

found that only the ectoderm and neural crest were dopa-positive in *Triturus torosus* embryos.

No definite answer can be given to the question of the significance of the dopa reaction in amphibian melanin formation. The reaction is not "absolutely specific" for melanoblasts in this group as Bloch¹ has claimed for mammals, but it is possible that the reactions of non-melanoblastic cells are adventitious.

The evidence in this paper is sufficient to show that a cell which gives a positive dopa reaction is not necessarily melanoblastic. Consequently, the dopa reaction is not an adequate criterion for distinguishing melanoblasts or prospective melanophores in amphibia.

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Effects of Various Diets, Cholesterol and Choline on Liver Lipids of the Rat.

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It has long been known that the diets high in fat cause a marked increase in the neutral fat of the liver. Best and his coworkers^{1, 2} have shown that this can be prevented or cured in the rat by administration of choline. Moderately fatty livers were produced by these workers when they fed rats exclusively on sucrose or on any other choline-free diets. Cholesterol in doses of 100 mg. daily had a similar effect. This change was found to be preventable, however, by the administration of as little as 5 mg. of choline daily. It has been shown by Schonheimer and Yuasa,³ and confirmed by Page and Menschick,⁴ Okey,⁵ Chanutin and Ludewig,⁶ Sperry and Stoyanoff,⁷ and others, that cholesterol feeding causes a marked increase in the cholesterol of the liver. Chanutin and Ludewig found that added carbohydrate accelerated while fat inhibited the deposition of cholesterol in the liver of cholesterol-fed rats. Little attention appears to have been given to the phospholipid fraction in the liver

¹ Best, C. H., and Ridout, J. H., *Am. J. Physiol.*, 1933, **105**, 6.

² Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1935, **83**, 255.

³ Schonheimer, R., and Yuasa, D., *Z. f. Physiol. Chem.*, 1929, **180**, 5.

⁴ Page, I. H., and Menschick, W., *J. Biol. Chem.*, 1932, **97**, 359.

⁵ Okey, R., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1003.

⁶ Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1933, **102**, 57.

⁷ Sperry, W. M., and Stoyanoff, V. A., *J. Nutrition*, 1935, **9**, 131.

under the various conditions mentioned, except for the finding by Chanutin and Ludewig that lecithin remained normal after cholesterol feeding. The specific effects of high-protein diets in this connection have not been recorded.

The results presented here supplement previous studies by supplying data on these points. The analyses reported were carried out on livers from rats used in a previously reported series of experiments on the influence of diet and other factors on the lipid content of the brain.⁸ Four rats from our own stock colonies were placed at the age of 3 weeks on each of the following special regimens: 1. standard control diet (Chanutin and Ludewig), 2. high-fat (85%), 3. high-carbohydrate (80%), 4. high-protein (80%), 5. Chanutin diet plus 5.0% added cholesterol, 6. Chanutin diet plus 2.5% cholesterol, 7. the latter plus 1.3% choline. The diets were made up from casein, lard, starch, cod liver oil, yeast powder, water and Osborne-Mendel salt mixture. The experiments extended over periods varying between 44 and 105 days. For each group of animals on special diet a control group was kept on a standard mixed diet for the same period of time. At the end of the experimental period the animals were killed by sudden decapitation, after which their livers were promptly removed and weighed. An aliquot portion of each organ was taken for determination of the water content. The remainder was immediately prepared for extraction by the procedure of Osato and Heki.⁹ The total fatty acids, cholesterol and phospholipids were then determined by the methods of Bloor.^{10, 11}

The results may be summarized briefly. Expressed in percentage of dry weight, the livers of the control rats fed on the standard diet for 105 days were found to contain on the average, total fatty acids, 14.34, cholesterol 1.03 and phospholipids 12.37. In comparison with these values those for the animals on special diets for the same length of time were as follows: *high-fat diet*, total fatty acids 33.05, cholesterol 1.45 and phospholipids 16.72%; *high-carbohydrate diet*, total fatty acids 24.32, cholesterol 1.25 and phospholipids 13.56%; *high-protein diet*, total fatty acids, 15.69, cholesterol 1.16 and phospholipids 33.92%.

Livers of animals taking the Chanutin control diet plus 5.0%

⁸ Stoesser, A. V., Petri, K. A., and McQuarrie, Irvine, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **82**, 761.

⁹ Osato, S., and Heki, M., *J. Biol. Chem.*, 1930, **87**, 543.

¹⁰ Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

¹¹ Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

cholesterol contained on the average no more of any of the lipids determined than those on the diet plus 2.5% cholesterol. However, all lipid fractions were higher in the livers of rats which were on the diet plus cholesterol 96 days than in those of rats which were killed on the 44th day. Values for the control animals for the two periods did not vary significantly. Contrary to the finding of Chanutin and Ludewig, the phospholipid content of the liver in our rats was found to be increased by approximately 50% as a result of prolonged cholesterol feeding. Choline effectively prevented any significant increase in the neutral fat and phospholipid content of the liver from cholesterol feeding but only partially inhibited the extra deposition of cholesterol.