК, цитохромную оксидазу, аскорбиновую кислоту, железо и липиды.

Авторы обнаружили снижение содержания кислых мукополисахаридов и РНК, а также увеличение содержания липидов в старших возрастных группах в нейронах и рост ПАС — позитивных веществ, цитохромной оксидазы и аскорбиновой кислоты, связанный с состоянием возбуждения секреторных клеток.

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NEUROPAT. POL. 1972, X, 2

J. MALÍNSKÝ

ONTOGENETIC DEVELOPMENT OF GLIAL CELLS IN THE GREY MATTER OF THE HUMAN SPINAL CORD

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The ultrastructure of glial elements during their development was studied only little (Tennyson, Pappas 1962, Fujita H., Fujita S. 1964, Wechsler, Meller 1967, Malínský, Brichová 1967, Malínský 1968). More attention was paid to the developing neuroblasts (Fujita H., Fujita S. 1963, Wechsler 1966, Herman, Kaufman 1964, Tennyson 1962, Malínský 1968). In man this topic remains unsolved and our previous papers (Malínský, Krajčí 1968) also concerned mainly the developing neuroblasts. Therefore the present paper will describe cytologic changes occurring in human glioblasts during their differentiation and evaluate them by means of the morphometric method.

Spinal cords of 75 human embryos and fetuses in the age of 3 to 20 weeks were fixed in glutaraldehyde and osmic acid, embedded in Araldite ACM, and ultrathin sections investigated under the electron microscope. Quantitative evaluation used the stereologic method of Weibel et al. (1966).

In the early embryonic period most cells in the primitive spinal cord are undifferentiated and immature — the so-called matrix cells, which are predecessors of both neuroblasts and glioblasts. They have large nuclei with more than one nucleoli; their cytoplasm contains many free ribosomes, solitary or in small groups. Both sacs of endoplasmic reticulum and the other organelles are rather scanty (Fig. 1).

The volume of the cytoplasm in the cells differentiating into neuroblasts increases and the proportions and the distribution of cell organelles change. Mitochondria grow both in number and size and so does the Golgi area. The most remarkable changes can be observed in the ultrastructure of the ergastoplasm. In those parts of the cytoplasm, which in

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the matrix cells are filled only with ribosomes, there appear sacs of endoplasmic reticulum of either reticular arrangement (Fig. 2) or parallel orientation (Fig. 3). During further fetal development these areas grow and get the appearance of typical Nissl clumps.

The ultrastructure of the cellular elements differentiating into glioblasts is clearly distinguishable from the ultrastructure of immature neuroblasts. Since their first transitional stages these cells are found to contain in their cytoplasm fewer ribosomes and the total density of their cytoplasm is much lower (Fig. 4). In the first half of the embryonic period the mantle zone has many cells representing transitional stages from undifferentiated matrix cells to neuroblasts and glioblasts. Only some of them can in this period with certainty be classified as neuroblasts and glioblasts. Towards the end of the embryonic period the easily classifiable cellular elements are more frequent and, moreover, the immature astroblasts can already be distinguished from immature oligodendroblasts.

The immature astroblasts have more cytoplasm (Fig. 5) forming wider cytoplasmic processes (Fig. 6). Its density is very low so that the cells are similar to mature astrocytes with their typical light „watery" cytoplasm and very few organelles. There are small mitochondria, Golgi areas, and rather numerous agranular sacs and vesicles. There is very little granular endoplasmic reticulum and a small number of free ribosomes.

The immature oligodendroblasts have rather little cytoplasm forming only a narrow rim round the nucleus. Their cytoplasmic processes are thinner, very often of alar form. The density of the cytoplasm is much higher than in astroblasts and sometimes even more so than in neuroblasts. Sacs of endoplasmic reticulum and free ribosomes are more numerous (Fig. 7). The Golgi apparatus is poorly developed and agranular sacs there are practically none. Some immature oligodendroblasts were observed to be closely related to neuroblasts adhering to their perikarya (Fig. 8).

The described ultrastructural differences between the various differentiating cellular types were quantitatively estimated by means of the stereological morphometric method. A system of regularly distributed test points served to calculate volume proportions between the cytoplasm and the nuclei and between individual organelles. The results are given in Diagrams 1 to 4.

These proportions were compared in immature matrix cells, neuroblasts, astroblast and oligodendroblasts of the six week old embryo (Diagram 1).

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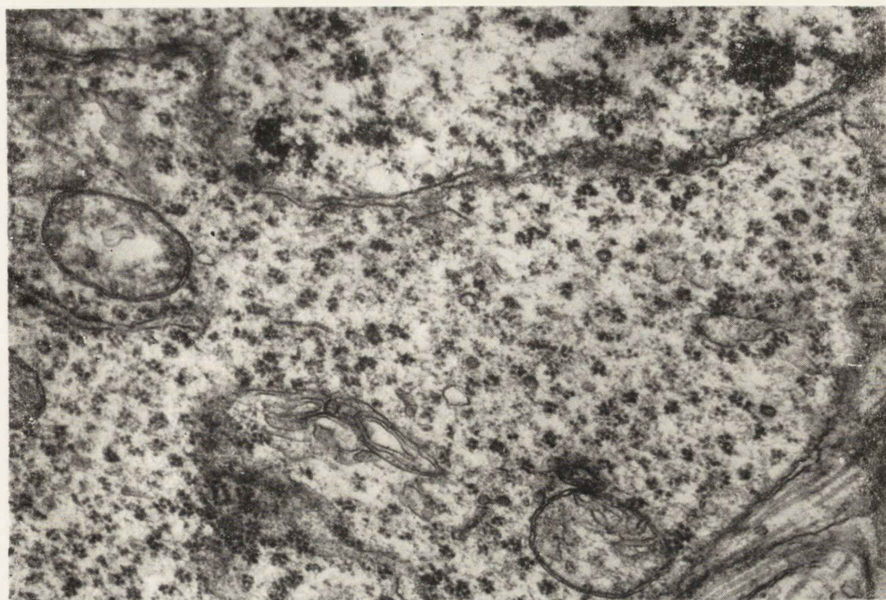

Fig. 1. Part of an immature matrix cell with many ribosomes and few organelles in its cytoplasm. × 30 000.

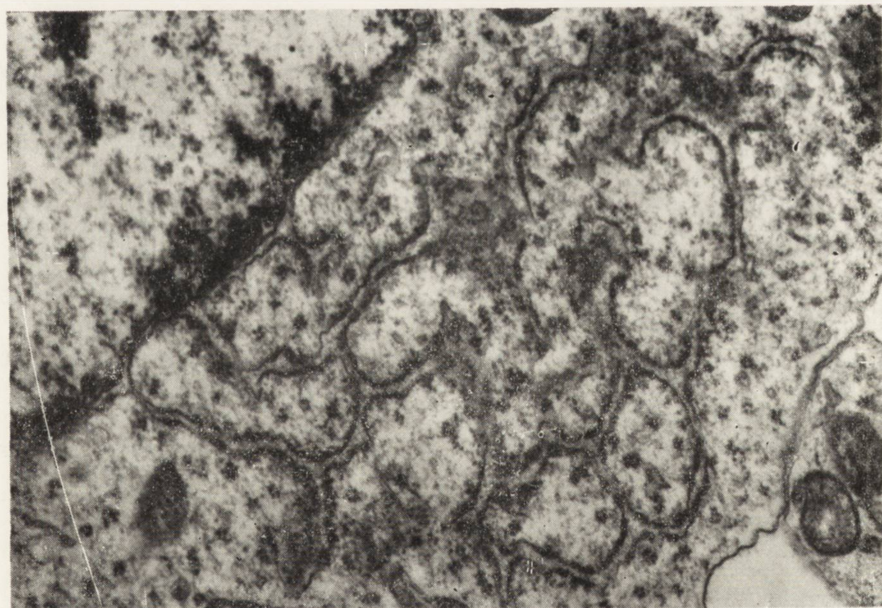

Fig. 2. An immature neuroblast with reticular arrangement of sacs of endoplasmic reticulum. × 30 000.

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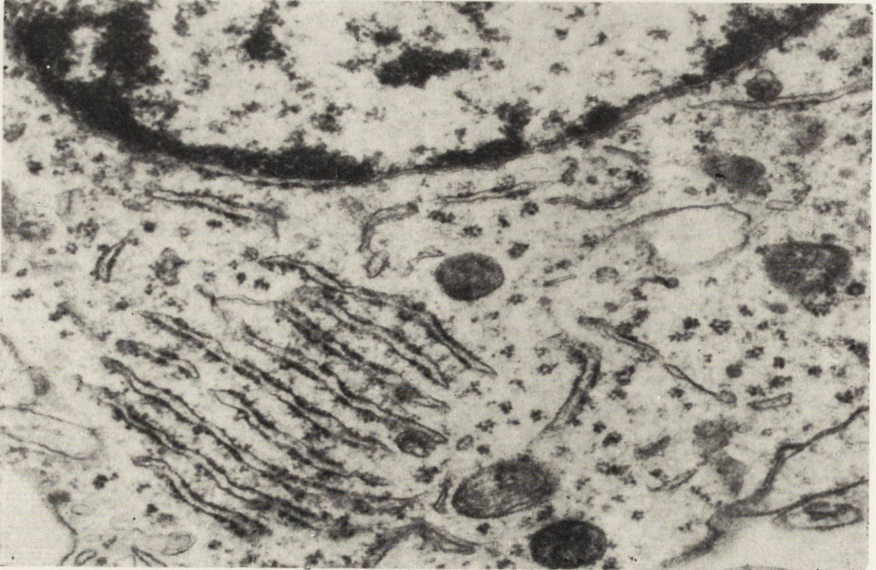

Fig. 3. Parallel orientation of sacs of endoplasmic reticulum in an immature neuroblast. × 30 000.

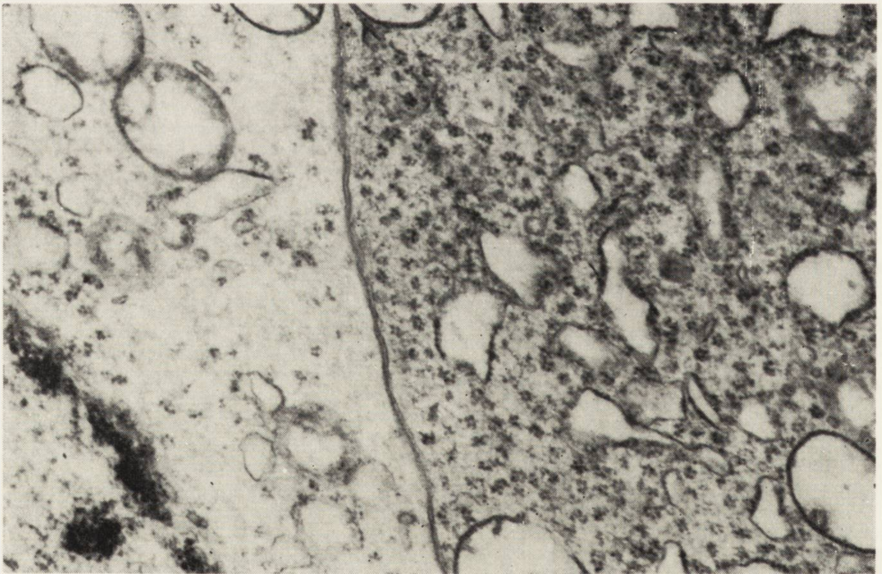

Fig. 4. Different density of cytoplasm in an immature neuroblast and glioblast. × 30 000.

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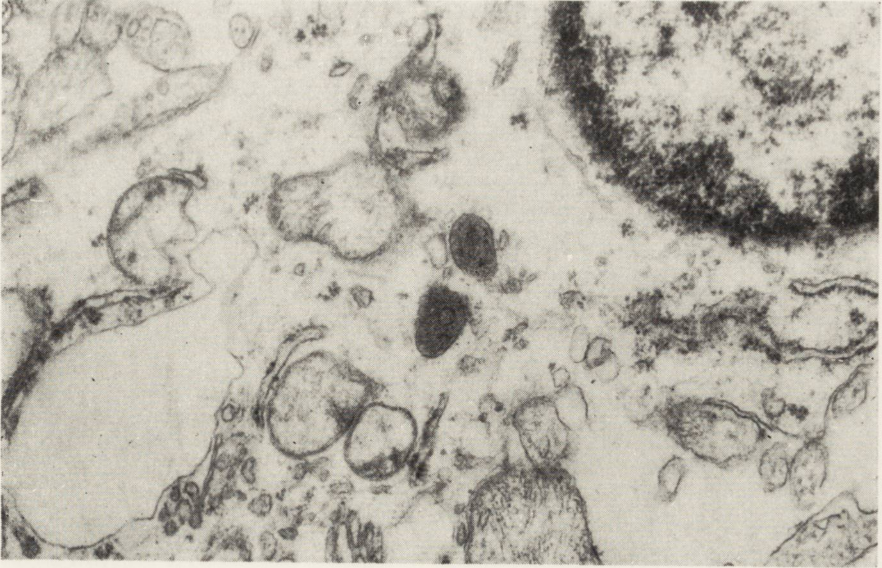

Fig. 5. Part of an astroblast with low density of its cytoplasm. × 30 000.

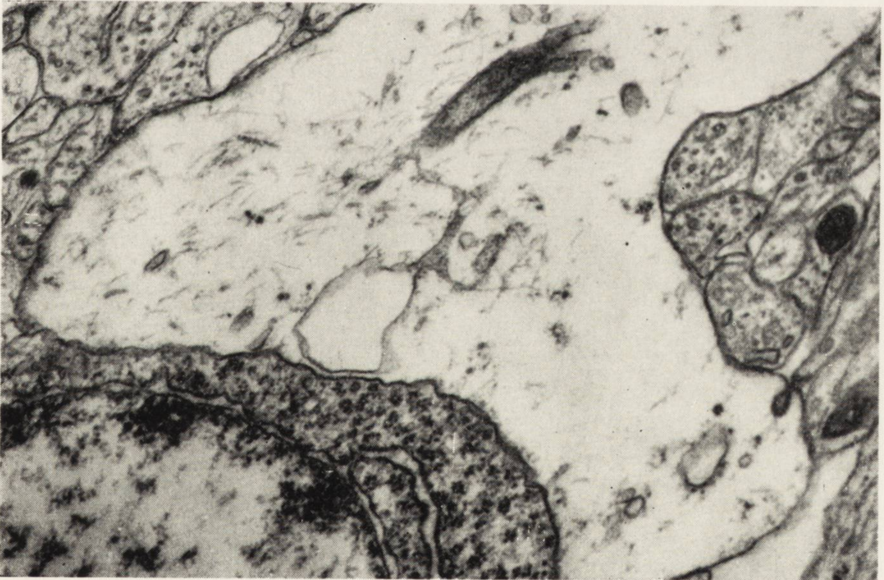

Fig. 6. Process of an astroblast containing very pale cytoplasm. × 30 000.

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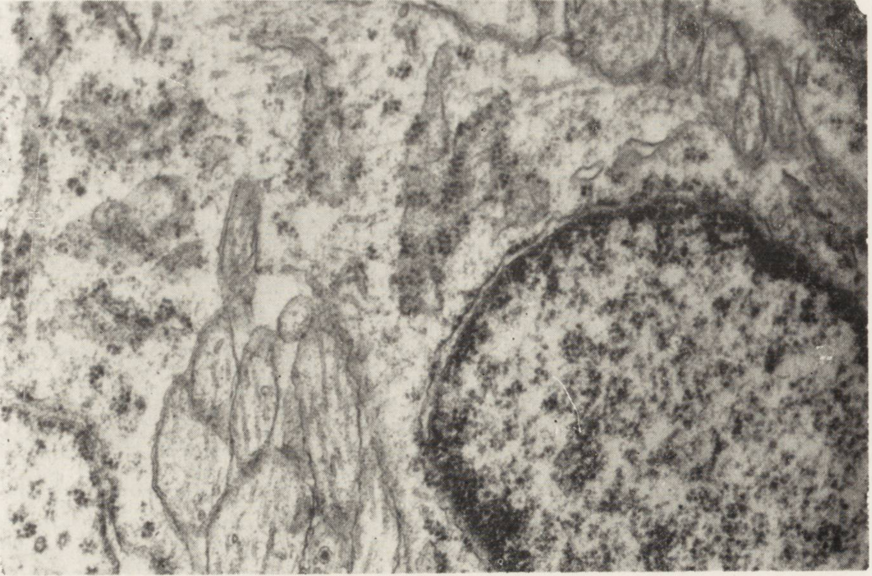

Fig. 7. Part of an oligodendroblast with a higher density of its cytoplasm. × 30 000.

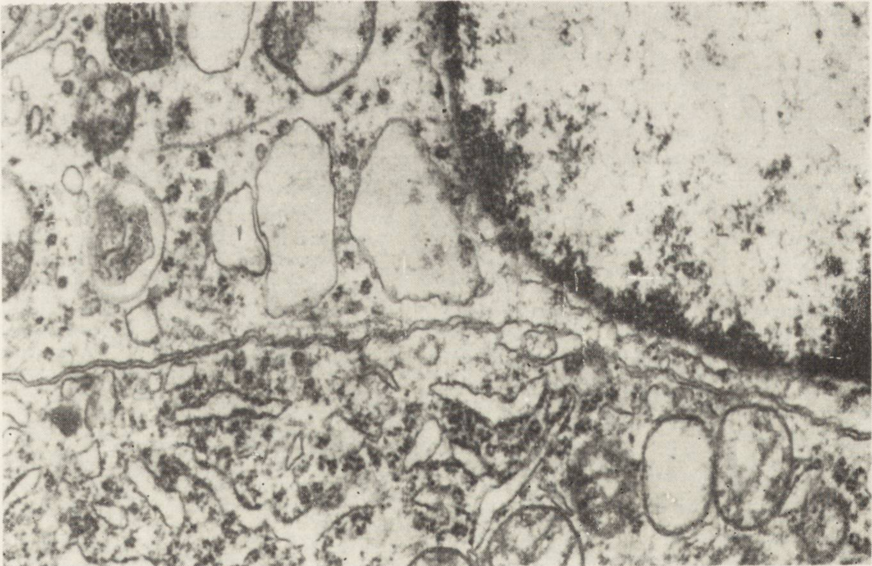

Fig. 8. An oligodendroblast in satellite position to neuroblast. × 30 000.

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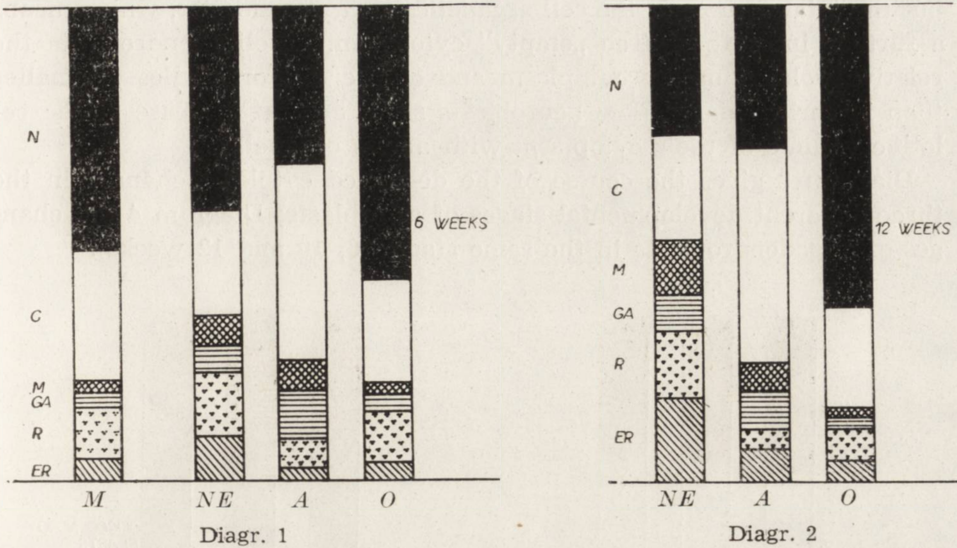

Diagram 1. Proportions of various cellular organelles in matrix cells, neuroblasts, astroblasts, and oligodendroblasts in 6 week old human embryo.

M — matrix cells	N — nucleus
NE — neuroblasts	C — cytoplasm
A — astroblasts	M — mitochondria
O — oligodendroblasts	GA — Golgi area
R — ribosomes	ER — endoplasmic reticulum

Diagram 2. Proportions of various cellular organelles in matrix cells, neuroblasts, astroblasts and oligodendroblasts in 12 week old human fetus.

M — matrix cells	N — nucleus
NE — neuroblasts	C — cytoplasm
A — astroblasts	M — mitochondria
O — oligodendroblasts	GA — Golgi area
R — ribosomes	ER — endoplasmic reticulum

From the diagram it is evident that already in this rather early embryonic stage there are clear differences in the proportions between the nucleus and the cytoplasm and even in the percentage of individual cell organelles. In the astroblasts the cytoplasm takes much greater portion than the nucleus, and the ergastoplasmic component takes less than the volume of the Golgi apparatus and agranular sacs together. In oligodendroblasts it is the other way round. The volume of cytoplasm is much smaller in proportions to the nuclear volume, and mitochondria and the Golgi areas with agranular sacs take very little. Neuroblasts have the highest proportion of cell organelles of all the examined cell elements. The volume of the ergastoplasm is the largest.

In the 12 week old fetus (Diagram 2) these differences are even more pronounced. The astroblasts increase the volume of their cytoplasm,

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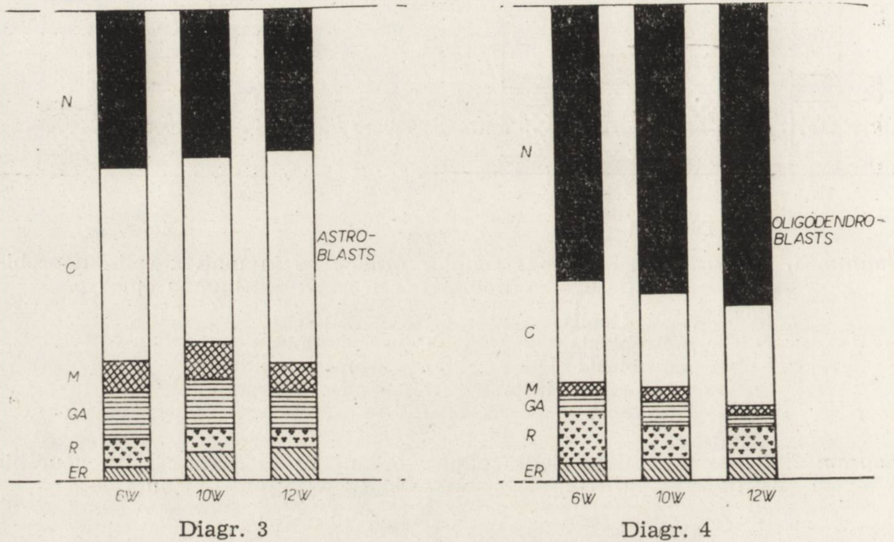
but the total volume of the cell organelles is rather smaller, which means a further increase of free „empty" cytoplasm. In oligodendroblasts the relative volume of the cytoplasm and of the cell organelles is smaller than in earlier stages. The neuroblasts show a great increase in the relative volume of their cytoplasm with all its organelles.

Diagram 3 gives the course of the described cytologic changes in the three different developmental stages of astroblasts; Diagram 4 the changes of oligodendroblasts in the same stages (6, 10 and 12 weeks).



Diagr. 3 Diagr. 4

Diagram 3. Age dependent proportions of various cellular organelles in differentiating astroblasts.

W — weeks

Diagram 4. Age dependent proportions of various cellular organelles in differentiating oligodendroblasts.

W — weeks

The other abbreviations in the diagrams 3 and 4 as in diagram 1.

J. Malínský

ROZWÓJ ONTOGENETYCZNY KOMÓREK GLEJOWYCH ISTOTY SZAREJ RDZENIA KRĘGOWEGO CZŁOWIEKA

Streszczenie

W pracy opisano ultrastrukturę astroblastów i oligodendroblastów istoty szarej rdzenia kręgowego u ludzi, we wczesnych okresach ich różnicowania i porównano ją z obrazem mikroskopowo-elektronowym neuroblastów. Różnice oceniano ilościowo w oparciu o metodę morfometryczną.

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Я. Малински

ОНТОГЕНЕТИЧЕСКОЕ РАЗВИТИЕ ГЛИАЛЬНЫХ КЛЕТОК СЕРОГО ВЕЩЕСТВА СПИННОГО МОЗГА ЧЕЛОВЕКА

Резюме

Сравнивается ультраструктура астробластов и олигодендробластов на ранних этапах их дифференцировки и сравнивается с электронно-микроскопической картиной нейробластов. Разницы оцениваются на основании морфометрического метода.

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NEUROPAT. POL. 1972, X, 2

J. MALINSKÁ

DEVELOPMENTAL CHANGES OF GLIAL ELEMENTS IN THE WHITE MATTER OF THE HUMAN SPINAL CORD

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Director: Prof. MUDr. J. Zrzavý

The number and structure of glial elements in the white matter change to a great extent during its development and maturation; these changes are most pronounced during the process of myelin formation. The number of glial cells was studied by Hillebrand (1966), Fleischhauer and Hillebrand (1966) in the corpus callosum of cat and by Wender and Mularek (1971) in the white matter of rat brain. References concerning the ultrastructure of the developing white matter are but few, e.g. Wechsler (1966), Wechsler and Meller (1967), Bergquist (1968) described its fine structure in chicken, and Gamble (1969) mentioned its ultrastructure in man.

Quantitative analysis of glial elements in human white matter was not performed at all and there is no sufficient knowledge about their fine structure either. Therefore we studied these problem on the spinal cord of man during its development. Some results of our work have been published in previous papers (Malinský 1968, Malinská, Malinský 1970, 1971, Malinský et al. 1971).

This time we examined spinal cords of human embryos and fetuses in the age of 3 to 20 weeks, and spinal cords of a newborn and an adult (20 years) man. Paraffin sections stained with hematoxyline and eosin and with cresyl violet were used for the optical microscope, and ultrathin sections from the material fixed with glutaraldehyde and osmic acid and embedded in Araldite ACM were examined under the electron microscope.

The marginal zone, forming the anlage of the future white matter, is in the early embryonic period completely acellular and contains only processes of both immature neuroblasts and glioblasts. Bodies of the

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glial cells appear later towards the end of the embryonic period, but their number is not too great. A rather massive development of glial cells is found in the fetal period; this being accompanied by the onset of myelin formation in the immature white matter. Later, in newborns and adults, the relative number of glial cells in the white matter is again smaller. The number of glial cells was counted in paraffin sections of the same thickness in a standard test area and the results calculated for the unit area of 1 sq. mm, but we did not try to estimate the absolute

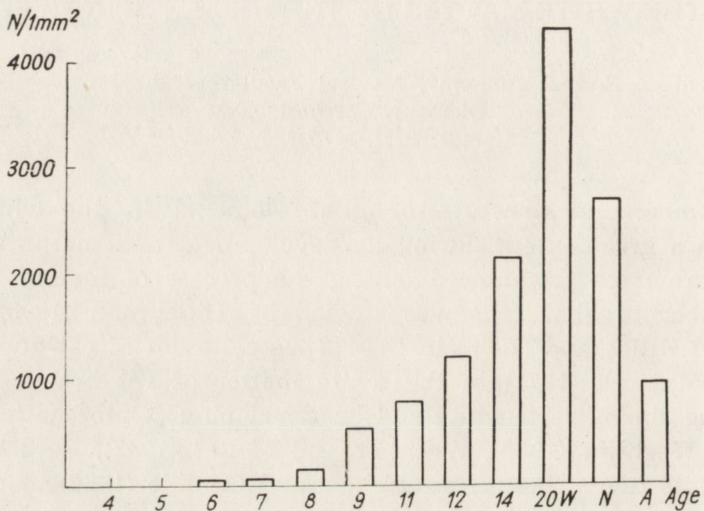

Diagram 1. Relative number of glial cells in the white matter in developing human spinal cord. *W* — week, *N* — newborn, *A* — adult.

number of cells or their number per unit volume. The obtained numerical values were used for the construction of diagram 1, which clearly shows that the relative number of glial cells in the developing white matter of human spinal cord has its maximum during the fetal period (20 weeks), the time when myelin formation shows also its great progress. Various areas of the white matter in the spinal cord do not exhibit the same degree of myelin formation in various developmental stages, and this fact is also manifested in the different number of glial cells. These differences are shown in diagram 2, which gives the relative numbers of glial cells in various funiculi, while in diagram 1 their relative number was counted only in the lateral funiculus.

The ultrastructure of the marginal zone and of the immature white matter changes very substantially during the prenatal development. As already mentioned above, the marginal zone in the early embryonic period does not contain any cell bodies and its processes are so poorly

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differentiated that even under the electron microscope it is not possible to make their exact classification.

The cells that appear in this zone later, belong to two different glial elements, macroglial and oligodendroglial. Macroglial cells are usually long, spindle-shaped elements, oriented in the marginal zone in radial direction, i.e. perpendicularly to the surface of the spinal cord. Their cytoplasm is pale, but there are fibrillar structures gradually appearing within it, and in the fetal period these cells change into fibrillary astrocytes.

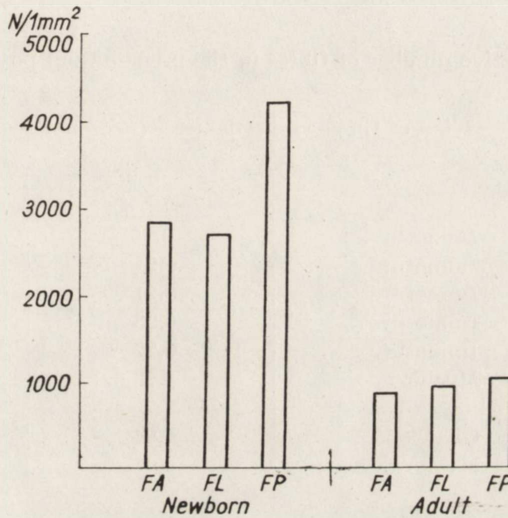

Diagram 2. Relative number of glial cells in various parts of the white matter of human spinal cord. *FA* — funiculus anterior, *FL* — funiculus lateralis, *FP* — funiculus posterior.

The elements of oligodendroglia undergo more prominent changes. Immature oligodendroblasts have their cytoplasm of granular structure which does not contain fibrillary structures or agranular sacs (Fig. 1). Their alar processes gradually develop characteristic relations to immature axons, enveloping them from both sides (Fig. 2) and forming typical mesaxones with spiral arrangement (Fig. 3). These structures are the first signs of the future myelin formation, but they occur even in pre--myelin stages. In later stages of the fetal period, when myelin formation reaches its maximum, the ultrastructure of oligodendroblasts is quite different (Fig. 4). The cells participating in the myelination have very dense cytoplasm and nuclei. Their alar proceses run to the surface of individual axons, where they form the typical myelin sheaths. In the fetal period it is possible to distinguish better fibres in the primitive white

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matter than in the embryonic marginal layer, but even here some fibres remain immature.

Changes of the fibres during their maturation were evaluated by a quantitative method. We calculated the relative number of fibres per unit area and measured their diameters. The results given in table 1 show that the diameter of immature fibres does not change substantially during the whole prenatal period, but their relative number per unit area increases greatly till the tenth week, but afterwards it falls down rapidly. This decrease can be explained by the transformation of immature fibres into specific mature processes, axons, and glial fibres.

Table 1. Size and relative number of fibres in the prenatal and postnatal white matter

Age (weeks)	Kind of fibres	Diameter (microns)	Number/1 mm²
5	Immature	0.25	10 040 000
7	Immature	0.21	12 000 000
10	Immature	0.21	19 000 000
12	Immature	0.23	13 000 000
	Immature	0.22	3 000 000
20	Myelin	1.06	160 000
Adult	Glial	0.84	400 000
	Myelin	3.61	27 500

The results of our studies of the white matter of adult man, were very interesting. The counts carried out on ultrathin sections demonstrated that the relative and absolute numbers of glial fibres were many times higher than the number of myelinated axons. Therefore we estimated volume proportions between the nerve and the glial components in this tissue by means of a quantitative morphometric method using a system of test points. We found (Table 2) that the glial elements take more than fifty per cent of the whole volume of the spinal cord white matter. But if we take into account that myelin is of glial origin too, it would be more than 75 p.c. We can also see that the proportion of axons is relatively small (less than 20 p.c.).

The demonstrated results show that glial elements are very important for the developing white matter of the spinal cord particularly in the period of myelin formation, when there appear special cellular forms of oligodendrocytes, found neither earlier not later. In the adult white

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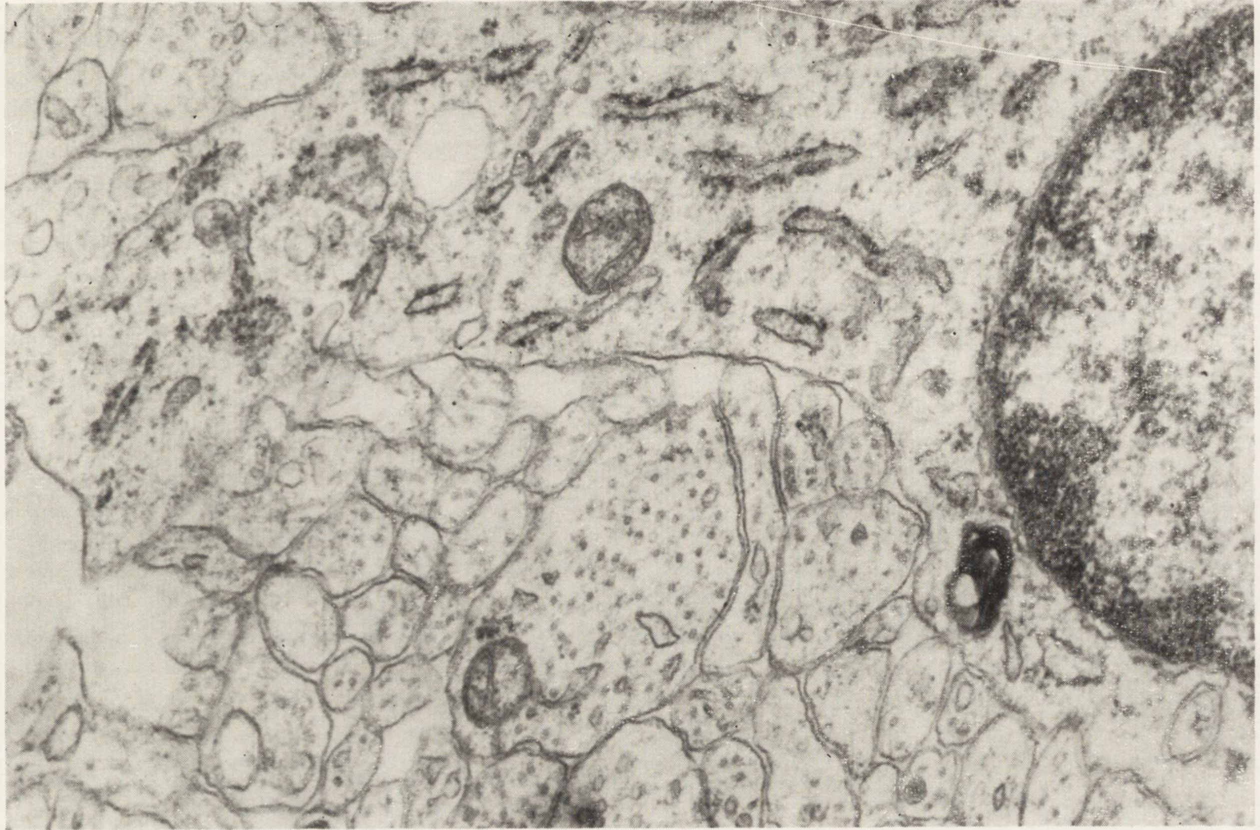

Fig. 1. Part of an immature oligodendrocyte in the white matter of 8 week old fetus. × 30 000.

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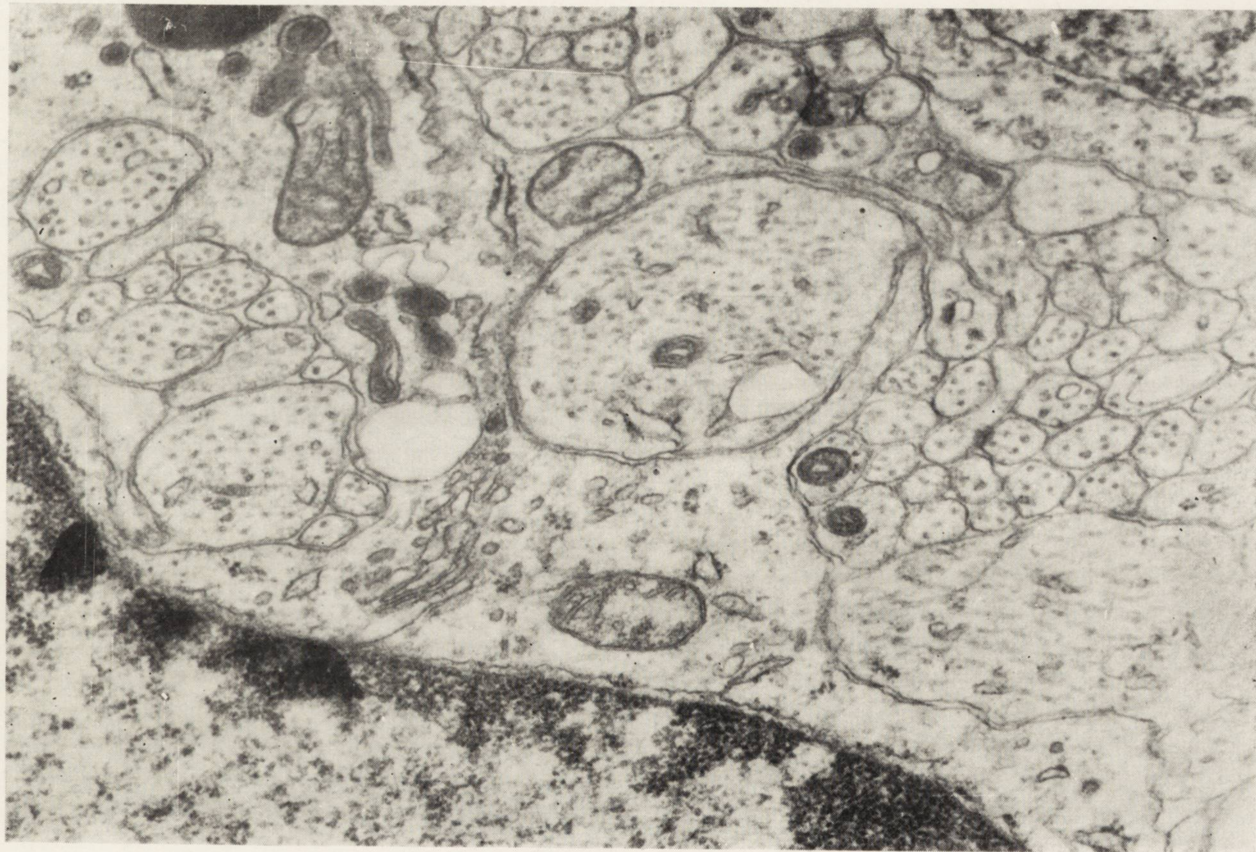

Fig. 2. Alar processes of an immature oligodendrocyte envelope a large axon. × 30 000.

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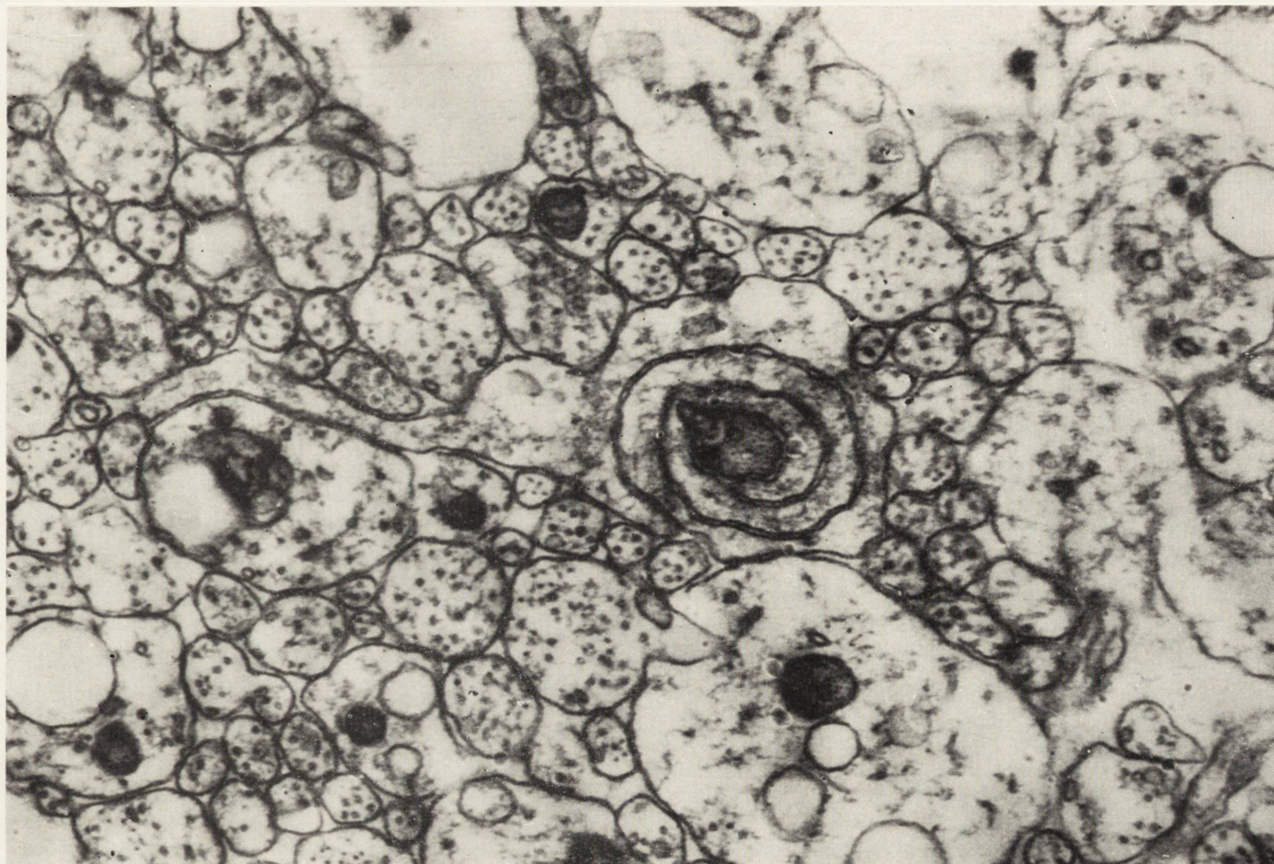

Fig. 3. Spiral arrangement of mesaxon round a small axon. × 50 000.

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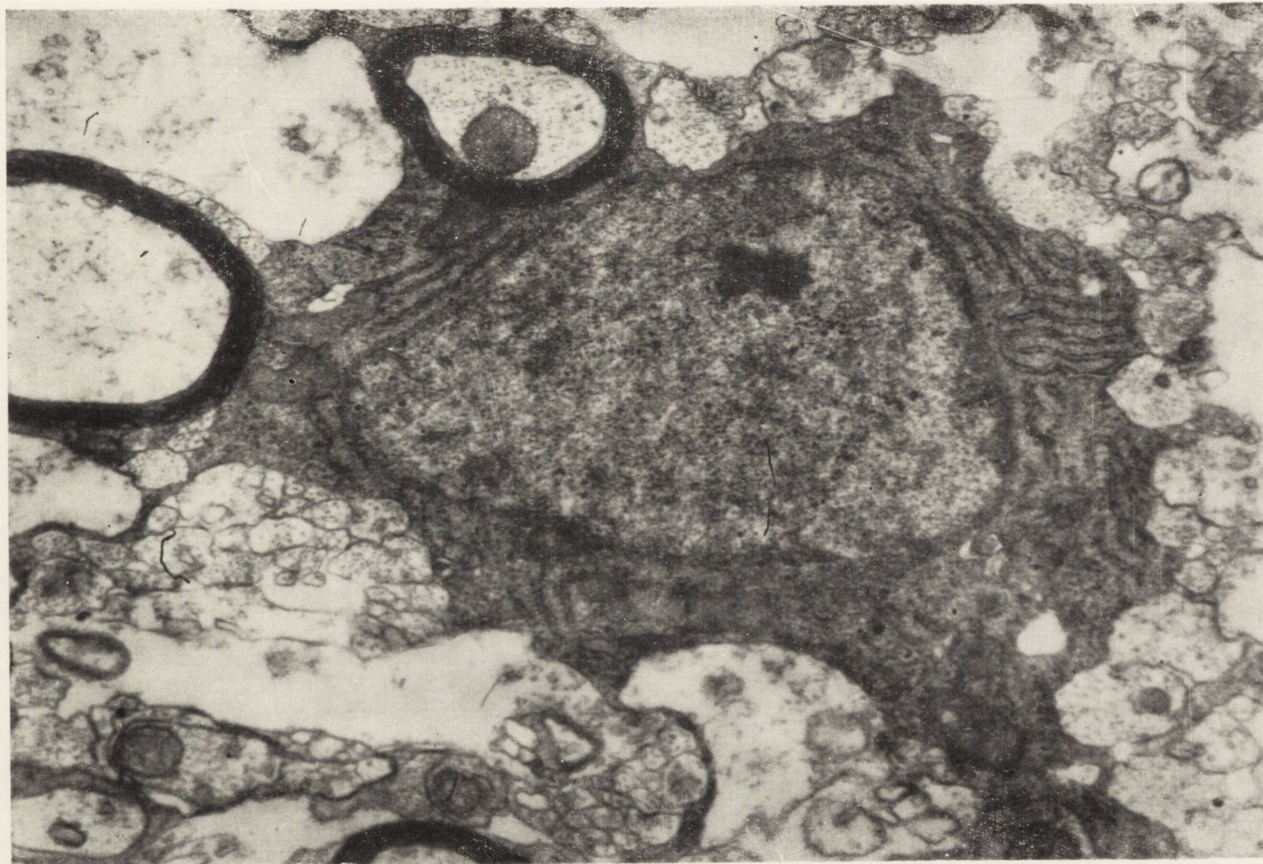

Fig. 4. Dark oligodendrocyte in the white matter of 30 week old fetus. Its processes participate on myelin formation round large axons. × 22.000.

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Table 2. Proportions (in per cent) of individual structures in the white matter of adult spinal cord

Myelinated fibres	41.0	
Axoplasm		18.5
Myelin sheath		25.5
Glial elements	52.5	
Glial fibres		38.6
Cell bodies : cytoplasm		8.5
nuclei		4.4
Mesenchymal elements	5.6	
Blood capillaries		2.6
Connective tissue		3.0
Intercellular spaces	0.9	0.9
	100.0	100.0

matter the volume proportion of glial elements is very high, so that their significance cannot be limited to some a passive supporting function.

J. Malinská

ZMIANY ROZWOJOWE ELEMENTÓW GLEJOWYCH ISTOTY BIAŁEJ RDZENIA KRĘGOWEGO CZŁOWIEKA

Streszczenie

Przebadano zmiany w ilości i obrazie ultrastrukturalnym komórek glejowych w istocie białej rdzenia kręgowego człowieka w okresie rozwoju i porównano je ze zmianami ilości, gęstości i średnicy włókien nerwowych i glejowych.

Я. Малинская

ИЗМЕНЕНИЯ РАЗВИТИЯ ГЛИАЛЬНЫХ ЭЛЕМЕНТОВ БЕЛОГО ВЕЩЕСТВА СПИННОГО МОЗГА ЧЕЛОВЕКА

Резюме

Исследовали изменения количества глиальных клеток и их ультраструктурного строения в белом веществе спинного мозга человека в течение развития и сравнивали их с изменениями количества, плотности и диаметра нервных и глиальных волокон.

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NEUROPAT. POL. 1972, X, 2

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RIBONUCLEIC ACID CONTENT IN NEURON-NEUROGLIA UNIT OF THE SPINAL CORD

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Advances in quantitative cytochemistry of the nervous system have resulted in a number of data concerning chemical composition and metabolic dynamics of individual neurons. Meanwhile, functional biochemistry of the neuron still needs intensive efforts to elucidate molecular mechanisms responsible for the specific activity of the neuron. Being the most important cellular structure of the nervous system, the neuron, nevertheless, is only a part of the whole morphological unit of the nervous tissue. The other part is constituted by neuroglial cells which morphologically represent a link between the neuron and the capillary network (for literature, see De Robertis, Gerschenfeld 1961).

Studies on the bioelectrical pattern of the nervous tissue have enabled neurobiologists to suggest a participation of glial cells in the specific phenomena of the nervous system such as excitation, inhibition, conductance, etc. (Galambos 1961, Tasaki 1965, Kuffler, Nicholls 1966, Roitbak 1968, 1969).

Basing on biochemical peculiarities of the neurons and glial cells Hydén (1959, 1960, 1964, 1967) put forward the idea of a single metabolic neuron-neuroglia unit. His most interesting data as well as some results obtained by Pevzner and his coworkers (Pevzner 1965, 1968, 1971; Pevzner, Haidarliu 1967; Saudargene, Pevzner 1969) indicate a possibility of parallel changes of RNA metabolism in the neurons and their glial satellite cells under some experimental conditions.

In experiments, the authors often applied rather drastic unadequate loads upon the nervous system such as an electric stimulation, hypoxia, administration of enzyme inhibitors, drugs, etc. Therefore, it seemed worthwhile studying the RNA metabolism within the neuron-neuroglia unit under more adequate, milder loads.

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Besides, it should be noted that, in general, studies on the functional biochemistry of the neuron have dealt mainly with effects of some factors rather than with the following reparation. In our opinion the analysis of the reparation metabolic dynamics might add a valuable information about the biochemical features of the neurons and neuroglia.

Therefore, the task of the present communication was to compare the dynamics of RNA changes within the neuron-neuroglia units which differ in their functional role under some loads and during the reparation period. Thus we studied the content of RNA per cell in the motor neurons of spinal cord anterior horns and sensitive neurons of spinal ganglia as well as in the glial cells adjacent to these neurons under conditions of hyper- and hypo-kinesia and during the restoration of normal motor activity.

MATERIAL AND METHODS

All the experiments were performed with male white mice weighing 28—32 g. As a model of hyperkinesia, swimming in water at 34—36° for 2—4 hr was applied. The animals were sacrificed immediately after the end of the swimming as well as 2—24 hr later.

To study hypokinesia, each mouse was placed into an individual narrow cage which restrained the movements of the mice without, however, their complete immobilisation. The restrained mice like the control ones, were given food and water ad libitum. There was a moderate excitation of the animals during the first 2—3 days of the restraint, later on the mice looked adapted. By the end of 3 weeks of the restraint there could be observed the so called "hypokinesia complex": weight loss (by 15—20%), paresis of hind legs, failure of locomotor coordination etc. The normal motor activity was restored in the animals 2—3 days after the cessation of 3 week restraint.

A group of mice was sacrificed immediately after 2 or 3 week restraint, another group of the animals, after 3 week restraint, was kept under conditions of free motor activity for 2—72 hr. As control, those mice were used which were constantly in a state of a relative physiological rest. Each group included 5—6 animals.

All the mice were decapitated without anaesthesia. The lumbar enlargement of the spinal cord as well as the adjacent spinal ganglia were fixed in a cold Brodsky solution (formol-ethanol-acetic acid, 3 : 1 : 0.3, by vol.) and embedded in paraffin. In 10 μ slices an optical density (extinction) of RNA was measured in the cytoplasm of neurons and in the bodies of glial satellite cells by means of two-wave length ultraviolet

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cytospectrophotometry at 265 and 280 mμ (Agroskin et al. 1960) using the two-wave length, one-beam cytospectrophotometer constructed by Agroskin (1964). The optical scheme of this photometer as well as details of measurements and calculations were described elsewhere (Pevzner 1959, 1963a, 1966a).

On measuring the optical density (D) of the cell studied the concentration of RNA was found according to Bouger-Lambert-Beer's law:

$$c = \frac{D}{\chi \times h}$$

where c is concentration (g/cm^3 or pg/μm^3), χ — extinction coefficient, h — thickness of sections (cm).

The absolute amount of RNA (pg) per cell was determined by multiplying the concentration value by the volume of the cytoplasm of neurons or by the volume of the body of glial cells. Cellular volume was calculated basing on the rotation ellipsoid formula. Large and small diameters of the cell were measured by means of an eye-piece micrometer in the nervous tissue sections stained with gallocyanin chromalum according to Einarson (1935, 1951).

RESULTS

Cytophotometric analysis of several methods of a selective RNA extraction from the sections of the nervous tissue

Absorption in UV is characteristic not only for nucleic acids but also for protein. The absorption coefficient of the former is 30—50 times as high as that of the latter (Caspersson 1936). However, the concentration of proteins in the cell body is as a rule much higher than that of the nucleic acids. Therefore the percentage of the absorption due to the proteins in the total UV-absorption of the fixed animal cell can be rather high.

Two different principles are used to measure separately nucleic acids in spite of them being in a mixture with cellular proteins. Caspersson (1936, 1950) suggested to subtract the absorption of the cellular protein from the total spectrum of the cell studied. The shortcoming of this suggestion consists in that UV-absorption of the protein is due to its aromatic amino acids (mainly tryptophan and tyrosine) of which concentration and ratio remain unknown. Caspersson (1950) used for his calculations a "standard" relation: 5 per cent of tyrosine and 1 per cent of tryptophan. However, the ratio of aromatic amino acids within the cellular proteins may fluctuate markedly.

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The correctness of the determination of the nucleic acids much depends also upon their ratio to the protein. If it is the protein which is mainly responsible for the cell absorption the error of the nucleic acid determination can be as high as 30—40% (Sandritter 1957, Sandritter et al. 1958).

Therefore, a number of authors, to beging with Pollister and Ris (1947) have used another method of separation of the absorbing substances. In this case, like in Caspersson's method, the spectrum of the absorbing structure is believed to be a sum of the absorption spectra of individual chemical components. So one of these components is eliminated to compare the total and residual absorption of the same structure. Thus, the optical density of RNA can be determined as a difference between the cell density values before and after the extraction of RNA.

The most specific method of RNA extraction is of course the treatment with RNA-ase solution. However, this treatment is shown by some authors to extract only a part of the RNA bulk from the fixed sections (see, for instance, Sandritter et al. 1957; Brodsky 1966). More available in this respect are methods of acid extraction of RNA from the sections. But conditions of this extraction presented in various papers differ to great extend and as has been pointed out the distinct extraction conditions must be applied to every tissue studied. Besides, the completeness of the extraction, as far as the nervous tissue sections are concerned, was checked only visually.

Therefore we considered it worthwhile to evaluate quantitatively several methods of the acid extraction of RNA from the nervous tissue sections by applying both visible and UV cytophotometry.

Determinations were made on 5—7 μ sections of Brodsky fixed superior cervical sympathetic ganglia and cerebral cortex of the cat.

Koenig and Stahlecker's (1952) scheme of extraction seemed rather suitable: a treatment of sections with 10% perchloric acid at 37° for 15 min. But these authors have noted that thereby DNA also begins to be extracted within 20 min. after the beginning of the perchloric acid extraction.

To check up this scheme of extraction we photometried the cytoplasm and nucleus of the neurons by means of UV-cytospectrophotometry at 265 mμ at various time intervales after the beginning of the perchloric acid treatment according to Koenig and Stahlecker (1952). It was found out that their scheme of extraction was not suitable for our object of studies: at the moment when the extraction of RNA had not yet been completed an intensive extraction of nuclear DNA already began (Fig. 1).

Milder conditions of acid hydrolysis of RNA with perchloric acid (treatment with 10% $HClO_4$ at 23—26°C for several hours) were suggested

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by Ogur and Rosen (1950). To check up this scheme of extraction we stained the sections with Einarson gallocyanin chromalum (Pearse 1962) and measured the optical density of cytoplasmic RNA by means of two--wave length photoelectrical cytospectrophotometry (Mendelsohn 1958, Agroskin et al. 1960) at 580 and 460 mμ using the aperture visible cytospectrophotometer constructed by Agroskin (1964). However, when trying Ogur and Rosen's method of extraction we failed to succesed in a complete extraction of cytoplasmic RNA, even as late as after 6 hr treatment of the sections with 10% $HClO_4$ at 23—26° (Fig. 2A). An attempt was also made to measure the content of nuclear DNA by means of visible cytospectrophotometry at 580 and 490 mμ of the Feulgen stained sections. The optical density of DNA was shown to be somewhat increased, what

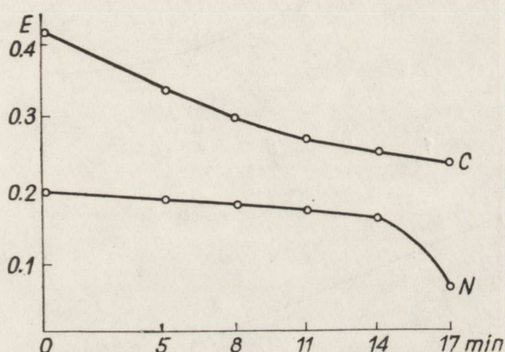

Fig. 1. Effects of perchloric acid (10% $HClO_4$, 37°C) on ultraviolet absorption of the cytoplasm and nucleus of nerve cells. Ordinate represents extinction in logarithmic units, abscissa represents duration of acid extraction (i.: min.). *C* — cytoplasm, *N* — nucleus.

might have been the result of a shrinkage of nuclei in the section or probably from alterations of properties of DNA molecules. Detailed study of this point seemed rather difficult because in the course of usual Feulgen hydrolysis with I N HCl, a majority of $HClO_4$-treated sections was lost from the object glasses, this phenomenon being one of the additional defects of the method of RNA extraction.

Ogur and Rosen (1950) also suggested another scheme of RNA extraction at lower temperatures (not higher than 4°) with a perchloric acid treatment lasting as long as 2—3 days. Cytospectrophotometric evaluation of this method has shown that 10% $HClO_4$ at 4° for 72 hr does extract RNA in fact completely without extracting cellular DNA (Fig. 2B). The disadvantage of this method, however, is its duration. Some success in this point can be achieved by using a more concentrated solution of the acid (Hess, Thalheimer 1965). Indeed, the treatment of sections with 16% cold perchloric acid has been found to result in a complete RNA extrac-

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tion in 48 hr while DNA content remained unchanged under these conditions (Fig. 2C).

It is of practical importance for the UV-cytospectrophotometry that the cellular proteins should not be extracted in the course of the extraction of nucleic acids. Using cytointerferometric method Pevzner et al. (1964) earlier showed that the value of the cell optical density at 280 mμ

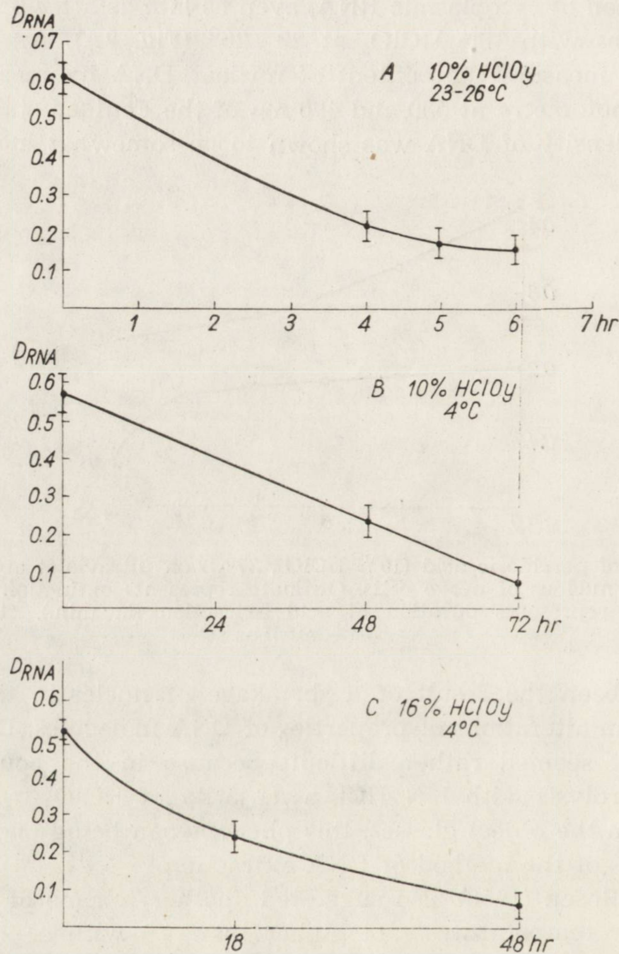

Fig. 2. Effects of perchloric acid on the content of RNA in the cytoplasm of nerve cells (visible photometry of preparations stained according to Einarson).

after the extraction of the sum of nucleic acids with a hot trichloracetic acid reflects rather well the content of total cellular protein. Therefore we compared the data concerning the transmissions at 280 mμ after the treatment with 5% trichloracetic acid and with the solutions of perchloric

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acid. As seen from Figure 3 the extraction with 16% $HClO_4$ at 4° for 48 hr did not influence the protein content in the nerve cells.

Basing on all these data we choose for further experiments the treatment of sections with 16% perchloric acid at 4° for 48 hr as a sufficiently reliable method of selective RNA extraction.

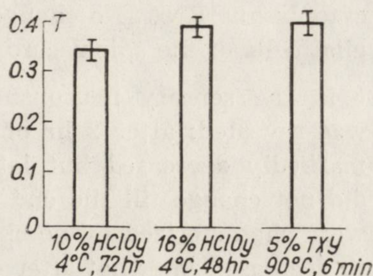

Fig. 3. Effects of perchloric acid on the content of protein in the cytoplasm of nerve cells (ultraviolet cytospectrophotometry). Ordinate represents transmission at 280 mμ after the acid extraction of RNA.

Absolute amount of RNA per cell in the neurons and their glial satellite cells of mouse spinal cord anterior horns and spinal ganglia in the state of relative physiological rest

The extraction scheme described above permitted us to compare the optical densities of cell before and after the selective extraction of RNA, this difference of the densities corresponding to the optical density due to RNA molecules. By calculating RNA concentration and multiplying it by corresponding values of cellular volumes, the absolute amount of RNA per cell (in pg) was found. As seen on the Table 1, in the mice which are in a state of relative physiological rest, the mean quantity of cytoplasmic RNA in the spinal ganglia neurons is about twice as high as that in the motor neurons of spinal cord anterior horns. The same holds true for their glial satellite cells: in the spinal ganglia, neuroglial cells are somewhat richer in RNA than in the spinal cord anterior horns (Table 1).

Table I. Amount of RNA per cell in areas of mouse spinal cord

Area of the spinal cord	Neurons			Glia		
	Number of animals	Number of cells	Mean RNA amount (in pg) ± S.E.M.	Number of animals	Number of cells	Mean RNA amount (in pg) ± S.E.M.
Anterior horns	50	974	113.8 ± 8.4	50	875	2.82 ± 0.16
Spinal ganglia	51	1119	255.4 ± 17.6	51	1064	4.00 ± 0.20

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Effect of swimming and following rest on RNA content within the neuron-neuroglia units of mice spinal cord anterior horns and spinal ganglia

In the motor neurons of anterior horns, the swimming for 3 hr and in particular for 4 hr induced a pronounced increase in the content of cytoplasmic RNA. No changes were observed in the RNA content in the glial cells of the spinal cord under these conditions (Fig. 4A).

In the sensory neurons of spinal ganglia, quite different dynamics was revealed: after 2 hr of swimming the content of cytoplasmic RNA markedly decreased but later it returned to normal and subsequently did not change till the end of the experiment. Their glial satellite cells were characterized by no changes in RNA amount after 3 hr swimming, to decrease somewhat after 4 hr swimming (Fig. 4B).

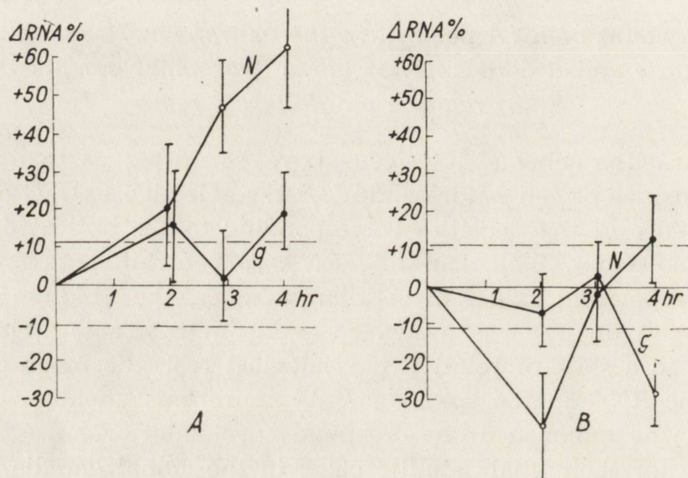

Fig. 4. Changes in the content of RNA in the neurons and neuroglia of spinal cord anterior horns *(A)* and of spinal ganglia *(B)* at swimming of various duration. Ordinate represents per cent deviation of the RNA content per cell from the corresponding control level, abscissa represents duration of the swimming. *N* — neuron, *G* — glial cells. Dotted lines at both sides from the control level (horisontal line) and vertical bars mean standard error of the mean multiplied by 1.96 (95% significance level).

At the end of 3 hr swimming, the content of cytoplasmic RNA still remained higher for 2 hr and subsequently returned to normal (after 6 hr rest). In the neuroglia of anterior horns, a decrease in RNA content was observed during the first hours of the rest, the return to normal proceeding at a slower rate than in the motor neurons (Fig. 5A).

The rest after the cessation of 3 hr swimming was characterized by no statistically significant changes in the content of RNA in the cyto-

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plasm of spinal ganglia neurons. In the perineuronal neuroglia of the ganglia, an increase of RNA content was revealed (Fig. 5B).

Effect of long-term restraint and following free motor activity on RNA content within the neuron-neuroglia units of mice spinal cord anterior horns and spinal ganglia.

As seen from Figure 6, no statistically significant changes in the content of cytoplasmic RNA was found either in the motor or in the sensory neurons studied by the end of 2- or 3- week restraint. There were no changes in RNA content either in the glial satellite cells adja-

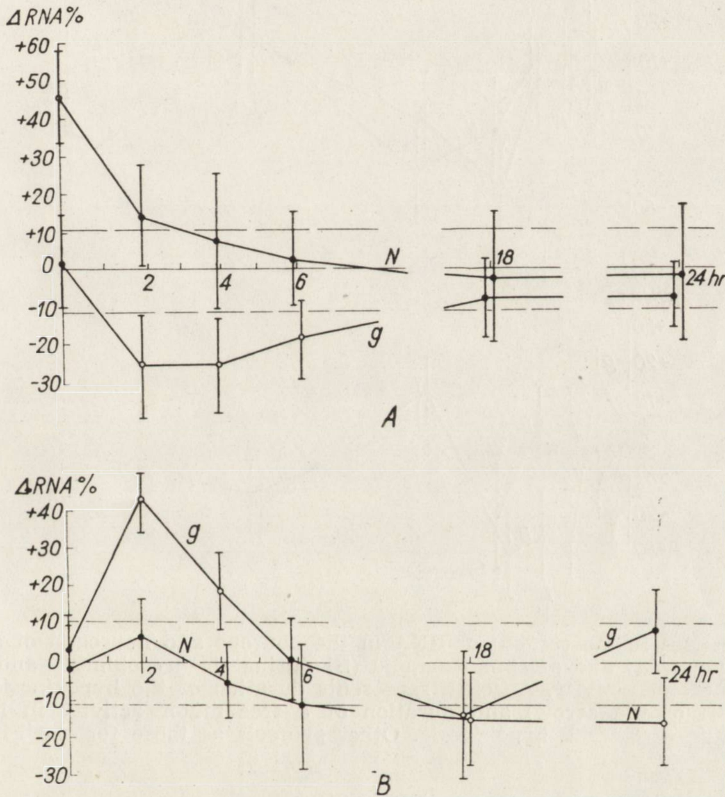

Fig. 5. Changes in the content of RNA in the neurons and neuroglia of spinal cord anterior horns *(A)* and of spinal ganglia *(B)* during rest after 3 hr swimming. Abscissa represents duration of rest. Other symbols as those for *Figure 4.*

cent to the neurons of spinal cord anterior horns. In the neuroglia of spinal ganglia the content of RNA was increased after 2 weeks and diminished after 3 weeks of restraint (Fig. 6).

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After the mice had been taken out of the restraint cages the beginning of a free motor activity was accompanied by quick and pronounced changes in RNA content. As early as after 2 hr, a decrease in the content of RNA occurred both in the neurons and in the glial satellite cells of spinal cord anterior horns and spinal ganglia, this change being somewhat higher in the neuroglia than in the neurons. Three days after the cessation of 3- week restraint, the content of RNA returned to normal in both kinds of neurons studied while in the glial cells a secondary change (a decrease) in RNA content took place (Fig. 6).

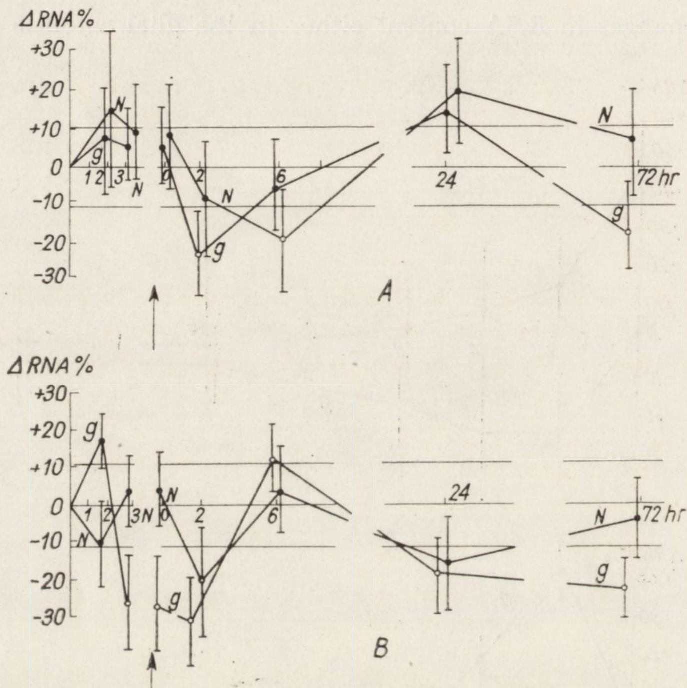

Fig. 6. Changes in the content of RNA in the neurons and neuroglia of spinal cord anterior horns *(A)* and of spinal ganglia *(B)* during the hypokinesia and the following free motor activity. Abscissa represents duration of the hypokinesia in weeks (to the left of the arrow) and duration of a free motor activity in hours after cessation of 3 week hypokinesia. Other symbols as those for the Figure 4.

DISCUSSION

A comparison of different methods of RNA extraction has permitted us to choose a scheme of selective extraction of RNA without touching cellular DNA and proteins. This has provided for the possibility of determining the content of cellular RNA by means of UV-cytospectrophotometry (Figs. 2 and 3). Of course, the conclusion both about the comple-

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teness of RNA extraction and about the absence of changes in DNA and protein content is true only within the limits of sensitivity of the cytospectrophotometric method. Besides, the suggested conditions of selective extraction of RNA can be used, in all probability, only for the nervous tissue sections fixed with Brodsky solution or with similar fixatives. The studies of other tissues as well as with other fixative will need perhaps different schemes of extraction.

The results of our studies have shown 3 hr and particularly 4 hr swimming to result in an accumulation of cytoplasmic RNA in the motor neurons of spinal cord anterior horns (Fig. 4). This is in agreement with many data of the literature (see reviews by Hydén 1962, 1964; Pevzner 1963b, 1966b; Brodsky 1966; Gaito, Bonnet 1971) which indicate that an intensive or prolonged functional load (until it induces an exhaustion, fatigue of neurons) is accompanied by an increase of neuronal RNA. At the same time, no statistically significant changes in the content of RNA have been observed under these conditions in the glial satellite cells adjacent to the motor neurons.

We can think that the functional load (swimming) used in our experiments is more adequate than the motor activity induced by electrical skin irritation (Pevzner, Haidarliu 1967) or by Metrazol (cardiazol) convulsions (Pevzner, Saudargene 1971), both these kinds of motor activity in rats causing a decrease of RNA as well in the motor neurons as in their glial satellite cells of spinal cord.

The cessation of swimming in our experiments resulted in two biochemical events. On the one hand, the content of neuronal RNA which was markedly increased owing the load, returned rather quickly to normal. On the other hand, the content of glial RNA which remained normal by the end of the 3 hr swimming decreased markedly and only later returned to the level at rest. In the neuroglia of spinal ganglia, the content of RNA was also characterized by no changes by the end of the swimming, but by pronounced changes after the cessation of the load (Figs 4 and 5). The character of the changes in the glial RNA was different in the motor and in the sensitive regions of the spinal cord.

Thus, whereas the increased level of functional activity of the nervous system was accompanied by RNA changes preferably in the neurons, the reparative changes in RNA content after the cessation of the load were localized mainly in the neuroglial cells.

Basing on the results of the experiments on restrained mice we may think that by the end of 2 or 3 week restraint a process of adaptation developed resulting in maintaining a new level of equilibrium between the catabolism and anabolism of RNA. This has seemed to be reflected

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particularly in the absence of RNA changes in the motor and sensory neurons by the end of restraint (Fig. 6).

On maintaining a stable adaptation, the beginning of a free motor activity of animals has proved to become a drastic stress-like action. It was biochemically reflected in a form of pronounced RNA decrease both in the motor structures of spinal cord and in the sensitive spinal ganglia. Differences in RNA dynamics between the motor and sensitive neuron--neuroglia units appeared later, during the reparation period. Biochemical differences appeared this time also between the neurons and their glial satellite cells (Fig. 6): the latter were characterized by a higher rate of the return of RNA content to normal.

In general, the data obtained have supported the conception put forward by Pevzner (1965, 1968, 1971): a marked stress-like change of the functional state of the nervous system is accompanied by similar changes of neuronal and glial metabolism, whereas a moderate, more adequate excitation of the nervous system results in the changes of RNA content which are different in the neurons and neuroglia. These two kinds of cells are particularly different during the period when the stress-inducing factor is eliminated, the reparation process in RNA metabolism proceeding in the neuroglia quicker than in the neurons. Perhaps, such a high rate of metabolic reparation of neuroglia provides for an important compensatory trophic role of the glial cells within a single functional-biochemical neuron-neuroglia unit.

V. A. Brumberg, L. Z. Pevzner

ZAWARTOŚĆ KWASU RYBONUKLEINOWEGO W JEDNOSTCE NEURON-NEUROGLEJ W RDZENIU KRĘGOWYM

Streszczenie

W pierwszej fazie badań porównano skuteczność różnych sposobów wybiórczej ekstrakcji kwasu rybonukleinowego. Badania prowadzono metodą cytospektrofotometryczną w ultrafiolecie i w świetle widzialnym (2 długości fal). Za najlepszą metodę, umożliwiającą całkowitą ekstrakcję RNA z komórek z materiału skrawkowego, utrwalonego w roztworze formol-etanol-kwas octowy i zatopionego w parafinie, uznano przeprowadzenie skrawków w 16% $HClO_4$ przez 48 godz. w temp. 4°. Przy tym sposobie ekstrahowania nie następuje utrata żadnej wymiernej ilości komórkowego DNA i protein.

Pływanie myszy w wodzie w 34—36° przez 3—4 godz. spowodowało znaczny wzrost ilości cytoplazmatycznego RNA w komórce w neuronach ruchowych rogów przednich rdzenia kręgowego. W glejowych komórkach satelitarnych, przylegających do tych neuronów, nie zauważono zmian znamiennych statystycznie. Podczas pierwszych godzin wypoczynku, po zakończeniu pływania, następował stopniowy powrót do normy zawartości RNA w neuronach, równolegle do zmniejszania się zawartości RNA w gleju.

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Trzytygodniowe pozbawienie myszy swobody ruchu w pojedynczych klatkach nie spowodowało wyraźnych zmian w ilości RNA w komórkach nerwowych i glejowych w rogach przednich rdzenia kręgowego, jak również w neuronach zwojów kręgowych. Spadek zawartości RNA wykazywały tylko komórki glejowe zwojów rdzenia.

W pierwszych kilku godzinach dowolnego ruchu po zaniechaniu unieruchomienia we wszystkich rodzajach badanych komórek ilość RNA znacznie się zmniejszyła. Obserwowany następnie powrót do normalnego poziomu RNA był szybszy w satelitarnych komórkach glejowych niż w odpowiadających neuronach.

В. А. Брумберг, Л. З. Певзнер

СОДЕРЖАНИЕ РИБОНУКЛЕИНОВОЙ КИСЛОТЫ В СИСТЕМЕ НЕЙРОН-НЕЙРОГЛИЯ СПИННОГО МОЗГА

Резюме

С помощью двухволновой цитоспектрофотометрии в ультрафиолетовой и видимой областях спектра были сопоставлены некоторые схемы избирательной экстракции РНК. Найдено, что наилучшим способом, позволяющим полностью экстрагировать РНК из фиксированных смесью формалин-этанол-уксусная кислота парафиновых срезов состоит в обработке этих срезов 16%-ной $HClO_4$ при 4°C в течение 48 час. Такая экстракция не удаляла никаких определимых количеств клеточных ДНК и белка.

Плавание мышей в воде при 34-36°C в течение 3—4 час. вызывало выраженное повышение количества цитоплазматической РНК (в расчете на одну клетку) в мотонейронах передних рогов спинного мозга. Не наблюдали статистически достоверных изменений содержания РНК в глиальных клетках-сателлитах, прилежащих к этим нейронам. В течение первых часов отдыха по окончании 3-часового плавания происходила постепенная нормализация содержания РНК в мотонейронах параллельно снижению содержания глиальной РНК.

3-недельное ограничение движений у мышей в индивидуальных клетках не приводило к значительным сдвигам количества РНК в нервных и глиальных клетках передних рогов спинного мозга, а также в нейронах спинальных ганглиев. Лишь в глиальных клетках спинальных ганглиев было выявлено некоторое уменьшение содержания РНК. В первые часы свободной двигательной активности после прекращения этой гипокинезии количество РНК снижалось во всех исследованных видах клеток. Последующее восстановление нормального уровня РНК характеризовалось более высокой скоростью в глиальных клетках-сателлитах, чем в соответствующих нейронах.

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N. ROBINSON

ENZYME HISTOCHEMICAL ALTERATIONS FOLLOWING INTRACRANIAL PENETRATION OF A MICRONEEDLE

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The technique of intracranial injection involves some traumatisation of nervous tissue and produces a non-physiological point of entry into the CNS.

The passage of a microneedle into the cerebrum will produce a front of compressed tissue and the distortion and displacement of cells which will result in disturbances to cellular metabolism. The subsequent injection of a small volume of fluid would be expected to result in changes of pH, electrolyte concentration, temperature and breaking of colloids. Changes in the histochemistry of enzymes controlling some metabolic pathways of the rat neocortex and corpus callosum resulting from these disturbances is reported.

MATERIAL AND METHODS

Adult male rats weighing 250—300 g were used. The animals were lightly anaesthetised, hair of the scalp shaved off and the animals placed in a stereotaxic instrument. A sagittal skin incision was made and the exposed skull scraped clean. Penetration of a sterilised fine glass needle, approximately 200 μ diameter, was made to predetermined depths of the cerebrum at Area 4 of Krieg (1946) and 5 μl saline injected over a period of 10 min. using a motor-driven micrometer. After injection the skin was sutured and penicillin administered. All the animals recovered and behaved normally within 30 min.

Animals were killed at 1, 2, 3, 5, 7 and 21 days after operation. Fresh frozen sections, 10 μ thick, were used for the histochemistry of the enzymes: $NADH_2$-diaphorase, succinate (SDH), glucose 6-phosphate (G6-PDH) and α-glycerophosphate (αGPDH) dehydrogenases, acid (AcP) and alkaline (AlkP) phosphatases, 5'-nucleotidase, adenosine triphospha-

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tase (ATPase), thiamine pyrophosphatase (TPPase), monoamine oxidase (MAO), and acetylcholinesterase (AChE). The techniques have been previously described (Robinson 1969).

RESULTS

The passage traversed by the needle was seen as intermittent areas of tissue necrosis; adjacent to the damage was a narrow region of traumatised tissue, approximately 200 μ wide on either side, where most histochemical changes were observed.

Within 24 hours the dehydrogenases exhibited an increase in activity in neocortex and white matter. $NADH_2$-diaphorase was raised in nerve cells and glia; in the corpus callosum the enzyme was raised in swollen glial cells (Fig. 1). Other changes in the dehydrogenases were less marked. A strong AcPase reaction was seen in nerve fibres (Fig. 2) and in some nerve cells. A raised 5'-nucleotidase activity was confined to nerve cells adjacent to traumatised tissue.

At 48 hours post-injection $NADH_2$-diaphorase, AcPase and 5'-nucleotidase had further increased in cortical nerve cells. In the corpus callosum αGPDH, G6-PDH and SDH exhibited some staining which is usually absent: these enzyme changes were accompanied by an increase in glial cell population.

At 3 days the dehydrogenases showed further increases in activity in swollen astrocytes and in round cells adjacent to injury in the neocortex (Fig. 3). A raised ATPase activity, not previously seen, was observed in some nerve cells. Abnormally high AcPase activity was localised in particles which were probably macrophages, lying between disrupted nerve fibres. Some ATPase activity, normally absent, was seen adjacent to injury in the corpus callosum.

At 5 days MAO, previously normal, was localised in round bodies and in fragmented axons within the neocortex. AChE which was also previously normal, showed a loss in nerve cells adjacent to injury. TPPase showed an increased diffuse reaction in neuropil; in nerve cells the characteristic cytoplasmic localisation was lost.

After 1 week 5'-nucleotidase activity was raised in callosal fibres adjacent to the region of injected saline; here degenerating fibres and compound granular corpuscles exhibited minute discrete deposits of reaction product (Fig. 4). AcPase was intense in the same region and also within cortical glial cells at the site of injury (Fig. 5). Some restoration of the low neuropil $NADH_2$-diaphorase activity was apparent and the raised level in nerve cells persisted. AlkPase raised in blood vessels along the path of the needle. ATPase was more intense in isolated nerve cells and

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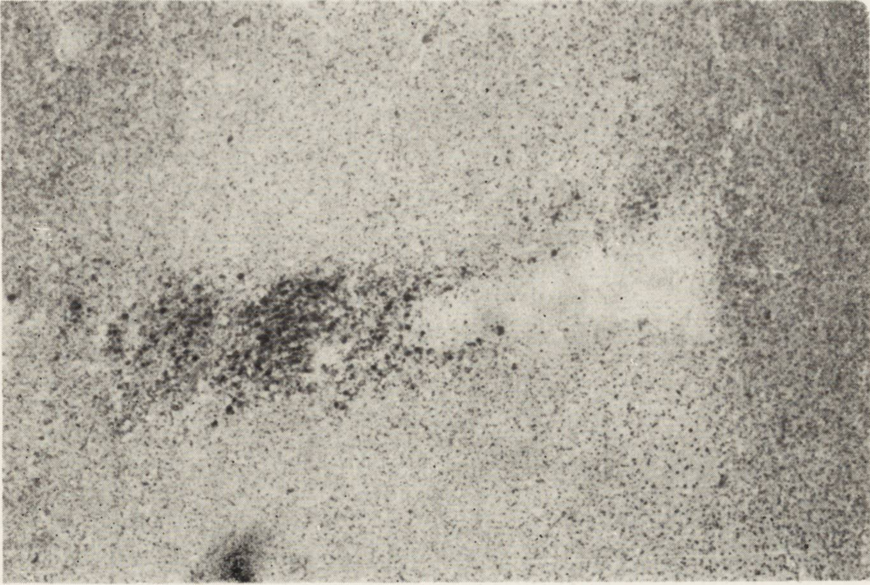

Fig. 1. 24 Hours. Raised $NADH_2$-diaphorase activity in corpus callosum glial cells. Neocortex above exhibited less response. No counterstain. × 100.

Fig. 2. 24 Hours. High acid phosphatase activity in glial cells and nerve fibres of the corpus callosum. No counterstain. × 250.

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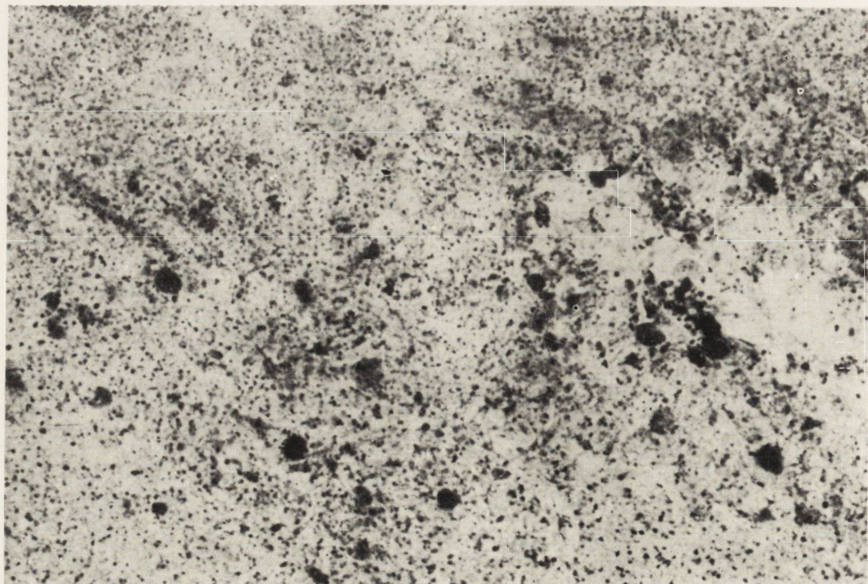
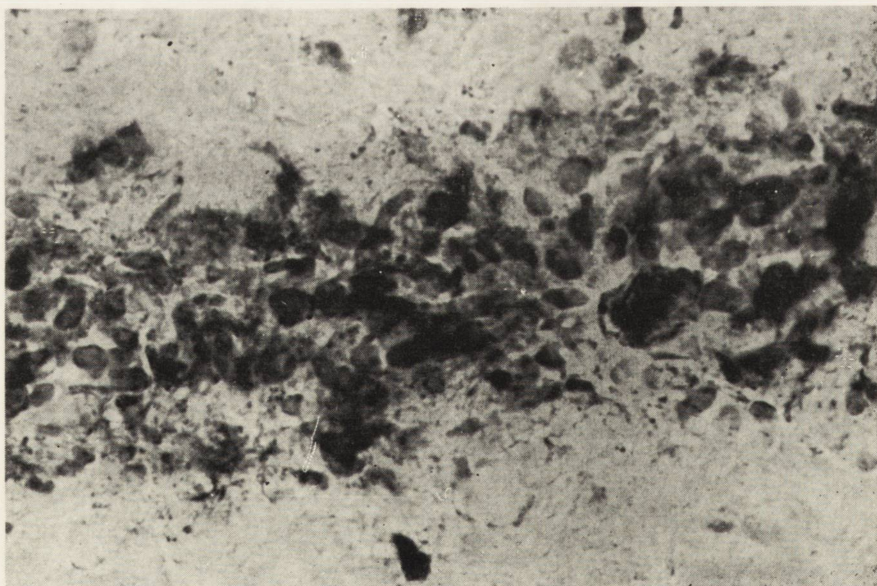


Fig. 3. 3 Days Raised $NADH_2$-diaphorase activity in some glial cells adjacent to injury. No counterstain. × 250.



Fgi. 4. 1. Week. High 5'-nucleotidase activity in glial cells and an inconsistent reaction in nerve cells of the neocortex. No counterstain. × 250.

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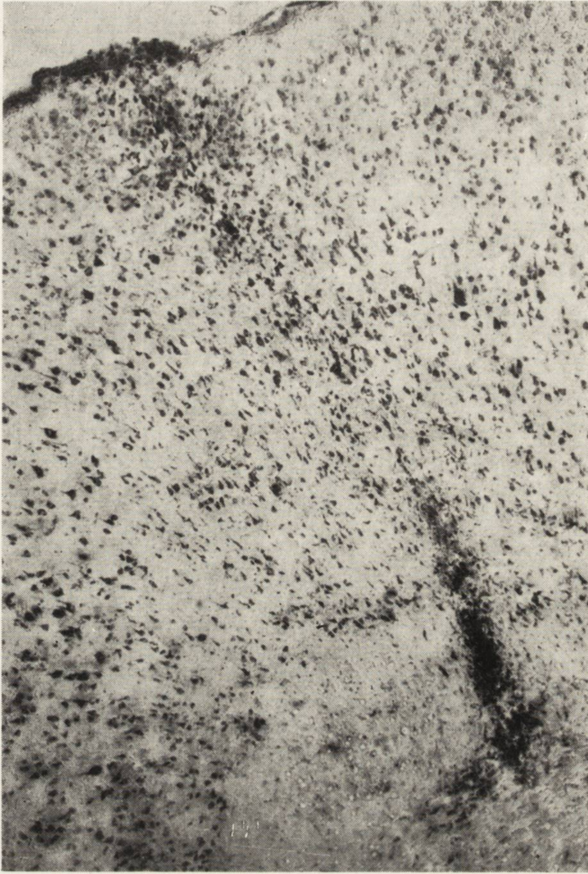

Fig. 5. 1 Week. Raised glial cell acid phosphatase activity along the site of needle penetration. No counterstain. × 40.

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Fig. 6. 3 Weeks. Diminishing adenosine triphosphatase reaction in glial cells and nerve fibres of the corpus callosum. No counterstain. × 400.

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numerous glial cells in the same well-defined region of injury in the neocortex.

At 3 weeks post-injection raised dehydrogenase activities still persisted and both AcPase and ATPase were above normal but poorly localised in the cytoplasm of nerve cells. The abnormally high reactions of AcPase and ATPase seen in cortical glial cells and damaged axons were still apparent; varying intensities of both enzymes were also observed in areas showing scarring at this time. The abnormal reactions of several enzymes localised in nerve fibres of the corpus callosum were still demonstrated (Fig. 6) and glial cells with enlarged displaced nuclei continued to exhibit a poorly localised but strong αGPDH reaction. Other enzymes including AChE, TPPase and MAO showed no further alterations since the small changes reported at 5 days.

DISCUSSION

The damage to tissue seen after penetration of the microneedle at Area 4 of Krieg was confined to a discrete narrow band of tissue necrosis, with injury diminishing rapidly away from the site of injection. Where fluid was injected into the tissue the area of necrosis was greater but usually within 400μ diameter, probably due to the slow rate of release and diffusion of the fluid. The changes in enzyme activity were generally confined to the periphery of this area indicating minimal disturbance to the metabolism of nerve and glial cells beyond the zone of tissue injury, particularly in the neocortex.

Changes in enzyme activity over a period of 3 weeks post-injection were most rapid within the first 24 hours. The increases in activities of the dehydrogenases indicated that increased demands were being made on oxidative processes in carbohydrate metabolism. The glial cell dehydrogenases were particularly responsive to disturbance but variations in the enzyme changes indicated that the pathways of glucose metabolism were involved to different extents. However, this and earlier histochemical and biochemical studies indicate that hyperactive astrocytes in both grey and white matter have a high metabolic turnover soon after injury.

The loss of enzyme activity within the neuropil of neocortex, where normally a moderate reaction was demonstrated, was probably a reflection of the sensitivity or some enzymes, for example, $NADH_2$-diaphorase and α-glycerophosphate dehydrogenase to disturbance. A similar response has been reported in human pathological conditions (Friede and De Jong, 1964) and after tissue contact with a cold probe (Rubinstein, Klatzo and Miquel, 1962).

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Phosphatases showed distinct variations in intensity with time. The early rapid and continuous response to injury of AcPase and also 5'-nucleotidase were in contrast to the negligible change of TPPase throughout the 21 days. TPPase is known to be plentiful in cerebral tissue where it is more resistant to depletion in a thiamine-deficient diet than other enzymes. The moderate increase in ATPase after 5 days post-injection did not parallel the response of the oxidative enzymes also concerned with energy metabolism and localised within mitochondria.

Two enzymes, MAO and AChE, distributed in the neocortex did not show any significant changes suggestive of increased metabolic requirements following tissue injury.

Enzyme changes in the corpus callosum, especially within the first few days, were more conspicuous and in some cases contrasted strongly with changes in the neocortex. Oligodendrocytes and swollen microglia in an increased cell population exhibited intense AcPase activity in the corpus callosum not so apparent in the neocortex; the dehydrogenases generally showed a similar pattern. Where nerve fibres in the corpus callosum had been severed the intense AcPase reaction adjacent to necrosis gradually diminished away from the site giving the impression of „damming up" of the enzyme as though migration of AcPase along fibres had been interrupted but this suggestion requires substantiation by other experimental evidence.

The enzyme reactions at 3 weeks post-injection indicated that the high abnormal levels of dehydrogenases and ATPase, particularly in glial cells, were at their zenith or even reverting to more normal levels, but the reactions for AcPase and 5'-nucleotidase persisted.

N. Robinson

ZMIANY HISTOENZYMATYCZNE PO ŚRÓDCZASZKOWYM WKŁUCIU MIKROIGŁY

Streszczenie

Przeprowadzono badanie aktywności enzymów toru metabolicznego glukozy i fosforanów, utleniania amin i acetylocholinesterazy w korze mózgu i w spoidle wielkim w mózgu dorosłego szczura po śródczaszkowym wkłuciu mikroigły.

W okresie 24 godzin komórki glejowe wykazywały zwiększoną aktywność dehydrogenaz i fosfataz. Włókna zmielinizowane charakteryzowała bardzo wysoka aktywność kwaśnej fosfatazy i 5-nukleotydazy, natomiast niektóre enzymy (acetylcholinesteraza, monoaminooksydaza i pyrofosfataza tiaminowa) nie wykazywały żadnych zmian.

Po 3 dniach zwiększona populacja komórek glejowych wykazywała dalszy wzrost w aktywności tych enzymów, zwiększona była również aktywność ATP. Mono-

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aminooksydaza, acetylocholinesteraza i pyrofosfataza tiaminowa wykazywały zmiany po 5 dniach, podczas gdy aktywność fosfatazy zasadowej wzrastała tydzień po injekcji.

Po 3 tygodniach utrzymywała się nasilona aktywność większości enzymów przy obniżeniu aktywności ATP.

Н. Робинзон

ГИСТОЭНЗИМАТИЧЕСКИЕ ИЗМЕНЕНИЯ ПОСЛЕ ВНУТРИЧЕРЕПНОГО ВВЕДЕНИЯ МИКРОИГЛЫ

Резюме

Иследовались изменения в гистохимии энзимов метаболического пути глюкозы и фосфатов, окисления аминов, а также ацетилхолинэстеразы в neocortex и corpus callosum мозга взрослой крысы после внутричерепного введения микроиглы. В течение 24 часов глиальные клетки обнаруживали увеличенную активность дегидрогеназ и фосфатаз. Миелиновые волокна характеризовала очень высокая активность кислой фосфатазы и 5'-нуклеотидазы, зато некоторые энзимы (ацетилхолинэстераза, оксидаза моноаминов и тиаминпирофосфатаза) не обнаруживали никаких изменений. После 3 дней увеличенная популяция глиальных клеток обнаруживала дальнейший рост активности этих энзимов, увеличенной также была активность АТФ. Оксидаза моноаминов, ацетилхолинэстераза и тиаминпирофосфатаза обнаруживали изменения спустя 5 дней, в то время когда щелочная фосфатаза увеличивалась неделю спустя от момента инъекции. После 3 недель удерживался увеличенный уровень большинства энзимов при снижении активности АТФ.

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NEUROPAT. POL. 1972, X, 2

A. PETRESCU

HISTOENZYMOLOGICAL ASPECTS OF REACTIVE ASTROGLIA AROUND CEREBRAL NECROTIC FOCI

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So far, the investigations on normal and pathologic enzymatic activity in neuroglia are not very numerous and the findings reported by various authors are still a matter of controversy. This leaves a widely open field for further research.

The present work is an attempt to study the enzymatic reactivity of astroglia. Our studies dealt with a wide spectrum of enzymes representing most of the metabolic cycles.

MATERIAL AND METHODS

A group of 8 rats was studied in which a necrotic foci had been produced in the brain by introduction of a heated dissection needle through the skull cap. The animals were killed at the 3 day and 1st, 2th, 3d, 4th and 6th weeks following experimental injury. The brain tissue fragments were frozen by immersion in liquid nitrogen; 6 — 8 μ cryostate coronal section through both cerebral hemispheres were cut and histoenzymological techniques were applied for the detection of the activity of the chosen enzymes representing the main metabolic cycles (Table 1), according to the methods used by Prof. R. Wegmann in the Paris Institute of Medical Histochemistry.

RESULTS

The brain lesion involved cerebral cortex, corpus callosum and sometimes putamen and caudate nucleus. From the morphological point of view the lesion consisted of 3 zones: the central necrotic zone, the peripheral zone (around the necrotic foci) comprising agglomerations of

*) The most part of the present work has been carried out during a WHO fellow scholarship (1970).

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Table 1. Histoenzymological and histochemical techniques used (with abbrevations)

UDPG-GT	— UDPglucose-glucosyltransferase
Dimedon + PAS	— glycogen
PHYLA I + II	— glycogen physphorylase I + II
G6P-Ase	— glucose-6-phosphatase
F-1, 6-P-Ase	— fructose-1, 6-diphosphatase
ALDA	— fructose-diphosphate aldolase
3 PGA-DH	— 3 glycero-phosphate-aldehyde dehydrogenase
LDH	— lactate dehydrogenase
ADH	— alcohol dehydrogenase
G6P-DH	— glucose-6-phosphate dehydrogenase
6PG-DH	— 6-phosphogluconate dehydrogenase
$NADPH_2$-TR	— reduced NADP tetrazolium reductase
LPDH	— lipoamid dehydrogenase
CA	— aconitate hydratase
ICDH	— isocitrate dehydrogenase
SDH	— succinate dehydrogenase
C-O	— cytochrome oxidase
MDH	— malate dehydrogenase
$NADH_2$-TR	— reduced-NAD-tetrazolium reductase
ATPase	— adenosino-triphosphatase
EST-Ase	— nonspecific esterase
N-EST-A	— naphtol acetate esterase
I-EST-A	— indoxyl acetate esterase
α-GP-Ase I and II	— alkaline and acid phosphatase
β-OHBDH	— β-hydroxybutyric dehydrogenase
α-GPDH	— α-glycerol phosphate dehydrogenase
OR	— oil red
NS	— Sudan black
DHO-DH	— dihydroorotate dehydrogenase
GDH	— glutamate dehydrogenase
ALP-A	— alanine amine peptidase
LEP-A	— leucine amine peptidase
MAO	— monoamine oxidase
GABA-TR	— aminobutyrate aminotransferase
GOT	— aspartate aminotransferase
RNA, DNA	— the Brachet method
AMPase	— 5'-nucleotidase
BT	— blue toluidine
PAS	— periodic acid Schiff reaction

macrophages and the outer zone, consisting of cerebral parenchyma in the vicinity of the lesion.

The necrotic zone did not reveal any enzymatic activity, except that of F-1,6-PAse, which gave a diffuse staining at the superficial border line of the zone. In the cicatrix mesenchymal network of this area the activity of some dehydrogenases such as ADH, G6P-DH, 6PG-DH, MDH in 2-week-old lesions and SDH in addition to the above mentioned in the

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3-week-old ones was observed. PAS-dimedont reaction for glycogen revealed the presence of red granules in the above mentioned network.

The peripheral zone around the necrotic focus displayed as early as at the 3rd day after production of lesion, the presence of numerous cells filled with granules of both hydrophilic and hypdrophobic (sudanophilic) lipids which according to our personal classification (Petrescu 1967, 1970) were identified as type II myeline-cholesterol-esters lipomacrophages. These cells revealed intense activities of various enzymes, identical in their nature and intensity with those described in a previous paper (Wegmann et al. 1970).

The modifications of the enzymatic activity in reactive astroglia were observed both in gray (cerebral cortex) and white matter (corpus callosum). Estimation of the enzyme activities of the reactive astroglia was always made in comparison with the normal picture in the corresponding areas of the opposite intact hemisphere.

Although the 3-day-old lesion involved the cerebral cortex only, the corpus callosum being spared, the astroglia in the white matter exhibited already evident enzymatic changes on almost the whole length of the corpus callosum. They were expressed by increased activity of the following dehydrogenases LDH, MDH, G6PDH, 6PG-DH, GDH, ICDH, CA, $NADH_2$-TR, $NADPH_2$-TR (Figs. 1, 2). These above changes persisted throughout the next stages studied. As regards the SDH, only the astroglia neighbouring directly the lesion exhibited some activity of this enzyme, which was completely absent in the opposite intact hemisphere. The activity of ATP-ase was not detected.

In the cerebral cortex no obvious enzymatic hyperactivity was noted in the astroglia around the 3-day-old lesion. However, due to diffuse granular enzymatic reaction in the neuropil the estimation of astroglial enzyme activity within cerebral cortex is difficult to carry out, irrespective of whether it is normal or slightly increased.

In the 7-day-old lesion and in all other older lesions an enzymatic hyperactivity of the reactive astroglia could be identified in the cerebral cortex concerning the same enzymes which were seen in the 3-day-old lesion and additionaly 2 other enzymes: LPDH and GABA-TR (Fig. 3).

In all the lesions, the enhanced enzymatic activities in the cerebral cortex were present only on a small distance from the necrotic focus. The number of astroglial cells in the vicinity of the cortical foci could be only roughly estimated, owing to the presence of other pathological elements and to the richness of the enzymatic activities in the neuropil.

As to the corpus callosum, the area of astroglial hyperactivity extented upon almost the whole length of the structure in all the lesions studied. In the white matter the astroglia, either normal or that with increased

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enzymatic activity, could be very readily detected, as no neuropil ground was present and, the cells themselves and their processes appeared very clearly (Figs. 4, 5).

The activity of some enzymes such as G6P-DH, 6PG-DH and ATP-ase (pH 7.4 and 8.5) seemed increased in the walls of the capillaries around the necrotic foci.

As to the enzymatic activity of the cortical neurons in the immediate vicinity of the necrotic foci, an increased activity concerned only $NADH_2$-TR and acid phosphatase.

DISCUSSION

Our studies reveal that some enzymatic activities present in normal astroglia become increased as soon as the latter becomes reactive. These enzymes are of the dehydrogenase group and are involved in a number of metabolic pathways a.o. in the glycolytic, in tricarboxylic and pentose cycle.

We would like to point out the particular character of SDH-activity which was not found in normal but was present in reactive astroglia. As for the presence or absence of SDH activity in astroglia, the results reported by the different investigators were contradictory. Wolfgram and Rose (1959), who attributed this contradiction to the differences in the techniques used, Romanul and Cohen (1959), Potanos et al. (1959), and Schiffer and Vesco (1961, 1962) have found a normal activity of this enzyme in normal and reactive astroglia both in human and in some animal species.

Rubinstein et al. (1962) studying the necrotic foci produced in the cerebral cortex of cat with so called cold lesion technique reported that there was no dehydrogenase activity in the necrotic focus, it was slight in the normal glia in the white matter and increased in the reactive astroglia. The authors found the following time sequences (from 16 hrs to 4 days), in the occurence of increased activities: GDH, $NADH_2$-TR, ICDH linked $NADH_2$, P-αGPDH, $NADPH_2$-TR, ICDH linked $NADPH_2$, SDH. The authors relate topographically the enzymatic hyperactivity to the presence of oedema in the white matter.

Mossakowski (1970), among others, considers that the normal astroglia has weak oxydative enzymatic activities which become increased under pathologic conditions (reactive hypertrophic astroglia).

It should be underlined that owing to the smallness of cytoplasm of normal astroglia, correct visual estimation of formazan quantity can hardly be performed; consequently we cannot judge with certainty whether the normal astrologia has a poor or a marked activity. Hydén

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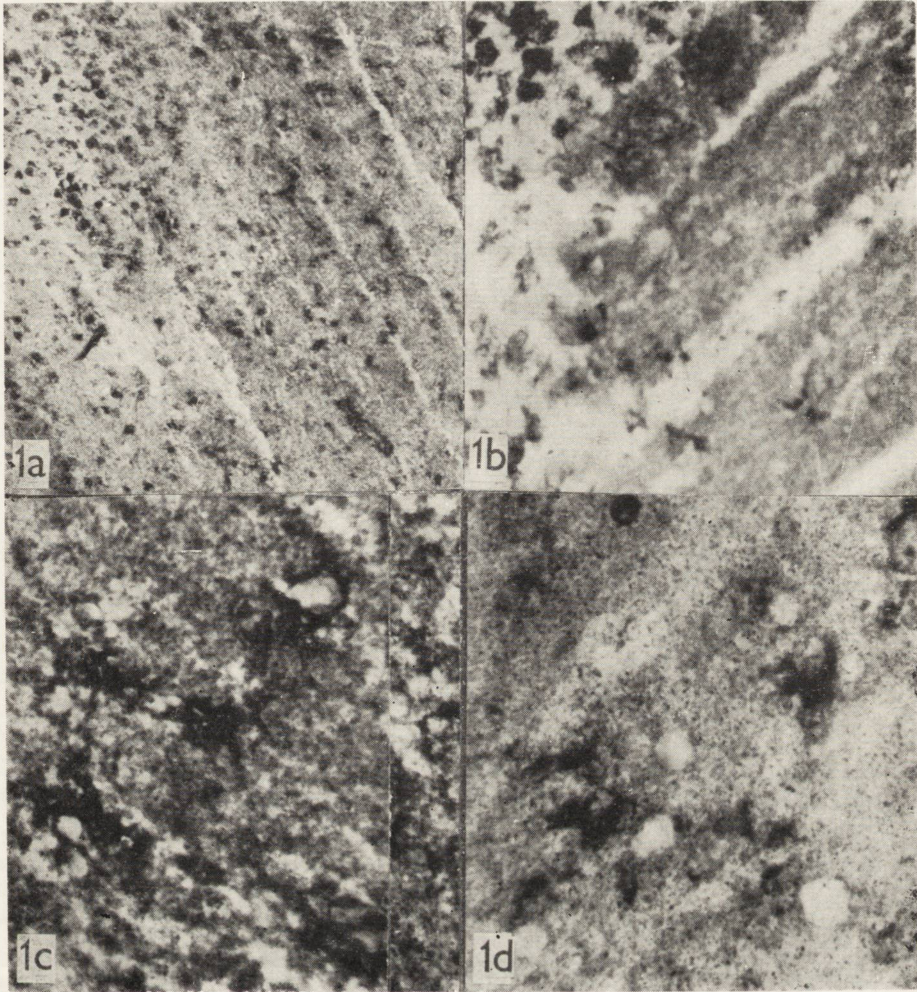

Fig. 1. Reactive astrocytes within cerebral cortex around the necrotic focus (3 day old lesion).

a — Glucose-6-phosphate dehydrogenase activity. The beginning of enzymatic hyyperactivity in astrocytes. Agglomerations of macrophages with intense enzymatic activity.

b — Acid phosphatase activity. Slight enzymatic activity in scarse astrocytes. Some round-shaped marcrophages densly filled with final product of enzymatic reaction are seen.

c — $NADPH_2$-tetrazolium-reductase activity. Strong enzymatic activity within perikarya and processes of astrocytes.

d — Isocitric dehydrogenase activity in astrocytes in the vicinity of necrotic focus.

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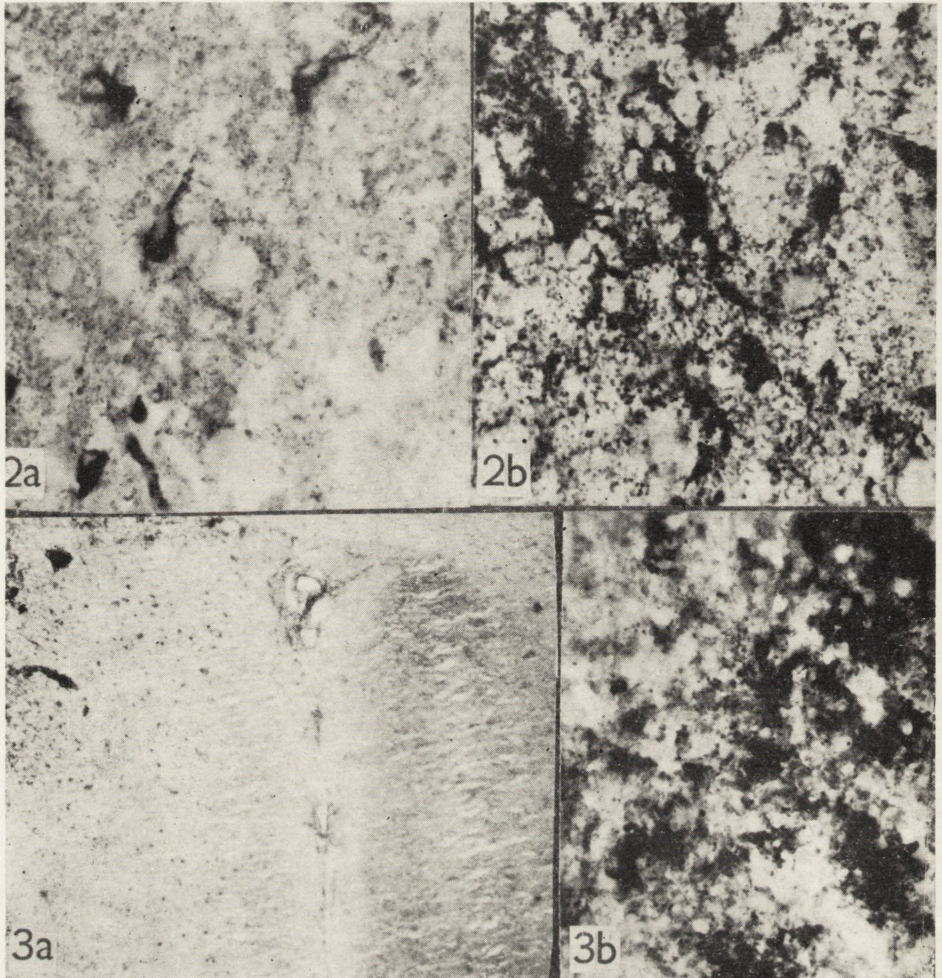

Fig. 2. Reactive astrocytes within corpus callosum underlying cerebral cortex with a necrotic focu (3 day-old lesion), a — Gluconate dehydrogenase activity. b — Lactate dehydrogenase activity.

Fig. 3. Reactive astrocytes within cerebral cortex, around the 1 week old necrotic focus.

a — Aconitate hydratase activity. Intense enzymatic activity around necrotic focus. No obvious enzymatic activity is seen in the opposite intact hemisphere. b — Acid phosphatase activity in the hypertrophied astrocytes.

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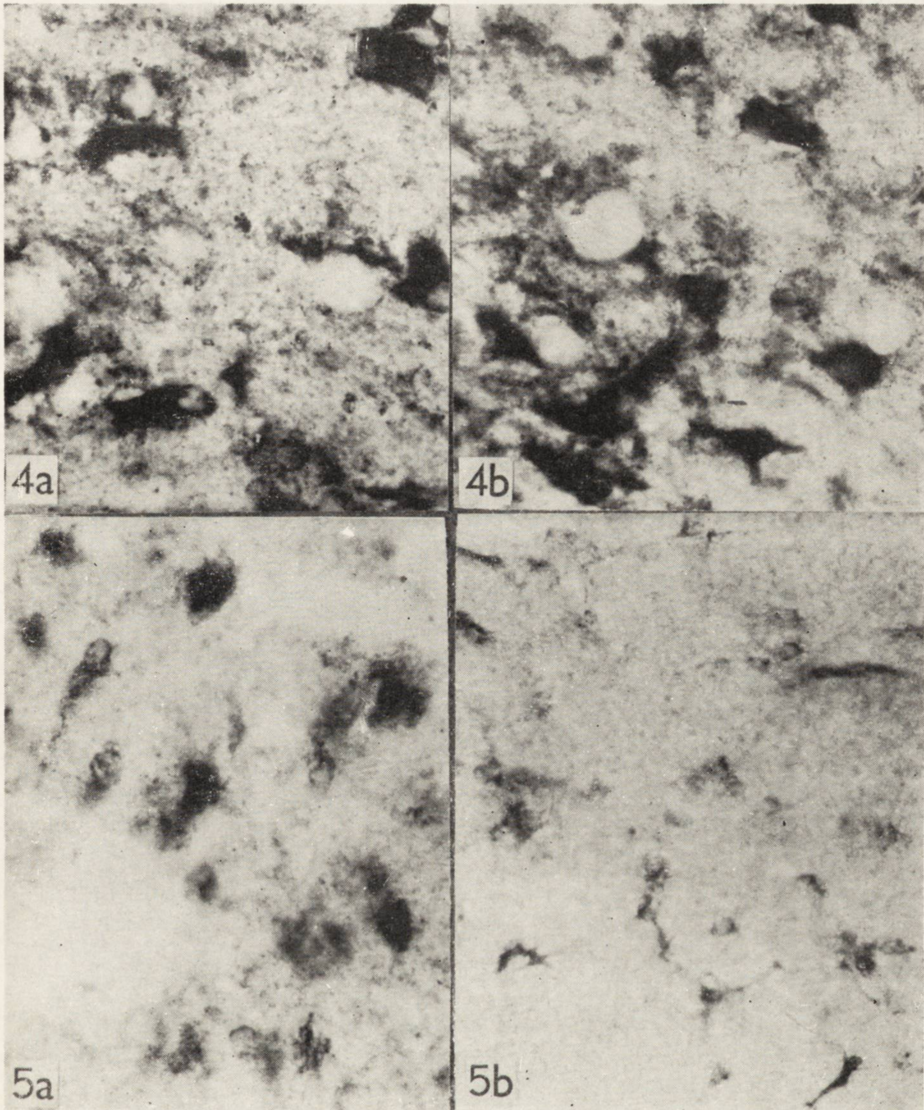

Fig. 4. Reactive astrocytes within corpus callosum, underlying cerebral cortex with 1 week old necrotic focus. a — Aconitate hydratase activity. b — Isocitric dehydrogenase activity.

Fig. 5. $NADH_2$-tetrazolium reductase activity in astrocytes of corpus callosum in the brain with 6 week-old necrotic focus. a — Reactive astrocytes close to the focus. b — Normal astrocytes in corresponding area of the opposite intact hemisphere.

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(1960) studying isolated cells by means of refined microchemical techniques has found that the succinic dehydrogenase and cytochrome-oxidase activities are more intense in the satellite glia than in the nerve cell.

The increase of enzymatic activities in the reactive astroglia is evident as early as on the 3rd day after lesion (first stage studied in this work) when the astroglia is not yet hypetrophied. However, in older lesions when astroglia becomes hypertrophic, estimation of these increased enzymatic activities in comparison with the activities in normal astroglia is difficult to perform only visually (on histochemical preparations), as the two cells (normal and hypertrophic astroglia) are different in size and shape. A correct answer to the question whether the hypertrophic astroglia is much more enzymatically active than the normal one can be given only by determinations performed per unit of cytoplasm surface or volume.

Concerning the minimum time interval after lesion during which an enzymatic hyperactivity in reactive astroglia might occur, some authors showed: 3 hours in experimental hypoxia for G6P-DH (Domańska 1970; cited by Mossakowski 1970), 6 hours after injury for the enzymes of the glycolytic cycle, somewhat longer for those of the tricarboxylic cycle (Friede 1966); 12 hours for $NADH_2$-TR and dehydrogenases linked to coenzyme I (Rubinstein et al. 1962).

Some authors found that the activities of the enzymes other than dehydrogenases were also increased in the reactive astroglia. This concerned acid phosphatase as reported by Koenig and Barron (1963), Schiffer et al. (1967) and Wender and Kozik (1969) in multiple sclerosis. The latter authors also found increased ATP-ase and TPP-ase in this disease.

Another finding of the present work is that the reactivity of astroglia was more spread in the white matter than in the gray matter as it included almost the whole length of corpus callosum already in the 3-day-old lesion (which had not penetrated this structure) whereas is was strictly confined around the focus in the gray matter even in the 6-week--old lesion.

A. Petrescu

HISTOENZYMATYCZNE WŁAŚCIWOŚCI ODCZYNOWEGO ASTROGLEJU, OTACZAJĄCEGO OGNISKO MARTWICY MÓZGU

Streszczenie

Ognisko martwicy tkanki nerwowej wywoływano poprzez wprowadzenie rozgrzanej igły preparacyjnej do półkuli mózgu szczura.

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Już w 3 dni od uszkodzenia tkanki (najwcześniejszy okres badań) stwierdzono wyraźne zmiany histochemiczne w astrocytach odczynowych. Polegały one na znacznym wzroście aktywności enzymów wykrywanych również w gleju prawidłowym. Należały do nich następujące enzymy: LDH, G-6-PDH, 6PGDH, GDH, GABA-TR, ICDH, CAH_2, LPDH, $NADH_2$-TR, $NADPH_2$-TR. Ponadto stwierdzono pojawienie się nieprawidłowej aktywności dehydrogenazy bursztynianowej i kwaśnej fosfatazy.

W istocie szarej (kora mózgu) odczynowe astrocyty obserwowano jedynie w bezpośrednim otoczeniu ogniska uszkodzenia tkanki, podczas gdy w istocie białej (spoidło wielkie) występowały one również w rozległym obszarze tkanki, oddalonym od ogniska (na całej długości spoidła wielkiego). Różnice rozległości odczynu w istocie szarej i białej utrzymywały się przez cały okres obserwacji (od 3 do 40 dnia po uszkodzeniu tkanki).

W astrocytach odczynowych stwierdzono wyłącznie wzmożoną aktywność tych enzymów, które występują w astrogleju prawidłowym.

А. Петреску

ГИСТОЭНЗИМАТИЧЕСКИЕ СВОЙСТВА РЕАКТИВНОЙ АСТРОГЛИИ ОКРУЖАЮЩЕЙ ОЧАГ НЕКРОЗА МОЗГА

Резюме

Введением нагретой препарационной иглы вызывался кровоточащий очаг некроза в мозгу крысы. Отчетливая активность иследованных дегидрогеназ (LDH, G-6-PDH, 6-PGDH, GABA-TR, ICDH, CAD, LPDH, MDH, $NADH_2$-TR, $NADPH_2$--TR) в нормальной астроглии увеличивается значительно в клетках реактивной астроглии уже на 3 день после образования очага повреждения (первый исследованный период). В реактивной астроглии обнаруживается также патологическая активность сукцинатдегидрогеназы и кислой фосфатазы.

В белом веществе реактивная астроглия (спайка мозга) обнаруживается не только в близком соседстве очага, как это имеет место в сером веществе (кора мозга), а почти вдоль целой длины большой спайки (в повреждениях длящихся от 3—40 дней).

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NEUROPAT. POL., 1972, X, 2

D. KRAJČI

THE ONTOGENETIC DEVELOPMENT OF SATELLITE CELLS IN SPINAL GANGLIA

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Ontogenetic development of spinal ganglia was in the first half of this century studied mostly under the light microscope. Summarizing reports were given by Streeter (1912) and Scharf (1958). Electron microscopy confirmed most of the earlier light microscopical findings supplying ultrastructural details.

The ultrastructure of spinal ganglia in rabbit embryos during their ontogenetic development was described by Tennyson (1965); the investigation was, however, centred upon developing neuroblasts. Prenatal development of satellite cells in the spinal ganglia of chicken embryos was studied by Pannese (1969) who divided ontogenetic development of satellite cells into four stages. In the course of this process there are changes in size and shape of the satellite cells, in their topographic relation to nerve elements and in the ultrastructure of the cytoplasm of these cells. In most animals the development of the satellite cells is not finished before birth and it goes on during their postnatal development.

We have been studying various phases of the ontogenetic development of spinal ganglia for some time (Malinský 1967, Krajčí, Malinský 1968, Krajčí 1971). Present work deals with the early period of prenatal development of satellite cells in human embryos (4 to 12 weeks old) and describes the satellite cells in final stages of prenatal period and during the postnatal development in the spinal ganglia of rat (14 and 20 days old embryos, newborns 5, 10, 28, 42 days old, adults), and of cat (newborns 5, 10, 21, 30, 90 days, adults).

Tissues were fixed either by immersion (human embryos) or by perfusion of the vascular bed. Fixation in 3 p.c. glutaraldehyde was follow-

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ed by second fixation in 1 p.c. osmic acid, dehydration and embedding in Vestopal W, Durcupan ACM or Epon 812. Semithin sections (0.7 μ) for light microscopy were stained with toluidine blue, ultrathin sections were contrasted with uranium acetate and with lead citrate after Reynolds. Electronograms were supplied by electron microscopes Tesla BS 242 and BS 613.

It the course of the development of spinal ganglia the differentiation of all cells does not take place at the same time so that very often in one ganglion we can find cells in different phases of their ontogenetic development. In the early embryonic period this asynchronous development of neuroblasts and glioblasts is not so obvious as later when among differentiated cells we can find neuroblasts or glioblasts of a very low degree of maturation.

The lowest degree of differentiation in cells of the spinal ganglia was found in 4 weeks old human embryos when ganglion and glial elements could not be reliably distinguished (Fig. 1). The cells are in a mutual close contact without any visible intercellular clefts (epitheloidal arrangement). They do not differ either in size or shape, the nucleoplasmic proportion is typical for young embryonic cells. The nuclei take the largest portion of the cellular volumes, they are round with fine chromatin and pronounced nucleoli. The ultrastructure of the cytoplasm completely corresponds to this low degree of cell differentiation. Out of the ergastoplasmic structures the granular components (ribosomes) are the most frequent, while sacs of endoplasmic reticulum are small and solitary. There are mitochondria, Golgi apparatus, and solitary dark bodies of lysosomal ultrastructure.

Only in some cells we could observe excentric nuclei and increase of cytoplasm on the opposite side of the cell. In this part of the cytoplasm there are more mitochondria and other organelles. These cells can with great certainty be denoted as the youngest neuroblasts, while glioblasts cannot still be identified.

In the next phase of the ontogenetic development the satellite cells can better be distinguished from neuroblasts (Fig. 2). The nuclei of glioblasts remain small, oval, with higher density of chromatin than in neuroblasts. The cells change their shape from the original round elements into a star or spindle shaped cells adhering to the surface of the neuroblasts and sending forth very thin processes penetrating among neuroblasts, which were before in close contact (Figs. 3, 4). These processes gradually become larger and separate neighbouring neurons by a stripe of satellite cell cytoplasm still common to both neurons. There are no intercellular clefts yet (Figs. 4, 5). Glioblasts get their typical satellite

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position, but characteristic for this transitional stage is the relation of one primitive satellite cell to several neuroblasts at the same time.

Ultrastructure of the cytoplasm of glioblasts is in this developmental phase clearly different from the cytoplasm of neuroblasts. While in neuroblasts there is an intensive increase of polyribosomes and there are more sacs of endoplasmic reticulum, the cytoplasm of glioblasts remains relatively light with solitary ribosomes and rare small sacs of endoplasmic reticulum (Figs. 2, 4, 5). One can also find here other organelles described in the preceeding stage (Golgi apparatus, mitochondria, lysosomes) and more frequent also in more differentiated cells. Centrioles, the less usual organelles in mature satellite cells, also occur in glioblasts of this developmental phase. Increasing number of glial cells is a further sign of the spinal ganglion immaturity. In mature spinal ganglia the satellite cells far outnumber the ganglion cells, because one neuron is always envoloped by many satellite cells.

Satellite cells in the second phase of ontogenetic development were found in spinal ganglia of human embryos from 6 to 12 weeks with increasing frequency. This stage was very frequent also in embryonic and newborn spinal ganglia of cat and rat. During their postnatal period the described immature satellite cells occurred with decreasing frequency up to 30 days after birth, when they were very exceptional.

In the early postnatal stage of the development of spinal ganglia the neurons are accumulated into clumps. It is the period of the long postnatal persistence of the original close epitheloidal relation of neurons without the interposed satellite cells and without intercellular clefts. For this reason we can in these places find even in postnatal period poorly differentiated satellite cells growing between the neurons, while on the periphery of these neuronal groups or on the free lying neurons the satellite cells are already mature.

The last phase of ontogenetic development of satellite cells is the time when the definit relation of these cells to neurons is formed. Each satellite cell is in contact with only one neuron and it is surrounded by the intercellular space varying in width (Fig. 6). So we see that the intercellular spaces are present between all neurons, they are however often visible only under the electron microscope. The corresponding process to these changes on ultrastructural level is the formation of the basal lamina on the membrane bordering the intercellular space. Collagen fibrils, present even in the narrowest intercellular clefts as well, get very close to this membrane.

Towards the end of ontogenetic development of the satellite cells we can also see the forming definitive very complicated relation between

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the neighbouring membranes of the satellite cell and the ganglion cell. The opposition of both the membranes of immature cells is usually straight, slight infoldings are exceptional (Fig. 5). In this third phase of the development of satellite cells both the mebranes are considerably undulated and so their surface increases many times between the satellite and the ganglion cell, which is so typical for mature elements (Fig. 8). There are cytoplasmic processes often sunk deep into the satellite cells budding out of the surface of the ganglion cell, or the other way round, there are processes of satellite cells found in the subsurface zone of the neuronal cytoplasm. In the cytoplasm of the maturing satellite cells, near their adneuronal membrane, there are small vesicles often arranged in rows (Figs. 6, 7). When fusing, these vesicles can give rise to new membranes of satellite cells and so take part in the dynamic changes on the surface between the satellite and the ganglion cells. The content of these vesicles can enter the neurons in the process of pinocytosis.

The ultrastructure of other cytoplasmic components does not show any distinct changes in this period. Mature cells have fine cytoplasmic filaments less distinct in younger cells. In the perinuclear portion of the cytoplasm there are all the organelles described in younger cells with the exception of the centriole, very rare in maturing or mature satellite cells.

In immature spinal ganglia the glial envelopes are thin, but in the last phase of the development of glial cells the perineuronal envelope thickens in some places and is formed by more layers of the satellite cells (Figs. 6, 7, 8). Membranes of the neighbouring satellite cells are here folded in a very complicated way which makes the connections of individual glial elements firm.

The described third phase of the development of satellite cells is the most difficult to limit in time. In our human embryonic material there were no satellite cells in this developmental stage. In the spinal ganglia of cat and rat there are the first totally differentiated satellite cells towards the end of prenatal period most frequently surrounding solitary neurons. In newborns the differentiation of satellite cells is in the third phase of the ontogenetic development when the cells acquire the mature morphology and ultrastructure.

The ontogenetic development of satellite cells can thus be summed up as follows:

In the first phase there is no morphological and ultrastructural difference between glioblasts and neuroblasts.

In the next developmental phase glioblasts get the satellite relation to neuroblasts, change into star shaped or spindle shaped elements with

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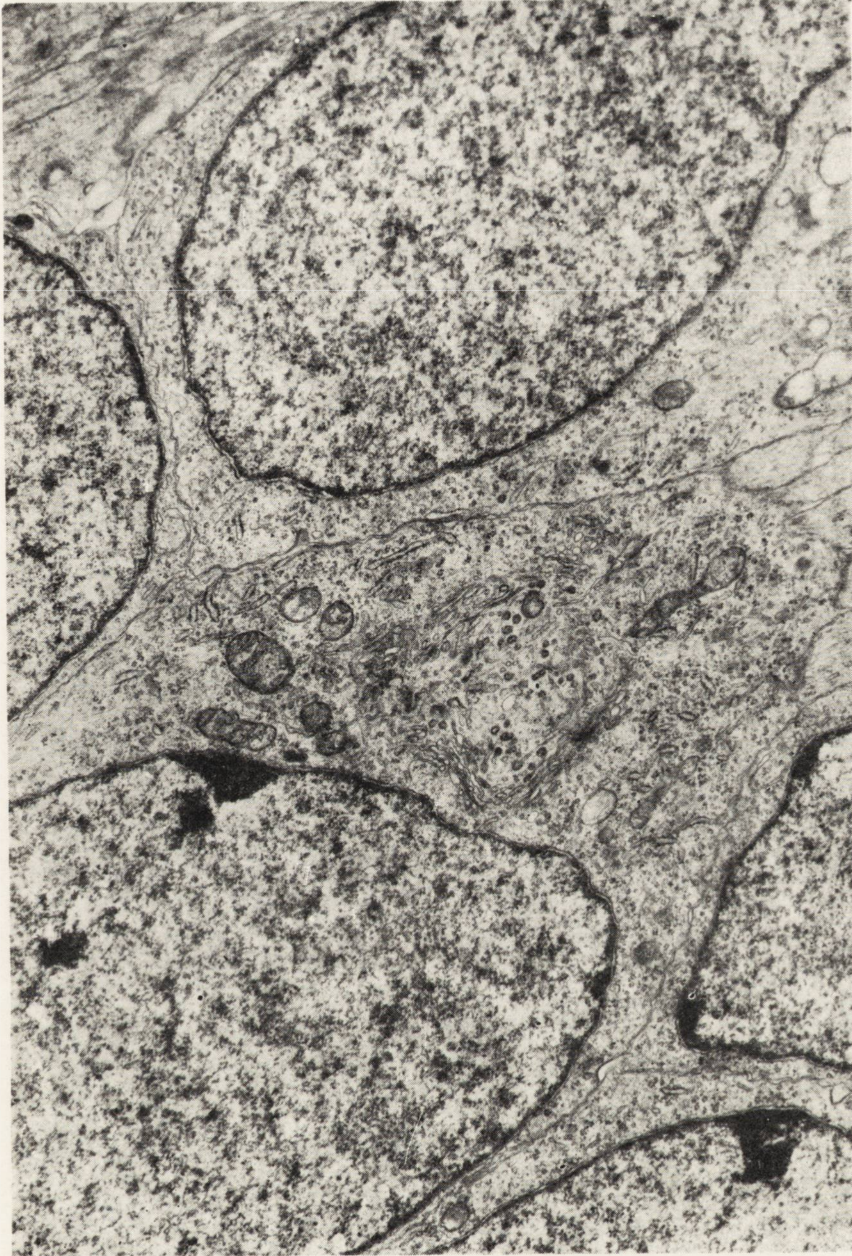

Fig. 1. Undifferentiated cells in mutual close contact without any visible intercellular clefts. It is not possible to distinguish the neuronal and glial elements, as to their size, shape and ultrastructure of their cytoplasm. Human embryo 4 weeks old. × 14 100.

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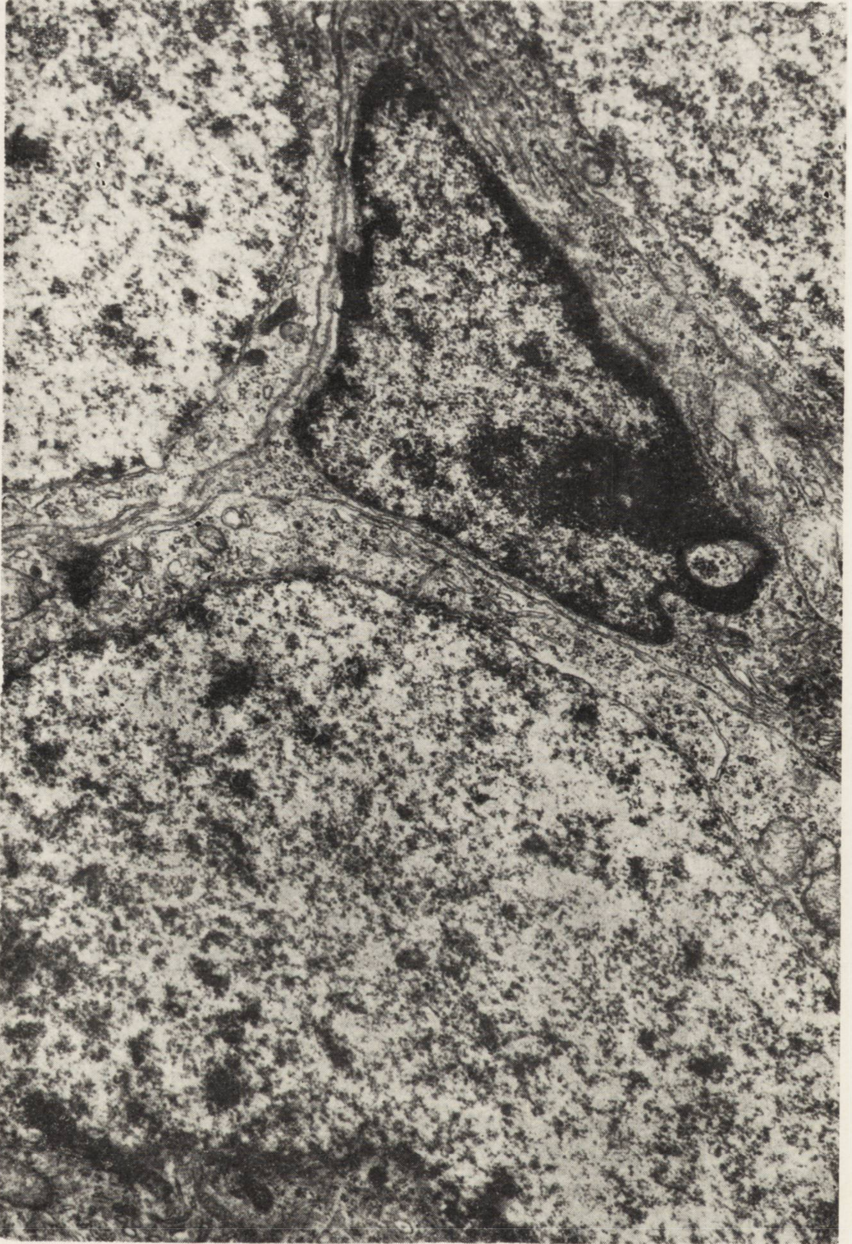

Fig. 2. Primitive satellite cell clearly differing from neighbouring neuroblasts. Its nucleus has an irregular shape and chromatin is condensed into caryosomes lying near the nuclear membrane. Cytoplasm of the satellite cell is in close contact with three neuroblasts. Human embryo 8 weeks old. × 16 450.

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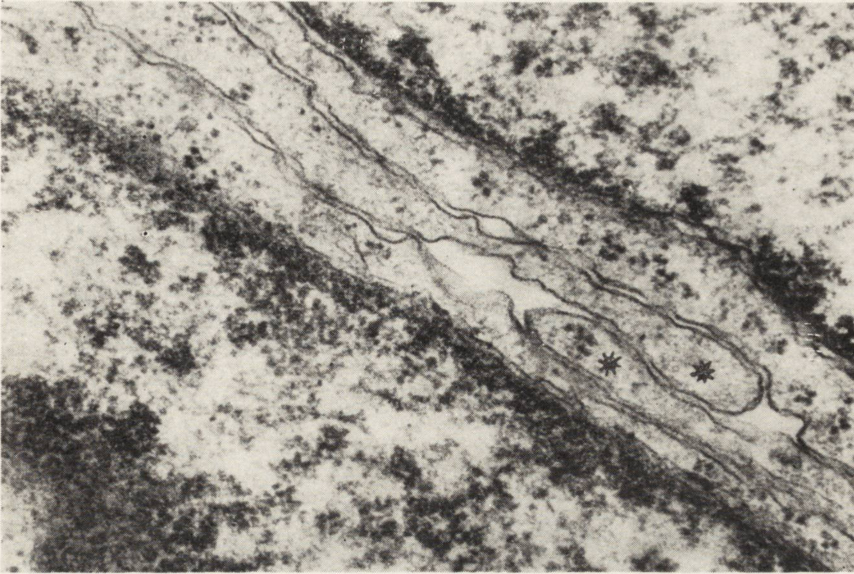

Fig. 3. Cytoplasmic processes of glial cells seen in longitudinal section (asterisks). These processes separate the neighbouring neurons, which were before in close contact. Human embryo 4 weeks old. × 66 000.

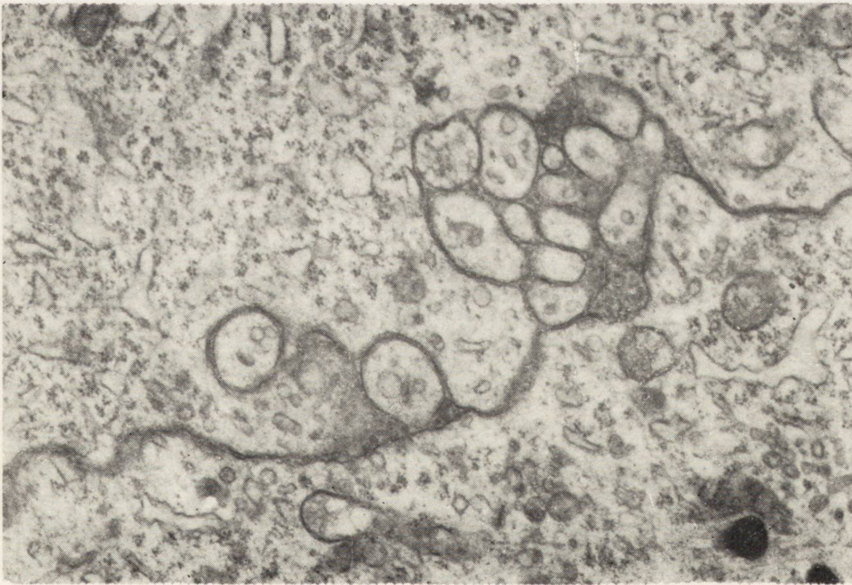

Fig. 4. Direct contact of two neurons without separating satellite cell sheath. Some glial processes penetrating between immature neurons are seen in cross sections. Cat 1 month old. × 30 000.

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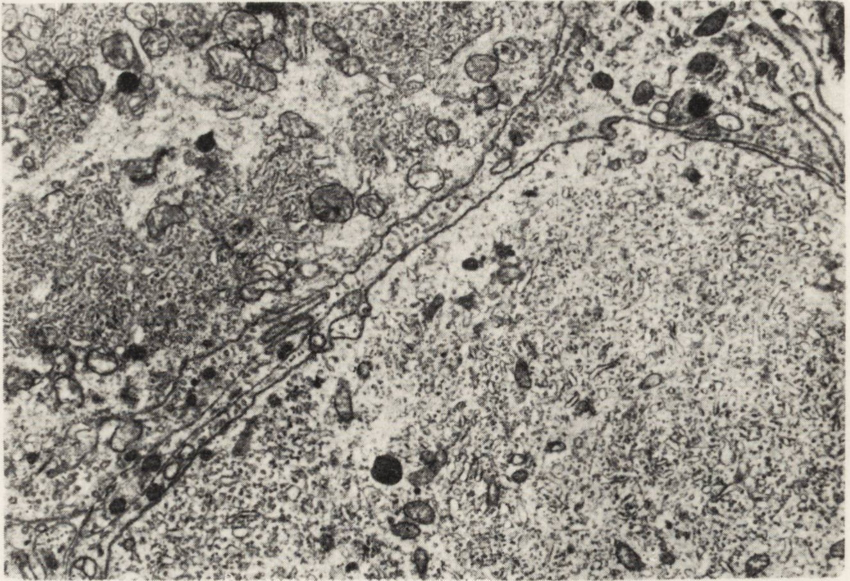

Fig. 5. Group of two neurons with a common satellite cell sheath. Intercellular spaces are not visible between them. The adjacent membranes of neuron and satellite cell are still straight without larger irregularities. In the cytoplasm of the maturing satellite cells there are small vesicles arranged in rows. Cat 1 month old. × 14 100.

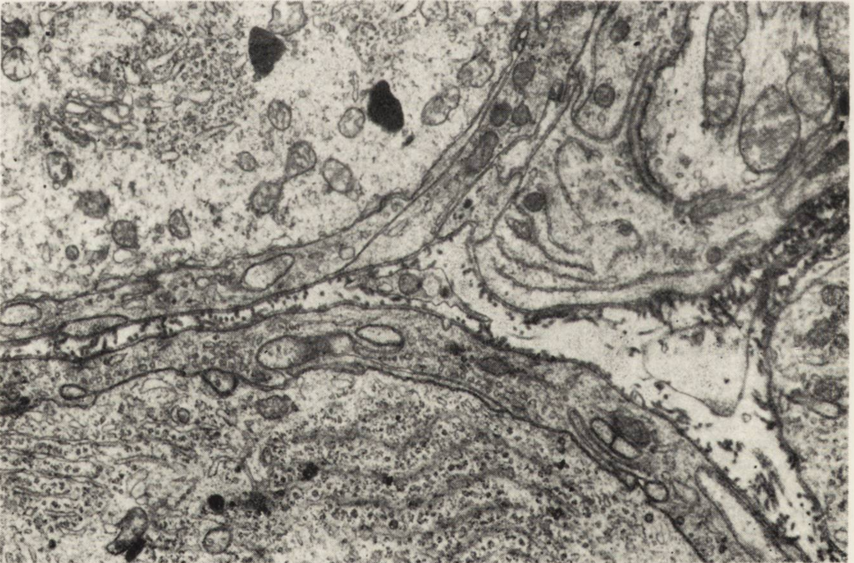

Fig. 6. Satellite cells in the last phase of their ontogenetic development. The definitive relation of these cells to neurons and intercellular spaces is formed. Membranes bordering the intercellular spaces are provided with a basal lamina. In its vicinity the collagen fibrills are seen. Cat 1 month old. × 14 100.

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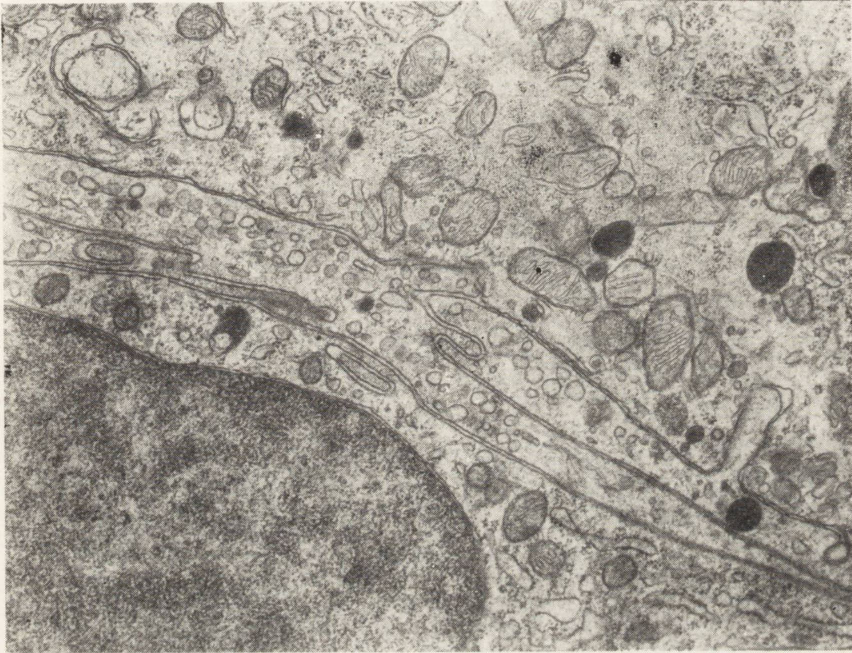

Fig. 7. The sheath of this neuron is formed by three layers of satellite cells. In this cytoplasm there are vesicles arranged in rows. The first cytoplasmic process of satellite cell penetrating deep into neuronal surface is visible (left). Cat 21 days old. ⨯ 30 000.

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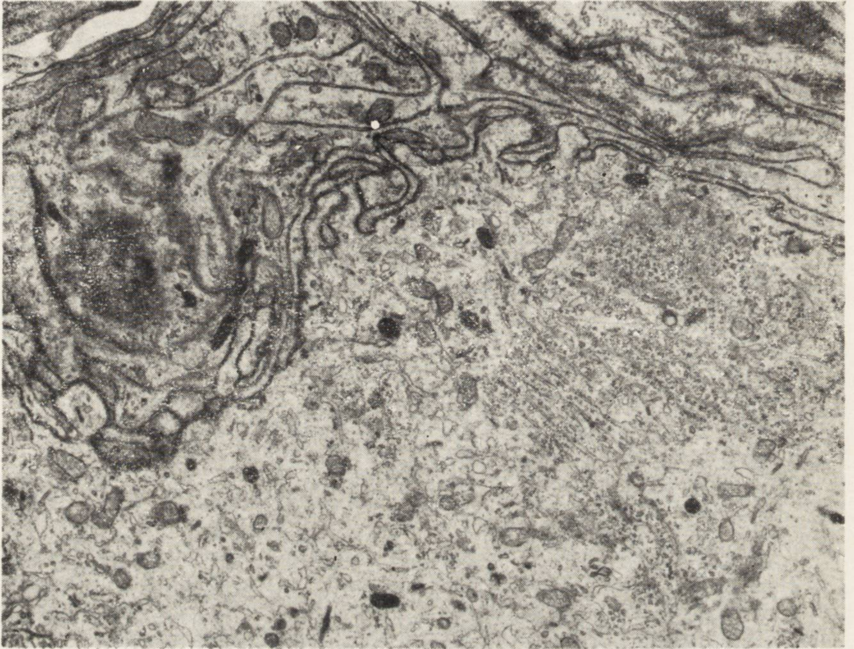

Fig. 8. Perinuclear region of a satellite cell forming indentation in the superficial part of the neuron. In the cytoplasm there are free ribosomes, solitary sacs of granular endoplasmic reticulum, some mitochondria, rod-shaped dense bodies and many fine filaments. Relations of neuronal and glial membranes are very complicated. Adult cat. × 14 100.

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cytoplasmic processes penetrating between the neuroblasts. Immature satellite cells are in this period common for several neighbouring neuroblasts. The adjacent membranes of the neuron and the satellite cell are straight without larger folds.

In the final phase each glial cell envelops only one neuron and between neighbouring satellite cells intercellular clefts appear, these varying in width and containing mesenchymal elements. The definitive ultrastructure is formed both of the satellite cells cytoplasm and of their adneuronal and abneuronal membranes.

The development of these relations and ultrastructure of the satellite cells does not take place in all glial elements at the same time so that in the spinal ganglia of one stage we can find satellite cells in different phases of ontogenetic development.

D. Krajči

ONTOGENETYCZNY ROZWÓJ KOMÓREK SATELITOWYCH W ZWOJACH MIĘDZYKRĘGOWYCH

Streszczenie

Przedmiotem badań był obraz ultrastrukturalny komórek satelitowych zwojów międzykręgowych w rozwoju osobniczym. Materiał badany obejmował zwoje międzykręgowe zarodków i płodów ludzkich (do 12 tygodnia życia śródmacicznego) oraz zwoje międzykręgowe szczurów i kotów w kolejnych stadiach rozwojowych od końcowych faz życia płodowego do wieku dojrzałego.

Wyodrębniono 3 fazy rozwojowe komórek satelitowych, wykazując ich odrębności morfologiczne i ultrastrukturalne oraz zmiany w stosunku między neuronami, a otaczającymi je komórkami satelitowymi.

Д. Крайци

ОНТОГЕНЕТИЧЕСКОЕ РАЗВИТИЕ САТЕЛИТНЫХ КЛЕТОК СПИННОМОЗГОВЫХ ГАНГЛИЕВ

Резюме

Авторы исследовали онтогенетическое развитие сателитных клеток межпозвоночных ганглиев человеческих зародышей и плодов (ранние периоды развития), а также крыыс и кошек от последнего периода плодовой жизни и околородового периода до зрелого возраста. Описаны 3 фазы развития сателитных клеток, отличающихся по морфологическому и ультраструктурному строению. Учитывается отношение сателитных клеток к нейронам.

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CONTENTS

BIOCHEMISTRY OF CEREBRAL TUMOURS

E. Grossi-Paoletti, S. Pezzotta, P. Paoletti: Metabolic characteristics of ethylnitrosourea-induced brain tumours 99
M. I. Prockorova, S. Y. Tumanova, T. V. Tchaika: Lipid metabolism of human brain tumours 111
W. Müller: DNA estimations in cerebral tumours of man 121
G. Viale, H. Kroh: Transfer and ribosomal ribonucleic acids in human gliomas 129
S. Kulesza, T. Borkowski: Characterization of nucleic acids isolated from human brain tumours 139
A. V. Archangelskaya, Z. S. Gershenovich, A. A. Krychevskaya: Brain nitrogen metabolism in rats with inoculated extracerebral sarcomas . . 147
R. Schröder: Experimental studies on the time course of necrobiosis in human gliomas 157
M. Wollemann, L. Gazsó, E. Róna: Changes of lactic dehydrogenases isoenzymes in tissue cultures of human brain tumours 167
I. A. Sytinsky, T. V. Tchaika, G. N. Michailova: Gamma-aminobutyrate-alpha--oxoglutarate transaminase (GABA-T) activity of human brain tumours of a various degree of anaplasia 175
D. Stavrou, M. Knedel, W. Weidenbach: Vergleichende Aspekte bezüglich Aktivität und Isoenzymmuster der Lactatdehydrogenase bei spontanen sowie experimentellen Hirngliomen 183
H. Schicha, W. Müller, K. Kasperek, R. Schröder: The occurrance of trace elements in cerebral tumours 189
W. Fischer: The proportion constancy of enzymes of the phosphotriose — phosphoglycerate group in brain tumours 199
E. Róna, A. Nagy, M. Wollemann, F. Slowik: Localization of various isoenzymes in different cell fractions of brain tumours 207

HISTOCHEMISTRY OF CEREBRAL TUMOURS

G. Osske, R. Warzok, W. Jänisch: Enzyme histochemical investigations of repeatedly transplanted brain and nerve tumours of the rat 223
D. Schiffer, L. Fornatto, G. Croveri, A. Fabiani: Histoenzymology of human and experimental brain tumours: remarks on the interpretation of hydrolytic enzyme reactions 229
H. Kroh: Morphological and enzymatic characteristics of experimental cerebral tumours in mice 235
I. Lolova, A. Ivanova, S. Bojinov, V. Christov: Some histochemical studies on medulloblastomas with references to their localization 241
J. Kałuża, H. Szydłowska: A comparative histochemical study of the functional groups in proteins and some oxidizing-reducing enzymes in reactive glia and glial tumours. I. Glial tumours 249
A. Fabiani, G. Croveri, L. Fornatto, D. Schiffer: Acid phosphatase activity in human brain tumours 255
A. Ivanova, I. Lolova, S. Bojinov, L. Karagjozov: Histochemical studies in pituitary adenomas with reference to type and clinical picture . . . 261
D. Felicetti, U. Bonk, C. Coutelle, R. Coutelle, F. W. Rath, F. Traub: Experimental investigations on invasive tumour growth 269

http://rcin.org.pl

BIOCHEMISTRY AND HISTOCHEMISTRY OF THE GLIA

E. Thomas: Contribution of enzyme histochemistry as to the nature and differences among mantle cells (satellite cells), amphycites, and Schwann cells in Remak fiber bundles and of myelinated fibers in dorsal root ganglia, sympathethic ganglia and in peripheral nerves of various animals 275

H. Szydłowska, J. Kałuża: A comparative histochemical study on functional protein groups and some oxidizing-reducing enzymes in reactive glia and glial tumours. II. Reactive glia 285

M. Wender, M. Kozik, O. Mularek: Oxidoreductases in developing neuroglia 295

L. Venkov: Isoenzymatic characteristics of some nonspecific esterases in the rabbit spinal cord 301

K. Renkawek: On the histochemical and enzyme-histochemical differences between various types of glial cells cultured in vitro 307

M. J. Mossakowski, B. Penar: Some aspects of the histochemistry of the reactive glia 317

S. Kasabyan, J. Romanchikov: Some aspects of histochemistry of glia and ganglion cells of hypothalamus in the age aspects 325

J. Malinský: Ontogenetic development of glial cells in the grey matter of the human spinal cord 331

J. Malinská: Developmental changes of glial elements in the white matter of the human spinal cord 337

V. A. Brumberg, L. Z. Pevzner: Ribonucleic acid content in neuron-neuroglia unit of the spinal cord 343

N. Robinson: Enzyme histochemical alterations following intracranial penetration of a microneedle 359

A. Petrescu: Histoenzymological aspects of reactive astroglia around a cerebral necrotic foci 365

D. Krajčí: The ontogenetic development of satellite cells in spinal ganglia . 373

TREŚĆ

BIOCHEMIA GUZÓW MÓZGU

E. Grossi-Paoletti, S. Pezzotta, P. Paoletti: Metaboliczne właściwości guzów mózgu wzbudzonych etylonitrozomocznikiem 99

M. I. Prockorowa, S. J. Tumanowa, T. W. Czajka: Metabolizm lipidów w guzach mózgu 111

W. Müller: Oznaczanie DNA w guzach mózgu u ludzi 121

G. Viale, H. Kroh: Kwas rybonukleinowy przenoszący i rybosomalny w glejakach ludzkich 129

S. Kulesza, T. Borkowski: Charakterystyka kwasów nukleinowych izolowanych z guzów mózgu u ludzi 139

A. V. Archangelskaya, Z. S. Gershenovich, A. Krychevskaya: Metabolizm azotowy w mózgach szczurów z wszczepionymi mięsakami pozamózgowymi . . . . , 147

R. Schröder: Badania doświadczalne nad czasowym przebiegiem nekrobiozy w glejakach u ludzi 157

M. Wollemann, L. Gazsó, E. Róna: Zmiany w izoenzymach dehydrogenazy mleczanowej ludzkich guzów mózgu w warunkach hodowli tkanek . 167

http://rcin.org.pl

A. I. Sytinskij, T. W. Czajka, G. N. Michajłowa: Aktywność gamma-aminomaślan-alfa-oksoglutaran-amino-transferazy (GABA-T) w guzach mózgu o różnym stopniu anaplazji 175
D. Stavrou, M. Knedel, W. Weidenbach: Badania porównawcze aktywności i składu izoenzymów dehydrogenazy mleczanowej w spontanicznych i doświadczalnych glejakach mózgu 198
H. Schicha, W. Müller, K. Kasperek, R. Schröder: Występowanie pierwiastków śladowych w guzach mózgu 183
W. Fischer: Stałość stosunku enzymów grupy fosfotriozy-fosfogliceranu w guzach mózgu 199
E. Róna, A. Nagy, M. Wollemann, F. Slowik: Lokalizacja różnych izoenzymów w poszczególnych frakcjach komórkowych guzów mózgu 207

HISTOCHEMIA GUZÓW MÓZGU

G. Osske, R. Warzok, W. Jänisch: Badania histoenzymatyczne wielokrotnie przeszczepianych guzów mózgu i nerwu u szczura 223
D. Schiffer, L. Fornatto, G. Croveri, A. Fabiani: Histoenzymologia ludzkich i doświadczalnych guzów mózgu — zagadnienie interpretacji odczynów enzymów hydrolitycznych 229
H. Kroh: Morfologiczne i enzymatyczne cechy doświadczalnych guzów mózgu u myszy 235
I. Lolova, A. Ivanova, S. Bojinov, V. Christov: Histochemiczne badania rdzeniaków o różnej lokalizacji 241
J. Kałuża, H. Szydłowska: Histochemiczne badania porównawcze grup czynnościowych białek i niektórych enzymów oksydacyjno-redukcyjnych w gleju odczynowym i nowotworach glejopochodnych. I. Nowotwory glejowopochodne 249
A. Fabiani, G. Croveri, L. Fornatto, D. Schiffer: Aktywność kwaśnych hydrolaz w guzach mózgu u ludzi 255
A. Ivanova, I. Lolova, S. Bojinov, L. Karagjozov: Badania histochemiczne gruczolaków przysadki w odniesieniu do typu i obrazu klinicznego . . 261
D. Felicetti, U. Bonk, C. Coutelle, R. Coutelle, F. W. Rath, F. Traub: Badania doświadczalne nad inwazyjnym wzrostem guzów 269

BIOCHEMIA I HISTOCHEMIA GLEJU

E. Thomas: Histochemia enzymatyczna jako czynnik różnicujący charakter komórek osłonkowych (satelitarnych), amficytów i komórek Schwanna włókien bezmielinowych i mielinowych w zwojach międzykręgowych, zwojach współczulnych i nerwach obwodowych u różnych gatunków zwierząt 275
H. Szydłowska, J. Kałuża: Histochemiczne badania porównawcze grup czynnościowych białek i niektórych enzymów oksydacyjno-redukcyjnych w gleju odczynowym i nowotworach glejopochodnych. II. Glej odczynowy 285
M. Wender, M. Kozik, O. Mularek: Oksydoreduktazy w rozwijającym się neurogleju 295
L. Venkov: Właściwości izoenzymów niektórych nieswoistych esteraz w rdzeniu kręgowym królika 301
K. Renkawek: Różnice histochemiczne i histoenzymatyczne pomiędzy różnymi typami komórek glejowych w hodowli in vitro 307
M. J. Mossakowski, B. Penar: Wybrane zagadnienia histochemii gleju odczynowego 317

http://rcin.org.pl

S. Kasabyan, J. Romanchikov: Niektóre zagadnienia histochemii gleju i neuronów podwzgórza w aspekcie wieku 325
J. Malinský: Rozwój ontogenetyczny komórek glejowych istoty szarej rdzenia kręgowego człowieka 331
J. Malinská: Zmiany rozwojowe elementów glejowych istoty białej rdzenia kręgowego człowieka 337
V. A. Brumberg, L. Z. Pevzner: Zawartość kwasu rybonukleinowego w jednostce neuron-neuroglej w rdzeniu kręgowym 343
N. Robinson: Zmiany histoenzymatyczne po śródczaszkowym wkłuciu mikroigły 359
A. Petrescu: Histoenzymatyczne właściwości odczynowego astrogleju otaczającego ognisko martwicy mózgu 365
D. Krajči: Ontogenetyczny rozwój komórek satelitowych w zwojach międzykręgowych 373

СОДЕРЖАНИЕ

БИОХИМИЯ ОПУХОЛЕЙ МОЗГА

Е. Гросси-Паолетти, С. Пеццотта, П. Паолетти: Метаболические свойства опухолей мозга вызванных этилнитрозомочевиной 99
М. И. Прохорова, С. Ю. Туманова, Т. В. Чайка: Обмен липидов в опухолях мозга 111
В. Мюллер: Определение ДНК в опухолях мозга у людей 121
Г. Виале, Г. Крох: Трансфер-рибонуклеиновая кислота и рибосомы в человеческих глиомах 129
С. Кулеша, Т. Борковски: Характеристика нуклеиновых кислот, изолированных из опухолей человеческого мозга 139
А. В. Архангельская, З. С. Гершенович, А. А. Кричевская: Мозговой метаболизм азота в развитии трансплантированной внемозговой саркомы 147
Р. Шредер: Экспериментальные исследования временного протекания некробиоза в глиомах у людей 157
М. Воллеманн, Л. Газжо, Е. Рона: Изменения в изоэнзимах лактатдегидрогеназы из человеческих опухолей мозга в условиях тканевой культуры 167
И. А. Сытинский, Т. В. Чайка, Г. Н. Михайлова: Активность гамма-аминобутират-альфа-оксоглутарат-амино-трансферазы (GABA-T) в опухолях мозга человека различной степени анаплазии 175
Д. Ставроу, М. Кнедель, В. Вейденбах: Сравнительные исследования активности и содержания изоэнзимов лактатдегидразы спонтанных и экспериментальных глиом мозга 183
Г. Шиха, В. Мюллер, К. Касперек, Р. Шредер: Наличие следовых элементов в опухолях мозга 189
В. Фишер: Постоянство соотношения энзимов группы фосфотриозы-фосфоглицерата в опухолях мозга 199
Е. Рона, А. Наги, М. Воллеманн, Ф. Словик: Локализация разных изоэнзимов в разных клеточных фракциях опухолей мозга 207

ГИСТОХИМИЯ ОПУХОЛЕЙ МОЗГА

Г. Осске, Р. Варцок, В. Яниш: Гистоэнзиматические иследования многократнопрививаных опухолей мозга и нерва крысы 223
Д. Шиффер, Л. Форнатто, Г. Кровери, А. Фабиани: Гистоэнзимология человеческих и экспериментальных опухолей замечания касающиеся гидролаз 229
Г. Крох: Морфологические и энзиматические свойства экспериментальных опухолей мозга мыши 235
И. Лолова, А. Иванова, С. Божинов, В. Христов: Гистохимические иследования медуллобластом с разной локализацией 241

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Я. Калужа, Г. Шидловска: Гистохимические сравнительные исследования функциональных групп белков и некоторых окислительно-восстановительных энзимов в реактивной глии и глиопроизводных опухолях. I. Глиопроизводные опухоли 249
А. Фабиани, Г. Кровери, Л. Форнатто, Д. Шиффер: Активность кислых гидролаз в опухолях мозга человека 255
А. Иванова, И. Лилова, С. Божинов, Л. Карагёзов: Гистохимические исследования аденом гипофиза по отношении к типу и клинической картине 261
Д. Фелицетти, Y. Бонк, Ц. Кутелл, Р. Кутелл, Ф. Рат, Ф. Трауб: Экспериментальные исследования инвазионного роста опухолей 269

БИОХИМИЯ И ГИСТОХИМИЯ ГЛИИ

Е. Томас: Энзиматическая гистохимия как фактор дифференцирующий характер сателитных клеток, амфицитов и клеток Шванна безмиелиновых и миелиновых волокон в межпозвоночных ганглиях, симпатических узлах и периферических нервах у разных видов животных 275
Г. Шидловска, Е. Калужа: Гистохимические сравнительные исследования активных групп белков и некоторых оксидационно-редукционных энзимов в реактивной глии и глиопроизводных опухолях. II. Реактивная глия 285
М. Вендер, М. Козик, О. Мулярек: Оксидоредуктазы в развивающейся нейроглии 295
Л. Венков: Характеристика изоэнзимов неспецифических эстераз спинного мозга кролика 301
К. Ренкавек: Гистохимические и гистоэнзиматические разницы между разными типами глиальных клеток в культуре ин витро . . . 307
М. Е. Моссаковски, Б. Пенар: Избранные вопросы гистохимии реактивной глии 317
С. Касабян, Е. Романчиков: Некоторые вопросы гистохимии глии и нейронов гипоталамуса в аспекте возраста 325
Я. Малински: Онтогенетическое развитие глиальных клеток серого вещества спинного мозга человека 331
Я. Малинская: Изменения развития глиальных элементов белого вещества спинного мозга человека 337
В. Брумберг, Л. Певзнер: Содержание рибонуклеиновой кислоты в системе нейрон-нейроглия спинного мозга 343
Н. Робинзон: Гистоэнзиматические изменения после внутричерепного введения микроиглы 359
Д. Крайци: Онтогенетическое развитие сателитных клеток спинномозговых ганглиев 365
А. Петреску: Гистоэнзиматические свойства реактивной астроглии окружающей очаг некроза мозга 373

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