EXPERIMENTS ON THE INJECTION OF PITUITARY BODY (ANTERIOR LOBE) EXTRACTS TO AXOLOTLS.*

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1. Introduction.

It has been shown that the removal of the pituitary body in mammals is generally fatal. Adler, whose work was extended by Allen, Smith, Atwell, and Hogben, has shown that such fatal results do not attend the removal of the amphibian pituitary body. The former investigators were able to destroy the hypophysial rudiment in the frog embryo, and prove that failure to metamorphose is the inevitable consequence of this operation. This work has been strengthened by the observations of Allen and Swingle on the acceleration of the metamorphosis in frog tadpoles by the implantation of the pituitary gland, and they concluded that the anterior portion (pars anterior) was responsible for this curtailment of the larval period. However, Gudernatsch and others found that pituitary feeding does not stimulate metamorphosis in Anura, and Huxley and Hogben obtained similar results with axolotls after feeding several months with anterior lobe. Later, Hogben found that by injecting commercial preparations of the extract of the anterior lobe axolotls could be induced to transform in a few weeks. Further metamorphosis was induced in thyroidless larvæ by the same method, suggesting the direct influence of the pituitary body on the metamorphic changes of the axolotls.

For these experiments, undertaken to test the efficacy of anterior lobe extracts as a means of inducing metamorphosis in the axolotl, twelve different products from various manufacturing chemists were obtained, some being in powder, others

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in tabloid form, and one a liquid extract.* The dried gland was extracted with Ringer's solution in each case, and each made up to the same concentration, using the manufacturer's estimate as the basis. The actual strength used was 1 gr. of dried substance in 5 c.c. of Ringer's solution, which was equivalent to 5 grs. of fresh gland in 5 c.c. Ringer's was also added to the liquid extract to make it up to the same strength as the others. Intraperitoneal injections were made tri-weekly, 0.5 c.c. at the time, so that the dose was equivalent to ½ gr. of fresh glandular substance, although in some series it was altered. The axolotls were kept in separate vessels of about 2 litres capacity. Feeding with raw beef, weighing and changing of water was carried out on days alternating with injections. In later stages feeding and change of water took place once a week.

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2. Injection Experiments.

The first series of experiments consisted in comparing the effects of tri-weekly injections of each extract. The individuals were treated in separate containers, each animal being injected with the same preparation each time, and conditions arranged to give uniformity throughout. Specimens not under treatment were kept in the same environment to act as controls.

* List of preparations used, the form in which they were supplied, the quantity of dried gland present and its equivalent of fresh gland, and amount of Ringer's solution added.

No. 1.—1 grain tablet = 5 grains fresh gland dissolved in 5 c.c. Ringer.
No. 2.—1 „ capsule = 2 „ „ „ 2 „
No. 3.—1 „ tablet = 5 „ „ „ 5 „
No. 4.—Powder 5 grains = 25 „ „ „ 25 „
No. 5.—2 grains tablet = 10 „ „ „ 10 „
No. 6.—1 grain tablet = 5 „ „ „ 5 „
No. 7.—1 aseptule = 1 c.c. of 20 per cent. extract—1 c.c. of Ringer added.
No. 8.—2 grains capsule = 2 grains fresh gland—dissolved in 2 c.c. Ringer.
No. 9.—5 „ tablet = 25 „ „ „ 25 „
No. 10.—2 „ „ = 10 „ „ „ 10 „
No. 11.—5 „ capsule = 5 „ „ „ 5 „
No. 12.—Powder 5 grains = 20 grains.

The range of temperature for the first part of the experiment was 17° to 19° C., but subsequently it was practically constant at 20° C. Previous to the first injection the weight and length of each animal were recorded, and these observations were repeated twice weekly during the period of the experiment. At the same time fresh water was placed in the container. 0.5 c.c. containing the equivalent of ½ gr. of fresh glandular substance was injected on each occasion, and these injections were continued, except in cases to be referred to later, until twenty injections had been made.

There was only one case of metamorphosis in all those animals experimented on in this series of operations (No. 7), and in this instance the first signs of change—a reduction of the dorsal fin—was noticed after twenty days at the ninth injection. The reduction of tissue continued until the twenty-sixth day, when the gills began to get smaller and the skin was cast, giving the animal a lighter appearance. The axolotl itself showed no signs of discomfort, being quite active and feeding normally, although there was a marked loss in weight as shown in table and curve. The length was unaltered. The skin had lost its soft velvety appearance, being quite smooth and of leathery texture, whilst the body itself was more rigid and firmer.

At this point owing to exhaustion of supplies, the injections ceased, but after a period of nine days, during which it remained under conditions identical with those of the early part of the work, an injection similar to that given to No. 8 of the series was administered. No further change had been noticed during this break, but soon after this fresh injection the animal became sluggish, much darker, and refused food, in fact it was in the same condition as specimen No. 8, which had died just previously after thirteen injections. Another injection was given, but as there were no signs of improvement the treatment was discontinued. Within three days the animal had regained its previous condition to all appearances, feeding well and weighing exactly the same. Further supplies having now been obtained, injections with the original extract were continued after twenty-one days, and the transformation proceeded, so that in seventeen days the gills disappeared

and the change was complete. The skin was shed several times, and two days later the animal was transferred to dry quarters. The results of the treatment with other extracts will be discussed in another part of this work, but meantime a report on a further series of experiments in which No. 7 extract was used only will be dealt with. In this series the changes induced, and conditions and other factors, were studied in much greater detail. Six sexually mature axolotls were taken, weighed and measured as before, arranged in three pairs of roughly the same weight, and placed in a small room kept at constant temperature of 24° to 25° C. One member of each pair was injected tri-weekly with 0.5 c.c. a fresh sample of No. 7 extract made up as in previous experiment, the other with 0.5 c.c. of Ringer's solution to act as a control. Weights and measurements were taken as before and general conditions kept as uniform as possible. After fifteen days the lightest member of the series showed signs of reduction of dorsal fin. This continued, a few sterile eggs being laid meantime, until the twenty-eighth day, when metamorphosis was practically complete. However, injections continued as the weight still decreased, but were stopped at twentieth injection, when weight became constant and animal taken from water. The injections in this experiment were continuous without a break, as happened with all members of this series. The control beyond losing a little weight was unchanged. Feeding was normal and the lengths unaltered.

In the second pair the first signs of reduction in the pituitary injected member also appeared after fifteen days, but change proceeded more slowly, probably due to greater bulk, and was not so advanced as first pair at the twenty-eighth day. The assumption of adult characters was complete on the forty-second day, just before the twentieth injection. Injections were stopped at this point and the animal taken out of the water. The weight decreased as in previous cases and was not constant until after twentieth injection. The control behaved as before, feeding normally, and there was the characteristic loss of weight. The third pair was the largest, and here the usual initial loss in weight was obtained, but from the fourteenth to thirtieth day it was practically constant.

All these changes in weight are shown on the curve. The first signs of tissue reduction appeared on the twenty-second day, and from then on the progress was extremely slow. On the thirty-eighth day the strength of the injection was increased by half. This produced an acceleration, but on increasing the dose on the forty-eighth day by another half, so that actually 0.5 c.c. of the original extract was given, the metamorphosis was considerably hastened and completed on fifty-third day. During the latter period the loss of weight was very rapid. The skin was cast at the twenty-first injection, and actually twenty-three injections had to be made. Here the metamorphosis was very much delayed, and it was only under the stimulus of the larger dose that it could be successfully completed. The control was quite normal and behaved in exactly the same way as the other controls.

The preparation used by Hogben was the desiccated product of the Armour Organotherapeutic Laboratory, that which proved efficacious in the experiments here described was supplied by Messrs Oppenheimer as sterile fluid extract. The results obtained are therefore compatible with the view that there is present in the anterior lobe a substance whose physiological activity is such as to promote developmental changes in Urodele larvæ. The question then arises why other preparations employed did not evoke the appropriate response.

In all the samples used the amount of gland extract present was given and the equivalent of fresh gland stated. The extracts for injection were made up accordingly, and only this particular extract (No. 7) proved successful in its action, so that in this manner it has been possible to test whether the amount of free extract was actually present in the preparation, and proceeding on these lines any such extract can be examined.

When the above series of experiments had been completed, a communication from the manufacturers showed that the extract No. 7 was stronger than stated, and on the new calculation 0.75 gr. of fresh gland had been given in each 0.5 c.c. instead of 0.5 gr. However, this does not in any way oppose the application of the above method for testing presence

of the active secretion. Experiments will be detailed later in which a double dose of all those extracts which failed in this series was administered but with no more success, showing that their failure was not a question of quantity but counteracting influences.

It is possible to give the approximate limit of the minimal dose. All animals used in these operations were sexually mature and were older than those employed by Hogben in his experiments. The majority were full-grown specimens and these results suggest that irrespective of age, size, and condition, the injection of sufficient quantity of extracts of the anterior lobe will induce metamorphosis. The upper limit would approximate to 1.5 gr. per 0.5 c.c. injection, from the amount necessary to complete the change of the largest member of the last series of experiments described. This amount decreases with the size as seen in the same series of experiments, but there is no uniformity in the decline nor definite relationship between the amount injected and the size. Another interesting point arising from these experiments is the relationship between the temperature and the rate of metamorphosis. It is the only factor in the environment whose effect could be determined. It was noticed in specimens kept below 17° C. that metamorphosis was appreciably retarded, whilst above 27° the animals became sluggish, refusing food, and losing weight with no noticeable increase in rate of change. Apparently the most suitable temperature is 21° to 23° C., at which the first signs of metamorphosis appear between the twelfth to fifteenth days, and the progress is complete by the fortieth day for average sized mature animals. In younger animals the first appearances were a little earlier and the transformation complete in about four weeks; but in the largest specimens the first signs appeared much later, and if the dose given was not below the minimal required for its size the transformation was rapid and complete in forty-eight days. However, as in the case of the minimal dose, it is impossible to give definite or exact data. The details regarding temperature and the length of the period of transformation were confirmed by an experiment on an average sized axolotl, injected triweekly with 0.5 c.c. of No. 7 extract containing 0.75 gr. of

gland, kept at temperature of 21° to 22°. These conclusions were justified in every way, the first change appearing at the twelfth day, and following a rapid sequence of changes it completed the assumption of adult characters on the fortieth day.

It seemed possible that increasing the amount of gland administered in the case of inactive preparations would result in metamorphosis. Accordingly several weeks after the first series of experiments the same animals (with exceptions to be mentioned later) were injected with twice the same dose of the foregoing extracts. Conditions were similar except the temperature was raised to 24° C. Beyond one or two variations the animals seemed to be the same and there was not one case of metamorphosis.

Before leaving this question of metamorphosis a note on the sequence of metamorphosis might be given as the writer's observations are not in entire agreement with those of Hogben. In each case the reduction of the dorsal fin at the anterior end was the first sign followed by a thinning of the tail fin. Absorption of the dorsal fin along the trunk region became marked and also protrusion of the eyes. In Hogben's series the latter was apparently one of the first signs. The reduction of the gills varied, becoming in some cases mere stumps fairly soon, starting before the disappearance of the dorsal fin. This was generally the case in older specimens, but they persisted right to the end being the last to disappear. In other cases the absorption commenced when the dorsal fin had gone. Meantime the body became considerably attenuated and stiffened, whilst the limbs seemed more rigid and stronger. There was no diminution in length as measurements will show, until completion, and even then it was very small. The larval skin was shed early on, soon after the beginning of the dorsal fin reduction. The succession of moults marked the gradual resorption of the gills. Pigmental changes followed as usual when the terrestrial form had been assumed. These variations are slight, however, and no doubt due to greater age and size, but the chief factor is the temperature. This also applies to variations in the times of the appearance of these changes and the period over which they extended.

Changes in body weight of the animals obtained in these

experiments were compared and considered in relation to previous work. In every instance there was an initial loss of weight during the first six or seven days, the loss being greater in the case of those animals kept at the higher temperature. The decline continued, but was only gradual until just after the first signs of change appeared, when a further marked decrease, continuing until the completion of metamorphosis, was noted. The weight then remained more or less constant. The transformation in the largest specimen was slower until the dose was increased, and the decline in weight was correspondingly less abrupt until the later phase. In no single instance was there an increase in weight or size although feeding was normal and regular. The controls, injected with saline, showed a gradual decline throughout the experiments, whilst the controls used in first series of experiments kept at room temperature showed no marked variations.

3. Investigation of Causes of Failure of Extracts.

Attention must now be directed to those extracts which failed to bring about metamorphosis when injected, and the endeavour to account for their action. There are several possibilities. First, an active principle may have been present, but not in sufficient quantity to bring about the transformation. Such a deficiency may have been brought about by the destruction or reduction of the potency of the active factors during manufacture, but the experiments in which the concentration of the dose was increased make this very improbable. Secondly, there is the question of impurities having an inhibitory effect. They may be either extrinsic or due to some other substance present in the pituitary itself.

As regards the presence of posterior lobe secretions, one may presume that within a short time of death the secretions diffuse from one part of the gland to another, so that the shorter the interval between the killing and extraction of the active principle the greater the chance of purity, and also the difficulty of separating the two portions (anterior and posterior) of the pituitary gland is well known, and hence it is quite easy to understand that portions of the posterior lobe appear

as impurities in anterior lobe preparations. A record of the actual experiments carried out with these different commercial extracts will demonstrate the deficiency in anterior extract and the probable presence and effect of the posterior lobe. The results obtained by using No. 7 have already been outlined, but the method of procedure was exactly the same. Tri-weekly injections were made with a dose of 0.5 gr. in 0.5 c.c. of Ringer's solution. They were measured and fed as before and other arrangements were the same, the operations in the first series being performed at room temperature. In all cases the water of the container clouded slightly after injection started compared with controls, and Nos. 1, 2, 5, 8, 9, 10, and 11 became very cloudy and a peculiar smell was detected. The following are records of each animal treated :—

No. 1. 20 injections.—Weight varied slightly, 60 gms. 1st day, 57 gms. 20th day, 70 gms. 45th day. Slight reduction in fin tissue at 23rd day but no further changes. Slight pigmentation effect. No increase in length. Fed normally except from 12th to 18th day when food refused. Normal again after injections ceased.

No. 2. 20 injections.—Weight constant till 36th day when weight increased by 10 gms. to 80 gms. After 12th day food refused. Slight pigmentation effect and slight tissue reduction at 23rd day. 36th day abdominal distension very marked and this increased. Small decrease in length. Animal sluggish after refusal to take food; state of coma and weakness of limbs followed. Injections stopped, but remained in comatose state till 64th day when it died. Post-mortem examination revealed well-developed ovaries and oviduct and organs apparently normal except liver, which was blackish grey in colour and showed hepatic degeneration. Abdominal distension due to excess of fluid smelling strongly of urine and contained some blood. This accumulation of fluid would account for increase in weight in spite of refusal of food. There were signs of tissue reduction and lungs were fairly well developed, pointing to activity of anterior lobe extract, but condition of animal apparently prevented further change.

No. 3. 20 injections.—Weight practically same throughout. Refused food from 12th to 20th day. No alteration in length, almost black towards end of experiment.

No. 4. 20 injections.—Fed normally. Length and weight same.

No. 5. 20 injections.—Weight and length same. Fed normally except between 12th to 20th days.

No. 6. 20 injections.—Slight loss in weight during experiment. Fed normally. Length same. Pigmentation changes.

No. 8. 13 injections.—No increase in weight till after 22nd day. 1st day,

136 gms.; 22nd day, 136 gms.; 27th day, 150 gms. No change in length. Refused food after 12th day. No signs of tissue reduction, but animal sluggish and distended. 28th day, 6 c.c. of abdominal fluid withdrawn for analysis. 2.1 per cent. urea present, phosphate in small quantities, no sugar. 30th day, animal died. Post-mortem examination showed well-developed lungs and organs normal except for liver, where hepatic degeneration was noted. Abdominal distension was apparently due to increase in amount of excretory fluid which renal system failed to cope with. Œdema resulted causing death. These symptoms were produced in No. 7 as stated when treated with this extract.

No. 9. 20 injections.—Weight and length constant. Feeding normal. Slight tissue reduction and pigmentation change.

No. 10. 20 injections.—Slight increase in weight of 5 gms. over period of experiment. Length same and feeding normal.

No. 11.—Gradual increase in weight from 55 to 70 gms. in 20 days. Refused food after 5th day. Water very cloudy indeed, yellow in colour with pungent odour. Acute inflammation of cloacal aperture and abdominal distension. Tissue reduction was apparent, but animal died after 12th day. Post-mortem showed same features as in previous specimens: hepatic degeneration, urea in abdominal fluid, etc.

Nos. 12 to 18.—Members of this series were injected with extracts of decreasing strength, the object being to investigate the minimal amount of anterior lobe extract necessary to bring about metamorphosis, but negative results were obtained. The normal dose was diluted to the following ratios 1/2, 1/5, 1/10, 1/25, 1/50, 1/100, and 1/200, and injections made as in other cases and under the same conditions. 20 injections were made. There was little variation in weight except in Nos. 14 and 16 which showed gradual increase and also pigmentation effect. No. 5 extract was diluted for the injections, except in those particular cases when a desiccated powder was used. No. 12 extract.—Length was constant and feeding normal.

A survey of these details show (1) that although no case of metamorphosis there were signs of tissue reduction in many; (2) there was no increase in weight except in specimens that died or showed abdominal distension due to excessive formation of urine; (3) no increase in length, hence no sign of gigantism; (4) apparent tissue reduction and refusal to take food occurred about the same time in each case, and no doubt due to same influence.

Following the results of the second series of experiments with No. 7 extract and controls treated with Ringer's solution, a third series were started at temperature of 24° to 25° with dose of twice concentration of former dose. Nos. 1, 3, 5, 6, 9, 10 only were used in this work, for No. 7 had already been utilised, and Nos. 2, 8, 11 had fatal results in the first series.

Injection of Pituitary Body Extracts to Axolotls

The same animals were used as in the first series, and during an interval of eight weeks the animals had acquired their original appearance. They had been kept at room temperature and fed and washed once a week.

The results can be recorded as before :—

No. 1. 12 injections.—Weight practically same; length constant; fed normally. Peculiar smell and cloudiness of water. Slight abdominal distension.

No. 3. 12 injections.—Weight constant, also length; feeding normal. Water cloudy and faint smell. Animal black.

No. 5. 12 injections.—Gradual reduction in weight; length constant; food refused latterly. Water very cloudy, yellow colour and pungent odour.

No. 6. 12 injections.—Slight reduction in weight; length constant; fed normally. Skin became rough towards end of experiment; water cloudy and faint smell.

No. 9. 12 injections.—Gradual decrease in weight; length constant; feeding normal; water cloudy and faint smell.

No. 10.—Animal found dead after 3rd injection, having burst overnight, making a post-mortem examination impossible.

On the fourteenth day all showed signs of tissue reduction. The tail fin was very thin, but as this change was so slight it cannot be maintained that it had any connection with metamorphosis, particularly as no further characteristic changes were noticed. As there was no alteration apparent the injections were stopped on the twenty-fifth day. During the operations measured quantities of the water in each container were tested for presence of urea, but although it was undoubtedly there, the quantity was too small to be successfully determined quantitatively.

These experiments, while suggesting lack of sufficient free active anterior lobe principle to bring about the assumption of adult characters, gave no evidence for increase in growth or gigantism. It is possible that the presence of posterior lobe secretion successfully countered effective action of the anterior portion in both this series of operation and that with smaller dose.

The effect of temperature in the case of No. 7 has already been discussed, but no reference was made to the slight cloudiness and smell of the water in the containers during the experiments at the higher temperature, a condition prevalent to a greater or less extent in all other cases treated with these extracts at high and low temperature, but not

obvious in controls. The state of the water at the higher temperature in some necessitated fresh water every other day. This contamination appeared to be of the same nature in each case, and the odour suggested the presence of urine. Hence it seems quite justifiable to conclude that the condition was due to the increased activity of a particular factor, which was the same in each case, and further, its greater activity was promoted by the higher temperature. The fact that the activity of this principle varied with different extracts suggests that it might be due to a posterior lobe secretion in the anterior lobe extract, the strength of its activity possibly denoting the amount of posterior lobe impurity, and further its action may have reduced the effective action of the anterior lobe.

Mammals injected with the posterior lobe have been observed to suffer from loss of appetite, increased peristalsis, mild enteritis, nervous manifestations, muscular tremors, and weakness of the hind limbs. Four axolotls died as noted before, and their condition previous to death agrees in a remarkable manner with that described in reference to mammals. In other cases loss of appetite and weakness of limbs was noted as soon as there was an apparent increase in the flow of urine followed by slight abdominal distension, and although those cases were not fatal the condition was obviously due to the same cause. This similarity again suggests presence of the posterior lobe, and the amount present on these occasions in the injections must have been very large, and the high percentage of urea in the sample of abdominal fluid extracted from No. 8, and the œdematous state of the fatal previous cases to death as well as the hepatic degeneration revealed by post-mortem examination, all bear evidence to the large amount present.

The above observations suggest that an impurity, possibly a posterior lobe secretion, was present to excess in Nos. 2, 8, 10, and 11. Animals treated with these extracts died. Nos. 1, 5, 6, and 12 have a large quantity which could be detected in the low as well as high temperature series of experiments, whilst Nos. 3, 9, and 7 probably contain quantity only detected at high temperature. It will be noted that although No. 7 successfully brought about metamorphosis, it contains some

of the impurity, but evidently the amount was not sufficient to affect the action of the anterior lobe.

4. Pigmentation Experiments.

Recent work on the effect of pituitary extracts on the melanophores of Amphibians suggested a means of testing the presence of this factor in the manufactured anterior lobe products used in these experiments. The ability of certain animals to change colour and so respond to their surroundings has long been familiar and aroused interest of biologists, but it is only of late years that the relation between pigmentation changes and internal secretions has been explored. Corona and later Lieben induced such changes in frogs with gland extracts, causing pigment cells to contract with adrenalin. M'Cord and Floyd Allen (1917) obtained contraction of melanophores in frog tadpoles by pineal administration. Huxley and Hogben and others have confirmed this, and further obtained melanophore expansion in axolotls after pituitary feeding, a result in agreement with the work of Allen (1916) Smith (1917), and Atwell (1919) who obtained melanophore contraction after pituitary extirpation. Later Swingle implanted the gland into pale tadpoles and obtained a darkening effect. The same reaction was noted by Hogben and Winton when pale adult frogs were injected with posterior lobe extract. In a series of papers a detailed and exhaustive study has been made recently by Hogben and Winton on the "Pigmentary Effector System of the Frog." The reaction of the frog's melanophores to this extract was explored and compared with the action of drugs administered in the same way, and these authors have been able to indicate the determining influençe of the endocrine secretions on the amphibian colour responses to environment by experimental extirpation of the gland in adult anura.

Further investigations have enabled the same authors to advance a quantitative method of estimating the strength of pituitary extracts by the sensitivity of the melanophore response of frogs, but the question has now arisen as to the exact localisation of the melanophore stimulant, since extracts made by Hogben from the three regions of the pituitary gave a reaction, so that the value of estimations of the strength of

posterior lobe extracts, or the posterior lobe present in anterior lobe extracts, based on this method, is doubtful. The bulk of evidence certainly shows the presence of more melanophore stimulant in the pars intermedia, and it is quite possible that diffusion will account for its presence in other parts, but further investigation is necessary before definite conclusions are drawn. Meantime, the method itself was successfully applied to determine the amount of melanophore stimulant present in the original extracts used in this work, and results compared with a view to ascertaining its effect on action of the anterior lobe extract.

Frogs for these experiments were prepared after weighing by washing and drying carefully, and then placing in dry glass jars with muslin cover in a dry warm place on a white background in bright light. In a few hours (three to four) they were quite pale, all the melanophores having contracted. A series of injections were first carried out with a sterile liquid extract containing 10 per cent. Pituitrin, kindly supplied by Messrs Parke, Davis & Co., and standardised with respect to an isolated uterus or its blood pressure raising property. Injections were made by various methods—intraperitoneal, intravenous, dorsal lymph sac—but all yielded the same results. Next 0.5 c.c. injections from a series of solutions of decreasing percentage strengths were made in pairs of frogs. Within ten minutes there was a response which reached its maximum in about half an hour, and the frogs remained dark for several hours if the dose was strong, but less with weaker samples. If the frogs were replaced in a favourable environment they regained the pale condition and were able to respond to further injections showing the same sensitiveness, so that their recovery was complete and they suffered no harmful effects. This agrees with the findings of Hogben and Winton. The response was found to depend on the weight of the frog, so that all results were expressed in terms of their reaction with a standard weight, thereby ensuring uniformity in the comparisons. The concentration of that solution of Pituitrin which just gave a visible darkening of the frog's skin was noted, and also the dose which just failed to give any response. In this way the minimal and sub-minimal doses

were determined after reference to the standard weight. The animals were examined microscopically, the skin being fixed in Bouin's fluid, dehydrated and mounted in balsam. The original extracts were next taken, and the minimal and sub-minimal doses found as in the case of the standard. Microscopic preparations were also made to confirm results. By comparison with the standard we can arrive at some idea of the amount of the secretion present in each injection made in the initial series of experiments. A preliminary experiment was carried out with Pituitrin to ascertain approximately the limits of doses giving response, followed by an exact determination.

The same procedure was adopted in the case of extracts themselves, a preliminary to find approximate limits followed by exact determinations. The following experiments were carried out with Pituitrin. Five frogs were injected with 0.5 c.c. of 0.1, 0.05, 0.01, 0.005, and 0.001 per cent. First three gave dark reaction, 0.005 per cent. slight reaction, and 0.001 per cent. no response. The following is the accurate determination:—

Weight.	Injection.	Dose in c.c.	Approx. Dose per 20 gms.	Result.
14 gms.	0·1 p. c. of Pituitrin	0·0005	0·00071	Very dark.
23 ,,	0·1 ,, ,,	0·0005	0·00043	,,
14 ,,	0·05 ,, ,,	0·00025	0·00036	,,
23 ,,	0·05 ,, ,,	0·00025	0·00022	,,
25 ,,	0·025 ,, ,,	0·000125	0·0001	Dark.
16 ,,	0·025 ,, ,,	0·000125	0·000156	,,
19 ,,	0·01 ,, ,,	0·00005	0·0000526	,,
24 ,,	0·01 ,, ,,	0·00005	0·000042	,,
20 ,,	0·0075 ,, ,,	0·0000375	0·0000375	Slight darkening.
25 ,,	0·0075 ,, ,,	0·0000375	0·00003	,, ,,
19 ,,	0·005 ,, ,,	0·000025	0·0000262	Very slight.
23 ,,	0·005 ,, ,,	0·000025	0·0000217	,,
25 ,,	0·0025 ,, ,,	0·0000125	0·00001	No change.
21 ,,	0·0025 ,, ,,	0·0000125	0·000012	,,
20 ,,	0·001 ,, ,,	0·000005	0·000005	,,
20 ,,	0·001 ,, ,,	0·000005	0·000005	,,
21 ,,	Ringer's solution	...	...	,,
19 ,,	,, ,,	...	...	,,

The *minimal dose* is 0·00002 c.c. and the *sub-minimal dose* 0·00001 c.c.

The following tables show the results of injections made with various dilutions of the extracts injected in the earlier experiments, and also the minimal and sub-minimal doses:—

Weight.	Injection.	Dose in c.c.	Approx. Dose per 20 gms.	Result.
	Extract No. 1.			
12 gms.	10 per cent.	0·05	0·083	Dark.
19 „	1 „	0·005	0·0053	No change.
34 „	0·1 „	0·0005	0·0003	„
19 „	5 „	0·025	0·0263	Slight darkening.
25 „	5 „	0·025	0·02	„ „
18 „	7·5 „	0·0375	0·0417	Dark.
18 „	7·5 „	0·0375	0·0417	„
23 „	10 „	0·05	0·0435	„
19 „	10 „	0·05	0·053	„
22 „	2·5 „	0·0125	0·0113	No change.
19 „	2·5 „	0·0125	0·013	„
Minimal dose = 0·02 c.c. Sub-minimal dose = 0·013 c.c.				
	Extract No. 2.			
16 gms.	10 per cent.	0·05	0·0625	Very dark.
28 „	1 „	0·005	0·00357	Dark.
28 „	0·1 „	0·0005	0·000357	Slight darkening.
17 „	5 „	0·025	0·0294	Dark.
17 „	5 „	0·025	0·0294	„
21 „	2·5 „	0·0125	0·0119	„
14 „	2·5 „	0·0125	0·018	„
20 „	1 „	0·005	0·005	„
19 „	1 „	0·005	0·00526	„
21 „	0·05 „	0·00025	0·00024	No change.
21 „	0·05 „	0·00025	0·00024	„
34 „	0·075 „	0·000375	0·00022	„
28 „	0·075 „	0·000375	0·00027	„
Minimal dose = 0·0004 c.c. Sub-minimal dose = 0·0003 c.c.				
	Extract No. 3.			
24 gms.	10 per cent.	0·05	0·0417	Very dark.
19 „	1 „	0·005	0·00526	Dark.
22 „	5 „	0·025	0·0227	Very dark.
20 „	5 „	0·025	0·025	„
20 „	2·5 „	0·0125	0·0125	Dark.
18 „	2·5 „	0·0125	0·0139	„
27 „	1 „	0·005	0·0037	Slight darkening.
26 „	1 „	0·005	0·0039	„ „
20 „	0·5 „	0·0025	0·0025	Dark.
21 „	0·5 „	0·0025	0·00238	„
18 „	0·1 „	0·0005	0·00056	Slight darkening.
25 „	0·1 „	0·0005	0·0004	„ „
29 „	0·25 „	0·00125	0·0009	„ „
18 „	0·25 „	0·00125	0·0014	Dark.
22 „	0·075 „	0·000375	0·00031	No change.
25 „	0·075 „	0·000375	0·0003	„
Minimal dose = 0·0004 c.c. Sub-minimal dose = 0·0003 c.c.				

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Weight.	Injection.	Dose in c.c.	Approx. Dose per 20 gms.	Result.
		Extract No. 5.		
23 gms.	10 per cent.	0·05	0·0435	No change.
23 „	1 „	0·005	0·00435	„
19 „	50 „	0·25	0·263	„
15 „	50 „	0·25	0·33	„
20 „	No. 5	0·5	0·5	Slight.
20 „	„	0·5	0·5	„
	Minimal dose = 0·5 c.c. Sub-minimal dose = 0·4 c.c.			
		Extract No. 6.		
14 gms.	10 per cent.	0·05	0·071	Very dark.
18 „	1 „	0·005	0·0055	Slight darkening.
18 „	5 „	0·025	0·0278	Dark.
20 „	5 „	0·025	0·025	„
19 „	0·5 „	0·0025	0·00262	Slight darkening.
22 „	0·5 „	0·0025	0·00227	„ „
23 „	2·5 „	0·0125	0·0109	Dark.
23 „	2·5 „	0·0125	0·0109	„
16 „	0·1 „	0·0005	0·000625	No change.
18 „	0·1 „	0·0005	0·00055	„
24 „	0·25 „	0·00125	0·00104	„
24 „	0·25 „	0·00125	0·00104	„
	Minimal dose = 0·0025 c.c. Sub-minimal dose = 0·0015 c.c.			
		Extract No. 7.		
17 gms.	10 per cent.	0·05	0·059	Very dark.
30 „	No. 7	0·5	0·33	„
18 „	1 per cent.	0·005	0·0055	Dark.
13 „	0·1 „	0·0005	0·00067	No change.
17 „	0·1 „	0·0005	0·00059	„
17 „	0·5 „	0·0025	0·00294	Dark.
18 „	0·5 „	0·0025	0·00278	„
19 „	0·25 „	0·00125	0·00132	Slight darkening.
19 „	0·25 „	0·00125	0·00132	„ „
	Minimal dose = 0·0013 c.c. Sub-minimal dose = 0·0007 c.c.			
		Extract No. 8.		
26 gms.	10 per cent.	0·05	0·0385	Very dark.
20 „	1 „	0·005	0·005	Slight darkening.
16 „	0·1 „	0·0005	0·000625	No change.
19 „	5 „	0·025	0·0263	Very dark
15 „	5 „	0·025	0·033	„
24 „	2·5 „	0·0125	0·0104	Dark.
17 „	2·5 „	0·0125	0·0147	„
15 „	0·5 „	0·0025	0·0033	No change.
16 „	0·5 „	0·0025	0·0019	„
	Minimal dose = 0·005 c.c. Sub-minimal dose = 0·0033 c.c.			

Weight.	Injection.	Dose in c.c.	Approx. Dose per 20 gms.	Result.
		Extract No. 9.		
19 gms.	10 per cent.	0·05	0·0526	Slight darkening.
32 „	1 „	0·005	0·003125	Very slight.
20 „	5 „	0·025	0·025	Dark.
21 „	5 „	0·025	0·0238	„
31 „	2·5 „	0·0125	0·008	Slight darkening.
28 „	2·5 „	0·0125	0·009	„ „
25 „	0·5 „	0·0025	0·002	No change.
18 „	0·5 „	0·0025	0·00278	„
Minimal dose = 0·0035 c.c. Sub-minimal dose = 0·0025 c.c.				
		Extract No. 10.		
18 gms.	10 per cent.	0·05	0·055	No change.
25 „	1 „	0·005	0·004	„
12 „	0·1 „	0·0005	0·0008	„
18 „	50 „	0·25	0·278	„
24 „	50 „	0·25	0·208	„
21 „	No 10	0·5	0·48	„
20 „	„	0·5	0·5	„
No trace whatever.				
		Extract No. 11.		
25 gms.	10 per cent.	0·05	0·04	Very dark.
25 „	1 „	0·005	0·004	No change.
20 „	0·1 „	0·0005	0·0005	„
19 „	2·5 „	0·0125	0·01316	„
22 „	2·5 „	0·0125	0·01136	„
21 „	5 „	0·025	0·0238	Slight darkening.
22 „	5 „	0·025	0·0227	„ „
Minimal dose = 0·025 c.c. Sub-minimal dose = 0·015 c.c.				
		Extract No. 12.		
21 gms.	10 per cent.	0·05	0·048	Very dark.
20 „	1 „	0·005	0·005	Dark.
20 „	0·5 „	0·0025	0·0025	„
15 „	0·5 „	0·0025	0·0033	„
30 „	0·25 „	0·00125	0·00083	Slight darkening.
20 „	0·25 „	0·00125	0·00125	„ „
22 „	0·1 „	0·0005	0·00045	No change.
24 „	0·1 „	0·0005	0·00041	„
Minimal dose = 0·00085 c.c. Sub-minimal dose = 0·0005 c.c.				

The temperature during these experiments was 20° C. It was found that with certain frogs the reaction was not satisfactory, and a sharply defined end point was not obtained. This may have been due to various factors, but in any case they were eliminated, and only those giving a well-contrasted

reaction used. A further selection was made at the start, when only those individuals with a skin having a uniform yellowish tint when pale were taken to facilitate observations. The relation between the body weight and minimal dose was found to vary a little, but as these were not intended as absolute estimations but merely for comparative purposes the small amount of error was of no importance. This error was therefore neglected, and by taking the mean of minimal and sub-minimal doses when referred to the standard weight, the amount of melanophore stimulant present in the dose injected during the metamorphosis series of experiments was determined sufficiently to allow comparisons. The following table shows effect of the injection of each extract, and the presence of melanophore stimulant as observed by pigmentation reaction :—

Extract.	Effect on Injection.	Melanophore Stimulant by Pigmentation Response.		
		c.c.	c.c.	c.c.
1	No effect . . .	M = 0·0005	S.M. = 0·0004	Mean = 0·00045
2	„ (died). .	M = 0·025	S.M. = 0·017	Mean = 0·021
3, 4	„ . . .	M = 0·025	S.M. = 0·017	Mean = 0·021
5	„ . . .	M = 0·00002	S.M. = 0·00001	Mean = 0·000015
6	„ . . .	M = 0·004	S.M. = 0·003	Mean = 0·0035
7	Change brought about in 4-6 weeks . .	M = 0·008	S.M. = 0·007	Mean = 0·0075
8	No effect (died). .	M = 0·002	S.M. = 0·0015	Mean = 0·00175
9	„ . . .	M = 0·003	S.M. = 0·002	Mean = 0·0025
10	„ (died). .	No trace	...	...
11	„ „ . .	M = 0·0004	S.M. = 0·0003	Mean = 0·00035
12	„ . . .	M = 0·012	S.M. = 0·011	Mean = 0·0115

Examination of this table shows (1) No. 7 extract induces metamorphosis in axolotls, but contains pigmentation factor; (2) No. 10 extract fails to induce metamorphosis, the injections having fatal results, but there is only a trace of the pigmentation factor ; (3) Nos. 8 and 11 have fatal results when injected, but yet have smaller quantities of this factor than No 7 ; (4) Nos. 1, 5, 6, and 9 have less pigmentation factor than No 7, but yet fail to bring about metamorphosis.

It seems evident from these details that the presence of a certain amount of the melanophore stimulant is of no consequence and does not prevent the activity of the anterior lobe secretion. The presence of the latter can be detected by the inducement of metamorphosis, providing there is at least a minimal amount

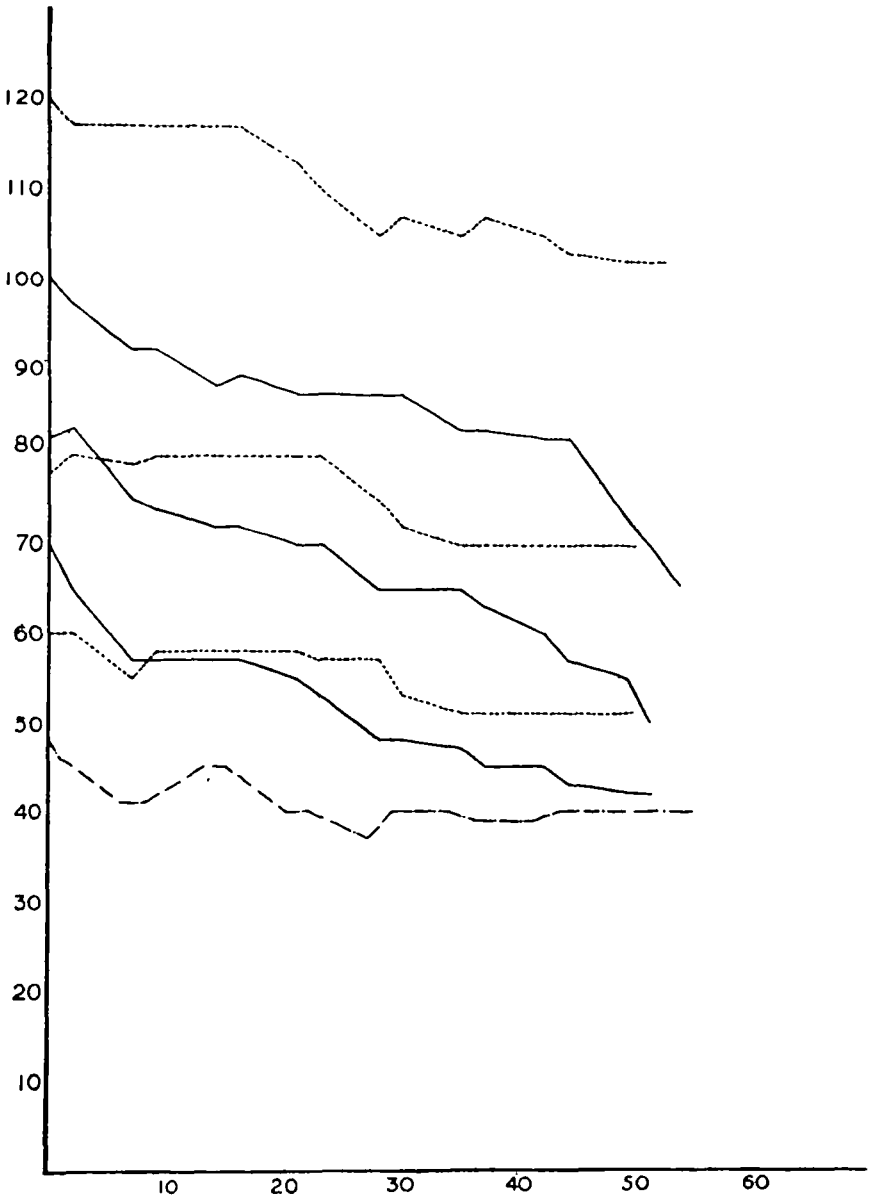
of free principle available. Further, it seems probable that the actual factor which inhibits the action of the anterior lobe and which has already been discussed is not identical with the melanophore stimulant, in fact, the latter may have very little, if any, counteracting influence. What this factor actually is, and its effect, is impossible to say from these experiments and further additions to our knowledge will be necessary before any light can be thrown upon the problem. Even so the presence of this particular factor below a certain quantity does not effect the transformation of larval Amphibians to adults. A definite value cannot be assigned to it yet, but in these experiments No. 7 is successful although this principle and pigmentation factor are present, whilst other extracts fail even when a double dose is taken and may even prove fatal, the amount of pigmentation factor present being smaller. There is little definite knowledge regarding activities of different factors in the pituitary body. They have never been isolated but their existence is assumed from experimental observations, and in this case it seems quite justifiable to conclude that these factors do exist, and further, that the pigmentation factor is not identical with that factor which seems mainly responsible for the inhibition of the anterior lobe secretion, when these results are reviewed.

In conclusion, I wish to acknowledge supplies of anterior lobe from manufacturing chemists which enabled me successfully to carry out this investigation.

Table showing (*a*) lengths and weights of animals at various stages of treatment with No. 7 at different temperatures, and (*b*) lengths and weights of controls at same stages :—

Temp.	Length.	Weight—1st Day.	Weight—15th Day.	Weight—30th Day.	Weight—Metamorphosis complete.
	cms.	gms.	gms.	gms.	gms.
20° C.	16·5	48	45	40	38
24°	17·8	70	57	48	42
24°	21	82	72	65	53
24°	21·6	100	89	87	66
22°	20·3	83	67	64	62
Controls.					Weight—Injections stopped.
24°	17·8	60	58	53	51
24°	19	78	80	72	70
24°	24·1	120	117	107	102

Injection of Pituitary Body Extracts to Axolotls



Curves showing decrease in weight of individuals during period of injection with No. 7 extract of anterior lobe until complete metamorphosis at various temperatures, and curves showing weight of controls injected with Ringer's during the same period.

Control at 24° C. – – – – – –

Pituitary injection at 24° C. ————————

Pituitary injection at 20° C. —— · —— · —— · —— ·

Table showing lengths and weights at same stages of individuals treated with other extracts :—

Temp.	Injection.	Length.	Weight— 1st Day.	Weight— 15th Day.	Weight— 30th Day.	Weight— Injections stopped.
		cms.	gms.	gms.	gms.	gms.
20° C.	1	17·8	60	58	63	66
20°	2	19·7	70	70	68	80
20°	3	16·5	40	42	42	41
20°	4	18·4	65	66	65	68
20°	5	19	73	71	72	72
20°	6	18·4	65	62	58	57
20°	8	28·6	136	135	150	...
20°	9	17·5	56	56	58	58
20°	10	19	63	68	67	68
20°	11	18·4	55	64	70	...
20°	12	20 3	80	77	78	80
20°	13	20·3	77	79	80	84
20°	14	19·7	75	76	84	92
20°	15	21	88	88	89	91
20°	16	18·4	60	61	63	66
20°	17	19	63	63	66	69
20°	18	21	89	88	83	90

Table showing lengths and weights at same stages as before of individuals treated with extracts of twice the concentration and at higher temperature :—

Temp.	Injection.	Length.	Weight— 1st Day.	Weight— 15th Day.	Weight— Injections stopped.
		cms.	gms.	gms.	gms.
24° C.	1	21·6	80	85	82
24°	3	17·8	54	50	49
24°	5	19·7	85	67	67
24°	6	19	62	60	58
24°	9	19	65	56	57
24°	10	20·3	70	...	...

5. References.

Adler (1914), *Arch. f. Ent. Mech.*, 39-40.
Allen (1917), *Biol. Bull.*, 32, 36.
„ (1918), *Journ. Exp. Zool.*, 24.
„ (1920), *Science.*
Atwell (1919), *Science*, 49.
Blair Bell, *The Pituitary.*
Goetsch, E. (1916), *Bull., Johns Hopkins Hosp.*, 27, No. 300.
Gudernatsch, J. (1912), *Arch. f. Ent. Mech.*, 35.
„ (1914), *Amer. Journ. Anst.*, 16.

Hewitt (1914), *Quart. Journ. Exp. Physiol.*, 7.
Hogben (1922), *Proc. Roy. Soc. B.*
„ (1923), *Quart. Journ. Exp. Physiol.*, 13
„ (1924). This *Journal*, vol. 1.
Hogben and Crew (1923). This *Journal*, vol. 1.
Hogben and Winton (1922), *Proc. Roy. Soc. B.*
„ „ (1923), *Proc. Roy. Soc. B.*
„ „ (1922), *Biochem. Journ.*
Hoskins, *Journ. Exp. Zool.*, 21, 29.
Huxley and Hogben (1922), *Proc. Roy. Soc. B.*
M'Cord and Allen, F. (1917), *Journ. Exp. Zool.*, 23.
Morse (1915), *Journ. Biol. Chem.*, 19.
Pearl (1916), *Journ. Biol. Chem.*, 19
Robertson and Ray (1916, 1919), *Journ. Biol. Chem.*
Schäfer (1909), *Proc. Roy. Soc. B.*
„ (1912), *Quart. Journ. Exp. Physiol.*, 5.
Smith (1920), *Amer. Anat. Mem.*, 11.
Spaul (1923), *Proc. Zool. Soc.*
Swingle (1918), *Journ. Exp. Zool.*, 24.
„ (1919), *Journ. Exp. Zool.*, 27.
„ (1919), *Journ. Gen. Physiol.*, 1, 2.
„ (1921), *Journ. Exp. Zool.*, 34.
Uhlenhuth (1919), *Journ. Gen. Physiol.*, 1.
„ (1921), *Journ. Gen. Physiol.*, 3.
„ (1922), *Biol. Bull.*, 42.
Wulzen (1914), *Amer. Journ. Physiol.*