physectomized in pregnancy rapidly regress, especially following parturition.[5]

8463 C

The Dopa Reaction in Amphibia.

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Considerable evidence has accumulated indicating that the dopa reaction is specific for distinguishing melanoblastic cells in mammals (Bloch[1]). According to Bloch the dopa is oxidized to dopamelanin by a specific oxidase which is present only in cells capable of forming melanin normally. The theory further assumes that dopa (3,4:dioxyphenylalanin) which is an intermediate oxidation product of tyrosine[2] is the normal precursor of melanin in animals. In mammals the reaction occurs only in pigmented regions of the epidermis, never in white areas. However, in adult amphibia dopa reacts in all regions of the epidermis whether pigment is present or not.[3] The reaction is most intense in the outer layers of the epidermis in contrast to the conditions in mammals where the reaction is most intense in the basal cells (*stratum germinativum*).

In the present work the skin of 4 different urodeles was used with results which confirm those of Schmidt (Table I). Laidlaw's technic[4] was used with the exception that frozen sections were not made. Small pieces of skin were fixed for 2 hours in 5% formalin, washed 10 minutes in distilled water and placed in 0.1% buffered dopa solution at a temperature of 36°C. for 6-11 hours. The controls were put in the phosphate buffer solution without dopa. The pieces of skin were then imbedded and sectioned in the usual way. Apparently, the dopa solution penetrates the epidermis rapidly, since the reaction is the same at the cut edges as elsewhere.

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5 Pencharz, R. I., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1131.

* National Research Fellow in the Biological Sciences.

1 Bloch, B., *Z. ges. exp. Med.*, 1917, **5**, 179; *Centralbl. Haut- und Geschlects-Krankheit*, 1923, **8**, 1; also, summary by Biedermann, W., *Ergebn. d. Biol.*, 1928, **4**, 360.

2 Raper, H. S., *Physiol. Rev.*, 1928, **8**, 245.

3 Schmidt, W. J., *Dermatol. Z.*, 1919, **27**, 284.

4 Laidlaw, G. F., *Anat. Rec.*, 1932, **53**, 477.

TABLE I.
The Dopa Reaction in Various Tissues of Amphibia.

		White Axolotl	Black Axolotl	*Triturus similans*	*Triturus pyrrhogaster*
1. Leydig cells	Occurrence	pres.	pres.	abs.	abs.
	Dopa reaction	—	—		
2. Epidermal melanophores	Occurrence	abs.	,,	,,	,,
	Dopa reaction		?		
3. Epidermal melanin granules	Occurrence	abs.	pres.	pres.	abs.
4. Epidermal cells	Dopa reaction	+	++	+++	++++
5. Mucous glands	Occurrence	abs.	abs.	pres.	pres.*
	Dopa reaction			+	+
6. Granular glands	Occurrence	abs.	abs.	pres.	pres.*
	Dopa reaction			—	—
7. Dermal melanophores	,, ,,	—	±	?	?
8. Blood cells	,, ,,	±	±	+	+

— = negative reaction.
+ = positive reaction (the number of plus signs in row 4 indicates the relative intensity of the reaction).
± = slight or questionable reaction.
? = no decision possible because of intense normal pigmentation (*cf.* dermal melanophores.)
*Some of the skin glands of this species are apparently compound, having both granular and mucous parts, while others are separate. Only the mucous portions give the reaction.

The strength of the reaction seems to be correlated with the total amount of pigment in the dermal melanophores. Thus,

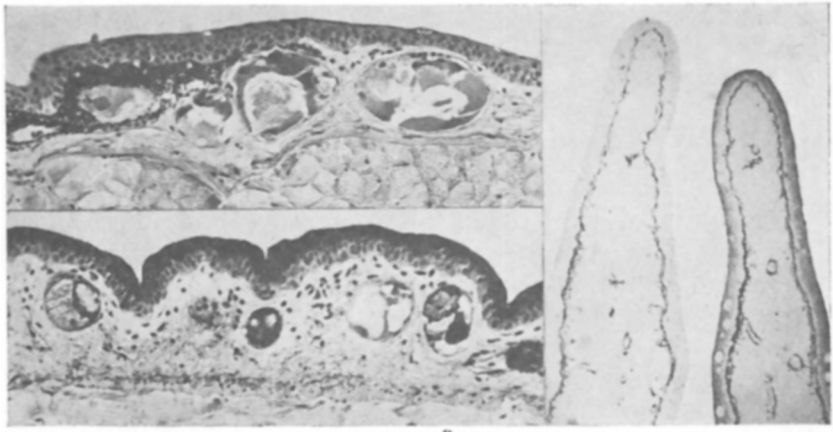

FIG. 1.
Sections of ventro-lateral skin of an adult *Triturus pyrrhogaster*. Above, control which shows dermal melanophores on the left. The epidermis contains no melanin. Below, section of skin which was placed in dopa solution for 11 hours. Heavy deposit of dopa-melanin in outer layers of epidermis and some skin glands (especially gland in center). Photos, x 75. Heidenhain's haematoxylin and eosin.

FIG. 2
Unstained sections through the dorsal fin of an adult black axolotl. Left, control; right, dopa-treated for 11 hours. The outer layers of the epidermis show a moderate reaction, except for the Leydig cells (circular colorless areas). The dermal melanophores seem to have shown a slight reaction. Photo, x 28.

the reaction increases in intensity in the epidermis of the 4 species in the following order (Table I, row 4): Albino axolotl, black axolotl (Fig. 2); *Triturus similans;*[5] *T. pyrrhogaster* (Fig. 1). While this general correlation seems to indicate that the reaction has some significance in regard to pigment formation, other results do not harmonize with this. The reaction takes place quite as well on the ventral sides where no dermal melanophores occur (pyrrhogaster, similans) as it does where melanophores are numerous. In addition, there is no relation between the presence or absence of epidermal melanin granules and the dopa reaction (compare rows 3 and 4). Thus, dorsal epidermis of *T. similans* contains melanin granules; ventral epidermis does not. Nevertheless, the reaction proceeds with the same intensity in both regions.

These results, while not in agreement with those in mammals, do not necessarily exclude the possibility that dopa may play some part in normal melanin formation in amphibia. For example, if it could be shown that the dark regions of the skin produced both the oxidase and the chromogen (dopa) and that the light skin produced the oxidase, as it apparently does, but no chromogen, then these experiments would fall into line with those on mammals.

Evidence from embryonic transplantations indicates that the epidermis is active in inducing melanin formation in prospective dermal melanophores.[6] Thus, it is not improbable that the epidermis of amphibia, even though not strictly melanoblastic, plays an important rôle in the enzyme-chromogen system responsible for melanin formation.

Further difficulties of interpretation are introduced by the fact that a number of other tissues which presumably have nothing to do with pigment formation give positive reactions. Thus, red blood cells (row 8), mucous skin glands (row 5) and the secretions of the unicellular glands of the intestine and rectum (similans) are dopa-positive. Preliminary experiments with embryos of *T. pyrrhogaster* show that not only the neural crest cells (prospective melanophores) and epidermis, but also some of the primitive blood cells in the mid-ventral blood-island and an occasional yolk cell are dopa-positive. As in adults, the reaction occurs in those cells which are of some importance in melanin formation as well as in other cells which have no such significance. This result is in contrast to those reported in abstract by Blanchard and McCurdy,[7] who

[5] New species. Twitty, V. C., *Copeia,* 1935, **2**, 73.

[6] DuShane, G. P., *Science,* 1934, **80**, 620; *J. Exp. Zool.,* 1935, **72**, 1.

[7] Blanchard, E. W., and McCurdy, H. M., *Anat. Rec.,* 1934, **60**, (Suppl.) 62.

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 Bloch[1] 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.

8464 P

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.