THE PIGMENTARY EFFECTOR SYSTEM IV.—A FURTHER CONTRIBUTION TO THE RÔLE OF PITUITARY SECRETION IN AMPHIBIAN COLOUR RESPONSE.*

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1. Introduction.

In a series of papers (Hogben and Winton, 1922-23) already published, evidence has been presented in favour of an interpretation of the regulation of colour response in Amphibia by the secretory activity of the pituitary gland. Previous con-

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tributions were based exclusively on the study of the common frog (Rana temporaria): the salient conclusions arrived at were as follows:—

(1) The synchronous pigmentary changes displayed by the common frog in consequence of the reciprocal "expansion" and "contraction" of the melanophores and xantholeucophores are conditioned by a variety of natural stimuli of which temperature, humidity, and illumination are the most significant: warmth, dryness, and bright light promoting the pallor associated with melanophore contraction, while cold, moisture, and shade induce the darkening of the skin which results from melanophore "expansion."

(2) Extracts of the pituitary gland of Mammals, Birds, Reptiles, Amphibia, and Fishes have a specific local action on the melanophores of the frog inducing the expanded condition; the reaction is an extremely delicate indicator of pituitary extract.

(3) While removal of the anterior lobe of the pituitary gland in the frog does not interrupt the normal sequence of pigmentary response to natural factors, frogs remain after removal of the whole gland uniformly pale with maximal contraction of the melanophores and complete expansion of the xantholeucophores in natural conditions, which invariably induce darkening of the skin in controls. After injection of pituitary extracts the characteristic melanophore expansion is evoked in hypophysectomised frogs as in normal pale individuals; but the former when subjected to such treatment regain pallor even when exposed to surroundings which represent the optimum conditions for pallor in normal frogs.

(4) The colour responses of the frog are gradual, requiring periods from an hour to several days to reach completion, a fact which converges with the negative effects of nerve section and stimulation, and with the action of drugs on the hypophysectomised animal to the conclusion that the rhythm of pigmentary change is regulated by endocrine rather than nervous influences.

Observations essentially confirmatory of the above but not yet published in full are mentioned by Krogh in his recent monograph on the Capillaries (1922). In this paper the
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attempt will be made to extend such conclusions to other Amphibia, and to supplement previous observations on minor issues which seemed to merit further investigation.

Acknowledgment is made to the trustees of the Dixon Fund for a grant in aid of the expenses incurred in this research.

2. The Bionomic Aspect of Amphibian Colour Response.

In setting forth an interpretation of colour response in the Amphibia as a class, brief attention is due to the bionomic aspect of the problem. The subject has been reviewed so thoroughly by Fuchs (1914) that there is, however, only need to refer to more recent evidence and such data as have emerged from the author's own observations.

(a) The Pigmentary Effector System of Amphibia.—As far as is known the cutaneous pigmentary effectors of the Amphibia consist of three practically ubiquitous types. These are (a) epidermal melanophores, (b) dermal melanophores, and (c) dermal xanthophores (xantholeucophores). In various species one or other of the above have been described as having lost the power of reacting by visible change; but this fixity may be due to the nature of the internal environment. Thus the melanophores of old black axolotls are always expanded in nature; but, as will be seen, the effects of hypophysectomy show that this state of affairs is not due to any inability to contract. In addition to the cutaneous pigmentary organs enumerated above there are other pigment effectors of more deep-seated situation, namely (a) the retinal pigment cells, and (b) the internal melanophores of many connective tissues, lining the peritoneum and lymph spaces. Except when otherwise stated subsequent remarks apply only to the cutaneous, more especially the dermal (or corial) melanophores, whose activity constitutes the dominant factor in bodily colour changes among Amphibia.

The exact manner in which the melanophores "contract" and "expand" has been the subject of much controversy. Some workers following v. Wittich (1854), Bimmermann (1878), Carnot (1896) hold that the melanophores are amœboid, contracting bodily. This view has been advocated recently
by Hooker (1914). Others following Lister (1858), Müller (1860), Kahn and Lieben (1907), Dawson (1920), affirm that the cell processes are not withdrawn in the contracted phase, which results purely from a migration of pigment granules from the cell processes into the central mass. The latter view claims more support from recent investigators. I have examined preparations of frog’s skin with melanophores contracted by the methods of Cajal, da Fano, and Golgi, and have never obtained clear evidence that the “cell processes” are not in reality preformed intracellular canaliculæ into which pseudopodia are protruded, as Hooker maintains, during expansion. But in all living and fixed preparations of the skin of the frog and axolotl, I have repeatedly found isolated clumps of granules left behind in the “cell processes” after the majority are seen to be aggregated in the central mass. Such appearances are not readily explicable on any supposition, but that contraction is due to a streaming of granules within cell processes which themselves remain static. Nevertheless, while inclining to the view that melanophores do not contract and expand sensu stricto, one may employ these terms as sanctioned by usage, without prejudice to the question which has been discussed, to describe the appearances respectively associated with pallor and darkening of the skin in Amphibia.

(b) Reactions of Melanophores to Natural Stimuli.—One of the most important factors involved in colour response in adult Amphibia is moisture. This of course does not enter into the responses of larval and aquatic forms as a significant element in the normal rhythm of pigmentary change. It seems to be generally agreed that humidity promotes darkening of the skin in adult Anura, and my own observations indicate that, in nature, moisture is the predominant factor which determines colour change, since, except at ordinary temperatures, light and darkness have no effect on frogs placed in dry surroundings. With the exception of Dawson’s observations on Necturus (1920), it is universally affirmed that warmth promotes the contracted phase and cold tends to induce darkening of the skin both in adult Anura (v. Wittich, Biedermann, Lieben, Hargitt, et al.), larval Anura (Cole, 1922), and adult Urodela (Flemming, Laurens). Another factor worthy of mention is
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the oxygen content of the surrounding atmosphere. It is generally agreed that oxygen deficiency induces darkening, and my own observations on the frog agree in this respect with those of others. In most adult Amphibia, a notable exception being Necturus (Dawson), light, if it has any affect, promotes the contracted phase, darkness or dull background the expanded. I am inclined to believe that the distinction between background and shade is greatly overstressed by some writers. The response appears to be under visual control both in adult (Lister, Rogers) and larval (Babak, Laurens) Amphibia. Anuran tadpoles with the exception of Rana pipiens (Hooker) react in the same way as the adult by contraction in strong light and expansion in darkness. In Urodele larvae (Laurens) there is a primary response to light by expansion and to darkness by contraction, followed after prolonged exposure by the more usual reaction of pallor in the presence and darkening in the absence of light. It is this secondary response which is abolished by removal of the eye and section of the optic nerves.

In order to make clear the precautions necessary in controlling experiments on colour response, reference may be made to the table given in the preceding paper of this series. My experiments on frogs and toads were performed by means of glass containers suitable to contain a single individual, and covered at the top with muslin held by a rubber band so that free interchange with the surrounding atmosphere was available, and any danger of vitiating the experiment by alteration in the gaseous content of the inspired air was avoided. Such containers were placed in a cold room and fitted with envelopes of black paper and moistened with addition of about 2 cm. depth of water to reproduce optimum conditions for darkening; or they were placed in a warm room, thoroughly dried and well illumined on a white background, when it was desired to reproduce optimum conditions for pallor. From 6° to 10° C. is a good "low" temperature, and 20° to 24° C. a suitable "warmth."

I have carried out experiments with normal and blinded frogs and find that, as would be expected, blind frogs react like the seeing animal independently of light in warm dry
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or cold wet situations. However, placed in water against a pure white background under bright illumination at moderate temperature 12° to 15° C., the mode of response in the blinded and seeing frogs is sharply contrasted: for the dermal melanophores of the latter expand, while those of the normal animal contract. My own observations on the adult frog are in agreement with those of Rogers (1906) on the adult Salamander, namely, that in the presence of moisture, within the limiting range of temperature for which light is a significant factor, the dermal melanophores of normal frogs contract in bright light, whereas those of the blinded frog remain permanently expanded in light or shade alike. "Adaptation to background" is thus under control of the organs of vision in Amphibia as in Fishes.

(c) Post-mortem Condition.—It is here necessary to add a further comment on the post-mortem condition of the melanophore, which is assumed by many other authors, following Lister, to be that of contraction. This is not an accurate statement of the case. It is true that in general melanophores are found contracted in dead frogs, the reason being that in live frogs the melanophores always tend, unless the illumination is very bright and the surroundings very dry, to be a little expanded. After observing the post-mortem condition of several hundred frogs exposed to optimum conditions for darkening or pallor, I can confidently affirm that melanophores
always tend to respond in the opposite sense to the condition prevailing at the time of death. If very pale animals die the melanophores invariably display partial expansion as the moribund state becomes advanced. I mention this fact because it seems to invalidate the method recently adopted by Uyeno (1922) for studying the action of CO₂ and oxygen upon the melanophores of the isolated limb.

(d) Colour Change in the Breeding Season.—It has long been noticed that an intense darkening of the skin is characteristic of the frog in the breeding season. I have pointed out elsewhere that there is no need to postulate any direct connection between this phenomenon and the physiological condition incident to reproduction, since the breeding season, when pairing takes place round the shady margins of muddy ponds at a comparatively low temperature, is pre-eminently the time when large numbers of frogs can be seen in nature subject to the optimum stimuli for melanophore expansion—moisture-cold, shade. This season I separated four pairs of copulating frogs in a white tank in bright light from the stock which were kept in shade on a black background, with the result that complete contraction of the dermal melanophores of the former ensued within forty-eight hours. It is a fact of common observation that during copulation the female is paler than the male. Examination of the skin microscopically shows, however, that the melanophores are equally expanded in normal conditions in both sexes, so that there would appear to be a secondary sexual distinction in the number of melanophores per unit area in the two sexes, an observation which I have not seen recorded elsewhere.

(e) Time Relations in Colour Response among Amphibia.—It is a remarkable fact that the extremely protracted latent period which intervenes between the application of the appropriate stimuli and the production of a particular colour change in consequence, has been studied by few investigators with great care or precision. Had more attention been paid to this aspect of the problem, it can hardly be doubted that the search for an alternative to nervous control would have been made at an earlier stage. In my experience darkening and pallor in response to the optimum conditions is never complete in
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frogs before at least one and a half hours, and more usually six to twenty-four hours have elapsed. The only exact observations I have been able to find are those of Laurens (1915) on Urodèle larvae. These are worth quoting as well to emphasise an important aspect of the problem which confronts us at a later stage. The following quotation is taken from Laurens's paper on Amblystoma larvae:

"1. Expansion of the melanophores of seeing larvae in the light one and a half to two hours.

2. Expansion of the melanophores of eyeless larvae in the light two to three hours, and contraction of the melanophores of seeing larvae in darkness two to three hours.

3. Contraction of the melanophores of eyeless larvae in darkness four to five hours.

4. 'Secondary' contraction of the melanophores of seeing larvae in the light three to five days, and 'secondary' expansion of the melanophores of seeing larvae in darkness five days or more."

Laurens does not give the temperature conditions to which such periods are subject.

3. Further Experiments on the Specificity and Localisation of the Melanophore Stimulant of Pituitary Extract

(a) Specificity.—As the excitatory action of pituitary extract upon the melanophores of the frog (or toad) is an eminently suitable subject for class demonstration, it is perhaps advisable to take this opportunity of insisting upon the elementary condition that to demonstrate the expansion of the melanophores it is first necessary that they should be in the contracted phase; *i.e.* experiments should only be performed on frogs kept in separate aerated containers (p. 253) in bright, warm, dry surroundings for several days before experiment, and, in any case, the web should always be examined under the low power of a microscope to ascertain whether the pigment cells are fully contracted initially.

The two following experiments were carried out to test the reactivity of the toad's skin to pituitary extract. Toads are perhaps more erratic in their pigmentary reactions than frogs,
but prolonged exposure—a week, for instance—to optimum conditions for pallor (bright illumination, warmth, and dryness) or darkening of the skin (shade, cold, moisture) displays as sharp a contrast between the extreme conditions which, as in the frog, are respectively buff or lemon yellow at one end of the scale and a fuscous grey or coal-black at the other.  

(i) Four pale toads (*Bufo vulgaris*) previously kept for twenty-four hours in dry well-illumined jars at 22° C. were injected into the peritoneum with 0.5 c.c. frog's Ringer solution. Four pale toads similarly treated received by intra-peritoneal injection 0.5 c.c. of a 1 per cent. boiled extract of ox pituitary (posterior lobe). At the end of an hour the melanophores were completely expanded in the latter series, the toads being dark grey to black in appearance. The others remained pale. The darkening of the pituitary series lasted twelve hours.  

(ii) Ten male toads of yellowish aspect were injected into the peritoneum as follows: Nos. 1 to 5 with 0.5 c.c. 1 per cent. (ox) pituitary extract; No. 6 with 0.5 c.c. saline; and Nos. 7, 8, 9, 10 respectively with 0.5 c.c. 1 per cent. extract of brain, spleen, liver, and salivary glands in Ringer (boiled). At the end of an hour Nos. 1 to 5 were darker than the rest which remained pale, while the pituitary series assumed maximum darkening of the skin within three hours from injection.  

The last experiment supplements previous observations on the tissue specificity of the pituitary melanophore response. The following additional experiment with frogs may also be included in this connection.  

(iii) A male rabbit was decapitated, boiled extracts as before being made of brain, spleen, liver, testis, and pituitary ground up with sterile sand in Ringer's solution. Eight pale frogs were respectively injected in pairs with 0.5 c.c. of a 1 per cent. and a 0.1 per cent. extract of brain, spleen, liver, and testis by intraperitoneal admission. Another pair were injected respectively with 0.5 c.c. of a 0.1 per cent. and 0.01 per cent. extract of the pituitary. After half an hour the characteristic darkening occurred in the last named pair, the others remaining pale.  

*6 Localisation of the Melanophore Stimulant.*—Many of the experiments in earlier contributions were carried out with Messrs Burroughs Wellcome's standardised liquid sterile extract.
of ox posterior lobe ("infundin"). In an experiment previously recorded no appreciable melanophore activity of anterior as compared with posterior lobe extracts of the mammalian gland was found. Since then I have made several tests, though difficulties in securing the glands within three hours of killing makes it difficult to interpret the results with confidence. In the following experiment the glands were removed from their capsules and immersed in acetone within twenty minutes of killing. By this means they are reduced to a leathery consistency within half an hour of immersion in the dehydrating fluid, so that the pars anterior, intermedia, and nervosa can be readily dissected without the contamination inevitably resulting from their viscous texture in the fresh gland. Portions of the three regions (pars anterior, intermedia, and nervosa) were separated from half a dozen pituitaries so treated, dried and weighed, boiled with a known volume of Ringer's fluid for testing. Three series each consisting of six pale frogs

<table>
<thead>
<tr>
<th>Amount of dried tissue in grams</th>
<th>Series I. (Pars anterior)</th>
<th>Series II. (Pars intermedia)</th>
<th>Series III. (Pars nervosa)</th>
</tr>
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<tbody>
<tr>
<td>0.01</td>
<td>Black Melanophores reticulate</td>
<td>...</td>
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<tr>
<td>0.005</td>
<td>Black Reticulate</td>
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<td>0.000025</td>
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<td>Pale Slightly stellate</td>
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weighing 17 to 20 gms. were used for injection of doses corresponding to different quantities of the three extracts. The melanophores of the web were in each case completely contracted at the beginning of the experiment. The gross and microscopic appearances exhibited three hours after injection are set forth in the table on preceding page.

A second experiment with different samples on the same three series of frogs gave comparable results to those set forth in this table. From this it would seem that while there is more of the melanophore stimulant in extracts of mammalian pars intermedia, it is by no means lacking from preparations of the anterior lobe. The melanophore test could not therefore be legitimately used, as it was at first hoped, to detect the presence of posterior lobe secretions in anterior lobe extracts.

The bearing of such experiments on the question of the localisation of the pituitary secretions is one which cannot be discussed profitably until comparative tests of the pressor, oxytocic, and melanophore activities of different samples have been carried out, as is intended, simultaneously. It must be remembered that the secretory products may not improbably diffuse with comparative facility from one region to another, and a relatively higher activity of extracts prepared from a given region does not necessarily imply that such a portion represents the seat of origin of the active substance. It is by implantation and extirpation rather than the administration of extracts that the localisation of the active constituents of pituitary preparations will be understood. Removal of the anterior lobe of the frog as recorded in the third paper of this series (Hogben and Winton, 1923), in agreement with the unpublished experiments of Krogh and Rehberg (op. cit.) does not result in the condition of permanent pallor consequent upon complete hypophysectomy. The melanophore stimulant is not therefore uniquely confined to the anterior lobe. It may then be manufactured either by the posterior lobe, diffusing into the anterior portion, or, indifferently, by both parts of the gland. The latter supposition is not welcome from the standpoint of histology; and Swingle's (1921) experiments on the implantation of the various portions of the amphibian pituitary into Anuran larvae favours the former alternative, viz., that the
melanophore stimulant originates in the pars intermedia and diffuses thence into the other portions of the gland.

One other point is of sufficient importance to merit brief treatment in this place. If the melanophore stimulant is normally present in relatively large quantities in the anterior lobe, its relation to the pressor and oxytocic substances emerges in a new form. It has already been pointed out that there are several indications which militate against identifying the melanophore with the pressor principle. In the light of Herring's work these considerations are enhanced; and the identity of the melanophore and oxytocic substances which remained a more open question becomes less likely. However, this question requires simultaneous examination of the relative potencies of extracts from different regions by all three tests, and on the evidence, so far available, it is inadvisable to state more than a suggestion.

4. The Effect of Pituitary Removal and the Subsequent Administration of Pituitary Extract to Urodele Larvae.

In experiments upon the effects of pituitary injections on axolotls (*Amblystoma tigrinum*), it was noticed that such procedure is followed by a very noticeable intensification of the normal black body colour of the melanic variety, in which the melanophores of older individuals (one to four years) in normal conditions display a condition of stellate expansion giving place to the reticulate condition after treatment with pituitary extract. It seemed therefore likely that pituitary secretion might be involved in the regulation of colour response in Urodeles as well as in Anura. To test this possibility the effects of pituitary removal on the pigmentary responses of axolotls were investigated.

(a) Technique of Hypophysectomy in Urodea.—I have not been able to find any reference to previous attempts to remove the pituitary in Urodea, despite the relative facility of the operation on these animals. The gape of the mouth in axolotls is extremely propitious for this purpose. When it is distended by spring forceps with pointed extremities bent outwards, the pituitary is readily visible as a prominent white spot beneath the thin integument and translucent cartilage of the roof of
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the mouth. The morphological characteristics of the organ have been made the subject of a recent study by Atwell, to whose paper the reader may refer for further information.

Ether was found to be the most satisfactory anaesthetic. The axolotl is placed on its back and its mouth distended in the manner indicated. To penetrate the thin parasphenoid above the gland by means of a dental drill fitted with a rose-burr of suitable size is a practically instantaneous proceeding. The pituitary now exposed is loosened by means of a fine cataract needle, and a pipette attached to a filter pump applied to the aperture sucks up the gland leaving a clear view of the brain uninjured below. The whole operation does not require more than two minutes, when anaesthesia is accomplished. Axolotls recover from the anaesthetic best, if placed in running water of insufficient depth to cover the body. The wound heals rapidly.

(b) Effects of Pituitary Removal.—Twenty axolotls of the melanic variety ranging from nine months to four years in age were treated by the above procedure. The results of complete removal are uniform. There was no operative mortality, and since several are alive six months after treatment, one may infer that viability is not impaired. Removal of the pituitary in Amphibia as a class is not attended by the characteristically fatal disturbances incidental to the operation in Mammalia.

Twenty-four hours after operation the animals are visibly more pale. When several days had elapsed, they assumed an aspect never encountered to the writer’s knowledge on nature—certainly not in aquaria. They now presented a pale grey appearance reminiscent of Smith’s, Allen’s, and Atwell’s descriptions of the hypophysectomised tadpole. In this condition, which on microscopic examination was seen to result from complete contraction of the melanophores (fig. 2), the axolotls have remained for several months in the laboratory.

(c) Subsequent Injection of Pituitary Extract.—Introduction of a very small trace of pituitary extract into the peritoneum was followed in every case by a complete general expansion of the cutaneous melanophores, so that the operated animals regained the appearance of normal black axolotls. The effect is manifest two to four hours after injection, and may last for several days with sufficiently large doses. To make the
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experiment more rigorous, the entire series of experimental animals were injected tri-weekly for a month with amounts (0.2 c.c. of a 1 per cent. saline extract) large enough to maintain continuously the black colour associated with melanophore expansion throughout the period of injections. Within a few days after cessation of the treatment, however, the former pallor was re-established.

As recorded elsewhere (Hogben and Crew, 1923—this Journal), hypophysectomised axolotls were employed to test for the presence of physiologically active substance in the foetuses of sheep and oxen at different ages from fecundation. Since then I have had opportunity of testing—with positive results—for the presence of the melanophore stimulant in an extract made from the pituitary of an abortive human foetus in the fourth month.

(d) Regulation of the Chromatic Function in Urodela.—From the foregoing observations it may be inferred that pituitary secretion in Urodela as well as Anura constitutes an essential factor in the regulation of colour response. From the survey of the bionomics of amphibian response at the beginning of this paper, it seems legitimate to state that there are no established phenomena relative to pigmentary response in Anura that necessarily presuppose the existence of any auxiliary mechanism. One may provisionally suggest that in Urodeles as in Anura pituitary secretion is controlled by various agencies, including thermo-receptors, in the skin, and that it is reflexly inhibited by light acting on the retina. This would explain why in the Salamander *Diemyctilus* (Rogers)—within the range of external conditions for which light is a significant

Fig. 2.—Dermal melanophores of the black Axolotl, age two years: on the left normal individual, on the right hypophysectomised animal.
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factor—section of the optic nerves resulted in permanent expansion of the melanophores, though transection of the cord did not interrupt the normal rhythm of colour response.

However, the phenomena described by Laurens and summarised in para. 2, seems to indicate a more complex problem in the case of young salamander larvae. For in these continued illumination operates in the reverse way on blinded individuals, which, instead of remaining permanently dark, react to light by melanophore expansion and to darkness by pallor. Laurens was not aware of the significance of pituitary secretion, and there is no need, therefore, to criticise his hypothesis of nervous control (vide infra, 5). The reversal of response implies (a) a regulating mechanism subordinate to the organs of vision, which we are now justified in identifying—predominantly at least—with pituitary activity; (b) a mode of reaction depending on the effect of light on the skin. Laurens explains (b) as due to the direct reactivity of the melanophores to light. This, however, does not seem to harmonise with the permanent pallor resulting from pituitary removal. Moreover, there is another possibility. Parker (1904) has indicated considerations in favour of the view that Amphibia possess photo-receptors in the skin. A possible explanation of the paradoxical results of blinding in young axolotls might therefore be attempted by supposing that colour response in Urodeles depends on a balance between pituitary activity and some other mechanism (possibly adrenal secretion) which promotes melanophore contraction, adjusted in such a way that light acting prepotently on the retina inhibits the former and activates the latter, while light acting on photo-receptors on the skin activates the former and inhibits the latter.

5. Further Experiments on the Relation of Nerves to Amphibian Melanophores.

In an earlier contribution (Hogben and Winton, 1922) the negative results through stimulation and section of peripheral nerves were recorded in connection with the cutaneous melanophores of the frog. No attempt was then made to review the state of the evidence for the existence of nervous regulation in amphibian colour response. After referring to
two experiments which throw some additional light on the question, further attention may be devoted to this possibility.

(a) Effect of Transection of the C.N.S.—Concerning the consequence of spinal transection as with the results of peripheral stimulation, the testimony of different observers is discordant. Needless to say any manipulations involving transection of the C.N.S. must avoid injury or disturbance to the pituitary. In two pale frogs the C.N.S. was divided respectively immediately behind the optic lobes and the medulla, with utmost precaution to leave the pituitary untouched. For three days they were kept under observation in warm dry surroundings, the skin retaining its pallor consistently. The melanophores remained contracted till the animals were replaced in cold moist surroundings, when the body uniformly assumed the darkening characteristic of melanophore expansion. The accompanying text figure (fig. 3) shows the state of the melanophores twenty hours after operation.

(b) Effect of Severing Whole Nerve Supply to the Hind Limb.—In agreement with Hooker it was found (op. cit.) that section of the sciatic did not produce local melanophore expansion in the limb on which the operation was performed. Hooker (1912) however, states, "Severing the lumbo-sacral plexus or removal of the lower sympathetic ganglia does not affect the normal and synchronous change of colour in Rana fusca. When, however, both these processes are carried out together, the lower part is at first slower in reacting (italics inserted). . . . These results demonstrate that there are two nerve centres for the control of the melanophores. . . ." It is not easy to see why these results demonstrate any such thing; but it seemed desirable to repeat the double procedure. When this was done on a pale frog, kept under warm dry conditions, no difference between the two limbs was observed during a period of two days, after which the animal was placed in cold wet surroundings. The pallor of the operated limb was evident on the following day in contrast to the rest of the body. The contrast disappeared after three days. It was evident also that the leg was much swollen. Microscopic examination showed almost complete stasis of the blood in the much dilated capillaries of the web. This might be
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anticipated from Krogh's work on the sympathetic control of capillary tone. Krogh goes so far as to state all "agencies which I have enumerated as giving rise to capillary dilatation will also, when applied in sufficient strength, cause oedema, or stasis, or both." It is little cause for perplexity that the endocrine mechanism is prevented from exercising its normal efficiency when the channels through which it operates are suspended by the indirect action of nervous decontrol of the circulation.

It is not easy to prove a negative, and to deny that amphibian melanophores are subject to direct innervation would not be justified; but in the light of the new evidence one can approach the question without a strong predisposition in favour of the positive attitude. The problem has been investigated in the past by methods which fail to discriminate between direct nervous control of the melanophores and concomitant vasomotor consequences of the experimental procedure. And Spaeth, than whom no modern worker has insisted more strongly on the nervous control of colour response in fishes, admits freely that there exist "no satisfactory demonstrations of the nerve endings in melanophores of reptiles and amphibians." In no case have those who record positive results from nerve section and stimulation carried out their experiments with rigid controls under optimum external

\[\text{Fig. 3.—Melanophores of the web of a frog twenty hours after transection of the C.N.S. immediately behind the mid-brain without damage to the pituitary: optimum conditions for pallor.}\]
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conditions operating in the opposite sense to the recorded effects. In no case have photographs of such observations been published for the enlightenment of other investigators. Moreover, on no relevant points relating to nerve section and stimulation is there unanimity in the testimony of different observers. The only argument that can be adduced in favour of the positive view with general assent is that derived from the opposite effects produced on amphibian melanophores respectively by those drugs which stimulate or prevent nervous impulses reaching plain muscle supplied by sympathetic nerves. And judging from the effect of drugs on the hypophysectomised frog (The Pigmentary Effector System III.), it would appear that even if a nervous mechanism exists it plays no significant rôle in the rhythm of normal colour response in Amphibia. But the cogency of applying an analogy based purely on experiment with plain muscle may be seriously questioned.

6. The Chromatic Function in Other Vertebrates.

Apart from Amphibia two other classes of vertebrates exhibit colour response. Of these there seems to be accredited experimental evidence with indisputable histological support for the view that in Fishes at least nervous impulses play a significant rôle in regulating the chromatic function. However, v. Frisch (1912), one of the most recent investigators in this field, points out that the consequences of nerve section and stimulation are not such as to suggest that nervous control can adequately interpret the entire range of normal responses, but seem to imply some auxiliary mechanism, the nature of which for the present remains obscure. If, as Spaeth (1917) claims, pituitary extract induces contraction of fish melanophores, it is clear that the term comprises structures which display important differences in their physiological properties; and unless Spaeth's observations are discredited, one can only conclude that the regulatory mechanism of colour response in Fishes and Amphibia is fundamentally dissimilar.

It only remains to be asked, therefore, whether the interpretation advocated for the Amphibia in this series of contributions can be legitimately extended to the Reptilia. As in the case of Amphibia there are no data derived from microscopic
The Pigmentary Effector System IV

examination to suggest that the melanophores are directly innervated. Unfortunately there has been little recent physiological enquiry into the chromatic function of Reptiles in spite of the fact that the proverbial (and grossly exaggerated) reactions of the chameleon prompted many earlier investigations. From the important work of Redfield (1918) on the American lizard Phrynosoma, it would seem that the response to light depends on the direct reactivity of the melanophores themselves and is a local phenomenon, while the response to nocuous stimulation ("excitement pallor") is due to adrenal secretion. Redfield's experiments do not, however, provide any justification for the view that nervous influence plays a direct part in these proceedings, though the action of adrenalin appears to be local. Neither of the conclusions stated above suggest a close resemblance between the chromatic function in Reptiles and Amphibia. In order to test the action of pituitary extract on Reptilian chromatophores, eight chameleons were placed by the author in a warm darkened chamber for two hours, by which time maximum pallor ensued. Four were then injected each with 0.5 c.c. of 1 per cent. "infundin" (tested simultaneously on the frog). After half an hour, one hour, two and four hours had elapsed no change was visible. The injected chameleons remained as pale as the controls. The experiment was repeated with similar results. It does not appear, therefore, that Reptilian melanophores respond like those of Amphibia to pituitary extract.

That there should be a different mechanism of colour response in the three groups of vertebrates which display the chromatic function is not so surprising as it might appear at first sight. For while in all vertebrate groups the predominant rôle in effecting changes in bodily colouration is discharged by the black pigment cells, the number of cutaneous pigmentary effector organs (xanthophores, erythrophores, etc.) is fairly numerous. In the same animal, e.g. the frog, there are two types of pigmentary effector organs—xantholeucophores and melanophores—whose reactions are diametrically opposite in most respects. It is not more remarkable that the "melanophores" of Reptiles or Fishes should respond differently from those of Amphibia, than that the xantholeucophores of the frog
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should respond in a manner different from the reaction of the melanophores of the same species.

7. Summary.

1. The contracted melanophores of both Anura (frogs and toads) and Urodela (salamanders) react by maximal expansion to pituitary extract; the active substance in the latter does not appear to be confined invariably to the posterior lobe of the mammalian gland. A positive melanophore response was obtained from the gland of a human abortus in the fourth month.

2. The removal of the whole pituitary gland may be accomplished in Urodele larvae (Amblystoma tigrinum) at any age (nine months to four years) without impairing their viability. After complete removal of the pituitary in Urodele larvae, as in adult and larval Anura, the melanophores remain contracted and a state of permanent pallor ensues. The normal colour resulting from melanophore expansion can be re-established by injection of pituitary extract; but such animals regain pallor, although exposed to conditions in which melanophore expansion invariably occurs in normal individuals.

3. It is legitimate to conclude that pituitary secretion is the main factor in regulating the chromatic function throughout the Amphibia as a class. Fluctuating pituitary secretion in correlation with those conditions that evoke colour response in the frog (cf. the third paper of this series) provides a satisfactory basis for the interpretation of all the accredited bionomic data concerning colour response in adult Amphibia. Possibly adrenal secretion or some auxiliary mechanism plays a subsidiary part; but there are no satisfactory grounds for believing that nervous agencies directly influence amphibian melanophores; and there is reason to believe that even if amphibian melanophores are directly innervated, nervous control is not significant to the normal rhythm of colour change.

4. The study of amphibian colour response provides evidence not only of the presence of physiologically active substances in the pituitary, but functional activity of the gland in the intact animal. It does not appear, however, that the interpretation of colour response here put forward for Amphibia can be extended to Reptiles and Fishes.
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