galactosphingoside. 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 auras 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.

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

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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

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, acid phosphatase and other 'lysosomal' enzymes.

Since larval tails include various structural elements, comprising very different types of tissue, the problem arises as...
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'\(^8\), 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 μm) 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\(^6\) 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 basilar 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 μm 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

\[\text{Burstone}^7\]

\[\text{Hess and Pearse}^6\]


\[\text{M. S. Burstone, J. nat. Cancer Inst. 21, 523 (1958).}\]