action of the intragranular electron transport system with the externally reduced coenzymes, as an hypothesis one may assume the involvement of some structural factor or/and of a carrier system (diaphorase, quinone) scarcely participating in the respiration of resting cells.

Riassunto. Gli autori dimostrano che l'aumento della respirazione dei leucociti durante la fagocitosi non è dovuto a fuoriuscita di una NADH-ossidasi dai granuli, ma alla maggiore attività NADH e soprattutto NADPH-ossidasica localizzata in granuli non lisati.

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September 18, 1963.

The Effect of ACTH on Cholinesterase Activity in Plasma, Whole Blood, and Blood Cells of Rats

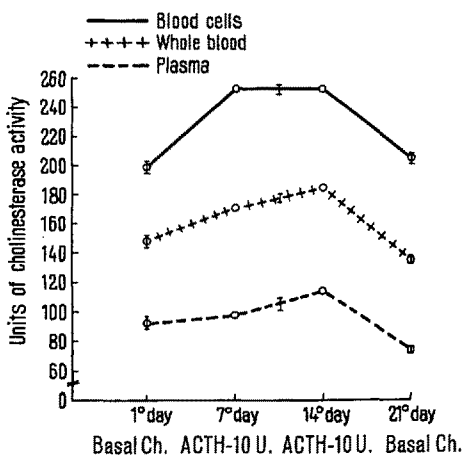
In 1960 one of us proved that when ACTH was administered to normal subjects and asthmatic patients, it produced increased cholinesterase activity in plasma, whole blood, and blood cells[1].

For this present study we used Wistar rats weighing about 250 g each. Blood was extracted by puncturing the jugular vein on four successive occasions: (a) under basic conditions, (b) and (c) after intramuscular and intraperitoneal injection of 10 units of ACTH, (d) under basic conditions. In this way the rats acted as their own controls.

Cholinesterase activity was determined in plasma, whole blood and blood cells by Bigg's colorimetric method[2].

ACTH was administered in the following way: 6 gel units intramuscularly and 4 units in a saline solution intraperitoneally.

Blood was extracted between 20 min and 120 min after the intraperitoneal injection of ACTH and from 3 to 5 h after its injection by the intramuscular route. This technique produced maximum stimulating effect of the corticotrophin on cholinesterase activity. Intramuscular and intraperitoneal injections were also given separately, but with this method we obtained less variation.



Cholinesterase activity in basal conditions and after ACTH.

Extractions were performed weekly and blood tests performed in order to avoid anaemia in the experimental animals.

The averages obtained under basic conditions, and expressed in units of cholinesterase activity were: 92 ± 3.7, 148 ± 3.9, and 199 ± 4.3 in plasma, whole blood, and blood cells respectively (Figure).

The values rose to 97, 171, and 252 after the first administration of ACTH. After the second administration, using the same technique, cholinesterase activity again increased, to 118, 185, and 253. The general average under the action of ACTH was 108 ± 4.5, 178 ± 2.4, and 255 ± 3.3 in plasma, whole blood, and blood cells respectively. Analysis of the statistics showed a highly significant increase, P: 0.001, in the different blood fractions.

Cholinesterase activity was again determined 7 days after the last administration of ACTH and the values were found to have dropped to the basic figures: 72, 135, and 205 units of cholinesterase activity in plasma, whole blood, and cells, respectively.

According to these findings ACTH possesses a stimulating effect on cholinesterase activity both in man and rats.

Résumé. On a déterminé chez des rats l'activité cholinestérasique dans le plasma, sang total et cellules sanguines avant et après l'administration d'ACTH. On a constaté une augmentation hautement significative: P: 0,001 après l'administration de cette hormone.

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August 19, 1963.

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[2] H. G. Biggs, S. Carey, and D. B. Morrinson, Amer. J. clin. Path. *30*, 181 (1958).

Stimulation of Dorsal Axial Differentiation in Ventral Explants of *Rana pipiens* Embryos by $CaCl_2$ and Guanidine HCl

It has been demonstrated that exposure of ectoderm of the early salamander gastrula *in vitro* to saline solutions of pH 4.0 and 9.7 for brief periods can stimulate the differentiation of parts of the brain[1], while brief treatment of ventral mesoderm at pH 12.0 promotes the formation of notochord, muscle and pronephric tubules[2]. The means by which the extreme pH levels cause prospective ventral tissue to form dorsal axial structures possibly may be allied with the provision of soluble protein, and possibly some ribonucleic acid, from some of the yolk platelets

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which are partially decomposed under these conditions[3]. Electron microscopic studies have shown that soluble protein fraction of guinea-pig bone marrow, which stimulates mesodermal differentiation of isolated salamander gastrula ectoderm, causes the loss of the surface layer of yolk platelets as the cells are induced to form mesodermal derivatives[4]. KARASAKI's[5] electron micrographs reveal that the surface layer of yolk platelets is normally lost at the time cells differentiate in the frog embryo.

ESSNER[6] has shown that concentrations of $CaCl_2$ above 0.02 *M* lyse isolated yolk platelets of ovarian eggs of *Rana pipiens*. In view of the previous evidence suggesting a correlation between the solubilization of yolk and the stimulation of differentiation of isolated gastrula ectoderm and ventral mesoderm, it was decided to test the effect of various calcium concentrations upon fragments of ventral mesoderm-ectoderm isolated *in vitro* in Niu-Twitty solution[7]. It has been suggested that the predominant yolk protein is a water-insoluble complex of a low molecular weight phosphoprotein and a high molecular weight protein[8,9]. Extremes of pH and calcium can solubilize yolk protein by disrupting electrostatic bonds between the phosphate groups of the phosphoprotein and amino groups of the high molecular weight protein, and the latter is then released and becomes water soluble. It is also possible that the binding of the two components of the complex is due to hydrogen bonds, and for this reason the effect of guanidine HCl was tested upon the isolated ventral mesoderm-ectoderm.

Approximately 2000 explants of ventral mesoderm-ectoderm were prepared by cutting early gastrulae (Shumway stage 10) in half, removing most of the yolky endoderm from the ventral halves and then cutting off the lateral edges of the ventral mesoderm-ectoderm fragments. These explants were cultured in groups of 10–12 for periods of 7–12 days upon a layer of 2% agar in small stender dishes filled with Niu-Twitty solution containing penicillin and streptomycin; the medium was changed daily. For periods of 4–6 h after the pieces of ventral mesoderm-ectoderm were extirpated, they were frequently spread flat with hair loops so as to prevent curling and allow the naked cells maximal exposure to the culture fluid before the less permeable surface coat covered the explants. A few explants from each type of culture were fixed, sectioned at 10 μ and stained with hemotyxylin-eosin at the termination of the period of culture. The explants in Niu-Twitty solution characteristically differentiated into ciliated epidermal vesicles which sometimes showed internal differentiation of mesenchyme and red blood cells. Over 200 explants were cultured in Niu-Twitty solution and only two or three instances of nerve and muscle differentiation were observed. In these instances well-developed tails usually formed indicating that some of the lateral edges of the chorda mesoderm were inadvertently included with the ventral mesoderm-ectoderm. Ventral mesoderm-ectoderm explants were isolated in the presence of various concentrations of acetylglucosamine, δ-aminolevulinic acid, rabbit and human erythropoietin, sodium iodide, diiodotyrosine, thyroid stimulating hormone, sodium thioglycolate, the four ribonucleoside triphosphates, phospho-enol-pyruvate and a complex mixture of amino acids, but the type of differentiation was similar to that observed in the control cultures in Niu-Twitty solution. Although not designed as

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[6] E. S. ESSNER, Protoplasma *43*, 79 (1954).
[7] R. A. FLICKINGER, J. exp. Zool. *112*, 465 (1949).
[8] L. G. BARTH and L. J. BARTH, *The Energetics of Development: A Study of Metabolism in the Frog Egg* (Columbia University Press, 1954).
[9] R. A. FLICKINGER, J. exp. Zool. *131*, 307 (1956).

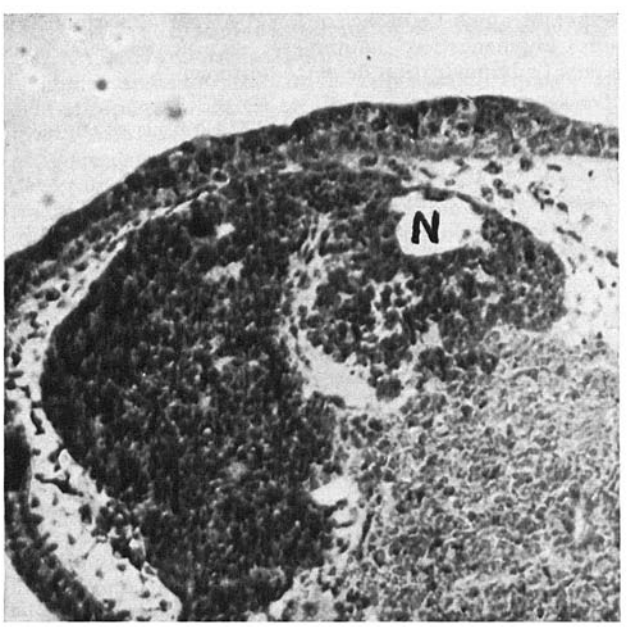

Fig. 1. Differentiation of neural tissue (N) in an explant of ventral mesoderm-ectoderm which was isolated from the frog gastrula, cultured in 0.05 *M* $CaCl_2$ in Niu-Twitty saline for one day and subsequently cultured in the saline medium alone for 10 days.

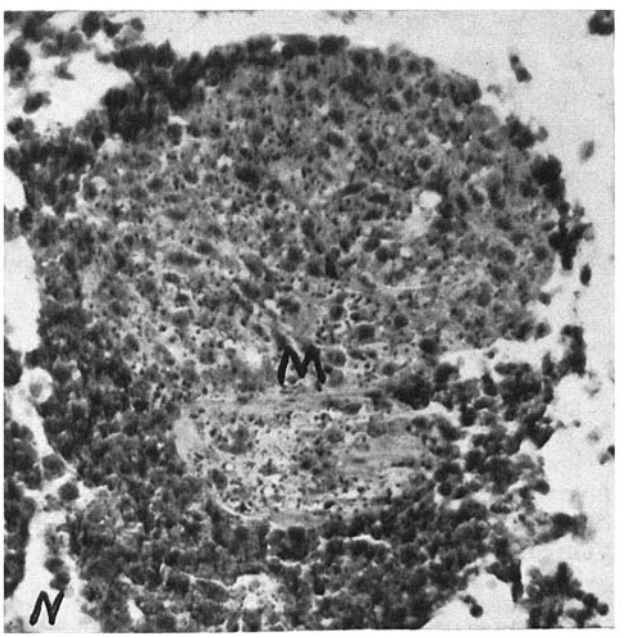

Fig. 2. Differentiation of nervous (N) and muscle (M) tissue in a ventral mesoderm-ectoderm explant which had been exposed to 0.5% guanidine HCl in Niu-Twitty saline for 12 h and then cultured in saline medium for 9 days.

controls, these experiments provide further instances of the normal type of differentiation of ventral mesoderm-ectoderm *in vitro*.

Over 600 cultures were isolated in Niu-Twitty medium containing concentrations of $CaCl_2$ ranging from 0.006 to 0.06 *M* for $^1/_2$–2 day periods, and then cultured in Niu-Twitty solution alone. Concentrations of calcium chloride below 0.025 *M* were not effective in initiating new pathways of differentiation, and 0.06 *M* $CaCl_2$ usually led to cytolysis of the explants. At concentrations above 0.02 *M* $CaCl_2$, the curling movements of the surface coat were retarded somewhat. After exposure to calcium chloride levels of 0.025–0.05 *M* for periods of 12–24 h, followed by culture in Niu-Twitty solution alone for 6–11 days, approximately 15% of the explants of ventral mesoderm-ectoderm developed nervous tissue. Muscle frequently was present in such explants, but notochord was usually absent. The presence of nerve and muscle tissue was not only recognized histologically, but also by twitching movements of these explants. An example of the induction of a mass of nervous tissue by 0.05 *M* $CaCl_2$ is illustrated in Figure 1.

Guanidine HCl (0.01, 0.1, 0.5, 0.75%) was added to the normal saline medium in 300 cultures of ventral mesoderm-ectoderm. The two lower concentrations were without effect and 0.75% guanidine HCl caused dissociation of the cells after 4–6 h. Exposure of 100 fragments of ventral mesoderm-ectoderm to 0.5% guanidine HCl stimulated the differentiation of nerve and muscle in approximately 25% of the explants as ascertained by twitching movements or histological examination (Figure 2).

Regarding the mechanism by which $CaCl_2$ and guanidine HCl can elicit dorsal axial differentiation from ventral explants, it has been mentioned that 0.05 *M* $CaCl_2$ lyses isolated ovarian yolk platelets *in vitro*[6], but little is known about the effect of guanidine HCl. Accordingly a suspension of ovarian yolk platelets in distilled water was prepared by homogenization and washing by low speed centrifugation. This washed suspension was divided into three 5 ml aliquots and guanidine HCl was added to two of the aliquots to give final concentrations of 0.5 and 1.0%. After 5 h at room temperature the three suspensions were centrifuged to sediment the intact yolk platelets and trichloroacetic acid was added to each of the three centrifugal supernatants to provide a final concentration of 5% TCA. These samples were then centrifuged in 12 ml graduated centrifuged tubes and the volumes of the TCA precipitates were obtained in order to provide an indication of the amount of yolk protein that was solubilized from the platelets. There was no TCA precipitate from the water suspension of yolk platelets, 0.45 ml of TCA precipitate from the 0.5% guanidine HCl suspension and 0.97 ml from the 1.0% guanidine HCl suspension. This indicates that 0.5% guanidine HCl, which can promote dorsal axial differentiation in ventral explants, can solubilize protein from yolk platelets.

Résumé. La culture de fragments isolés de mésoderme et d'ectoderme ventraux des gastrula de grenouilles (*Rana pipiens*) en présence de $CaCl_2$ 0,025–0.05 *M* pendant un jour ou en présence de HCl guanidine 0,5% pendant un demi jour, et suivi d'une période de culture dans un salin physiologique induit une différentiation de tissus nerveux et musculaires dans 15 à 25% des cas. Des concentrations de $CaCl_2$ et de HCl guanidine pareilles à celle qui induit cette différentiation peuvent solubiliser des plaquettes vitellines isolés.

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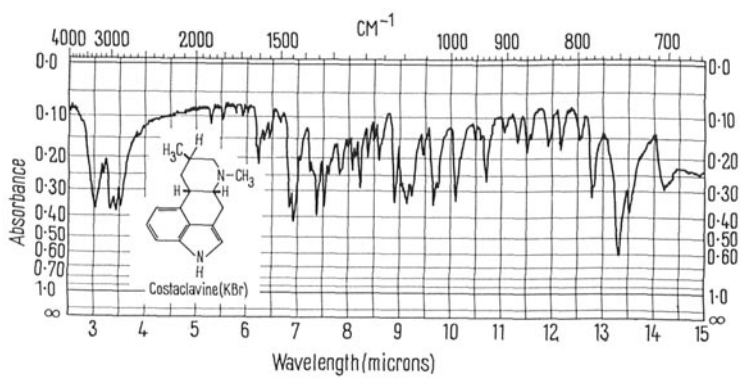
Department of Zoology, University of California, Davis (U.S.A.), August 15, 1963.

Costaclavine from *Penicillium chermesinum*

During a 'screening' of fungi for their ability to convert clavine type ergot alkaloids, a strain of *Penicillium chermesinum* Biourge was found to be capable of synthesizing on its own small amounts of a clavine alkaloid, behaving chromatographically as costaclavine. This latter alkaloid has previously been isolated by Abe[1] from saprophytic cultures of *Agropyrum* type ergot, together with the isomers festuclavine and pyroclavine.

P. chermesinum Biourge PC 106-I was grown in 500 ml Erlenmeyer flasks on 100 ml of a medium containing malt extract 'Difco' 10%, lactose 5% and asparagine 0.5% in distilled water. Addition of tryptophan had no positive effect on the alkaloid yield. The alkaloids (11 mg) were isolated from 60 culture flasks and separated as described earlier[2]. A number of pigments were removed by extraction with chloroform of the aqueous acidic alkaloidal extract.



The major alkaloid was recrystallized from ether-acetone, m.p. 182–184° (costaclavine[1] 182°). It gave the van Urk reaction and showed the ultraviolet absorption at 275, 283 and 293 mμ, typical[2] of the dihydro ergot alkaloids. The infrared spectrum of the isolated alkaloid was identical with that of authentic costaclavine. Further, the paper and thin layer chromatographic[2] behaviour of the isolated costaclavine was the same as that of authentic costaclavine.

The occurrence of ergot alkaloids in nature, previously restricted to *Claviceps* and *Aspergillus* among the fungi, therefore, also has been extended to the genus *Penicillium*. However, it should be noted that ergot alkaloids have been suspected earlier in *P. roqueforti*[3]. Quite interesting is the fact that

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