NEUROPAT. POL. 1990, 28, 3-4
PL ISSN 0028-3894

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DYNAMICS OF PATHOMORPHOLOGICAL CHANGES IN THE BRAIN OF RATS AFTER CLINICAL DEATH

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Progress in studies on functional, metabolic and structural aspects of cerebral ischemia turned the attention of numerous authors to the late sequelae of the ischemic incident. The milestones on this way have been marked by observations concerning metabolic abnormalities resulting from temporary ischemia which may play the role of both compensatory and secondarily damaging factors (Siesjö 1981), by description of the maturation phenomenon (Ito et al. 1975; Klatzo 1975; Yamagushi, Klatzo 1984) and of the feature, known under the name of the delayed neuronal death (Suzuki et al. 1983 a, b; 1985), with its implication concerning the possibility of a neurotransmitter-induced excitotoxic mechanism of neuronal lesions (Pulsinelli 1985 a, b). Two opposite phenomena underlay studies on late sequelae of cerebral ischemia — the first of which is the question of reversibility of tissue damage due to ischemia, the second — the progressive nature of ischemic encephalopathy stressed by reanimatologists.

Studies of those problems require an appropriate experimental model, preferentially of global cerebral ischemia. In searching for such one we decided to apply the model of experimental clinical death in rats, described by Korpachev and his collegues in 1982. The main advantage of the model consists in the possibility of long survival of animal after the resuscitation procedure, related with relatively little experimental trauma. In addition it does not require deep anesthesia and pharmacological treatment. Its disadvantages consist in high mortality during and immediately after the experimental procedure, connected to a great extend with the duration of cardiac arrest and above all in the fact that it is not a model of isolated cerebral ischemia, but of experimental clinical death with all consequences resulting from generalized ischemia of all body organs (Safar 1986).

Paper presented at Symposium on Neurobiology of Cerebral Ischemia and Hypoxia. Poznań, June 29-July 1, 1989.

MATERIAL AND METHODS

The experiments were performed on adult albino rats of both sexes, weighing ca 180 g in which clinical death was induced according to the method described by Korpachev et al. (1982). Compression of the heart vascular bundle by a special hook inserted into the thorax led in the course of 1.5-2 min to complete cardiac arrest and cessation of the respiratory function lasting till resuscitatory management was undertaken. In the case of our experiments this was done either after 10 or 15 min of complete cessation of brain bioelectric activity. Following resuscitation, which included external heart massage and controlled respiration, the experimental animals survived for 3, 6 and 24 hrs, 3, 7, 14 and 28 days as well as 6, 9 and 12 months. Their age mates not subjected to any experimental procedures formed the control group.

The brains of control and experimental animals were examined histologically and immunomorphologically. Histology was done on paraffin sections stained with hematoxylin-eosin and according to the Klüver--Barrera's method. Immunomorphology included immunostaining of glial fibrillary acidic protein (GFAP). Detailed description of methods applied is presented in previously published papers of Mossakowski et al. (1986), Mossakowski and Krajewski (1988) and Zelman and Mossakowski (1988).

As detailed physiopathological characteristics of the experimental model are given elsewhere (Mossakowski et al. 1986; Kapuscinski 1987; Majkowska 1989), only basic data concerning cerebral bioelectric activity and cerebral blood flow are presented here.

Control electrocorticographic activity preceding compression of the heart vascular bundle was typical for anesthetized animals with waves of 6-8 Hz and amplitude to 300 mvolts. Efficient vascular compression resulted in appearance of slow waves with decreasing amplitude and frequency. The isoelectric line was usually observed after 15-20 seconds of compression. The first burst of bioelectric activity in case of 10 and 15 min cardiac arrest appeared 26.8 $\pm$3.9 and 39.0 $\pm$2.8 min respectively from the beginning of vascular compression. Continuous ECG activity with numerous slow waves and spikes usually appeared 40.0 $\pm$5.2 min after beginning of resuscitation.

Cerebral blood flow, measured by the 133-Xenon clearance method showed a total stop at the time of cardiac arrest followed by an increase to 161.6 $\pm$38.5% in 35 min after resuscitation and average drop to 85% two hours after resuscitation.

RESULTS

Brain light microscopy revealed structural abnormalities in all examined animals, although their extent and intensity showed wide individual differences. A characteristic feature of the cerebral pathology

consisted in the involvement of practically all the brain structures and differences in their nature and intensity depending on the survival time after experimentally induced cardiac arrest. Less striking were differences between animals surviving 10 or 15 min cardiac arrest. This was even more obvious in the light of the above mentioned individual variances of the extensiveness of pathological changes.

The early changes, appearing during the first postresuscitation day consisted in moderate widespread neuronal degeneration involving various structures of the central nervous system, prevailing specially in the cerebral and cerebellar cortex and basal ganglia. However, they were also present in areas relatively resistant to ischemic damage, such for instance as substantia nigra, some nuclei of the reticular formation and cranial nerves. The degenerative changes involved usually single neurons or their groups lying against a backround of otherwise unchanged neuronal population.

The earliest neuronal degeneration took the form of microvacuolization localized either intracytoplasmically or pericellularly (Fig. 1). As known from electron microscopic study, the former corresponds to swelling of cytoplasmatic structures, mostly mitochondria, the latter to swelling of perineuronal astrocytic processes. The other forms of neuronal abnormalities consisted in chromatolysis leading to pictures described in classical neuropathology as acute neuronal swelling (Fig. 2) and central or peripheral chromatolysis, respectively. At the same time typical ischemic neuronal changes appeared (Fig. 3). Purkinje cells showed characteristic homogenous degeneration. Some slight focal tissue spongiosis was present in the cerebral cortex and basal ganglia. In the white matter some loosening of tissue texture with acute swelling of oligodendrocytes were seen.

Features of neuronal loss appeared starting from the third postresuscitation day. At first they were superimposed on the above mentioned degenerative changes of the nerve cells, which at that time became even more intense and diffuse (Fig. 4). In later stages the number of neurons with degenerative changes became steadily reduced, being replaced by neuronal loss. The latter was localized mostly in typical selectively vulnerable areas such as hippocampus, mostly CA_1 sector, ganglion cell layer of the cerebellar cortex, IIIrd neocortical layer and striatum, with particular involvement of larger nerve cells. Degenerative changes, if present, at that time were mostly localized in the same brain structures. Borderline zones of the cerebral cortex were also the site of severe changes.

In the 6th postresuscitation month in addition to localized neuronal loss, different types of degenerative changes involving nerve cells were present. Several groups of them could be distinguished. The first one, taking the form of chronic nerve cell degeneration and their calcification

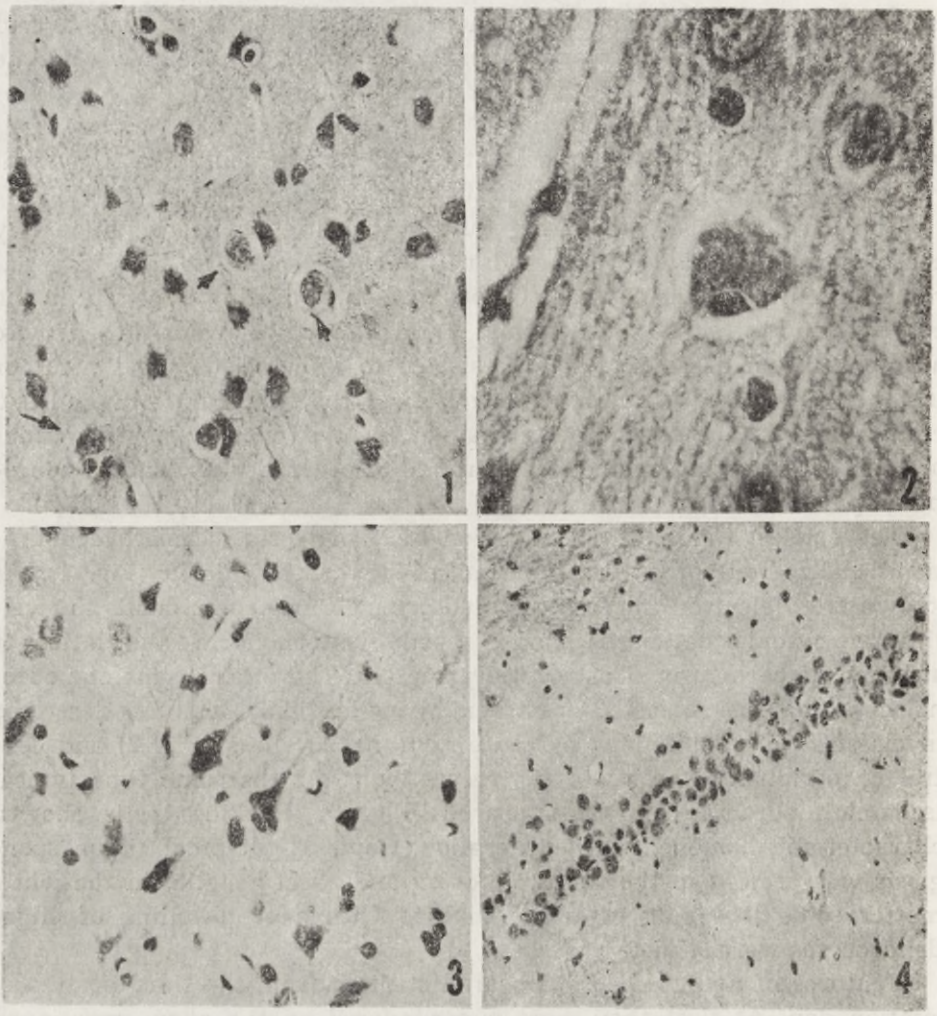

Fig. 1. Perineuronal vacuolization of neuropil (arrows) in early postischemic stage. Experimental animal, survival 72 hours after ischemia. HE. × 200

Ryc. 1. Okołoneuronalna wakuolizacja neuropilu (strzałki) we wczesnym okresie po niedokrwieniu. Zwierzę doświadczalne. 72-godzinne przeżycie po niedokrwieniu. HE. Pow. 200 ×

Fig. 2. Cortical neuron with features of acute swelling of cytoplasm. Experimental animal, survival 24 hours after ischemia. HE. × 1000

Ryc. 2. Neuron piramidowy kory mózgu z cechami ostrego obrzmienia cytoplazmy. Zwierzę doświadczalne, czas przeżycia 24 godz. po niedokrwieniu. HE. Pow. 1000 ×

Fig. 3. Typical ischemic neurons in the IIIrd cortical layer. Experimental animal, survival 24 hours after ischemia. HE. × 400

Ryc. 3. Neurony III warstwy korowej z typowymi zmianami niedokrwiennymi. Zwierzę doświadczalne, czas przeżycia 24 godz. po niedokrwieniu. HE. Pow. 400 ×

Fig. 4. Neuronal loss and degeneration of Ammon's horn pyramidal neurons. Experimental animal, survival 7 days after ischemia. HE. × 200

Ryc. 4. Ubytki neuronalne i zwyrodnienie w warstwie piramidowej rogu Amona. Zwierzę doświadczalne, czas przeżycia po niedokrwieniu 7 dni. HE. Pow. 200 ×

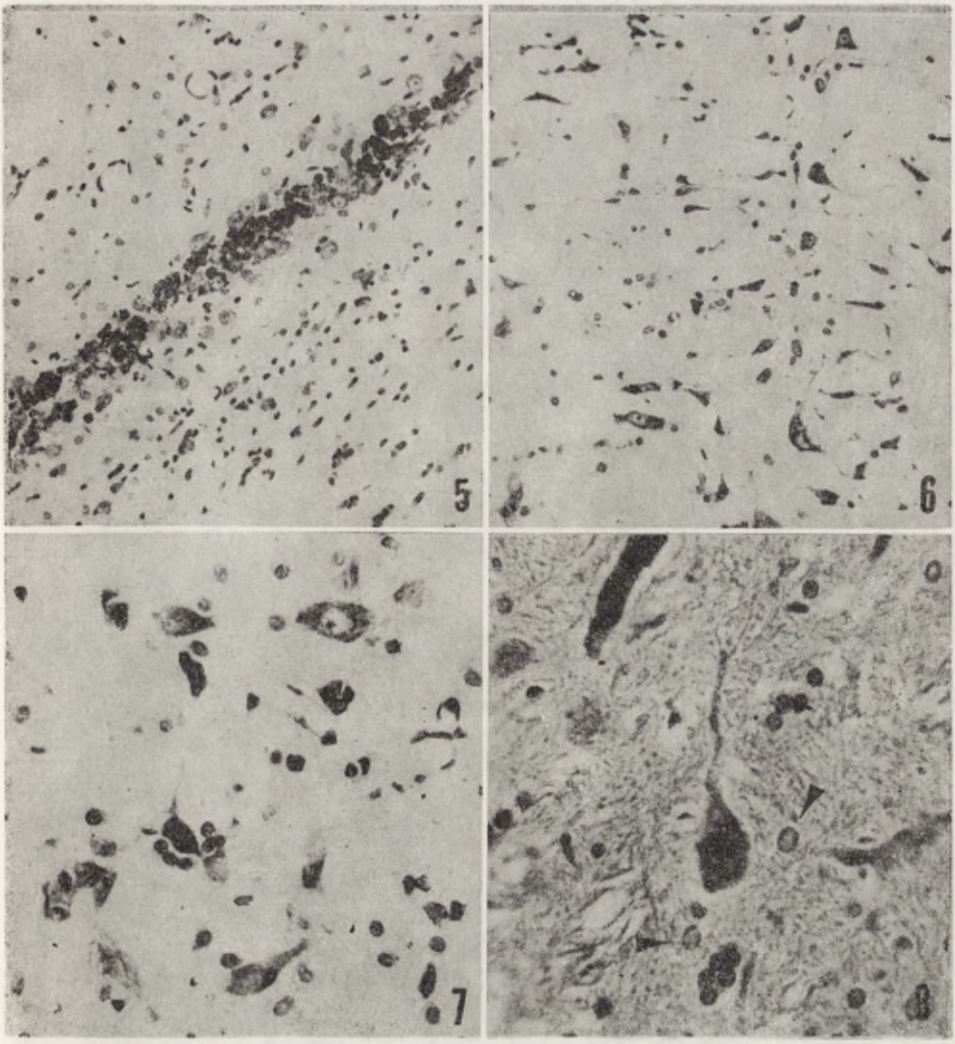

Fig. 5. Dense calcifications in the pyramidal cell layer of Ammon's horn. Experimental animal, survival time 6 months after ischemia. HE. × 100

Ryc. 5. Obfite zwapnienia w warstwie piramidowej rogu Amona. Zwierzę doświadczalne, czas przeżycia 6 miesięcy po niedokrwieniu. HE. Pow. 100 ×

Fig. 6. Shrunken dark neurons in pontine nucleus raphe centralis. Experimental animal, survival 6 months after ischemia. Cresyl violet. × 200

Ryc. 6. Obkurczone ciemne neurony w mostowym jądrze szwu. Zwierzę doświadczalne, czas przeżycia 6 miesięcy po niedokrwieniu. Fiolet krezylu. Pow. 200 ×

Fig. 7. Degenerating neurons from brain stem reticular formation. Note increased perineuronal glial satelitosis. Experimental animal, survival 6 months after ischemia. Cresyl violet. × 400

Ryc. 7. Wyrodniejące neurony z tworu siatkowatego pnia mózgu. Uwagę zwraca wzmożona okołoneuronalna satelitoza glejowa. Zwierzę doświadczalne, czas przeżycia 6 miesięcy po niedokrwieniu. Fiolet krezylu. Pow. 400 ×

Fig. 8. Nodular glial proliferation replacing broken down neurons. Two astrocytic nuclei resembling Alzheimer cells type II (arrows). Experimental animal, survival 6 months after ischemia. HE. × 900

Ryc. 8. Grudkowa proliferacja gleju w miejscach ubytków neuronalnych. Dwa jądra astrocytarne, przypominające komórki Alzheimera typu II (strzałki). Zwierzę doświadczalne, czas przeżycia 6 miesięcy po niedokrwieniu. HE. Pow. 900 ×

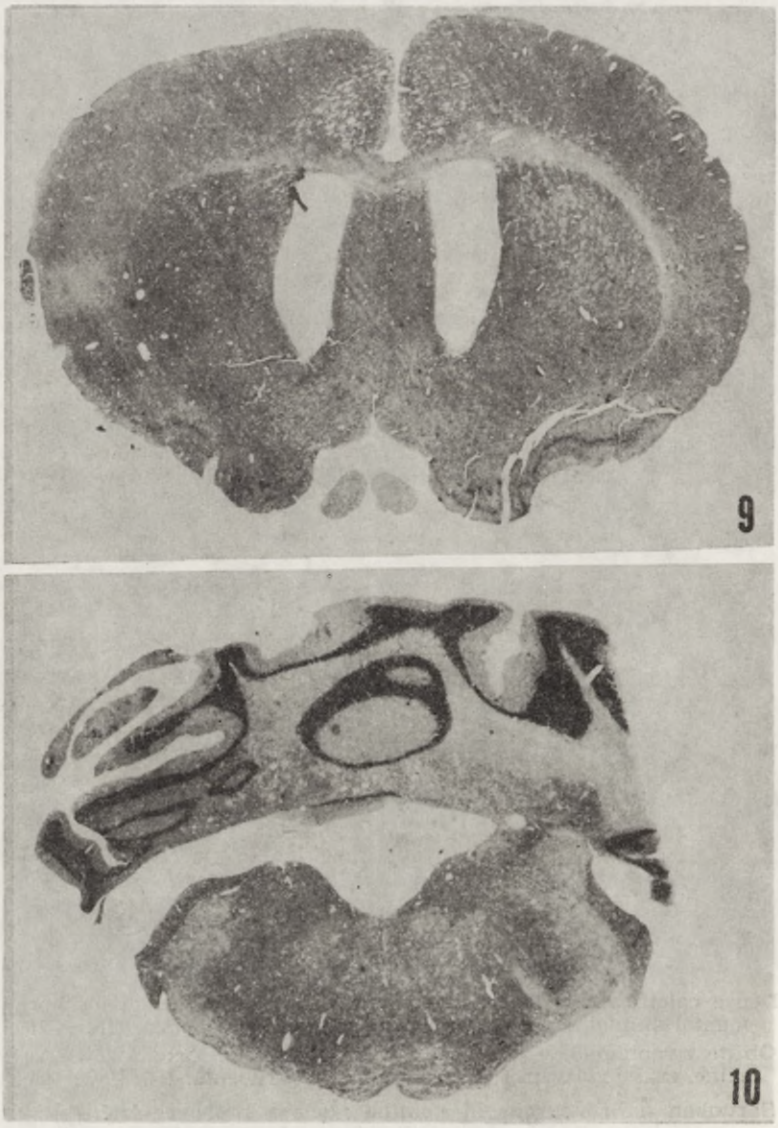

Fig. 9. Enlarged lateral ventricles in an experimental animal with 9 months survival after ischemia. HE. × 9

Ryc. 9. Poszerzone komory boczne mózgu zwierzęcia doświadczalnego z 9-miesięcznym przeżyciem po niedokrwieniu. HE. Pow. 9 ×

Fig. 10. Dilated IV ventricle in an experimental animal with 12 months survival after ischemia. HE. × 9

Ryc. 10. Poszerzona komora IV u zwierzęcia doświadczalnego z 12-miesięcznym przeżyciem po niedokrwieniu. HE. Pow. 9 ×

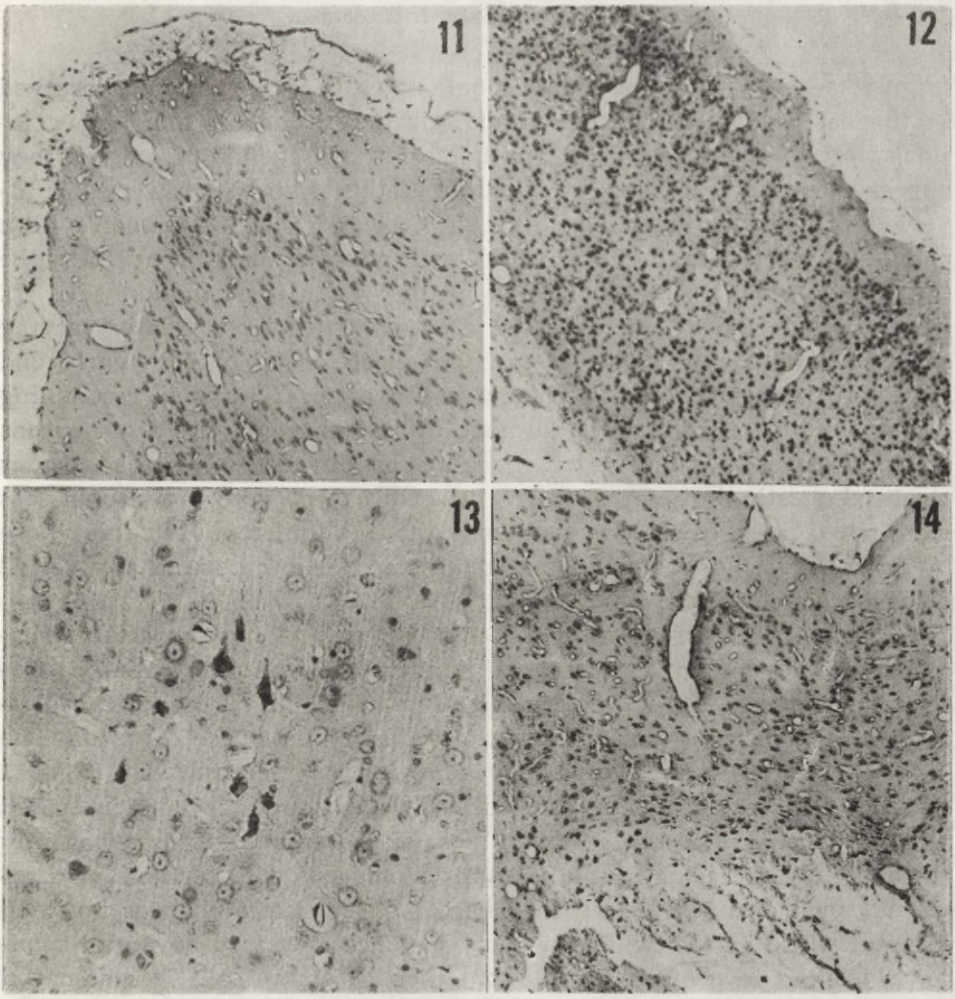

Fig. 11. Considerable dilatation of subarachnoid space with uneven outlines of the cerebral cortex. Experimental animal, survival 12 months after ischemia. HE. × 60

Ryc. 11. Znaczne poszerzenie przestrzeni podpajęczynówkowej z nierównym obrysem powierzchni kory mózgu. Zwierzę doświadczalne z 12 miesięcznym przeżyciem po niedokrwieniu. HE. Pow. 60 ×

Fig. 12. Thinned densely populated cerebral cortex with uneven outlines and total disintegration of subcortical white matter. Experimental animal, survival 12 months after ischemia. HE. × 200

Ryc. 12. Zcieńczała, bogatokomórkowa kora mózgu z nierównymi obrysami powierzchni i całkowitym rozpadem istoty białej podkorowej. Zwierzę doświadczalne, czas przeżycia 12 miesięcy po niedokrwieniu. HE. Pow. 60 ×

Fig. 13. Group of shrunken dark neurons in the IIIrd cortical layer, surrounded by otherwise normal neuronal population. Experimental animal, survival 9 months after ischemia. HE. × 200

Ryc. 13. Grupa ciemnych, obkurczonych neuronów w otoczeniu niezmienionej populacji komórek nerwowych. Zwierzę doświadczalne, czas przeżycia 9 miesięcy po niedokrwieniu. HE. Pow. 200 ×

Fig. 14. Cavernous disintegration of subcortical white matter in an experimental animal with 9 months survival after ischemia. HE. × 100

Ryc. 14. Jamisty rozpad podkorowej istoty białej u zwierzęcia doświadczalnego z 9-miesięcznym przeżyciem po niedokrwieniu. HE. Pow. 100 ×

(Fig. 5) seemed to represent residual states of abnormalities observed in the early postresuscitation period. Curiously enough some other lesions were of a nature typical for the early postischemic period but they appeared in those areas and structures of the brain which were not involved previously, for instance sector CA_2 and CA_4 of Ammon's horn and some specific brain stem nuclei, such as serotoninergic nuclei raphe (Fig. 6) or gigantocellular nucleus of the reticular formation (Fig. 7). Neuronal loss and degeneration were accompanied by a greatly varying glial reaction, taking different morphological forms. In both early and relatively late stages after ischemia naked astrocytic nuclei, resembling Alzheimer type II cells and glial nodules replacing broken down neurons appeared (Fig. 8). Intensive proliferation and hypertrophy of astrocytes localized mostly but not exclusively, in areas of severe neuronal loss were noted later. In some cases mixed astrocytic-microglial nodules were present. Hematogenous cellular reaction was not a feature at any period of postischemic pathology.

Morphological observations performed 9 and 12 months after resuscitation following both 10 and 15 min cardiac arrest revealed hydrocephalic features. They were expressed by widening of both the ventricular system (Figs 9 and 10) and the subarachnoid space (Fig. 11). The brain surface outlines were uneven indicating atrophic processes (Figs 11 and 12). Cerebral cortex in most areas was narrow, showing increased cellular density (Fig. 12), although most of the neurons were apparently normal. Only occasionally small groups of neurons with features of chronic neuronal changes were seen (Fig. 13). More severe changes involved cerebral white matter which in many instances revealed advanced spongiosis leading to profound cavitation (Figs 13 and 14). In less damaged areas features of glial proliferation were noted. Diffuse astrocytic proliferation was seen also in grey matter formations.

DISCUSSION

The results of our studies indicate clearly that global cerebral ischemia resulting from experimentally induced 10 or 15 min cardiac arrest in rats is followed by widespread neuronal damage, involving practically all brain structures, belonging or not to selectively vulnerable areas of the central nervous system. In general the intensity of changes, revealing marked individual variability, was moderate as compared with similar conditions in other mammalians; this being probably the result of a relatively high resistance of the rat central nervous system to ischemia. It seems worth mentioning that the extent and intensity of structural brain abnormalities, as well as their nature, depended more on the survival time after the ischemic incident than on the duration of the latter. Due to the above mentioned individual differences in res-

ponse to cerebral ischemia, it was not exceptional to find more severe tissue alterations in animals with 10 min clinical death than in those in which total cerebral blood flow stop lasted 15 min.

The postresuscitation encephalopathy observed represents a gradually progressing process extended over a long period after ischemic incident. This nature of the process is indicated by the pathological changes in the brain occurring during the whole observation period, including one year. They revealed characteristics of not only residual features resulting from early tissue damage, but of an active pathological process even in very late postischemic stage.

The pathological process is characterized by specific dynamics consisting of early widespread neurodegenerative changes followed by localized neuronal loss, confined mostly to selectively vulnerable areas of the central nervous system. The longer period of the postischemic stage displays non-specific degeneration of the nerve cells, accentuated specially in the brain stem formations. After one year the process ended in generalized cerebral atrophy, expressed by features of internal and external hydrocephalus and narrowing of the cerebral cortex, showing exponents of increased cellular density. Brain antrophy occurring only in the experimental animals, in contrast to their age-mates not subjected to any experimental procedure, permits to reject an age-dependent origin of the atrophic process. In addition, one more element of late cerebral pathology is worth pointing out. This is diffuse and severe cerebral white matter damage, taking the form of breakdown and cavitation. This type of changes in a rather rare phenomenon in postischemic rat pathology. Neither does it appear frequently in human pathology (Brucher 1962). This type of the white matter alteration is usually considered as the result of severe and prolonged vasogenic brain edema, which was not the case, at least to such a degree, in the experimental conditions under study (Mossakowski et al. 1986; Kapuściński 1988; Zelman, Mossakowski 1988).

The mechanism of postresuscitation encephalopathy, leading to generalized cerebral atrophy requires elucidation. The question arises as to whether the pathological process initiated by acute global cerebral ischemia and progressing for one year can be considered as an exponent of the maturation phenomenon in the sense described by Klatzo (1975), and, if so, what is the factor or factors responsible for its appearance and course. As one of the possibilities, the autoimmune reaction, evoked by massive neuronal breakdown with accompanying damage of the blood-brain system occurring in the early period of postresuscitation, should be taken into consideration (Mossakowski, Krajewski 1988). The appearance and increase in the content of antineuronal antibodies in the sera of experimental animals found in the course of the postresuscitation period may suggest a mechanism of neuronal loss similar to that sug-

gested by Nandy (1975, 1983) in the case of aging processes. In part of the experimental animals appearance of antimyelin antibodies complementing antineuronal ones was revealed. In such a case autoimmune mechanism may also concern white matter damage.

DYNAMIKA ZMIAN PATOMORFOLOGICZNYCH W MÓZGU SZCZURÓW PO DOŚWIADCZALNEJ ŚMIERCI KLINICZNEJ

Streszczenie

Przeprowadzono ocenę obrazu patomorfologicznego mózgu szczurów poddanych doświadczalnej śmierci klinicznej trwającej 10-15 minut. Okres przeżycia po incydencie niedokrwiennym zawierał się w granicach od 3 godzin do 1 roku.

Wykazano postępujący charakter zmian rozwijających się w następstwie przebytego incydentu niedokrwiennego, cechujących się typową i powtarzalną dynamiką. Wczesne nieprawidłowości wyrażały się uogólnionymi zmianami zwyrodnieniowymi, na które w dalszym okresie nakładały się ubytki neuronalne zlokalizowane przede wszystkim, choć nie wyłącznie, w obszarach wybiórczej wrażliwości na niedokrwienie. W dalszej ewolucji procesu obserwowano narastanie zwyrodnienia neuronów w obszarach nie zajętych we wczesnej fazie procesu, w tym przede wszystkim w strukturach pnia mózgu. W końcowym stadium obserwacji stwierdzono cechy uogólnionego zaniku mózgu, wyrażające się wodogłowiem zewnętrznym i wewnętrznym oraz znacznym zcieńczeniem kory mózgu. W znacznej części przypadków towarzyszyło temu uszkodzenie istoty białej prowadzące do jej rozpadu. Wysunięto hipotezę, opartą na wcześniejszym spostrzeżeniu, że postępujący proces encefalopatyczny, kończący się uogólnionym zanikiem mózgu i rozpadem jego istoty białej, może mieć charakter procesu autoimmunologicznego, związanego z pojawianiem się we krwi przeciwciał przeciw neuronalnych i przeciw mielinowych.

REFERENCES

1. Brucher J. M.: Neuropathological problems posed by carbon monoxide poisoning and anoxia. Prog Brain Res, 1962, 24, 96-100.
2. Ito U., Spatz M., Walker J. T. Jr., Klatzo I.: Experimental cerebral ischemia in Mongolian gerbil. I. Light microscopic observations. Acta Neuropathol (Berl), 1975, 32, 209-223.
3. Kapuściński A.: Mózgowy przepływ krwi w doświadczalnym modelu śmierci klinicznej. Neuropatol Pol, 1987, 25, 287-298.
4. Kapuściński A.: Bariera krew-mózg w modelu śmierci klinicznej u szczurów. Neuropatol Pol, 1988, 26, 175-183.
5. Klatzo I.: Pathophysiological aspects of cerebral ischemia. In: Nervous system. Vol. 1. The basic neuroscience. Ed.: Tower B. Raven Press, New York, 1975, pp. 313-322.
6. Korpachev V. G., Lysenkov S. P., Tiel L. Z.: Modelirowanije kliniczeskoj smierti i postreanimationnoj bolesni u krys. J Patol Fiziol Exp Ter, 1982, 3, 78-80.
7. Majkowska-Wierzbicka J.: Pathophysiological characteristics of clinical death in rats. Neuropatol Pol, 1989, 27, 83-96.
8. Mossakowski M. J., Hilgier W., Januszewski S.: Ocena zmian morfologicznych

w ośrodkowym układzie nerwowym w doświadczalnym zespole poreanimacyjnym. Neuropatol Pol, 1986, 24, 471-489.
9. Mossakowski M. J., Krajewski S.: Antineuronal antibodies in blood sera of rats subjected to global cerebral ischemia. Neuropatol Pol, 1988, 26, 37-48.
10. Nandy K.: Significance of brain reactive antibodies in serum of aged mice. J Gerontol, 1975, 30, 412-416.
11. Nandy K.: Neuroimmunology and the ageing brain. Exp Brain Res, 1982 (Suppl. 5), 123-127.
12. Pulsinelli W. A.: Selective neuronal vulnerability: morphological and molecular characteristics. Prog Brain Res, 1985a, 63, 29-31.
13. Pulsinelli W. A.: Deafferentation of the hippocampus protects CA1 pyramidal cells against ischemic injury. Stroke, 1985b, 16, 144-146.
14. Siesjö B. K.: Cell damage in the brain. A speculative synthesis. J Cereb Blood Flow Metab, 1981, 1, 155-185.
15. Suzuki R., Yamaguchi T., Kirino T., Orzi F., Klatzo I.: The effect of 5-minute ischemia in Mongolian gerbils. I. Blood-brain barrier, cerebral blood flow and local glucose utilization changes. Acta Neuropathol (Berl), 1983, 60, 207-216.
16. Suzuki R., Yamaguchi T., Li C. L., Klatzo I.: The effects of 5-minut ischemia in Mongolian gerbils. II. Changes of spontaneous neuronal activity in cerebral cortex and CA_1 sector of hippocampus. Acta Neuropathol (Berl), 1983, 60, 217-222.
17. Suzuki R., Yamaguchi T., Inaba V., Wagner H. G.: Microphysiology of selectively vulnerable neurons. Prog Brain Res, 1985, 63, 59-68.
18. Yamaguchi T., Klatzo I.: Maturation of cell damage fallowing transient ischemia in Mongolian gerbils. In: Cerebral ischemia. Eds.: A. Bes, P. Braquet, R. Paoletti, B. K. Siesjö. Elsevier, Amsterdam, New York, Oxford, 1984, pp. 13-24.
19. Zelman I. B., Mossakowski M. J.: Remote pathological brain changes in rats following experimentally induced clinical death. Neuropatol Pol, 1988, 26, 151-162.

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