LXIII. STUDIES ON THE PITUITARY. I.

THE MELANOPHORE STIMULANT IN POSTERIOR LOBE EXTRACTS.

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(Received July 4th, 1922.)

INTRODUCTION.

To the manifold physiological responses evoked by extracts of the posterior lobe of the pituitary gland, it has lately become necessary to add that of inducing pigmental changes in those lower vertebrates (Fishes, Reptiles, Amphibia) which possess considerable capacity for temporary modification of bodily colour through the activity of a special type of effector organ, the chromatophore (melanophores, etc.). Recent work on the part played by endocrine organs in amphibian metamorphosis has in particular brought to light a number of interesting data relative to the factors which control pigment response in these organisms. One of the most characteristic consequences of pituitary removal in tadpoles noted by Allen [1917] and others was a condition of extreme pallor resulting from general contraction of the dermal melanophores (black pigment cells). More recently Swingle [1921] has shown that implantation of the pituitary in pale tadpoles induces a darkening of the skin, a result shown to follow injection of posterior lobe extracts into adult frogs by the writers [1922]. The present communication attempts to explore the use of frog melanophores as indicators of pituitary extracts and to emphasise the remarkable specificity of this reaction. Few people are aware how marked is the capacity for pigmental change possessed by the common frog (*R. temporaria*), the same individual varying from a light yellow to a coal black or dark grey tint within comparatively short time limits: the difference is mainly due to the "expansion" and "contraction" of the dermal melanophores. In general between temperatures of 0-25° frogs placed in bright illumination are pale if kept dry whether on a light or dark background. They may become dark if placed on a dark background in water. For the study of pigmental darkening by pituitary injection, it is best to leave the animals singly in dry glass dipping jars for a few hours in bright light on a white background to ensure maximal contraction of the melanophores. Observations can be supplemented by microscopic preparations of skin, fixed in Bouin's fluid, dehydrated, cleared and mounted without more elaborate treatment. In earlier experiments [Hogben and Winton, 1922] decerebrated

animals were used. For injection Burroughs Wellcome's all glass tuberculin syringe graduated in 0.1 cc. was found to be most convenient: unless otherwise stated the intraperitoneal method was adopted.

In these experiments the frogs were stored in white porcelain tanks with glass tops, and were thus always ready for use. To facilitate observation individuals in which the skin is uniformly of a yellowish hue when pale, rather than the varieties with irregular markings, spotted or speckled aspect should be selected.

QUANTITATIVE EXPERIMENTS.

The following experiments were carried out to determine how far the melanophore response might be utilised to estimate the activity of posterior lobe extracts. The preparations employed were Burroughs Wellcome's 0.5 cc. phials of liquid sterile posterior lobe (21 %) extract ("infundin") stated to be standardised by action on the isolated mammalian uterus. Injection with 0.5 cc. of 1/1000 solution of this preparation invariably invoked the characteristic darkening of the skin, while 0.5 cc. of a 1/10,000 solution failed to do so in every case. The following preliminary experiments in which the animals employed were not weighed may be quoted to indicate the range of sensitivity of the frog's melanophores to pituitary extracts and the relative sensitivity with different methods of injection. The average temperature was 72° F.

Sample A. Three series of six frogs received an intraperitoneal injection equivalent to 0.00031, 0.00025, 0.00019, 0.00012, 0.00009 and 0.00006 cc. of infundin respectively. In two series the animals which received 0.00012 cc. and upwards underwent darkening of the skin and in the other series those which received 0.00019 cc. and upwards gave a positive reaction.

Sample B. Two series of six frogs were injected with 0.00025, 0.0002, 0.00015, 0.0001, 0.000075, 0.00005 cc. of infundin. In one series (intraperitoneal) the individuals which received 0.00015 cc. and upwards showed visible darkening of the skin accompanied, as seen on microscopic examination, with full expansion of the melanophores. The individual which received 0.0001 cc. displayed the melanophores in a slightly expanded (stellate) condition, while in the remaining frogs the melanophores were fully contracted. In the remaining series (dorsal lymph sac injection) microscopic examination revealed a precisely similar seriation, but the frog which received 0.0001 cc. showed a slight visible darkening which was not noticeable in the corresponding animal of the preceding group.

Sample C. Six frogs received an intraperitoneal and six an intravenous injection equivalent respectively to 0.0005, 0.0004, 0.0003, 0.00025, 0.0001 and 0.00005 cc. of B.W. liquid sterile extract. In both series the animals which received 0.0005, 0.0004 and 0.0003 cc. doses reacted with full expansion of the melanophores; a slight darkening of the frogs injected with 0.00025 and 0.0001 cc. was visible, but microscopic examination revealed the expansion of the melanophores in the former though not in the latter case. The animals injected with a dose of 0.00005 cc. did not react at all.

In these experiments summarised in Table I the frogs used were in no way selected, and it may be inferred that such methods are suitable for detecting the presence of posterior lobe extracts in quantities exceeding that equivalent to 5×10^{-5} g. of fresh gland (ox) substance, *i.e.* about 0.05 % of a clinical dose. In view of the relation between sensitivity and size indicated below it will be seen that by selecting sufficiently small individuals (or species of Amphibia), it should be possible to detect posterior lobe secretions in considerably smaller quantities than this and therefore in amounts that lie much beyond the limits of sensitivity of methods at present employed. We have found no evidence of a difference in the sensitivity of the reaction with the three methods of injection described.

Table I.

Sample	Method of injection	Minimal dose cc.	Subminimal dose cc.
A	Intraperitoneal	0.00012	0.00009
A	Intraperitoneal	0.00012	0.00009
A	Intraperitoneal	0.00019	0.00012
B	Intraperitoneal	0.00015	0.0001
B	Dorsal lymph sac	0.00015	0.0001
C	Intraperitoneal	0.00025	0.0001
C	Intravenous	0.00025	0.0001

Sample D. Two series, each of six frogs, were weighed and arranged in a heavy and a light series. Doses were injected so that pairs, one from each series, received the same dose per unit body weight—the results are shown in Table II, and demonstrate proportionality between body weight and the minimal effective dose.

Table II.

Approximate dose per 20 g. weight cc.	Heavy series			Light series		
	Wt. g.	Dose cc.	Response	Wt. g.	Dose cc.	Response
0.00025	31	0.00037	Darkening	16	0.0002	Darkening
0.0002	30	0.0003	"	15	0.00015	"
0.00015	25	0.0002	"	15	0.00011	"
0.00015	23	0.00018	"	14	0.0001	"
0.0001	23	0.00012	—	13	0.00007	—
0.00005	21	0.00005	—	12	0.00003	—

Sample E. (See Table III.) Eight frogs of 18–20 g. body weight received 0.0002, 0.00016, 0.00014, 0.00012, 0.0001, 0.00008, 0.00006, 0.00004 cc. infundin (intraperitoneal). The first five showed darkening: the rest remained pale, *i.e.* the minimal dose to produce maximal expansion of the melanophores lay between 0.0001 and 0.00008 cc. Six other frogs weighing 15–16 g. were injected *via* the peritoneum with 0.0002, 0.00015, 0.0001, 0.000075, 0.00005, 0.000025 cc. infundin. The first four only showed darkening of the skin, *i.e.* the minimal dose lay between 0.000075 and 0.00005 cc. A third set of eight frogs weighing 13–14 g. were tested from the same sample of infundin with the following doses (intraperitoneal): 0.0002, 0.00015, 0.0001, 0.00008, 0.00006, 0.00004,

0.00002, 0.00001 cc. The melanophores were fully expanded in the first three and contracted in the last three, while the frogs which received 0.00008 and 0.00006 cc. were found to display the pigment cells in a condition of partial expansion.

Sample F. (See Table III.) The following doses were administered to twelve individuals. A 1 (30 g.) and A 2 (25 g.), 0.00035 cc.; B 1 (30 g.) and B 2 (26 g.), 0.0003 cc.; C 1 (30 g.) and C 2 (26 g.), 0.00025 cc.; D 1 (29 g.) and D 2 (27 g.), 0.0002 cc.; E 1 (29 g.) and E 2 (28 g.), 0.00015 cc.; and F 1 (28 g.), and F 2 (28 g.), 0.0001 cc. infundin. The pairs A, B, C, D, uniformly displayed an intense darkening of the skin after half an hour had elapsed. The two pairs E and F on the other hand remained pale. This set of animals was deliberately chosen for large size; none of the females were carrying ripe eggs at the time.

The results obtained from the three foregoing experiments, although obtained with three different samples of "infundin," and therefore heterogeneous, present sufficient uniformity to be instructive in connection with the problem of what order of error is introduced in the method. It will be seen that the interval between minimal and subminimal doses, representing the margin of uncertainty, can usually be reduced to 25 %–30 % of the minimum effective dose; occasionally however, as in the third series of experiment (*E*), unsuitability of frogs may extend this even to 60 %.

That the simple correlation between body weight and minimal dose does not invariably obtain, is illustrated by a further experiment which was undertaken with a view to determining the sensitivity of the reaction to a dose injected about two hours subsequent to a previous dose in the neighbourhood of the minimal dose.

Sample G. (See Table III.) A preliminary experiment with four frogs showed that this sample was somewhat more potent than some used previously, and suggested the doses described. 32 frogs were weighed, and divided into four groups.

Eight frogs (9–11 g. body weight) were injected respectively with 0.0002, 0.00015, 0.000125, 0.0001, 0.000075, 0.00005, 0.000025, 0.00001 cc. infundin. Visible darkening of the skin occurred in the first five, the remaining three were unaffected. The minimal dose was in this case between 0.000075 and 0.00005 cc.

Eight frogs (12–13 g. body weight) were injected with 0.00025, 0.0002, 0.00015, 0.000125, 0.0001, 0.000075, 0.00005, 0.000025 cc. infundin. The first three only showed visible darkening of the skin. The minimal dose was thus between 0.00015 and 0.000125 cc.

Eight frogs (14–15 g. body weight) were injected with the same series of doses. The first four in this instance displayed visible pigmental change, the dose necessary being between 0.000125 and 0.0001 cc.

Eight frogs (17–19 g. body weight) were injected with 0.00035, 0.0003, 0.00025, 0.0002, 0.00015, 0.0001, 0.000075 and 0.00005 cc. The first three

only showed darkening of the skin, *i.e.* the minimum dose for this series was between 0.00025 and 0.0002.

Table III.

Sample	Wt. g.	Actual dose		Wt. g.	Dose per 20 g. body weight			Error in min. %
		Min. cc.	Submin. cc.		Mean min. cc.	Min. cc.	Submin. cc.	
<i>D</i>	23	0.00017	0.00012	23	0.00015	0.00015	0.0001	0
<i>D</i>	14	0.0001	0.00007	13				
<i>E</i>	18	0.0001	0.00008	20	0.00012	0.0001	0.00008	- 17
<i>E</i>	15	0.000075	0.00005	16		0.0001	0.00007	- 17
<i>E</i>	13	0.0001	0.00004	14		0.00015	0.00006	+ 25
<i>F</i>	29	0.0002	0.00015	29	0.000145	0.00014	0.0001	- 3
<i>F</i>	27	0.0002	0.00015	28		0.00015	0.00011	+ 3
<i>G</i>	19	0.00025	0.0002	17	0.000215	0.00025	0.00023	+ 14
<i>G</i>	15	0.000125	0.0001	14		0.00017	0.00015	- 21
<i>G</i>	12	0.00015	0.000125	12		0.00025	0.00021	+ 15
<i>G</i>	8	0.000075	0.00005	10		0.00019	0.0001	- 11
<i>G</i>	8	0.000075	0.00005	10		0.00019	0.0001	- 11
(repeat) <i>G</i>	17	0.0002	0.00015	18		0.00023	0.00017	+ 7
(repeat)								

Mean deviation 11.8 %.

Effect of subsequent doses. After injection with concentrated extracts the condition of melanophore expansion may last 12 hours or more. After injection with minimal doses it passes off in about $1\frac{3}{4}$ – $2\frac{1}{2}$ hours. After $1\frac{3}{4}$ hours following the first injection, the last series injected with sample *G* received corresponding doses of the same magnitude. The minimal dose was slightly lower, 0.0002–0.00015 cc. On injecting the first series two hours after the initial treatment the minimal dose was the same as before. It thus appears: first that given sufficient time for recovery the sensitiveness of the melanophores is not appreciably changed by previous treatment, second, that the effect of repeated doses is, in contrast with the action of the pressor principle [Howell, 1898], identical.

Like Dale and Laidlaw's original method¹ for estimating the uterine stimulant, this method is appropriate to comparison rather than absolute standardisation of extracts. Several factors which may lead to anomalous results might be considered, such as possible seasonal variation of sensitivity², and variations depending on the immediate previous history of the frogs with respect to moisture, temperature, etc. Again, different strains of frogs may respond with varying facility, and we have certainly noted that some are more and others less satisfactory in giving a sharply defined end point and well contrasted reaction. Furthermore, isolated frog's skin placed in Ringer shows the characteristic response to posterior lobe extracts, and may react with more constant sensitivity than an intact animal; these and other relevant considerations we have at present no opportunity to examine. The specificity,

¹ In Dale and Laidlaw's [1912] standard method for estimating the activity of pituitary extracts by their action on the isolated guinea pig's uterus, the minimal dose of infundin which produces a maximal contraction varies between .003–.005 cc. which is diluted to about 100 cc.

² Compare the striking variation in the electrical phenomena of frog's skin which occurs during the breeding season—[Bayliss and Bradford, 1886].

certainly and delicacy of the melanophore response will recommend it for such biochemical and pharmacological purposes as do not require a degree of accuracy higher than that we have described, namely consistency of minimal dose, to the extent of a mean deviation of 12 %. In each of the foregoing experiments the actual number of frogs used was the minimal number requisite for demonstrating the end point in a series. It is evident that in testing samples on a large scale the degree of activity might be compared with greater confidence by employing a larger number of individuals. In any case a simple and direct method is provided for testing the retention of activity of pituitary products employed in the dispensary or hospital.

SPECIFICITY OF THE MELANOPHORE STIMULANT.

Concerning the mode of action of the melanophore stimulant there is no need to add anything further in this place to our previous observations except to emphasise the local response obtained on isolated strips of skin placed in Ringer's extracts. It is our intention to publish in the near future a comprehensive account of the reactions of frog melanophores to drugs; but it is of special interest to insert at this point a few observations under this heading to lay stress on the highly specific nature of the response evoked in amphibian melanophores by posterior lobe extracts. The physiological effects hitherto described as characteristic of posterior lobe extracts are equally characteristic of a group of other well-known drugs. In the case of the melanophore reaction described in this communication we have to deal with a property of the extract which is not precisely simulated by any of the more familiar pharmacological reagents, as the accompanying table signifies. The case of digitalis merits special comment. The water-soluble digitalin (Parke Davis) was employed. In a dosage of 1 mg. death followed 30–45 minutes after injection *via* the dorsal lymph sac and no colour change resulted. 0.5 mg. was generally fatal after a few hours and accompanied in one case by a partial expansion of the melanophores. Doses of 0.3 mg. (*i.e.* one half the minimum lethal dose)

Table IV.

Pale frogs were injected with doses of the drugs undermentioned.

(a)	Pilocarpine	...	...	25 mg., 12.5 mg., 2.5 mg.	...	...	Remained pale
(b)	Atropine	...	...	10 mg., 3 mg., 1 mg.	...	...	"
(c)	Strychnine	...	...	1 mg., 1/25 mg., 1/100 mg.	...	...	"
(d)	Curare (decerebrated)	...	...	Curarised	...	...	"
(e)	Histamine	...	...	3.6 mg., .36 mg., .036 mg.	...	...	"
(f)	Ergatoxin	...	...	6.5 mg., .65 mg., .065 mg.	...	...	"
(g)	Tyramine	...	...	20 mg., 2 mg., .02 mg.	...	...	"
(h)	Veratrine	...	...	1/4 mg., 1/20 mg., 1/100 mg.	...	...	"
(i)	Digitalis	...	...	Numerous doses of varying range below M.L.D. (cf. text)	...	...	"
(j)	Barium chloride	...	...	1 mg., 1/5 mg.	...	...	"
(k)	Sodium nitrite	...	...	5 mg.	...	...	"
(l)	Caffeine	...	...	10 mg., 2 mg.	...	...	"
(m)	Apocodeine	...	...	{ 2.5 mg.—5 mg. without motor paralysis 10 mg. motor paralysis within 30 mins.	...	...	Slight darkening
(n)	Nicotine	...	...	{ 1/10 mg. no motor paralysis 1/5 mg.—1 mg. with motor paralysis	...	...	Remained pale Partial darkening

or less produced absolutely no effect on coloration. But no effect was obtained on the isolated skin. A partial expansion usually but not invariably occurs with nicotine and apocodeine only in dosage sufficient to produce motor paralysis without immediate lethal effects. In none of these cases is the reaction readily comparable with the pituitary reaction.

SOME CHEMICAL PROPERTIES OF THE MELANOPHORE STIMULANT.

Experiments were carried out to test how far the chemical properties of the pituitary melanophore stimulant agree with those of the other active constituents of the posterior lobe extracts.

Inactivation by boiling with dilute HCl. There appear to be at least two autacoid substances contained in extracts of the posterior lobe, since it is possible to destroy or reduce the pressor activity of "infundibular" extracts without diminishing in a corresponding manner their power to excite plain muscle. Dudley [1919] by extraction of dried and powdered infundibulum with acidified water, treatment of the solution with colloidal ferric hydroxide, and subsequent continuous extraction of the filtrate at reduced pressure with butyl alcohol, succeeded in separating a residue containing all the uterine stimulant ("oxytocic" principle) together with a portion of the pressor substance. The latter has been shown by Abel and Nagayama [1920] and Dale and Dudley [1921] to be rapidly destroyed by boiling with 0.5 % HCl. The oxytocic principle on the other hand is only slowly destroyed by such treatment. In this respect it appears that the melanophore stimulant is like the uterine principle and is not identical with the pressor substance.

The effect of continued boiling with dilute HCl was investigated as follows. A 0.5 % solution of the commercial extract was made up in 0.5 % HCl. This mixture was subjected to continuous boiling for five hours, a sample being removed at the end of thirty minutes. At the conclusion of the experiment a sample of the unboiled mixture, the portion which had been subjected to only half an hour's hydrolysis, and the residue were respectively neutralised with soda and diluted to a concentration approximately isotonic with frog's Ringer. From each of the three solutions, A (unboiled), B (boiled half an hour), C (boiled five hours), 0.5 cc. was injected into a pair of frogs whose pigment cells were fully contracted. The macroscopic and microscopic examination of the six animals at the conclusion of an hour revealed a marked contrast. The A and B pairs were dark and showed the typical pituitary reaction. The pair C remained pale. Microscopic preparations of the skin showed that in the C pair the melanophores were fully contracted, and the A and B pairs expanded. The result of the experiment indicates that pituitary extracts retain a considerable potency to induce melanophore response after half an hour's boiling with 0.5 % HCl; hence the melanophore stimulant is not identical with the pressor substance. According to Abel and Nagayama the oxytocic activity of the pituitary extracts after being reduced to about one-fifth by hydrolysis

for 30 minutes resists further destruction. Dale and Dudley however did not find that the residue was stable to prolonged boiling with acid; and the behaviour of the melanophore stimulant in this experiment conforms to their experience of the uterine stimulant.

The oxytocic potency of the samples taken after 30 minutes' boiling with 0.5 % HCl was found by Dale and Dudley to be between a quarter and a fifth of the previous activity. The potency of pituitary extract to induce melanophore expansion after half an hour's hydrolysis appears to be reduced in somewhat similar proportions. In a further experiment an approximation to the amount of melanophore stimulant remaining after half an hour's boiling with 0.5 % HCl was investigated thus. Three portions *A*, *B*, *C* (15 cc.) were taken from a 2 % "infundin" solution. *A* was boiled for half an hour, after which 15 cc. 1 % HCl and sufficient 1 % soda to neutralise it were added. To *B* were added 15 cc. of 1 % HCl, which was neutralised before boiling for 30 minutes. *C* was acidified with 15 cc. of 1 % HCl, boiled for half an hour, and subsequently neutralised. Each solution thus contained originally 0.3 cc. of sterile extract. *A* and *B* were made up to two litres so that an injection of 0.3 cc. would be equivalent to 0.00015 cc. infundin. *C* was made up to half a litre: one-half of this (*C* 1), representing a dilution one quarter of *A* and *B*, was used for injection, the other half was diluted one in four (*C* 2). Twenty-eight frogs were injected as in the following table from which it appears, on making the correction necessary for body weight, that the samples *A* and *B* (controls) were about *four* times as potent as *C*, the solution which had been subjected to boiling with 0.5 % HCl.

The positive sign signifies darkening of the skin, the numbers in brackets refer to the body weight in grams, and the extreme left hand column gives the dosage in cc. injected.

Table V.

	<i>A</i>	<i>B</i>	<i>C</i> 1	<i>C</i> 2
0.8 cc.	+ (12)	+ (12)	+ (12)	+ (11)
0.7 "	+ (13)	+ (13)	+ (13)	— (11)
0.6 "	+ (14)	+ (13)	+ (14)	— (11)
0.5 "	+ (16)	+ (16)	+ (16)	— (11)
0.4 "	+ (17)	+ (18)	+ (17)	
0.3 "	— (18)	+ (19)	— (18)	
0.2 "	— (20)	— (20)	+ (20)	
0.1 "	— (23)	— (23)	— (23)	

Action of proteoclastic enzymes. Perhaps the most significant fact indicative of the type of chemical compounds to which the infundibular autacoids are allied is furnished by the action of proteoclastic ferments. Schafer and Herring [1906] stated that pepsin destroys the pressor activity of pituitary extracts leaving intact the diuretic principle. They denied further that trypsin affected either. Later work by Dale [1909] and Dudley [1919] indicates that trypsin rapidly destroys both the pressor and oxytocic principles. To test the action of proteoclastic ferments on the melanophore stimulant six solutions were made up: *A* (0.5 % infundibular extract in 0.2 % HCl and

0.5 % pepsin); *B* (0.5 % infundin in 0.2 % HCl); *C* (0.2 % HCl and 0.5 % pepsin); *D* (0.5 % infundin in 0.5 % saline trypsin); *E* (0.5 % saline trypsin); *F* (0.5 % infundin in 0.5 % *boiled* trypsin). After two hours' digestion *A*, *B* and *C* were neutralised, boiled and diluted till isotonic with Ringer. *D*, *F* were boiled. On injection into pairs of pale frogs, the pairs injected with *A*, *B* and *F* showed darkening of the skin with expansion of the melanophores. The rest remained pale with the melanophores contracted. Thus the melanophore stimulant is rapidly destroyed by trypsin and is not rapidly destroyed by pepsin. In this connection evidence is provided that the melanophore stimulant is not identical, as Abel and Kubota [1919] once believed the uterine principle to be, with histamine, since the latter (Dudley) is not destroyed by tryptic digestion. As a matter of fact histamine does not in any concentration cause melanophores to expand.

Relation to other pituitary autacoids. In view of the possible identity of the diuretic and pressor substances, and the likelihood that the oxytocic stimulant is responsible for the galactagogue action of posterior lobe extracts, it is not necessary to postulate the existence of more than two distinct autacoids secreted by the juxtaneural epithelium. It is possible that the melanophore stimulant is not identical with either the pressor or the oxytocic autacoid, but there is strong evidence that it is not identical with the former, namely (*a*) its slow rate of inactivation by boiling with dilute hydrochloric acid; (*b*) no reversal of response after successive action; (*c*) failure of drugs which agree with pituitary extracts in their effect on the blood pressure to evoke the same reaction. For the present therefore we may provisionally attribute the melanophore response to the oxytocic substance.

SOURCE OF THE PITUITARY MELANOPHORE STIMULANT.

In Swingle's [1921] experiments upon the implantation of the pituitary gland in amphibian larvae, the pars intermedia was found to be responsible for the darkening of the skin. The results obtained from injections are consonant with the conclusion that the juxtaneural epithelium is the source of the secretion which induces melanophore expansion.

The demarcation between the three parts of the gland in the case of ox pituitaries is very striking; and ox pituitaries secured within an hour of killing were therefore employed in the following experiment. The three portions of the gland were carefully separated: each portion was weighed, ground up with sand, and extracted with Ringer's solution at 35° for two hours. A 0.1 % and a 0.02 % solution were made up from each extract so prepared. A pair of frogs were injected with each of the six solutions. At the end of half an hour the two pairs injected respectively with the strong and weak anterior lobe extracts remained pale. The pair injected with a weak extract (0.02 %) of pars nervosa extract also remained pale. While the pairs injected with both weak and strong extracts of pars intermedia as well as the pair injected with the stronger pars nervosa extract were conspicuously

darkened through, as microscopic examination displayed, the general expansion of the dermal melanophores. In view of the non-glandular character of the pars nervosa, and the higher concentration of the melanophore stimulant in the pars intermedia, it seems likely that the melanophore stimulant is secreted by the latter portion of the gland, diffusing rapidly into the former.

PHYLETIC DISTRIBUTION OF THE MELANOPHORE STIMULANT.

The following observations indicate the occurrence of the same melanophore stimulant in the pituitary gland of various classes of Vertebrata.

Mammalia. An adult female rabbit was decapitated, and its pituitary gland together with pieces of muscle, brain, ovary, pancreas, liver, suprarenals and spleen removed instantly. The tissues mentioned were severally ground with sand and extracted with Ringer in the usual way, after having been first weighed. A pair of frogs was injected with 0.5 cc. per individual of a 1 % solution of each extract. An additional pair received a corresponding amount of a putrid meat extract, while another pair received 0.5 cc. of a 0.1 % solution of the same pituitary extract. The two pairs injected with 1 % and 0.1 % pituitary extracts alone displayed a visible darkening of the skin, and, upon microscopic examination, the expansion of the melanophores.

Birds. Pituitaries were removed from eight ducks, weighed, ground up with sand, extracted and made up to 2 %, 0.4 %, and 0.08 % solutions which were administered respectively to pairs of pale frogs (0.5 cc. per individual). The animals injected with the two stronger solutions displayed the characteristic pigmentary response: the pair injected with the 0.08 % extract however remained pale.

Reptiles. Into each of a pair of frogs an injection of an extract of the pituitary of the lizard (*Lacerta viridis*) was made. The characteristic reaction was evoked. Two other frogs injected with an extract of brain tissue as controls, remained pale.

Amphibia. The experiment with frog's pituitary is of special interest as bearing on the rôle of the juxtaneural epithelium in normal pigmentary responses. Six frogs' pituitaries were removed and made up to 5 cc. in Ringer's solution. An injection of 0.5 cc. into each of a pair of pale frogs evoked an intense darkening of the skin. The remaining solution was diluted 1.5 in 10; and a pair of frogs injected with 0.5 cc. of this solution also underwent darkening of the skin. The 1.5/10 solution was again diluted 1/5. A pair of frogs injected each with 0.5 cc. of the latter showed in one case a darkening, while the other individual remained pale. On further dilution no response was induced by injection. It appears from the foregoing data that the amount of pituitary melanophore stimulant in the gland of one frog is not only sufficient to account for the darkening of the skin in one animal of the same species but in as many as fifty-six individuals.

Fishes. Pituitaries were removed from six cods (*Gadus*) and were made up to 2 %, 0.2 % and 0.04 % extracts. Two pairs of frogs injected with the usual amount of the two stronger solutions displayed the characteristic

darkening of the skin. Only one of two individuals injected with 0.5 cc. of the 0.04 % extract underwent pigmentary darkening: a pair injected with a 5 % extract of cod's brain remained pale.

Tunicata. The widespread distribution of the pituitary melanophore stimulant in the Vertebrate series encouraged the search for a similar substance among the lower Chordata; and extracts of the so-called dorsal tubercle or subneural gland of the Tunicate, regarded by morphologists as the homologue of the Vertebrate hypophysis, were accordingly prepared with this end in view. *Ascididiella*, a solitary Ascidian about $1\frac{1}{2}$ inches in length, was selected for the purpose. The dorsal tubercle in this form is very large and quite conspicuous through the translucent mantle after the gelatinous test has been removed. The organ in question was removed from twenty-four individuals and a 10 cc. extract was made. Injection into pale frogs was not followed by any pigmentary change.

REACTION OF CHROMATOPHORES TO PITUITARY EXTRACT.

The observations above mentioned refer to the behaviour of the dermal melanophores of the frog, and in their reactions to pituitary extracts these resemble those of other Amphibia, of which we have examined *Hyla*, *Bombinator* (*Anura*) and *Amblystoma* (*Urodela*). The frog possesses in addition at least three other types of pigmentary effector organs: (a) epidermal melanophores, responding to pituitary extracts in a manner similar to the dermal melanophores, though their sensitivity may be different; (b) the dermal xantholeucophores (yellow pigment cells) which respond *in the opposite sense*, i.e. contracting after pituitary injection—this property not being shared by *Hyla* in which the xantholeucophores appear to be non-contractile; (c) the retinal pigment cells. Fujita and Bigney [1918] have shown that these expand after treatment with adrenaline, an observation which we have confirmed. Pituitary injection has no effect upon them either in the expanded or contracted condition.

The fact that the frog itself possesses two types of chromatophores which differ diametrically in their mode of response to posterior lobe extracts is of interest in view of the fact that Spaeth [1917] describes the contraction of the melanophores of the fish (*Fundulus*) after action of the pituitary autacoid in certain circumstances. In view of the conformity of data derived from glandular extirpation and injection in the case of Amphibia, Spaeth's observations require in our belief reconsideration, though the above-mentioned fact does not permit one to regard his conclusions as necessarily improbable.

SUMMARY.

1. The pars intermedia of the mammalian pituitary secretes a specific stimulant inducing expansion of the dermal melanophores in Amphibia: this property is shared by extracts from the pituitary of birds, reptiles, amphibia and fishes: it was not found in the subneural (hypophysial) gland of Tunicates. Sufficient can be extracted from the gland of one frog to induce intense visible darkening in more than fifty other pale individuals of the same species.

2. The action of pituitary extracts on melanophores is one of extreme delicacy, and can be used to detect with certainty the presence of posterior lobe secretions in quantities equivalent to 5×10^{-5} g. fresh glandular substance (*i.e.* about 1/2000th ordinary clinical dose) by their power to induce intense darkening of the skin in pale frogs.

3. A method of approximate quantitative estimation of potency of posterior lobe extracts is indicated by experiments on the sensitivity of the melanophore response of frogs, which further show within what limits of error such a procedure may be successful. The delicacy of response does not depend on the mode of injection, nor is it affected by previous injection of minimal doses of the extract; it is however correlated with body weight.

4. Whereas the physiological effects hitherto described as characteristic of posterior lobe extracts are equally characteristic of a group of other well-known drugs (*e.g.* digitalis, BaCl_2 , histamine), the melanophore response described above is a property which is not precisely simulated by any of the more familiar pharmacological reagents. An effect in the same sense is indeed produced only by nicotine and apocodeine in doses sufficient rapidly to produce motor paralysis, and even here the darkening is much less conspicuous.

5. The melanophore stimulant in pituitary extracts is rapidly inactivated by tryptic but not by peptic digestion; its activity is diminished by boiling with 0.5 % HCl at about the same rate as is that of the uterine stimulant, but much more slowly than that of the pressor substance. The probable identity of the uterine and melanophore stimulants may be inferred.

6. In view of this probable identity of the uterine and melanophore stimulants, the accessibility of the materials, facility of manipulation, specificity and shortness of time implied by the method involving this response, it would seem to be serviceable for testing the activity of posterior lobe extracts in use in the laboratory and hospital.

The experiments were carried out in Prof. MacBride's laboratory at the Imperial College of Science, and the writers were advised on several points by Dr H. H. Dale, F.R.S., to whom grateful acknowledgment is made. The expenses of the research were defrayed by a grant made by the Government Grants Committee of the Royal Society.

REFERENCES.

- Abel and Kubota (1919). *J. Pharm. Exp. Ther.* **13**, 243.
Abel and Nagayama (1920). *J. Pharm. Exp. Ther.* **15**, 347.
Allen (1917). *Biol. Bull.* **32**, 117.
Bayliss and Bradford (1886). *J. Physiol.* **7**, 216.
Dale (1909). *Biochem. J.* **4**, 427.
Dale and Dudley (1921). *J. Pharm. Exp. Ther.* **13**, 27.
Dale and Laidlaw (1912). *J. Pharm. Exp. Ther.* **4**, 75.
Dudley (1919). *J. Pharm. Exp. Ther.* **14**, 295.
Fujita and Bigney (1918). *J. Exp. Zool.* **27**, 391.
Hogben and Winton (1922). *Proc. Roy. Soc. B.* **93**, 318.
Howell (1898). *J. Exp. Med.* **3**, 2.
Schafer and Herring (1906). *Proc. Roy. Soc. B.* **77**, 571.
Spaeth (1918). *J. Pharm. Exp. Ther.* **11**, 209.
Swingle (1921). *J. Exp. Zool.* **34**, 119.