D and DA injections duodenal CA fluorescence was as in controls.

Reserpine (5 mg/kg) administered 24 h before D and DA injections was not able to inhibit the binding of the above-mentioned biogenic substances in Paneth cells and had no effect on the fading of their fluorescence. The pretreatment of mice with Niamid® prolonged the disappearance of administered drugs in granules of Paneth cells as well as in other intestinal structures.

The ability of Paneth cell granules to bind CA is surprising, since, at least in the rat, these cytoplasmic organelles fulfil the criteria of lysosomes[7] defined by DE DUVE[8]. However, Paneth cell granules have many specific staining and enzymatic characteristics and therefore they cannot be regarded as typical lysosomes found in other types of cells. In the rat[7], as well as in the present study on the mouse, Paneth cell granules exhibit histochemically a MAO activity. Obviously the detoxification mechanism of monoamines by MAO operates in the mouse, since the fading of administered biogenic substances was so rapid and prolonged by MAO inhibitors in the present study.

The specificity of the histochemical fluorescence reaction used has been well established chemically[9–11]. The similar accumulation of D and DA by Paneth cells supports a similar binding mechanism for these biogenic substances but the possibility that D is first converted in vivo to DA and that this amine accumulates in Paneth cell granules is not excluded. Reserpine is known to block the CA storage mechanism in many types of organelles of nervous origin[12,13] but, in the Paneth cells of the present study as in certain other cells which normally do not contain CA[14], reserpine has no effect on the amine binding or fading. On the basis of the present study, the binding type between histochemically identified protein-polysaccharide matrix of granules[7] and administered substances remains obscure.

The physiological function of Paneth cells is largely obscure but they participate in the production of digestive enzymes[15]. The present results indicate that granules may also participate in the binding of biologically active amines and in their elimination by oxidative detoxification mechanism[16].

Zusammenfassung. Nach spezifischer i.v. Injektion wird gefunden, dass Dopamin und 3,4-Dioxyphenylalanin-aminosäure im Zytoplasma der Panethschen Zellen des Mäuseduodenums angehäuft sind. Während Niamid® das meist rasche Verschwinden der Formaldehyd-verursachten Aminfluoreszenz in den Granula von Panethschen Zellen verzögerte, blieb Reserpin wirkungslos.

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Response of Neuronal Lysosomes to Anoxia in Tissue Culture of Mammalian Cerebellum

Cytopathological changes in the brain due to oxygen deficiency have been extensively studied although the precise mechanism is still not understood[1]. More recent experiments have demonstrated enzyme loss in the brain of experimental animals following periods of anoxia[2–4].

It has also been reported that neuronal lysosomes showed cytopathological changes following exposure to anoxia[4,5].

The present communication deals with similar changes demonstrated in mammalian cerebellar neurons grown in tissue culture as exhibited by histochemical acid phosphatase reaction.

Material and methods. Tissue culture explants were made of new-born kitten cerebellar cortex using the flying coverslip-roller tube method as previously described[6–8]. The cultures were fed once a week with nutrient fluid consisted of 50% Gey's solution, 45% human cancerous ascitic fluid and 5% embryonic extract of 8-day-old chick embryo.

After 2–4 weeks in vitro, cultures were divided into 2 groups (control and experimental). The experimental cultures were then exposed to anaerobic conditions for times ranging from 30 min to 24 h, while the control cultures continued to grow under normal condition.

To produce an anaerobic condition, the alkaline pyrogallic acid method was used[9,10]. A cotton plug was pushed into the test tube so that the upper portion of the plug was 1.5 inches below the lip of the tube. 1 g of pyrogallic acid and 1 g of sodium carbonate were mixed and inserted on top of each cotton plug. A rubber stopper was placed tightly in the tube and the boundary of the tube and stopper was sealed with paraffin.

The basis of the alkaline pyrogallic acid method lies in the fact that pyrogallic acid, when placed in alkaline pH, will absorb a large quantity of oxygen. At varying intervals, cultures of both the experimental and control groups were fixed in cold formol-calcium for 5 min at 4 °C and then placed in Gomori's glycerophosphate-lead medium for 30 min at 37 °C. After incubation they were

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immersed briefly in dilute ammonium sulphide solution and mounted in glycerine-jelly. The control of enzyme reaction, in the absence of substrate in the incubating medium, was always negative.

Results. In normal control cultures, acid phosphatase (AcPh) positive sites were shown as dark brown lead sulphide granules varying in size from 0.5–0.8 μ in neuronal cytoplasm. These AcPh granules were present uniformly throughout the nerve cell soma (Figure 1). They were also seen to extend into the dendrites.

In the experimental cultures, an initial increase in the number of AcPh positive granules was noted. This increase was evident after 1 h and continued to 2 h at which time it plateaued until 4 h (Figures 2 and 3). A striking feature in the neurons of the experimental cultures at this time as compared to the controls, is the presence of AcPh granules in axonal processes (Figure 5). At 6–8 h there was a noticeable decrease in the number of AcPh granules. And at 24 h the granules were so few as to result in the loss of cell delineation (Figure 4).

Discussion. The loss in enzyme activity that was noted after hours of exposure to anoxia supports the finding of BECKER and BARRON[4] in their work on experimental anoxic and anoxic-ischemic encephalopathy in rats.

Although the appearance of cytolysome (swelling of lysosome)[11] after a short exposure to anoxia was a characteristic picture in the work of aforementioned authors, AcPh preparations of the present study did not reveal those swelling of lysosomes even after a prolonged exposure to anoxia. It seems that the shorter incubation time lessens the fusion or clumping of lysosomes into large patches, conventionally called cytolysome[12].

Since the increase in AcPh positive granules occurred after the exposure of cultures to anoxia for more than 1 h, it is unlikely that it can be due to neo-formation of the lysosome but must be the result of lysosomal membrane damage which alters the permeability, resulting in the passage of substrate into the lysosome identifiable by histochemical method for AcPh.

Continued exposure to anoxia finally causes rupture of the damaged membrane and release of the hydrolytic enzymes resulting in loss of their histochemical demonstrability.

NOVIKOFF and ESSNER[13] described a gradient in the distribution of lysosomes along the neuronal dendrites and axons but this was absent in this study.

Figure 5 illustrates a linear arrangement of AcPh positive granules indicating that they are arrayed in the axon without a gradient.

Although it is still unclear what factor is responsible for the induction of lysosome migration from the neuronal soma into the axon after an anoxic treatment, there must be a relationship between such discharge and 'axonal transport' of WEISS and his associate[14,15]. Also there are many arguments on the disadvantages of Gomori's glycerophosphate-lead method because of its lead adsorption artefacts[16,17].

Nevertheless in the present communication, the results have shown a sharp localization of reaction products with a brief fixation and a short incubation time.

The present communication has shown that in the course of anoxia in cultured cerebellar neurons, the initial rise in AcPh activity in neuronal soma was evident after 1–4 h of anoxia, and the subsequent loss of enzyme activity following continued anoxic treatment up to 24 h was demonstrated. It is reasonable to interpret these findings as an indication for the active participation of lysosomes in the process of nerve cell degeneration and its subsequent lysis. The histochemical study of cultured nervous tissue in vitro in various experimental conditions might be useful in providing structural correlations for functional as well as pathological alterations of the neurons in given environments.

Zusammenfassung. In Gewebekulturen wurde die zytochemische Veränderung der Lysosomen durch Sauerstoffentzug an Säugetierkleinhirnnervenzellen mit der Gomori-Säurephosphatasemethode studiert.

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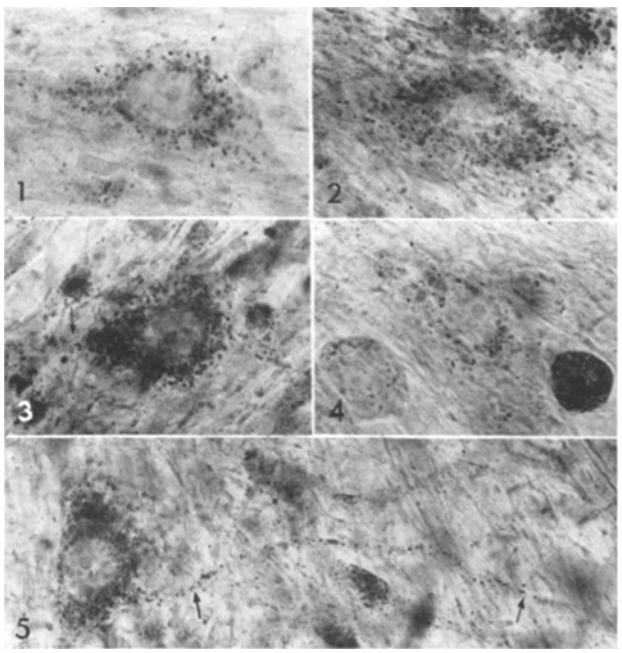

Fig. 1. Normal kitten cerebellar neuron. AcPh granules are seen diffusely in the neuronal soma. × 1000.

Fig. 2. Anoxia 1 h. AcPh granules are increased in number comparing with normal neuron of Figure 1. × 1000.

Fig. 3. Anoxia 2 h. The number of granules (lysosomes) are markedly increased in the neuronal soma and also extend into the dendrite (arrow). × 1000.

Fig. 4. Anoxia 24 h. Since few AcPh granules are left in the neuronal soma, the neuron is not clearly outlined. × 1000.

Fig. 5. Anoxia 3 h. The lysosomes extend into an axon (arrows) as far as 150 μ. There is no visible gradient of lysosomal structures. × 1000.

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