ANTERIOR PITUITARY AND GROWTH IN THE AXOLOTL (*AMBLYSTOMA TIGRINUM* (GREEN) NEOTENIC FORM)

I. THE EFFECTS OF INJECTION OF GROWTH-PROMOTING EXTRACTS OF THE ANTERIOR PITUITARY

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(With Two Text-figures)

I. INTRODUCTION

It is well known that removal of the mammalian pituitary causes inhibition of growth and that the administration of certain extracts of the anterior lobe of the pituitary will induce resumption of growth in hypophysectomized mammals and acceleration of growth in intact mammals. On the other hand, species of *Amblystoma* appear to continue to grow after hypophysectomy, although at a lower rate than before (Blount, 1935), and administration of anterior pituitary to these animals has not given clear results. There are now many preparations of anterior pituitary available which give clear and repeatable results with mammals, and we thought it worth while to investigate their effects upon growth in intact axolotls and to see whether factors influencing growth also affected metamorphosis.

The problem of the growth-promoting action of the anterior pituitary in amphibia has been investigated by feeding pieces of anterior lobe (Smith, 1918, 1920; Uhlenhuth, 1921), by injection (Smith & Smith, 1923; Burns & Buyse, 1931) and by transplantation (Swingle, 1921; Allen, 1928; Burns, 1930; Burns & Buyse, 1931; Blount, 1935). Smith (on tadpoles) and Uhlenhuth (*Amblystoma tigrinum* and *A. opacum*, both after metamorphosis) obtained an increased growth rate after feeding anterior lobe, but since feeding pituitary gives negative results with mammals, it appears that this method of administration is not likely to prove the most fruitful in investigating the action of the gland in Amphibia. The effect of transplantation of the pituitary has been extensively investigated by Blount (1935), who found that transplantation of two extra pituitary rudiments from donors of the same species (*A. punctatum*) caused a partial inhibition of growth, while Swingle (1921) and Burns (1930) found that implants of adult anterior lobe produced a slight increase in the growth rate.

It seemed to us that the most satisfactory method of investigating the problem would be by the injection of extracts, since Smith & Smith (1923) obtained growth stimulation by injection of saline extracts of anterior lobe into tadpoles, and also because this method permits of adequate control and of more accurate quantitative work than either of the others.

We have used weight as a measure of the growth, but realizing that weight may be an imperfect measure we have also photographed our animals under standard conditions at regular intervals. The main objection to taking increase in weight as a criterion of growth is that experimental treatment may produce profound alterations or deformities in the body shape of the animals which cannot be called "growth" in the normal sense. In our experiments, the animals have preserved their normal proportions throughout, and we consider our use of weight as an index of growth as completely justified. We have chosen the following extracts for injection: (*a*) Schockhaert's (1931 *a*), which is simply a saline suspension of anterior lobe; (*b*) Van Dyke & Wallen-Lawrence's (1930) phyone, a more purified preparation giving marked growth effects in mammals; and (*c*) prolactin (Bates & Riddle, 1935), which may be a component of the growth-promoting complex, if this eventually proves to be a mixture of several hormones. The experiment has been controlled in two senses, first, by treating each animal as an individual and establishing its growth rate before and after injection, and secondly, by running a batch of animals injected with muscle extract parallel with those receiving anterior pituitary.

II. MATERIALS AND METHODS

(*a*) *Animals.* The axolotls were bred in the laboratory, and were the progeny of two pairs of adult animals only. Larvae hatched between 15 and 19 June 1936 and were fed on mixed Protozoa, and later on *Daphnia.* As soon as possible the animals were trained to take small pieces of food offered to them with forceps, and until the experiments were started living enchytrae were put into the tanks twice weekly as well. 120 animals were reared. We wish to express our thanks to Miss R. M. Renton for giving us the newly hatched larvae and also for her advice as to how they should be reared.

(*b*) *Extracts.* The following extracts were used in the experiments:

(1) Extract of ox muscle.

(2) Schockaert's (1931 *a*) extract of anterior pituitary.

(3) Van Dyke and Wallen-Lawrence's (1930) growth extract of anterior pituitary (phyone).

(4) Prolactin (Bates & Riddle, 1935).

The anterior lobes of ox pituitaries were used in the preparation of extracts 2, 3 and 4.

Schockaert's extract was made according to the instructions given in his paper (1931 *a*). The essentials of the method are the grinding of the sterilized anterior lobes with sand, followed by a 10 min. extraction with saline. The liquid was decanted after centrifugation, and the strength adjusted so that 1 ml. of extract

was equivalent to 600 mg. of fresh anterior lobe. This extract was prepared freshly every week and was kept frozen solid until just before use, when it was melted and diluted with twice its volume of saline, so that the liquid injected contained the equivalent of 200 mg. of fresh anterior lobe per ml. Since there is a possibility that the concentrations of different hormones in the anterior pituitary may vary with the season, we would point out that the glands used in this preparation were obtained between 25 November and 10 February.

The muscle extract was prepared in exactly the same way as Schockaert's extract, but ox muscle was substituted for anterior pituitary.

Growth hormone was prepared according to the method of Van Dyke & Wallen-Lawrence (1930). This consists of extracting anterior lobes of ox pituitaries with dilute caustic soda at 0° C., precipitating the hormone at *p*H 7·2 and purifying it by repeated precipitation with sodium sulphate. The precipitated growth hormone is finally dissolved at *p*H 7·5 to make a solution containing the equivalent of 200 mg. of fresh anterior lobe per ml. The extract was stored in small quantities in sealed glass tubes, the contents of which were kept frozen until immediately before use.

The prolactin was prepared by iso-electric precipitation from an alkaline alcoholic extract of anterior lobe. A mixture of prolactin, thyrotrophic and gonadotrophic hormones was first obtained (Bates & Riddle, 1935, p. 367) from which prolactin was separated by precipitation from a solution of the mixed hormones at *p*H 3 to 4 in the presence of sulphates (Bates & Riddle, 1935, p. 368). Before use, the powder so obtained was dissolved in decinormal caustic soda, the *p*H brought to between 7·4 and 7·6 with hydrochloric acid, and the solution diluted with saline so that 1 ml. contained the equivalent of the amount of prolactin in 200 mg. of fresh anterior pituitary. The glands used in the preparation of the growth extract and prolactin were obtained in November and January.

(*c*) *Food.* The animals were fed on liver, beef and chopped worms from which the gut contents had not been removed. They were given pieces of food held in forceps and at every meal time food was offered until they either refused it or expelled it without swallowing. They were fed twice daily, once in the morning with either beef or liver and again in the evening with beef or liver followed by worm. Usually liver was fed for four days and beef for three each week. We believe that the animals were thus kept in a state approaching repletion throughout the experiment.

It is obviously important in experiments on growth, that the food given to the animals shall provide a physiologically complete diet and that, not only shall there be an adequate supply of necessary vitamins and mineral salts, but also that shortage or absence of an essential amino acid be avoided. The mixture of foods given was chosen with this in view. Patch (1927) has shown that in late larval life, neither liver nor beef alone is adequate for healthy growth in *A. tigrinum*. Liver feeding alone causes loss of appetite and a weakening of normal peristaltic action of the gut, while animals given beef alone grow rapidly but show signs of mineral deficiency, and especially of a calcium lack which may lead to a tetanic condition. Patch found that best growth of all was obtained on a diet consisting of the two meats, together with earthworms, with their cuticle broken and the contents of their guts removed. We

have not thought it necessary to remove the contents of the guts of earthworms fed to our animals, but otherwise the diet given was similar to that of Patch and confirmed her observations that upon it the animals remain healthy and grow satisfactorily.

While we consider the mixture of foods an essential part of our technique, we now know that feeding to repletion every day is unnecessary. Digestion is very slow in the axolotl: an individual piece of meat remains in the alimentary canal for at least a week before it is completely digested, wherefore an axolotl which has not been fed for a period up to a week is still receiving nourishment and is not starving. Feeding to repletion on alternate days would have been adequate.

We have not attempted to discover the conditions, for example with regard to food, temperature, number of animals per unit volume of water, etc., necessary for maximum growth, and it is probable that in these experiments no individual animal attained the rate of growth possible to it under optimal conditions. The experiments were, however, carefully controlled and the control animals have shown a regular increase in weight throughout.

(*d*) *Dosage.* The extracts were administered by intraperitoneal injection. Preliminary experiments with Schockaert's (1931 *a*) extract showed that injection of the equivalent of 1 mg. of fresh tissue per gram of axolotl per day caused a marked increase in the rate of growth and this dosage was used throughout the experiments. We have no evidence that this dose is not supramaximal. Daily administration of the extract prevented the animals from feeding and accordingly injections were made thrice weekly, Tuesday and Thursday doses containing the equivalent of 2 mg., while the Saturday dose contained the equivalent of 3 mg. of fresh gland per gram of axolotl. The strength of all extracts was adjusted so that each ml. contained the equivalent of 200 mg. of fresh tissue and hence, with the exception of one experiment (tank 5), animals of the same weight were injected with the same volume of fluid. For tank 5, equal volumes of phyone and prolactin solutions were mixed, and each animal given a double dose of this fluid. The doses were readjusted after each weekly weighing.

(*e*) *Experiments.* When the 120 animals reared were 9 weeks old (weight about 5 g. and length from head to tip of tail about 9 cm.), i.e. on 20 August 1936, the fifty which had increased in weight most rapidly were selected and divided into five groups of ten animals each. Since there were fairly considerable differences in growth rate even among these fifty, as far as possible each batch was set up to contain animals covering the same range of growth rates. Each batch was kept in a cylindrical glass tank, 32 cm. in diameter and 22 cm. high. The amount of water in each tank was kept constant at 6 l. throughout the experiment, giving a depth of 7·5 cm. Allee (1931) has shown that the number of animals per unit volume of water may have either a stimulating or retarding action on growth, according to the concentration of animals. By keeping the volume of water constant in all the different tanks, we hope to have eliminated this factor. The tanks were kept side by side on a bench under approximately identical conditions of illumination in a room whose temperature fluctuated between 15 and 20° C.

It was found that if ten animals were kept together under these conditions, they were liable to eat portions of each other, but that if the tanks were subdivided into smaller compartments this habit was circumvented. Accordingly, throughout the experiments described here, ten animals were kept in one tank in 6 l. of water, but the tanks were subdivided into three equal compartments by varnished perforated iron partitions and the animals were segregated into one group of four and two groups of three. The water was changed daily.

The animals in these tanks were fed as described above and were weighed on Wednesday each week. Each one was taken out of the water and carefully dried with a linen cloth before weighing. In order to test the accuracy of the method, animals were treated in this manner once, replaced in water, and then the whole process repeated. It was found that the weights so obtained agreed within 1 % with animals of 2 g. and more closely with heavier animals.

It proved possible to differentiate between the animals by means of the black and yellow markings on their skin, more especially in the tail region, where the marks remained more or less constant. It was therefore practicable to keep records of the changes in weight of each animal individually. These weekly weighings were carried out on the untreated animals for twelve weeks in order to establish the normal growth rate. At the end of this period the animals were injected with the different extracts as follows:

Tank 1 with meat extract;
Tank 2 with Schockaert's extract;
Tank 3 with growth extract (phyone);
Tank 4 with prolactin;
Tank 5 with prolactin together with growth extract;

but otherwise the treatment was continued as before. The injections were made for a period of 12 weeks. At the end of this time the animals in each of the tanks, 2, 3 and 5, were found to fall into five pairs, both members of a pair having about the same growth rate. In each case, one animal was killed and the other kept alive without further injection, so that the after-effects could be investigated on a representative half of the animals. Similarly, injections of prolactin were continued with half the animals in tank 4, while the other half were left untreated. The weighings were continued for a further 13 weeks on the remaining animals.

During the whole period of the experiments the animals were photographed under standard conditions every four weeks.

III. RESULTS

(*a*) *The effects of the injections*

The effect of the injection of the different extracts upon the weight of the animals is shown in Figs. 1 and 2. In Fig. 1 the mean weekly weights of each batch of animals is graphed against time, and in Table I these weights are given together with the standard error of the mean.

All the animals showed a slight loss of weight during the first week of injection, but this is a characteristic reaction of the axolotl to any change of a routine to which it has become accustomed, and this loss was immediately followed by a continual increase in weight for the rest of the period.

The axolotls treated with Schockaert's extract reacted very variously to the injections (Fig. 2*a*). Eight of the ten animals grew about as rapidly as those given growth extract, but two of these stopped growing before the end of the injections, one after 9 and the other after 11 weeks' treatment, and the two remaining axolotls grew more slowly than the rest of the group.

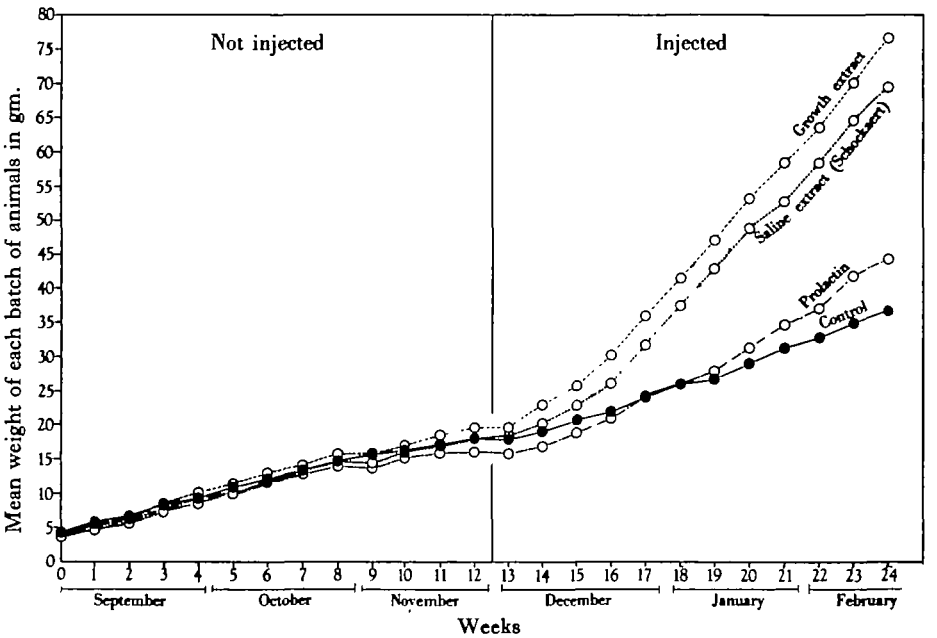

Fig. 1. Mean weekly weights, before and during injection, of sets of ten animals injected with muscle extract (controls), Schockaert's extract, phyone and prolactin respectively.

All except one of this group exhibited some of the changes usually associated with metamorphosis in the axolotl, namely, bulging eyes, growth of eyelids, some reduction in size of the dorsal and ventral fins, and thickening of the gill filaments followed by absorption of the gills; but only the two which did not gain in weight rapidly showed absorption of the entire ventral fin and any marked reduction of the gills: even in these animals there was no complete absorption of the dorsal fin, and after 5 weeks' injection the changes proceeded no further. At post-mortem examination animals of this group were invariably found to have enlarged and very vascular thyroids, and there was evidence of a fracture in about the centre of the shaft of the long bones which had healed with the formation of a cartilaginous callus.

The animals injected with phyone alone, or together with prolactin, gave identical results; they grew very much more rapidly than before and than the controls, but

showed absolutely no change in shape whatsoever. At post-mortem examination, animals of these groups showed no macroscopic abnormalities in any organ, except signs of a healed fracture of the long bones.

Table I. *Mean weekly weights of each batch of* (10) *animals in grams, before and during injection*

Every case where the deviation of the weight of any individual animal from the mean was greater than 2 × S.D. is indicated by an asterisk. In no case did the deviation exceed 3 × S.D.

Date	Controls (muscle injections)	Schockaert injections	Phyone injections	Prolactin injections	Prolactin + phyone injections
2 Sept.	4·2 ± 1·4	3·6 ± 0·7	4·2 ± 1·3	3·9 ± 0·5	4·3 ± 1·2
9	5·7 ± 1·7	4·7 ± 0·8	5·6 ± 1·4	5·1 ± 0·7	5·5 ± 1·4
16	6·6 ± 2·0	5·6 ± 1·1	6·5 ± 1·7	6·2 ± 0·7	6·4 ± 1·5*
23	8·3 ± 2·1	7·2 ± 1·3	8·5 ± 2·0	7·6 ± 0·7	7·9 ± 1·4*
30	9·3 ± 2·6	8·5 ± 1·7	10·1 ± 2·4	9·1 ± 0·8*	9·4 ± 2·2*
7 Oct.	10·8 ± 2·7	9·9 ± 2·0	11·4 ± 2·6	10·1 ± 0·7	10·8 ± 2·4*
14	12·1 ± 2·6	11·6 ± 2·1	13·0 ± 2·6	11·6 ± 1·1*	12·0 ± 2·4*
21	13·3 ± 2·8*	13·1 ± 2·3	14·1 ± 2·6	12·9 ± 1·3	13·3 ± 2·6*
28	14·6 ± 2·8	14·6 ± 2·7	15·7 ± 2·5	14·0 ± 1·5	14·4 ± 2·6*
4 Nov.	15·6 ± 3·1	14·4 ± 2·5*	15·7 ± 2·4	13·7 ± 1·6	14·3 ± 2·5*
11	16·2 ± 3·0	16·0 ± 2·8*	17·0 ± 2·2	15·2 ± 1·5	15·8 ± 2·8*
18	17·1 ± 3·2	16·9 ± 2·9	18·5 ± 2·3	15·8 ± 1·5	15·9 ± 2·6*
25	18·0 ± 3·4	18·1 ± 3·0	19·6 ± 2·8	16·0 ± 1·5	16·7 ± 2·9*
		Injections started			
2 Dec.	17·9 ± 3·3	18·5 ± 3·1	19·6 ± 2·5*	15·7 ± 1·7	17·0 ± 2·8
9	19·0 ± 3·6	20·2 ± 3·2*	23·0 ± 3·2	16·8 ± 1·9	19·4 ± 3·4
16	20·7 ± 4·2	22·9 ± 3·9	25·7 ± 3·8	18·8 ± 1·8*	21·9 ± 3·0
23	21·9 ± 4·7	26·1 ± 4·7	30·2 ± 4·1	21·0 ± 2·2	25·8 ± 4·0*
30	24·1 ± 5·5	31·7 ± 6·4	35·9 ± 4·5	24·3 ± 2·4	31·5 ± 4·6
6 Jan.	26·0 ± 5·7	37·5 ± 8·4	41·5 ± 5·5*	25·9 ± 2·2	37·2 ± 5·7
13	26·7 ± 5·7	42·9 ± 8·1	47·1 ± 6·2	27·9 ± 2·1	41·6 ± 6·7
20	29·0 ± 6·1	48·9 ± 10·2	53·2 ± 8·1	31·3 ± 2·4	48·3 ± 7·2
27	31·3 ± 6·4	52·9 ± 11·2	58·5 ± 8·1	34·7 ± 3·3	58·3 ± 7·9
3 Feb.	32·8 ± 6·0	58·5 ± 12·9	63·6 ± 8·9	37·1 ± 3·3	57·2 ± 8·1
10	34·9 ± 6·8*	64·7 ± 16·0	70·2 ± 9·6	41·8 ± 3·8	64·3 ± 10·2
17	36·7 ± 6·9	69·5 ± 18·6	76·6 ± 11·2	44·4 ± 4·0	68·1 ± 10·5*

The axolotls treated with prolactin grew somewhat faster than did the controls, but the acceleration in the growth rate was slight compared with that caused either by phyone or Schockaert's extract. No abnormalities were found post-mortem.

Apart from a slight reduction in the length of the gills, an enlarged photograph of a given animal taken at the beginning of the experiment could be accurately superimposed on one taken at the end, hence none of the experimental animals showed any of the symptoms usually associated with the injection of growth-promoting hormones. There was no change in the shape of the head along any axis, and there were no swellings at the limb joints. It has been shown (Howes, 1938) that for a long period in late larval growth the axolotl undergoes no change in bodily proportions, and we consider that the more rapid increases in weight induced in these experiments were due to simple acceleration of a normal process.

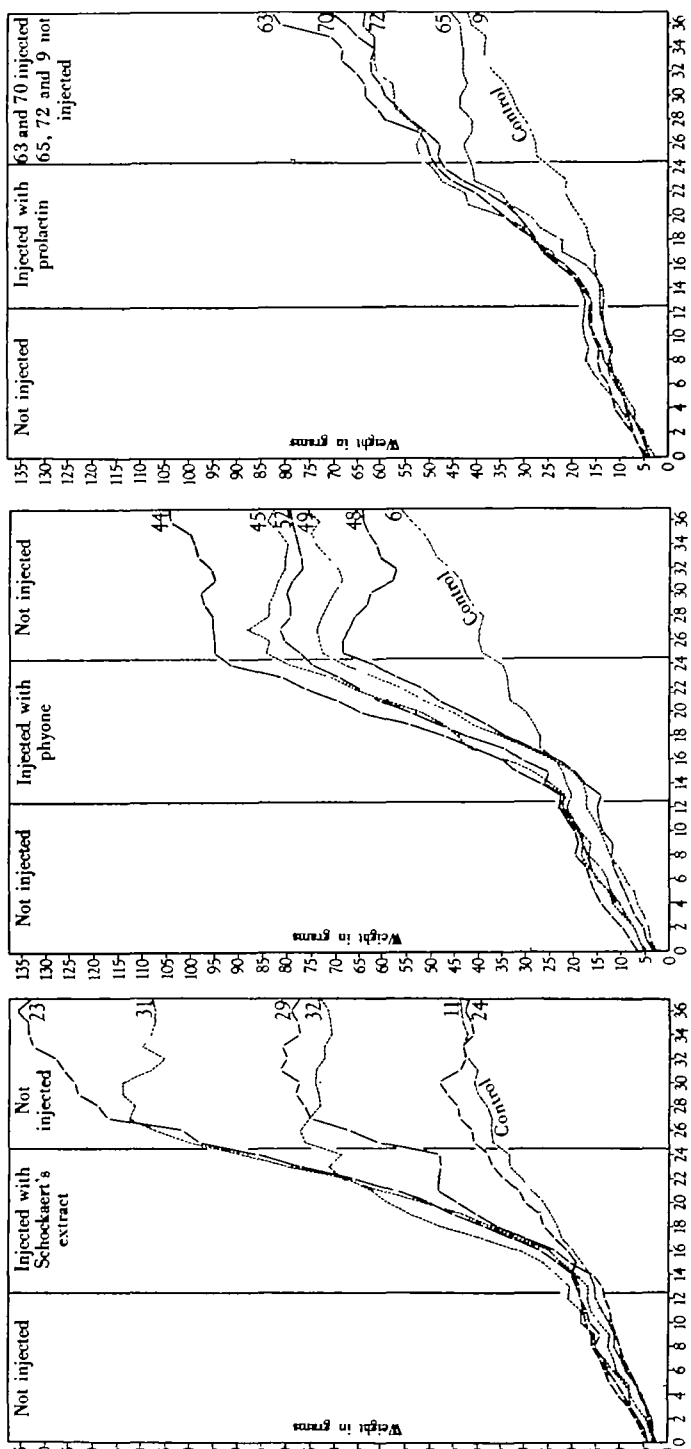

Fig. 2. Curves showing the changes in weight of individual axolotls before, during and after injection with (*a*) Schockaert's extract; (*b*) phyone; (*c*) prolactin. In each case the weights of a single control (Nos. 11, 6 and 9) are included for comparison.

(*b*) *The after-effects of the injections*

The axolotls which were kept alive after the injections were stopped, showed interesting after-effects (Fig. 2). Those given Schockaert's extract continued to grow at the rate established during injection for about 3 weeks; then in four cases the animal lost weight and did not grow again during the remaining weeks in which the experiment was continued. The fifth animal grew at its established rate for 3 weeks, after which the rate of growth gradually fell off.

Eight of the ten animals given phyone, with or without prolactin, continued for 1 week only at their accelerated growth rate. After this their weight either decreased or remained stationary for from 6 to 9 weeks, and growth was subsequently resumed at a rate approximately equal to that of the animal before injection. The remaining animals showed similar phenomena with different time relationships.

The prolactin-injected animals gradually resumed their pre-injection growth rate.

IV. DISCUSSION

Before discussing the effects of injection, it is necessary to consider what hormones are likely to be contained in the extracts. Schockaert's preparation is partly a suspension of finely ground cellular material of the anterior pituitary and probably also an extract. It may therefore be assumed to contain all the hormones present in the gland, although not necessarily in their natural proportions. Further, there is strong likelihood that any hormone in this extract will occur normally in the anterior pituitary, since the tissue is not treated with hydrolysing agents such as acid or alkali, and autolysis is reduced to a minimum by keeping the extract frozen and by making a fresh preparation each week. Suspensions of anterior pituitary promote growth in hypophysectomized and in thyroidless tadpoles (Smith & Smith, 1923) and in rats, both intact (Evans & Long, 1921) and hypophysectomized (Smith, 1930). Schockaert (1931*b*) found that his extract caused hyperplasia of the thyroid in ducks and (1931*a*) stimulated testicular growth, and Bellerby (1933) found that ovulation without oviposition could be induced by injection of saline suspensions of anterior lobe into the common frog. Schockaert's extract may therefore be assumed to contain growth-promoting, thyrotrophic and gonad-stimulating substances. The action of the last is not clear, since Smith (1930) showed that while a saline suspension of fresh anterior lobe restored the normal rate of growth of rats dwarfed by hypophysectomy, it had no restorative effect upon their atrophied reproductive organs; and Evans & Long (1921) found that saline suspensions, while causing increased growth, delayed sexual maturity in that animal. Our results confirm previous findings in that the extract had growth-promoting and thyrotrophic actions on the axolotls; our data are inadequate for any decision to be made on its gonad-stimulating action, but there was no obvious indication of enlargement or activity of the gonads. Possibly such changes can only be determined on histological examination. Burns & Buyse, in *A. tigrinum*, found macroscopic changes in the testes but not in the ovaries after injection, although histological examination revealed effects in the gonads of both sexes.

Phyone also contains growth-promoting and thyroid-stimulating hormones. Adams (1934) and Adams & Martindale (1936) found phyone to have a thyrotrophic action and to stimulate ovulation in *Triturus viridescens*. Schockaert (1931 *a*, *b*) found phyone to induce both histological changes and increases in weight in the thyroid of ducks, and to stimulate the growth of their testes, but to have no effect upon their ovaries. Riddle *et al.* (1932), using pigeons, found phyone to contain traces of prolactin and thyrotrophic hormone. Hertz *et al.* (1932) found phyone to produce marked follicular enlargement and extensive luteinization in the ovaries of juvenile rabbits, but to be without effect upon the ovaries of immature rats. Our results give no indication of either a thyrotrophic or gonadotrophic action of phyone on axolotls.

The preparation of prolactin was probably free from both thyrotrophic and gonad-stimulating hormones.

The experiments described above are thus comparisons between the effects of mixtures of growth-promoting, thyroid-stimulating, gonadotrophic and lactogenic hormones in three different proportions and of prolactin alone. The salient features of the results are: (*a*) the growth-promoting effects of all the extracts, together with (*b*) the complete absence of change in proportion of the different organs of the experimental animals, i.e. normal growth and no appearance of the specific arthritis of acromegaly; (*c*) the absence of any gonad-stimulating action; (*d*) the varying effects of Schockaert's extract on individual animals; (*e*) the suppression of growth as an after-effect of injection of Schockaert's extract and of phyone.

The injection of saline or alkaline extracts of anterior pituitary or of growth hormone is frequently described as causing "acromegalic symptoms" in intact animals. Burns & Buyse (1931) state that injection of simple alkaline aqueous extracts of macerated anterior lobe into *A. tigrinum* caused "a noticeable acromegaly" in some experimental animals, but it is questionable whether the term "acromegaly" is aptly applied to skeletal changes induced by the injections, since there are no changes in the skin and soft parts of axolotls resembling those in human acromegaly. It seems clear that the skeletal response is usually one of bony overgrowth and widening changes in the skull (Burns, 1934; Evans *et al.* 1933). Since we anticipated responses of this nature in our experimental animals, they were closely watched for symptoms and were photographed at 4-weekly intervals during the 37 weeks of the experiment. Our results are at variance with those of Burns & Buyse and of Burns (1934) with implantation experiments, in that we obtained no widening of the skull and no prognathism whatsoever. How far this is due to the nature of our extracts, to the environmental conditions under which our experiments were carried out, e.g. temperature, feeding, chemical composition of the water in which the animals lived or to the genetic constitution of our axolotls, it is impossible to say. That this last factor may be of importance is suggested by the work of Evans *et al.* (1933) who showed that different breeds of dogs responded differently to injections of the same growth hormone.

The injection of prolactin also accelerated the rate of growth but not to such a great degree as phyone alone or plus prolactin. This agrees with the results of

Bates *et al.* (1935) and of Kemp & Marx (1936), who worked with hereditary dwarf mice. Our doses were standardized in relation to the amount of dry prolactin equivalent to a given weight of fresh pituitary tissue and hence do not afford any information as to the relative potency of prolactin as a growth-promoting substance. The injection of prolactin together with phyone caused no further increase of growth rate over that induced by phyone alone; we attribute this result to the dosage of phyone being already maximal.

The varying effects of Schockaert's extract, ranging from growth stimulation strictly comparable with that obtained with phyone, to scarcely any effect upon growth and an obvious tendency towards metamorphosis, are interesting. We are of the opinion that by manipulating the experimental conditions it would have been possible to enhance either the metamorphic or growth-promoting effect of this extract. Spaul (1924) suggested that there was some relationship between rate of metamorphosis and temperature. He found that most rapid metamorphosis occurred between 21 and 23° C. and that there was appreciable retardation below 17° C., although Huxley & Hogben (1922) metamorphosed animals between 8 and 15° C. It will be remembered that the temperature during our experiments fluctuated between 15 and 20° C. The inverse correlation between rate of growth and the magnitude of the metamorphic signs shown by our animals, is in agreement with the findings of Spaul that axolotls lost weight during metamorphosis.

When an extract of anterior pituitary containing a mixture of hormones is injected into an axolotl with its own pituitary intact, it seems probable that the effects obtained will be considerably influenced by the degree of deviation of the proportions of the hormones in the extract from the proportions of the same hormones being secreted by the animal's own pituitary. Thus, if the mixture injected into a growing axolotl approximates closely in composition to the secretions of the animal's own gland, then it is to be expected that growth will continue at an enhanced rate but that there will be no changes in proportion of any organ or organs, e.g. no widening of the head or prognathism. It is clear that the composition of the animal's own secretion will vary slightly from animal to animal: the discrepancy between this and the injected material will therefore also vary. We would suggest that Schockaert's extract contains an excess of thyrotrophic hormone as compared with the animals' own secretions, and therefore induces hyperactivity in the thyroid gland to a degree varying from animal to animal. If this hyperactivity causes an increase in the secretions of the thyroid over a threshold concentration, then metamorphic signs will appear and a further increase might even cause complete metamorphosis. Similarly, we believe that the composition of our preparation of phyone resembled the secretions of the animals' own glands closely enough to produce accelerated growth without abnormality.

The recent work of Bates and Riddle and their co-workers (Bates *et al.* 1935; Bates *et al.* 1937) has led to considerable doubt as to the existence of a hormone acting directly upon the cells as a growth stimulant, i.e. as a growth hormone in the strictest sense, and suggests that the growth-promoting action of anterior pituitary extracts may be due, at any rate in part, to hormones acting through other endocrine

glands. These workers have also shown that highly purified preparations of prolactin "give good body growth in pigeons and but little in dwarf mice, while thyrotrophic hormone decreases body weight in pigeons and increases it in dwarf mice" (Bates *et al.* 1937, p. 603). There are thus differences in the growth reaction between different species, especially in relation to prolactin and to thyrotrophic hormone; but since Bates *et al.* (1935) have shown that prolactin and thyrotrophic hormone have a synergistic action upon body growth, it may be assumed that these two hormones, at least, are necessary constituents of any extract producing accelerated "normal" growth. The very existence of the thyrotrophic hormone as a separate entity implies that if growth-promoting substances not containing this hormone are injected into animals which then grow at an increased rate, these animals will, in effect, suffer from hyposecretion of their thyroids. Adams & Martindale (1936) injected newts with dilute phyone and found that after 20 daily injections the thyroids of these animals became hyperplastic and depleted of colloid, but that after 30 days the response of the glands was less. In our experience there is always a latent period (more than 21 days in sexually mature animals (Clements & Howes, unpublished)) between the beginning of injections of phyone and the first evidence of accelerated growth. It seems to us that the results of Adams & Martindale may be explained by the existence of a longer latent period in the reaction of the animals to prolactin than to thyrotrophic hormone, so that at first there was hyperplasia of the thyroid but when growth of the rest of the body commenced, the greater activity of that gland was balanced by the increase in growth rate. This agrees with our findings that animals injected with phyone for 12 weeks showed no enlargement of the thyroids.

We therefore tentatively conclude that our results may be interpreted as showing the effects of different proportions of prolactin and thyrotrophic hormone on growth in the axolotl. Prolactin does not fully accelerate growth in the absence of thyrotrophic hormone, but when these two are combined in the proportions in which they are present in phyone, they promote normal growth. There is an excess of thyrotrophic hormone in Schockaert's extract and hence the animals show a tendency to metamorphose.

The effects upon weight changes when the administration of growth-promoting extracts is stopped is striking, as are also the different after-effects of Schockaert's extract as compared with those of phyone. Evans & Simpson (1931), working with rats, found a similar effect after administration of growth hormone: when daily injections were stopped, there was a rapid fall in weight for the first 10–15 days, followed by a continued gradual loss. We attribute our effect to the production of anti-hormone by the animals. Collip & Anderson (1935) have postulated that for each hormone "there is an opposite, an antagonistic or antihormone substance", and that "the absolute amount of a hormone and its respective antagonist determines the degree of stability of the subject as far as this particular endocrine function is concerned". We interpret the after-effect of the injection of phyone into axolotls as being due to a compensatory output of anti-growth substance in response to the presence of an excess of growth-promoting substance owing to the injections. The

action of the growth-promoting substances persists for some time after injections are stopped, but owing to the production of an excess of anti-growth substance which more than neutralizes the growth-hormone secreted by the animals' own glands, a period ensues during which weight is actually lost. The later re-establishment of the original growth rate may be due either to a compensatory hypersecretion of the anterior lobe or to a diminishing production of anti-growth substance. The more varied after-effects of the injection of Schockaert's extract may be attributed to the production of several anti-hormones and hence to a phenomenon resembling that of competition of antigens.

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V. SUMMARY

1. The effects of injection of (*a*) Schockaert's extract of anterior pituitary, (*b*) Van Dyke & Wallen-Lawrence's phyone and (*c*) prolactin, upon the growth of larval neotenic *Amblystoma tigrinum*, have been investigated. The normal growth-rate was established before injections were begun and controls were injected with muscle extract.

2. The dosage adopted in all cases was equivalent to 1 mg. of fresh tissue per gram of axolotl per day. This was probably supramaximal.

3. Schockaert's extract and phyone caused a marked increase in the rate of growth, prolactin a slight increase.

4. Of the ten animals injected with Schockaert's extract, all except one showed some of the signs usually associated with metamorphosis in *A. tigrinum*. The other experimental animals did not.

5. In no case were there any abnormal changes in the bodily proportions or in the shape of the skeleton.

6. The growth of half the animals after injections had been stopped was also investigated. In the case of most of the animals injected with Schockaert's extract or with phyone, there was a loss of weight, followed by recovery and growth in the case of the latter only. The prolactin-injected animals showed no such reaction.

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