the mode of stimulation of RNA polymerase activity by histones and polymers of basic amino acids is not clear, they may function as polyamines at low concentrations and stimulate RNA polymerase activity by displacing newly synthesized RNA from DNA, by dissociating or preventing polymerase-RNA complex formation or by influencing the conformation of DNA[8–12].

The following polypeptide hormones also stimulated the RNA polymerase activity of *E. coli* (Figure 4): adrenocorticotropin (Sigma Chemical Co.), growth hormone (Armour Lab.), insulin (Eli Lilly & Co.), luteinizing hormone (Endocrine Study Section, NIAMD), and oxytocin (Sigma Chemical Co.). Growth hormone possessed the greatest stimulatory capacity and oxytocin was least effective. When adrenocorticotropin and growth hormone were treated with maleic anhydride[7], they lost their ability to stimulate the RNA polymerase activity. The above results suggest that polypeptide hormones may act as polyamines and stimulate RNA polymerase activity. The stimulation of cyclic AMP formation by peptide hormones may be a function of their capacity to act as polyamines[13].

Zusammenfassung. Decalysin, Polyarginin, Polylysin, Polyornithin, Kalbsthymushistone und Polypeptidhormone stimulieren in niedriger Konzentration die DNA-abhängige RNS-Polymerase-Aktivität von *Escherichia coli*, während sie in hoher Konzentration die Enzymaktivität hemmen.

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Demonstration of Neurofibrillary Degeneration Induced by Anoxia in Spinal Motor Neurons in vitro

Anoxic effects on the central nervous system are commonly encountered in a variety of human conditions. Characteristic morphological changes have been well documented[1]. In general, neuronal disintegration leading to extensive cell loss is observed. The processes leading to these changes are less well known. In an attempt to elucidate some of these processes resulting from anoxic conditions, we turned to use of tissue culture method in order to be able to examine the changes under more carefully controlled conditions. During the course of these studies, in which silver impregnation technique was utilized, prominent neurofibrillary changes were observed in the anoxic cultures.

Explants of spinal cords of chick embryos, 10–14 days in ovo, were placed on collagen coated coverslips and maintained in roller-tubes[2]. The cultures were fed once a week with a nutrient medium consisting of 32% human placental cord serum or fetal calf serum, 32% Eagle's medium, 32% Simms' balanced salt solution and 4% 8-day-old chick embryo extract, to which glucose was added to give a final concentration of 600 mg per 100 ml medium. After 2–4 weeks in vitro, cultures were exposed to anoxic conditions for periods of from 30 min to 48 h using alkaline pyrogallol method[3]. At various intervals anoxic and control cultures were taken from the roller-tubes, fixed in formol-ammonium bromide solution and silver impregnated by a modification of Bodian's protargol method[4].

During the second week of culture large motor neurons could be identified by means of their large vesicular nuclei and prominent nucleoli (Figure 1)[5]. Myelin sheaths could also be seen as early as 6 days in vitro, but active myelin formation usually started in the middle of the second week in vitro, and thereafter neurons and myelin sheaths matured considerably. These neurons were prominent in fixed cultures impregnated with silver and showed normal, delicate, argentophilic neurofibrils in the perikarya and dendrites (Figure 2). In the anoxic cultures, neurons appeared to contain thicker, strongly argentophilic neurofibrils in their perikarya and dendrites. In anoxic neurons, the neurofibrils were found in 3 kinds of configurations: 1. A tangle of thickened neurofibrils forming a ball-like structure within the perikaryon (Figure 3). Sometimes the neurofibrillar balls occupied the entire perikarya (Figure 4). 2. A whirl-like arrangement of concentrically distributed fibrils in a neuron devoid of recognizable nucleus (Figure 5). 3. A condensed neurofibrilar mass which retained the outline of a neuron but lacked any non-argentophilic nuclear structure and was, therefore, termed 'ghost cell' (Figures 6 and 7). The earliest neurofibrillary changes could be observed after 1 h of exposure to anoxia and consisted of tangles of the neurofibrils (Figure 3). Subsequently, the changes proceeded to whirls, and finally to the last stage of degeneration, the 'ghost cells'. The degree of neurofibrillary alterations increased with the length of exposure to anoxia.

The findings in this study are in general agreement with the observations of the effects of anoxia in certain other conditions. HORNET and NEREANTIU[6] described the appearence of neurofibrillary changes after experimental induction of microemboli in the brain of a dog. Similarly, MINAGAWA et al.[7] have reported apparently

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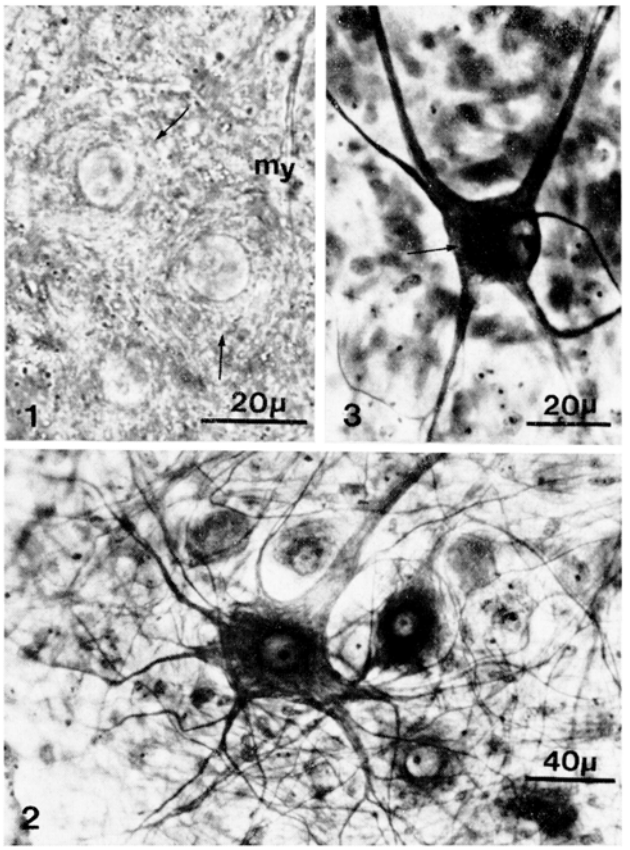

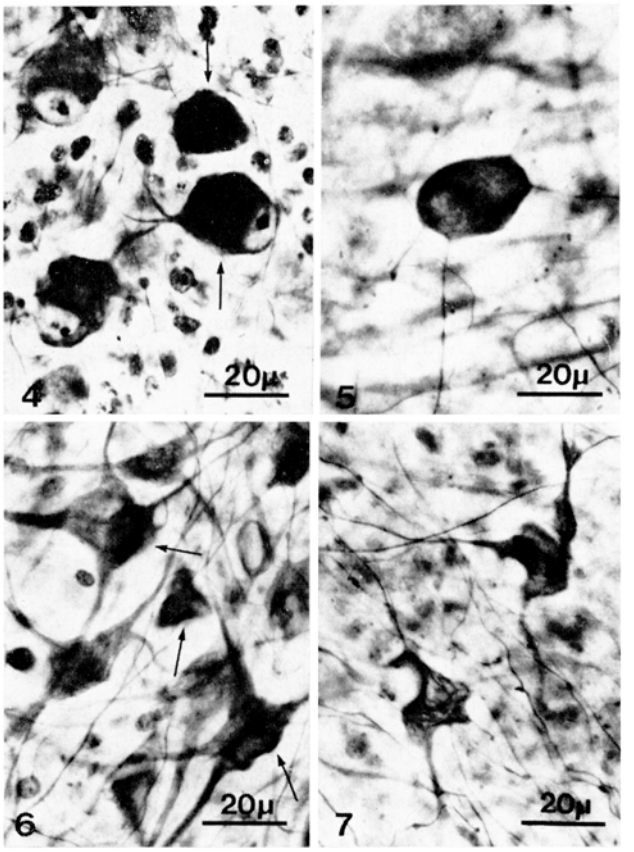

Fig. 1. Large motor neurons (arrows) of chick spinal cord in a living control culture of 24 days in vitro. They are identified by large vesicular nuclei. Myelin sheath (my) can also be seen nearby.

Fig. 2. A group of large motor neurons in a control culture demonstrated by Bodian's protargol method. Fine neurofibrillary networks are seen in neuronal perikarya and in dendrites.

Fig. 3. Note the eccentric positioning of neuronal nucleus and strongly argentophilic neurofibrillary tangle in the perikaryon (arrow). 1 h exposure to anoxia. Bodian method.

Fig. 4. Neurofibrillary tangles occupy entire neuronal perikarya (arrows). 2 h exposure to anoxia. Bodian method.

Fig. 5. A whirl-like arrangement of neurofibrils. Nuclear structure is not any more recognizable. 18 h exposure to anoxia. Bodian method.

Fig. 6. Condensed masses of neurofibrils localize in neuronal perikarya (arrows). 24 h exposure to anoxia. Bodian method.

Fig. 7. Final stage of neurofibrillary alteration. Neurofibrillary masses occupy entire perikarya and deform their contours. They are termed as 'ghost cells'. 48 h exposure to anoxia. Bodian method.

identical alterations in a human patient after carbon monoxide poisoning. Recently similar neurofibrillary tangles have been demonstrated in nervous tissue after exposure to a variety of mitotic inhibitors[8,9] and aluminum compounds[10-12]. Anoxia may now be added to the list of agents resulting in neurofibrillary changes.

The basic mechanism of neurofibrillary degeneration in neurons is still not fully understood. It is reasonable to assume that such neurofibrillary proliferation or excess protein synthesis might be induced after cell injury or other possible damaging impacts. In this connection, it is noteworthy that some neurons grown in poorly controlled culture conditions also occasionally show similar neurofibrillary masses with highly argentophilic character. Anoxia might be a main underlying cause for the changes occurring in such poor culture conditions. To clarify the basic mechanism of these changes, further ultrastructural study of neurofibrillary changes brought about by anoxia is underway[13].

Zusammenfassung. Die neurofibrilläre Degeneration durch Anoxie wurde an Nervenzellen des Rückenmarks von Hühnerembryonen mit der Bodian-Silberimprägnation in Gewebekultur studiert.

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[13] Our preliminary electron microscope study indicates that dramatic increase of neurofilaments is observed both in neuronal cytoplasm and dendrites in cultures exposed to anoxia.