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DISTURBANCES OF CORTICAL DEVELOPMENT

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The development processes coinciding in formation of cerebral cortex were presented. On this background may arrrise the cortical developmental abnormalities. The review of principal cortical malformations include the 1) agyria-pachygyria – lissencephaly type I, 2) polymicrogyria, 3) disorganized cortical structure – lissencephaly type II, 4) minor cortical developmental anomalies and 5) other cortical dysplasia syndromes. It will help to reconstruct the pathomechanism of cortical abnormal development from early occurring primary errors to late changes in which coincide necrotic lesions. The presented material illustrate the role of time of occurrence and intensity of damage in final result of pathologic developmental processes.

Key words: *cortical development, cortical malformations.*

Four closely connected developmental processes coincide in the formation of the cerebral cortex. They are consecutive, in part they overlap one another:

1. Neuron migration from periventricular germinal centres to the cortical plate starts during the 2nd and 4th month, diminishing during the 5th month, it is completed around mid 7th month, but the last nerve cells from the subplate zone may probably reach the cortex not earlier than after birth (Kahle 1969; Sidman, Rakic 1973; Evrard et al. 1989; Kostovic et al. 1989). During this period the regional differences in onset and rate of migration follow the phylogenetically determined sequence of cortical development.

2. Formation of the glial-pial barrier occurs at about the 6th week and it matures very early in gestation. It creates an important factor of control of neuronal migration (Choi 1988).

3. Vascularization of the hemispheric wall begins in the 7th week of gestation, when the endothelial channels appear, penetrating from the primitive endothelial plexus (Kuban, Gilles 1985; Norman, O'Kusky 1986). Development of the vascular network coincides with the appearance and maturation of the cortical structure.

4. Formation and maturation of the cortical layers, which starts in about the 6th developmental month, after the end of major waves of neuronal migration, overlaps the final phase of this process.

Cortical developmental abnormalities may arise during each phase of cortical development as the result of disturbances or impairment of one or more of the processes cited above at any moment of their course. The review of principal cortical malformations (Table 1) will follow as far as possible the time of their origin. It will help to reconstruct the pathomechanism in which early occurring primary errors of development may overlap during its further course necrotic changes resulting in abnormalities of the final phase of maturation.

Table 1. Principal cortical malformations

1. Agyria-pachygyria – lissencephaly type I
2. Polymicrogyria
3. Disorganized cortical structure – lissencephaly type II
4. Minor cortical developmental anomalies
5. Other cortical dysplasias

Agyria-pachygyria, known also as lissencephaly type I, is a rare malformation and the earliest in respect to time of appearance (Dąmbska et al. 1983b; Friede 1989). Its pathomechanism is attributed to the arrest of neuronal migration. The hemispheric wall is flat, deprived of gyri and sulci. Microscopic examination reveals that a great number of nerve cells never reached the cortical plate. The hemispheric wall presents in the majority of agyric brains a four-layered structure consisting of: 1) molecular, 2) cellular, containing mainly pyramidal neurons, 3) sparcely cellular and 4) deep cellular layer containing cells arrested in

Fig. 1. The four-layered structure of agyric hemispheric wall. Cresyl violet. ×60

migration (Fig. 1). A less often observed type of agyria includes a more or less continuous mass of neurons under a thin molecular layer with poorly demarcated borderline between its external and internal parts. Jellinger and Rett (1976) analysed both types according to migration timing established by Sidman and Rakic (1973). They proposed for the second of described types of agyric wall the teratogenic period at 10 – 11 weeks of gestation, when the cortical plate increases in thickness as the result of a great wave of neuronal migration. The first, most frequently seen four-layered type of agyria seems to arise later, around the 11 – 13th weeks of development, when the inner and outer part of the cortical plate are well demarcated.

Pachygyria is a more attenuated anomaly of the same type. It presents broad, abnormal gyri with four-layered or even six-layered structure. Both types of agyria and pachygyria may be found in the same case, with topography corresponding to the development time of various cortical regions, pachygyria being in some cases localized in the phylogenetically older paleocortex (Jellinger, Rett 1976). Pachygyria may also be combined with normal cortex. In such cases the internal layer of pachygyric cortex did not find its prolongation in the neocortical internal layer. This could be considered as an indirect morphological confirmation of an arrest of neuronal migration. The abnormal cortical structure could also impede the formation of normal interneuronal connections. It is probably the cause of an aberrant, inverse of oblique position of several cortical neurons visualized by the direction of their principal dendrites (Tanaka et al. 1988). The cortical malformations described above coincide often with other, rather mild CNS anomalies. They are secondary to cortical anomalies, such as lack of claustrum and capsula extrema, abnormal structure of thalamus and underdevelopment of cortico-spinal tracts, or arise at a similar time of development, like anomalies within the inferior olives, dentate nucleus and cerebellar cortex.

Subcortical heterotopias have to be mentioned together with the agyria-pachygyria syndrome as belonging to the same group of malformations. Particularly rare laminal heterotopias could be located near the pachygyria, when even some transitional cases between both anomalies were reported (Jacob 1936). They are usually bilateral and present a layer of cells arrested in migration between the ventricular wall and the cortex separated from both by layers of white matter. More common are nodular heterotopias located around the corners of lateral ventricles, and presenting clusters and islands of neurons. They comprise nerve cells in various stages of maturation, but never presenting the bizzare forms characteristic for tuberous sclerosis often seen with similar localization.

The agyria-pachygyria syndrome may occur in genetically determined familial syndromes (Miller 1963; Dicker et al. 1969; Jellinger, Rett 1976) or in sporadic cases (Münchoff, Noetzel 1965; Dąmbska, Schmidt-Sidor 1971; Dobyns et al. 1985). The best known is the Miller-Dicker syndrome occurring in cases with chromosomal abnormality – microdeletion of band 17p13.3 (Dobyns 1989; Kwiatkowski et al. 1990). This syndrome is characterized by particular clinical

features including microcephaly, profound mental retardation, cranio-facial defects, epilepsy, and decerebration at the end of life which is rarely longer than 2 years. Other cases with familial occurence were also seen (Pavone et al. 1990). Some were described with extreme micrencephaly and severe cerebellar malformations (Barth et al. 1982). Sporadic cases incline to mention the suggestion of Steward et al. (1975) concerning a possible role of early necrotic changes in the pathomechanism of agyria.

Polymicrogyria (PMG) called also microgyria or micropolygyria consist in excessive folding of all or of external cortical layers without separation of gyri. It may be generalized in one or both hemispheres or may present only focal anomaly. The gyri on the external surface of hemispheres look mostly coarse and too broad. Microscopically two types of PMG may be differentiated: Unlayered PMG, when small, fused gyri contain only a molecular and one cellular layer. The fusion of gyri is often underlined by a line of vascular profiles (Fig. 2). Four-

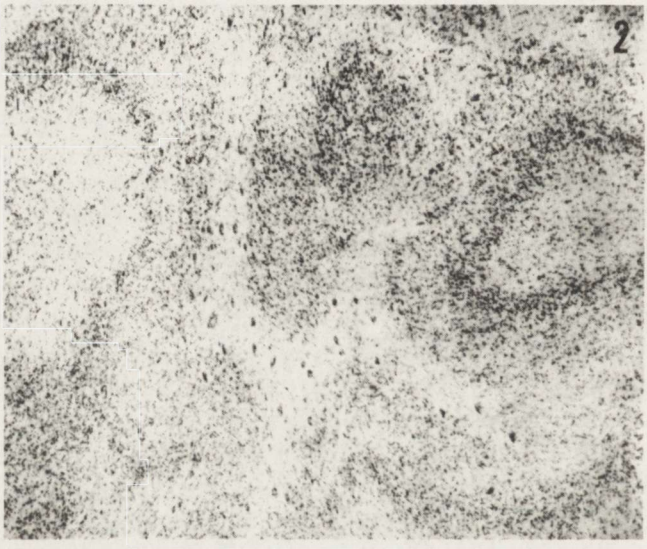

Fig. 2. Polymicrogyria. Fusion of gyri underlined by vascular profiles. Cresyl violet. ×60

-layered PMG include: 1 – a molecular layer, 2 – an external cell layer, 3 – a sparce cell layer and 4 – a deep cell layer. The 3rd and 4th layers may be unfolded (Fig. 3). The subpial surface overlying the PMG cortex may sometimes include a layer of myelinated fibers. More often damage of the glial-pial barrier results in glial and neuronal heterotopias into the meninges. They seem to facilitate the fusion of abnormally folded gyri, and in this way play supplementary role in PMG formation.

In the pathomechanism of this cortical anomaly the role of various factors complementary to each other seems to appear. The morphological observations allow to admit, that PMG presents the result of disturbances in cortical deve-

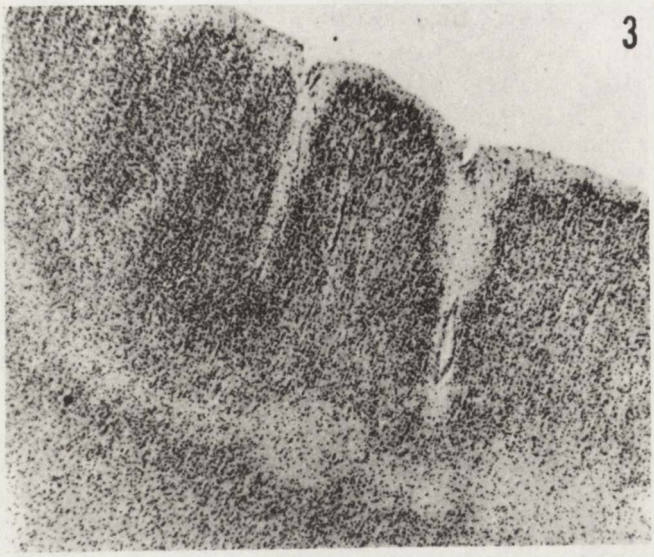

Fig. 3. Four-layered polymicrogyria. Cresyl violet. × 60

lopment arising after migration of the main waves of nerve cells, when only the last ones have to reach the external cortical layers, and when cortical stratification begins. The role of partial necrosis induced at that time by transient, generalized or local perfusion failure became generally accepted as the basic pathomechanism of PMG (Barth 1987; Friede 1989). It was confirmed by the experimental study on rats performed by Dvorak and Feit (1977) and by Dvorak et al. (1978).

The changes in meninges overlying the polymicrogyric cortex such as inflammatory infiltrations (Dąmbska et al. 1983a) allow to suppose the failure of perfusion by meningeal vessels, forming the cortical vascular bed, parallelly to the maturation of the cortex (Kuban, Gilles 1985; Norman, O'Kusky 1986). The vascular malformation or leptomeningeal lipomatosis (Dragojevic et al. 1973) if topographically corresponding to PMG-cortex may be also suspected of playing a similar role at a given moment of cortical and vascular development. Lesion of the distal terminal blood vessels in the subcortical area may damage the glial fibers involved in the last phase of neuronal migration, leading in this way to formation of well demarcated foci of PMG (McBride, Kamper 1982; Ferrer, Catala 1991). This type of changes may similarly occur as consequence of periventricular lesions due to CMV infection (Friede, Mikolasek 1978; Dąmbska et al. 1983a, Marques-Dias et al. 1984). Generalized perfusion or other disturbances have to be taken into account when PMG in the cerebral cortex coincides with similar type lesions in the cerebellar cortex. Many authors confirmed the role of perfusion failure in four-layered PMG (Levine et al. 1974; Williams, Caviness 1976; McBride, Kamper 1982) and the same was concluded for unlayered PMG (Ferrer 1984; Ferrer, Catala 1991). The teratogenic time for unlayered

PMG is proposed between the 13–19th week of gestation, and for four-layered PMG between 20–22 week. It was calculated according to the phases of cortical development and occurrence of pathological events during pregnancy, followed by formation of PMG (Bankl, Jellinger 1967; Norman 1980; Barth 1987; Ferrer, Catala 1991). The cases in which unlayered and four-layered PMG coincide support the idea of a common pathologic mechanism of both types of this anomaly. The differences in time of development of various cortical areas may result in such coincidence after one pathologic accident.

Polymicrogyria may appear in familial cases often combined with other malformations like the Meckel syndrome (Pactau et al. 1985) in adrenoleukodystrophy (Barth 1987), in the Aicardi syndrome (Ferrer et al. 1986) or in the Zellweger cerebro-hepato-renal syndrome (Volpe, Adams 1972). It may be also due, as previously mentioned to many exogenous factors.

Disorganized cortical structure – lissencephaly type II. The lissencephalic hemispheric wall may contain cortex abnormalities essentially differing from the agyric mantle (Dąmbska et al. 1983). Therefore, morphological differential diagnosis between both malformations, resembling by gross external features has to be taken into account. In lissencephaly type II the neocortex is thick and its structure is disorganized (Fig. 4). Pyramidal and small neurons, sometimes immature for their age, form the islands and clusters. Subsequently, no lamination of the cortex is seen. Intracortical proliferation of glial and mesodermal elements gives in some segments the impression of a scar. In other segments some

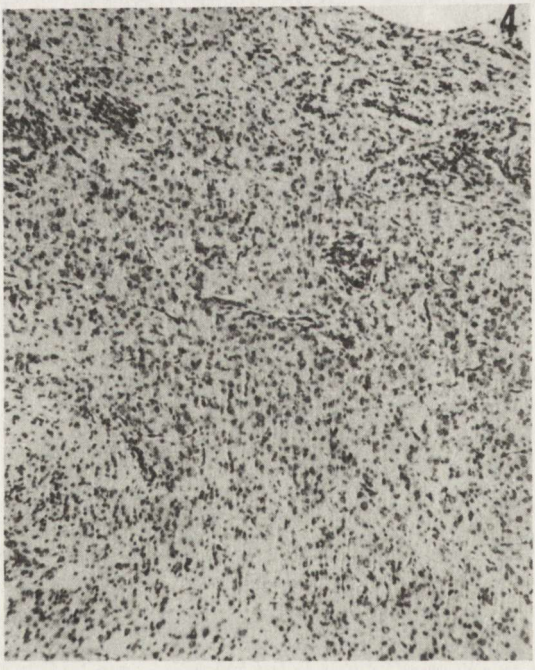

Fig. 4. Disorganized cortical structure with total lack of layering and break of glial-pial barrier. Cresyl violet. × 100

elements of polymicrogyric structure could be detected. The glial-pial barrier was in the majority of so far described cases damaged on long segments, and glio-neuronal heterotopias were abundant (Dąmbska et al. 1986). The role of a break in the glial-pial barrier relatively early in development was proposed by Choi and Matthias (1987) as leading to abnormal migration to the molecular layer and meninges and consecutive disorganization of cortical layering. The rare cases with similar cortical disorganization (Norman et al. 1976 and our unpublished case) without or with only minimal disruption of the glial-pial barrier call attention to the destructive processes within the cortex during its development as an other possible or additional mechanism of this anomaly.

Its teratogenic time has uptill now never been discussed. The glial-pial barrier matures before the end of the second gestational month, the primary major neuronal migration occurs during the third month. It is possible to speculate that so early starts the abnormal localization of neurons resulting in disorganized cortical structure after a prolonged time of teratogenesis until the end of migration in the last months of development.

Lissencephaly type II was described by Walker (1942), then by other authors and at present is known in the Walker-Wartburg syndrome, Fukuyama syndrome (Dąmbska et al. 1982) and in other syndromes of cerebro-oculo-muscular dystrophies. The relations between them are uptill now under discussion (Takashima et al. 1987; Dobyns et al. 1989), as far as appearance of similar changes in other cases.

Minor cortical developmental anomalies. They constitute a group suggesting in the majority of cases, disturbances of the last phase of neuronal migration to the external cortical layers, sometimes combined with minor lesions of the glial-pial barrier. They are restricted mostly to external cortical layers. The classic "brain warts" (Jacob 1940) or "dysgenésie nodulaire disséminé de l'écorce" (Morel, Wildi 1952) consists in small nodules containing neurons of the second layer intruding into the molecular layer. The cortex around the nodules is usually normal. Ectopies of neurons and glial cells into layer I may also be not typical, as described above, consisting of a less organized collection of neurons and glia, sometimes intruding into the meninges as a glio-neuronal heterotopy. The adjacent cortex may also present focal anomalies. Kaufman and Galaburda (1989) found such changes in dyslectic brains, significantly more often than in normal ones. The glio-neuronal heterotopias in the leptomeninges overlying the neocortex may be also focal, minimal without disturbances of cortical organization (Dąmbska et al. 1986). Sometimes persistent Cajal-Retzius cells are seen within them.

Other developmental anomalies of neocortex. Some rare cases with particular morphological features of developmental anomalies of neocortex did not fit into any of the above presented groups. Among them very rare cases in which immaturity presents the principal abnormality of the cortex were observed. The particular immaturity of neocortex with a picture corresponding to the V–VI months of developmental age in the newborn at term was observed in the Neu-

-Laxova syndrome (rare lethal autosomal recessive disorder) as the main trait of CNS malformation (Ostrovskaya, Laziuk 1988). The authors proposed that it may be the IIIrd type lissencephaly. The immaturity of dysgenetic cortex was also sygnallized in the cerebro-renal Galloway syndrome (Kozlowski et al. 1989) and in a single sporadic case (Laure-Kamionowska, Majdecki 1982). The observation of minor cortical developmental anomalies as compared with the previously described severe malformations and eventual disturbances of maturation of cortical structure illustrate well that the final result of pathologic developmental processes depends on the time of occurrence and intensity of the lesions.

ZABURZENIA ROZWOJU KORY MÓZGU

Streszczenie

W pracy przedstawiono procesy rozwojowe zachodzące w czasie powstawania kory mózgu, będące podłożem jej ewentualnych zaburzeń rozwojowych. Przegląd głównych typów wad kory mózgu obejmuje: 1) bezzakrętowość – szerokozakrętowość – gładkomózgowie typ I, 2) drobnozakrętowość, 3) dezorganizację struktury kory – gładkomózgowie typ II, 4) małe zaburzenia rozwoju kory oraz 5) inne dysplazje korowe. Analiza morfologiczna nieprawidłowości rozwojowych umożliwia odtworzenie ich patomechanizmu, począwszy od wcześnie występujących pierwotnych zaburzeń do zmian późnych, w których współdziałają procesy martwicze. Przedstawiony materiał ilustruje znaczenie momentu wystąpienia zaburzeń i nasilenie uszkodzenia dla charakteru i końcowego efektu nieprawidłowo przebiegających procesów rozwojowych.

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AMMON'S HORN CHANGES IN FOCAL BRAIN ISCHEMIA IN HUMANS

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Neuronal changes in Ammon's horn were examined immunocytochemically in 20 patients aged from 51 to 101 years, deceased in the course of ischemic lesions localized within the area supplied by vessels derived from other then Ammon's horn vascularization (middle cerebral artery). Numerous neurons within various sector of the pyramidal layer, in the dentate gyrus, subiculum and entorhinal cortex were immunopositive in reaction with antibodies to serum proteins (albumin, IgG, alpha-1-antitrypsin), indicating their damage. The distribution of damaged Ammon's horn pyramidal cells differed from the location of injured Ammon's horn neurons in experimental investigations of brain ischemia and did not indicate a selective vulnerability of pyramidal cells in the human h_1 area, corresponding to the CA_1 sector in animals. Contrary to experimental material, changes in the human Ammon's horn are caused by numerous overlapping factors.

Key words: *human brain ischemia, Ammon's horn, neuronal changes.*

In our previous immunocytochemical investigations, in various pathological processes, a positive immunocytochemical reaction with serum proteins of a part of cortical neurons was found in areas distant from the lesion. In HE staining affected cortical neurons had an ischemic appearance. These changes were observed both in multiple sclerosis (Rafałowska et al. 1990) and ischemic brain stroke (Krajewski et al. 1988; Rafałowska et al. 1990). Particularly interesting were Ammon's horn changes, incidentally seen in cases with infarcts localized within the area of the middle cerebral artery (MCA) supply. Injured neurons reacting to serum protein antibodies were scattered in various sectors within the pyramidal layer of Ammon's horn, but not only in the h_1 sector, contrary to experimental brain ischemia in which most distinct changes of Sommer sector (CA_1) are observed. The divergence of Ammon's horn injuries in the experimental models and in humans led us to evaluation of Ammon's horn changes in human focal ischemia of the area supplied by the MCA.

MATERIAL AND METHODS

The materials comprised 20 cases of ischemic brain infarct within the area of middle cerebral artery supply, deceased in 2nd, 4th, 5th, 6th, 7th, 8th, 9th, 13th,

14th and 20th day of the stroke. Material was divided into 2 groups: middle--aged group (M) from 51 to 65 years, and senile group (S) from 80 to 101 years. Within the ischemic brain hemisphere the immunoreactivity of neurons in every hippocampal part was compared in both age groups. Ammon's horn of the contralateral "healthy" hemisphere was used as a control. In all the cases autopsy was performed within 24 h after death. Formalin-fixed and paraffin-embedded sections of hippocampal structures were investigated histopathologically and immunocytochemically. For histopathological examination sections were stained with hematoxylin and eosin and with luxol fast blue cresyl violet. The peroxidase-antiperoxidase method of Sternberger's et al. (1970) for visualization of albumin, IgG, alpha-1-antitrypsin was applied. Immunocytochemical reactions were performed in the following way: 8 – 10 μm thick sections deparaffinized and preincubated with 2% normal swine serum diluted with trisma-base (Sigma, USA) at pH 7.6. Thereafter, they were incubated overnight with antibodies against albumin (1:5000, Biomed, Poland), IgG (1:5000, Biomed, Poland), alpha-1-antitrypsin (1:1000, Dakopatts, Denmark). After rinsing the section with PBS, pH 7.6, they were incubated with the following secondary reagents for 1 h: swine antibodies against rabbit IgC (1:50) and rabbit--PAP-complex (1:200) (all antisera from Dakopatts, Denmark). The immune reaction was developed for 15 min incubation in 0.05% Diaminobenzidine tetrachloride (Sigma, USA) with addition of 0.01% H_2O_2. Then, the sections with hemalum counterstaining were dehydrated and mounted with DePex (Serva, FRG).

RESULTS

Histopathological investigation

In staining with hematoxylin and eosin a moderate neuronal loss within subiculum and h_1 sector was observed. A slight neuronal loss in the remaining sectors a pyramidal layer was also noted. In senile cases neuronal loss was more marked. Some nerve cells had an ischemic appearance. The dentate gyrus showed a slight loss of granular cells in 5 senile cases, among which in 4 cases arterial hypertension was manifested clinically or by hypertophy of the left ventricle in autopsy. In some cases loosening of tissue structure in various Ammon's horn layers was noted. It was observed in cases with cerebral edema, which was a common finding in both groups, but predominated in middle-age cases.

Immunocytochemical investigations

1) Table 1 indicates that a considerable part of the middle-aged group cases (7/10) showed a distinct intracellular reaction with albumin, IgG and alpha-1-antitrypsin (Figs 1 – 4). In majority of the senile group cases (7/10) a weak intracellular reaction with albumin, IgG and alpha-1-antitrypsin was observed (Fig. 5).

2) A little higher immunoreactivity with albumin in the middle-aged group was noted in rather early periods of the stroke.

Table 1. Immunoreactivity of Ammon's horn pyramidal cells in middle and senile age groups

Immunoreactive cells		
Very numerous	Numerous	Single or areactivity
M 2*	S 2	S 6
	S 4	S 7
M 5	S 5	S 8
M 7		S 9
M 8		S 13
M 9		S 14
M 13		S 20
M 14		

M – middle age case, S – senile age case.

* Numbers indicate survival time in days. M 4, M 6, and M 20 cases were excluded because of not complete immunocytochemical reactions.

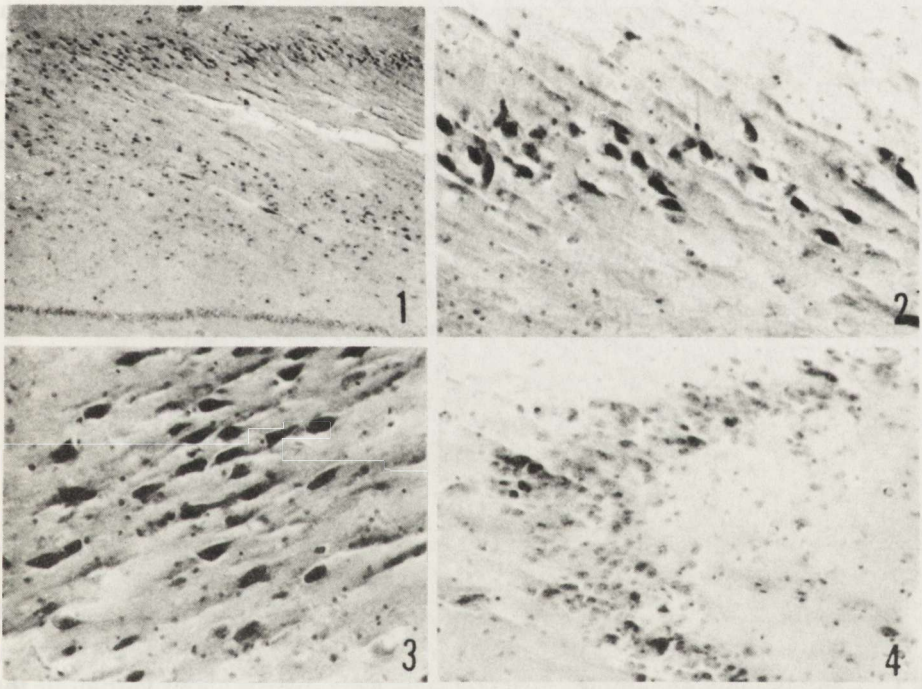

Fig. 1. M – 53 years, 2nd infarct day. Numerous immunoreactive cells within h_3, h_4 and h_5 areas of the pyramidal layer. Some of the dentate gyrus cells are weakly immunoreactive. Alpha-1-antitrypsin. × 13

Fig. 2. M – 53 years, 2nd infarct, day. Immunoreactivity of numerous neurons within h_3 area. Alpha-1-antitrypsin. × 64

Fig. 3. M – 53 years, 2nd infarct day. Immunoreactivity of numerous cells within h_4 area. Alpha--1-antitrypsin. × 64

Fig. 4. M – 53 years, 2nd infarct day. A weak reactivity of rather numerous neurons within hilus of dentate gyrus. Alpha-1-antitrypsin. × 64

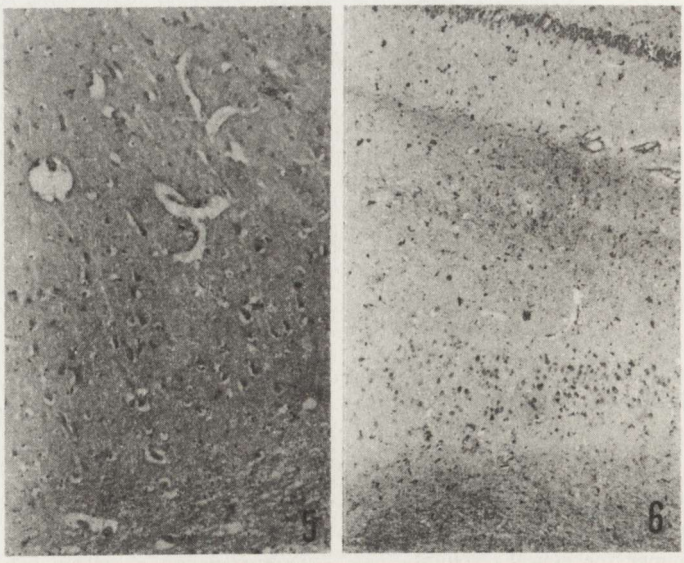

Fig. 5. S — 83 years, 8th infarct day. Loss of neurons and very weak immunoreactivity of h_1 pyramidal cells. Albumin. ×32

Fig. 6. S — 86 years, 4th infarct day. Border zone of h_1 and h_2 area. More immunoreactive cells within h_2 area. Diffuse reactivity of the *stratum oriens, stratum radiatum* and *stratum lacunosum moleculare*. Albumin. ×13

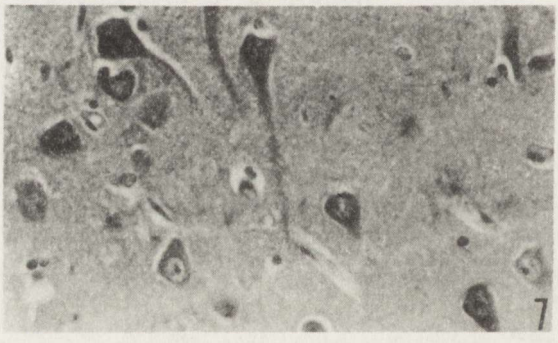

Fig. 7. S — 57 years, 5th infarct day. Pronounced immunoreactivity of h_3 pyramidal cells. IgG. ×12

3) Immunoreactivity of the h_1 sector was weaker than of h_2-h_5 areas (Fig. 6) in 9 cases, particularly in the first 5 infarct days in both groups of the cases. In the remaining cases immunoreactive cells were disseminated within all the sectors of the pyramidal layer (Fig. 7), in subiculum and dentate gyrus (Figs 8, 9).

4) In the subiculum the changes were similar to the h_1 changes in the majority of cases. More distinct changes within the subiculum than in the h_1 area were found in 5 mainly senile cases.

5) In the dentate gyrus the smallest changes were observed in the first infarct days of the middle-aged cases, and then increased from the 6th day of stroke

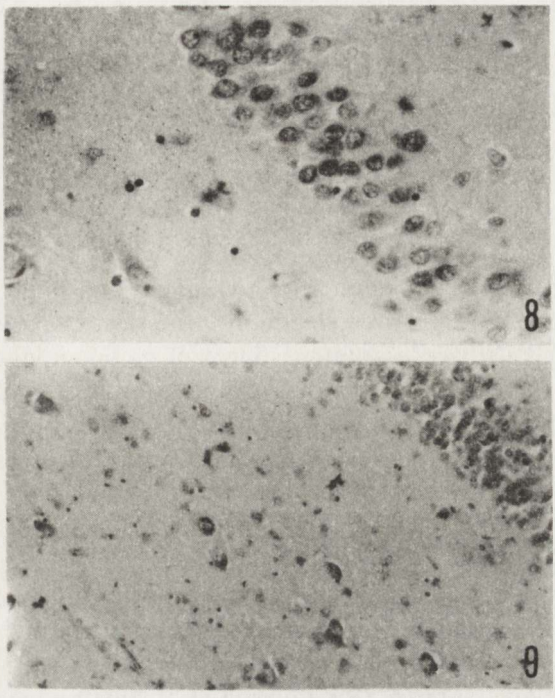

Fig. 8. M — 57 years, 5th infarct day. Weak immunoreactivity of some cells within dentate gyrus. IgG. × 112

Fig. 9. S — 86 years, 4th infarct day. Immunoreactivity of rather numerous neurons within granular layer and h_5 area. Albumin. × 64

Table 2. Immunoreactivity of dentate gyrus granular cells in middle-aged and senile group

Immunoreactive cells					
Numerous, disseminated		Rather numerous		Non numerous or single	
M 7*	S 2	M 6	S 7	M 2	S 7
M 9	S 4	M 8	S 13	M 4	S 9
	S 5	M 13		M 5	S 14
	S 8	M 14			S 20

M — middle age case, S — senile age case.
* Numbers indicate survival time in days.

(Table 2). In the senile cases more distinct changes were observed in the first infarct days. In later periods of disease they were less clear.

6) Considering the anatomical connections, changes within the h_1 area and subiculum, h_1 area and dentate gyrus were compared. A similar reaction of the h_1 area and dentate gyrus was noted in the majority of senile cases (8/10).

It is necessary to emphasize that: a) immunocytochemical reactions were not complete in some cases; b) with weak immunoreactivity of cells, a small difference

in slice thickness may cause estimation of a weak positive (+) or a very weak positive (±) reaction.

Owing to the lack of morphometric evaluation of immunoreactive cells, only greater groups of cases with similar immunocytochemical reaction could be taken into consideration.

DISCUSSION

Our studies revealed, that numerous pyramidal and granular cells in Ammon's horn were immunopositive in reaction with 3 serum proteins, although the infarct was located within an another source of blood supply than Ammon's horn. It is necessary to assume that injury of hippocampal cells was not connected directly with ischemic damage of Ammon's horn structures. Probably two mechanisms are involved in the development neuronal changes in the material under investigation.

An important factor is the increase of blood-brain barrier (BBB) permeability. In immunocytochemical investigations of human brain ischemia a diffuse reaction of brain tissue is a manifestation of increased blood vessel permeability (Krajewski et al. 1988) and it is not limited to infarct area only. Disturbances of the BBB permeability are more distinct and increase quicker in middle-aged humans than in senile subjects (Rafałowska et al. 1990).

The BBB dysfunction is followed by cerebral edema with hemodynamic and metabolic disturbances leading to impairment of nerve cells. Acute hypertensive episodes cause a transient opening of the BBB, and neurons may be damaged even after a short opening of the BBB (Johansson et al. 1990; Sokrab et al. 1990); the infarct area can be also widened (Duverger, MacKenzie 1988; Grabowski et al. 1988). In BBB disturbances numerous transmitters participate (Wahl et al. 1988). The noxious influence on nerve cells of extravasated plasma constituents is also taken into consideration (Nordborg 1990).

Dysfunction of the BBB may be attributed to three different mechanisms: hemodynamic, cytotoxic and neuroexcitatory (Nagashima et al. 1990). Transport of blood-borne proteins through the Golgi apparatus and lysosomes (Broadwell et al. 1988) is carried out in three sequential steps: receptor-mediated endocytosis, diffusion through the endothelial cell cytoplasm and receptor-mediated exocytosis (Kumagai et al. 1987; Pardridge 1990).

The second mechanism of nerve cell damage may be activation of phospholipase A_2 during ischemia (Jesse, Franson 1979) and severe injury of the cell membranes, which became permeable to various molecules. Probably, all factors mentioned above may play a role in the immunopositive reaction of Ammon's horn nerve cells, distant from the infarct lesion, but corresponding to ischemic cells in the HE staining.

Our material indicated that the distribution of damaged pyramidal cells of Ammon's horn considerably differs from the location of injured Ammon's horn pyramidal cells in experimental investigations. It is known, that the CA_1 sector

(corresponding to human h_1 area) is selectively vulnerable in experimental ischemia, particularly in short-term (5 – 7.5 min) one. In this sector delayed death of neurons (Kirino et al. 1984) is preceded by their bioelectrical hyperactivity (Suzuki et al. 1983). The very gist of this phenomenon is not completely clarified. The role of neuroexcitatory amino-acids glutamate and aspartate, is emphasised. Ischemia, causing a release of glutamate leads to hyperactivity of synapses containing excitatory amino acids transmitters (Olney 1978). Vulnerability to ischemia or hypoxia in the tissue culture is connected with the already formed and active synapses (Rothman 1983).

Neurons of the CA_1 have numerous glutamate receptors (Kirino et al. 1985) among them there are quisqualate (Woodruff et al. 1990) and NMDA (Wieloch 1985) receptors, so their excitotoxic damage and delayed death is more ready. The electron microscopic picture also idicates cell activation (Kirino et al. 1985; Mossakowski, Gadamski 1987; Mossakowski et al. 1989). In the cerebral ischemia ion changes are manifested, i.a. by influx of Ca^{2+} to the cell (Nedergaard 1987), and glutamate and other excitatory amino acids can stimulate this phenomenon in postsynaptic cells (Pulsinelli 1985). Calcium ions accumulate within dendrites and in the cell soma of CA_1, both following transient ischemia of 10-min duration (Martins et al. 1988) and after repeated brief cerebral ischemic insults in gerbils (Araki et al. 1990). Calcium accumulation is correlated with signs of neuronal damage (Martins et al. 1988; Araki et al. 1990), and with progression of ischemic cell changes with time (Dienel 1984). It is assumed that disturbance of calcium ion homeostasis is the cardinal cause of cell death (Sakamoto et al. 1986).

The mechanism of calcium ions uptake by the cell is not clear. Following cerebral ischemia calcium ions accumulate also in the rat dentate hilus of Ammon's horn but before irreversible cell damage. This seems to contradict passive calcium influx across the plasma membrane (Benveniste, Diemer 1988). The influence of nimodipine and APV (2-amino-5-phosphonovalerate) upon the reduction of extracellular calcium redistribution indicates calcium channels coupled to NMDA-sensitive glutamate receptors play a role in ischemic calcium influx to neurons of the hippocampus (Salińska et al. 1989, 1991).

The action of excitatory neurotransmitters and intracellular calcium accumulation, leading to a delayed death of CA_1 neurons, provoked numerous investigations on the possibility of preventing excitotoxic damage of CA_1 pyramidal cells. It was found that deafferentation of the CA_1 protects against injury of neurons in this area (Gage et al. 1984; Pulsinelli 1985; Wieloch 1985). Similarly operate prostacyclin and nimodipine – calcium channels blockers (Mossakowski, Gadamski 1987; Łazarewicz et al. 1989, 1990; Pluta et al. 1991) and excitatory amino acid antagonists (Croucher, Meldrum 1984).

The above mentioned findings indicate the important role of the excitotoxic factor in the selective delayed death of the CA_1 neurons. However, our material showed changes other than the data obtained in experimental investigations. In the h_1 area the immunocytochemical reaction of nerve cells, indicating their damage, was weaker for the first 5 days of infarct than within the remaining areas

of the pyramidal layer. In later periods immunopositive cells were disseminated within the entire pyramidal layer and even relatively less vulnerable dentate gyrus. Explanation of this phenomenon is very difficult. It is possible, that changes in the first days of infarct indicate delayed death of the h_1 neurons. However, we have no data, how long was the period of ischemia. After experimental long-term cerebral ischemia, CA_1 changes appear faster than in the case of brief ischemia, and injury of all the other sectors occurs also faster and in more severe form, with an almost total loss of pyramidal cells (Kirino et al. 1985). In our material such alterations were not observed. It is necessary to suppose that the patient dying during ischemic stroke had suffered from several ischemic incidents (transient ischemic attacks and, other less or more short-term disturbances of the cerebral blood flow). If the basic pathogenetic mechanisms are similar in animals and humans then "ischemic tolerance" (Kitagawa et al. 1990) should develop in man. This phenomenon occurs after minor, 2-min ischemic attacks repeated at 1-day intervals before 5-min ischemia. "The ischemic stress" (Kitagawa et al. 1990) disturbs cell metabolism, but simultaneously leads to increased synthesis and, similarly to heat shock protein, protects against RNA destruction (Yost, Lindquist 1986) leading to thermic tolerance (Barbe et al. 1988; Riabowol et al. 1988). It is possible that in our material disseminated changes, without a dramatic loss of pyramidal and granular Ammon's horn neurons are an exponent of i.a. the "ischemic tolerance" phenomenon. It seems that the vascular factor should not be neglected. Probably, the lower perfusion pressure and circulating blood volume in the CA_1 sector in gerbils (Imdahl, Hossman 1986) influence also the selective vulnerability of this area.

In people with a long duration case history or in very old patients the vascular system probably adapts to a slowly and gradually changing homeostasis, mobilizing adaptive mechanisms. It is possible, that these adaptive mechanism are the cause of small damage and weak immunocytochemical reactions in the majority of senile cases in our material. Pulmonary gase exchange in dying, often uncoscious patients is perturbed and then, hypoxia is an additional acting factor. Therefore, it is necessary to surmise that Ammon's horn changes in humans are not caused by a single, but by numerous overlapping factors. The participation of each factor seems to be impossible to estimate.

ZMIANY W ROGU AMONA W OGNISKOWYM NIEDOKRWIENIU MÓZGU U CZŁOWIEKA

Streszczenie

Oceniano immunocytochemicznie neurony rogu Amona u 20 zmarłych wskutek zawału mózgu w innym niż róg Amona obszarze unaczynienia (tętnica środkowa mózgu). Liczne neurony w różnych częściach warstwy piramidowej, zawoju zębatym, podkładce i korze entorinalnej wykazywały reakcję z przeciwciałami przeciwko białkom osocza krwi (albumina, IgG, alfa-1-antytrypsyna), świadczącą o ich uszkodzeniu. Lokalizacja uszkodzonych komórek rogu Amona była inna niż w doświadczalnym niedokrwieniu mózgu i nie wykazywała szczególnej wrażliwości na niedokrwienie neuronów

pola h_1, odpowiadającego sektorowi CA_1 u zwierząt. W przeciwieństwie do materiału doświadczalnego zmiany w rogu Amona u człowieka w przebiegu niedokrwienia mózgu wywołane są licznymi nakładającymi się czynnikami patologicznymi.

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CYCLIC GMP LEVELS IN THE RAT BRAIN AND PLASMA DURING CLINICAL DEATH AND AFTER RESUSCITATION

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Changes of cGMP content in the rat brain and plasma have been evaluated by means of the radioimmunologic method after 5-min clinical death and up to 2 hours after resuscitation. Ischemia produced a decrease of cGMP in the brain, however, at the 15th min after resuscitation a reversible significant rise of nucleotide concentration was noted. In plasma at the end of ischemia and in the postischemic period a significant decrease of cGMP level was observed. The mechanisms of cGMP regulation in the central nervous system and the significance of the obtained results are discussed.

Key words: *cGMP, ischemia, brain, plasma.*

In a number of different cell types, cellular responses to circulating hormones are mediated by the intracellular second messenger, adenosine-3', 5'monophosphate (cAMP). Cyclic guanosine monophosphate (cGMP) has also been implicated as a mediator of extracellular signals, second messenger that accounts for many actions of neurotransmitters and hormones in the nervous system. Cyclic AMP and cGMP play important roles in the regulation of vascular contractility and thus, blood supply to the brain (Northup 1989; Pearce 1989). Intracellular levels of cGMP in nerve tissue are regulated by many of the same neurotransmitters that increase Ca^{2+}. Hydrolysis of cGMP is efected by the same family of cyclic nucleotide phosphodiesterases as for cAMP. At least two distinct types of guanylate cyclase synthesize cGMP from CTP (Waldman, Murad 1987). The metabolism of arachidonic acid derivatives e.g. prostaglandins and thromboxanes, results in the generation of hydroxy- and endoperoxides, and this may be the link to hormonal regulation of the soluble guanylate cyclase. Thus, the cGMP message is generated in response to extracellular signals controlling the production of prostaglandins. This may underline the correlation between the elevation of intracellular Ca^{2+} and cGMP (Northup 1989). An additional form of guanylate cyclase, bound to plasma membranes, has recently been isolated (Waldman, Murad 1987) and proved to be directly activated by the atrial natriuretic peptide

(ANP) (Kuno et al. 1986). At present, this is the only humoral factor known to stimulate the synthesis of cGMP directly (Steardo, Nathanson 1987; Hamprecht et al. 1990). The role of cGMP in phototransduction is extensively described in intracellular messengers in vertebrate photoreceptors (Matthews, Lamb 1992).

In this report, the aim of the study was to examine the effect of clinical death and recovery after resuscitation on the levels of cyclic nucleotide cGMP in the rat brain and plasma.

MATERIAL AND METHODS

Under ether anesthesia 5-min clinical death was induced in 35 adult female Wistar rats, weighing 170–180 g, by intrathoracic compression of the cardiac vessels bundle at the base of the heart, with a hook-like device without major surgery (Korpachev et al. 1982). Cardio-pulmonary resuscitation was performed by external cardiac massage and artificial ventilation. The animals were sacrificed in groups of five at the end of ischemia and 5, 15, 30, 60 and 120 min after resuscitation. Five animals served as control group in which under ether anesthesia the sham-operation was performed.

Preparation of plasma samples: blood was drawn from the right ventricle into cooled polyethylene tubes and mixed with 1% of its volume of 0.5M EDTA, pH 7.5. Plasma was separated by centrifugation at 4°C (5000 g, 5 min) and stored at −20°C for assay later. EDTA acted both as an anticoagulant and as a phosphodiesterase inhibitor to prevent degradation of cGMP by plasma enzymes. Before the assay, 1 ml ethanol was added to 0.5 ml of plasma (mixed and left for 5 min at room temperature) to coagulate protein, centrifuged, the supernatant evaporated to dryness at 55°C under a stream of nitrogen, and the residue dissolved in 0.25 ml of assay buffer (Tris-EDTA).

Preparation of brain samples: brains were removed from the skull in less then 30 s, frozen in liquid nitrogen, and stored at −20°C until analyzed. Brain samples — right cerebral hemisphere — were cut off, weighed (approx. 500 mg), and extracted by homogenization in buffer containing 4 mM EDTA, followed by heating for 3 min in a boiling water bath to coagulate protein. After contrifugation the cGMP in the supernatant was assayed.

The sensitive and specific ^{3}H cyclic GMP radioimmunoassay (RIA) kit (Amersham) was used. The RIA sensitivity was within the range from 0.04 pmol up to 8 pmol per incubation tube and was performed according to Amersham's assay protocol. All samples were assayed in duplicate including standards to construct the linear calibration curve for calculations. Radioactivity was counted for 4 min in an LS 5000 TA Beckman liquid scintillation system in Bray's liquid scintillant mixture. All chemical reagents were chromatographic grade and water was deionized and double distilled in glass. Results were expressed as mean ± SD and were analyzed by Student's t-test.

RESULTS

The changes in cGMP content in the rat brain and plasma after clinical death and after resuscitation are presented in Table 1.

Table 1. Cyclic GMP in the rat brain and plasma during clinical death and after resuscitation. The values represent means ± SD from 5 animals, in pmol/g of wet brain or pmol/ml of plasma

	Control	End of clinical death	Period after resuscitation				
			5 min	15 min	30 min	60 min	120 min
Brain	130±7.9	60±9.3*	126±17.1	213±65.3*	111±9.6	114±9.6	121±34.9
Plasma	10.7±0.3	3.6±1.1*	2.8±1.0*	3.3±1.6*	3.5±0.7*	4.1±1.2*	4.2±0.5*

* Significant from control $p < 0.05$.

At the end of 5-min clinical death cGMP levels in the brain significantly decreased, on the average to 46% of control values. Five minutes after resuscitation cGMP levels significantly increased as compared with the end ischemic values and did not differ from control. At 15 min after resuscitation the levels of cGMP in the brain significantly increased reaching on the average 163% of control values. At 30 min after resuscitation the levels of cGMP decreased to control values, and did not differ significantly from the control after 60 and 120 min of recovery.

In the plasma at the end of 5-min clinical death cGMP levels significantly decreased, on the average to 34% of control values. During the whole 2 hrs period after resuscitation cGMP levels were still significantly lower reaching, on the average, 26% of control values 5 min after resuscitation and 39% after 120 min.

DISCUSSION

There were substantial alterations in content of cGMP in the brain and plasma during clinical death and after resuscitation. The changes in the brain were qualitatively similar to those found in previous studies of Lust et al. (1977) and Mrsulja et al. (1986, 1989). They evaluated changes of cAMP and cGMP, in the gerbil cerebral cortex, hippocampus, striatum, cerebellum and spinal cord following bilateral common carotid artery occlusion. In the brain structures they observed a significant decrease of cGMP levels to less than 40% of control after 1, 5, and 20 min of ischemia. In the first minute of the post-ischemic period, the cGMP levels increased to 284% of control in the 1-min ischemic group. After 5 min of resuscitation, the cGMP levels in the 5- and 20-min ischemic groups increased more than 2-fold. The most striking consensus from a variety of stroke models, techniques and species is the rapid fluctuations in brain cyclic nucleotide content following acute episodes of primary and secondary ischemia (Palmer

1985). The profound decrease of cGMP levels in plasma after resuscitation from clinical death observed in our studies, could not be confronted with results of other authors because the above mentioned data were not available in the literature.

Guanylate cyclase, the key enzyme for formation of cGMP is present in the luminal and basal membranes of rat brain capillary endothelium (Karnushina et al. 1980), the main structure of the blood-brain barrier, and cGMP modulates the function of this barrier (Joó et al. 1983).

Much recent interest in the role of endothelial cells has resulted from the discoveries that they produce relaxing factors such as prostacyclin and the endothelium-derived relaxing factor (EDRF) and vasocontrictor factors such as endothelin (Stoclet 1989). Vascular smooth muscles are relaxed by all agents which either increase cGMP production or decrease its breakdown by phosphodiesterases. In blood vessels, basal production of EDRF increases cGMP content, and stimulation of EDRF release induces a large increase in cGMP level (Vanhoutte et al. 1986). The finding that nitric oxide (NO) synthesized from arginine directly activates guanylate cyclase (Palmer et al. 1987) and the effects of EDRF (NO) in blood vessels are inhibited by guanylate cyclase inhibitors support the hypothesis that cGMP is responsible for EDRF-induced vasodilation (Stoclet 1989). It is worth emphasizing that in he clinical death model a reversible increase of cGMP levels in the brain was observed at the same time as the postischemic reactive hyperemia (Kapuściński 1987). According to Pearce (1989) hypoxia significantly increased cGMP content in the brain arteries and promoted the release of an EDRF. Cyclic nucleotides and related membrane enzyme systems might be used as target molecules to develop future therapeutic strategies for prevention or treatment of stroke (Palmer 1985).

Mechanisms of regulation of cGMP in the nervous system are at present unknown and new data are still being reported. The elevated levels of cGMP in the brain would indicate a hyperactive condition. It has been known for several years that activation of excitatory pathways in the brain can lead to large increases in the levels of cGMP, particularly in the cerebellum (Danysz et al. 1989). Recent data showed that Ca^{2+} entry leads to an increase of NO synthesis which is powerful activator of soluble guanylate cyclase in the brain, and elsewhere, and plays an important role in the regulation of blood flow (Garthwaite 1990).

On the other hand, the atrial natriuretic factor (ANF) strongly raises the level of cGMP in neuronal and glial cultured cells, independently of the presence of calcium ions, and is accompanied by release of the nucleotide (Hamprecht et al. 1990). On the basis of the presented and discussed data it might be assumed that the functions of cGMP are comparable to neurohormonal activity.

A close relationship between ANF and cGMP suggested the necessity to evaluate changes of the atrial netriuretic factor in the rat brain and plasma in the same experimental model.

POZIOM CYKLICZNEGO GMP W MÓZGU I OSOCZU SZCZURA PODCZAS ŚMIERCI KLINICZNEJ I PO RESUSCYTACJI

Streszczenie

Stosując metodę radioimmunologiczną oceniono zmiany zawartości cGMP w mózgu i osoczu szczura po 5-minutowej śmierci klinicznej i do 2 godzin po resuscytacji. W mózgu ischemia powodowała zmniejszenie zawartości cGMP, natomiast w 15 minucie po resuscytacji występował przejściowy znamienny wzrost nukleotydu. W osoczu zarówno podczas ischemii, jak i w okresie poischemicznym występowało znamienne zmniejszenie zawartości cGMP. Przedyskutowano znaczenie uzyskanych wyników na tle mechanizmów regulacji cGMP w ośrodkowym układzie nerwowym.

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ULTRASTRUCTURAL EVALUATION OF NEURONAL DAMAGES INDUCED BY LOW DOSES OF QUINOLINIC ACID IN DISSOCIATED HIPPOCAMPAL CULTURE

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The effect of a subtoxic level of quinolinic acid (QUIN) on the morphological picture of the rat hippocampus in dissociated cultures was investigated. Addition of 50 μM of QUIN to the culture medium induced only slight advanced and reversible changes of the postsynaptic elements, whereas the majority of pyramidal neurons were undamaged. The results suggest that subtoxic concentration of QUIN is insufficient to produce stable depolarization of cell membranes and severe neuronal damage.

Key words: *QUIN (low concentration), neuronal damage, tissue culture.*

Quinolinic acid (QUIN), an endogenous excitotoxic agent in the kynurenine metabolic pathway, exerting a potent neurotoxic effect, has been widely studied in different experimental models (Schwarcz et al. 1983; Wolfensberger et al. 1983; Stone, Connick 1985). The exact mechanism causing nerve tissue damages is as yet not fully understood, however, the activation of the N-methyl-D-aspartate (NMDA) receptor seems to play a crucial role in the development of neuronal injury (Stone et al. 1987). It has been also proposed, that the excitatory effect of QUIN is mediated *via* activation of NMDA receptors, although the presence of a QUIN-specific subpopulation of these receptors could not be excluded (Stone et al. 1987). A great deal of evidence indicated that QUIN might represent a putative pathogenic factor in some neurodegenerative disorders, epilepsy and aging processes in the central nervous system (Olney 1978; Coyle et al. 1981; Schwarcz et al. 1984; Stone et al. 1987). The neurotoxic effect of QUIN was evidenced in our previous studies performed both in organotypic and dissociated cultures of the hippocampus – a structure highly vulnerable to QUIN intoxication (Khaspekov et al. 1989; Kida, Matyja 1990).

The aim of the present study was to evaluate the neurotoxic effect of low, subtoxic concentration of QUIN on hippocampal neuronal elements in dissociated cell culture.

MATERIAL AND METHODS

The experiments were performed on dissociated cell cultures of hippocampus prepared from fetal mice at 15 to 17 day gestational age. Cultures were prepared as previously described by Khaspekov et al. (1989).

Dissociated cells (about 10^6 cells/dish) were plated in 15-mm plastic Falcon dishes coated with polylysine. Plating medium consisted of Eagle's Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum, glutamine (1%) and glucose (1%). Cultures were maintained at 37°C in a humidified CO_2-containing atmosphere. On the 13th to 17th day of growth *in vitro* (DIV) selected cultures were exposed to the medium supplemented with quinolinic acid (QUIN, Sigma Co) in a concentration of 50 μM. Sister cultures were kept in standard conditions. 1, 2 and 3 days post QUIN exposure the cultures were processed for electron microscopy. Selected cultures were fixed in 2.5% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in alcohols and embedded in Epon 812. Ultrathin sections were counterstained with lead citrate and uranyl acetate and examined in a JEM 1500 XB electron microscope.

RESULTS

The control, 14-day-old dissociated cultures of the hippocampal formation, revealed mature pyramidal (Fig. 1a) and granular nerve cells and more or less densely packed neuronal and glial processes with numerous synapses (Fig. 1b).

The cultures exposed to 50 μM of quinolinic acid (QUIN) showed slightly pronounced changes consisting of typical axon-sparing postsynaptic damages. One day post QUIN exposure some postsynaptic dendrites became swollen with enhanced electron lucency (Fig. 2). Their majority revealed the presence of vacuoles and/or vesicles of various size. Large vacuoles could be seen in the longitudinal sections of the dendrite in close proximity to the synaptic cleft. Sometimes, one axonal bouton formed synaptic contacts with several dendrites, each of them containing one or more vacuoles (Fig. 3). The axonal terminals did not show morphological abnormalities. The other striking feature of postsynaptic changes were mitochondrial damages. Some dendrites contained elongated, dark mitochondria with condensed matrix (Fig. 4a), whereas the others displayed severely enlarged, swollen mitochondria with disrupted, short cristae and light matrix (Fig. 4b).

The cultures exposed to 2 days of QUIN action exhibited a similar pattern of ultrastructural changes involving predominantly postsynaptic elements. Dendritic swelling accompanied by presence of vacuoles and vesicles was still the most prominent feature (Fig. 5). However, some of the postsynaptic dendrites were most severely affected and revealed, beside vacuoles and vesicles, delicate floccular material nearly filling the entire dendritic process. A few dendritic profiles

Fig. 1. Control cultures of hippocampal formation for 14 days *in vitro*: *a)* Fragment of pyramidal neuron. ×10000; *b)* Neuropil with numerous synaptic contacts (arrows). ×24000

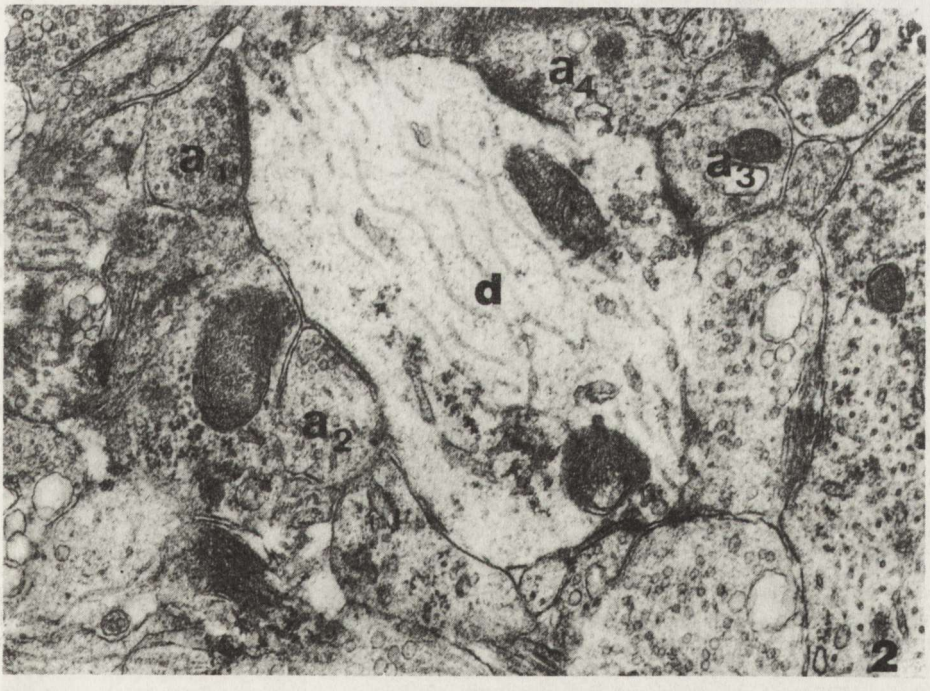


Fig. 2. One day post QUIN exposure. Slightly swollen dendrite (d) surrounded by several axonal boutons (a_{1-4}). ×20000

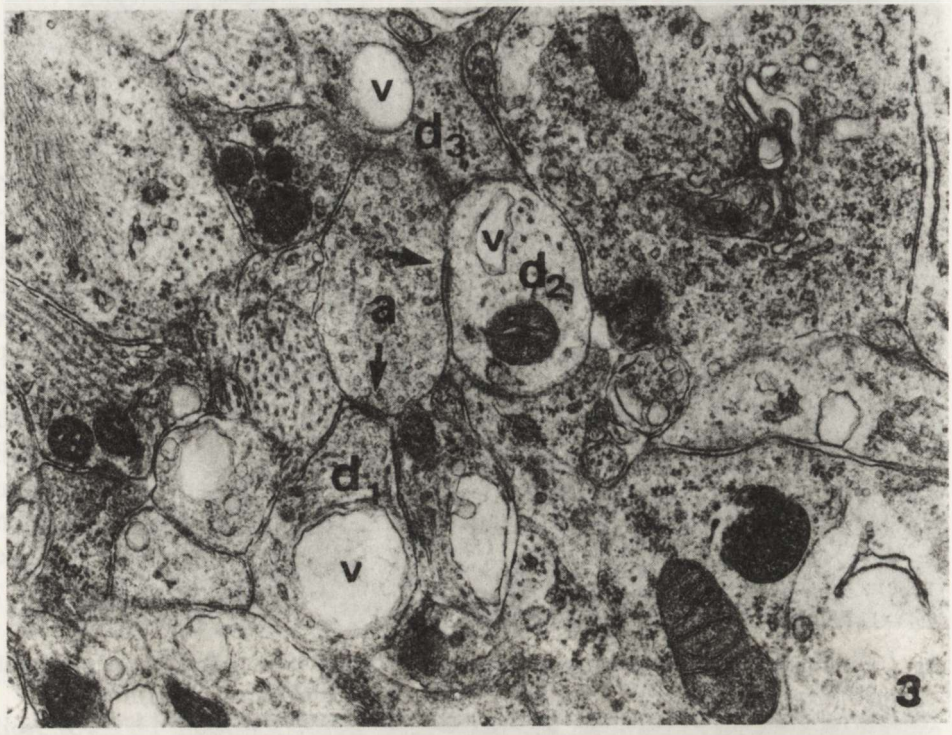


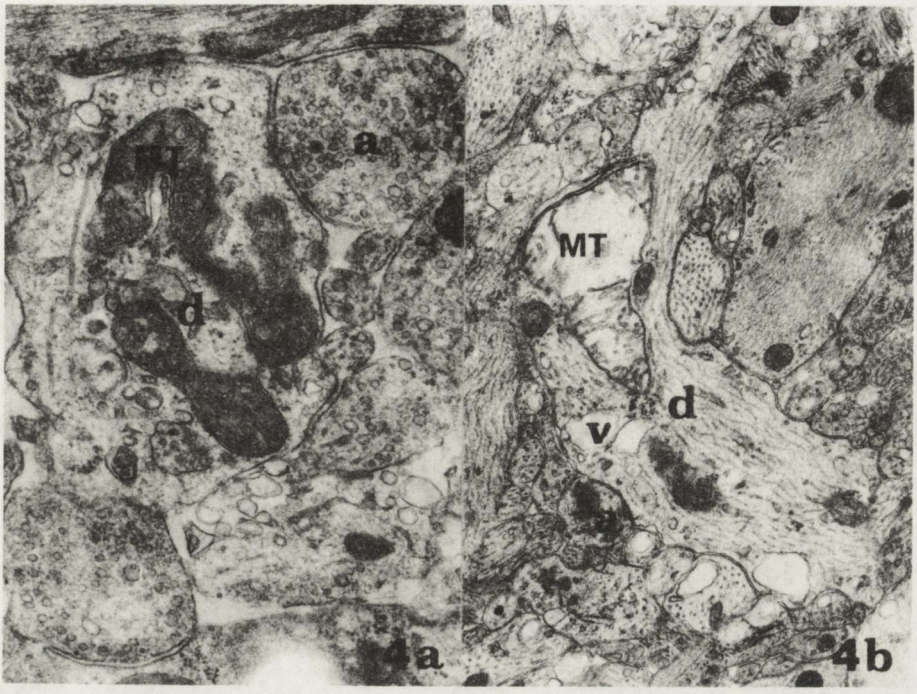
Fig. 3. The same culture. Axonal ending (a) forming synaptic contacts (arrows) with several dendrites (d) containing large vacuoles (v). ×24 000

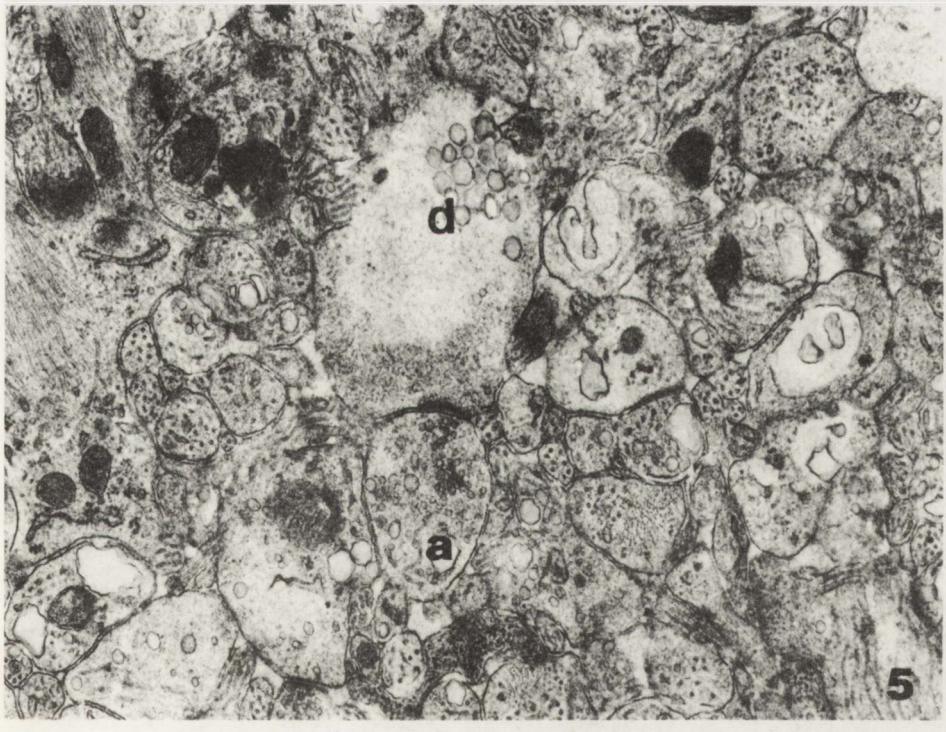
contained damaged mitochondria, vacuoles and multivesicular bodies (Fig. 6). The others were completely filled with vesicular and membraneous structures. In these two experimental groups, the nerve cells were quite well preserved and displayed normal ultrastructural features (Fig. 7). Neither were glial cells damaged, slight swelling of glial cytoplasm and processes was seen only occasionally.

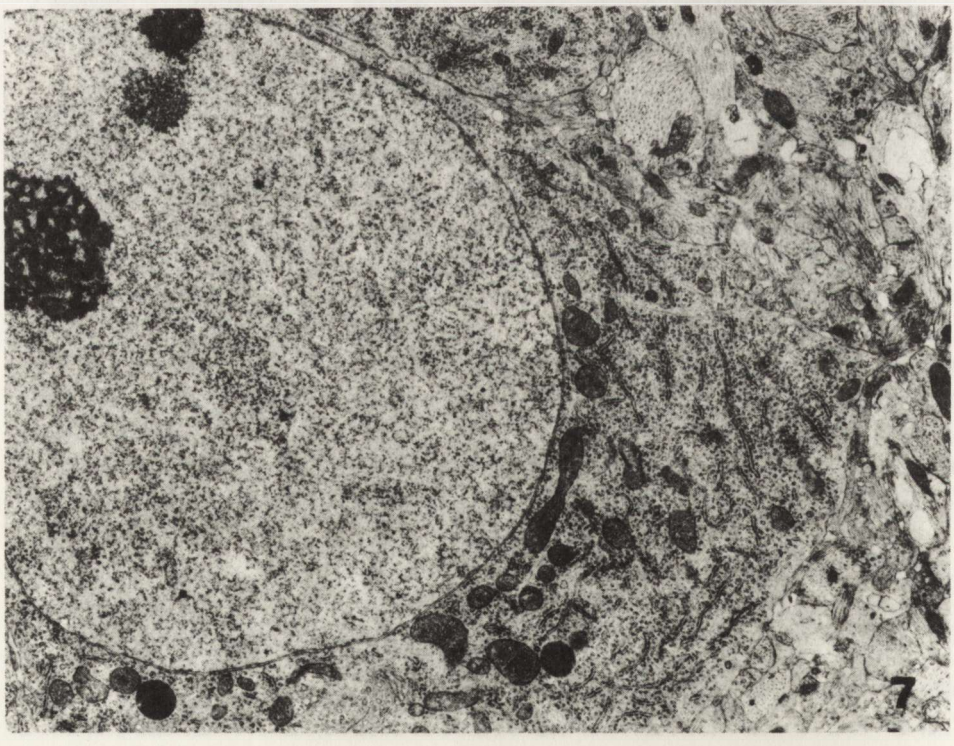
The cultures exposed to 3 days of QUIN action showed normally looking nerve cells and densely packed neuropil containing numerous well preserved synaptic contacts. A great majority of both pre- and postsynaptic elements remained intact (Fig. 8). More or less damaged dendritic processes could be seen sporadically.

Fig. 4. a) The same culture. Elongated, dark mitochondria (MT) and vesicles in the dendrite (d). Axonal bouton (a) filled with synaptic vesicles. ×24 000; *b)* Postsynaptic dendrite (d) with severely enlarged, swollen mitochondria (MT) and vacuoles (v). Small bouton (a) with synaptic contact. ×15 000

Fig. 5. Two days post QUIN exposure. Swelling of postsynaptic dendrite (d). Unchanged axon terminal (a). ×24 000







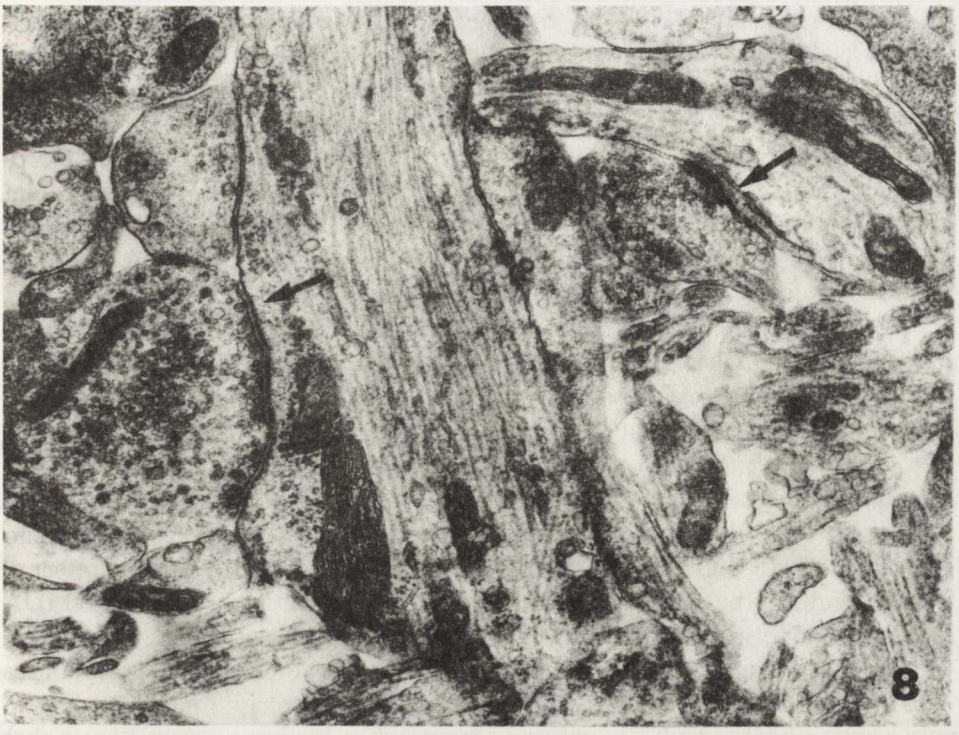


Fig. 8. Three days post QUIN exposure. Neuropil with well preserved pre- and postsynaptic endings (arrows). × 24 000

DISCUSSION

Three present ultrastructural study showed, that QUIN, applied even at a relatively low, subtoxic level, is able to induce postsynaptic changes which have been described as typical for this neurotoxin (Schwarcz et al. 1983; Whetsell, Schwarcz 1983). During the first 2 days the morphological changes consisted mostly of the presence of vacuoles and damaged mitochondria in the postsynaptic elements, whereas the neurons persisted intact independently of the time of observation. The postsynaptic changes occurred reversible and disappeared within 3 days after QUIN exposure.

The precise mechanism of the neurotoxic effect of QUIN is still not clear. The hypothesis that this agent, as other exciatatory amino acids, acts via activation of the NMDA subclass receptor has been widely discussed (Perkins, Stone 1983; Stone, Connick 1985). However, more recently the excessive calcium influx has been considered as a factor implicated in the QUIN toxicity (Coyle et al. 1981; Retz, Coyle 1984; Mayer, Westbrook 1987).

Fig. 6. The same culture. Postsynaptic dendrite (d) with damaged mitochondria, vacuoles (v) and large multivesicular body (MVB). Several well preserved axonal boutons (a). × 20 000

Fig. 7. The same culture. Well preserved pyramidal cell. × 8000

Our previously performed experiments with dissociated hippocampal cultures showed acute swelling and destruction of postsynaptic elements and severe degeneration of individual nerve cells, both being age-dependent (Khaspekov et al. 1989). The present experiment performed on a well differentiated and most sensitive to QUIN toxicity cell culture revealed a similar pattern of morphological damages, limited, however, to postsynaptic dendrites. Thus, the extent of abnormalities and their severity were not so advanced as after an axcess of this neurotoxic compounds producing both irreversible dendritic changes and pyramidal cell injury.

It could be suggested that the low subtoxic concentration of QUIN is sufficient for activation of the NMDA receptor, reflecting relatively discrete ultrastructural changes of postsynaptic dendrites. This concentration of the drug, however, proved insufficient to produce stable depolarization of the postsynaptic membrane and activate voltage-dependent calcium channels and induce Ca^{2+} influx causing leathal cell injury. It has been proved that a calcium-dependent mechanism is involved in the chronic and permanent toxicity of excitatory amino acids (Choi 1985; 1987; Choi et al. 1987), so the most potent excitotoxic efect of QUIN seemed to be connected with excessive calcium entry through the NMDA receptor channel (Tsuzuki et al. 1989). Ion substitution experiments have suggested two mechanism of excitatory amino acid toxicity: cell swelling and lysis due to osmotic imbalance by prolonged depolarization and calcium influx leading to activation of lipases and proteases and irreversible damage of organelles (Faber et al. 1981; Griffiths et al. 1983; Olney et al. 1986; Rothman 1985). The effect of the subtoxic level of QUIN might be connected with the first suggested mechanism of their neurotoxicity.

ULTRASTRUKTURALNA OCENA USZKODZEŃ NEURONALNYCH WYWOŁANYCH MAŁYMI DAWKAMI KWASU CHINOLINOWEGO W DYSOCJOWANEJ HODOWLI HIPOKAMPA

Streszczenie

Badano wpływ niskiego stężenia kwasu chinolinowego (QUIN) na obraz morfologiczny dysocjowanej hodowli hipokampa myszy. Kwas chinolinowy, dodany do medium hodowlanego w stężeniu 50 μM, wywoływał jedynie niezbyt zaawansowane i odwracalne zmiany w obrębie elementów postsynaptycznych, podczas gdy większość neuronów piramidowych pozostawała nie uszkodzona. Wyniki sugerują, że subtoksyczne stężenie QUIN jest niewystarczające do wywołania trwałej depolaryzacji błony komórkowej oraz znacznego uszkodzenia elementów neuronalnych.

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HEMIBALLISMUS IN PATIENT WITH BLASTIC PHASE OF CHRONIC MYELOGENOUS LEUKEMIA

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The authors report the clinical course and neuropathological findings in the case of hemiballismus due to leukemic infiltration of the subthalamic nucleus. The symptoms were observed in a 19-year-old patient with blastic phase of chronic myelogenous leukemia, two weeks before death, when what is called the "critical point" developed (white cell count beyond 100 G/l and thrombocytopenia less than 50 G/l). The investigation are considered to confirm the role of the "critical point" in the development of central nervous system leukemic complications.

Key words: *hemiballismus, leukemia, subthalamic nucleus.*

Hemiballismus is a very uncommon extrapyramidal syndrome consisting in damage of the subthalamic nucleus (SN) or its surroundings (Crossman 1989; Hanaoka et al. 1990). The exact pathomechanism of this syndrome remains unclear, it is suggested, however, that it is due to the disappearance of the inhibitory influence on SN of other basal ganglia such as striatum, pallidum (Dewey, Jankowic 1989; Hidaka et al. 1989; Destee et al. 1990). The onset of hemiballismus is usually explosive, because it occurs mostly as sequel of vascular disorders in, or near the SN. Other causes of this syndrome are only rare. They are: lupus erythematosus, neoplasms (meningiomas, angiomas, metastases from gastric cancer), toxic agents, degenerative changes (Gilon et al. 1990; Hanaoka et al. 1990; Havsager, Carstensen 1991; Burnett, Jankowic 1992; Laing, Howell 1992). Occasionally, SN destruction has been found in brains of patients without previous clinical manifestations of hemiballismus (Oppenheimer, Esiri 1992).

We report the clinical course and results of neuropathological examination in a case of hemiballismus with leukemic infiltrates within the SN in a patient with blastic phase of chronic myelogenous leukemia. We will try to explain the evolution of leukemic infiltrates in the brain and their relation to this rare neurological syndrome.

CASE REPORT

A 12-year-old boy was hospitalized in 1984 with a diagnosis of chronic myelogenous leukemia. In spite of systemic chemotherapy with busulfan and hydroxyurea, hematological remission was not achieved. During the chronic phase of leukemia the white cell count was elevated, but less than 30 G/l. After 65 months duration, the disease transformed to the blastic phase. Leukocytosis was permanently higher than 100 G/l, in the range of 132 to 240 G/l (81–95% of myeloblasts). Due to chemotherapy with 6-merkaptopurine, vincristine and prednisone the white cell count was periodically reduced to 14–20 G/l, but not for longer than a few weeks. The growth fraction (Ki-67) of leukemic cells in peripheral blood was low (2.5–3.5%). Platelet count ranged from 350–110 G/l, but within two weeks before death it markedly decreased to 65–36 G/l, leading to clinical bleedings. Throughout the blastic phase were noted anemia and a chronic disseminated intravascular coagulation syndrome. Serum laccid dehydrogenase ranged from 580–290 u/l. The patient died after 5 months duration of the blastic phase, at the age of 19, owing to cerebral bleeding, within a one-week lasting period of coexistence of hyperleukocytosis 280 G/l and thrombocytopenia 35 G/l.

Two weeks prior to death, involuntary movements of the left shoulder and upper limb appeared, including abduction, adduction, internal and external rotation of the arm and extention of the forearm. The onset of the extrapyramidal syndrome was insidious and gradually developed during a couple of days. The movements were moderate with fluctuation in their intensity, and disappeared

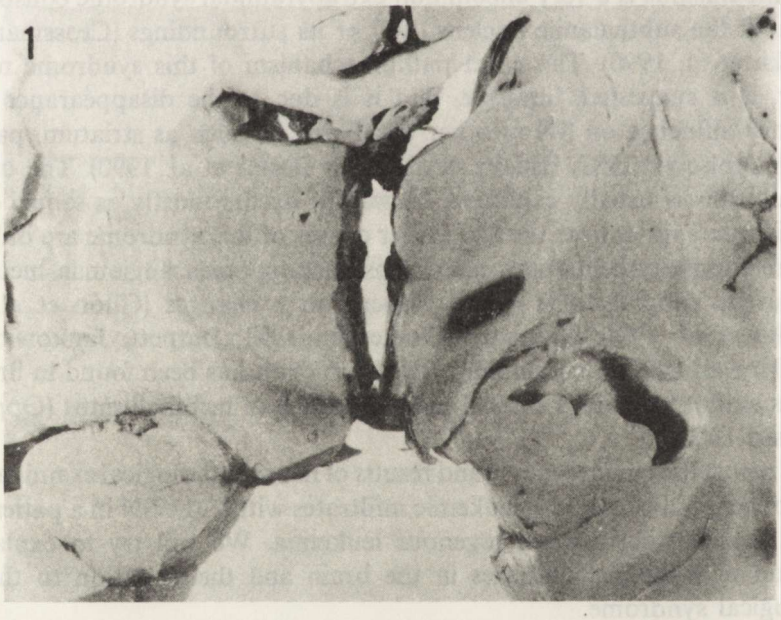

Fig. 1. Coronal section of basal ganglia revealing hemorrhage in the subthalamic nucleus. The hemorrhage has a central pale area representing intracerebral leukemic infiltrate

during sleep. They persisted until death. Neurological laboratory examinations were within normal limits.

Discharge diagnoses were blastic phase of chronic myelogenous leukemia, left-side hemiballismus probably due to leukemic infiltrate within the right SN, and CNS-bleeding. Autopsy (M. Markiewski M. D., Department of Pathology) revealed leukemic infiltrates within the spleen, lungs, heart, liver, kidney and lymphnodes, as well as numerous petechias. Multiple coronal sections of the brain showed multiple variable-sized hemorrhagic lesions in the white matter of both cerebral hemispheres, brain stem and cerebellum. One of them was located in the right SN (Fig. 1).

Microscopic examination was performed on paraffin-embedded material stained with hematoxylin-eosin, cresyl violet, van Gieson, Holzer, and Heidenhain methods, and immunostaining with DAKO-elastase in the indirect immunoalkaline phosphatase-antyalkaline phosphatase method (APAAP). It revealed that all hemorrhages were filled with massive infiltrates of leukemic cells (Fig. 2). The cells were usually well-preserved, in spite of SN hemorrhagic lesion

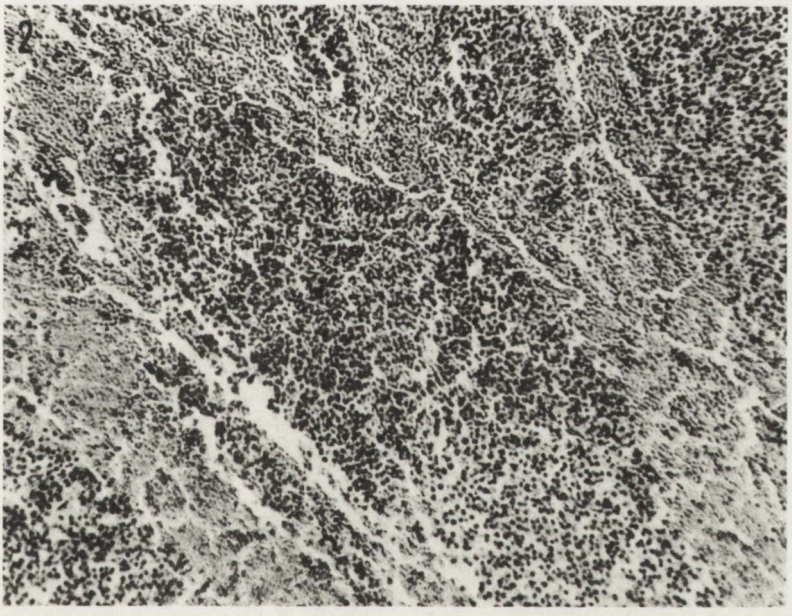

Fig. 2. Well-preserved leukemic infiltrates within brain hemorrhage. H.E. × 200

in which areas of pronounced blastic cell degeneration were seen (Fig. 3). In the neighbourhood of hemorrhagic lesions appeared areas of tissue edema. In all vessels marked leukostasis was present in the brain and leptomeninges, whereas perivascular leukemic infiltrates in the brain were seldom observed, mainly around thin-walled veins located in white matter. Leukemic cells penetrated from the vessel lumen across the wall into the perivascular space, but they did not

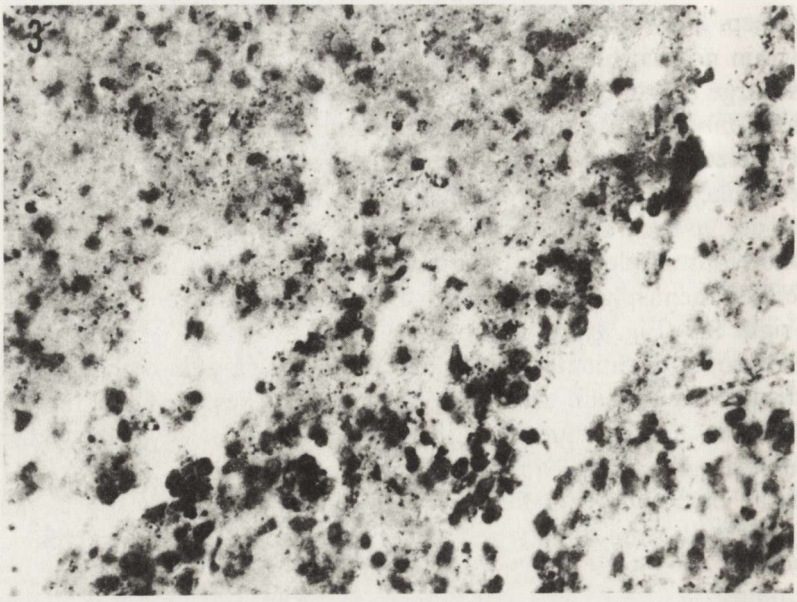

Fig. 3. Necrotic changes within leukemic infiltrate observed in hemorrhage of subthalamic nucleus. Cresyl violet. ×400

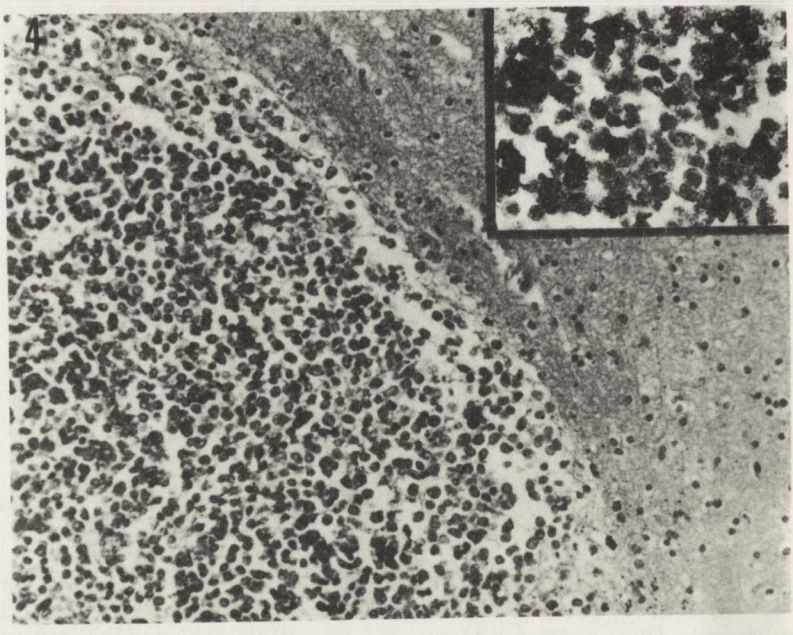

Fig. 4. Intracerebral leukemic infiltrate of left frontal lobe. H.E. ×200. Top right: neutrophil elastase-positive leukemic cells. ×400

infiltrate nervous tissue. In subcortical white matter of the left frontal lobe there was leukemic infiltrate with clear-cut border (Fig. 4) surrounded by fibrillary gliosis (Fig. 5). Meningeal leukemic infiltrates were but little pronounced. They were usually observed between the cerebellar lobules and at brain base. The growth fraction of leukemic cells (Ki-67) within the CNS was absent.

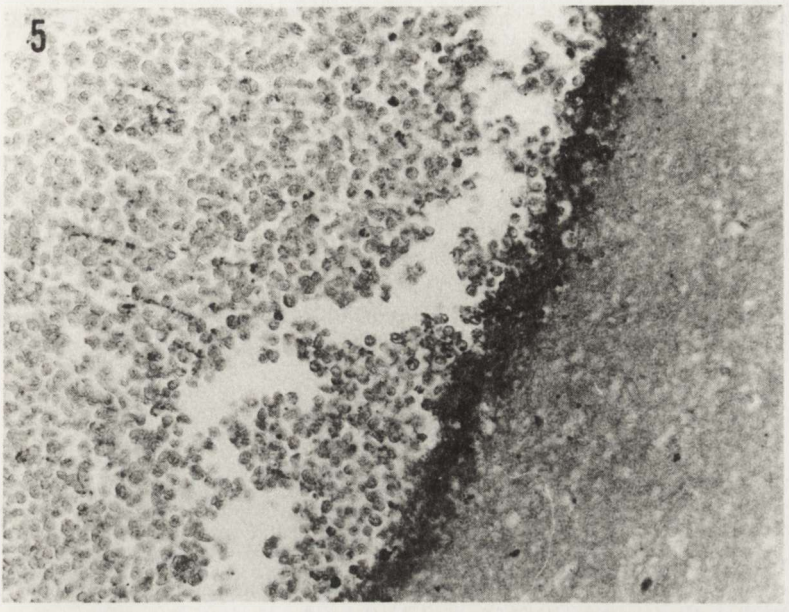

Fig. 5. Fibrillary gliosis around leukemic infiltrate presented in Fig. 4. Holzer. ×200

DISCUSSION

I our case involuntary movements of the left arm and upper limb appeared two weeks before death. The onset of hemiballismus was insidious, and clinical symptoms were moderately manifested contrary to the violent and very expressive clinical course of ballismus related to bleeding in the SN (Burnett, Jankowic 1992; Laing, Howell 1992). It would seem, that in our case the extrapyramidal syndrome was dependent on the leukemic infiltrate which developed in the SN within the last several weeks of the patient's life. Degenerative changes of blastic cells within fresh bleeding confirm the possibility of relatively long-lasting blastic infiltrates within the SN. It may be confirmed by the fact that neurological disturbances appeared during pronounced hyperleukocytosis (168 G/l), distinctly exceeding 100 G/l – of the white cell which is considered to be responsible for CNS leukemia. Thrombocytopenia was lower than 50 G/l – thus the period could be named "critical point" (Nowacki, Zdziarska 1993).

Apart from the leukemic infiltrate in the frontal lobe all remaining leukemic foci, including SN lesion coincide with hemorrhagic changes. The clinical course and neuropathological examinations allow to suppose, that all CNS leukemic

infiltrates with the exception of the SN, appeared during the last days of the patients life and were initially too small to cause clinical manifestations. The infiltrates probably induced hemorrhagic changes before death, as it was previously observed (Nowacki 1987), and beside coma, they were not able to produce "focal" neurological symptoms. This sequence of events based on our observation presented above may explain the appearance of hemiballismus, a rare extrapyramidal syndrome in a patient with fatal outcome of leukemia.

HEMIBALIZM U CHOREGO W FAZIE BLASTYCZNEJ PRZEWLEKŁEJ BIAŁACZKI SZPIKOWEJ

Streszczenie

Przedstawiono obraz kliniczny i zmiany neuropatologiczne w przypadku hemibalizmu na tle nacieku białaczkowego w jądrze podwzgórzowym. Objawy wystąpiły u 19-letniego chorego w fazie blastycznej przewlekłej białaczki szpikowej na dwa tygodnie przed zgonem, w okresie tak zwanego punktu krytycznego (współistnienie leukocytozy ponad 100 G/l z małopłytkowością poniżej 50 G/l), co potwierdza jego rolę w powstawaniu powikłań białaczkowych w ośrodkowym układzie nerwowym.

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EFFECT OF CHRONIC ADMINISTRATION OF SODIUM VALPROATE ON THE MORPHOLOGY OF THE RAT BRAIN HEMISPHERES

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Effective doses of sodium valproate (200 mg/kg) applied in rats chronically (1, 3, 6, 9 and 12 months) evoked the first morphological changes in the brain hemispheres after 9 months of drug administration. Structural abnormalities of the brain tissue consisted in disseminated nonspecific neuronal lesions and patchy nerve cell loss, more pronounced in the final phase of the experiment. The neuronal lesions were localized predominantly in the 3rd and 5th layers of the neocortex and in the pyramidal cell layer of the hippocampus. They were accompanied by vascular wall alterations, perivascular tissue damage, as well as by microvacuolar changes and spongy degeneration of the subpial and periventricular regions. Vasogenic character of parenchymal changes is stressed by the authors. The possible influence of liver damage on the development of brain pathology is discussed.

Key words: *sodium valproate, brain damage.*

A long-term therapy with valproic acid (VPA), an anticonvulsant drug, or its derivatives bring about functional disturbances in the central nervous system (CNS), manifested by, i.e. bilateral intermittent arm tremor, exacerbated by action, postural tremor, head shaking while drinking, and they were found not to be without harmful effect upon the nerve tissue (Mattson et al. 1978; Hyman et al. 1979; Lautin et al. 1979; Cramer et al. 1984; Majkowski 1986; Sobaniec 1991). Sobaniec (1991) in group of 60 children chronically treated with sodium valproate in 5.1% of cases observed neurological side effects like tremor, ataxia, paresthesias, and in 17% behavioural disturbances (drowsiness, hyperexcitability). According to Cramer et al. (1984) almost in 45% of patients receiving VPA, EEG recordings showed an increase in the mean number and duration of spike wave discharges at one hour post-dose compared to pre-dose.

The presented paper is a continuation of our earlier studies on the clinical, pathomorphological and biochemical effects of chronic application of VPA in epileptic children and its administration to animals under experimental conditions (Sobaniec 1989; 1991; Sobaniec et al. 1987; 1988; 1989a, b). Previously we

have described structural changes in the cerebellum and brain stem of rats to whom VPA was chronically administered (Sobaniec et al. 1989a). In both investigated structures tissue alterations were found after 6 months of drug application becoming more intensive in rats treated with VPA for 9 and 12 months. Neuropathological abnormalities were expressed by neuronal alterations, swelling of endothelial cells and tissue spongiosis. Pathomorphological evaluation of the brain hemispheres seems to us purposeful as in the available literature we did not find sufficient data on this subject.

MATERIAL AND METHODS

Hundred fifty male Wistar rats (body weight 160 – 180 g) preselected according to clasical pharmacological screening tests were used for experiments. Hundred rats divided into 5 groups (each consisting of 20 animals) were given VPA for 1, 3, 6, 9 and 12 months. Control group (6th) consisted of 50 rats matched according to the age and body weight with those of the experimental group animals. Sodium valproate (Vupral, Polfa) dissolved in physiological saline was administered through a metal gastric tube at the dose of 200 mg/kg of the body weight, once daily, before feeding (Frey, Janz 1985). More details have been reported in our previous reports (Sobaniec et al. 1989a, b). Neurologic and somatic state of the animals was checked during the whole experiment. The body weight of rats was inspected fortnightly making adequate correction of the drug dose. At the end of experiment every animal out of the 6 groups was subjected to an intracardiac perfusion with a neutralized 10% formaldehyde solution in the physiological saline under the pressure of 80 to 100 mm of Hg after which the rats were decapitated and the heads were placed in the perfusion fluid for 24 hours. Then, the brains were removed from the skulls and cut frontally at the levels of optic chiasm, fully developed basal ganglia, mesencephalon, pontine region and at the bulbar region, close to the cerebellum. The tissue blocks were proceded routinely to paraffin-embedded sections. Histological staining methods were Mayer's hematoxylin and eosin (HE), Klüver-Barrera, Heidenhain, Kanzler-Arendt's and Gomori impregnation.

RESULTS

Gross brain examination revealed moderate thickening, edema and hyperemia of leptomeninges in most of the rats of the groups 3rd, 4th and 5th.

Microscopical examination: the tissue changes in the brain hemispheres were found in groups 4 th and 5th, i.e. after 9 and 12 months of VPA application. Due to their similarities they are described jointly. The structural abnormalities were disseminated, nonspecific and of various intensity in particular hemispheric regions. Total tissue disintegration was rare. Neuropathological changes consisted in neuronal degeneration, alteration of blood vessel wall and perivascular tissue damage, microvacuolization and spongy tissue degeneration.

Neuronal changes were mostly expressed by the appearance of shrunken and hyperchromatic neurons (Figs 1 – 3) with features of chronic nerve cell degeneration, less frequently of cell sclerotization. They occurred either separately, or they

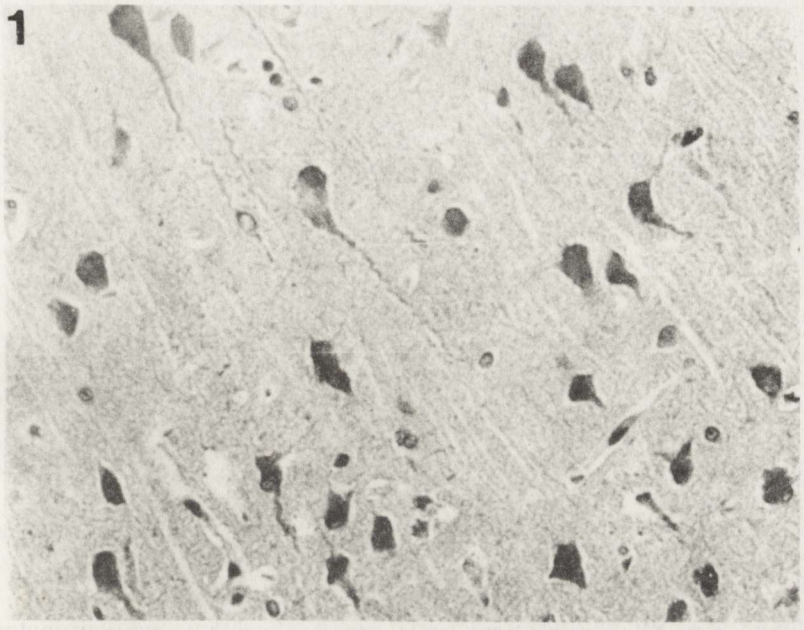


Fig. 1. Shrunken and hyperchromatic neurons in temporal cortex. 9 months of VPA administration. Klüver-Barrera. × 160

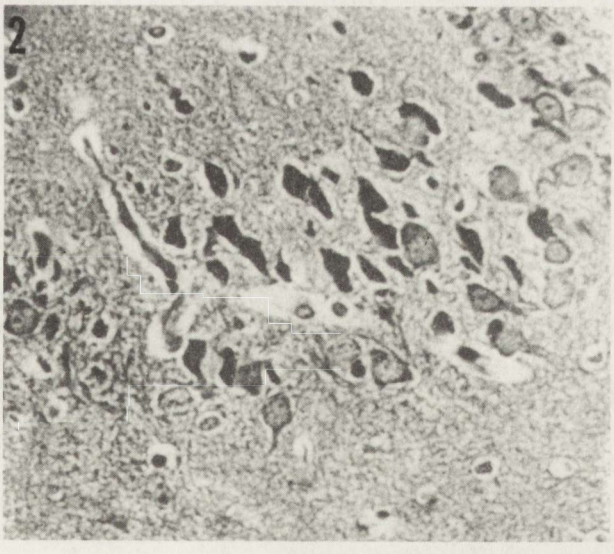


Fig. 2. A group of markedly shrunken hyperchromatic neurons in pyramidal layer of the hippocampus. 12 months of VPA administration. Klüver-Barrera. × 160

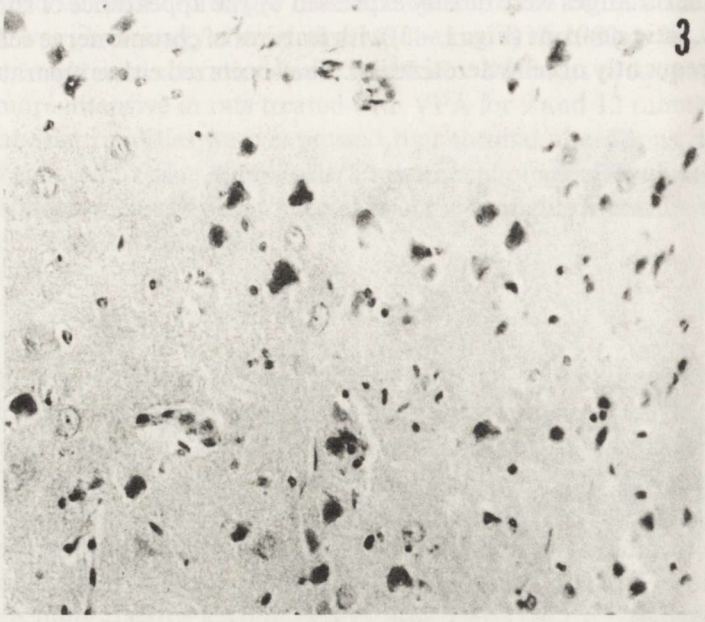
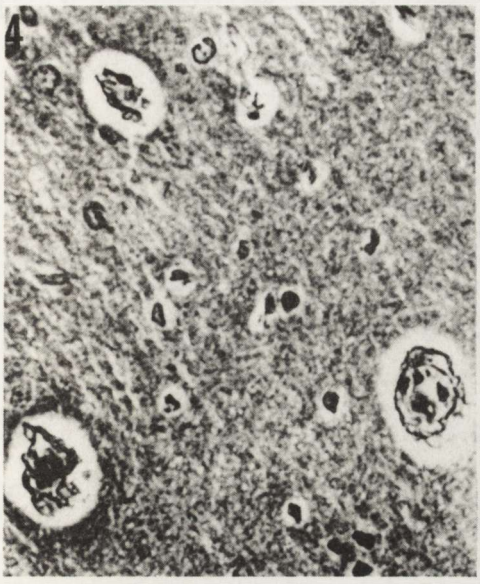


Fig. 3. Temporal cortex. Hyperchromatic neurons in close vicinity to blood vessels with markedly widened perivascular spaces. 12 months of VPA administration. HE. ×80

were spread or grouped among unaltered nerve cells. Damaged neurocytes were found in neocortex, mainly in the 3rd and 5th layers, and particularly in temporal cortex (Fig. 1) and pyramidal cell layer of the Ammon's horn (Fig. 2). They were also observed in the vicinity of blood vessels (Fig. 3). Beside hyperchromatic neurons shadows of nrve cells (ghost cells) were observed as well as nerve cell loss in the form of microhollows after brokendown neurons, small areas of nerve cell rarefactions or areas completely devoid of nerve cells. More extensive loss of cortical neurons was seen mainly in rats of the 5th experimental group. In the subcortical nuclei moderately hyperchromatic neurocytes appeared occasionally and did not alter markedly a general morphological picture of the structural abnormalities of the cerebral hemispheres. A concomitant glial reaction was rather scanty. It was expressed by the appearance of activated astroglial and microglial cells. Sometimes enlarged and depleted of chromatin astrocytic nuclei with well defined nuclear membrane resembled Alzheimer glia type II.

An essential morphological abnormality found after 9 and 12 months of VPA administration was the damage to the vascular walls, mainly of arterial vessels in the white and grey structures. It was expressed by swelling and proliferation of endothelial cells what caused thickening of the vessel wall and narrowing of the vascular lumen (Figs 4 and 5). In this cases the vascular wall structure was often blurred. Around these altered blood vessels perivascular spaces were frequently enlarged (Figs 4 and 5) and contained exudate fluid and blood morphotic elements, while surrounding tissue structure was loosened (Fig. 6), with or



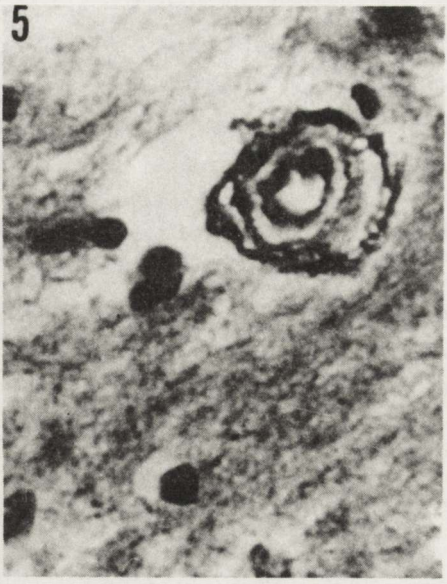


Fig. 4. Small arterioles with swollen endothelial cells and narrowed lumen in cortical marginal layer. 9 months of VPA administration. Gomori impregnation. × 120

Fig. 5. Small artery with thickened wall and narrowed lumen in temporal white matter. 12 months of VPA administration. × 260

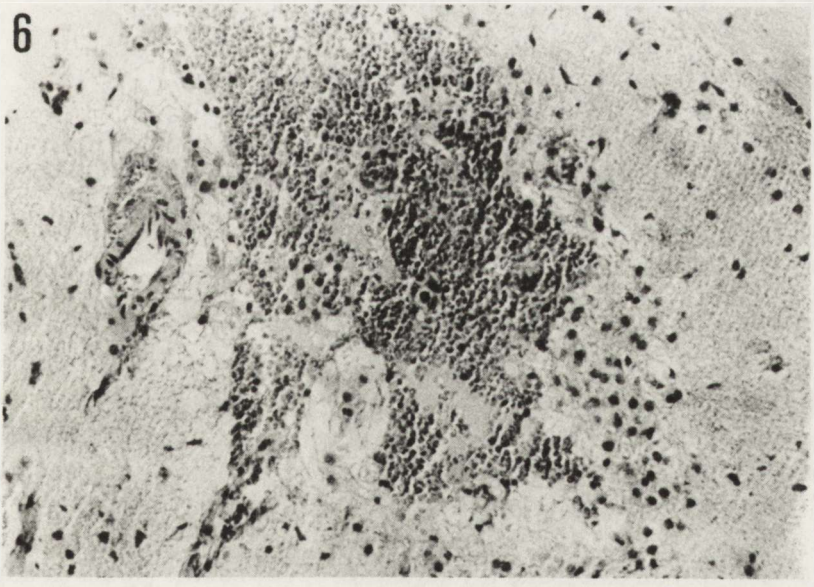


Fig. 6. Recent hemorrhagic focus in white matter. An artery with thickened wall is visible. 12 months of VPA administration. HE. × 80

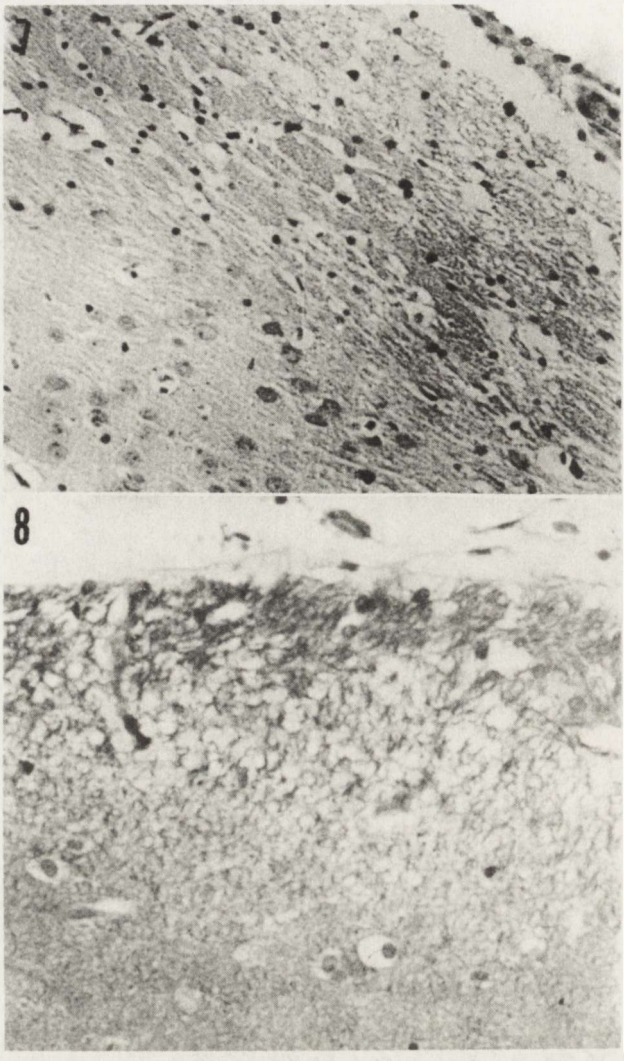


Fig. 7. Microvacuolization in marginal layer of temporal cortex. 9 months of VPA administration. HE. × 160

Fig. 8. Spongy degeneration in subependymal white matter around lateral ventricle. Klüver-Barrera. × 160

without alterations of nerve fibers and their myelin sheaths. In single animals recent perivascular erythrorrhagies or small perivascular hemorrhages (Fig. 6) were noted. The subpial marginal layer of cerebral cortex and subependymal white matter around lateral ventricles exhibited microvacuolization or even spongy changes (Figs 7 and 8). The subependymal spongy degeneration was associated by myelin pallor and myelin sheath abnormalities (swelling, fragmentation, finegranular breakdown). Quite often hemispheric white matter showed loosened structure with the presence of microvacuoles in which shrunken oligodendroglial nuclei could be found.

Choroid plexus of the lateral ventricles often was swollen and congested (Fig. 9).

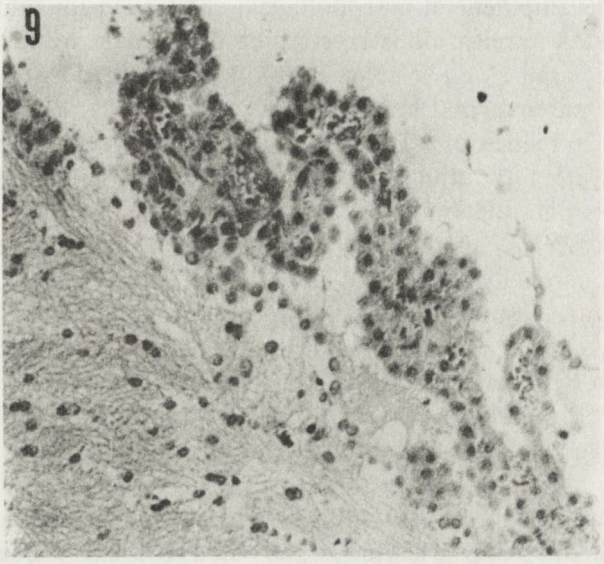


Fig. 9. Swollen and congested choroid plexus of the lateral ventricle. HE. ×80

DISCUSSION

Our studies revealed that VPA given to rats in an effective dose during experiments lasting from 1 up to 12 months exerts damaging effect on the animal's brain hemispheres barely after 9 months, whereas cerebellum and brain stem became injured by this drug earlier, i.e. after 6 months (Sobaniec et al. 1989a), probably due to their greater sensitivity to the VPA action.

Structural abnormalities of the brain hemispheres, likewise in the cerebellum and brain stem, were predominantly disseminated. They had features of nonspecific neuronal lesions which became intensified during the final stage of the experiment. Neuronal changes coexisted with lesions of the blood vessel walls in both grey and white matter, perivascular tissue damage, microvaculization of some brain areas and spongy degeneration of the subpial and subependymal regions. The above and previously described (Sobaniec et al. 1989a) morphological abnormalities evidence toxic action of long-term VPA application upon the brain tissue. The described brain abnormalities are nonspecific and were observed in various experimental models: after long-term administration of neuroleptics (oxazepam and proptylin) (Herman et al. 1978), following application of cytostatic CCNU (Lomustine) (Maziarz, Szczech 1984), in rats subjected to cobaltous acetate intoxication (Bugera, Śmiałek 1986) as well as in hepatogenic encephalopathies (Mossakowski 1981), in which however, the appearance of astrocytic changes with naked nuclei formation is the most remarkable patho-

logical abnormality. Irrespective of qualitatively similar structural alterations different pathogenic mechanisms are engaged in their development in various types of experimental models. Our results seem to indicate that an important effect on the development of morphological changes in rat brain hemispheres after chronic VPA application is excerted by the damage to the blood vascular system and increased blood vessel permeability, evidenced by the character and topography of parenchymal lesions.

The direct influence of VPA or its metabolites as the cause of vascular damage seems rather doubtful, as the long-term drug administration was without harmful effect upon the brain. Many author share the opinion that undue concentrations of VPA can indirectly damage the central nervous system exerting toxic effect on parenchymal organs like kidneys and liver, that in turn evokes hyperammonemia responsible for valproate encephalopathy (Coulter, Allen 1980; Thurston et al. 1981; Imler et al. 1982; Warter et al. 1983a, b; Matsumoto et al. 1984; Strolin-Benedetti et al. 1984; Compostrini, Muclow 1985). Scheffner et al. (1988) observed fatal failure of liver in 16 VPA treated epileptic children and the role of kidneys in valproate-induced hypermmonemia was also considered (Warter et al. 1983a, b).

The influence of valproic acid-induced hepatic damage on the development of brain damage in our material should be considered too, as in the same experimental condition the first VPA-induced morphological lesions in the liver appeared after three months of drug application, i.e. markedly earlier than in the brain (Sobaniec-Łotowska et al. 1993). The most prominent microscopical alterations consisted in degenerative changes involving principally hepatocytes in periportal regions (extensive steatosis and vacuolar degeneration, focal hepatocyte necrosis) associated with chronic inflammatory infiltrates and vascular disturbances. Similar fatty degeneration of hepatic cells of rat liver found Lewis et al. (1982) after short-term application of high VPA doses (750 mg/kg). Liver lesions of the same type, but differently localized were described also in patients treated chronically with valproic acid or it salts. In biopsy and autopsy material in the centrilobular zone hepatic cells steatosis and lysis, erytrorrhagies and inflammatory infiltrates were noted (Jeavons 1983; Scheffner et al. 1988; Zimmerman, Ishak 1982). One can not, however, exclude still other mechanisms of neurotoxic action of VPA used in chronic experimental model that have to be elucidated in further studies.

CONCLUSIONS

1. The first neuropathological changes in the rat brain hemispheres caused by a long-term VPA administration appear not earlier than 9 months after the start of experiment and become intensified after longer drug application.

2. The neuropathological picture was dominated by vascular changes and lesions of vasogenic origin. Neuronal alterations confined mostly to the cerebral cortex were rather related to vascular damage and tissue edema than to direct VPA action.

3. The valproate-induced encephalopathy seems to be secondary to blood vessel damage caused by factor or factors originating in the course of toxic VPA action on the internal organs, i.e. liver or kidneys.

WPŁYW PRZEWLEKŁEGO STOSOWANIA WALPROINIANU SODU NA OBRAZ MORFOLOGICZNY PÓŁKUL MÓZGU SZCZURA

Streszczenie

Przeprowadzone badania histologiczne wykazały, że lek przeciwpadaczkowy – walproinian sodu (Vupral, Polfa), przewlekle stosowany u szczurów w dawce 200 mg/kg m.c. na dobę, powoduje pierwsze zmiany morfologiczne w półkulach mózgu dopiero po upływie 9 miesięcy. Stwierdzone nieprawidłowości strukturalne miały charakter rozsiany i były bardziej nasilone po 12 miesiącach podawania leku.

U zwierząt doświadczalnych obserwowano nieswoiste uszkodzenia neuronów, przede wszystkim III i V warstwy kory nowej oraz zakrętu hipokampa, o cechach schorzenia przewlekłego i stwardnienia neuronów oraz ubytki komórek nerwowych. Towarzyszyło im uszkodzenie łożyska naczyniowego oraz naczyniowopochodne uszkodzenie tkanki w otoczeniu zmienionych naczyń, mikrowakuolizacja i zgąbczenie podoponowego obszaru kory i podwyściółkowej istoty białej w otoczeniu komór bocznych, z uszkodzeniem elementów strukturalnych tkanki w ich obrębie.

Autorzy zwracają uwagę na naczyniopochodny charakter obserwowanych zmian i sugerują ewentualny udział uszkodzenia wątroby w patomechanizmie rozwoju zmian w mózgu.

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ABSTRACTS

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Z. ADAMCZEWSKA-GONCERZEWICZ, J. DORSZEWSKA, M. WENDER, A. GROCHOWALSKA

IDENTIFICATION AND DISTRIBUTION OF FREE STEROLS IN THE RAT BRAIN WHITE MATTER AFTER MODERATE HYPOXIA

Department of Neurology, School of Medicin, Poznań

The effect of moderate hypoxia on the content and composition of free sterols in the brain white matter was studied. The experiments were performed on female Wistar rats which were kept for 30 minutes in a chamber filled with a mixture of gases containing 7% oxygen. The animals were sacrificed 4 and 24 hours and 14 and 60 days after the experimental hypoxia.

From the isolated myelin, the neutral lipids were extracted and separated by means of gas chromatography coupled to mass spectrometry, the DB-1701 column being coupled to Hewlett-Packard mass detector.

The applied GC-MS method demonstrated for the first time, in the myelin of rats subjected to experimental hypoxia, the occurrence of number of free sterols with molecular masses ranging from 368 to 426. Cholesterol was dominating sterol species present in the myelin in all the studied periods, its percentage decreasing until the latest period under investigation, i.e. 2 months after the experimental hypoxia. The next free sterol in range, found in appreciable amounts, was probably 4,4,14α-trimethyl-5α-cholest-24-en-3-one, with a molecular mass of 426, its percentage being the highest in the latest period studied. Other sterols, such as desmosterol, di- and trimethyl sterols were found in the isolated myelin fraction during all the studied periods following the hypoxia at much lower concentrations, almost in trace amounts.

This persistence of appreciable amounts, of rare free sterols, which under physiological conditions are present only in trace amounts, is indicative of some inefficiency of certain metabolic steps, such as the isomerisation of sterols at the delta^{8} and delta^{7} position to delta^{5}, as well as of a deficient reduction of the double bond at delta^{24}. These effects might have been caused by free radicals generated by the hypoxia.

The results also indicate that the degradation processes occurring in the lipid rich brain membranes immediately following the hypoxia state persist over a long period of time (at least up to 2 months, the longest period under study).

D. ADAMEK[1], K. STACHURA[2]

BALOON CELL MELANOMA OF CEREBRAL HEMISPHERE: A CASE REPORT AND THE MORPHOLOGICAL ANALYSIS OF 30 MELANOMA CASES OF THE BRAIN

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The case of cerebral hemisphere tumor of unusual histopathological type, localized in cortex and subcortically is presented. The superficial cortical multilocular and diffuse infiltrate consisted of uniformly looking cells rich in melanin. In the subcortical white matter just beneath the cortical infiltrate there was a main, well demarkated spherical tumor mass formed by almost indentical large cells with very small centrally placed nuclei and ample, bright, slightly foamy cytoplasm devoid of any pigment. These cells were tightly packed and had very distinct margins resembling epithelium. In some of them mitotic figures were noted. The results of immunohistochemical investigations of these unusual cells were as follows: EMA, vimentin and desmin: negative; S-100 protein: positive; antigen CD68 (macrophage marker): negative. The patient at the time of surgery had no suspected melanotic dermal or mucosal changes. No other features of neoplastic disease except the brain tumor were noted. Baloon cell, epithelioid, unpigmented infiltrate may become the source of diagnostic mistakes falsely suggesting either carcinoma or massive macrophageal infiltration. The histogenetic considerations on baloon cells were based on the analysis of 30 melanoma cases of the brain.

M. BARCIKOWSKA

ORGANIZATION OF BRAIN ISCHEMIC INFARCTION. AN IMMUNOHISTOCHEMICAL STUDY

Department of Neuropathology, Medical Research Centre, PASci, Warszawa

The main task of the study was to describe brain necrotic focus organization with astroglial and microglial cells participation, as revealed by immunohistochemistry.

Forty cases (mean age 83.4 years) of brain ischemic infarction divided in 4 groups according to the time of onset were examined. Data from 24h, 2–7, 8–16, 19–29 and 31–102 days after the stroke were analyzed. Tissue samples from the margin of infarction with the necrotic focus were taken from each brain. After routine stainings (H&E and Klüver-Barrera) ABC immunohistochemistry with diaminobenzidine as chromogen was performed. To visualize astroglia GFAP and to visualize microglia anti-ferritin serum and RCA-1 lectin were chosen.

It was of interest that two stages of astroglial reaction could be observed. One, very severe, during the first week after artery occlusion and another 4 weeks after the stroke onset. Ramified microglia presented similar pattern of appearance. Contrary to astroglial and ramified microglial proliferation in time, macrophages appeared in the necrotic tissue soon after the 2nd day with maximum intensity between the 7th and 28th day from the beginning. Anti-ferritin positive macrophages were noted in smaller number after 28 days when compared with RCA-1 positive macrophages.

On the margin of infarct, proliferated vessels were labeled by RCA-1 from the 7th to the 28th day of organization.

Anti-ferritin antibody can be used as a marker for the most active stage of macrophage cell, lasting from 2 to 28 days of stroke. Contrary to more stable period of macrophages activation after 4 weeks which is better labeled by RCA-1.

M. BARCIKOWSKA

PUTATIVE ROLE OF GLIA IN BRAIN AMYLOIDOSIS

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Glia participation in cascade of events leading to the appearance of amyloid within neuropil was a subject of a study.

Seven Creutzfeldt-Jakob (CJD), three progressive supranuclear palsy (PSP), three Gerstman--Straüssler syndrome (GSS), ten Alzheimer's disease (AD), ten Parkinson's disease (PD) and six normal aged cases were examined.

Routine stainigs (H&E, Klüver-Barrera) and Yamamoto silver impregnation, preceeded avidin--biotin immunohistochemistry. The following antibodies were used: 4G8 for A 4, GFAP, RCA 1, LN 1, anti-ferritin, for visualization of astroglia and microglia, respectively. Anti-tau 1 and 3.39 anti--ubiquitin antibodies were chosen to label cytoskeletal alterations.

The results showed proliferation of microglia as previous to the appearance of diffuse and focal amyloid within areas free of senile plaques (SP) in the AD and without PrP deposits in CJD. This first stage of "inflammatory" type was followed by the focal glial proliferation in the vicinity of SP. Scanty glial reaction was noted within diffuse amyloid deposits.

The presence of microglia in the center and astroglia in the periphery of SP (A4/PrP) is well known as well as their function in lysosomal phagocytosis and extracellular proteolysis of amyloid. The latter seems to lead to the appearance of the fibrilar amyloid form. Through interleukin secretion the microglia cells also stimulate the proliferation of astroglia, which create a "scar", isolating the toxic amyloid from neuropil. Diffuse amyloid deposit seems to be more dangerous.

M. BARCIKOWSKA[1], A. FRIEDMAN[2]

CLINICOPATHOLOGICAL STUDY OF 5 CASES WITH DEMENTIA AND PARKINSONIAN SYNDROME

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The aim of this study was an assessment of the possibilities of immunohistochemical differential diagnosis of Parkinson's disease (PD) and Alzheimer's disease (AD) in patients who died with clinical symptoms of both dementia and parkinsonian syndrome.

Five cases with long history of parkinsonian symptoms and good response to L-Dopa and coexistence of clinical signs of dementia – assessed by use of Reisberg's GDS (Global Dementia Scale) as 5–6 stage were examined.

Representative specimens from frontal, temporal, hippocampal and parahippocampal regions and from substantia nigra (SN) and locus coeruleus (LC) were taken. Routine stainings (H&E, Klüver-Barrera) and silver impregnation (Yamamoto) were performed before immunohistochemical labeling. Following antibodies were used: 4G8 directed against beta-peptide, anti-tau 1 and 3.39 anti-ubiquitin to visualize cytoskeletal degeneration, neurofibrillary tangles (NFT) and Lewy bodies (LB).

In all cases numerous senile plaques (SP) were observed within frontal cortex, hippocampus and a few in SN. In 4 cases they were also present in brain stem nuclei. Additionally, NFT were noted in neocortex, hippocampus, SN and LC. In 3 cases changes were assessed as severe, in one as moderate. LB occurred in all cases within SN, in LC in 4 cases. In two cases LB were also found focally (by 3.39 antibody staining) within frontal and temporal cortex.

In view of these findings the neuropathological diagnosis of AD and PD was made in 3 cases and in 2 cases we made diagnosis of coexisting Diffuse Lewy Body Disease. In no case was it possible to make diagnosis of a single disease either clinically or neuropathologically. We suggest that this is the result of an overlap of these disorders, which probably represent a kind of clinicopathological continuum of neurodegenerative diseases.

E. BERTRAND[1], G. SZPAK[1], E. LEWANDOWSKA[1], J. GAJDA[3], A. CZŁONKOWSKA[3], T. KRYST-WIDŹGOWSKA[2]

MORPHOLOGICAL AND ULTRASTRUCTURAL ANALYSIS OF THREE CASES OF WILSON'S DISEASE

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Three patients with Wilson's disease, who died at the age of 27, 33 and 33 years, are described. The duration of the disease was 1.5, 8 and 10 years, respectively. In all cases typical clinical symptoms of Wilson's disease were observed: extrapyramidal syndrome, cerebellar signs, psychoorganic syndrome, epileptic seizures and in two cases symptoms of hepatic insufficiency. The diagnosis was confirmed by ceruloplasmin and copper level evaluation in serum and urine in two cases. In the third case the test with radioactive copper was pathological. MRI showed extensive, symmetrical foci of tissue damage in striatum, pallidum, thalamus and dentate nucleus in all the cases. Slides from cerebral hemispheres were stained with HE, PTAH, Klüver-Barrera and van Gieson methods; selected specimes were impregnated with Cajal and Bielschowsky techniques.

The most severe changes were found in putamen bilaterally. Lacunar disintegration of tissue with intensive glial reaction in the form of numerous Alzheimer type II cells, non numerous Alzheimer type I cells and single Opalski cells was observed. Diffuse rarefaction of white matter of both cerebral and cerebellar hemispheres was found with concomitant diffuse or nodular gliosis. Patchy loss of neurons in the cerebral cortex was present.

Ultrastructural study revealed glial cells with very clear cytoplasm in striatum, pallidum, thalamus and dentate nucleus. They contained degenerative mitochondria and residual channels of endoplasmic reticulum. Numerous axons showed vacuolization of varying degree. Their myelin sheaths exhibited separation of lamellae. Although duration of disease differed, no essential differences in the topography and pattern of changes were found between the three described cases.

J. BOROWSKA-LEHMAN[1], E. IŻYCKA[1], L. PIKIEL[2], W. DUŻYŃSKI[2]

PRIMARY AND SECONDARY EXTRACRANIAL MENINGIOMAS

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The authors examined 5 cases of extracranial meningiomas diagnosed from biopsy material. Three of them were secondary to intracranial lesions, the other two were unrelated to cranial cavity. The first three were: two men and a woman aged 49, 29, and 33 with 20, 2 and 1 year of disease history, respectively. Both male patients had a major part of neoplasm localized in soft tissues of the head, causing its marked deformation. Tumors infiltrated epicranial aponeurosis, skeletal muscles, bones of cranium convexity and dura. In the female patient the tumor was localized in the upper part of nasal cavity, expanding to frontal sinus and through a bone defect to the cranial cavity. The other two cases occurred in two female patients, aged 15 and 71 years with the history of disease of 18 and 12 months. Tumors were localized in the orbit and penetrated the paranasal sinuses, and additionally in the last case, to oronasal part of pharynx, approaching the base of cranium. Diagnostic difficulties and aggressive expansion of tumor out of a cranial cavity has been discussed.

J. BOROWSKA-LEHMAN[1], W. M. NYKA[2], L. CHROSTOWSKI[1], E. IŻYCKA[1]

SUBACUTE SCLEROSING PANENCEPHALITIS WITH LESIONS OF SPINAL CORD AND SPINAL GANGLIONS COEXISTING WITH GRAY MATTER NECROSIS

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The case of 20-year-old woman with diagnosed subacute sclerosing panencephalitis (SSPE), of more than half year of disease history, was presented. The disease started with progressing damage of vision and decrease of mental abilities. In final stages paresis of limbs occurred. Cerebrospinal fluid

and blood samples showed increased level of anti-measles antibodies. The patient was transferred to the Neurology Clinic, School of Medicine, in Gdańsk from a District Hospital, with respiration disorders, in state of unconsciousness. After some days deep coma developed. The mechanical respiration was applied. The patient died after two weeks, with symptoms of circulatory failure. The autopsy showed obliteration of the brain stem and cerebral cortex structures. Microscopic examination revealed changes characteristic for SSPE of chronic course within brain hemispheres, cerebellum, brain stem, spinal cord and spinal ganglia. Additionally, a focal and diffuse ischemic necroses of different stages were found in the cerebral cortex, subcortical nuclei and brain stem. Interpretation of morphological changes in relation to clinical history was discussed. Difficulties in identification of inclusions in necrotic neurons and disturbances in cerebral blood flow due to the presence of several thrombi closing the blood vessels lumina were stressed.

M. DĄMBSKA, M. MUZYLAK, D. MAŚLIŃSKA

DAMAGE OF AXONS AND MYELIN SHEATHS IN THE PERIPHERAL NERVOUS SYSTEM OF RABBITS AFTER VINCRISTINE INTOXICATION

Laboratory of Developmental Neuropathology, Medical Research Centre, PASci, Warszawa

Peripheral neuropathy is the most frequent complication of vincristine treatment, limiting successful treatment with this antimitotic drug.

We attempted to follow the course of ultrastructural changes within peripheral nerves of rabbits in various age groups after prolonged vincristine administration. Early changes appeared in axons, in myelin sheaths, and Schwann's cells. Axonal shrinkage occurred with agglomeration of fibrillary elements. Disjoining of myelin lamellae was followed by their severe disruption and finally by disintegration of myelin sheaths. Several Schwann cells contained lipid deposits, or revealed degenerative changes.

After three months of survival the lessions were less severe, particularly in young animals. Myelination or remyelination of some nerve fibers was seen. Our observation may suggest a partial reversibility of changes after vincristine intoxication.

M. DĘBIEC-RYCHTER[1], J. ALWASIAK[1], W. PAPIERZ[2], P. P. LIBERSKI[1]

CYTOGENETIC ANALYSIS IN HUMAN MALIGNANT GLIOMAS*

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Cytogenetic analysis of human gliomas including 13 glioblastomas, 2 anaplastic astrocytomas, 2 astrocytomas and 2 oligodendrogliomas were performed using short-term culture method. The study revealed extra copie of chromosome 7 and monosomy of chromosome 10 as the most prevelent abnormality in glioblastomas versus low-grade astrocytomas and oligodendrogliomas, which had predominantly normal karyotypes. Additionally, loss of chromosome 14 occurred in 4 tumors (in 3 cases in polyploid, clonal metaphases), and partial loss of chromosome 14, due to translocation in 4 other glioblastoma cases. The most frequent structural abnormalities of chromosome 7 were deletions involving 7p12, 7p15 and 7p22. Loss or alterations of chromosome 9 occurred in 3 tumors in complex clonal karyotypes. Other frequent abnormalities were alterations of 2q, 6q, 11p, 17p, 18q and 22q in which the precise breakpoints of these chromosomes varied from tumor to tumor. Double minutes were observed in 3 cases. Our study indicates that in addition to the loss of chromosome 10 a nonrandom loss or rearrangement of chromosome 14 may be involved in the evolution of glioblastomas. In patients with cerebral gliomas, cytogenetic analysis provides independent information, however, its value as a prognostic factor remains to be proved.

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M. DĘBIEC-RYCHTER[1], J. ALWASIAK[1], W. PAPIERZ[2], P. P. LIBERSKI[1]

N-*myc* AND C-*erb*B-1 AMPLIFICATION IN HUMAN PRIMITIVE NEUROECTODERMAL TUMORS (PNET)*

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High-molecular-weight DNAs from 29 human primitive neuroectodermal tumor tissues were examined for possible rearrangement and/or amplification of the c-*erb*B-1 and N-*myc* protooncogenes by Southern blot hybridization. Imumunohistochemical staining revealed varied degree of neuronal differentiation in all tumors. In one case the N-*myc* and c-*erb*B-1 genes were found to be simultaneously amplified 5 and 20 times, respectively. All remaining tumors showed lower than 2:1 ratios of N-*myc* to control gene and were not considered as representing amplification. 10- and 30-fold amplification of c-*erb*B-1 gene were observed in two other cases. There was no rearrangements of either studied protooncogene. Our results suggest that N-*myc* and c-*erb*B-1 protooncogene amplification is a relatively uncommon mechanism of their activation in PNETs and provide molecular evidence for heterogeneity in this class of tumors.

J. DORSZEWSKA, Z. ADAMCZEWSKA-GONCERZEWICZ, M. WENDER, A. GROCHOWALSKA

CEREBRAL STEROLS IN EXPERIMENTAL SEVERE HYPOXIA

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Free sterols of cerebral myelin under severe hypoxia were studied.

Rats of Wistar strain were placed in the chamber with a gas mixture containing 2% O_2 for 3 min. The animals were sacrificed after 4 and 24 hours, and 2 or 8 weeks after hypoxia. Total free sterols of the myelin fraction were extracted and then separated by GC-MS (gas chromatography and mass spectrometry).

Results showed that the level of the main typical myelin sterol, i.e. cholesterol decreased 14 days and 2 months after hypoxia. 4,4,14α-trimethyl-5α-cholest-24-en-3-one came second in quantity and its level was found decreased 4 and 24 hours after hypoxia and increased 2 months after the experiment. Sterols of molecular weight 412, 414 and 426 were observed in all experimental groups in smaller amount in comparison to control group and the remaining derivatives only in trace amount.

Free sterols persisting for a very long time after hypoxia may be the result of some disturbances in their metabolism, which are responsible for their transformation into cholesterol. The second possibility is the creation of free radicals as the result of hypoxia.

D. DZIEWULSKA

AGE-DEPENDENT CHANGES IN ASTROGLIAL REACTIVITY IN HUMAN ISCHEMIC STROKE

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The influence of aging on astroglial reactivity in human ischemic infarcts was examined. Astroglial hypertrophy and proliferation within tissue adjacent to the necrotic focus in the area of middle cerebral artery supply were compared in two groups: senile (80–101 years old, 32 cases) and middle age (42–63 years old, 33 cases) during 32 days of disease.

Astroglial reactivity was evaluated using immunocytochemical methods with antibodies against the protein S-100, GFAP and vimentin, as well as morphometric methods.

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Our investigations revealed that astroglial reactivity was less pronounced, more diffuse and of shorter duration in the senile than in the middle age group. GFAP-positive cells were less numerous and the degree of hypertrophy, examined by computer analysis and morphometry, was smaller in the senile group. In addition, proliferation examined indirectly by means of antibodies against vimentin, was observed half as often and was less pronounced.

The author suggests that age may modify astroglial reactivity. Differences in the astrocytic reaction may be caused by the decrease of mitogenic and morphogenic factor activity, the decrease of astrocytic susceptibility to stimulating factors, and/or ischemic tolerance phenomenon.

J. DYMECKI

PROGRESS IN METHODS OF SUPPLYING BRAIN WITH DOPAMINE

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The aim of the study is to present the recent progress in neurotransplantation, both in experimental techniques and in therapeutic trials in Parkinsonian patients. Neural transplantation can promote functional recovery by replacement of degenerated nerve cells, release of specific neurotransmitters, reconstruction of lost nerve pathways or production of the nerve growth factor (NGF).

The world review of therapeutic transplantation in Parkinson's disease shows a slow decrease in number of adrenal medulla transplantation and successive increase in number of fetal substantia nigra grafts. That is the result of longer survival and better function of fetal tissue grafts in the patients's brain, corresponding to long-life clinical recovery.

The new technique is the "cograft", the peripheral nerve fragment implanted together with adrenal medulla or fetal tissue into the patient's brain. The advantage of this technique consists in peripheral nerve NGF release to the graft.

Similar technique was applied in Alzheimer's disease. NGF was introduced directly to the brain by osmotic minipomp. Temporary improvement in mental faculties of the patient was observed.

In order to avoid ethical and legal problems involved in obtaining fetal tissue, the liquid nitrogen cryopreservation of tissue from spontaneous abortion or extrauterinal pregnancy was used. Banking of fetal tissue makes it constantly available for transplantation.

Genetic engineering seems to be very promising technique. The host tissue, for example fibroblasts, is transformed to synthesize dopamine. The genetically engineered cells can replace the dopaminergic fetal neurons.

A. FIDZIAŃSKA

DESMINOPATHIES – MORPHOLOGICAL STUDY

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By using antibodies against the intermediate filaments (desmin and vimentin) we have analysed the distribution of desmin and vimentin in the muscle biopsy specimens consisting of large cytoplasmic inclusions of intricate ultrastructure. The cytoplasmic inclusions were characterized by presence of both granular and filamentous structures. Immunocytochemical study revealed the presence of desmin decorated inclusions in the affected muscle. Clinically examined patients showed stationary or slowly progressive proximal myopathy.

These cases may represent a new disease resulting from an unknown myofibrillar protein defect which produces abnormalities in the structure of the muscle contractile elements.

B. GAJKOWSKA[1], M. J. MOSSAKOWSKI[2]

ELECTRON-MICROSCOPIC LOCALIZATION OF GABA AND GLUTAMATE-LIKE IMMUNOREACTIVITY IN CA 1 SECTOR OF HIPPOCAMPUS IN MONGOLIAN GERBILS AFTER TRANSIENT ISCHEMIA

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We have examined the subcellular distribution of glutamate and GABA in synapses of Mongolian gerbils hippocampus using postembedding immunogold staining method for electron microscopy. Immunolabeling was performed by 10 nm gold-antibody complex for glutamate and GABA. The gold particle densities gave useful information about the relative concentrations of these amino acid neurotransmitters. Our results indicate that ischemia leads to the temporal decrease of GABA content in some synapses of interneurons, with glutamate content remaining unchanged.

Six hours after ischemia GABA-like immunoreactivity in symmetric synapses is very low and most of these synapses are swollen. Twenty four hours after ischemia the level of GABA-like immunoreactivity in ultrastructurally unchanged symmetric synapses is comparable to the level of the control. This suggests reversible insufficiency of GABA-ergic synapses after ischemia.

Also the level of glutamate-like reactivity in asymmetric ultrastructurally unchanged synapses in slightly enhanced or comparable to the control. Presence of high concentration of glutamate immunoreactivity in glial cells is striking. It is conceivable that the glia capacity to metabolize the excess of glutamate is disturbed after ischemia.

In conclusion our results indicate that ischemia leading to disturbances in neurotransmitters turnover and thus in the balance between neuronal excitation and inhibition can play a role in hippocampal injury.

K. HONCZARENKO, T. JEŻEWSKI, P. NOWACKI, A. FABIAN

CORRELATIONS BETWEEN NEUROPATHOLOGICAL PICTURE, CT IMAGE AND CLINICAL MANIFESTATION IN BRAIN METASTATIC NEOPLASMS

Laboratory of Neuropathology, Neurological Clinic, Pomeranian School of Medicine, Szczecin

Fifty four patients with metastatic tumors in the central nervous system were analysed. The patients were treated in the Neurosurgical Clinic between 1982 and 1992. In 37% of patients neurological signs were the first clinical manifestation of the malignant disease, in other 17 patients the neurological signs appeared after diagnosis of the primary neoplasm. Most frequently lung and breast cancer were diagnosed. In 3 cases CT image revealed numerous brain metastases. In 27 cases CT demonstrated one solid tumor within the brain. In other 24 cases CT image of the brain neoplasm was polymorphic and resembled arachnoidal cyst of brain abscess. In 28 cases brain edema of various degrees was observed in the neighbourhood of the tumor. Histopathological investigations of the material received intraoperatively revealed metastatic character of the neoplasm. In patients with no diagnosis of the primary neoplasm, metastases from lung or kidney were usually suggested on the basis of neuropathological examination. Clinical manifestation of the metastatic tumors usually correlated with their location in the brain and surrounding edema manifested in CT image. In many cases CT image and intraoperative macroscopical picture of the neoplasm suggested the brain abscess.

K. HONCZARENKO, P. NOWACKI

DEGENERATIVE CHANGES IN THE CEREBELLUM IN ACUTE NON-LYMPHOBLASTIC LEUKEMIAS AND NEOPLASMS OF INTERNAL ORGANS

Laboratory of Neuropathology, Neurological Clinic, Pomeranian School of Medicine, Szczecin

The aim of the study was the neuropathological evaluation of the degenerative changes in the cerebellum in acute non-lymphoblastic leukemias (ANLL) and neoplasms of internal organs (NIO), with special reference to the analysis of influence of various factors on etiopathology of cerebellar changes. Neuropathological investigations were done in two groups of patients. The first group consisted of 81 patients deceased due to ANLL and blastic phase of chronic, myelogenous leukemia. They were treated accordingly to chemotherapeutic protocols differing in arabinoside cytosine dose. The second group contained 60 patients deceased due to NIO. It was usually lung, ovarian and gastric cancer. These patients were treated with neither chemotherapy nor x-ray therapy. Most evident changes were observed within the cerebellar granular layer and dentate nuclei. Diffuse granular layer rarefaction or atrophy and focal rarefaction of neurons in dentate nuclei were usually observed. Granular layer degeneration usually coexisted with segmental atrophy of Purkinje cells and Bergmann's glia proliferation. Moderate myelin swelling and astro- and microglia proliferation were also observed in the examined cases. On the basis of the above-mentioned changes cerebellopathy type II was diagnosed neuropathologically. In the first group of patients cerebellopathy was observed in 32% of cases, whereas in the second group in 38.3% of cases. The cause of the development of cerebellopathy type II is discussed. Authors suggest that in patients with ANLL it is mostly due to chemotherapy, especially arabinoside cytosine application, while in patients with NIO cerebellopathy it rather depends on autoimmunologic factors.

L. IWANOWSKI[1], H. KROH[2,3]

ARTERIOVENOUS MALFORMATION AS THE MOST FREQUENT FORM OF CEREBRAL VASCULAR DYSPLASIAS

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On the base of biopsy and postmortem investigations of specimens from 27 patients, a review of different forms of cerebral vascular dysplasias was made. The aim of the authors' study was to compare the incidence of particular forms of malformations in their material with earlier reports.

Four teleangiectasis, 11 arteriovenous malformations, 7 cavernous angiomas and 5 mixed forms of cavernous angioma with arteriovenous malformation were found. Arteriovenous malformations prevailed. Clinical symptoms appeared most frequently in the age interval between 15 and 45 years. The observations support the theory of Kaplan et al. that all cerebral vascular dysplasias originate as a congenital maldevelopment of the small blood vessels. Other results were consistent with those generally known but not yet presented in Polish literature.

J. KAŁUŻA

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF CELLULAR RESPONSE IN HUMAN BRAIN PRIMARY AND SECONDARY NEOPLASMS

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Cellular responses of the brain caused by primary or secondary neoplasm may differ largely both qualitatively and quantitatively. For the immunological assessment of reactive cells either inside of a tumor or in its vicinity, antibodies against the following antigens were applied: CD68 – macrophage marker, OPD4 – for lymphocytes T helper/inducer and thymocytes, CD45RO, UCHL1 – for

resting T cells encompassing both subgroups CD4 and CD8 of class II MHC and class I MHC. Moreover, antibodies against CD21, 1F8 – lymphocyte B and, first of all, dendritic cell marker – were applied.

In gliomas most reactive cells were CD68-positive, which speaks for the presence of the cells with macrophageal features. The CD45, UCHL1-positive cells were less numerous. Only very few cells were OPD4-positive and just single CD21, 1F8-positive. In metastatic neoplasms the results of investigations were qualitatively similar to primary neoplasms. Quantitative measurements in metastatic neoplasms disclosed higher numbers of reactive cells in each particular immunological phenotype as compared with the primary ones. The statistical significance of the morphometric analysis will be presented.

On the base of the results of the performed immunohistochemical tests the explanation of quantitative differences observed in cellular response to the neoplasm in brain has been attempted.

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MORPHOLOGICAL AND CLINICAL ANALYSIS OF COMPLICATIONS IN THE POSTSURGICAL TREATMENT OF RECIDIVING GLIA-DERIVED BRAIN TUMORS

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The investigations were performed on 12 patients who died in the Clinic of Neurosurgery of Medical Academy in Kraków because of reccurring brain glioma. All the patients after the first surgery were treated with both surgery and radio- and chemotherapy. The time lapse between surgeries varied from 6 months to 5 years. In postmortem examinations samples from central nervous system, lungs, kidneys, and liver were taken.

Vascular changes in the vicinity of the remains of neoplastic infiltrate and in regions remote from surgical defect have been noted. The most frequent alterations in blood vessels were the thickening and hyalinization of arterial wall, endothelial edema, microthrombi and histiocytic metaplasia of medial (muscular) layer of arterial wall. Lymphocytic infiltrations were noted in the vicinity of postsurgical defect and in remains of the tumor as well as in remote places. Most frequently remote lymphocytic infiltrations were noted in meninges and periventricular region, very rarely though in the white matter of centrum semiovale. Among other organs the lungs were the most frequent site of pathological changes, i.e. hyaline membranes in alveoli and lymphocytic infiltrations in interalveolar septa. Cellular responses in brain areas remote from the site of surgical resection of tumor are likely to be sequel to the postsurgical treatment. The lymphocytic infiltrations alone may point to generalization of cellular reaction in the brain neoplasm.

S. KASPEREK, A. WĘGLARZ

A CASE OF RADIATION MYELOPATHY OF THE THORACIC CORD

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This condition is most often found in patients with bronchial carcinoma. A further example is presented here.

A 62-year-old man with an advanced carcinoma in the right hilus was treated with chemo- and radiotherapy in a total dosis of 40 Gy in 10 fractions. About 8 months later a progressive paraparesis with sensory disturbances began, a typical Brown-Sequard syndrome on level Th10 developed. It evoluated to complete spastic paraplegia (level Th7). Repeated CSF examinations and myelography were normal. A massive pulmonal hemorrhage led to death one year after the onset of the spinal symptoms.

On autopsy malacia was found among others in a part of the thoracic spinal cord but no metastases. Microscopy revealed marked coagulative necrosis without perivascular infiltrations or astroglial response limited to the irradiated segments (in neighbourhood of the hilus). Striking

thickening, hyalinisation and fibrosis of vessels were visible. Conglomerations of small vessels resembled the teleangiectasias. The structure of the spinal cord was preserved above and below the necrotic segments.

The clinical and postmortem data of this case correspond to known descriptions. Diagnostic procedures of the condition are not straightforward and there is a possibility of false clinical diagnosis of spinal metastases. Changes in MRI are not regarded as sufficient to warrant the diagnosis. The investigations of Rubin et al. (1988), who found the elevation of myelin basic protein in the CSF 100–1000 times the normal value in rabbits with induced radiation myelopathy, may announce the solving of diagnostic problems connected with radiation myelopathy in man.

A. KĘDZIA

DEVELOPMENT OF HUMAN BRAIN VENOUS SYSTEM IN THE LIGHT OF CONTEMPORARY INVESTIGATIONS

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The study aimed in morphological and topographical analysis of the cerebral veins and dura mater sinuses in the prenatal period. The following questions were taken into consideration: degree of venous blood drainage in both cerebral hemispheres, localization of anastomoses and their characteristics, angioarchitecture of dura mater. Evaluation of clinical aspects of the venous system morphology was also considered. A variety of methods was used: injections, Pickworth's, evaluation in infrared (IR), ultraviolet (UV), as well as luminescence in IR and UV. The obtained data were analysed with the use of three computer systems: BVS 6471, BVS 6472 and Cytochromics. The investigations were performed on 200 fetuses aged from 3 to 8 months and the material was quantatively analysed by the Anna Krefft's method. In the prenatal period the tentorial and occipital sinuses, which subsequently disappear, play an important role in the venous blood drainage. The superior saggital sinus is created by coalescence of both marginal sinuses. Veins enter sinuses via the so called bridge veins, which start developing from the third month of fetal life on. It was noticed that primarily all veins and sinuses have netlike nature, whereas the large veins have linear character. Veins develop in the following order: first surface veins of the brain are appearing on the brain convexity, then on its basis, internal cerebral veins are formed as the last. The white matter veins are parallel to the white matter fibres, and are characterized by a very small diameter facilitates intra- and periventricular hemorrhages. Differentiation of cerebral vessels depends on the function and energy demands of the growing structures; it occurs very intensively in the fifth and sixth months of prenatal life. It was noticed that the surroundings the lateral fossa and lateral sulcus of the brain hemispheres are the brain regions of the richest multidimensional venous drainage. Every brain region has its specific angioarchitecture. Valves in the cerebral and leptomeningeal veins in dura mater sinuses were observed. Stricking correlation between morphology and function of the venous system was noted. The investigations were performed with the use of the new techniques which made observation of hidden parts of the brain possible. The use of IR and the computer image analysis system enabled us to present three-dimensional vascular network. Many details invisible in other methods can be visualized by the use of linear transformation and mathematical morphology.

J. KOWALSKI, S. KASPEREK

INTRAMEDULLARY METASTASIS OF BRONCHIAL CARCINOMA – CASE REPORT

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MRI has solved problems connected with intravital diagnosis of intramedullary tumors – a rare site of metastatic neoplasm. In the present case the condition was diagnosed according to classic data only, because of limited access to the new method.

In a 52-year-old farmer with no previous neurological history, a complete spastic paraplegia, urine retention and sensory loss on levels Th10 – 12 developed within 2 months. Then the palsy changed to flaccid paresis (on level Th7). Myelography and 2 CSF examinations were normal. X-ray showed a so-called *tumor rotundum* in the upper lobe of the right lung, spondylotic thoracic and lumbar changes and calcifications of abdominal arteries. In differential diagnosis either vascular lesion of the spinal cord or an intramedulary metastasis was suspected. Death after 6 months of spinal syndrom was due to cachexia, anemia, fever and bedsores.

Autopsy revealed a dissemination of metastases of bronchial carcinoma, among others to the brain. In the 5 – 7th segments of thoracic cord malacia was suspected. In addition multiple abscesses in the brain and in subdural thoracic space were found. Microscopy showed a microcellular carcinoma infiltration of the whole cross-section in the middle thoracic spinal cord segments. In the meninges purulent changes were present.

P. B. KOZŁOWSKI[1], M. DĄMBSKA[2]

NEUROPATHOLOGY OF ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) IN CHILDREN*

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In recent years, the spread of HIV infection has resulted in increased incidence of AIDS in women of child-bearing age and subsequently in the children born to these women. Congenital HIV infection in infants and children has produced central nervous system (CNS) lesions quite different from those seen in adults with AIDS, including more common CNS involvement by HIV-related pathology and severe microencephaly/brain atrophy, which are relatively rare opportunistic CNS infection.

In material collected in NYS Institute primary microencephaly (brain to small for developmental age) or brain atrophy, which are particularly severe complications of congenital HIV infection, are seen in the majority of children with AIDS (approximately 60%), and in some cases, the deficit of brain weight is 55% smaller then the brain weight normal for the given age of child.

HIV encephalitis with multinucleated giant cells is seen almost as often. However, there is only a partial overlap of cases with microencephaly/atrophy, and cases with HIV-encephalitis.

CNS opportunistic infections are not common – they are seen in approximately 10% of cases. The most common agents include cytomegalovirus and *Candida albicans*. Other, rarely seen infectious agents include *Aspergillus*, *Mycobacterium tuberculosis*, *Mycobacterium avium intracellulare*, *Pseudomonas*, and *Streptococcus*. Certain CNS infections such as toxoplasmosis or progressive multifocal leucoencephalopathy, which are common in adults with AIDS, are not seen in children with AIDS. Vascular CNS pathology, including large and microscopic infarcts and hemorrhages, is observed in approximately 20% of children with AIDS.

The severity and the frequency of CNS involvement in children with HIV infection places the picture of AIDS in new perspective. It appears that HIV-induced CNS damage may begin early in the nonsymptomatic silent phase of disease (possibly even as early as intrauterine life in congenital infection), and damage may occur even in the absence of clinical immune deficiency. Eventually, a fatal immune deficiency develops. In the absence of effective anti-viral therapy that can effectively stop systemic and neural replication and spread of HIV, the medical care of these children will extend their lives, but may not affect the progression of the CNS damage.

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I. KUCHNA, M. DĄMBSKA, D. MAŚLIŃSKA

TISSUE REACTION IN MULTIFOCAL ENCEPHALOPATHY AND CYSTIC LEUCOENCEPHALOPATHY IN INFANTS

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Leucoencephalopathy with formation of subcortical cavities presents a particular type of fetal and newborn brain damage. We compared the cases of cystic leucoencephalopathy with multifocal encephalopathy in brains of the newborn. The infants were born at 28 to 40 weeks of developmental age and survived 1 – 3 months. Anoxic-ischemic episodes were clinically confirmed in all analyzed cases. Neuropathological examination was performed on representative slices stained with routine methods and examined by the immunohistochemical method of glial fibrillary acidic protein (GFAP).

In the examined cases we found various of tissue necrosis, particularly in periventricular white matter. The intensity of reactivity of macrophages, glial cells and vascular proliferation differed from case to case. This was related to the topography of the lesions. White matter damage presented less intensive disintegrative-reparative reactions than grey matter necrosis. The wide-spread necroses of the white matter led to formation of cavities, their walls presenting scarring with fibrous astroglial proliferation. The differences in tissue reactions in the examined cases seem to be related to the developmental age at the moment of damage.

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EFFECT OF CRYOPRESERVATION METHOD ON THE CELL STRUCTURE OF FETAL SUBSTANTIA NIGRA

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Successful cryopreservation of fetal neural tissue in liquid nitrogen for its prolonged storage as a donor material for neurotransplantation depends on many variables such as composition of the freezing medium, freezing and thawing rates and the type of cryoprotectant. The aim of the present study was to evaluate the effect of some cryoprotectants and the chosen cryopreservation method on the structure and ultrastructure of mesencephalic tissue as well as cells immunoreactivity against tyrosine hydroxylase (TH). These data are essential for establishing the best possible conditions for survival of dopaminergic cells.

The ventral part of mesencephalon containing fetal substantia nigra cells was removed from 15-day-old rat fetuses and dissected into 0.5 – 1.0 mm^3 blocks, immersed in 1 cm^3 of Ringer solution for about 30 min and transferred into the freezing medium containing the following types of cryoprotectants: 10% v/v dimethyl-sulphoxide (DMSO), 10% v/v 1,2-propylene glycol (1,2-PG) or 10% v/v glycerol (GL). The tissue was cooled according to previously examined technique in a computer-controlled freezer at a rate $-1°C$/min until $-70°C$ and then plunged into liquid nitrogen for storage during 7 – 30 days. Rapid thawing of the tissue was carried out in water bath at 37°C for 3 min according to previously tested technique. After removal of cryopreservation medium the material was processed with standard methods for light or electron microscopy.

Preliminary studies proved that cryopreserved fetal mesencephalic cells stored in liquid nitrogen were able to survive and to develop in tissue culture after thawing. Histological analysis showed that cryopreservation of fetal mesencephalic tissue in the presence of GL resulted in marked loosening of tissue structure and in the majority of cells damages due to intracellular ice nucleation were clearly visible (lack of nuclear envelopes, damaged cytoplasm membranes). The ultrastructure of the few cells that successfully survived the procedure of cryopreservation is however very similar to control ones. Morphology of fetal tissue cryopreserved in the presence of DMSO and 1,2-PG resembles the control one and damages of nuclear envelope as well as changes of cytoplasm and nuclei structure are much less frequently visible as compared to GL. The ultrastructural organization in the majority of fetal

mesencephalic cells cryopreserved in the presence of DMSO is similar to control ones, whereas in the case of 1,2-PG numerous cells lack nuclear envelope and damages of ER membranes and cytoplasm are visible ("pseudovacuoles", most probably caused by ice micronucleation). Immunocytochemical staining showed TH-positive dopaminergic cells in fetal mesencephalon cryopreserved by the chosen technique independently of cryoprotectant used.

The results indicate that among the tested the 10% DMSO is the best cryoprotectant for fetal mesencephalic tissue cryopreservation. Both tissue morphology and cells ultrastructure as well as sustained immunoreactivity to TH suggest that the technique of cryopreservation in the presence of DMSO described above may be a valuable method of fetal mesencephalic tissue banking for neurotransplantation.

E. LEWANDOWSKA[1], J. KULCZYCKI[2], G. SZPAK[1]

SOME ASPECTS OF MICROSCOPIC AND ULTRASTRUCTURAL CHANGES IN THE SSPE BRAIN

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The report compares neuropathological changes in four cases of a subacute sclerosing panencephalitis (SSPE) with differing clinical course. According to commonly used criteria, two among our cases were evaluated as acute, one as subacute, and one as a chronic case. The survival time was 6 weeks, 3 months, 2 years and 7 years, respectively.

Since the autopsy material was taken from all cases very early after death it was possible to evaluate morphological changes in the brain under both, light and electron microscope. The microscope and ultrastructural analysis permitted to follow the pathological process up from the early stage of inflammatory infiltration throughout increasing reaction of neuroglia and demyelination to the late encephalophatic changes including appearance of Alzheimer's neurofibrillary degeneration.

Moreover, the intranuclear inclusions were found in all stages of the disease. In the structural analysis the typical nucleocapsids of paramyxoviruses were observed as well as some of the ultrastructural changes occasionally present in various phases of the inflammatory-degenerative process of nervous tissue in SSPE.

P. P. LIBERSKI

TRANSMISSIBLE AND NON TRANSMISSIBLE BRAIN AMYLOIDOSES. ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF AMYLOID PLAQUES: HOW MUCH IS THE SAME AND HOW MUCH IS DIFFERENT?

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Ultrastructural and immunohistochemical similarities between transmissible (Creutzfeld-Jakob disease — CJD, and Gerstmann-Sträussler-Scheinker syndrome — GSS) and non-transmissible (Alzheimer's disease) brain amyloidoses (TBA and N-TBA) are reported here. Synthesis and processing of amyloid precursor followed by accumulation of a final deposits (PrP^c and beta A4 are final deposits in TBA and N-TBA, respectively) are the central pathogenetic events in both types of amyloidoses. The amyloid plaque composed of amyloid fibers, dystrophic neurites, astrocytes and microglia cells in varied proportion is the crucial neuropathological entity. The role of microglial cell as amyloid producer/processor cell seems to be analogous in both types of amyloidoses. The impairment of slow axoplasmic transport leading to the accumulation of neurofilament triplet protein in the TBA and of tau protein in the N-TBA causes the development of dystrophic neurites (Liberski et al., in Alzheimer's disease and related disorders, Iqbal K et al., eds. Alan R Liss, Inc: 549). Other findings may only be secondary and non-specific phenomena.

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ULTRASTRUCTURAL STUDIES IN A CASE OF GERSTMAN-STRÄUSSLER-SHEINKER (GSS) SYNDROME FROM THE ORIGINAL AUSTRIAN FAMILY*

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We report here ultrastructural findings in the brain of a 40-year-old female with GSS. The patient came from the original Austrian family first described in 1936. Several types of amyloid plaques were seen: 1) "kuru" plaques; 2) multicentric plaques; 3) senile plaques; 4) "purely" neuritic plaques. Abundant astroglial reaction at the periphery of plaques and microglial involvement were observed. Proliferating vascular basal laminae were seen in close proximity to plaques. Abnormal subcellular organelles and neurofilaments accumulated in dystrophic neurites, either associated with plaques or distant from plaques, were frequently seen. Tubulovesicular structures (TVS)[1] were detected.

[1] Liberski et al., Acta Neuropathol., 1992, 84, 238–243.

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ULTRASTRUCTURAL NEUROPATHOLOGY OF A CHILEAN CASE OF TROPICAL SPASTIC PARAPARESIS (HTVL-1 ASSOCIATED MYELOPATHY)

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Ultrastructural findings of Chilean case of tropical spastic paraparesis (TSP), an encephalomyeloneuropathy caused by HTVL-1 retrovirus is reported here. Axonal degeneration accompanied by extensive astrocytic gliosis, microglial activation and lymphocytic infiltration were observed in anterior and posterior horns. Axons accumulated increased number of neurofilaments and Hirano bodies.

A few samples of cryostalloids, previously labeled "Hirano-like bodies"[1] or "multilamellar bodies"[2] were observed. They consisted of stacks of 30 to 40 electron-dense lamellae separated by electron-lucent spaces. Lamellae were "immersed" within amorphous "substance". Dorsal root ganglia neurons contained abundant lipofuscin. A few myelinated axons were demyelinated and surrounded by concentric arrays of Schwann cell membranes. Axons of dorsal roots accumulated an increased number of neurofilaments.

[1] Liberski et al., Ann. Neurol., 1988, 23, suppl. S185.
[2] Liberski et al., Retrovirus Humanos, Ed.: V. Zaninovic Cali Colombia: 133.

P. P. LIBERSKI[1], A. H. WELLS[2]

ULTRASTRUCTURAL STUDIES OF EXPERIMENTAL BOVINE SPONGIFORM ENCEPHALOPATHY TRANSMITTED TO PIGS**

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Bovine spongiform encephalopathy (BSE) is a scrapie-like disorder in cattle[1]. The ultrastructure of experimental BSE in domestic pigs[2] is reported here. Vacuoles in neuronal processes, mostly in dendrites, and neuroaxonal dystrophy, such as in other subacute spongiform virus encephalopathies (SSVE)[3], constituted important ultrastructural findings but no amyloid plaques were seen. The topo-

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graphy of vacuolation, particularly the severe involvement of cerebral cortex, was different from that in cattle[4]. Mild astrocytosis and proliferation of microglial cells were seen. Tubulovesicular structures, regarded as the only disease-specific virus-like particles observed *in situ* in the SSVE[5], were abundant. In conclusion, experimental BSE in pigs recapitulated all the important features of other SSVE.

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[1] Wells et al., Vet. Rec., 1987, 121: 419–420.
[2] Dawson et al., Vet. Rec., 1990, 227: 338.
[3] Liberski et al., Exp. Neurol., 1989, 106: 133–141.
[4] Liberski et al., J. Comp. Pathol., 1992, 106: 361–381.
[5] Liberski et al., Acta Neuropathol., 1992, 84: 238–243.

A. MAJCHROWSKA[1], M. BARCIKOWSKA[2], P. P. LIBERSKI[1]

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF BRAIN TISSUES REACTION TOWARD MALIGNANT TUMOR

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Ultrastructural and immunohistochemical (GFAP, NFP, ferritin and CD68 (MHC II)) studies of the pattern of brain tissue reaction toward the presence of brain tumor are reported here. Eleven specimens (4 glioblastoma, 3 medulloblastoma, 1 astrocytoma, 1 malignant lymphoma and two samples of tumor periphery) were studied. Despite significant differences in tumor histopathology, brain tissues reaction was virtually the same. Astrocytic reaction was detected in all specimens. It was associated with abundant activated ferritin-immunopositive microglia cells both at the tumor periphery and within the tumor mass. A significant fraction of microglia expressed MHC II (CD68) antigen suggestive of antigen presentation. The degenerative phenomena of severe Wallerian degeneration (2/11), intramyelin vacuoles (11/11) and neuroaxonal dystrophy (5/11) were observed. Macrophages containing cellular debris accompanied degeneration of myelinated axons.

The reaction proved to be highly uniform and consisted of coexistent reactive and degenerative phenomena.

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NUMEROUS PARANEOPLASIC SYMPTOMS PREVIOUS TO THE LUNGS CANCER

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In a 31-year-old man first transient gynecomasty and impotence occurred, next deep senso-motoric polyneuropathy developed. The changes of blood were present (hyperglobulia up to 6.5 M/μl, HGB 19 g/dl, platelets 760 K/μl). The prolactine level was raised – 20 ng/μl. The cachexy proceeded.

Clinical changes in CNS: papilloedema with no other symptoms of intracranial hypertension. EEG and CT were normal. The patient suffered from girdle pains of thorax. The deep polyneuropathy was accompanied by ischemia of the lower legs and feet. In the secretion from the bronchial tree there were cells of planocellular cancer. Immunoelectrophoresis of the CSF showed pesence of IgM, raised IgA level and lack of IgG. Two weeks before death ischemic stroke of the left hemisphere with the right hemiparesis occurred.

Anatomopathologic micro- and macroscopic examinations revealed no neoplasmatic changes in lungs. Ischemic focus was found in the left frontal lobe. In the spinal cord atrophy and degenerative changes of anterior horns were observed. Spinal meninges were thickened and fibrosed.

The authors discuss the problem of early hormonal symptoms, changes in blood, disturbances in the composition of proteins in CSF and senso-motoric polyneuropathy, which can appear many months before the manifestation of the primary focus.

D. MAŚLIŃSKA

DIFFUSE BETA-AMYLOID PLAQUES IN KUFS DISEASE

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Monoclonal antibodies (mAbs) against amyloid beta-protein (beta A/4) are believed to be markers of brain amyloid fibres. However, they also reveal beta/A4 epitopes in neurons of aging brains and brains of people affected by neuronal ceroid lipofuscinoses (NCL). Kufs disease is the only form of NCL in which beta A/4 epitopes were detected not only in neuronal storage material but also in extracellular immunoreactive deposites (plaques).

In the present study various mAbs, which are markers of different fragments of amyloid precursor protein (APP) and beta/A4, were used to characterize the composition of these plaques. Additionally, brain sections were used for immunohistochemistry with Thioflavine S and for staining with Bielschowsky and Bodian methods. Glial cells were visualized with anti-ferritin or anti-glial fibrillary acidic protein (GFAP) antibodies. The intensive immunoreactivity of Kufs' plaques with two beta/A4 mAbs were observed. Reactions of plaques with all other APP markers and anti Thioflavine S were negative. The beta/A4 immunoreactive Kufs' plaques were not detectable with either Bielschowsky or Bodian method. No characteristic accumulation of glial cells were found in plaques sites.

Our results provided evidence that betaA/4 immunoreactive Kufs' plaques share the characteristic features of diffuse beta-amyloid plaques demonstrated previously in aging and Alzheimer's brain.

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ADJACENT MENINGIOMA AND GLIAL TUMORS – CASUAL OR CAUSAL COEXISTENCE

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The occurrence of multiple primary brain tumors of the diverse germinal origin, apart from central form of neurofibromatosis, is uncommon and in most cases this curious coexistence was discovered only at autopsy.

Five cases of meningioma and glioma established in biopsy material from one or more than one operation are reported here. Two cases of an originally benign meningioma were followed by the development of anaplastic glioma in close juxtaposition to the site of operation. The third case revealed a glioblastoma assciated on recurrence with the development of anglioblastic meningioma. In the fourth case two types of neoplastic tissue exhibited continuity in the place of contact between meningioma and glioma. The last case demonstrated conspicuous "island" of meningothelial cells inside the glial tumor of the oligodendroglioma type.

The juxtaposition or continuity of two histologically different tumors suggests that one of them might lead to local proliferation and independent growth of the other.

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UNUSUAL BRAIN DAMAGE IN A TERM NEWBORN WITH SEVERE ASPHYXIA

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A case of cardiac arrest encephalopathy with unusual findings in brain stem in a term newborn with asphyxia after prolonged labour (maternal/fetal disparity) is presented. After resuscitation with artificial respiration initiated he survived 16 days in a deep coma with areflexia.

Widespread hemispheric necrosis of gray and white matter with total loss of neurons and with adjacent reactive astrocytes, microglial cells, macrophages and proliferating vessels was found in autopsy of the brain. Calcification of neurons was present in partially preserved basal ganglia. Symmetrical necrosis of gray matter nuclei of the dorsal brain stem extending from the midbrain to the medulla was observed. In addition to total neuronal and astroglial loss intensive proliferation of vessels and large number of giant multinucleated cells originated probably from monocyte/macrophage lineage was found. They were weakly positively reactive with RCA-1 and immunoreactive with anti-ferritin and anti-muramidase. No evidence of inflammation was found.

Unusual reaction of giant cells and striking intensity of proliferating vessels in the brain stem is noteworthy.

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ASSESSMENT OF THE BLOOD-BRAIN BARRIER BY BIOCHEMICAL EXAMINATIONS IN PATIENTS WITH NON-HODGKIN LYMPHOMA OR ACUTE LEUKEMIA

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The blood-brain barrier (bbb) was evaluated in 18 patients; 10 men and 8 women, aged 16–78 years (mean age 35 years). There were 12 cases with high grade malignancy non-Hodgkin lymphoma, 3 cases with acute myeloblastic leukemia and 3 with acute lymphoblastic leukemia. Four patients had CNS involvement manifested by clinical symptoms and tumor cells appearance in cerebrospinal fluid (CSF). One patient had neuropathy without CNS involvement. The CSF was taken during lumbar puncture, which was done for CNS prophylaxis or treatment. Fifty two samples of serum and CSF were drawn simultaneously. The level of total protein were measured in serum and CSF; IgG, albumin and transthyretin were analysed by rate nephelometry. Albumin concentration quotient (QAlb = CSF alb/serum albumin $\times 10^3$) and IgG concentration quotient (QIgG = CSF IgG/serum IgG $\times 10^3$) were calculated for assessment of the bbb.

The bbb was damaged in all patients with CNS involvement, however, in 3 cases the bbb improved during intrathecal treatment. The disturbance of the bbb in the patient with neuropathy was also noticeable. Minor disturbances of the bbb were noticed in 4 out of sampled cases during intrathecal prophylaxis. In 2 cases without CNS involvement there were locally synthesized IgG in CNS. Measurement of transthyretin provided additional information about the bbb status, because the majority of CNS transthyretin seems to be synthesized specifically by choroid plexus.

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LOCALIZATION AND INTENSITY OF THE PATHOLOGICAL CHANGES IN THE HIPPOCAMPAL FORMATION IN AGING, SENILE DEMENTIA AND ALZHEIMER'S DISEASE

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The intensity and topography of the pathological changes that are observed in the hippocampal formation (which include the hippocampus, entorhinal cortex and subicular complex) are important for understanding of the pathogenesis of dysmnesia in aging, senile dementia, and Alzheimer's disease.

The hippocampal formation from the brain of 7 individuals without dementia, 5 – with senile dementia, and 10 – with Alzheimer's disease, were studied. All the brains were fixed in a 10% solution of formalin for at least a three months. Then a block of hippocampal head was taken and cut into 8-μm-thick paraffin sections. The sections were either stained with cresyl violet or were immunocyto-

chemically stained using the following monoclonal antibodies: against β-amyloid (4G8) or neurofibrillary tangles (Tau-1).

The number of neurons, amyloid deposits and neurofibrillary tangles were estimated using morphometric methods. A characteristic and similar topographical distribution of the pathological changes was observed in all groups, and their greatest development was seen in the CA1 sector, in layer II of the entorhinal cortex and the molecular layer of the dentate gyrus.

The intensity of these changes is much less in the groups of "physiological" aging and senile dementia than it is in the Alzheimer's brains.

So long as dysmnesia in Alzheimer's disease can be linked with severe damage of the hippocampal formation, its relatively small intensity in senile dementia gives rise to the presumption about its different mechanism of pathogenesis in a lot of cases.

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CEREBRAL MICROCIRCULATION DISTURBANCES IN EXPERIMENTAL CARDIAC ARREST

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Microcirculation abnormalities represent an important component of ischemic pathology of the central nervous system. Resulting from cerebral ischemia, they play the role of a secondary pathogenic factor underlying postischemic lesions of the brain tissue. Their nature has been widely described in the neuropathological literature. A large series of experiments in this area have been carried out among others in our laboratories. So far most of attention concerned abnormalities involving, on the one hand, capillary vessels and segments of arterioles and venules which directly connected with them, and larger brain arteries and veins, on the other hand.

Studies described in the present paper aimed at evaluating ultrastructural abnormalities concerning vessels the caliber of which, exceeds that of pre- and postcapillaries. The studies were carried out on adult albino rats, which were subjected to clinical death for the periods of 5 – 10 min, resulting from experimental cardiac arrest performed according to the method described by Korpachev et al. (1982). Scanning and transmission electron microscopic analysis was done on the brains of animals which survived the ischemic incident for the time ranging from 5 min to 24 h. Two types of structural abnormalities were observed. The first one was represented by changes identical with those seen in capillary vessels. These included features of increased micropinocytic activity, enhanced number and length of endothelial microvilli, damage or disintegration of endothelial cells as well as indicators of the blood-brain barrier alteration. The second group of changes, involving exclusively small arteries consisted in the appearance of morphological features of vasospasm. The possible mechanism of the latter as well as its pathogenic role in the development of postischemic tissue damage was discussed.

M. J. MOSSAKOWSKI, I. B. ZELMAN

TOXOPLASMOSIS OF THE CENTRAL NERVOUS SYSTEM IN THE AQUIRED IMMUNE DEFICIENCY SYNDROME

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The aim of the study was to analyse the patomorphology of the brain lesions due to infection with Toxoplasma gondii in the course of aquired immune deficiency syndrome (AIDS). Toxoplasmosis is one of the most common opportunistic pathology of the central nervous system in AIDS. In our material pathological process was found in 12 cases of 45 brains of patients with clinically diagnosed AIDS.

The pathomorphology of the process revealed great variability. Necrotic foci of different size surrounded by a ring of inflammatory reaction were the most common findings. Evolutional stages of inflammatory necrotic process corresponded to all three phases described by Navia et al. (1986)

In most cases the process was multifocal with a characteristically variable advancement of changes in different foci, ranging from small inflammatory granulomas to large chronic toxoplasmic abscesses. Micronodular encephalitis involving practically all parts of the central nervous system was relatively common. In these cases differentiation with other forms of encephalitic processes, including HIV-encephalitis was necessary. Appearance of occasional pseudocysts filled with parasitic cytozoids, not accompanied by any inflammatory reaction or concomitant with pathological processes not related with toxoplasmic infection was found in some other cases. Toxoplasmic involvement of the central nervous system in some cases was the only pathology found in the brain, in others it was accompanying procesess related directly with HIV or different forms of opportunistic processes.

M. MUZYLAK, D. MAŚLIŃSKA

CHANGES IN CENTRAL NERVOUS SYSTEM OF RABBITS AFTER ACUTE AND PROLONGED VINCRISTINE INTOXICATION

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The antitumor effect of vincristine (VCR) is critically dependent on both concentration and duration of exposure. However, neurotoxicity is frequently encountered and is the limiting factor in the drug clinical use.

Since the data concerning the effect of VCR on the central nervous system appeared controversial, the present study was undertaken to examine the ultrastructure of brain parenchyma after the development of neurotoxic side effects in young and adult rabbits. Animals received a single injection of vincristine or were treated five weeks with the drug.

After single injection of vincristine the biphasic reaction of brain vessels (vasoconstriction, increased activity of pinocytotic process) and degeneration of cells (apoptosis) were observed. Following prolonged treatment the consecutive stages of degeneration of neurons (including axons and nerve terminals) were found. Affected astroglial cells were mainly swollen, but some of them developed fibrillary reaction (perivascular). Changes of myelin sheaths were secondary to the changes in axons.

The results provided evidence that the biphasic reaction of brain vessels and apoptosis are crucial, new phenomena which should be included in the pathomechanism of vincristine neurotoxicity.

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CHEMOTHERAPY AND CNS-INTRAVASCULAR COAGULATION IN MYELOPROLIFERATIVE DISEASES

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Studies in hemostasis and neuropathology have been done on 121 patients deceased due to acute myeloblastic leukemia (AML) type M1 and M2 and in 30 patients dead due to blastic phase of chronic myelogenous leukemia (BPCML). Patients with AML were treated with chemotherapy according to protocols: COAP (cyclophosphamide, oncovin, cytosine arabinoside, prednisone), AR (cytosine arabinoside, daunorubicin), TAD (6-thioguanine, cytosine arabinoside, daunorubicin), VAPA (vincristine, adriamycin, prednisone, cytosine arabinoside). Eleven patients with AML were not treated with chemotherapy. During BPCML hydroxycarbamid or 6-mercaptopurine and methotrexate were administered *per os*. Intravascular coagulation (IVC) was common in AML-patients who died within 14 days after chemotherapy (64.1% of cases – group I), less frequent in patients deceased after longer period (53.1% – group II) and rare in AML-patients untreated with chemotherapy (36.4% – group III) and in patients dead due to BPCML (33.3% out of BPCML-cases – group IV). Differences between groups I and III, II and III, I and IV, II and IV were statistically significant. IVC was

more frequent in patients treated according to TAD (81.3% of cases) and VAPA (78.5%) than in patients treated accordingly to AR and COAP protocols or with BPCML-chemotherapy (54.5%, 53.8% and 33.3% of cases, respectively); the difference was statistically significant.

The athors have concluded that polychemotherapy, especially according to TAD and VAPA protocols, may cause IVC in the central nervous system in patients with AML. IVC is more seldom in BPCML due to less "aggressive" chemotherapy, applied orally.

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NON-NEOPLASTIC INFILTRATIONS IN THE CNS AS A MANIFESTATION OR PARANEOPLASTIC PROCESS IN MYELOBLASTIC LEUKEMIAS

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The clinical trials and neuropathological investigations were done in 30 patients of both sexes, deceased due to acute myeloblastic leukemia or chronic myelogenous leukemia in the blastic phase. In all cases groups of non-neoplastic cells, located in the vicinity of CNS blood vessels were observed. For immunologic cells characterization the following monoclonal antibodies were used: anti-human B cell, anti-human T cell, anti-human neutrophil elastase and anti-GFAP. Immunostaining was performed with APAAP technique. The number of vessels surrounded by cell groups ranged in particular cases from 2 to 38. Cell agglomerations appeared as infiltrations in the perivascular space and in the vessels wall or as glial nodules located in the neighbourhood of vessels. The above described abnormalities we observed in the white matter of all cases and in the grey matter only in 25% of cases. The cell agglomerations were present most frequently in frontal and occipital lobes. They were more seldom in brain stem and in cerebellum. The glia nodules and perivascular infiltrations appeared in the vicinity of small veins, more rarely in the neighbourhood of larger veins and most seldom around capillary vessels. The results suggest that the non-neoplastic cell accumulations are the manifestation of paraneoplastic mechanisms of interaction between nervous tissue and leukemia. They were less frequent in cases with leukostatis and leukemic infiltrations in the CNS. The mean number of cellular accumulations was: 11 in cases with CNS leukemia and 16 in cases without CNS leukemia. The ineffective immune response due to malignancy may, therefore contribute to the expansion of leukemia.

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DURATION OF BLASTIC PHASE OF CHRONIC MYELOGENOUS LEUKEMIA AND PARENCHYMATOUS CHANGES IN THE CNS

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The clinical trials and neuropathological investigations have been done on 31 patients of both sexes, deceased due to blastic phase of chronic myelogenous leukemia (BPCML). The cases were divided into groups according to BPCML duration. Group I consisted of 17 cases with BPCML duration from 1 to 10 weeks (on the average 4.8). Group II contained 14 cases with BPCML duration from 12 to 99 weeks (on the average 34.3). During the chronic phase the patients in both groups were treated with Busulfan or Hydroxycarbamid. During the BPCML Hydroxycarbamid or 6-Mercaptopurine and Methotrexate were used. Cytostatics were administered *per os*. The investigation revealed that parenchymatous changes in the CNS were similar in both groups. Degenerative changes of neurons and focal or diffuse neuronal loss were observed, especially in the frontal and hippocampal cortex and less frequently in the cerebellar cortex and in dentate nuclei. The evident changes were also observed within the ependyma, namely the swelling of ependymal cells, their segmental or diffuse loss and granular ependymitis. The ependymal changes were more frequent in the lateral and in the third

ventricles. The intensity of parenchymatous changes in the CNS was similar in both groups in spite of significantly different BPCML duration. On the strength of these results, the authors suggest that BPCML duration doesn't influence the CNS parenchymatous changes.

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IMMUNOHISTOCHEMICAL STUDIES IN A CASE OF PURKINJEOMA

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We report here a case of 55-year-old female in whom a posterior fossa tumor was clinically suspected but not confirmed in 1989. In 1992, she was readmitted because of aggravated headaches and vertigo. CT scan revealed a cerebellar bihemispheric mass which was surgically removed. The histopathological diagnosis was *Purkinjeoma* or *dysplastic gangliocytoma.*

Gangliocytomas are slowly growing childhood tumors with generally good prognosis. They occur most commonly in cerebral hemispheres and may be cystic or calcified. Certain peculiar lesions, not clearly neoplastic in nature, have been reported in adults. These include expanding cerebellar lesions alternatively named *Purkinjeoma, dysplastic gangliocytoma, gangliomatosis* of the cerebellum, diffuse hypertrophy of the cerebellar cortex or Lhermitte Duclo's disease (Russel and Rubinstein, 1971). In these lesions, the cerebellar architecture is distorted. There is thickening of folia over limited areas consisting of an outer layer of well-developed myelinated fibers and an inner layer of abnormal neurons superficially resembling the Purkinje cells. Abnormal neurons are delineated by SF-immunoreactive structures. The cerebellar white matter is either reduced or completely absent.

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IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES OF NEURONAL DIFFERENTIATION IN PRIMITIVE NEUROECTODERMAL TUMORS*

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Twenty eight medulloblastomas and 7 desmoplastic medulloblastomas were studied by immunohistochemistry with antibodies against neuron specific enolase (NSE), 200 kDa partially phosphorylated neurofilament protein (NFP) and synaptophysin (SF) in all cases and by electron microscopy in 15 cases. Ultrastructurally, the most frequent feature of neuronal differentiation found in all specimes was the presence of neurotubules and pleomorphic dense core vesicles. Neuronal rosettes were observed in 70% of cases and synaptic specializations in 3 cases. NSE immunoreactivity was found in all samples in most of the cells. SF and NFP immunoreactivity was observed in 27 tumors. In contrast to NSE immunoreactivity, only a fraction of tumor cells showed NFP and SF immunoreactivity ranged from isolated immunopositive cells to larger immunopositive areas.

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COMPLETE CEREBRAL ISCHEMIA – PLATELETS – BRAIN BETA-AMYLOIDOSIS

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Occurrence of local microcirculation disorders in different brain structures in various periods of recirculation after cerebral ischemia is commonly known. In order to evaluate the possible involvement of blood platelets and their cytotoxic substances in the afore-mentioned phenomena, thrombocytes accumulation in the cerebral vessels was studied using the scanning and transmission electron microscopy. The experiments were carried out in rats in which complete central nervous system ischemia was produced by cardiac arrest[1]. The ischemia lasted 5 or 10 minutes and the survival time was 3, 5 and 15 minutes; 1, 3, 6, 24 and 48 hours; 6, 10 and 12 months. Neither accumulation nor aggregation of platelets was observed in the control rats, while in the experimental animals aggregation and accumulation of blood platelets and their adhesion to vascular wall was noted irrespectively of the survival time. Numerous platelet aggregates varying in size and stage of disintegration were seen in the lumen of the arterial and venous vessels. Thrombocytes localized in the vicinity of the endtothelial cells were often degranulated, with altered shape and numerous projections. Platelet aggregates were noted in various structures of the central nervous system: the cerebral cortex, thalamus, basal ganglia, hippocampus and cerebellum. Single or aggregated thrombocytes were also seen in the perivascular space. The platelet aggregation occurred both in animals with a short survival span after the ischemic episode and in those with a long one (e.g. 1 year). The aggregates tended to localize at the site of vascular branching or bifurcation which corresponded closely with changes in blood-brain barrier permeability. Our results were consistent with the hypothesis of persistence or even irreversibility of the endothelial cell damage initiated by the ischemia. Continuous stimulation of the endothelial cells by the activated thrombocytes may lead to their dysfunction resulting in a *circulus vitiosus* of development of disseminated microfocal tissue damage. Superimposition of these changes, which may be a clinically asymptomatic progressive process, may present an important factor in the pathomechanism of a slowly progressing postischemic encephalopathy, and possibly of other degenerative central nervous system diseases involving accumulation of beta-amyloid. Under these circumstances beta-amyloid may originate from intra- and extravascular aggregates of thrombocytes containing its precursor.

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[1] Acta Neuropathol., 1991, 83, 1–11.

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ON THE PARTICIPATION OF BLOOD VESSELS IN THE PROCESS OF HUMAN SPINAL CORD MYELINATION

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Appearance of the reactive astrocytes during myelination of human spinal cord was characterized immunocytochemically in our previous investigations[1]. A question arised whether vascularization of the spinal cord undergoes changes at the same time.

The material comprised spinal cord segments C8 or Th1 of 5 human fetuses aged from 18 to 34 weeks and of 6 infants aged from one day to 3 years. Peroxidase-antiperoxidase Sternberger's et al.

(1970) method for visualization of myelin basic protein (MBP) and endothelial cells (lectin *Ulex Europeus* and Factor VIII) was used on formalin-fixed paraffin-embedded sections.

Endothelial cell immunoreactivity to factor VIII appeared later than immunoreactivity to lectin. The latter appeared during myelination gliosis within the posterior funiculi and spinal proper tracts in the 18th week of fetal life. Then, in parallel to increasing immunoreactivity to MBP, *Ulex Europeus* exhibited numerous small blood vessels in other spinal cord tracts. Increasing vascularity during myelination was clearly manifested within pyramidal tracts, which underwent myelination later than phylogenetically older tracts. In the fetal period, the pyramidal tracts, especially lateral ones, were hypovascularized. Within anterior pyramidal tracts nonnumerous vessels were observed in the 34th week of fetal life and in the 1st postnatal day – within lateral pyramidal tracts. In the period of the full or almost full morphological maturity of myelin, white matter of the spinal cord became again hypovascularized.

The mechanisms of a temporary increase of blood vessel supply in the spinal white matter undergoing myelination require further investigations.

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[1] Neuropatol. Pol., 1991, 29, 41–47.

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JUVENILE MOYAMOYA DISEASE IN THE COURSE OF PRIMARY IMMUNODEFICIENCY. CASE REPORT

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An eight-year-old girl (A.B., 85/93) with inborn immunodeficiency suffered from repeated severe infections. She was treated by transplantation of cultured thymus epithelium cells in three-year--interval, then with Thymostimulin (Serono). After short improvement hemolytic anemia and pronounced neurological symptoms appeared. Arteriographically, occlusion of the left internal carotid artery (ICA) was noted. Shortly after, the patient died.

Macroscopic pathology: Besides hyperplasia of the thymus other main changes involved the cranial portion of both ICA and the circle of Willis arteries. All these vessels showed irregular thickening, were hard and with irregular, narrow, sometimes occluded lumen. Two aneurysm-like formations were found connected to the anterior communicating and to the left anterior cerebral artery. These changes associated with colliquative necrosis of the left and disseminate necrotic foci in the right cerebral hemisphere with brain stem herniation, causing brain death.

Light and electron microscopic examination: Twenty specimes from brain base arteries, stained with H&E, van Gieson, and Masson methods, demonstrated pathological changes. They were manifested by: a) generalized thickening of the *tunica interna* due to fibrosis with pronounced reduction of the vessel's lumen, b) thickening, or splitting, and discontinuity of the internal elastic laminae, c) atrophy or fibrosis of the *tunica media* and *adventitia*, d) formation of new blood passages between fibres of the elastic lamina and layers of connective tissue creating pseudoaneurysm, e) the recanalization of organized thrombi.

Conclusion: The limitation of pathological changes to the cranial portion of the internal carotid and circle of Willis arteries suggest the Moyamoya syndrome which have developed as a result of autoimmune processes in the course of a T cell defect.

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CANDIDA ALBICANS INFECTION IN NEONATES AND INFANTS REQUIRING PROLONGED INTENSIVE CARE

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Fifteen neonates and infants who required neonatal intensive care developed systemic candidiasis. Then of them were premature (26–27 weeks of gestation, mean 30 weeks) with low or very low birth weight (900–2250 g, mean 1278 g). Clinical diagnosis was: sepsis (11/15), necrotizing enterocolitis (5/15), meningitis (1/15), mucoviscidosis (1/15), DIC (4/15). Positive candida cultures (blood, urine) were obtained in six cases. At autopsy systemic *Candida albicans* infections with multiple-organ involvement were diagnosed: lungs (11/15), kidneys (5/15), heart (5/15), jejunum (1/15). Neuropathologically meningeal and brain candidiasis was diagnosed in all cases. In 13/15 cases inflammatory changes (polymorphonuclear leukocyte infiltrations, multiple abscesses, granulomas, glial reaction) were very intensive and widespread with heavy fungal infiltration. In the other two, very premature cases, inflammatory changes were connected with a widespread brain necrosis.

The purpose of our report is to indicate the discrepancy between clinical detection of fungal infection and extensive autopsy changes and also to indicate the importance of autopsy investigations for clinic at the time when autopsy is so often discontinued.

B. SCHMIDT-SIDOR[1], K. SZYMAŃSKA[2], M. PAKSZYS[3]

A CASE OF NON-KETOTIC HYPERGLICYNEMIA WITH OCCLUSIVE CHANGES OF MENINGEAL AND BRAIN VESSELS. A CLINICO-NEUROPATHOLOGICAL CASE REPORT

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Non-ketotic hyperglicynemia is an autosomal recessive metabolic disease with high level of glycine in plasma and cerebro-spinal fluid. In the infantile form of the disease neurological symptoms (hypotonia, myoclonic seizures, apnea, progressive obtundation) begin during the neonatal period. Affected infants who survive neonatal period are profoundly retarded. Neuropathological picture discloses reduction of white matter with disturbed myelin formation and extensive spongy degeneration with marked gliosis.

A case of an infant girl born with signs of intrauterine dystrophy is reported. Physical and psychomotor development was arrested from the beginning of life. Tonic-clonic and myoclonic seizures started at the age of two months. The EEG demonstrated a burst-supression pattern. At six months she was microcephalic, deeply retarded with spastic quadriplegia. Glicyne plasma level was 0.373 μmol/ml (N 0.208–0.071), glicyne CSF level was 0.022 μmol/ml (N 0.006–0.002). She died at 12 months of age. Neuropathological investigation disclosed: 1. Symptoms of brain development delay (gyral pattern as in 38–40 week of fetal life, delay of myelination). 2. Degenerative changes (loss of cortical neurons with laminar spongiosis and moderate gliosis, spongy degeneration of myelinated tracts). 3. Vascular changes (intimal thickening of many meningeal and brain vessels with multiple infarcts and hemorrhages of different age and distribution). Large recent cerebellar hemorrhage had been the cause of death.

Till now vascular changes in non-ketotic hyperglicynemia had not been reported. Vascular changes similar to described in our case are known to occur in homocystinuria, a metabolic disease excluded in our case.

S. M. STEFANKO

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY WITH AN UNUSUALLY LONG CLINICAL COURSE

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Patient JvdH, 44-year-old, was known to have chronic lymphocytic leukemia and was in remission after fluaradabine therapy[1]. He was admitted in September 1990 after an epileptic episode. MRI examination revealed multiple white matter lesions in the cerebral hemispheres. Neurologic examination was normal. Brain biopsy and ultrastructural examination showed abnormalities characteristic of progressive multifocal leukoencephalopathy (PML), with groups of closely spaced paracrystalline forms typical of Papova virus. Numerous reactive perivascular lymphoplasmocytic infiltrates were also present. The patient's condition remained unchanged during next 12 months. After one year he died due to complications. The long survival time without clinical manifestation was striking.

A few cases of PML with long survival time or spontaneous remission, have been described[2]. In these cases, as in our case, a lymphocytic perivascular reaction was found, together with typical PML changes. The stabilization of this process can be explained by a high degree of immunologic resistance, manifested morphologically by perivascular inflammatory reaction.

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J. STROSZNAJDER

ISCHEMIA MEDIATED LIPID MEDIATORS RELEASE AND THEIR ROLE IN MODULATION OF NEURONAL SIGNAL TRANSDUCTION

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Brain ischemia induces a massive release of neurotransmitters that bind to the surface receptors and stimulate elevation of several second messengers including these of lipid origin. Brain ischemia was induced by ligation of both common carotid arteries for 10 min in gerbil (*Meriones unguiculatus*). We have observed that brain ischemia in gerbils induces liberation of inositolphosphate(s) (IP_n), diacylglicerol (DAG) and arachidonic acid (AA).

It was found that DAG and AA might modulate not only protein kinase C activity but also phospholipase A, C, and D. AA accumulated during brain ischemia, can be responsible for disturbation of $GABA_A$/Cl-channel receptor function. Moreover arachidonic acid similarly as inositol 1, 4, 5-triphosphate (IP_3) might release Ca^{2+} ions from endoplasmic reticulum. Our studies indicate that liberation of these lipid derived second messengers occur as a consequence of phosphoinositides degradation by phospholipase C action coupled to muscarinic, cholinergic receptor (mAChR) and serotonergic $5HT_2$ receptor. However, arachidonic acid is liberated also directly from membrane lipids, particularly from phosphatidylinositol, by the action of phospholipase A_2 (PLA_2). Our studies on PLA_2 have shown that Ca^{2+}-dependent PLA_2 is also stimulated by mACh and $5HT_2$ receptor. The ischemic induced polyphosphoinositides depletion cannot be restored. Resynthesis of polyphosphoinositides in ischemic brain is significantly impaired. Ischemia decreases lipid phosphorylation through reaction of phosphoinositides kinases. The ischemic induced polyphosphoinositides degradation and depletion, particularly phosphatidylinositol 4, 5-biphosphate (PIP_2), might be in consequence responsible for alteration of neurotransmission and for the modification of cytoskeletal dynamics in neuronal cells.

It was observed in our studies that application of specific antagonists to serotonergic $5HT_2$ receptor and muscarinic cholinergic receptor or agonists of serotonergic $5HT_{1A}$ and GABA receptors before ischemia may have ameliorating effect on postreceptor processes activated by ischemia. These compounds applicated intraperitoneally several minutes before ischemia protect the brain against liberation and accumulation of lipid mediators.

J. SZCZECH, M. WENDER, L. MAZIARZ, K. KUSZA

OLIGODENDROGLIA-MYELIN COMPLEX IN THE POSTRESUSCITATION SYNDROME

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In the experimental model of clinical death in rats the oligodendroglia-myelin complex was evaluated 1, 2, 9 and 14 days after resuscitation. Karyometric measurements of oligodendroglia cell nuclei were performed on Feulgen-stained slices using an automatic analyzer of microscopic pictures. Planimetric measurements of myelin fibres were performed on EM prints.

Changes in the myelin fibres and in the oligodendroglia cells found at different time intervals after ischemia in various brain region varied. Twenty four hours after resuscitation the parameters of oligodendroglia nuclei size showed an increase of section circumference and area of cross-section, together with changes of form factors, demonstrating an increase of nuclear membrane foldings, what may be the sign of nuclei edema. After 9 and 14 days the changes in nuclei form remained, which can be explained by the persisted lesions of membrane structure. This conclusion is supported by the occurrence of regional differentiation of DNA extinction and indices of nuclear chromatine state, seen in the late period after resuscitation. The changes of oligodendroglia may be primarily responsible for the structural lesions of myelin observed in the postresuscitation syndrome.

M. SZENBORN, A. BRZECKI

NEUROPATHOLOGICAL STUDY ON THE NUCLEUS BASALIS OF MEYNERT IN MATURE AND OLD AGE

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The nucleus basalis of Meynert (nbM) is the single major source of cholinergic innervation of the entire cerebral cortex. Data from human and animal studies suggest that brain cholinergic activity diminishes as a consequence of aging. The aim of this study was the description of the morphological changes in the nbM in normal aging.

Our patients, who died of non neurological diseases and without dementia, were divided into three comparison groups accordingly to the age at death; the young group (A) – 10 brains of patients (mean age $x = 34.7$), the mature group (B) – 15 ($x = 55.2$), the old group (C) – 18 ($x = 75.0$) and additionally 2 Alzheimer's brains.

Lipid affinity was characteristic of neurons of the nbM. It occurred in young cases and reached maximum in the mature age. Astrocytic gliosis (increase in GFAP-positive astrocytes) dominated in the group B and decreased in the group C. Fibrillary gliosis showed significant positive linear correlation with age ($p < 0.001$). The remarkable feature of the aging was significant cell loss in the nbM in advanced age group ($p < 0.05$).

The cellular loss in the nbM, which projects to entire cortical mantle, may be responsible for the cortical atrophy and disorders of higher cortical function that appear during normal aging.

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GLIOMA OF THE SPINAL CORD AND FOURTH VENTRICLE ASSOCIATED WITH SYRINGOMYELIA AND SYRINGOBULBIA

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A 28-year-old patient with intramedullary and intraspinal (C1–C4) tumor penetrating the fourth ventricle associated with syringomyelia (C5–Th6) diagnosed with MRI is presented. The duration of illness from the first signs and symptoms until tetraplegia was 4 months. The patient died 8 months later. Surgical spinal decompression performed 8 months before death did not improve the neurological symptoms.

The specimens from the brain and spinal cord were stained with HE, cresyl violet, van Gieson's, PAS, PTAH, Cajal's, Bielschowsky's and Klüver-Barrera's methods. Immunoreactions with GFAP and vimentine were also performed. Microscopical analysis showed that the intraspinal tumor originated from glial stem over the syringomyelic cavities and was growing up into the fourth ventricle. The tumor was composed mainly of elongated cells forming multilayer pseudorosettes with fibrillated tail attached to a central blood vessel. Nonnumerous tubules with rosettes were also found as well as dot-shaped juxtanuclear PTAH-positive blepharoplasts. Several areas of anaplastic giant cells with bizzare nuclei, single or multiple, were observed. These GFAP-negative cells were not stained positively in Cajal's method as well. In these polymorphic areas large necroses and pathological vessels with endothelial proliferation were found.

Syringomyelic cavities were multilocular. They were not connected with the spinal central canal. They were irregular in their course along the spinal cord below the glial stem. Their walls were composed of thick layer of glial fibres and monstrual cells with very long processes. The syringobulbia cavities were bilateral, localized in latero-dorsal part of medulla. At different levels both tumor and syringomyelia caused compressive damages of the surrounding tissues including the anterior and posterior horns. Secondary demyelination and degeneration of spinal tracts and roots was observed.

The morphological analysis suggests the diagnosis of anaplastic ependymoma converted into the glioblastoma multiforme probably connected with clinically asymptomatic, earlier existing, syringomyelia.

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CORRELATION BETWEEN ADENYLATE CYCLASE (AC) ACTIVITY AND LOCALIZATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN RAT BRAIN AFTER CLINICAL DEATH

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Presence and distribution of vasoactive intestinal polypeptide (VIP) and changes in the activity of adenylate cyclase were evaluated in the brain of rats subjected to 10 min clinical death due to cardiac arrest. Experiments were performed in rats in which clinical death was induced according to the method of Korpachev et al. (1982). Ten minutes after cardiac arrest resuscitation procedure was undertaken until full recovery of spontaneous heart action was observed. Animals were sacrificed 3 and 24 hours, 3 days and 3 weeks after ischemic incident.

Peroxidase-antiperoxidase method (PAP) with DBA was used for immunocytochemical investigation of VIP. Adenylate cyclase activity was studied by histochemical method for light and electron microscopy based on the precipitation of strontium. Isoproterenol and GppNp were used as activators and AMP-PAP as substrate for detection of the enzyme.

In control rats brains cut on the level of dorsal part of hippocampus VIP immunoreactivity was observed in many brain regions. This was the highest in the neurons of cerebral cortex, in the nerve fibres and cell bodies of some thalamic nuclei, in ependymal cells and choroid plexus. Moderate

VIP-positive staining was observed in the walls of microvessels and around them. In experimental animals remarkable decrease of the reaction intensity was observed in various regions and structures of the brain. The activity of adenylate cyclase was encountered in the brain vascular network. Progressive decrease of enzyme activity was noted in the early survival stages reaching the lowest intensity 24 hours after ischemic incident. Electron microscopic study revealed disturbances of normal polarity of AC distribution in vascular endothelium.

The presented data indicating simultaneous decrease of VIP-immunoreactivity and changes in localization and intensity of histochemically detected AC-activity, resulting of brain ischemia, may suggest relation between these abnormalities. This implies a connection of AC-alteration with changes in vasomotor function of VIP.

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EFFECT OF SODIUM TELLURITE ON THE MYELIN IN THE ADULT RAT BRAIN

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Previous studies on the intoxication with tellurium performed in rats aged less then 3 weeks revealed a transient peripheral neuropathy with segmental demyelination and remyelination.

These studies aimed in morphological and ultrastructural characteristics of the neurotoxic effect of sodium tellurite on the myelin of the CNS and the optic nerve in the adult animals. Female 25 Wistar rats weighing about 130 g were used in the experiment. Single 2.5 mg/kg Te^{+4} dose was administered intraperitoneally. Morphological and EM studies were carried out on the 7th and on the 30th day after the intoxication. Sodium tellurite caused disappearance of almost all myelinated fibres in the cerebral cortex, in the white matter, and in the corpus callosum, while myelin staining in the pyramidal tract and in the optic nerve appeared only slightly lighter than in the controls after 7 days from the intoxication. Almost complete remyelination was observed in the examined structures after 30 days.

EM studies showed pathological changes in some examined structures in the CNS. In the optic nerve some sheaths of the myelinated fibres were thinner and swelling of their axoplasm was noted. After 7 days swelling was also observed in some oligodendrocytes. Abnormalities of the remyelination process was shown on the 30th day after the intoxication with sodium tellurite. Pathological changes in the cerebral white matter were similar in character and intensity to those described in the optic nerves. Ultrastructural changes were also noted in the synapses of the cerebral cortex adjacent to the white matter.

The pathology of the transient demyelination and of the remyelination following sodium tellurite intoxication may be explained by changes in the activity of some enzymes, as blockage of the cholesterol biosynthesis pathway at the level of convertion of squalene to the 2,3 epoxysqualene.

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ULTRASTRUCTURAL BRAIN CHANGES IN YOUNG RATS WITH MORRIS HEPATOMA AND WITH OR WITHOUT FARMORUBICINE TREATMENT

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The aim of the study was the evaluation and comparison of ultrastructural brain changes in young Buffalo rats with experimentally induced neoplastic disease and in rats subjected to anti-neoplastic chemotherapy. The experimental material included 3 groups of animals: 1) rats implanted subcutaneously at 14th day of life with Morris hepatoma 7777 and examined after 3 weeks of tumor

growth; 2) healthy 14-day-old rats treated with farmorubicine (3 injections at weekly intervals); 3) rats with implanted Morris hepatoma 7777 and farmorubicine treatment. Specimens for electron-microscopic study were taken from the corpus callosum, subcortical white matter and cerebral cortex of experimental and controls rats.

Previous light microscopic study performed on the same experimental model suggested that in rats with neoplastic disease subjected to chemotherapy the damaging effects of both can interfere leading to enhacement of brain changes. The present study demonstrated striking differences in ultrastructural feature between the first and second animal groups. In the group 1 the most frequent changes were noted in mitochondria, which were augmented with hypertrophied cristae and increased matrix density, and in axons displaying shrinkage and condensation of axoplasm. In the group 2 the changes were characterized by swelling of glial cells and neuropil processes. However, in the group 3 an increased incidence of degenerated cells and nerve fibres together with changes characteristic of two other groups were found. These results indicate an increased susceptibility of immature nervous tissue to noxious action of the cytostatic in rats affected with neoplastic disease.

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CEREBELLAR CHANGES IN MICE WITH SPONTANEOUS MAMMARY TUMORS

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Morphological changes in the cerebellum of mice with spontaneous mammary neoplasms of varied malignancy were estimated for comparison with paraneoplastic cerebellopathies in humans with mammary carcinomas.

Cerebellar changes in neoplastic mice, in contrast to control animals, concerned most often the Purkinje cells, but extensive, complete degeneration of neurons in cortical cerebellar layers were never oberved. However, neuronal changes were also frequently noted in cerebellar nuclei. Coexisting GFAP-positive perivascular glial reaction, increased cellular gliosis in the molecular and Purkinje cell layers, vascular congestion and changes of edematous origin may suggest the influence of humoral factors (indirectly or directly connected with the tumor) on the impairment of neuroectodermal elements in murine cerebellar tissue.

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ASTROCYTIC MARKERS IN RAT SPINAL CORD IN EXPERIMENTAL HYPERAMMONEMIA

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Hyperammonemia (HA) was induced in Wistar rats by 3 i.p. administrations of thioacetamide (259 mg/kg body weight) at 24 h intervals. Control animals received 0.9% NaCl according to the same protocol, and the animals were decapitated 24 h after the last administration of the drug. Kryostat sections of the cervical region of spinal cord were immunostained for two astroglia-specific proteins: glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS), using a double fluorescence method. The first layer included monoclonal mouse antibodies against pig spinal cord GFAP (1:400), or rabbit anti-GS antibodies (1:50). The second layer consisted of fluorescein (FITC)-conjugated rabbit antimouse antibodies or rhodamine (TRITC)-conjugated pig antirabbit antibodies, respectively, both 1:20. Incubations were carried out at room temperature.

In control sections, moderate GFAP reaction in astroglial processes and only rudimentary GS reaction confined to astrocytic cytoplasm were noted in both dorsal and ventral horns of spinal cord. HA intensified GFAP reactivity of the individual astrocytes, but also increased the number of GFAP-positive cells. HA enhanced GS reactivity in the cytoplasm and made it discernible in the astrocytic processes, which may indicate increased *de novo* synthesis of the enzyme.

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MORPHOLOGICAL STUDY ON MICROGLIA IN HUMAN MESENCEPHALON DURING THE FETAL DEVELOPMENT AND IN THE PROCESS OF AGING

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The ontogeny and the morphology of microglia in the mesencephalon has been the focus of attention of neuropathologists interested in the central nervous system degenerative diseases. To evaluate the cytogenesis and the structure of these glial elements, we studied the mesencephalons in 47 human fetuses in the 7th—40th weeks of postmenstrual gestation age and in the 18 adult brains from 20 to 70 years old.

The microglia was identified and characterized by morphological criteria using immunocytochemistry for ferritin and Ricinus communis agglutinin-1 (RCA-1). As early as the 8th week of gestation, RCA-1 positive cells were detected in fetal mesencephalon. Up to the 9th weeks of gestation they were visible as rounded perivascular cells without processes. In the later period microglia cells were rounded with short processes also in the areas remote from the vessels. In the 18th—40th week of gestation age, there was variability in the morphology of microglia. In fetuses born alive these cellular elements were more ramified. In adult mesencephalon these glial elements could be classified into three categories: the rounded cells with short thick processes, detected near vessels; long branched cells, found along the fibre tracts and the radially branched cells found in the area of the substantia nigra. These glial elements did not show any features characteristic for human brain aging. These results reveal that mesencephalon microglia cells appear very early in the ontogeny and they do not change significantly during aging of the brain.

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THE CASE OF POLYGLUCOSAN BODY DISEASE (APBD)

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A 45-year-old unconscious woman was admitted to hospital, where she died 3 days later. Before she had only suffered from headache during the previous month. She had had no past medical history. Cerebrospinal fluid pressure was increased, there were 350 mg/100 of protein, and 105 mg/100 of glucose. Brain specimens were stained with histological methods: H&E, PAS, PAS-dimedon, cresyl violet, Bielschowsky and immunohistochemical techniques using anti-glial fibrillary acidic protein (GFAP) and *Ricinus communis* agglutinin-1 (RCA-1). The main microscopic abnormality was massive accumulation of round or oval polyglucosan bodies (BP) of various size in the cerebral hemipsheres, brain stem and cerebellum. These bodies were found most frequently around vessels in the white matter and under the pia in the cortex. The PB were found in the processes of nerve cells, astrocytes, microglial cells, but not in their perikarya. The stored material stained strongly in PAS, PAS-dimedon, weakly in H&E, cresyl violet, Bielschowsky, but reaction for GFAP and RCA-1 occurred only in the peripheral part of PB. The PB stained with cresyl violet and the Bielschowsky's methods fall into three categories: the light bodies, the dark bodies, and the light bodies with dark cores. These clinicopathologic features were consistent with the diagnosis of adult polyglucosan body disease (APBP) and were different from other conditions in which PB may accumulate.

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BRAIN TISSUE FROM BOTH NEURONAL CEROID LIPOFUSCINOSES AND MUCOPOLISACCHARIDOSES, BUT NOT LONG TERM FIBROBLAST CULTURES SHOW INCREASED EXPRESSION OF SUBUNIT C OF MITOCHONDRIAL ATP SYNTHETASE

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Recent data showed specific storage of a proteolipid, subunit c of mitochondrial ATP synthetase in late infantile, juvenile and adult forms of neuronal ceroid lipofuscinosis (NCL). At present, we have investigated the expression of subunit c in long term cultures of fibroblasts from patients with NCL, mucopolisaccharidosis (MPS) and normal controls. It was aimed to evaluate the usefulness of this model to study accumulation of the storage material under definely modified conditions. Western blot analysis showed that the expression of subunit c in long term fibroblasts in culture, both grown in standard conditions and after leupeptin and ammonium chloride treatment (inhibiting lysosomal function) was not increased compared to MPS and normal controls. Moreover, subunit c immunoreactivity was distinctly higher in brain tissue homogenates, even from young healthy controls, than in NCL fibroblasts. Following our finding of high expression of subunit c in brain homogenate not only from the NCL, but also from MPS case, we performed immunohistochemical study on brain tissue section from MPS type I and type III, NCL and controls. We have observed significant neuronal immunoreactivity of subunit c in MPS, although weaker than in LINCL, but markedly stronger than in agematched controls. Therefore, we conclude that NCL fibroblasts in long term cultures, due to yet undefined factors, loose their ability to accumulate subunit c. Moreover, increased subunit c immunoreactivity found at present in MPS, other lysosomal storage disorder, favors the hypothesis that accumulation of this proteolipid observed in NCL may be caused by its altered degradative pathway.

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CEREBROSPINAL FLUID BETA-2-MICROGLOBULIN IN PATIENTS WITH NON-HODGKIN LYMPHOMA OR ACUTE LEUKEMIA TREATED WITH CHEMOTHERAPY

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Beta-2-microglobulin (B2m) was measured in the cerebrospinal fluid (CSF) and serum in order to detect early central nervous system (CNS) involvement. The studies were prospective and included 18 patients, 10 men and 8 women, aged 16–78 years (mean age 35 years) with high grade malignancy non-Hodgkin lymphoma (12 pts), acute lymphoblastic leukemia (3 pts) and acute myeloblastic leukemia (3 pts). The CSF was taken during lumbar puncture, which was done for CNS prophylaxis or treatment. Fifty four samples of serum and CSF were drawn simultaneously. CSF and serum B2m were measured by enzyme immunoassay. Four patients had CNS involvement manifested by neurologic symptoms and tumor cells in the CSF. One patient had neuropathy without CNS involvement.

The level of CSF-B2m in three patients with CNS involvement and in the patient with neuropathy was below 3 mg/l. The ratio CSF-B2m/serum-B2m in the three patients with CNS involvement, in the patient with neuropathy and in two patients undergoing intrathecal prophylaxis was below 1. An increase of CSF-B2m level during intrathecal prophylaxis in the patient with neuropathy and a decrease of CSF-B2m level after intrathecal treatment in two cases with CNS involvement were observed.

The authors conclude that determination of CSF-B2m may be useful in early diagnosis of CNS involvement and in monitoring intrathecal chemotherapy.

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INTRACEREBRAL UPTAKE OF PLASMA PROTEINS AFTER CARDIAC ARREST IN HUMANS

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We investigated intracerebral localization of selected serum proteins in brains of ten subjects, aged 44—66 years, who had died in the postresuscitation period following cardiac arrest in various times after acute incident, ranging from 1 to 36 days. Formalin-fixed, paraffin-embedded sections from the frontal and temporal regions were used and immunocytochemical ABC and PAP methods were applied vor visualization of serum proteins.

Accumulation of extravasated albumin and fibrinogen was detected intracellularly in ependymal cells, neuronal and astrocytic bodies and processes and as a diffuse reaction without relation to any CNS structural elements, predominantly in the subpial and subventricular regions. The distribution of intracerebral immunoreactivity both intracellular and diffuse, presented characteristic irregular, disseminated pattern with accentuation of changes in the vicinity of blood vessels and in areas of more advanced tissue alterations. No protein extravasation into extended perivascular spaces was detected.

Positive staining of both ischemic and histologically unaltered neurons during the whole observation period indicated the progressive development of neuronal damages. Taking into account the functional differences between albumin and fibrinogen, the latter as a representative of acute phase protein, may exhibit protective action in the process of nerve cell injury. The astrocyte immunostaining was indicative for preexisting edema and active participation of astrocytes in resolution of extravasated proteins. The discrepancy between intensity of vasculogenic tissue damage and relatively poor astroglial immunoreactivity was considered to be related to profuse damage of a part of astroglial population.

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