FURTHER OBSERVATIONS ON THE PIGMENT CHANGES FOLLOWING REMOVAL OF THE EPITHELIAL HYPOPHYSIS AND THE PINEAL GLAND IN THE FROG TADPOLE

(With 4 figures in text)

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That the early removal of the epithelial hypophysis in the frog tadpole results in the production of an individual with a greatly disturbed pigmentary system has been shown by P. E. Smith, B. M. Allen and the writer. The operated animal exhibits a much lighter color than its normal mates and has been termed a "silvery" individual or an "albino." Apparently considerable difference of opinion has existed concerning the nature of this pigmentation change and the altered condition of the various types of pigment cells which may be responsible for it. Since the publication of a previous brief contribution bearing on these points additional observations have been made and the total results are now presented in a more comprehensive form, together with photomicrographs from the living animal.

HISTORICAL

Smith ('16) states that the differences in color begin to be noticeable in the operated tadpole before a length of 15 mm. has been reached. In his paper Smith holds that "these pigment differences are referable chiefly, if not solely, to the condition of the epidermis." Counts of the melanophores of corresponding areas in the 'albino' and in the controls showed that in the epidermis the number of these cells was reduced in the former. It was further observed that the melanophores of the albino specimens contain fewer pigment granules than do those of the controls, and thus have a distinctly lighter appearance. Smith states that "the melanophores are equally expanded in the two types, consequently the lighter appearance of the albinos cannot be due to the con-

tracted condition of the chromatophores." The free pigment granules which are so noticeable in the superficial layer of the epidermis in normal animals are much reduced in the operated tadpole. The deeper or subcutaneous pigment is present in as great quantity, if not greater, than in the normal animals.

Allen ('17) noted that the color change occurs when the operated tadpole is about 8 mm. in length. He also records the observation that only a few pigment cells are to be found in the epidermis of the operated animal. It is suggested that the pigment cells have migrated to deeper positions. As regards the deeper chromatophores, he states that "there is a constant difference in that they are expanded in the normal tadpoles and much contracted in the operated ones."

At this stage of an apparent controversy the writer ('19) obtained evidence, of an experimental nature, which strongly supported Allen's contention that the lighter color of the operated tadpole is due largely to an altered state of contraction of the melanophores, rather than to a reduction in the amount of pigment material present. This evidence consisted, briefly, in the ability to darken appreciably the silvery tadpole by subjecting it to extracts or emulsions of pars intermedia, coupled with the direct observation in both living and fixed material that the deeper melanophores, which in the silvery tadpole are contracted, expand under this treatment.[1]

Smith ('19) strongly controverted the statement that the silvery tadpole may be darkened by treatment with appropriate pituitary solutions, stating that "albinous larvae have never in the hands of the writer been appreciably increased in depth of pigmentation." And in regard to the view of Allen and the author that the deeper melanophores are contracted in the silvery animal he stated: "In the experience of the writer no constant deviation exists from the various stages of relaxation or expansion which may occur normally in the subepidermal or deeper melanophores as contrasted

[1]At the time of this former brief article the writer failed to recognize that any controversy existed concerning the role of the light-colored pigment cells, the xantholeucophores. By use of the low powers of the binocular microscope their changed condition in the operated tadpole as compared with the normal is readily apparent, even to the casual observer. All statements in the previous article refer only to the melanin-bearing chromatophores.

with normal larvae." At this time he directed attention to the role of the silvery, or light-colored pigment cells, the xantholeucophores.[2] These cells, which are contracted and inconspicuous in normal tadpoles, are widely expanded in the operated animal. They are responsible for the metallic lustre which the silvery tadpole displays. They are held to mask, to a considerable degree, the deep melanophores and thus to reduce to a minimum any color effect which might be produced by the latter.

In his recent memoir Smith ('20) has considerably modified the views expressed by him in previous writings. In fact, he is able to confirm the writer in both of the points formerly controverted by him, admitting that "the deep melanophores exhibit a contracted condition" at least in the young albino, and further agreeing "as pertains to the darkening of these larvae and the expansion of the corial melanophores when placed in the pars intermedia solution." He states also that in the albino tadpole darkened by immersion in pars intermedia solutions the xantholeucophores suffer a great contraction.

The prolonged feeding of albinos[3] with posterior lobe substance was found by Smith to result in a considerable degree of darkening. So far as analyzed by him, this darkening is to be accredited to the restoration of melanin granules in the epidermal chromatophores and epithelial cells. The xantholeucophores were found to be refractory and were not reduced to their normal state of contraction. The condition of expansion or contraction obtaining in the deep melanophores is not recorded.

McCord and Allen ('17) found that the normal frog tadpole is made very transparent by immersion in solutions or emulsions

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[2]The term 'xantholeucophore' as used by Gaupp ('04) has been objected to recently by Schmidt ('19-'20) who finds that the two pigments (xanthin and guanin) exist separately in two kinds of cells, xanthophores or lipophores, and guanophores or leucophores, respectively. In the present article xantholeucophore is used to designate the pigment cell, or cells, containing light-colored pigments, without attempting to analyze the varieties. The 'interference cell' of Biederman is homologous.

[3]The continued use of the term 'albino' (Smith) seems confusing and undesirable. It is probably to be considered a relic from the time when the light color of the hypophysis-free tadpole was believed to be due principally to a reduction in the amount of pigment material. Such reduction is now known to be confined to the pigment of the epidermis. Hereafter in this article the term 'silvery' will be employed as being more accurately descriptive.

of beef pineal substance in water. A study of the pigment cells showed that the epidermal chromatophores are not perceptibly modified, but the deep melanophores undergo a very striking contraction. The effect is transitory, passing off in a few hours unless pineal material is repeatedly added. Smith ('20) confirms most of the results obtained by these observers. He notes further that the xantholeucophores are not changed from their usual state of strong contraction.

OBSERVATIONS

Pineal Solutions. Treatment of normal frog tadpoles with pineal solutions or emulsions, first done by the author in the spring of 1918, following McCord and Allen, gave results which confirmed those writers. The change by which the normal dark tadpole becomes transparent is accomplished in a short time, usually 15 minutes to one hour. The condition is transitory and disappears within two to four hours, or sooner if the tadpole is removed to fresh water. While the animal is in the transparent state the eyes, which apparently do not suffer reduction in pigmentation, are very distinct. as are also the liver and other large viscera. The animal does not display the silvery metallic lustre so characteristic of the hypophysectomized tadpole. The appearance of the animal is dull and drab and the term *ashen* has been chosen to designate it.

A critical examination of the pigment cells in the ashen tadpole discloses the following points which may be briefly summarized:

(1) The free epidermal melanin is not changed.

(2) The epidermal melanophores apparently are not changed from their normal, expanded, dark condition.

(3) The deep melanophores are reversed from their normal expanded condition to one of very strong contraction.

(4) The xantholeucophores are not modified from their normal state of marked, pin-point contraction.

Pineal Gland and Hypophysis Removal. Removal of the pineal gland alone in the frog tadpole was found to produce no change in the depth of pigmentation. Epiphysectomy under chloretone anesthesia was performed when the larvae were about 8 mm. in length. It was found that previous to this stage the cavity of the pineal is in open communication with

the third ventricle. Consequently earlier removal of the gland results in a temporary cerebral fistula. Shortly before a length of 8 mm. is attained the gland closes off from the brain and, if care is exercised, it may be removed without perforating the brain wall. Such an accident rarely proved fatal, however. Epiphysectomized tadpoles responded to treatment with beef pineal quite as readily as the unoperated.

Having in mind the effect of pineal solutions on the pigmentation of normal larvae, it was assumed, subject to experimental determination, that perhaps the main factor in the production of the silvery tadpole following hypophysectomy was an unrestrained activity of the pineal. It was held that if this be true removal of the pineal, as well as the hypophysis, would prevent the characteristic silvery condition of the hypophysis-free animal. Accordingly the double operation was performed: first the removal of the hypophysis at the stage of 3.5 to 4 mm. and six or seven days later, when the animal was approximately 8 mm. in length, the removal of the pineal. The hypothesis on which the double operation was undertaken was entirely disproved, since these tadpoles turned silvery, as well as those which had undergone hypophysectomy alone.

Condition of the Chromatophores in the Silvery Tadpole. Repeated observations made on both living and fixed material confirm the previous observations of the writer as regards the condition of the melanophores in the silvery or hypophysectomized tadpole. The amount of pigment in the epidermal melanophores is reduced as is apparently also their actual number. Some of them are contracted, or at least the pigment material is concentrated in them. The pigment granules of the epithelial cells are reduced in number, as stated by Smith. The deep melanophores exhibit various stages of contraction. They are not so uniformly or so strongly contracted as in the case of the temporary transparency of the ashen larva (normal tadpole under influence of beef pineal).

In the silvery tadpole the xantholeucophores are readily seen to be so widely expanded that the neighboring cells are almost contiguous; in the normal they are contracted to minute points. That they do not mask entirely the deeper melanophores may be seen by reference to fig. 1, which was photo-

graphed from a living tadpole 15 mm. in length. Many of the melanophores may be seen as rounded black spots. These are particularly noticeable in the vicinity of the eye and at the root of the tail. The xantholeucophores appear in this figure as relatively large irregular white spots. Their arrangement in two layers becomes more distinct when the tadpole has at-

Fig. 1. Living, unanesthetized, silvery tadpole, 15 mm. in length, photographed through one tube of the binocular microscope. The deep melanophores, strongly contracted, may be seen as rounded black spots in the vicinity of the eye and at the root of the tail. The expanded xantholeucophores show as larger, irregular white spots.

tained a length of 25 to 30 mm. It is not so apparent in the younger stages.

Objection has been made to the tail as a site for study of pigment cells, on the ground that this region is atypical. It has been our observation that the midrib (not the fin) of the tail contains the different varieties of chromatophores found in the skin of other regions of the body, that they exist in the

same relative states of contraction or expansion, and respond, to certain stimuli at least, in the same manner as those of other parts of the body. True, they are not nearly so numerous nor so richly branched as on the dorsum of the body, but this is an advantage rather than otherwise, especially in observations with high power objectives. Figure 2 represents a photomicrograph, taken by reflected light, from the midrib of the tail of a living silvery tadpole while held in a Clark chamber and immobilized by a 1:5000 solution of chloretone. Previous observations had shown that chloretone of itself does

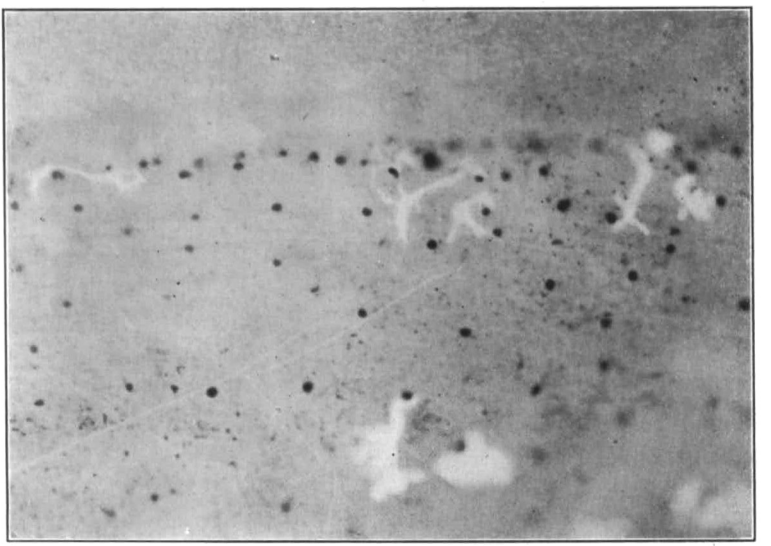

Fig. 2. An area from the mid-rib of the tail of a 16 mm. silvery tadpole, held in the Clark chamber under chloretone anesthesia; photographed with 16 mm. objective and reflected light. Deep melanophores, contracted; xantholeucophores, expanded.

not bring about the contraction or the expansion of the chromatophores, nor prevent the reactions ordinarily obtained by the use of other agents. Figure 2 shows the deep melanophores and the xantholeucophores. The former are the more numerous and exist as dense rounded spots, showing marked contraction. The latter are relatively scanty and exhibit great expansion. Their processes are often more delicate than those found in the coresponding cells of the dorsal body region. From these observations, combined with the study of *toto* skin

mounts prepared after careful fixation, the condition of the various types of pigment cells in the silvery tadpole may be summarized as follows:

(1) The free epidermal pigment is reduced in amount.

(2) The epidermal melanophores are reduced in number and apparently in pigment content. Contraction of the cells, or concentration of pigment in them, is often apparent.

(3) The deep melanophores are in various stages of contraction. They are not all in such complete contraction as may be induced by such agents as pineal gland solutions, for example, but they are definitely contracted when compared with the same cells in the normal dark tadpole.

(4) The xantholeucophores are widely expanded.

The Darkening of the Silvery Tadpole by Action of Pituitary Extracts. That the silvery tadpole may be made considerably darker for a short time by treatment with certain pituitary solutions was reported previously by the writer. Although denied by Smith at that time ('19) confirmation has recently been given by that author ('20). Further observations have verified our earlier results. It has been possible to obtain a darkening with pars intermedia substance of the beef hypophysis, with aqueous extracts of the posterior lobe prepared according to a method described elsewhere (Atwell and Marinus), and with 'oral' pituitrin (Parke, Davis and Co., serial number 2413685). Darkening did not result from dilute extracts of the anterior lobe of the beef hypophysis. It was sometimes obtained by the use of very strong anterior lobe extracts, but these proved rapidly fatal. It is possible that when the stronger extract was employed there was sufficient contamination from the posterior lobe to cause the effects seen. It was ascertained that when an appropriate strength of anterior lobe extract failed to produce darkening, the effect could always be produced by use of posterior lobe extract of the same strength. This seems to give support to the assumption that the posterior lobe is the part responsible for color changes in the silvery tadpole.

Critical observation of the living animal, unanesthetized, under the binocular miscroscope, shows that in the darkened tad-

pole the deeper melanophores are expanded. A limited, partial contraction of the widely expanded xantholeucophores has been observed in some cases. This has not in any case been great enough to be considered reciprocal to the expansion of the deep melanophores. In these temporary periods of darkening the epidermal pigment, both that found free in the epithelial cells and that contained in the epidermal melanophores, is not restored. Confirmatory results were obtained by examination of the living tadpole in the Clark chamber. It was possible to select a certain field and to study it while the animal was in the

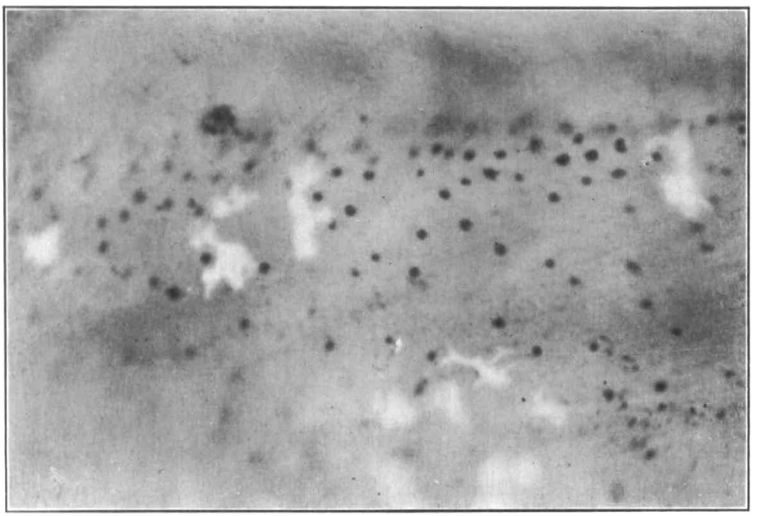

Fig. 3. From the mid-rib of tail of a silvery tadpole; same as figure 2.

silvery state and later when darkened by a 1:100 solution of 'oral' pituitrin. Photographs by both transmitted and reflected light were obtained. Transmitted light does not differentiate well the xantholeucophores from the melanophores, but reflected light brings out the two types of chromatophores in a beautiful manner. Photographs obtained with a 16 mm. objective, showing the same field before and after darkening, are presented in figures 3 and 4. The field chosen was from the midrib of the tail. Epidermal melanophores are not shown, being for the most part out of focus. In figure 3 the deep melano-

phores may be seen as numerous dense, rounded spots. The xantholeucophores are less numerous and are seen in the figure as widely expanded white cells. In figure 4, taken after the silvery tadpole had been darkened by 'oral' pituitrin, the same deep melanophores and xantholeucophores shown in figure 3 may be identified. The melanophores are seen to have expanded by sending out numerous processess. The xantholeucophores have changed but very little, although perhaps a slight degree of contraction may be noted. This is not marked enough to be considered reciprocal to the expansion of the melanophores. The condition of the several kinds of pigment cells in the silvery

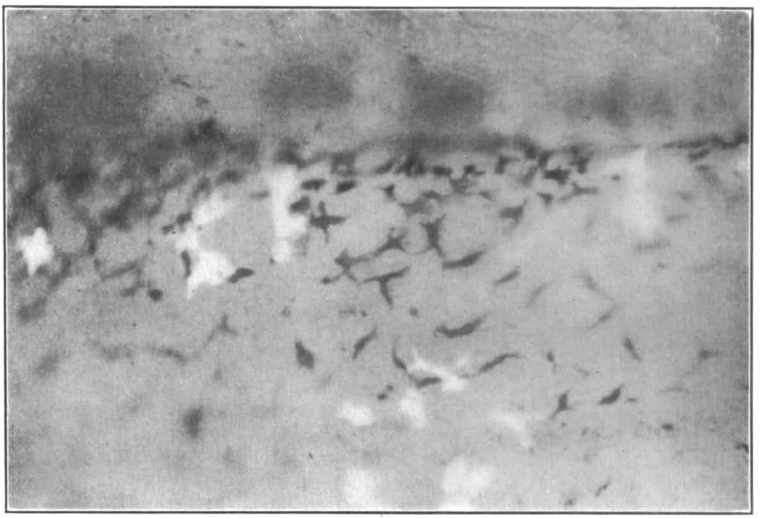

Fig. 4. Same field as in figure 3, showing change in pigment cells when animal is darkened by action of 'oral' pituitrin. Same cells shown in figure 3 may be identified. Deep melanophores are expanded; xantholeucophores not greatly changed.

larva which has been darkened by the action of pituitary extracts may be summarized briefly as follows:

(1) The free epidermal pigment is not restored.

(2) The epidermal melanophores are not restored.

(3) The deep melanophores are expanded, but ordinarily not so completely as in the normal untreated control.

(4) The xantholeucophores are perhaps somewhat contracted. This reaction is not so marked as the expansion of the melanophores.

DISCUSSION AND SUMMARY

It is evident that the appearance of a lighter color in the frog tadpole following removal of the epithelial hypophysis, is not a simple phenomenon. It is not due to the reaction of any one type of chromatophore alone. As concerns the melanin-bearing type of cell, of which there are two varieties, epidermal and deep, reduction and contraction (or concentration) occurs in the former and contraction in the latter. It is also to be noted that reduction of the amount of 'free' melanin in the epithelial cells takes place. The light-colored pigment cells or xantholeucophores become widely expanded.

In attempting to evaluate the roles of the different chromatophores in the causation of the characteristic appearance of the hypophysis-free tadpole, experimental methods have been of much value. It has been shown that when the normal dark tadpole is treated with a pineal solution the principal reaction is a contraction of the deep melanophores. The animal in this condition presents a light colored, semitransparent appearance which has been described as *ashen*. The silvery tadpole, in addition to being light colored, possesses a characteristic metallic lustre. This latter feature is due, without doubt, to the expansion of the xantholeucophores which may be observed in the silvery but not in the ashen tadpole. That the condition of the deep melanophores is of importance is amply shown by the fact that these cells are not by any means completely obscured by the expanded xantholeucophores. If the deep melanophores were not contracted the silvery tadpole would be considerably darker. In fact just such a darkening takes place when posterior pituitary extract is used. The principal reaction is found to be an expansion of the deep melanophores. The writer cannot agree with Smith that the xantholeucophores contract sufficiently to be largely responsible for the darkened appearance.

The reason why the darkened silvery tadpole is not so dark as the unoperated control is the fact that the xantholeucophores do not markedly contract, as well as the fact that the epidermal melanin is not restored in so short a time. Authors are pretty well agreed that the xantholeucophores are more refractory to the action of agents than are the melanophores. This is clearly shown by the use of pineal substance. Furthermore, the long-

continued feeding of silvery tadpoles with posterior lobe substance results in the restoration of the epidermal melanin but does not produce contraction of the xantholeucophores (Smith).

CONCLUSIONS

1. The silvery tadpole is light-colored because of the condition of its melanophores, and lustrous because of the condition of its xantholeucophores. The light color is due to the fact that the deep melanophores are contracted and the epidermal melanophores and free melanin are reduced in amount. The metallic silvery appearance is due to a wide expansion of the xantholeucophores.

2. When the silvery tadpole is temporarily darkened by the action of posterior lobe extracts the deep melanophores expand. There is in some cases a slight contraction of the xantholeucophores, but this is never complete. The epidermal melanin is not restored.

3. Removal of the pineal gland alone does not produce a pigmentary disturbance in the frog tadpole.

4. Removal of the pineal gland soon after the removal of the epithelial hypophysis in the frog tadpole does not prevent the silvery reaction characteristic for the removal of the hypophysis alone.

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