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Cataracts in *Amblystoma tigrinum* Larvae Fed Experimental Diets.

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Lens opacities in larvae of the tiger salamander were first observed by the author in members of an Illinois strain fed a semi-synthetic diet containing 45% of purified casein (repeated treatment with dilute alkali), 37% of powdered whole milk, cooked cornstarch, cod-liver oil, and bakers' yeast. Mature cortical cataracts developed in 40% of the animals living for 2 months or longer on this diet, while the eyes were normal in larvae of the same lot fed a diet of powdered beef muscle, cooked cornstarch, fat, minerals, and the vitamins. The disease did not appear in larvae fed the casein diet supplemented by cystine[1] (1.25% of the total protein of the diet), a

[1] Patch, E. M., *Science*, 1934, **79**, 57.

result confirmed by repetition of the work with 2 sets of Wisconsin larvae. Cystine has also been found a preventative of cataracts[2, 3] produced in rats by casein diets.

Cataracts were next observed in *Amblystoma tigrinum* larvae that had been fed for 3 months or more on synthetic rations of the separate proteins prepared from fresh muscle (extraction by an alkaline-buffered KCl solution and precipitation by graded acid dialysis). Seven rations were made like the earlier one of powdered whole muscle, each with a different muscle protein: beef myosin, beef globulin x, these 2 proteins and myogen from the white muscle of chicken, and the extraction residue (largely stroma protein) from both kinds of meat. All of these rations produced cataracts in larvae from both Tennessee and Wisconsin, with a total occurrence of 51%, while the disease did not appear in larvae of the same sources fed raw muscle or liver or synthetic diets of these tissues. The lens changes produced by the diets of the separate muscle proteins were like the changes caused by the diet of purified casein: rarely there was visible an early stage of radiating striae, which was followed by complete cortical disintegration and final disappearance of the shrunken lens into the vitreous chamber. Dietary supplementation with riboflavin[4] (1.25 γ per gram of ration) did not prevent these cataracts. Twice the quantity of cystine which had been effective with the casein diet, was preventative with 2 of the muscle-protein diets, but not with 2 others. The latter may have needed a higher amount of free cystine; or possibly, during the preparation of the separate muscle proteins, some additional constituent of whole muscle necessary for lens health may have been lost.

Cortical cataracts with a very different history appeared in the carnivorous salamander larvae when they were fed synthetic diets of dried whole blood (beef or turtle), defibrinated blood, or hemoglobin. After 4 or 5 weeks on these rations, all of the larvae showed an easily visible black band of zoning in the outer cortical region of the lens and parallel to its surface. In 37% of the animals, whitening of the cortex followed, accompanied by a large fluid content within the lens capsule during a prolonged period of maturity. The lens zoning developed in larvae fed a diet of globin without heme, but not in those fed a diet of heme (3 times the amount in the hemoglobin diet) combined with powdered beef muscle instead of with globin. Also, no lens disturbance developed in larvae whose

[2] Bourne, M. C., and Pyke, M. A., *Biochem. J.*, 1935, **29**, 1865.

[3] Yudkin, A. M., and Geer, H. A., *Arch. Ophth.*, 1940, **23**, 28.

[4] Day, P. L., Darby, W. J., and Cosgrove, K. W., *J. Nutrition*, 1938, **15**, 83.

sole source of protein was fibrin. Addition of riboflavin to the faulty blood diets did not prevent the cataracts, and cystine alone was not preventative when it made 7% of the dietary protein. There was, however, a definitely lower occurrence and intensity of the disease when both cystine (3.3%) and glutamic acid (15%) were added to the diet; and this preventative effect was still greater when glycine (3%) accompanied them. Incomplete prevention with the liberal supply of the three acids suggests inadequacy of some additional dietary component.

Summary. Two types of cortical cataract have been produced in salamander larvae by synthetic diets differing in their protein constituents. The first type, rarely detectable in the early stages and with a marked final shrinkage, was caused by a diet of purified casein and by diets of the separate muscle proteins. The second type, with an easily visible early zoning, large fluid content at maturity, and halted later changes, was caused by diets with high hemoglobin content. Prevention of the first type of cataract was attained by dietary supplementation with cystine, while prevention of the second type required all 3 components of glutathione and apparently some additional factor.

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Ferrous and Ferric Iron in Liver Extract.

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In recent years much attention has been paid to the relative efficiency of absorption and utilization of ferrous iron as compared with ferric iron in treatment of the iron deficiency anemias. It is not our purpose further to discuss this question, but rather to present the results of an investigation carried out on solutions of liver extract to which iron salts had been added.

In general, ferrous ions in solution are rapidly oxidized to the ferric state unless protected by some reducing agent. Borgen and Elvehjem[1] commented on the power of homogenized chicken liver extracts to reduce ferric iron. We had previously noted considerable reducing power in a simple aqueous liver extract. These facts sug-

[1] Borgen, D. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **119**, 725.