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Nerve Cell Changes in the Experimental Occlusion of the Middle Cerebral Artery

Histological and Histochemical Study

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With 3 Figures in the Text

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Experimental ways of producing a true cerebral anoxia are few and technically difficult. In most cases, a complete transitory ischemia of the whole brain has been carried out. Among the procedures employed, we can mention the compression of all the neck arterial vessels (KABAT and DENNIS 1938—dog), the occlusion of the pulmonary artery (WEINBERGER, GIBBON and GIBBON 1940—cat), and the circulatory arrest induced by ventricular fibrillation (FISCHER and PELĚSKA 1957—dog).

Other investigators interrupted the arterial circulation, temporarily or definitively, in vessels whose supply area was not provided with collateral circulation enough to prevent local ischemia. In this way, TUREEN (1938) clamped the descending aorta in the dog and BLASIUS and ZIMMERMANN (1957, 1958) in the rabbit; RASMUSSEN (1938) accomplished the ligation of the middle cerebral artery in the dog; DENNY-BROWN et al. (1956) provoked reflex arterial spasm in the cortex of the monkey by means of an abrupt venous distention with physiologic saline. BLASIUS and ZIMMERMANN (1957, 1958), in their work, made comparative physiological, morphological and histochemical investigations on the ganglion cells of the anterior horns of the spinal cord submitted to transitory ischemia.

In order to undertake a cytological and cytochemical study of the nerve cells deprived of oxygen, we chose, among all those experimental modalities, the occlusion of the middle cerebral artery in the dog. Besides its simple technique, such an intervention permits the localization of the lesions within an area of fairly constant limits; this, on the other hand, facilitates the distinction between the anoxic alterations of the neurones and the artefactual changes of the same.

The occlusion of the middle cerebral artery is, at present, well documented as to the anatomo-physiological mechanisms involved and the macroscopical anatomy and pathology of the lesions. The neuronal cytology of the latter, however, has not been fully investigated, nor has their cytochemistry been studied as yet.

We shall, then, describe in detail the morphological and some histochemical changes occurring in the cortical nerve cells after experimental ligation of the middle cerebral artery. It is hoped that the cytochemical study will contribute towards a better understanding of the formation of the ischemic alterations.

Material and methods

Sixty-four normal adult dogs were used. Anesthesia was induced by intravenous nembutal, 0.04 g per kg i.v., and a left temporal craniectomy carried out. In fifty-four dogs the left middle cerebral artery was occluded with silver clips at its origin. In some cases, the anterior cerebral, posterior cerebral and internal carotid arteries were also ligated[1]. Of the ten controls, in six animals the vessels were only exposed and intense cerebral compression carried out; in the other four, only the anterior[2] or the posterior[3] cerebral arteries were occluded.

We recorded the neurological behavior of the dogs until the experiment was interrupted. Dogs were killed by perfusion with the fixative (500—1000 cm^3) through the common carotid arteries in the cases not intended for the study of enzymes, or by bleeding of the same vessels when both histological and enzymic examinations were to be done. In this case the brain was sectioned in coronal slices which were distributed into various fixatives. The sections from the damaged side and the symmetrical ones were always studied comparatively.

For the histological study, 10 μ paraffin sections were stained by cresyl violet (differentiation in acetic alcohol at pH 3.8—4), by the Heidenhain-Woelcke method for myelin, and by hematoxylin and eosin. For the histochemical study we performed in similar sections the PAS (controlled by omission of periodic acid, amylase and acetylation) and the Sudan black B (in 70% alcohol) stainings. Alkaline phosphatase was demonstrated by the Gomori method in 5 μ paraffin sections after fixation in cold acetone; acid phosphatase by the Gomori method in 10 μ frozen sections after fixation in cold formalin. Controls were incubation without substrate, immersion in distilled water at 80° C ten minutes prior to incubation, and addition of inhibitors to the incubating media (CNK 0.01 M for the alkaline phosphatase and FNa 0.01 M for the acid phosphatase). All the histochemical methods were carried out according to PEARSE (1960).

Results

I. Histological study

A. Experiments of long duration[4]

With the exception of dogs 21 and 211, all the others showed ischemic cerebral lesions. In the majority of them, infarcts were present in the Sylvian region, the underlying white matter and the striated nuclei, chiefly in the caudate nucleus and the amygdala (Table 1). As to the histopathology, three types of lesions were found: (a) *Areas of devastation*—usual in the shortest survivals but sometimes encountered even on the eighth day. Some necrotic nerve cells very faintly stained, but frequently still retaining the morphology of the ischemic cell change, could be distinguished in these areas. (b) *Softening*—observed for the first time on the fifth day and henceforth even more frequently. (c) *Gliosis*—noticed in two dogs only (245 and 247). Now and then the three types were present together in the same brain as shown by dog 247. Type (a) lesions seem to correspond to the initial stage of changes which afterwards become those of type (b).

B. Acute experiments[5]

3 hours. In the cerebral cortex numerous shrunken nerve cells, strongly basophilic, were observed. Among them three morphologic types could be discerned: (a) Dark homogeneous cells of indented contour; (b) Hyperchromatic cells, rather elongated but still of regular shape (Fig. 1 A). These two types appeared bilaterally and were not limited to any well defined area,

[1] Dogs 58, 60, 61, 74, 79, 80, 82, 87, 90, 91, 95, 96, 220, 222, 224 had occlusion of the four indicated arteries; in dogs 26, 216, 219 only the middle and anterior cerebral arteries were ligated (see Table 2, acute experiments).

[2] Dogs 241, 242.

[3] Dogs 243, 244.

[4] Experiments were called acute or of long duration, according to the period of survival which lasted from three to twenty-four hours in the former, and was longer than twenty-four hours in the latter.

[5] Animals were classified according to survival periods, and results summarised in Table 2.

which denotes their artefactual origin, type (b) cells corresponding to the common chromophilic cells. (c) Cells also shrunken but only existing in the left Sylvian cortex from dog 224. These were elongated and sometimes a little swollen, with a pycnotic nucleus either dark and homogeneous or light purple, the nucleolus often being apparent. Their cytoplasm, not very dark, appeared finely granular or delicately reticulate as if honey-combed (Fig. 1B). The intercellular background was light greenish, a little hemorrhagic, and contained hyperplastic glia cells and some neutrophils. On account of such features, we considered this type of change due to anoxia and called it pathological shrinkage. In the cortex of some dogs of this series numerous nerve cells were observed, on both sides, with the typical aspect of the cell edema or hydropic change[1]. Confined, mainly, to deep layers and to the depth of sulci, they seem, like types (a) and (b), caused by artefact.

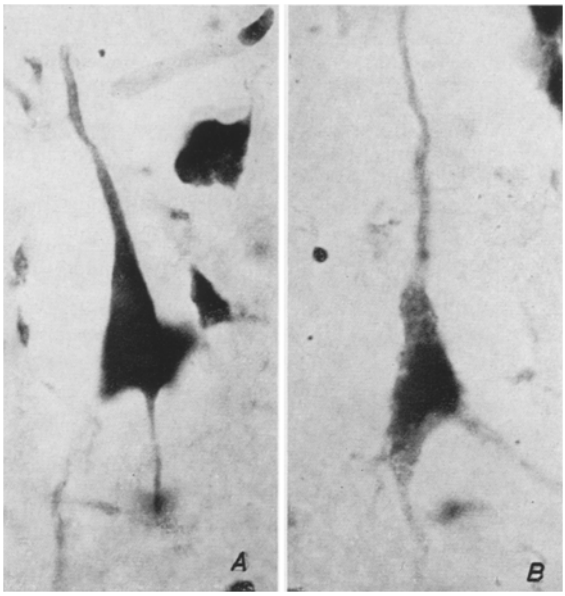

Fig. 1 A and B. Cerebral cortex. A—Dog 48, artefactually shrunken (chromophilic) nerve cell. B— Dog 224, pathologically shrunken nerve cell. Cresyl violet. ×800

6 hours. In almost all the cases, numerous pathologically shrunken cells were observed throughout the left Sylvian gyri. We saw edematous cells in two brains (dogs 58 and 79) but only in the cortex of the operated side and accompanied by satelitosis. The left caudate nucleus of dog 222 showed a well defined line of separation between the area of healthy neurones and that of the injured ones which exhibited either intense retraction of the pathological shrinkage type, or extensive cytoplasmic vacuolization as in the hydropic change (Fig. 2A). It is thus clear that, at this survival time, both changes assumed an obvious focal character. In dogs 29 and 82 some cortical cells displayed an appearance similar to the ischemic change, although not absolutely typical of this state.

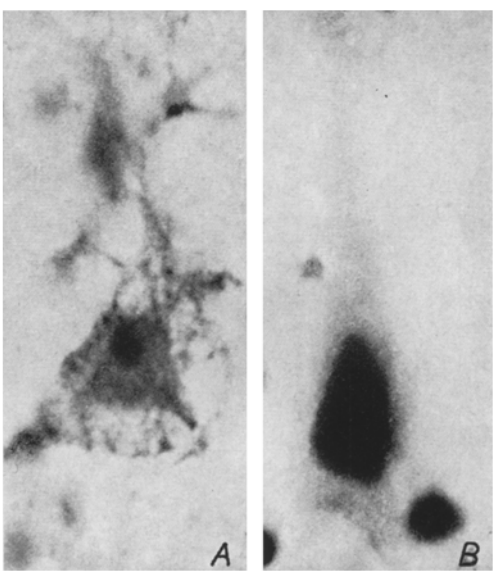

Fig. 2 A and B. A—Dog 222, *N. caudatus*, hydropic cell change. B—Dog 28, *G. Sylv. a.*, ischemic cell change. Cresyl violet. ×800

9—20 hours. Same aspects as in the preceding series. In the cerebral cortex of dog 49, more and more shrunken cells filled with small vacuoles could be seen as we came nearer to

[1] *Wasserveränderung Nissls* (JAKOB 1927), liquefaction necrosis (MORRISON 1946), water-laden cells (CHORNYAK 1948).

Table 1. *Experiments of long duration*

Dog	Survival	Localization and type of lesions	Histochemical study
25	39 h	*G. Sylv. a.* and *p.*, *G. ectos. a.*, *C. semi-ovale*, *Crus cer.*, *Tract. opt.*, *N. caudat.*, *Amygd.* (a)	PAS, Sudan
39	2 days	*C. pirif.*, *Amygd.* (a)	PAS, Sudan
40	2 days	*G. Sylv. a.* and *p.*, *G. orbit.*, *G. preor.*, *C. semi-ovale*, *Claustr.* (a)	
45	3 days	*G. Sylv. a.*, *G. ectos. a.*, *G. orbit.*, *G. coron.*, *R. preopt. l.* (a)	
209	3 days	*G. Sylv. p.*, *G. ectos. p.*, *C. semi-ovale*, *Nu. ventrs. thal.*, *Amygd.*, *N. subthalam.* (a)	Acid phosphatase
16	4 days	*C. pirif.*, *R. preopt. l.* (a)	
24	4 days	*G. Sylv. a.* and *p.*, *G. ectos.*, *G. ectos. a.*, *G. coron.*, *P. tempor.*, *C. semi-ovale*, *Crus cer.*, *Tract. opt.*, *Nu. ventrs. thal.*, *Amygd.*, *Hippoc.* (a)	
23	5 days	*Caps. int.*, *N. caudat.* (a); *G. Sylv. a.* (b)	
81	5 days	*Amygd.* (b)	Acid phosphatase
84	5 days	*Amygd.* (a)	Alkaline phosphatase
22	6 days	*G. Sylv. a.* and *p.*, *G. ectos. a.*, *C. semi-ovale*, *N. caudat.*, *Gl. pall.*, *Putamen* (a, b); *Amygd.* (b)	PAS, Sudan
195	6 days	*G. Sylv. a.* and *p.*, *C. semi-ovale* (b)	Alkaline phosphatase
185	7 days	*G. Sylv. a.* (a); *C. semi-ovale*, *N. caudat.* (b)	Acid phosphatase
186	7 days	*G. Sylv. a.* and *p.*, *G. ectos. p.*, *P. tempor.* (a); *C. semi-ovale* (b)	Acid phosphatase
187	7 days	*G. Sylv. a.* and *p.*, *N. caudat.*, *Gl. pall.*, *Putamen* (a, b)	Alkaline phosphatase
190	7 days	*Amygd.*, *Gl. pall.*, *Putamen*, *Nu. ventrs. thal.* (b)	Acid phosphatase
192	7 days	*G. Sylv. p.*, *G. ectos. p.*, *Gl. pall.*, *Caps. int.* (a, b)	Acid phosphatase
245	8 days	*G. Sylv. a.*, *C. pirif.*, *Gl. pall.*, *Putamen*, *Amygd.*, *N. supraopt.*, *N. hypothal. l.*, *N. entopedunc.*, *Claustr.* (a, b); *N. caudat.* (c)	
247	8 days	*G. Sylv. a.* and *p.*, *G. ectos. a.*, *G. orbit.*, *G. coron*, *P. tempor.*, *C. semi-ovale*, *Caps. int.*, *Amygd.*, *Claustr.* (a); *N. paraventr.* (b); *N. caudat.*, *Gl. pall.*, *Putamen* (c)	
20	14 days	*G. Sylv. a.* and *p.*, *C. semi-ovale*, *N. caudat.*, *Gl. pall.*, *Putamen*, *Amygd.*, *Claustr.* (b)	

Lesioned areas in left cerebral hemisphere are mentioned. Letters (a), (b), (c) indicate the histological type of infarct according to the text. Dogs 21 (7 days survival) and 211 (6 days) had no lesions.

the Sylvian gyri. The vacuoles grew bulkier and in the center of the lesion the nerve cell cytoplasm appeared completely empty as in the exploded type of CHORNYAK (1948). Such images suggest a definite relationship between the cell shrinkage and the hydropic change, which seem just different stages of the same degenerative process. In certain brains, some ghost cells were observed.

Table 2. *Acute experiments*

Dog	Survival	Localization and type of lesions	Histochemical study
224	3 h	*G. Sylv. a.* and *p.* (d)	PAS, Sudan, acid phosphatase
29, 223	6 h	*G. Sylv. a.* (29-d, f; 223-d, e)	PAS, Sudan (29); alkaline phosphatase (223)
58, 79, 83	6 h	*G. Sylv. a.* and *p.* (58, 79-e; 83-d)	Alkaline phosphatase (83)
80	6 h	*G. Sylv. p.* (d)	
82	6 h	*N. caudat., G. coron., G. ectos. a.* (d, f)	PAS, Sudan
222	6 h	*N. caudat.* (d, e)	PAS, Sudan, acid phosphatase
49	9 h	*G. ectos., G. Sylv. a.* and *p.* (d, e)	PAS, Sudan
27	10 h	*G. ectos., G. Sylv. a.* and *p., N. caudat.* (d, e)	PAS, Sudan
74	11 h	*G. Sylv. a.* (d)	Alkaline phosphatase
219, 220, 221	12 h	*G. Sylv. a.* and *p.* (219, 220-d, f; 221-d, e)	PAS, Sudan (220); acid phosphatase (219, 220); alkaline phosphatase (221)
217	19 h	*G. Sylv. a.* and *p., G. ectos. a.* and *p. G. supras.* (d, e, f)	Alkaline phosphatase
216	20 h	*G. coron., G. ectos. a., G. Sylv. a.* and *p.* (d, f)	PAS, Sudan, alkaline phosphatase
28	24 h	*G. coron., G. ectos. a., G. Sylv. a.* and *p.* (d, f)	PAS, Sudan
43	24 h	*G. ectos., G. Sylv. a.* and *p.* (d)	PAS, Sudan
44	24 h	*G. Sylv. a.* and *p.* (d)	
95, 213	24 h	*G. Sylv. p.* (d, f)	PAS, Sudan, acid phosphatase (213)
96	24 h	*G. ectos. a., G. Sylv. a., G. orbit.* (d)	
214	24 h	*G. ectos., G. ectos. p., G. Sylv. a.* and *p.* (d, f)	Acid phosphatase

Cerebral areas referred to above do not indicate the exact boundaries of the lesions but only the zones in which neuronal changes were observed. Types of nerve cell change: d = pathological shrinkage, e = hydropic change, f = ischemic change. Dogs 26, 48, 87, 225 (3 h survival), 90 (12 h), 60 (13 h), 61 (14 h), 91 (24 h) showed no histological lesions. Both phosphatases were studied in dogs 87 and 90, alkaline phosphatase in dog 225, acid phosphatase in dog 91.

24 hours. The Sylvian region of three animals—dogs 28, 95 and 213—displayed lesions of the type of devastation and had a large infiltration of neutrophils. Nevertheless, the changed nerve cells could still be well recognized especially in dog 28 where most of them were in the state of ischemic change. Although elongated they appeared not so funnel-shaped as the shrunken cells, the nuclei being very dark in comparison with the hollow, glassy and well outlined cytoplasm (Fig. 2B). In dog 213 ischemic cells were found together with pathologically shrunken cells, and with some forms of severe cell disease. In the other animals of the series, no further case was observed of coagulation necrosis. At this survival period, both the pathological shrinkage and the hydropic change become rarer than before.

C. Control experiments

Regarding the dogs with compression of the cerebral cortex[1], two thin superficial layers of gliosis were observed in two brains, one in the *Cortex piriformis*, the other in the *Polus temporalis*. The *Polus temporalis* of a third animal possessed a small destructive focus with microhemorrhages and gliosis. In the occlusion of the anterior or posterior cerebral arteries[2] lesions were only reported in one case of the second modality. We noticed in this animal (dog 243) a big infarct occupying the ventral nuclei of the thalamus, of the histological type (a) but with intense peripheric gliomesodermal reaction. So it seems that these ligations are not always harmless. It should be noted that small traumatic hematomas in the zone overlying the clips and provoked by them, can occasionally be seen in any dog that was operated on.

II. Histochemical study[3]

A. PAS and Sudan black B

In pathologically shrunken cells both the nucleus and the cytoplasm were very faintly positive to PAS and Sudan, but the cytoplasmic granulations displayed the same coloring as those found in normal neurones. Edematous cells had a few small pale granulations placed along the intervacuolar network. The cytoplasm of the ischemic cells was completely unstained

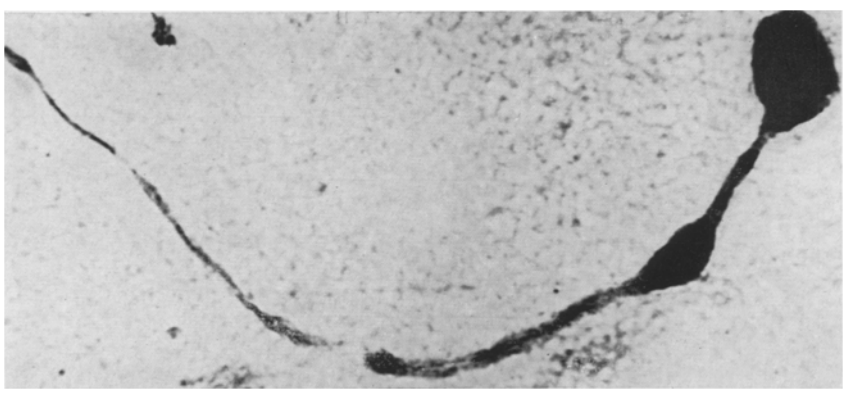

Fig. 3. Dog 214, cerebral cortex. Acid phosphatase activity (Gomori), incub. 1 h. A damaged nerve fiber. ×800

and the nucleus scarcely positive. On the contrary, the artefactually shrunken (chromophilic) cells showed always to be strongly stained by both methods.

B. Phosphatases

Alkaline phosphatase. In the acute lesions with survival periods of 3 h no enzymic alterations were detected, but after 6 h a diminished activity was noticed both in the neuropil of gray matter and in blood vessels present in anemic areas. The injured pyramidal nerve cells were retracted, exhibiting a pale gray cytoplasm and a uniformly dark nucleus. In the chronic infarcts, only the proliferating blood vessels and the nuclei from granule cells, glia cells and fibroblasts showed a positive reaction.

Acid phosphatase. Around the infarcts (experiments of long duration) and throughout the anemiated areas of some acute cases (213, 214, 219), numerous strongly positive nerve fibers showing interruptions, varicosities, and ending frequently in bulgy swellings (Fig. 3) were already observed, while in normal brain regions with the same incubation time nearly all the nerve fibers still remained negative. In the pericarya of changed cells, nuclei displayed a uniform dark staining and the cytoplasm a slight lead sulfide precipitate. Here, as in the case

[1] Survival period of 10 days.

[2] Survival of 8 days.

[3] Dogs which were studied by these techniques are indicated in Tables 1 and 2.

of the alkaline phosphatase, it was impossible to distinguish the type of change, and the chromophilic cells had a stained nucleus and a negative cytoplasm. Nuclei of glia and mesenchymatous cells and the granulations of granule cells were stained dark in the experiments of long duration.

Discussion

For the first hours following the occlusion of the middle cerebral artery two prevailing types of nerve cell changes were found, the pathological shrinkage and the hydropic change. In the longer survivals both continued to be present, but became less frequent. Such changes were really focal, entirely different from similar images due to artefact, and appeared with various degrees of intensity from the slight reversible stage to the very advanced one, approaching terminal disintegration.

As a rule, no great attention has been paid to them. However, JAKOB (1927) mentions the hydropic change as caused by circulatory troubles and MORRISON (1946), in his experiments of exposure to atmospheres of low oxygen concentration, described shrunken cells in deep cortical layers as well as cell edema[1] in the superficial ones. CHORNYAK (1948) regards the hydropic change as resulting from intracellular acidosis due to hypoxemia.

The ischemic cell change was seldom observed in the first hours, only becoming widespread at 24 h of survival and afterwards, since the neurones described in the chronic infarcts also certainly belonged to this type. We never saw any kind of transitional morphological stage between shrinkage or cell edema and the ischemic change, which would be very unlikely on account of their quite different morphology. For this reason, and still having in mind the strong cellular alterations observed from the very first hours after the experiment, we put aside the idea that an initial hypoxia, causing the two first changes, would have been replaced later by a true anoxia which had provoked the third one. According to SCHOLZ (1957 b) this image becomes evident only after a period double or triple of that ascribed to it by SPIELMEYER (1922)—6 h, that is, only 12 to 18 h after the onset of the ischemia.

Other forms of degeneration such as the severe cell disease and ghost cells appeared more rarely. The acute cell disease was never recorded.

With the Nissl method the damaged cells and the chromophilic ones can be mistaken; with PAS and Sudan black, on the contrary, the former always remain faintly colored or uncolored, while the latter stain strongly. Our findings differ, at this point, from those of DIXON (1953) who, in a two-day old human cerebral infarct after occlusion of the middle cerebral artery, saw the necrotic neurones shrunken, with the nucleus and the cytoplasm PAS-strongly positive. The cytoplasmic granulations showed the usual staining and, in this particular, we confirmed the same fact. In our sections, however, the granulations of the diseased neurones were, especially in the edematous cells, nearly always more delicate and scattered than those in the normal nerve cells, contrary to what happened to the chromophilic cells in which they frequently appeared bulkier.

Neuronal chromophobia or chromophilia towards basic dyes can result from experimentally induced cellular hyperactivity with loss of RNA or hypoactivity with accumulation of RNA (EINARSON 1949; DIXON 1955). JARLSTEDT (1962),

[1] "Liquefaction necrosis".

however, demonstrated that Purkinje cells, either chromophilic or chromophobic, possess an altogether identical RNA content. There is, on the other hand, no doubt that chromophilia may be present in histological sections due to technical artefact (Scholz 1957a). In this way, Cotte (1957) discussed the appearance of non saturated fats of lipofuscin type in the cytoplasm of nerve cells turned chromophilic by traumatism during its removal prior to fixation. It seems also probable that the carbohydrate-protein complexes responsible for the PAS-positivity, by concentrating within the chromophilic cells, would strenghten not only the size and colorability of the neuronal granulations, but also the positivity of the cytoplasmic background.

It is quite a different matter with the nerve cells damaged by ischemia. In fact, the intensive intracellular edema of the hydropic changed cells, as well as the milder one occurring at the beginning of the pathological shrinkage, seem the result of a rise in intracellular acidity due to the increased cellular activity and to the anaerobic glycolysis (Chornyak 1948). Because of this, the intracellular concentration of carbohydrate-proteins, associated or not with unsaturated lipids, should have decreased, the PAS and Sudan black diminished positivity being thus explained. Glycogen was never detected in the damaged areas, in spite of the presumably intensified anaerobic glycolysis (Shimizu, Morikawa, and Ishi 1957).

Blasius and Zimmermann (1957, 1958) reported a progressive and finally complete loss of the glycogen content (Best's carmine stain) in the nuclei of the anterior horn nerve cells of the spinal cord when the arterial blood flow was transitorily interrupted in the rabbit aorta for more than 15 minutes. The DNA, RNA and the cytoplasmic PAS-positive material of those cells were also found to have decreased, whereas the plasmal reaction became positive after 10 minutes of ischemia and some little Sudan black-positive dots appeared after ischemia of 15 and 20 minutes.

The decreased alkaline phosphatase activity of the neuropil in the anemic areas was noticed by us after 6 h of survival. Colmant (1962), in experiments of elective parenchymatous necrosis in rats, verified the same fact but only from 16 h onwards. Blasius and Zimmermann (1958) also found a diminished activity of this enzyme in the spinal cord motor cells submitted to transitory ischemia, as well as a striking decrease of the succinic dehydrogenase activity. In our preparations, both the damaged and the chromophilic nerve cells never were alkaline phosphatase positive, and the same occured in relation to the acid phosphatase. Anderson, Song, and Christoff (1962) also registered a reduced acid phosphatase activity in severely ischemically injured neurones from human and experimental material.

Most interesting was the strong acid phosphatase reaction exhibited by the nerve fibers around the chronic infarcts and throughout areas affected by acute ischemia. It may have been caused by anatomical section of the fibers due to the infarct, by direct ischemia of the same, or by their secondary degeneration following the nerve cell body necrosis.

The first hypothesis will better explain the pictures exhibited round the margin of the chronic lesions and is well stressed in the literature. Thus, Heinzen (1947) observed, biochemically, an acid phosphatase increase on both stumps of the rat transected sciatic nerve during the first week, and Gould and Holt (1961)

confirmed the same fact histochemically. According to them, twenty-four hours after the transection a striking rise in acid phosphatase and esterase activities was already to be found in the axoplasm of the distal stump for a distance up to 0.5 mm, and it remained until the twelfth day, in spite of the axon breaking up. LASSEK and HARD (1945) noted, also histochemically, increased acid phosphatase activity along the pyramidal tract of cats and monkeys, in which the motor cortex had been removed.

Nevertheless, we would rather adhere to the second hypothesis, for the direct ischemia seems to us the more logical explanation for the rapid increase in enzymatic activity displayed by the acutely damaged nerve fibers.

Summary

Morphological and histochemical changes occurring in nerve cells after experimental occlusion of the left middle cerebral artery have been studied. Material consisted of fifty-four dogs with ligation of that artery and ten controls with either only retraction of the cortex or ligation of the anterior or posterior cerebral arteries. Techniques employed were cresyl violet, PAS, Sudan black B and Gomori methods for alkaline and acid phosphatases.

In twenty-two animals which survived 39 h to fourteen days, the distribution of chronic lesions and their histological type have been examined, infarcts being nearly always localized within the Sylvian region and the striated nuclei. Thirty-two other dogs had survivals inferior to or of 24 h and in these acute experiments nerve cell changes were carefully studied. Histologically, it was possible to detect three types of acute nerve cell change: (1) shrinkage and (2) hydropic change, the first appearing after 3 h, the second after 6 h, both with focal localization, thus easily distinguishable from similar bilateral artefactual images as the common chromophilic cells; (3) ischemic cell change, encountered for the first time after 6 h and having its peak at 24 h, whereas (1) and (2) became less frequent at this survival period.

PAS and Sudan black B stained the three types of acutely damaged nerve cells faintly but were strongly positive in the chromophilic ones. Alkaline and acid phosphatase activities were absent both from changed and chromophilic neurones. However, nerve fibers existing throughout acutely lesioned areas as well as around chronic infarcts had strong acid phosphatase positivity and showed morphological changes such as interruptions, varicosities and terminal swellings. Owing to the rapid appearance of that increased activity, the A. considered it directly due to the ischemia of the nerve fibers.

Zusammenfassung

Die in Nervenzellen nach experimentellem Verschluß der A. cerebri media auftretenden morphologischen und histochemischen Veränderungen wurden studiert. Das Untersuchungsmaterial bestand aus 54 Hunden mit Unterbindung der A. cerebri media und 10 Kontrolltieren entweder mit Rindenentfernung oder Unterbindung der A. cerebri anterior oder posterior. Kresylviolett, PAS, Sudanschwarz B-Färbung sowie die Gomori-Methode auf alkalische und saure Phosphatase wurden angewendet.

Bei 22 Tieren mit einer Überlebenszeit von 39 Std bis zu 14 Tagen wurde die Verteilung chronischer Läsionen sowie ihr histologisches Bild untersucht. Infarkte waren fast immer in der Sylvischen Region sowie im Striatum lokalisiert. 32 Hunde hatten Überlebenszeiten von weniger oder bis zu 24 Std. Die Nervenzellveränderungen dieser akuten Experimente wurden sorgfältig untersucht. Histologische konnten drei Arten von akuten Nervenzellveränderungen festgestellt werden:

1. Schrumpfung, frühestens nach 3 Std; 2. Wasserveränderungen nach 6 Std, wobei beide deutliche Herdlokalisation zeigten, und daher leicht von ähnlichen bilateralen Artefakten — wie den üblichen chromophilen Zellen — zu unterscheiden waren; 3. ischämische Zellveränderungen, die frühestens nach 6 Std auftreten und ihr Maximum bei 24 Std erreichen, wobei 1. und 2. zu diesem Überlebenszeitpunkt abnehmen.

Mit PAS und Sudanschwarz B werden diese drei Arten von akut geschädigten Nervenzellen schwach gefärbt, die Chromophilen hingegen reagieren stark positiv. Alkalische und saure Phosphataseaktivität konnte weder in den veränderten noch in den chromophilen Nervenzellen festgestellt werden. Jedoch zeigten Nervenfasern in akut geschädigten Arealen als auch um chronische Infarkte stark positive saure Phosphataseaktivität und ließen morphologische Veränderungen, wie Unterbrechungen, Schwellungen und Endauftreibungen erkennen. Der Autor vermutet, daß die rasch auftretende Aktivitätssteigerung in direkter Beziehung zu der Ischämie der Nervenfasern steht.

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