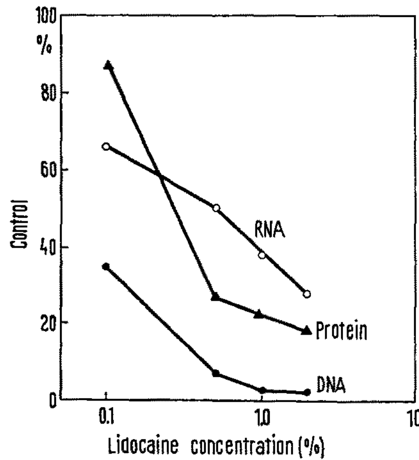

Fig. 1. Effect of lidocaine on macromolecular syntheses.

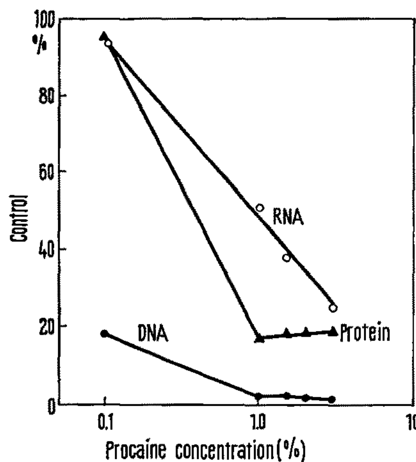

Fig. 2. Effect of procaine on macromolecular syntheses.

Ehrlich ascites cells propagated as previously described[7] were washed, resuspended in Waymouth medium (6×10^7 cells/ml) and distributed into screw-cap tubes containing premeasured amounts of the drug and radioactive precursors. [Thymidine-^{3}H for DNA ($7.7\times10^{-6}M$, 0.5 μc/ml); uridine-5-^{3}H for RNA ($2.0\times10^{-4}M$, 2.4 μc/ml); and lysine-^{3}H for proteins ($5.6\times10^{-4}M$, 0.3 μc/ml)]. The cultures were incubated at 37°C for 2 h and processed for the determination of radioactivity incorporated into acid-insoluble form (Figures 1 and 2)[8,9].

Zusammenfassung. Nachweis, dass in Ehrlich-Ascites-Zellen die DNS-Synthese durch Lokalanaesthetika stärker gehemmt wird als die RNS- und die Proteinsynthese.

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Stimulation of RNA Polymerase Activity by Histones, Polyamino Acids, and Polypeptide Hormones

DNA-dependent RNA polymerase from various sources is stimulated by polyamines and inhibited by histones and polylysine[1-5]. We wish to report that at low concentrations histones, polymers of basic amino acids and polypeptide hormones may act as polyamines and stimulate RNA polymerase of *E. coli*.

DNA-dependent RNA polymerase of *E. coli* was purchased from Biopolymers, Chagrin Falls, USA. Decalysine was a generous gift of Dr. G. L. TRITSCH, Roswell Park Memorial Institute, Buffalo, USA. RNA polymerase activity was assayed by mixing histones, polyamino acids or polypeptide hormones (0.1–20 μg/25 μl) with RNA polymerase (1.25 units/25 μl). To the mixture was added highly polymerized calf thymus DNA (20 μg/50 μl) and bovine serum albumin dissolved in 0.1 *M* *Tris*-HCl buffer, pH 7.9 (50 μg/50 μl). After incubating the mixture at 37°C for 3 min 100 μl of a solution containing 100 μmoles each of ATP, CTP and GTP, 1 μCi of [^{3}H]UTP, 1.25 μmoles of $MgCl_2$, 0.5 μmoles of $MnCl_2$, 1.0 μmoles of β-mercaptoethanol, 3.0 μmoles of *Tris*-HCl buffer (pH 7.9) were added and incubated at 37°C for 10 min. The reaction was terminated by the addition of 2.5 ml of cold 5% TCA. The precipitate was washed 3 times, heated at 100°C to dryness, and dissolved in 0.5 ml of Soluene 100 (Packard Instrument Co.) overnight. Radioactivity was measured as previously described[6].

Figure 1 shows the effect of calf thymus histones on the RNA polymerase activity. At high concentrations, histones inhibited the polymerase activity as was reported by FOX and WEISS[1]. However, when less than 4.0 μg was

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added to the assay system, a stimulation of the polymerase activity was observed. Similar effects were obtained with polylysine, polyarginine and polyornithine. Polyglutamic acid did not influence the polymerase activity nor did it compete with the stimulatory influence of polylysine. Decalysine and copolymer of Gly-Lys (60:40, mole %) had a stimulatory capacity equivalent to that of polylysine while the copolymer of Glu-Lys-Ala (42:28:30, mole %) was significantly less effective. Lysine and arginine did not influence the polymerase activity and Lys-Lys had a very slight stimulatory effect. Calf thymus histones treated with maleic anhydride[7] were less effective in influencing the polymerase activity than the untreated histones and equivalent to that of the copolymer, Glu-Lys-Ala, suggesting that the stimulatory capacity was partially dependent upon the content of lysine residues. These results suggest that histones at low concentrations behaved like polyamines and stimulated RNA polymerase activity.

The combined addition of histones and spermine to the RNA polymerase system stimulated the activity to the same degree as spermine alone but was effective at lower concentration (Figure 2), suggesting that histones and spermine affected the RNA polymerase system by a common mechanism. On the other hand, NH_4Cl and polylysine had a potentiating effect on the RNA polymerase activity (Figure 3) suggesting that they influence the polymerase system by different mechanisms. Although

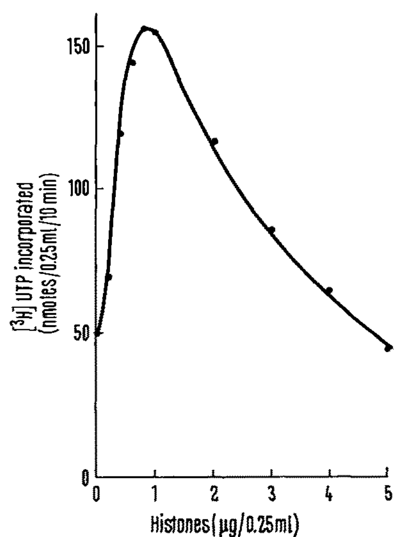

Fig. 1. Effect of varying concentration of calf thymus histones on RNA polymerase activity. The polymerase activity was equivalent to 1 nmole of [^{3}H]UTP incorporated into RNA in 10 min in a total volume of 0.25 ml. The results with lysine-rich and arginine-rich histones were similar.

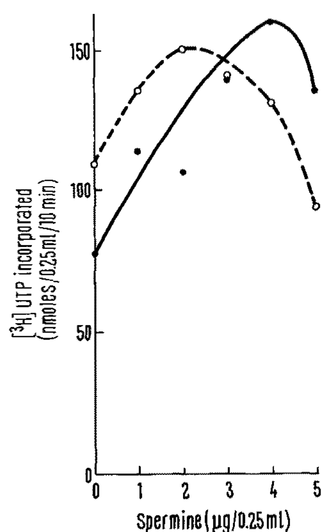

Fig. 2. Effect of spermine and calf thymus histones on RNA polymerase activity. The control mixture contained varying concentrations of spermine (●—●). The test mixture contained in addition to spermine, 0.8 µg of calf thymus lysine-rich histones in a total volume of 0.25 ml (○---○).

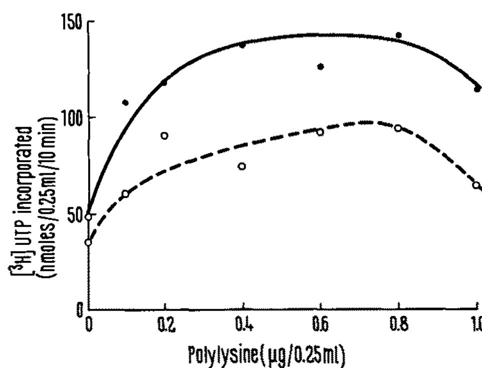

Fig. 3. Synergistic effect of NH_4Cl and polylysine on RNA polymerase activity. The concentration of NH_4Cl was 0.4 *M*. With NH_4Cl (●—●); without NH_4Cl (○---○).

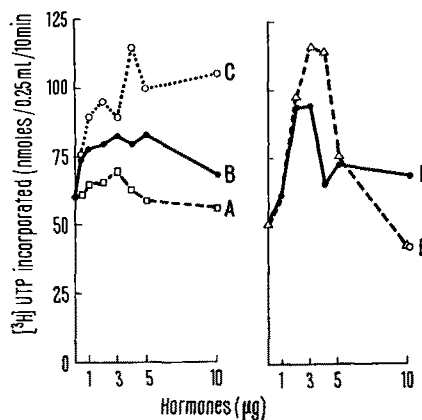

Fig. 4. Effect of polypeptide hormones on RNA polymerase activity. The following hormones were added to the assay system: oxytocin (A); insulin (B); luteinizing hormone (C); adrenocorticotropin (D); growth hormone (E).

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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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