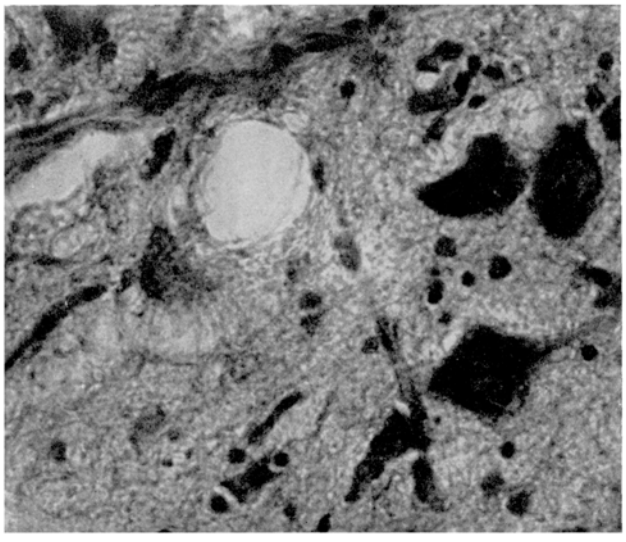

Fig. 7. C 479. A space apparently left by a degenerated neuron. This chick was killed when 12 days of age after being on the Torula yeast diet with galactose for 52 h. LFB-Nissl stain, ×435.

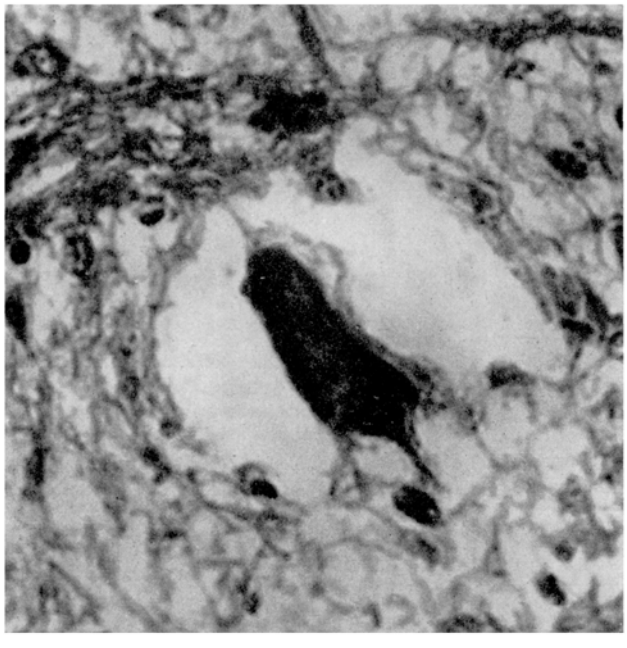

Fig. 8. C 512-4A. This nerve cell is pyknotic and has an adjacent pericellular clear space that probably is edema. This day old chick was fed the synthetic diet containing 40% galactose; 48 h later it was weak, had a tremor, convulsions and died. LFB-Nissl stain ×616.

galactosphingoside[9]. Apparently little is known of the mechanism which produces tremor, convulsions and death in young chicks fed an excessive amount of galactose. The morphologic changes in the brain, resulting from galactose intoxication, are characterized by acute degenerative changes in the neurons. Accompanying this degenerative change is pericellular edema. The nerve cells injured by excessive amount of galactose usually are within the basal ganglia. It is suggested that an excessive amount of galactose in young chicks in some manner injures the neurons, probably by acting through some local enzyme system. The early effect of galactose apparently is that of cell stimulation, as manifested by tremor and convulsions. This effect is reversible without any residual damage. Death, however, may occur in chickens fed the higher concentrations of galactose and they may have severe damage to the nerve cells in the basal ganglia.

The occurrence of convulsions often preceded by aurae is the characteristic clinical manifestation of galactose intoxication in the chicken. Convulsions may occur in man and animal in a wide variety of circumstances, among which may be mentioned hypoglycemia and hypoxia. Blood glucose levels in these chickens fed excessive amounts of galactose are within the range of normal. There is nothing clinically to support anoxia in the birds.

The mechanism of damage to the neurons in the basal ganglia of young chicks fed galactose in concentrations above 10% is unknown. However, the ease by which convulsions and degeneration of the nerve cells can be produced offers an excellent opportunity for the neurochemist, anatomist and pathologist to study specific nerve cell injury and the occurrence of edema[10].

Résumé. Des poussins nourris avec une ration contenant plus de 10% de galactose développent des tremblements, des convulsions et meurent. Une dégénération des neurons et de l'oedéma péri-cellulaire se présente dans la zone des ganglions basals, dans la zone médullaire et dans les lobes optiques.

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[9] W. E. STONE, chap. 18 in *Neurochemistry. The Chemical Dynamics of Brain and Nerve* (Ed. by K. A. C. ELLIOT, IRVINE H. PAGE, and J. H. QUASTEL; Charles C. Thomas, Springfield, Ill. 1955), p. 485.

[10] A 16 mm Kodachrome movie illustrating the clinical and histologic changes in galactose intoxication is available for those who are interested.

Histochemical Localization of Acid Phosphatase and Cathepsin-like Activities in Regressing Tails of Xenopus Larvae at Metamorphosis[1]

The atrophy of the larval tail, which occurs during anuran metamorphosis, represents a striking example of tissue regression. At the biochemical level this process coincides with a marked increase in the activity of cathepsins[2,3], acid phosphatase[4] and other 'lysosomal' enzymes[5]. Since larval tails include various structural elements, comprising very different types of tissue, the problem arises as

[1] This work was supported by the 'Swiss National Foundation for Promotion of Scientific Research' (Project 1613).

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to whether the observed activation of acid hydrolases reflects a general or a tissue-specific reaction. The investigation of this problem is not only important to the elucidation of the metamorphic response at the cellular level but also bears upon the mechanism of tissue regression in general. According to the 'lysosome concept'[6], in which it is suggested that a release of bound acid hydrolases at cell death occurs, one would actually expect a uniform and diffuse distribution of these enzymes in the regressing tails. In order to obtain direct evidence, advantage was taken of the recent improvements of enzyme histochemistry. Thus the present report is concerned with the distribution of acid phosphatase and cathepsin-like esterase activities in tails of Xenopus larvae at very advanced stages of metamorphosis, indicating macrophages as the most important sites of activity.

Tail rudiments of frog-like Xenopus larvae were fixed for 16 h in cold 4% formalin (previously neutralized with $CaCO_3$) in the presence of 10% sucrose. After rinsing in tap water, the tails were embedded in pieces of fresh rat muscle and immediately frozen in liquid nitrogen (18 sec). Sections (7 μ) were cut in a cryostat (−20°C), mounted on cover slips, and then put into a chilled solution of 10% sucrose for transfer to room temperature (+20°C). By this procedure it was possible to circumvent enzyme diffusion, which results from the deposition of water films on the tissue. This invariably occurs if sections are brought unprotected from the cryostat to room temperature. The sites of activity of acid phosphatase were revealed by the azo-dye coupling method of BURSTONE[7], using naphthol AS-TR phosphate as substrate and the diazonium salt Fast Dark Blue R at pH 5.2. In order to demonstrate cathepsin-like activity, the staining reaction for organophosphorus-resistant esterase as described by HESS and PEARSE[8] was relied upon. After pretreatment with E_{600} (inhibitor) and cysteine (activator), the sections were incubated with O-acetyl-5-bromo-indoxyl at pH 7.0 in the presence of E_{600} and cysteine.

The preparations obtained with these methods reveal very similar staining patterns, which clearly demonstrate a *differential distribution* of both acid phosphatase and cathepsin-like esterase in regressing tails (Figure 1). Thus, in tail rudiments, in which the muscle cells are almost completely resorbed, a very intense staining reaction is found in the sub-epidermal connective tissue, confined to macrophages. Some activity, especially of esterase, is also demonstrable in the basal portion of the epidermis. On the other hand, several tail structures such as the notochord, the neural tube and the remaining muscle cells – although undergoing regression – do not give any appreciable staining reaction. In macrophages it is possible to distinguish intracellular inclusions. These are best recognized in preparations stained for acid phosphatase, but sometimes are also seen by the esterase reaction (Figure 2). These inclusions, 1–10 μ in size, belong to the category of lysosomal structures and presumably represent 'phagosomes', although their evolution remains yet to be investigated. It is, however, noteworthy that in tails of premetamorphic tadpoles there occur only few macrophages

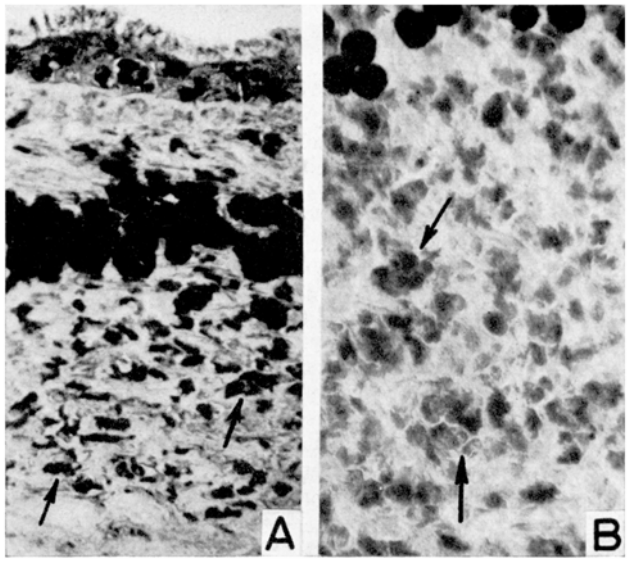

Fig. 1. Differential distribution of acid hydrolases in regressing tails. Cryostat-sections of very advanced stages of regression stained for acid phosphatase (A) and cathepsin-like esterase (B) respectively. In both cases the most intense reaction is confined to cells (↑) of the sub-epidermal connective tissue. 270 ×.

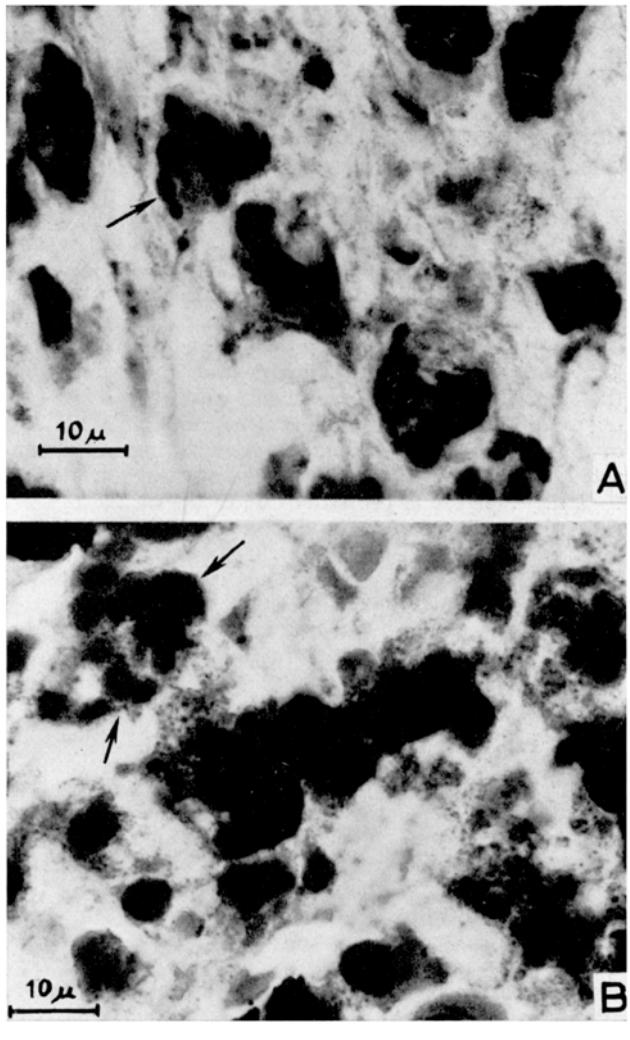

Fig. 2. Localization of acid hydrolases in tail macrophages. Small portion of the sub-epidermal connective tissue showing cell-specific staining reactions for acid phosphatase (A) and cathepsin-like esterase (B) respectively. (↑) indicates intracellular inclusions presumably representing 'phagosomes'.

[6] C. DE DUVE, in *Subcellular Particles* (Ed. T. HAYASHI, The Ronald Press Co., New York 1959), p. 128.
[7] M. S. BURSTONE, J. nat. Cancer Inst. *21*, 523 (1958).
[8] R. HESS and A. E. G. PEARSE, Brit. J. exp. Path. *39*, 292 (1958).

which are scattered in the connective tissue and between muscle cells. According to our present observations, these macrophages contain fewer inclusion-bodies than those in regressing tails.

The evidence obtained from enzyme histochemistry leads to the conclusion that the increase in activity of acid phosphatase and cathepsins is due to the local activation of macrophages in the regressing tail. This view is also supported by our observation[9] that during tail atrophy there is a change in the pattern of cathepsins. It is therefore conceivable that the tail macrophages are the sites of this activity. Future work will be directed to the elucidation of those events which initiate the activation of macrophages in the tail tissue[10].

Zusammenfassung. Histochemische Reaktionen an regredierenden Schwänzen von Krallenfroschlarven haben ergeben, dass die saure Phosphatase und die kathepsinartigen Esterasen vorwiegend in Makrophagen des subepidermalen Bindegewebes angereichert sind. Somit dürfte die erhöhte Aktivität dieser Fermente im regredierenden Schwanzgewebe auf der lokalen Aktivierung von Makrophagen beruhen.

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Abteilung für Zellbiologie, Zoologisches Institut der Universität Bern (Switzerland), April 5, 1963.

[9] R. WEBER, in preparation.

[10] We are greatly indebted to Dr. R. HESS (CIBA Basel) for generous advice on histochemical techniques. Our thanks are also due to Prof. E. LÄUPPI (Gerichtlich-medizinisches Institut, Bern) for permission to use his cryostat and to Mr. B. NICK for assistance in microphotography.

Reactivity of Cat Skeletal Muscle Vessels to Noradrenaline During Induced Respiratory Alkalosis

Several workers[1-3] have shown that alterations in the acid-base balance of the blood will change the pressor responses to i.v. adrenaline or noradrenaline injections. Usually, uncompensated acidosis diminishes and uncompensated alkalosis increases the pressor responses. However, the site and mechanisms of these effects are to a large extent still obscure[4]. The present study shows that the overall response of a peripheral vascular bed, consisting mainly of skeletal muscle vessels (the skinned hind leg of the cat) to noradrenaline increases during uncompensated respiratory alkalosis. The results suggest further that this increase is primarily due to the increase in pH rather than the decrease in carbon dioxide tension.

Methods. Ten cats (weighing 2.0–4.3 kg), anaesthetized with pentobarbital, were used. One leg was tied off at the upper part of the thigh with only the large vessels and nerves remaining untouched. This leg was subsequently skinned and the paw tied off just above the ankle joint, leaving the femoral artery to perfuse the skeletal muscles. The blood in the femoral artery was exteriorized in a polyethylene loop and the flow maintained by means of a constant output pump (Sigmamotor Inc., U.S.A.). Changes in perfusion pressure during the course of an experiment thus reflected changes in the vascular resistance of the perfused region. The preparation also included resection of the ipsilateral sympathetic chain at the midlumbar level and the splanchnic nerves on both sides.

The following physiological functions were continuously recorded on a Grass polygraph: (1) systemic blood pressure (femoral artery), (2) perfusion pressure (in the loop, distal to pump), (3) arterial pH (in the loop, proximal to pump) and (4) carbon dioxide tension (in end tidal air). The blood pressures were recorded via transducers (Statham Inc., U.S.A.). The arterial pH was recorded via a pH-meter (PHM 22, Radiometer Inc., Denmark). End tidal carbon dioxide tension was determined by drawing the expired air through a gas-analyzer (Beckman Spinco model LB-1). Spontaneous respiratory movements were abolished by a slow i.v. drip of succinylcholine iodide (Celocurin, Vitrum Inc., Sweden) in a low-molecular weight dextran (Rheomacrodex Mw 40000, Pharmacia Inc., Sweden) and the animals were ventilated artificially.

The animals were initially ventilated with oxygen and the degree of the ventilation was adjusted to maintain pH approximately 7.4. Blood flow to the perfused leg was such that the perfusion pressure equalled the systemic blood pressure. The animals were, at this stage, in a state of compensated metabolic acidosis with a low carbon dioxide tension in the end tidal air. This was later compensated by a slow i.v. infusion of 0.6 N sodium bicarbonate in an amount sufficient to restore the normal carbon dioxide tension of 40 mm Hg. To maintain a constant ventilation and blood pH, at the same time, the percentage of carbon dioxide in the inspired gas was increased. Later respiratory alkalosis could easily be produced by again administering oxygen. With this experimental procedure it was possible to follow the peripheral effect of i.a. noradrenaline injections during three different types of alterations in the acid–base balance: (1) Compensated metabolic acidosis with a low carbon dioxide tension and a normal blood pH; (2) 'Normal' stage with a normal carbon dioxide tension and normal blood pH; (3) Uncompensated respiratory alkalosis with a low carbon dioxide tension and a high blood pH.

To obtain larger changes in the carbon dioxide tension, the compensated metabolic acidosis (1) was aggravated in some experiments by a slow infusion of 5–10 cm³ 0.2 N hydrochloric acid i.v.

The effect of noradrenaline in different doses (administered i.a. in the loop) on the perfusion pressure was tested repeatedly during the experiments, and the recorded changes were expressed in two different ways: (1) The percentual change in the response of a noradrenaline dose sufficient to produce a 50 mm Hg increase of the perfusion pressure at stage 2; (2) The relative increase or decrease of the dose required to maintain a constant 50 mm Hg pressure response.

Results. In 8 experiments out of 10, uncompensated respiratory alkalosis increased the overall response of muscular blood vessels to i.a. injections of noradrenaline. An increase of the pH to approximately 7.56 (5 cats) increased the response of an i.a. noradrenaline injection to an average of 114%. A more severe alkalosis (pH 7.80; 5 cats) increased the response to 132%. The dose necessary

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[3] I. H. PAGE and F. OLMSTED, Circulation (N.Y.) *3*, 801 (1951).
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