(protein). With diarrhea sulfur does not appear to be quite as well absorbed as the nitrogen. Although normally very little chloride is eliminated by the intestine, the amount found in the stools may be considerably increased in diarrhea. About one third of the total phosphorus output of the intestine and kidney is found in the stools. The percentage output in the feces of both calcium and magnesium is high, due, as we believe, to the lacto-vegetarian diet, which resulted in a poor absorption of compounds of these elements. As might be anticipated from our knowledge of potassium salts, a very appreciable amount of this element is eliminated in the feces, and diarrhea considerably accentuates this elimination. In general it may be said regarding the intestinal diarrhea, that, although it very definitely reduces the absorption of nitrogen, sulfur, chlorine and potassium, it appears to be almost without influence on the phosphorus, calcium and magnesium.

43 (1418)

The pigment changes in frog larvæ deprived of the epithelial hypophysis.

By **P. E. SMITH** (by invitation).

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It has been known for a considerable time that in the fishes, amphibia and reptilia remarkable changes in color pattern or external appearance occur. It is also generally known that these effects are due to the reciprocal interplay of alterations in at least two great systems of pigment-bearing cells, the bearers of dark pigment (melanophores) and the bearers of various other pigments, many of them metallic lustered (xantholeucophores). To the latter class of pigments the trout owes its silvery appearance.

Somewhat over two years ago the author, followed shortly by B. M. Allen, showed that peculiar silvery frog larvæ were invariably produced when the epithelial portion of the hypophysis was removed in early embryonic stages. For the sake of brevity these individuals were designated "albinos" and are always in conspicuous contrast to the darker, normal specimens. As might

be expected from our introductory remarks, both of the great groups of pigment-bearing cells contribute to produce this strange effect; but no mention of the iridescent cells (the xantholeucophores) was made by the writer at the time of his early communications on this subject[1] and it is noteworthy that in both of the subsequent papers by B. M. Allen[2] and in the recent communication by W. J. Atwell[3] these cells have received no attention whatever.[4]

It can be demonstrated easily that the silvery, or albinous, condition, as is the case in so many instances of the color change in animals, is participated in by both groups of cells, melanophores and xantholeucophores. Furthermore, it is indeed a fact that under normal conditions no change in the condition of one of these sets of cells takes place without a reciprocal alteration in the other. This conception was forced home to the writer not merely by a reëxamination of the anatomical causes for the albinos, but by extensive physiological and pharmacological experiments on normal and albinous specimens. It is also a fact that pigment changes, other than those associated with true pigment cells, have escaped the notice of most observers, although the writer demonstrated and commented upon the conspicuous reduction of the superficial "free" pigment possessed by the epithelium at the time of his communication to the American Association of Anatomists in December, 1917. Since Atwell has raised the whole

[1] "Experimental Ablation of the Hypophysis in the Frog Embryo," *Science*, N. S., vol. 44, no. 1130, August 25, 1916 and "The Effect of Hypophysectomy in the Early Embryo upon the Growth and Development of the Frog," *Anatomical Record*, vol. 11, October, 1916.

[2] "Extirpation Experiments in *Rana pipiens* Larvæ," *Science*, vol. 44, November 24, 1916, and "Effects of the Extirpation of the Anterior Lobe of the Hypophysis of *Rana pipiens*," *Biological Bulletin*, vol. 32, no. 3, March, 1917.

[3] "On the Nature of the Pigmentation Changes Following Hypophysectomy in the Frog Larvæ," *Science*, N. S., vol. 49, No. 1254, January 10, 1919.

[4] That a satisfactory analysis of the condition of these cells has not hitherto been made may perhaps be attributable to two causes, first, the fact that the tail is atypical in this respect, and it would appear that Atwell on account of the advantage of employing Clark's beautiful mechanism for observing the living tail has paid too much attention to this locality; secondly, the fact that the xantholeucophores lose their pigment content and are hence impossible to detect in sections after many fixing fluids. Formalin, Zenker's fluid and Bouin's fluid, for instance often produce this effect after a short interval, although Helly's fluid and some other chrome mixtures fortunately preserve them.

question in his recent paper, it seems desirable, in advance of a more extensive presentation, to describe briefly the various anatomical findings which it can now be stated underlie the albinous condition.

The study of great numbers of such larvæ produced by experiment during the last three years, conducted, it is to be emphasized, both on living and on appropriately fixed specimens, has given uniformly concurrent testimony that these hypophysis-free albinos are produced by three chief alterations of the pigment mechanism. These may be enumerated as, (1) Reduction in the system of epidermal melanophores, consisting of greatly lessened numbers of these cells and in the contracted and pigment-poor condition of those cells which are present; (2) a marked reduction in the number of so-called free pigment granules of melanin in the epithelium; (3) an invariable expansion of the xantholeucophores situated in both deep and superficial strata of the dorsum of both head and body.

The condition of the melanophores is especially interesting, due to Atwell's contention that previous investigators have overlooked what he feels to be the major contribution towards the albinism made by a contraction of the subepidermal melanophores. Atwell bases his contention on three lines of evidence: first, an increase in the pigmentation, producing almost a normal depth of color, when albinous larvæ are treated with a solution of dried pars intermedia substance; secondly, the preparation of many whole mounts of the skin of albinous larvæ in which he claims to have discovered an invariable contraction of the deep melanophores; thirdly, observations on the living tail fin of albinous larvæ treated with an extract of pars intermedia where an expansion of these cells was observed. It is only fair to state that full admission is made of the reduction of the epidermal melanophores, though this is rated as of secondary importance. Albinous larvæ have never in the hands of the writer been appreciably increased in the depth of pigmentation even with the use of the one procedure most potent in expanding the subepidermal melanophores—the sunlight. Moreover, it is extremely difficult to understand Atwell's contention that the contraction of these cells is the main cause of albinism when we are confronted with the anatomical

relationships involved in all the main body area, aside from the ventral region, for a layer of xantholeucophores intervenes between the epidermis and the subepidermal melanophores, a layer which in the albino so completely screens off the subepidermal melanophores as to make their observation in life extremely difficult. It will readily be understood that this effect is greatly emphasized by the great expansion of the xantholeucophores which the author has invariably found in albinism.

As regards the second point, it is necessary to remark that every precaution must be taken (temperature, background, speed of fixation, etc.) in the preparation of material for study in order to justify any certainty that the condition of the pigment cells has not been changed by a complicating extraneous factor. Comment has already been made on the impropriety of applying observations made on the tail fin to the very differently constituted skin of the dorsum of the body. Moreover, it is not to be wondered at that an expansion of the subepidermal melanophores could be observed after the application of pars intermedia extract to animals in a Clark chamber where the strong illumination is probably alone sufficient to produce this result.

In the experience of the writer no constant deviation exists from the various states of relaxation or expansion which may occur normally in the subepidermal or deeper melanophores of albinos as contrasted with normal larvæ.

There is no need to comment on the writer's contention that the epidermally situated melanophores of albinos are greatly at fault; both subsequent observers have confirmed him in the great reduction of the number of these cells; and Atwell has observed the reduction in the pigment content of the remainder, which appeared to have been denied by Allen. Though we have felt free to contest the view of Allen that a contraction pertains in the deep melanophores of the body, corroboration must be given to his views as pertains to the physiological state of the epidermal melanophores. The epidermal melanophores are scanty in number, reduced in pigment content, and most of them exhibit varying degrees of contraction.

As regards the great reduction in the so-called free pigment in the superficial layer of the epidermis, the writer wishes to reiterate

his statements made in 1916. While it is impossible to attribute more than a minor rôle to this, nevertheless, it is just as striking and constant as the other pigment effects. This alteration in the epithelial "free" pigment would appear to have escaped detection along with the decided changes in the xantholeucophore cells. Attention has already been called to the solvent action of many fixatives on these cells. Their identification is not interfered with by the use of Helly's fluid and is rendered easiest of all when whole mounts preserved in this way are explored with the polariscope with which the doubly refractive powers of the guanin substance is brought out. It is proper here to call attention to the fact that the maximal expansion which these cells enjoy in albinous larvæ can also be overlooked because of a subsequent contraction in them which occurs with the use of anesthetics and in many conditions of impaired vitality occurring through disease or intentionally experimentally produced as with too strong doses of adrenalin. In another place comment will be made on the change in the physiological and pharmacological reactions of both types of pigment cells; but it may be stated here that the widely expanded xantholeucophores of albinos are singularly unamenable to most experimental influences and in this they are in striking contrast to the iridescent cells of normal animals. That the expanded xantholeucophores contribute decidedly to the albinous appearance is shown by the behavior of those albinous larvæ fed on posterior lobe substance. These animals exhibit a partial recovery of the melanin deficiency which may indeed approach the normal. In spite of this, they are always conspicuously lighter than their normal controls, a fact readily explainable by failure of this treatment to influence the persistent expansion of the iridescent cells.

44 (1419)

On the reaction of the pigment cells in normal and albinous frog larvæ.

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In the experimentally produced albinous frog larvæ which follow a successful early extirpation of the epithelial portion of