LXXXI. HORMONES OF THE ANTERIOR PITUITARY LOBE.

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It has been recognised for some considerable time that the anterior lobe of the pituitary gland plays an important part in the organism. Disturbances of growth (gigantism, dwarfism, acromegaly) and the sexual system (dystrophia adiposo genitalis) have been ascribed to faulty functioning of the anterior pituitary.

Evans and Long [1923-1924] were first to obtain from the anterior lobe substances which affected growth and the female reproductive cycle. They found that suspensions of anterior pituitary lobes ground up with saline, when injected intraperitoneally, produced increase in the post-pubertal growth of rats and inhibited the female oestral cycle. This inhibition of oestrus has been confirmed by Bellerby [1928], using an opalescent suspension obtained by grinding up anterior pituitary lobes with alkali, neutralising and freeing from cell debris by centrifuging. These suspensions also interfere with the normal birth mechanism [Teel, 1926]. Clear sterile extracts obtained by filtering the suspensions through a bacterial filter produced increase in growth [Putnam, Teel and Benedict, 1928] but were not carefully examined further.

On the other hand, when fresh anterior pituitary lobes are transplanted into females, immature animals are brought to premature sexual maturity and display oestrus within a few days [Smith, 1927; Zondek and Ascheim, 1927]. This same premature maturity is also produced by injections of urine of pregnancy and has been suggested as a test for pregnancy [Zondek and Ascheim, 1928]. These results are confirmed by Evans and Simpson [1928], who claim that the hormone affecting increase in the rate of growth and inhibition of oestrus is not only different from that producing premature sexual maturity but is definitely antagonistic to it. Further, they state that the hormone producing premature sexual maturity is obtained from anterior lobes of pituitary glands by acid extraction, but they give no experimental details.

Briefly then the following effects are ascribed to the anterior pituitary lobe: (1) growth-promoting and oestrus-inhibiting (alkaline extracts of anterior pituitary lobes); (2) premature sexual maturity-producing (occurring in urine of pregnancy; produced by implantation of anterior pituitary lobes and by undescribed acid extracts of the lobes).

This communication details preliminary attempts to repeat and amplify some of the work described above.

Filtered alkaline extracts.

Ox anterior pituitary lobes were worked up a few hours after death of the animal. The author is deeply indebted to Mr Vallet and Messrs Oppenheimer, Son and Co. for the gift of the glands.

On each occasion 50 ox anterior lobes weighing about 75 g. were ground up with sterile sand in a sterile mortar and a little water was added to make a paste, then 100 cc. of 0.1 *N* sodium hydroxide were added and the mixture was well shaken. After standing in the ice-chest for 18 hours the mixture was carefully neutralised to phenol red by addition of 0.1 *N* hydrochloric acid. The suspension was centrifuged for an hour and the supernatant fluid pipetted off and filtered through a sterile 14 cm. Seitz filter; this filtration was slow and continued in an ice-chest at reduced pressure overnight. In this way a clear yellow or pink-brown liquid was obtained and transferred to 1 cc. ampoules. Such extracts retained their activity when kept in an ice-chest for at least 6 weeks.

1 cc. of each extract was injected daily (Saturdays and Sundays being excepted) intraperitoneally into sexually mature, female white rats weighing from 128–146 g. (no toxic symptoms or abscesses were observed even after 6 months' treatment). Each animal was at first maintained on a separate extract, but later when the growth-promoting properties of each extract had been proved in this way all the animals were treated with the same extracts. The animals were weighed daily and the vaginal smears examined daily. Small cotton-wool swabs, wound on small wire loops, were inserted carefully into the vagina and the smear was transferred to a warmed microscope slide and stained with Sebrazi's stain, being covered with a cover slip and examined whilst the stain was still moist. This method was found most satisfactory for rapid cytology.

When injected the rats immediately began to grow more rapidly. On the average the injected rats grew at the rate of over 12 g. per week whilst control animals never grew at a rate exceeding 6 g. per week, the average growth being about 3 g., and the usual rate of adult animals is much less (0.2–2 g. per week). The actual figures obtained are given in Table I and the weekly growth curves in Fig. 1. It will be seen that one injected rat weighed 300 g. whilst the heaviest control animal did not exceed 180 g.

The animals were not injected at the week-ends and after every week-end a drop in weight of 4 to 16 g. was observed. During Christmas week the animals were not injected for 6 days and the weights fell on an average 23 g. (extremes 21–26 g.); weight was regained immediately injections were continued. Now it is known that when the pituitary gland is removed from tadpoles the thyroid gland atrophies and almost completely disappears, and in acromegaly the thyroid gland is frequently increased in size. This suggests a close relationship between the pituitary and thyroid glands, and it is suggested that the injection of anterior pituitary lobe extracts stimulates the

thyroid gland, and that this stimulation to over-activity persists for a time after discontinuance of injections, thus resulting in increased basal metabolism and hence reducing the weight by increased combustion. Despite this marked effect on growth no cessation of oestral cycles was observed. Vaginal smears demonstrated normal recurrence of oestrus in the female rats.

Table I. *Rat weights.*

<i>Injected Series:</i>		October				November					December				Jan. 5
Rat No.	Ex-tract No.	5	12	19	26	2	9	16	23	30	7	14	21*	28*	1929
1	5	128	142	159	162	174	192	190	195†	215	232	253	266	242	270
2	6	—	146	156	170	184	199	214	235	248†	266	274	290	268	298
3	7	—	—	137	150	158	177	192	200	209†	230	246	258	232	269
4	8	—	—	—	144	168	185	190	202	213	222†	232	246	225	253
<i>Control Series:</i>															
5	—	102	110	115	118	122	127	128	133	133	141	144	142	149	145
6	—	102	112	119	122	130	137	—	—	—	—	—	—	—	—
7	—	127	131	146	153	160	157	156	158	163	162	169	165	175	182
8	—	142	151	153	156	158	161	162	162	165	166	173	176	—	—
9	—	154	157	161	163	163	167	171	175	163	172	174	174	180	180
10	—	152	162	165	163	165	168	173	—	—	—	—	—	—	—
11	—	140	146	152	160	162	173	175	—	—	—	—	—	—	—
* No injections.															
† Rats transferred to Extract A 14

Fig. 1. Mean weekly growth.

In order to facilitate filtration, some of the extracts were rendered definitely acid, filtered and neutralised. This resulted in agglutination of the precipitate and accelerated filtration. The growth-promoting properties of these extracts were, however, variable—one extract promoted good growth

whilst the next did not. The ovaries of animals treated with it were, however, filled with corpora lutea and resembled the so-called "mulberry ovary." These acidified alkaline extracts, therefore, possessed little growth-promoting activity but had a follicle-ripening and corpus luteum-producing effect.

Filtered lime extract.

50 fresh ox anterior lobes were ground up with sand and 300 cc. of saturated lime water were thoroughly mixed with the mash. After standing overnight carbon dioxide was led in and the mixture was shaken until neutral to phenol red. After a further adjustment of the neutrality after standing, the mixture was centrifuged rapidly for 20 minutes and the clear red supernatant fluid was filtered through a sterile Seitz filter. The filtration proceeded rapidly and was complete within a few minutes. This fluid was injected intraperitoneally in 1 cc. daily doses into female rats and was well tolerated. Increased rate of growth was produced with this extract. In one rat the rate of growth for 3 months prior to injection was less than 0.3 g. *per diem*, in the first 23 days after injection the rate had risen to 1.6 g. *per diem*. For a time four other mature rats also received this extract and grew at the increased rate of about 2 g. a day. The oestral cycles were not affected in any animal and proceeded normally. This constitutes a most rapid method of preparing a potent growth-promoting extract without harmful effects to the body-wall (no abscesses produced) but occasionally a potent extract is not obtained.

The solid residue left after extraction, neutralisation and centrifuging was extracted with 100 cc. 0.1 *N* acetic acid. This extract was neutralised and centrifuged yielding an almost colourless clear liquid which filtered rapidly through a small Seitz bacterial filter and yielded an extract which caused increase of growth but no cessation of oestrus. The alkaline extraction, therefore, had not removed all the growth-promoting hormone from the gland paste.

Unfiltered alkaline extracts (A 9, A 10, A 11).

In each case 50 fresh ox anterior lobes were immersed in 40 % alcohol for 10 minutes, rinsed with three changes of 0.9 % saline, ground up with washed sand in a mortar with a little (30 cc.) water and 100 cc. of 0.1 *N* sodium hydroxide; the mixture was allowed to stand in an ice-chest overnight and then neutralised to phenol red with 0.1 *N* hydrochloric acid, all the operations being conducted with precautions for sterility. The neutralised material, which tended always to become more alkaline, was centrifuged rapidly for an hour and the supernatant opalescent pink fluid was mixed with a sterile concentrated aqueous solution containing 1.5 g. of sodium benzoate, and kept in small tubes in an ice-chest. Each day 1 cc. of this suspension was injected intraperitoneally into sexually mature rats. Sterile abscesses tended to form in the abdominal wall, especially when the extracts

remained subcutaneous. The rate of growth was accelerated by these extracts, as shown in Table II. When injections were discontinued an immediate drop in weight after 2 days was observed.

Table II.

Extract No.	No. of animal	Pre-injection		During injection		Post-injection	
		Period days	Mean daily growth g.	Period days	Mean daily growth g.	Period days	Loss in weight g.
9	12	47	0.6	43	1.6	Killed for dissection	
10	13	55	0.7	29	1.7	6	27
11	14	—	—	29	2.1	7	24

Fig. 2. Growth curve of rat 13.

No oestrus was observed in rat 5 (Extract A 9) during the whole 6 weeks of injections, although the pre-injection period was marked by normal oestral cycles of duration 6, 8, 6, 5, 8, 8, 7 days respectively. With rat 6, oestrus was not affected (Extract A 10), whilst with rat 7 (Extract A 11) the smears were mixed, that is to say, on no occasion were purely cornified epithelial cells alone observed in the daily smear, but the continuous appearance of large quantities of polymorphonuclear leucocytes in the smear, as with rat 5, was absent.

Maturity-producing hormone.

Zondek and Ascheim [1928] have shown that urine from pregnant women contains a hormone which produces premature sexual maturity in mice 3 weeks old. Implantation of fresh anterior pituitary lobes has the same effect [Smith, 1927; Zondek and Ascheim, 1927], but no extract of anterior lobes with these effects has been described in any detail.

Urine of pregnancy. 3 weeks old mice (weighing 6–9 g.) and 3 weeks old rats (weighing 30–40 g.) were injected with 5–6 doses in all, during 3 days, of 0.2–1.0 cc. of catheter specimens of urine (rendered slightly acid) obtained from pregnant women. When the animals were killed on the fifth day evidence was obtained in each case of the production of premature sexual maturity; the uterus was enlarged, the ovaries contained haemorrhagic spots and ripe follicles or corpora lutea and the vaginal smear contained cornified epithelial

cells. In the case of the rats the uteri and ovaries were weighed after dissection, and, whilst in the two control litter-mate sisters the weight of the ovaries and uterus together was in each case 0.06 g., in the rats injected with urine of pregnancy the weights were 0.1–0.15 g. (mean = 0.13 g.).

Anterior pituitary lobe extracts. Attempts were now made to obtain the premature maturity-producing hormone from anterior lobes of pituitary glands. As has been described, the growth-producing hormone is obtained by alkaline or neutral extracts and it was anticipated that acid extracts would contain this maturity hormone.

Acid extract. 50 anterior pituitary lobes were ground up with washed sand and a little water, 100 cc. of 0.1 *N* acetic acid were added and the mixture was allowed to stand in the ice-chest until next morning, when it was centrifuged for 30 minutes. The clear red supernatant fluid was rendered neutral to phenol red by addition of 0.1 *N* sodium hydroxide, and filtered through a sterile Seitz bacterial filter. The filtration was completed within a few minutes. This extract produced considerable increase in rate of growth (*e.g.* an injected adult female rat grew 60 g. in 4 weeks) of mature rats but did not inhibit oestrus. It had no apparent effect on 3 weeks old mice when injected intraperitoneally in 5 doses of 0.1–0.4 cc. in the course of 3 days. The uteri remained thread-like and infantile as distinct from litter-mate sisters receiving injections of urine from pregnant women.

Adsorption. The extraction was conducted as described in the previous section, but after centrifuging the clear red supernatant fluid was shaken thoroughly with pulverised acid-washed kaolin and after standing the suspension was centrifuged and the supernatant fluid decanted. One portion was placed in a tested collodion membrane and dialysed against distilled water for a few hours, and the clear colourless dialysate was filtered through a small sterile Seitz bacterial filter. Another portion was ultrafiltered through fairly thick cellophan, and the colourless ultra-filtrate was filtered through a sterile Seitz bacterial filter.

These two extracts were injected intraperitoneally into females of a litter of 3 weeks old rats, the former in six doses of 1 cc. and the latter in six doses of 0.25 cc. Both these animals showed enlarged uteri and ovaries and cornified epithelial cells in the vaginal smear on the fifth day. Three other females of the litter which received injections of urine from pregnancy also showed the same effects, whilst a control litter-sister remained in the usual infantile condition.

Hydrochloric acid anterior pituitary extract. 50 fresh ox pituitary lobes (80 g.) were ground up with sand in a mortar and made into a paste with 20 cc. of water and 100 cc. of 0.1 *N* hydrochloric acid. The mixture was allowed to stand 18 hours in an ice-chest and then centrifuged rapidly for 30 minutes. The very opalescent supernatant fluid was pipetted off and shaken up with washed kaolin. After standing, the mixture was centrifuged rapidly for 1½ hours. The cloudy supernatant fluid was neutralised with 0.1 *N* sodium

hydroxide (40 cc. were required) and centrifuged for 0.5 hour after standing an hour. The perfectly clear, practically colourless supernatant fluid was decanted and filtered through a Seitz filter in a few minutes. An adult rat injected daily intraperitoneally with 1 cc. of this fluid showed no increase of weight in a fortnight. No appreciable amount of growth-hormone was, therefore, present.

Three female rats, aged 24 days, were taken:

- (1) was injected with five doses of 1 cc. of the extract spread over 3 days;
- (2) was given five injections of 1 cc. of urine of pregnancy;
- (3) was the control.

All were killed on the fifth day with coal-gas. Vaginal smears of (1) and (2) revealed pure cornified epithelial cells indicating oestrus whilst those of (3) did not; (3) possessed a thread-like uterus and undeveloped ovaries whilst (1) and (2) possessed distended large uteri and developed ovaries. The extract therefore possesses, in common with urine of pregnancy, the power of producing premature sexual maturity in young female rats.

Experiments on female rabbits. Extracts of anterior pituitary lobes were made with acetic acid and dilute hydrochloric acid, shaking with kaolin and neutralisation. The sterile filtered extracts were clear and yellow in colour and were injected intraperitoneally in four consecutive daily doses of 2 cc. into healthy female rabbits. The animals were killed on the day after the fourth injection and in every case large clear follicles were found in profusion in the ovaries, many of the follicles being haemorrhagic. The uteri were highly vascularised and pink in colour.

One pregnant rabbit was treated in the same way and the ovaries contained both clear and haemorrhagic Graafian follicles in every stage of development side by side with the corpora lutea of pregnancy.

DISCUSSION.

It has been found possible to obtain extracts of anterior lobes of pituitary glands with the following effects:

- (1) growth-promoting (filtered alkaline extracts);
- (2) growth-promoting and oestrus-inhibiting (unfiltered alkaline extracts);
- (3) premature maturity- and ripe follicle-producing (acid extracts treated with kaolin, etc.).

It would seem therefore that three effects may be produced by extracts of anterior lobes. Evidence from histological, surgical, pathological and biochemical fields presents a strong case for the existence of hormones in the anterior pituitary lobe controlling growth and the female reproductive cycle. Two inferences may be drawn: theoretically, one is faced with the presumption that the anterior pituitary lobe may control the ovarian cycle, and practically, one may hope for the establishment of replacement therapy in disturbances of growth and the female reproductive system.

Discontinuance of injections of growth-promoting extracts is followed by an immediate and rapid fall in weight which reaches its lowest level (a fall of over 20 g. in rats) in about one week. When injections are recommenced weight is rapidly regained. It appears possible that the extract had two effects: (a) to stimulate growth, and (b) to stimulate the thyroid gland. That the thyroid gland is affected by the anterior pituitary lobes is shown by the fact that ablation of the anterior pituitary lobe in tadpoles is followed by atrophy of the thyroids [Smith, 1922]. Anterior pituitary deficiency results in depression of metabolism, temperature, etc. [Reye, 1928]. Anterior pituitary hypertrophy is frequently associated with increase in the activity of the thyroid.

Possibly growth stimulation ceases directly injections are discontinued, but thyroid stimulation, which is over-weighted by growth stimulation during the injections, continues for a time, increases the metabolic rate and hence causes the loss of weight observed. The possibility arises therefore that anterior pituitary medication might improve the condition of intractable cases of hypothyroidism.

SUMMARY.

1. The preparation is described of extracts of anterior pituitary lobes which (i) promote growth, (ii) inhibit oestrus, (iii) produce precocious sexual maturity and cause ripening of Graafian follicles.

2. When rats, which have been treated with growth-promoting extracts, are no longer injected a rapid fall in weight is observed. A tentative explanation lies in stimulation of the thyroid gland.

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