

# The William Henry Welch Lectures

## Some Recent Advances in the Physiology of the Anterior Pituitary<sup>1</sup>

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The history of pituitary physiology has passed through various stages. The earliest period in the development of our knowledge on the functions of this gland is that of the anatomists. In this era pure morphological observation and theoretical speculation were the only means by which scientists tried to attack these problems, and the results they obtained would certainly seem rather ingenuous today. First Galen (1) thought the function of the gland to be filtration of a mucous fluid produced by the brain and its secretion into the nasopharynx. Therefore he called the gland "glandula pituitaria." Piccolhomini (2), in 1636, maintained that the function of the pituitary is to close the lower aperture of the infundibulum, and thus to prevent the spirits of life from escaping.

The next period in the history of pituitary physiology was that of clinical observation of hypophyseal diseases initiated by Pierre Marie's classical papers on acromegaly. These observations first called attention to the role of this gland in the regulation of somatic growth.

The modern era in the study of this gland began with the introduction of experimental methods. Just as in the study of other endocrine glands, it has been of great value to observe the changes following the removal of the pituitary from the organism. This operation, which was first performed by Sir Victor Horsley in 1886 (3), has proved to be of great assistance in the solution of problems concerning pituitary physiology. The technical difficulties connected with it account for the poor results of earlier workers,

which led to erroneous conclusions. Most of their experimental animals died shortly after removal of the gland and therefore they concluded (Vassale and Sacchi (4); Biedl (5) and others) that the hypophysis is absolutely essential for life. Improvements of the surgical method by later investigators such as Cushing, Aschner, Smith and others have now definitely established the fact that an animal may live for very long periods after complete hypophysectomy.

By far the most satisfactory approach is the oral, or parapharyngeal route, which allows the removal of the gland without injury to the adjacent hypothalamic centres of the brain. This method has been used throughout in our laboratory and enabled us to study, during the past two years, the effects of hypophysectomy on mice, rats, guinea-pigs, cats, dogs and monkeys.<sup>2</sup> We found it very helpful to use ether as an anaesthetic, given through a tracheal cannula, or an intratracheal tube. The danger of meningitis is comparatively slight, even though an open communication is left between the sella turcica and the nasopharynx, for this communication is closed very soon after operation by a clot.

I should like to give you now a short description of the symptoms of pituitary deficiency which appear after surgical removal of the gland, and also of the effect of replacement therapy.

### GROWTH

In accordance with observations of other investigators, we can confirm the observation that removal of the pituitary stops growth, but we found that there is one rather remarkable excep-

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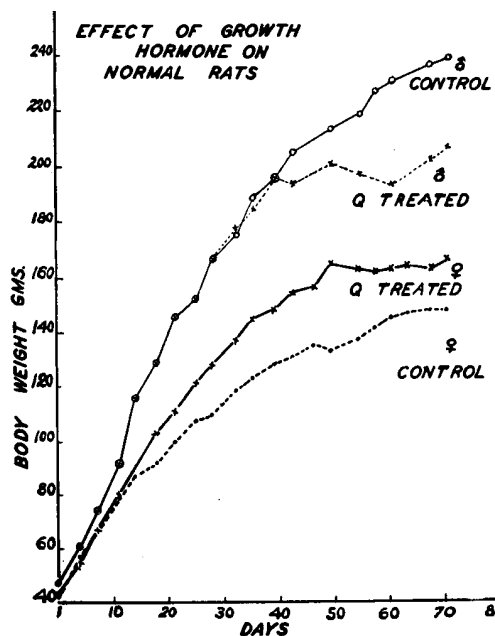
<sup>2</sup> Doctor Hans Selye has carried out most of the hypophysectomies.

tion to this rule. If hypophysectomy is performed in very young animals for instance, in rats about three weeks of age, growth does not stop immediately after operation, even though the gland is completely removed. Thus we have seen that rats which weighed approximately 25–35 grams at the time of operation proceeded to grow until they reached a weight of approximately 60 grams (6). Serial sections proved that no trace of pituitary tissue was left behind. The possibility of growth in the absence of pituitary tissue in very immature animals is of particular interest, because it shows that in early stages of ontogenetic development the organism must have an extrahypophyseal source of growth-stimulating substances. Here again we see once more the striking parallelism between ontogenetic and phylogenetic development, for it has been shown that the growth in length of reptiles and amphibia is also largely independent of the pituitary and therefore will not be completely inhibited by hypophysectomy.

The most satisfactory test animal for the standardization of hypophyseal growth hormone has proved to be the hypophysectomized rat of about 100 grams in weight. If the pituitary is removed at this period, growth will stop immediately unless growth-hormone-containing extracts are administered.

#### *Physiological effects of the growth hormone*

Hypophysectomized rats treated with a purified growth hormone (Q extract) put on weight immediately. This is in marked contrast to the effect which we obtained in our earlier work when crude alkaline extracts of anterior lobe were being used. We observed then that a period of about ten days elapsed before any appreciable growth took place. This we know now was due to the presence of the thyrotropic principle in the extracts used at that time, since simultaneous administration of the purified growth and thyrotropic principles does not result in growth until late in the second week. This apparent inhibitory action of the thyrotropic substance upon the growth substance is due probably to the increased metabolism offsetting the growth effect and is not a direct antagonistic action of the thyrotropic and growth principles. We agree with Evans that adult rats whose growth curves are plateaued may be caused to grow with the growth extract. We have been unable to affect appreciably the growth curve of normal male rats treated daily with the Q extract from the 4th week of life. Female animals so treated do show some acceleration of their growth rates as compared with their controls.



**Chart 1.** Growth curves of normal rats treated with growth hormone (Q extract) compared with untreated controls, male and female. Note the positive effect in the treated females (see Evans).

Hypophysectomized rats treated with the Q extract have been caused to increase their weight at the rate of about 2–3 grams per day for 35 days. This rate of growth cannot be maintained, however, and in the course of some weeks they cease to grow and may actually lose weight in spite of continuous treatment with a known potent extract.

Hypophysectomized rats treated with Q extract show in addition to actual increase in body weight a very definite change in their nitrogen and calcium metabolism. The nitrogen balance changes from a negative to a positive one, as shown by the studies of Mr. Peter Black, and the calcium balance is influenced in the same direction, as has been shown just recently by Dr. Leonard Pugsley. Skiagrams of the skull made under especially devised and controlled conditions in our laboratory by a guest worker, Dr. Hector Mortimer, show clearly that the Q extract produces very marked changes. The failure of growth of the cancellous elements in the hypophysectomized animal is not only made good, but in certain cases these structures may even become hyperplastic by the suitable exhibition of Q extract.

#### *Chemistry of the growth hormone*

The alkaline extract of anterior lobes first described by Evans is very rich in the growth hor-

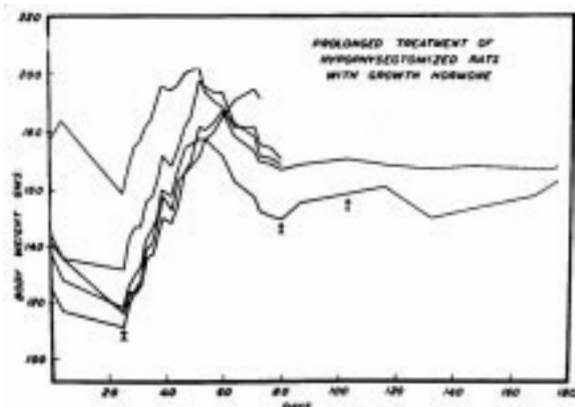


Chart 2. Growth curves of hypophysectomized rats treated with Q extract and fed on adequate diet. Note development of resistance after about 35 days of treatment.

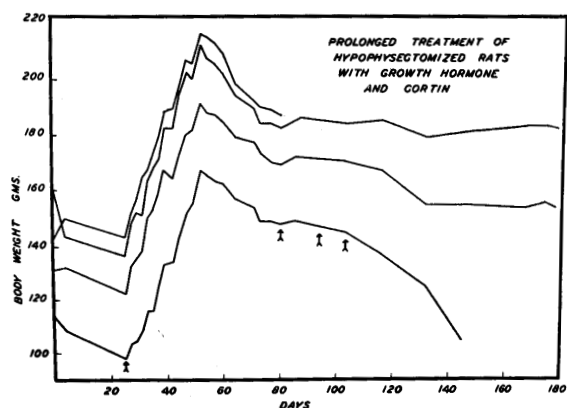


Chart 3. Like Chart 2, but illustrating non-effect of cortin.

It contains so much of the other pituitary principles, both active and inert, that an attempt was made to prepare an extract from which as much as possible of these contaminating substances would be removed, at the same time leaving the growth hormone in an active state. Many methods have been tried. It is possible to obtain potent extracts of the growth principle by a variety of procedures, such as extraction of the acetone-dehydrated fresh lobes with either aqueous alkaline or aqueous-acid media, or acid-acetone or alcohol of 60 per cent concentration, followed by subsequent fractionation with salt, alcohol and acetone, and isoelectric precipitation. If one is desirous of obtaining a relatively pure hormone extract rather than quantitative recovery of the total active principle present in the original gland tissue, the following method may be employed (8).

Desiccated acetone-dehydrated anterior lobes of cattle are ground to a pulp and suspended in ten volumes of distilled water. The mixture is vigorously stirred and then is made alkaline to the extent of 0.25 per cent NaOH by the addition of

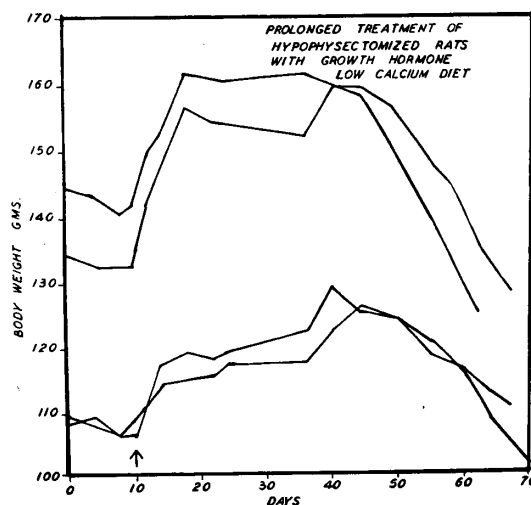


Chart 4. Growth curves of hypophysectomized rats treated with extract and fed a low calcium diet. Note the rapid loss in weight after resistance has developed.

the requisite amount of a saturated aqueous solution. The mixture is allowed to stand for two hours. This preliminary treatment with dilute alkali serves a twofold purpose: (1) destruction of any contaminating posterior lobe principles, and (2) facilitating solution of the tissue. Also, the mild hydrolytic action of the dilute alkali is probably of definite value.

The alkaline mixture is treated next with sufficient acetic acid to give a marked flocculation (isoelectric precipitation of the proteins). The pH

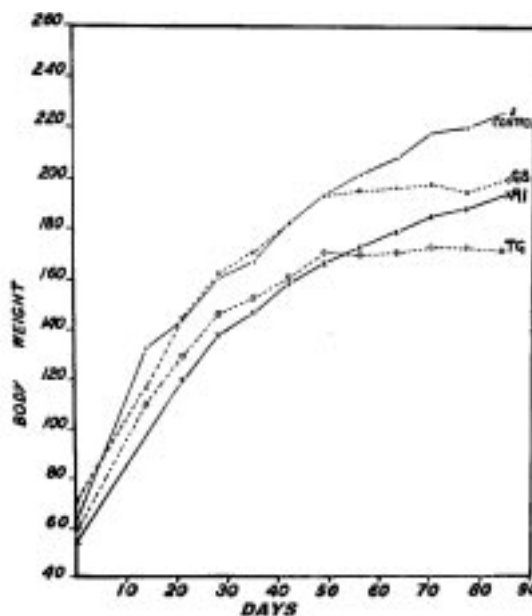
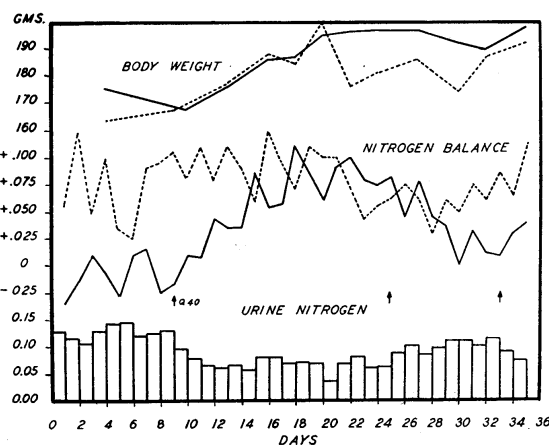


Chart 5. Growth curves of normal male rats treated with: T.G.—thyrotropic hormone; A.I.—adrenotropic hormone; G.S.—a mixture of anterior lobe principles. Note that prolonged treatment with pituitary principles definitely slows the growth rate.

should be about 5.5. Under these conditions filtration of the mixture can be carried out quite rapidly. The residues are suspended in ten volumes of distilled water; the mixture is made alkaline (0.1 per cent NaOH) and again precipitated at the isoelectric point as in the first instance. After filtration the residues are again treated as above, and for a thorough extraction of the gland tissue this part of the process should be repeated five times. The combined filtrates are concentrated at low temperature and pressure to 1/25 to 1/50 the original volume. The concentrate contains the growth hormone, which tends to precipitate out as an isoelectric protein fraction during the concentration process; the thyrotropic hormone; the adrenotropic hormone; a maturity factor; some prolactin; a ketogenic and a diabetogenic substance. Both of these latter may not be definite entities, as will be seen in the subsequent discussion.

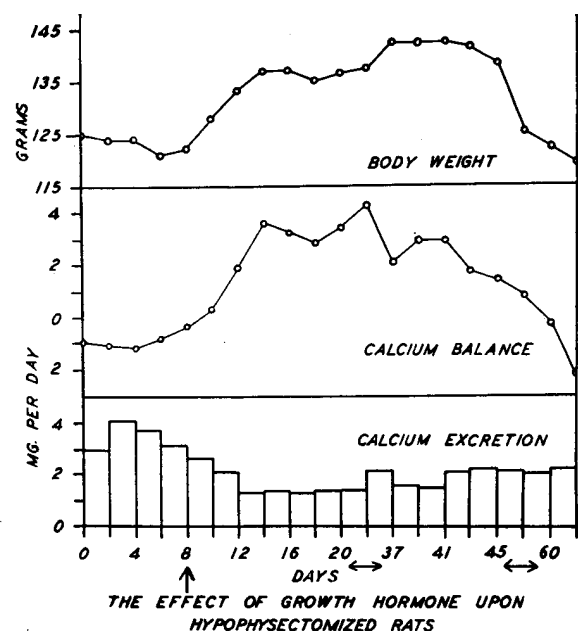
A very satisfactory growth hormone preparation can be obtained by filtering off the flocculence which appears as a result of the vacuum distillation. This precipitate should be dissolved in 0.1 per cent NaOH and reprecipitated at the isoelectric point by careful addition of dilute acetic acid. Flocculation of the isoelectric precipitate is aided by further concentration of the mixture at low temperature and pressure. This protein-like fraction should be further purified by repeated isoelectric precipitation.



**Chart 6.** Showing the effect of the growth hormone upon growth, nitrogen balance and urinary nitrogen of completely hypophysectomized rats (solid line) and of normal animals (dotted line). At the first arrow the injections were started; at the second, injections were stopped; and at the third, they were started again.

The filtrates obtained in this process are combined and worked up for the thyrotropic and adrenotropic active principles (9, 10).

The best growth hormone preparations are made by treating the concentrate of the combined original filtrates with  $\text{Ca}_3(\text{PO}_4)_2$  in the presence of 1 per cent ammonia (8). The suspension of calcium phosphate in the concentrate is best produced by adding separately, during vigorous agitation,  $\text{CaCl}_2$  and  $\text{NaH}_2\text{PO}_4$  in appropriate amounts. Agitation of the mixture is maintained by further concentration in the vacuum still. The mixture is filtered and the filtrate is washed with dilute ammonia water (0.05 per cent) until the filtrate is colorless. The  $\text{Ca}_3(\text{PO}_4)_2$  mass is suspended in water and made alkaline with NaOH (0.5 per cent). The mixture is filtered and the whole process is repeated three times. The combined filtrates are neutralized with acetic pH 6 and concentrated at low temperature and pressure to a small volume. The precipitate is removed by filtration and after solution in dilute soda again precipitated at the isoelectric point. The solution of the precipitate in dilute alkali represents the final product. One such preparation sterilized by Berkefeld filtration at pH 9 has shown no evidence of losing its activity over a period of one year. This has been stored in a refrigerator at about 1°C.



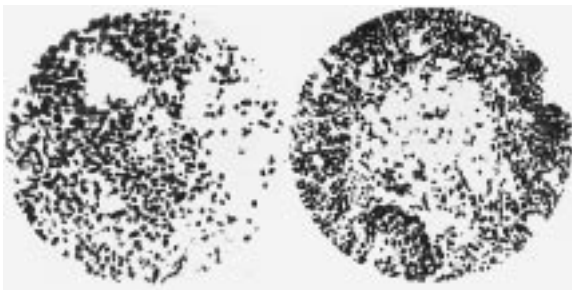
**Chart 7.** Showing the favorable effect of Q extract upon calcium balance in hypophysectomized rats maintained on a low calcium diet (Dr. Leonard Pugsley).

The growth hormone may be standardized by noting the growth effect upon recently hypophysectomized and otherwise untreated rats. We have taken as an arbitrary unit the amount of extract necessary, when administered twice daily

by intraperitoneal injection, to cause a weight increase of 1 gram per day in 100 gram rats, over 10 to 15 days (8). Extracts made by the method outlined above may contain from 25 to 100 units per cubic centimeter representing 1 gram of original pituitary tissue.

### SEX

Another important effect of hypophysectomy is the atrophy of the genital organs. The germinal epithelium of the testis atrophies and sperm cell formation stops. In the ovary follicle maturation and corpus luteum formation is inhibited and the animals become continuously dioestric. But the effect of the pituitary on the ovary is not limited to the maturation of follicles; in fact we have been able to show that the so-called "maturity hormone" is as important for the immature ovary as it is for the mature one. Before puberty, there is no follicle maturation, nor oestrus, nor corpus luteum formation, but the ovaries of prepubertal hypophysectomized animals still show definite signs of hypophyseal deficiency (39).



**Fig. 1.** Ovary of hypophysectomized rat two months after hypophysectomy. The theca cells are changed into theca-deficiency cells and their nuclei show the typical wheel shape.

**Fig. 2.** Involved corpus luteum in ovary of hypophysectomized post pubertal rat 265 days after hypophysectomy.

The thecal cells, which ordinarily have a spindle-shaped nucleus and a fine granular chromatin structure, change their appearance. The nuclei become circular and their chromatin aggregates in large, dark lumps, peripherally arranged, leaving white, chromatin-free spaces in between, so that the nucleus comes to present a somewhat wheel-like appearance. In order to stress the origin and significance of these cells, we called them "theca deficiency-cells" (11). It has been possible to prepare suitable extracts, which will prevent or cure the effects of hypophysectomy on the gonad, and the study of replacement therapy has also led to some interesting findings concerning the difference between the various gonadotropic extracts.

Thus we have found, in confirmation of P. E. Smith and others, that gonadotropic extracts prepared from pituitary tissue will lead to follicle maturation and corpus luteum formation in the hypophysectomized rat. In this respect the gonadotropic hormone of the pituitary differs from the anterior-pituitary-like hormone prepared from pregnancy urine or placenta (A.P.L.); in fact we have never been able to stimulate follicle maturation or corpus luteum formation in the absence of the hypophysis with A.P.L. (7, 40). It would be erroneous, however, to think that A.P.L. is quite ineffective in the hypophysectomized rat. Although its administration does not produce true corpora lutea, it leads to the luteinization of thecal cells and to the production of so-called "thecal corpora lutea." The main difference between the two types of gonadotropic hormones appears to be, therefore, that the pituitary hormone acts on the granulosa and on the theca, while A.P.L. luteinizes the theca only. In this respect our findings are not quite in accordance with those of P. E. Smith, who has obtained true corpora lutea by the administration of pregnancy urine preparations. It is interesting to note, however, that we repeated his experiments in our laboratory with commercial "antuitrin S" without being able to produce any results other than thecal luteinization (12). We have no explanation to offer for this discrepancy, except possibly that the "antuitrin S" used by Dr. Smith—which, as he told us, was a special preparation—was different from the commercial "antuitrin S." The difference in the morphogenetic effect of the two gonadotropic hormones is not the only one, for it has been possible to show that A.P.L. leads to continuous oestrus in the hypophysectomized adult female, while this is not the case after administration of the pituitary extract. In the case of the immature hypophysectomized female, oestrus has not been obtained after A.P.L. administration, although the thecal luteinization was very marked. These observations led to more detailed study of the effects of hypophysectomy and hypophyseal hormones on the theca cell.

The theca deficiency-cells, as described above, can easily be brought back to normal by the administration of A.P.L.; in fact, we have seen that they may be stimulated to such a degree as to take on the appearance of corpus luteum cells. The confinement of A.P.L. action to the theca cells is observed not only in the hypophysectomized animal but also in the very immature rat. Administration of A.P.L. to these during the first days of life results in thecal luteinization. It is generally known that rats under 14 days of age

are not suitable for the Aschheim-Zondek reaction, for they will not form corpora lutea under the influence of pregnancy urine. An histological study of ovaries of such animals, showed, however, that their gonads are by no means insensitive to A.P.L. The only difference between the somewhat older rat and the very immature animal is that the former reacts with corpus luteum formation, while the latter with the formation of thecal corpora (13). These observations led us to the conclusion that the pituitary of the very immature rat is probably not fully functional, and that this accounts for the fact that it reacts to A.P.L. in the same way as the hypophysectomized older animal.

The conclusion which we drew from all these experiments was that A.P.L. needs a complementary factor produced by the pituitary in order to act on the granulosa cell, while its effect on the theca is not necessarily dependent upon the pituitary (14, 15). This conception has also been substantiated by other experimental series, in which we found that continuous administration of A.P.L. leads to an enlargement of the pituitary and of the thyroid, as well as to development of the mammary gland to the stage corresponding to that of late pregnancy (16, 17).

#### *The preparation of extracts containing the maturity principle of the anterior lobe*

The preparation of even a relatively pure maturity hormone extract of the anterior pituitary has not yet been accomplished. There are more difficulties to be overcome in relation to the purification of this principle than in the case of any other. The great variation in the yield of active principle seen in the glands of different species is of especial interest. In our hands ox glands have been quite unsatisfactory as a source of potent extracts, whereas glands of sheep and hogs have been found to be relatively very rich in maturity principles. Others have had a similar experience in their work on pituitary gonadotropic hormones. If frozen rat pituitary glands are implanted into immature females, the gonadotropic effect is only a fraction of that obtained when fresh glands are implanted in a similar manner. Since an alkaline extract of the anterior lobes of the ox has been shown to be capable of neutralizing the oestrus effect of a potent alkaline extract of the rat's pituitary when both are administered simultaneously to the immature rat, one must recognize the existence of inhibitory substances of either a specific or non-specific nature in the fresh glands and accept as a possibility that a negative gonadotropic effect of a

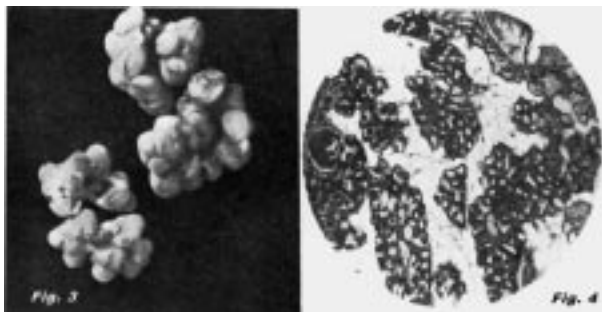
certain extract may be due not to absence of a maturity factor but to an inhibitory substance neutralizing the effect of an oestrogenic factor. We have frequently failed to obtain any vaginal oestrus reaction with certain pituitary extracts which have produced a definite A.P.L. ovary in immature rats. Methods of preparing gonad stimulating extracts of the anterior pituitary have been published by Van Dyke and Wallen Lawrence (18, 19, 20); Evans, Meyer and Simpson (21); Hisaw, et al. (22, 23); and others (24, 25, 26, 90).

Our best maturity extracts have been made by extracting the anterior lobes of sheep or hog with 30 per cent acetone containing 1 per cent  $\text{NH}_4\text{OH}$ . The maturity factor appears to follow the thyrotropic principle, and methods useful in purifying the latter are applicable to the purification of the former. In general these consist in isoelectric precipitation of relatively inert protein materials and concentration of the active principle by ammonium sulphate precipitation of the isoelectric filtrates, solution in water of the ammonium sulphate precipitate and precipitation from this by alcohol or acetone (70 per cent) of the active material. Salts can be removed successfully from the final extracts by dialysis. Extracts which have been made in this manner can be boiled at pH 5 for one hour without any appreciable change in the potency as regards maturity principle, whereas such treatment causes almost a complete destruction of the thyrotropic substance. This is the best evidence we have to submit that the thyrotropic and maturity factors are different substances. We do not feel, however, that it is fully adequate to settle entirely this question.

#### *Pregnancy*

In all probability the pituitary plays an important role during pregnancy. It has been possible, however, to show that hypophysectomy during the second half of pregnancy does not interfere with the maintenance of gestation. The only effect of hypophysectomy, when performed in the second half of pregnancy in rats or mice, is a marked prolongation of the gestation period (27, 28). This is most probably due to a delayed involution of the corpus luteum of pregnancy. We have found, however, in confirmation of Pencharz and Long (29), that hypophysectomy in the early stages of gestation leads to resorption of the embryos in the rat. We have been able to show that the same is true in the mouse, while the guinea-pig seems to behave differently; for we have seen that pregnancy was not interfered with in a guinea-pig hypophysectomized during a very early stage of gestation.

If the maintenance of the corpus luteum of pregnancy is not interfered with by hypophysectomy, the question arises—what is responsible for keeping this corpus luteum in a functional condition? It is very likely that the pregnant uterus itself is responsible for this, since removal of the fetuses leads to immediate involution of the corpus luteum of pregnancy. In order to determine whether the presence of the embryo as such is essential, or whether the distention of the uterine wall is the important factor, Dr. Selye (30) performed the following experiment: In a series of pregnant rats, the uterus was emptied and melted paraffin was introduced into its cavity, so as to distend it. When these rats were killed six weeks after the operation their ovaries were considerably enlarged (fig. 3) and contained a large number of corpora lutea comparable to those of pregnancy. The mammary glands, however, had completely retrogressed. It seems from this experiment that the mere distention of the uterine wall as such has a great effect upon the ovary. The involution of the mammary glands is not necessarily an indication of a deficiency in corpus luteum hormone production, since it is well known that even the continuous administration of corpus luteum hormone by injection cannot maintain the mammary gland in a fully developed condition for more than a few weeks. These experiments are insufficient to draw any definite conclusion concerning the role that uterine distention plays during pregnancy, but they certainly prove that the distention of the uterus has a marked effect on the ovary. We should be inclined to assume that this effect is also attained through a stimulation of the pituitary, since the hypophyses of our experimental animals—the uteri of which had been distended—were greatly enlarged. These experiments remind us of those performed by Loeb (31) on the guinea-pig, which



**Fig. 3.** A pair of greatly enlarged ovaries, taken from a rat the uterus of which had been distended with paraffin, beside a pair of normal ovaries.

**Fig. 4.** Fully lactating mammary gland in a suckling pseudo-pregnant mouse.

showed that hysterectomy greatly prolongs the life-span of the corpus luteum in this species. Since hysterectomy has no clean-cut effect on the ovaries or the cycle in the rat, we assume that the mechanism at work after hysterectomy differs from that after uterine distention.

### *Lactation*

The development of the mammary gland is not interfered with (27, 32) in rats and mice hypophysectomized in the latter half of gestation. Milk secretion starts normally at parturition. This, however, lasts for but a few hours, then definitely stops. The mammary gland involutes. If hypophysectomy is performed during the course of an already established lactation, milk secretion stops within a few hours (6). As you know, it has been possible to produce a lactation-promoting hormone from the pituitary. Oscar Riddle, whose work on this subject has been of considerable importance in the elucidation of the problems of lactation and crop milk secretion, has called this hormone "prolactin." It is likely that this prolactin stimulates the secretion of the mammary gland (33, 34). Our experiments—showing that a short period of lactation may be observed soon after delivery, in animals from which the pituitary has been removed a long time before—seem to indicate that the hypophysis is not absolutely essential for the secretion of milk. There might possibly be an extra-hypophyseal source of the lactation-promoting hormone. We have considered the possibility that the pregnant uterus may also yield prolactin, and that the short lactation period observed after delivery in the hypophysectomized animal may be the result of a release of prolactin from the pregnant uterus. We know that the corpus luteum inhibits lactation, and therefore a lactation-promoting hormone would probably not lead to milk secretion during the life of the corpus luteum of pregnancy; but as soon as this corpus luteum ceases to function, lactation starts.

In this connection we performed the following experiments. A group of rats were injected with A.P.L. until their mammary glands were fully developed, and then castrated. Forty-eight hours later a histological study of the mammary glands showed that lactation had set in. In this case, the removal of the considerably luteinized ovaries initiated milk secretion in the mammary gland of the non-pregnant animal.

In another series, we treated a group of rats with A.P.L. in exactly the same manner as in the previous experiment. We then castrated them, and removed the hypophysis at the same time. Under

these conditions, no milk secretion was observed. Our conclusion was that the removal of the ovary, with its corpora lutea, stimulated the secretion of prolactin from the pituitary, and thereby initiated milk secretion. If the pituitary was removed, milk secretion could not occur. If we compare the results of these experiments with those on pregnant animals, as previously described, we see that the only difference between them is the presence of a pregnant uterus. It is suggestive, therefore, to assume that this pregnant uterus was responsible for the initiation of milk secretion following parturition (17, 35).

In order to determine whether the pregnant uterus is able to produce prolactin at any time during gestation, we removed the fetuses and the pituitary simultaneously during the second part of pregnancy, and we found that Caesarian section, which ordinarily initiates milk secretion, is unable to do so in the absence of the pituitary. Animals impregnated at the postpartum cycle immediately after delivery of a litter (thus pregnant and lactating at the same time) were hypophysectomized. Milk secretion ceased immediately. Thereupon we came to the conclusion that the substance which stimulates milk secretion in the hypophysectomized animal is probably released from the pregnant uterus only shortly before parturition (36).

It is well known that weaning stops milk secretion. The explanation of earlier workers was that the accumulation of the products of secretion within leads to pressure atrophy of its epithelium, and thereby stops secretion. In the light of the "prolactin" theory, we were interested to see whether the stimulus of suckling has any direct effect on the production of prolactin in the pituitary. If this were so, one would have to assume that the mere nervous stimulus of suckling maintains milk secretion during the whole lactation period, through an effect on the prolactin production of the hypophysis. In order to test this, Dr. Selye trancised all the galactophores in lactating mice and rats while the stimulus of suckling was maintained. In this experiment no milk could escape from the gland and secretion still persisted, as long as the litter was suckling. This experiment indicates that the accumulation of milk in the gland is not the immediate cause of inhibition of secretion after weaning. In the animal from which the young have been weaned milk secretion stops because the nervous stimulus of suckling is absent (37).

The fact that the nervous stimulus of suckling has so definite an effect upon the pituitary throws an entirely new light upon the question of lactation dioestrus and lactation amenorrhoea. The fact

that sexual cyclicity stops during lactation, both in experimental animals and in humans, has been interpreted as the result of the drain upon metabolism during this period. It has also been thought that it is not lactation which interferes with menstruation, but that the initiation of menstruation has an adverse effect upon lactation. In the above experiments it seems that the oestrous cycles do not appear in nursing mothers, even though the galactophores are cut. In this condition, there is no milk secretion and thus no drain on the metabolism. We thought, therefore, that the inhibition of sexual cyclicity during lactation may also be due to the nervous stimulation of suckling.

Another group of experiments was done to see whether the stimulus of suckling would also inhibit sexual cyclicity in the non-lactating animal. In a group of adult normal female rats and mice we studied the effect of the stimulus of suckling upon the oestrous cycle. We found that an adult rat, if given a litter of young animals, will build a nest and try to nurse the litter in many cases. If the mammary gland of such a rat is examined about ten days later, growing mammary epithelium is seen. At the same time the ovaries contain large corpora lutea similar to those seen during pregnancy and pseudo-pregnancy. No follicle maturation occurs and vaginal smears show continuous dioestrus. This condition is very similar to the so-called pseudo-pregnancy produced by sterile copulation or stimulation of the vagina with a glass rod. During the later stages of this pseudo-pregnancy produced by suckling, the mammary gland is well-developed and frequently numerous large placentomata are found in the uterus (figs. 4, 5). Still later, lactation sets in (Selye and McKeown (38)).

This type of pseudo-pregnancy is particularly interesting because it may have a parallelism in humans. While sterile copulation does not interfere with the cycle in women, the stimulus of suckling has a definite inhibitory influence as shown by lactation amenorrhoea. We would be inclined to interpret this condition as the result of the nervous stimulation of the nipple. These experiments may also give a more satisfactory explanation of the fabulous accounts of various investigators who claim that milk secretion may be initiated in virgin girls who have attempted to quiet an infant by offering it the breast.

#### *Testis*

We have confirmed the observations of previous investigators concerning testicular atrophy which follows removal of the hypophysis in

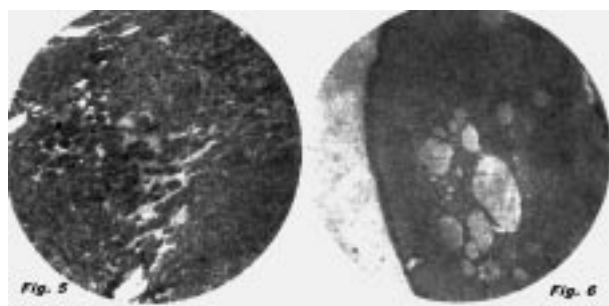


Fig. 5. Placentoma in the uterus of a pseudopregnant mouse.

Fig. 6. Cysts in the adrenal cortex of a pseudopregnant mouse.

experimental animals. We also found, in confirmation of Philip E. Smith, that this atrophy can be prevented by the daily administration of hypophyseal implants. Our results differ, however, from those of Smith, insofar as the effect of A.P.L. is concerned. Administration of even large doses of A.P.L. has consistently failed to stimulate sperm cell formation in hypophysectomized rats. The interstitial tissue hypertrophy which follows A.P.L. administration in the normal animal is not influenced by the removal of the gland, but—just as in the normal—this hormone has no effect on the seminal epithelium after hypophysectomy (7).

In this connection it is interesting to note that the very immature male rat seems to react in the same manner to A.P.L. as does the older animal, the only clean-cut effect being proliferation of the interstitial tissue. Unlike the ovary, the testis reacts in the same manner after A.P.L. administration in the immature or hypophysectomized rat as it does in the mature male.

### THYROID

That there is a very close relationship between the anterior pituitary and the thyroid glands has been very clearly established both experimentally and clinically. Cushing (1912) (40) observed in the dog that, following a transient hyperplasia occurring about 24 hours after hypophysectomy, involution of the gland ultimately resulted. Allen reported that the thyroid of tadpoles became atrophic following removal of the pituitary, and he noted that the implantation of the pituitaries of frogs caused a restoration of the thyroid to normal (41). P. E. Smith obtained similar results in the case of the white rat (43).

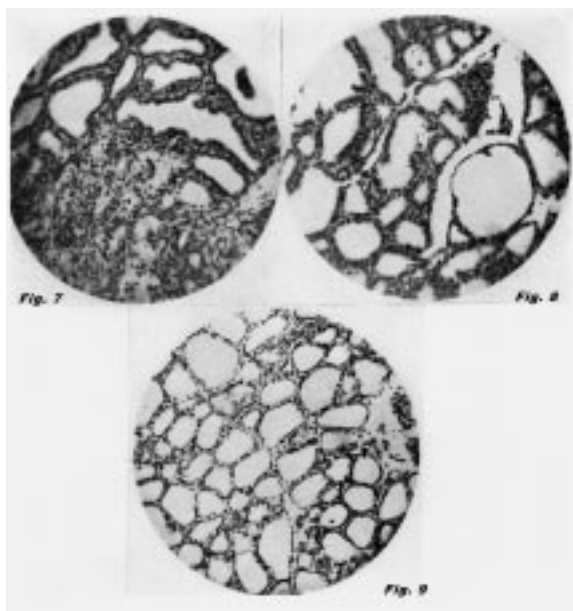
Clinical evidence pointing to the control of the thyroid by the pituitary has been abundantly available. Graubner (44), in a review of 34 cases of hypophyseal cachexia (Simmonds' disease)

reported in the literature, found that in all cases the thyroid was reported as being small and atrophic and sometimes even sclerotic. As a result of the considerable experience which we have had in our own laboratory with hypophysectomized animals, we can confirm these findings in regard to the atrophic changes in the thyroid of dogs, monkeys, guinea-pigs, mice and rats from which the hypophysis has been removed.

Hyperplasia of the thyroid gland has been produced in a number of different species of animals by injections of anterior pituitary extracts. This fact completes the chain of evidence proving that the anterior pituitary normally exercises a controlling influence over the thyroid by means of an internal secretion.

Animals in which the thyroid stimulating action of the anterior pituitary has been conclusively demonstrated are the guinea-pig (Loeb and Bassett, 1929, 45; Aron, 1930, 46, 47; Janssen and Loeser, 1931, 48; Krogh, 1932, 49; Grab, 1932, 50), the cat (Schittenhelm and Eisler, 1932, 51), the dog (Houssay, 1932, 52), the rabbit (Baumann and Marine, 1932, 53), the pigeon (Riddle and Polhemus, 1931, 54), the duck (Schockaert, 1932, 55), and the rat (Anderson, 56, and Anderson and Collip, 1933, 9). Some of these workers reported failure to produce hyperplasia in the thyroid of the rat. It is of particular interest therefore to note that Dr. Evelyn Anderson, working with us, *was able to produce marked thyroid hyperplasia with hyperthyroidism, in the rat treated with crude pituitary extract plus Staphylococcus aureus vaccine*. Junkmann and Schoeller have found that the pituitary of the rat is relatively very rich in the thyrotropic substance compared with the pituitary of the guinea-pig, as determined by implantation into test guinea-pigs, and that the rat is much less sensitive than the guinea-pig to the thyrotropic hormone. In this connection, I should venture to suggest that it *may follow as a general rule that the sensitivity or responsiveness of an animal to injected hormone varies inversely with the hormone content or production of the animal's own gland*.

Hyperplasia of the thyroid of the rat has been produced by other means than the injection of highly potent thyrotropic hormone. Thus McCarrison has observed thyroid enlargement and hyperplasia in rats to which have been fed cultures of bacteria from the faeces of goitrous persons. We have seen extreme hyperplasia without hyperthyroidism in certain rats obtained from a dealer and kindly supplied to us by Dr. C. N. H. Long of Philadelphia, who had noted the condition and was



**Fig. 7.** Control thyroid of goitrous rat. Weight 143 mg., metabolic rate 86 per cent of normal.

**Fig. 8.** Thyroid of goitrous rat after 9 days treatment with thyreotropic hormones. Note large vesicle, with flattened epithelium, filled with dense colloid. Weight 138 mg., metabolic rate 230 per cent of normal.

**Fig. 9.** Thyroid of normal rat after 74 days treatment with thyreotropic hormone. Note similar appearance to that seen in untreated hypophysectomized rat.

so kind as to send them on to us for use in our work.

The use of a non-specific agent such as a killed culture of *Staphylococcus aureus* in conjunction with anterior pituitary extracts in order to produce thyroid hyperplasia and hyperthyroidism became quite unnecessary after we had succeeded in preparing the thyreotropic principle in concentrated and purified form.

#### *Preparation of the thyreotropic hormone*

The presence of the thyreotropic principle can be demonstrated in almost any type of simple extract of anterior pituitary glands. A variety of methods are therefore available for the preparation of extracts rich in this hormone. Loeser (58) described a method for preparing an active extract in the form of a dry powder. His process consisted of aqueous ammoniacal extraction of the acetone treated glands followed by removal of proteins with trichloroacetic acid and precipitation of the active substance by acetone from the trichloroacetic filtrate. Alcohol and acetone were subsequently used as precipitation reagents. Junkmann and Schoeller (59) have reported on the chemical properties of the thyreotropic extract.

The method which we use to prepare an

active extract is as follows: The combined filtrates obtained at the  $\text{Ca}_3(\text{PO}_4)_2$  adsorption stage in the Q process for growth principle are concentrated and saturated with  $(\text{NH}_4)_2\text{SO}_4$ . The precipitate is taken up in distilled water and again precipitated with  $(\text{NH}_4)_2\text{SO}_4$ . Alcohol is added to the neutral aqueous extract of the precipitate to make 70 per cent by volume of this reagent in the mixture. The precipitate is collected on a filter and extracted with distilled water. An equal volume of acetone is added and the precipitate is collected on a filter and extracted with distilled water. This solution represents the final product, but further purification for the purpose of reducing the content of contaminating adrenotropic principle can be accomplished by repeated precipitation from 50 per cent acetone.

Most excellent preparations of the thyreotropic plus some adrenotropic hormone can be prepared by working up the combined filtrates from the above process by concentration and precipitation from 90 per cent alcohol.

#### *The physiology of the thyreotropic hormone*

The administration of adequate amounts of the thyreotropic hormone to normal animals results in the course of a few days in enlargement and hyperplasia of the thyroid. There is an increase in the metabolic rate (Siebert and Smith, 57, Verzar and Wahl, 61), increased heart rate (Schittenhelm and Eisler, 51), exophthalmos (Schockaert, 55, Loeb and Friedmann, 61), a reduction of the iodine content of the gland (Loeser, 58; Schockaert and Foster, 62), an increase in the alcohol-insoluble iodine of the blood (Closs, Loeb and MacKay, 63; Grab, 64; Schittenhelm and Eisler, 51), a depletion of liver glycogen and an increase in the acetone bodies in the blood Eitel and Loeser, 65; (Eitel, Löhner and Loeser, 66). Verzar and others have shown that none of the signs of hyperthyroidism can be produced in thyroidectomized animals by injections of thyreotropic hormone extracts (60).

Since the guinea-pig is exceedingly sensitive to the thyreotropic hormone, we made use of this animal as a test object in our earlier work on the purification of extracts.

The metabolic effect of bi-daily injections of an active extract into a group of 16 prepubertal guinea-pigs consisted of an average increase of 129 per cent on the 4th day and 134 per cent by the 6th to 8th day. The animals were killed after one week of injections, and the thyroid glands were more than doubled in size as compared with the controls; and the histological picture was that

of extreme hyperplasia. Our Q extract and A.P.L. tested in a similar manner gave negative results.

From a study of the changes in metabolism from day to day in guinea-pigs and rats injected with the thyreotropic hormone, it is evident that there is a preliminary rise sometime within the first four days. This is followed by a secondary rise in about a week. This latter rise is not maintained, and a decline to normal or even subnormal levels gradually takes place. Since it has been shown by a number of workers that, following the injection of the thyreotropic hormone, there occurs an outpouring of thyroid secretion, one might suggest that the first rise in metabolism is due to the sudden discharge of existing stores of thyroid hormone into the blood stream, while the second rise follows after the hyperplasia of the gland, induced by the hormone, has advanced sufficiently to give an increased production of the thyroid hormone. The subsequent decline in metabolic rate has been made a subject of special study in our laboratory, but I shall defer until a little later a full discussion of our results.

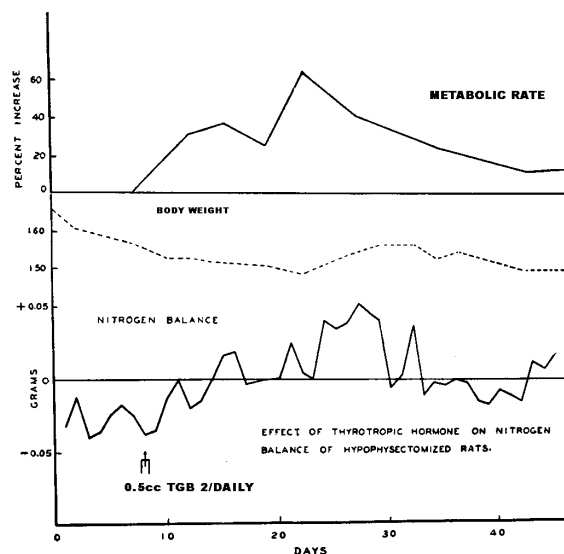
The average increase in metabolic rate which we have seen in normal rats of our colony, treated with the thyreotropic hormone, has been +35 per cent. The administration of even massive doses of the hormone does not result in higher values. We obtained quite different results, however, with a small series of goitrous rats to which reference has already been made. A control animal in this group had a thyroid gland weighing 142 mg as compared with a normal rat's gland of 20 mg. The gland proved, on microscopic examination, to be extremely hyperplastic. The control metabolic rates of these animals was slightly subnormal. Immediately following treatment with thyreotropic hormone the metabolic rate rose rapidly. One animal showed a 95 per cent increase within 24 hours. The highest rate observed in the group was 262 per cent above normal. This was on the ninth day of injections. All of the rats showed definite symptoms of hyperthyroidism, irritability, weakness, exophthalmos and excessive sweating, and there was a 20 per cent loss of weight in the course of nine days.

Hypophysectomized rats show a decline in the metabolic rate to an average of 74 per cent of normal as determined on a series of 118 of our experimental animals. The thyroid gland decreases in weight and the microscopic picture is one of marked involution of the cellular elements. We have found that these animals are much more sensitive to injections of the thyreotropic hormone than are normal animals. They compare very favorably in this respect with the guinea-pig.

The metabolic rate has frequently been restored to normal within a week with bi-daily doses of as little as 0.005 cc. of a purified thyreotropic extract. The normal rat has required at least 0.08 cc. bi-daily to give a similar response. We have chosen as an arbitrary unit of the thyreotropic hormone the minimum amount, administered daily in two injections, which will cause a rise of 20 per cent in the metabolism of the hypophysectomized rat by the fourth day. We believe that this unit compares fairly well with the Junkmann-Schoeller unit as determined by the use of the normal guinea-pig, but it has this advantage that the metabolism of the hypophysectomized animal, both before and during treatment, is far less variable than the histological picture of the guinea-pig's thyroid. The normal guinea-pig thyroid is extremely sensitive to the thyreotropic hormone stimulus, but unfortunately control animals all too frequently, in our experience, have shown mild thyroid hyperplasia.

We have no evidence of our purified thyreotropic extract causing growth in hypophysectomized animals that have been treated for some weeks. Thus, 40 hypophysectomized rats had an average weight of 121 gm before injections of the thyreotropic extract were commenced, and an average weight of 110 gm after 3–4 weeks.

#### *Effects of the thyreotropic hormone on metabolism*



**Chart 8.** Effect of the thyreotropic principle upon nitrogen balance in hypophysectomized animals (Mr. Peter Black).

It is well known that thyroid feeding causes a creatinuria (Cramer and Krause, 1913). Dr. David Barr observed an increased creatine excretion in a

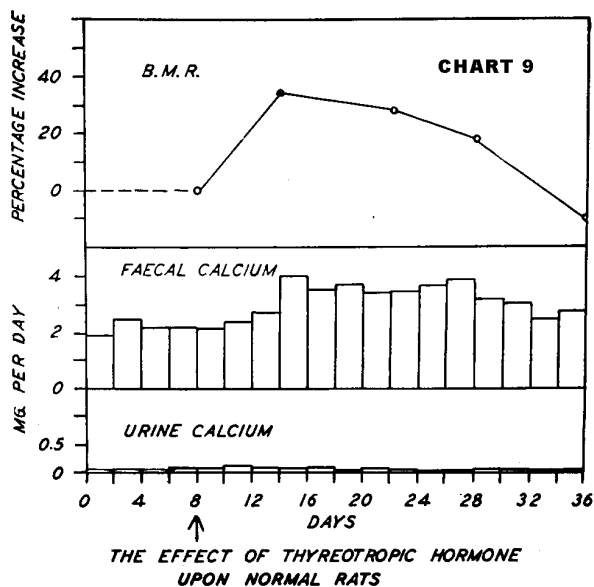
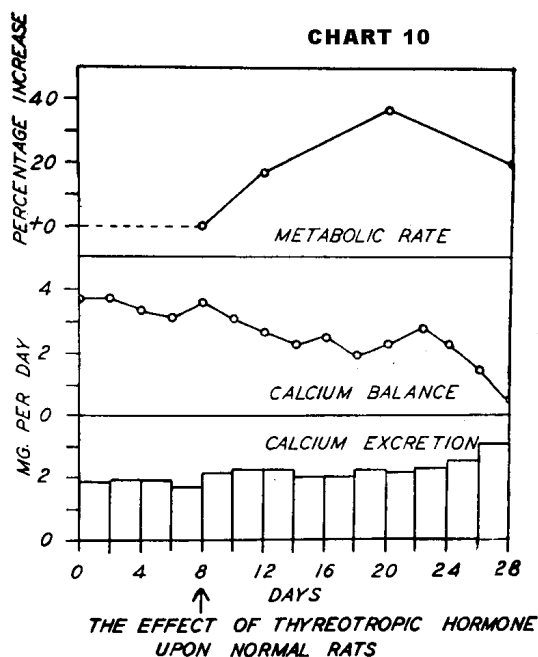


Chart 9. Effect of the thyreotropic extract upon calcium excretion (Dr. Leonard Pugsley).

Chart 10. Effect of the thyreotropic hormone upon the cal-



cium balance of normal rats (Dr. Leonard Pugsley).

patient given our T.G. extract. It is of interest, therefore, to compare the effects of thyroid feeding and of thyreotropic hormone injections upon the creatine excretion in relation to the changes produced by each in metabolic level. This part of the work has been carried out by Dr. L. Pugsley and Dr. Evelyn Anderson. The feeding of desiccated thyroid to normal rats caused a rise in the

creatinine excretion which reached a peak on the 10th day. The creatinine excretion then gradually decreased, while the metabolic rate was still rising. The thyreotropic hormone caused a temporary increase in creatinine excretion, both in normal and hypophysectomized rats. As in the case of the metabolic rate response, the former were less sensitive than the latter. Six units caused a definite increase in excretion in the normal rats, and one unit acted similarly in the hypophysectomized animals. An increased creatinine excretion was likewise noted in the dog treated with the thyreotropic extract. Purified extracts of the growth and adrenotropic hormones had no effect on the creatinine excretion of normal rats.

We see here two definite examples of the principle which I enunciated above; namely, *that the responsiveness of an animal to an administered glandular extract may vary inversely with the hormone content or production of the animal's own gland.* The principle may be illustrated by a specific example. The pituitary of the rat is relatively rich in the thyreotropic substance; the thyroid gland of the normal rat is relatively very active and the metabolic rate is accordingly relatively high. The normal rat is relatively insensitive to thyreotropic hormone. The hypophysectomized animal cannot produce thyreotropic hormone; the thyroid gland of the animal is relatively inactive and the metabolic rate is depressed, *but the sensitivity to injected thyreotropic hormone is at least ten times greater than in the case of the normal rat.*

Mr. Peter Black has extended the study of the effects of thyreotropic hormone extracts to fish. Here the effects are in general as they are in the mammal. The interesting observation was made, however, that untreated fresh fish placed in the water in which injected fish had previously been kept likewise showed an increase in the metabolic rate. The addition of the hormone to the water in which untreated fish were kept had no effect. It appears, therefore, that, in the case of the fish (cat-fish) the injection of thyreotropic hormone causes the liberation into the water of a substance which can be taken up by another fish with a resultant increase in metabolism of the latter animal. Further investigation along these lines will be carried out to determine if possible whether it is the true secretion of the thyroid which is passed into the water from the thyroid gland of the injected fish.

One of the effects of thyroid feeding is an increase in the calcium excretion by the bowel (91). Doctor Pugsley has been able to demonstrate a similar, though not so marked, effect in

normal animals injected with the thyreotropic hormone which occurred only during a period of elevated metabolic rate.

A remarkable demonstration of the fact that the thyreotropic hormone has a direct action upon the thyroid cells has been given by Eitel, Krebs and Loeser, who produced hyperplasia in sliced thyroid tissue treated in vitro with an active thyreotropic extract. Houssay, Biasotti and Magdalena, and Marine and Rosen (92) have likewise obtained a reaction in transplanted thyroids.

### ADRENAL

While the earlier workers, like Aschner, found no adrenal cortical atrophy in hypophysectomized dogs, more recent observations (Smith and others) have shown that definite atrophy of the adrenal cortex follows the removal of the hypophysis in rats. We were able to confirm these observations on the rat, and furthermore found that similar atrophy of the adrenal cortex may be produced in mice, guinea-pigs and monkeys. It is remarkable that the cortical atrophy which is so obvious after removal of the hypophysis in rats, mice and cats is not very marked in the hypophysectomized dogs so far examined by us. In the case of a dog hypophysectomized one month before autopsy, the width of the adrenal cortex was not markedly less than that of the normal control litter mate.

Histologically this cortical atrophy is characterized first by an intense hyperaemia of the cortico-medullary junction; then the zona fasciculata and zona reticularis of the cortex show definite signs of atrophy (fig. 10). The cells degenerate, and the cortex becomes progressively narrower. Numerous pigment cells appear in the zona reticularis, which contain yellowish green granules. Such cells may also appear in this zone in the normal rat during old age, but they are never found in young animals, and even in the senile rat they are never as frequent as they are after hypophysectomy.

#### *Preparation of the adrenotropic hormone*

The alcohol and acetone filtrates which are obtained in the preparation of the thyreotropic principle are combined and concentrated at low temperature and pressure. The aqueous concentrate is saturated with ammonium sulphate and the precipitate is removed. It is extracted with 1 per cent aqueous ammonia and the extract is dialyzed, to remove the  $(\text{NH}_4)_2\text{SO}_4$ . After dialysis the extract is treated with ten volumes of absolute alcohol and the precipitate is removed and

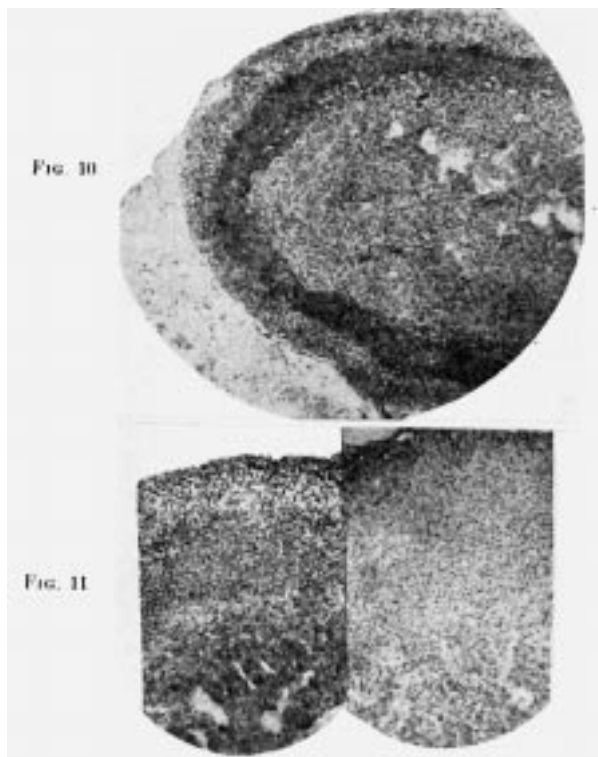


Fig. 10. Cortical atrophy in rat 265 days after hypophysectomy.

Fig. 11. Cortical repair with adrenotropic hormone 139 days after hypophysectomy. Left—control biopsy weight 3.5 mg. Right—6 days after treatment weight 7 mg.

extracted with dilute aqueous alkali (0.05 NaOH). This extract is neutralized to pH 6 with dilute acetic and allowed to stand at 1°C for some days. The precipitate which has formed is removed and dissolved in either dilute acid pH 3 or dilute alkali pH 9. This isoelectric fraction which is obtained in the above manner is almost free of thyreotropic hormone and can be shown to be rich in adrenotropic principle by the following method of assay devised by Dr. Evelyn Anderson.

The left adrenal is removed from hypophysectomized rats ten days to two weeks following the operation. This gland is weighed and sectioned for microscopic examination. The animal is injected twice daily for six days with the extract to be tested. The animal is killed and the right adrenal is weighed and sectioned, as is also the thyroid gland. Control experiments have shown that there is no compensatory hypertrophy of the remaining gland after unilateral adrenalectomy in the hypophysectomized animal. Increase in weight of the remaining adrenal over that of the one taken at biopsy, together with microscopic evidence of cortical repair is taken as positive evidence of adrenotropic activity in the extract. The presence or absence of thyreotropic hormone can

be determined in the same test animals by noting whether there is any increase in metabolism during the period of injections and if there is any evidence of increased cellular activity in the thyroid, taken at the post-mortem examination. It has been only in isoelectric fractions that we have obtained negative tests for thyrotropic hormone.

The purified adrenotropic hormone extract has been shown to withstand prolonged boiling at pH 5.

#### *Physiological effects of the adrenotropic hormone*

As yet we have very little detailed information on the physiological effects of the adrenotropic hormone. No appreciable improvement has been seen in the cachectic condition of the hypophysectomized rat treated with the active extract, even though a substantial degree of repair of the atrophic cortex has taken place (fig. 11). Normal animals injected with the extract have not shown any marked changes in the size or histological appearance of the adrenals. Dr. Pugsley has obtained, in a preliminary study of the effect of the adrenotropic extract upon the calcium and potassium balance of normal rats, very suggestive evidence that, during the first four days of the administration of the hormone, the potassium balance tends to become less positive, whereas no effect was noted on the calcium balance.

### PARATHYROID GLANDS

Hypophysectomy has produced no detectable changes in the parathyroid glands of the rats in our series. We can confirm Houssay that the parathyroids of the hypophysectomized depancreatized dog become atrophic. We have seen in one

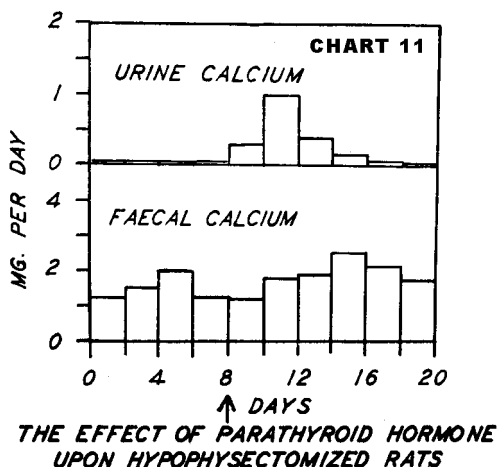


Chart 11. Showing that parathyroid hormone produces a hypercalcaemia in the hypophysectomized rat (Dr. Leonard Pugsley).

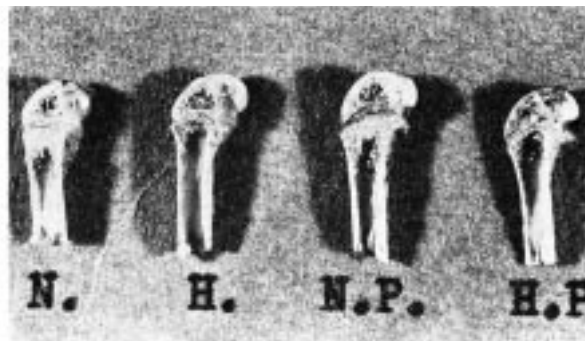


Fig. 12. Action of parathyroid hormone on bone of normal as compared with hypophysectomized rat. N-Normal. H-Hypophysectomized. N.P.-Normal treated. H.P.-Hypophysectomized treated.

case a large focus of necrosis at the centre of one parathyroid, while the other showed definite signs of atrophy (fig. 13). We have seen definite enlargement of the parathyroids of the cat within two weeks of hypophysectomy. It is possible that there may be an early stage of parathyroid enlargement and over-activity which is later followed by atrophic change. Blood serum calcium of the hypophysectomized rat is normal or slightly increased. The calcium balance tends to be negative, but it has been restored to a definite positive value by treatment with the growth hormone (Pugsley) (67).

Hypophysectomized rats treated with the parathyroid hormone respond to it in the same manner as normal animals (Pugsley) (68) insofar as the early hypercalcaemia is concerned (chart 11).

The effect of parathyroid hormone on the bone is quite different when applied in very large doses in acute experiments and in chronic experiments. While this hormone leads to bone resorp-



Fig. 13. Necrosis in the parathyroid of a pancreatectomized hypophysectomized dog, 10 weeks after hypophysectomy.

tion and formation of many osteoclasts in the acute experiment, the same substance leads to the formation of osteoblasts and to bone apposition when administered daily over long periods. We found that the same dose of parathyroid hormone which would lead to bone apposition and osteoblast formation in the normal animal leads to bone resorption in the hypophysectomized one. It seems, therefore, that the formation of new bone tissue is interfered with in the absence of the hypophysis (fig. 12).

### THYMUS

The literature on the effect of hypophysectomy on the thymus is somewhat contradictory. P. E. Smith (1930, 69) saw no specific changes in the thymus of the hypophysectomized rat. Richter and Wislocki (1930, 70) noted enlargement of the thymus and lymph glands of the rat. Koster (1930) (71) found the thymus enlarged in the hypophysectomized dog. Sakamoto and Saito (1932, 72) described a condition of fatty degeneration in the thymus of hypophysectomised rabbits. Kapran (1932, 73) observed involution of the thymus of the dog after hypophysectomy. Our observations on the thymus of the hypophysectomized rat are in agreement with P. E. Smith's. We have frequently seen enlargement of the thymus in rats treated with certain extracts of anterior lobe for some weeks. The new tissue which could be separated from the old on account of the slightly lighter color of the new lobes proved on examination to be lymphatic in type. This effect has been noted in chronic experiments in which extracts rich in the adrenotropic hormone have been used. The significance of this is not yet understood, but it may be stated that there is sug-

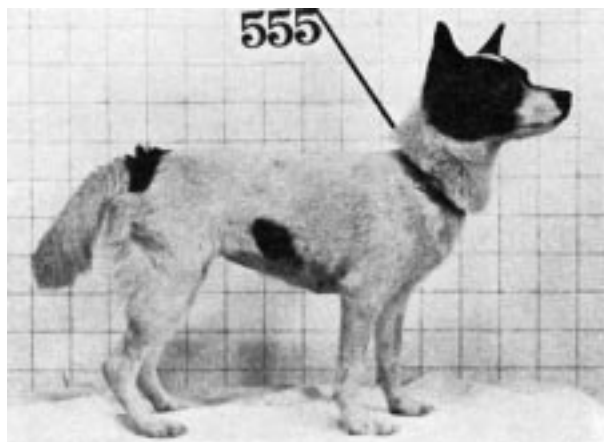


Fig. 14. A typical Houssay dog 11 weeks after second operation

gestive evidence that certain of the blood-forming organs may be influenced by anterior pituitary principles. Miss Margaret Hill is studying this phase of the problem.

### *The diabetogenic and ketogenic effects of anterior pituitary extracts*

Burn and Ling (1930) (74) demonstrated that rats on a salt butter diet, injected with an alkaline extract of anterior lobes, excreted large amounts of acetone bodies in the urine. Hoffmann and Anselmino (1931) found that certain anterior lobe extracts caused an increase in the acetone bodies of the blood and also lowered the basal metabolism (75, 76). Funk (1932, 77) confirmed Hoffmann and Anselmino and stated that the fat hormone could be obtained from both normal and pregnancy urine. Magistris (1932, 78, 79) found an increase in the acetone bodies of the blood of rabbits treated with an aqueous extract of acetone dried powder pituitary preparation. He reported also a lowering of the metabolic rate. He was of the opinion that the fat metabolism hormone was not identical with the thyretropic, growth or maturity factors.

Riatti (1932, 80) noted a decreased acetone excretion following either pancreatectomy or phloridzin administration to hypophysectomized as compared with normal dogs.

Evans (1933) reported that he had produced glycosuria in a female dog and obesity in a male animal by injections with his preparation of the growth hormone.

Houssay, et al. (1933, 82) reported that ante-



Fig. 15. Two hypophysectomized monkeys 10 weeks after operation

rior lobe extracts inhibited the hypoglycaemia of hypophysectomized animals and increased the glycosuria, diuresis, and acetone body excretion

of hypophysectomized depancreatized animals. Barnes has reported somewhat similar results.

It is impossible to know from the earlier work whether the so-called diabetogenic principle and the so-called ketogenic principle are one and the same. It is possible that in each case we are dealing with a special physiological effect of one of the known pituitary hormones. The lowering of metabolism that has been observed by Hoffmann and Anselmino and by Magistris may have been due to the secondary effect of the thyreotropic principle. Using the Burn-Ling test object as a method of assay, Mr. Peter Black has been studying the ketogenic action of the various extracts of anterior lobe with which we have been working. This work has not yet advanced to the stage where final conclusions can be drawn, but the results so far obtained would tend to support the view of Magistris that the ketogenic substance is distinct from the thyreotropic substance.

The diabetogenic substance can be studied to best advantage in the Houssay dog, i.e., in the depancreatized and hypophysectomized animal. Dr. Barnes very kindly tested some of our extracts on his Houssay animal last year. Diabetogenic activity was found by him in both the growth and the thyreotropic fractions. We are at present in our own laboratory attempting to build up a colony of Houssay dogs together with a number of hypophysectomized animals of different species. We have been able to confirm Houssay, Barnes and others in regard to the effect of hypophysectomy upon the diabetic condition of totally depancreatized animals.

We have lost some of our completely hypophysectomized monkeys from hypoglycaemia and one Houssay dog died presumably from hypoglycaemia. This particular animal was an exception to the general rule as regards the blood sugar. Fasting values of 50 mg had been observed previous to death. The treatment with extracts to which this animal had been subjected no doubt contributed in part to the unusual blood sugar values which had been observed over a period of many days. These points will be discussed in detail later. I would like to mention here that we have lost four of our hypophysectomized cats approximately ten days after hypophysectomy all of which showed histological signs of liver degeneration.

### ANTI-HORMONES

It has been a matter of great interest to those working in endocrinology to note in the case of many laboratory animals and also in the case of

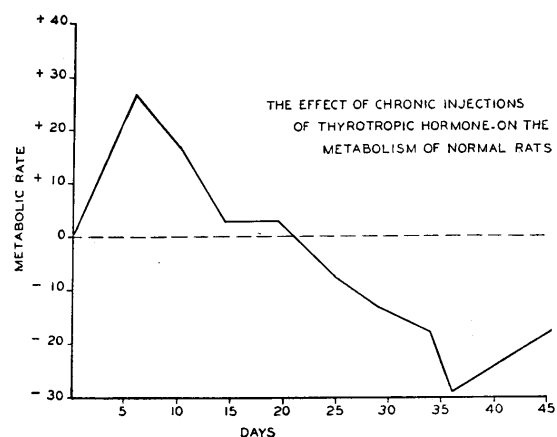
many patients, that a state of lowered reactivity, of increased resistance or of actual non-responsiveness may gradually become manifested in those that have been treated for a long period with some glandular extract. Likewise, it has been observed that certain previously untreated animals or patients may be non-responsive to injections of a known potent glandular extract. We have only to think of the failure of the parathyroid hormone to affect appreciably the blood serum calcium of the rabbit or of the patient who is resistant to enormous doses of thyroid extract.

It was therefore a matter of more than usual interest to us when we found in chronic experiments on animals in which the thyreotropic hormone was injected twice daily over a long period that the animals not only failed absolutely to give a positive metabolic response, but actually showed a lowering of the metabolism to the level of that of hypophysectomized animals (83). The thyroid glands of the chronically injected animals became atrophic and the thyreotropic hormone was absent from their own pituitary glands (fig. 16). In my Washington lecture to the Congress of Physicians and Surgeons published last September, I asked the following questions anent this remarkable fact:

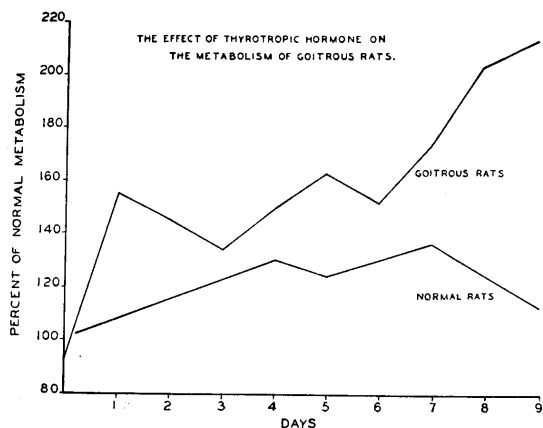
“Does the thyroid gland develop an immunity to the injected hormone?”

Is some inhibitory mechanism brought into action by the continued stimulation with the thyreotropic hormone?”

Does the purified extract used contain as well a true inhibitory principle which has a definite latent period before its effects become manifested?”

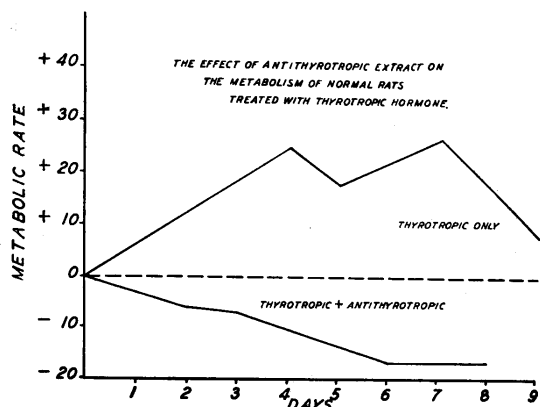


**Chart 12.** The average metabolic rate curve of a group of normal rats injected daily with the thyreotropic hormone. Note the fall to subnormal levels after 30 days treatment.

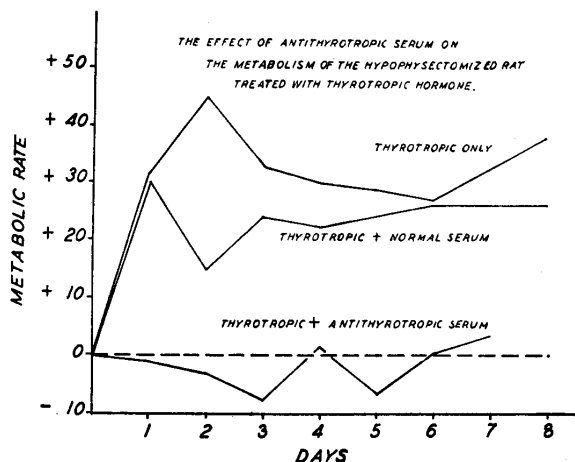


**Chart 13.** A comparison of the metabolic response in normal and goitrous rats treated with the thyretropic hormone.

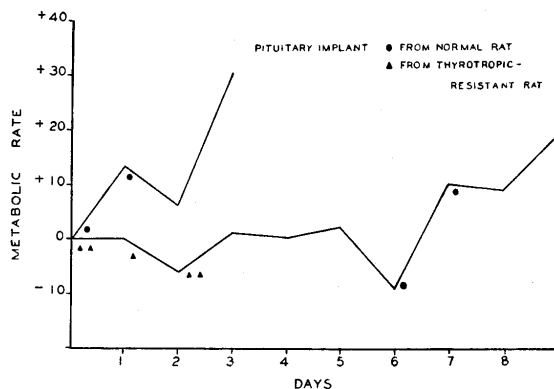
In the light of our recent experiments, our second question would appear to be the most pertinent. Dr. Evelyn Anderson and I have been able to demonstrate most positively that the blood serum of the thyretropic hormone resistant rats contains a substance, which we propose to call the antithyretropic hormone, which when injected into normal rats confers upon them, for a time, resistance to the elevating effect of the thyretropic hormone on the metabolic rate. Furthermore, we have extended our studies to other species, and we have produced the antithyretropic hormone substance in the blood of the rabbit, goat, dog and horse, by daily injections of adequate amounts of the thyretropic hormone extract. Dr. Carl Bachman has obtained negative results with the active serum using the complement fixation and precipitin tests. This encouraged us to prepare serum-protein-free extracts of the antithyretropic blood. This proved to be entirely feasible and now concentrated and highly active antithyretropic hormone extracts are a fact (83).



**Chart 14.** The demonstration of the anti-thyretropic effect of the purified extract of horse serum from an animal injected with the thyretropic hormone.

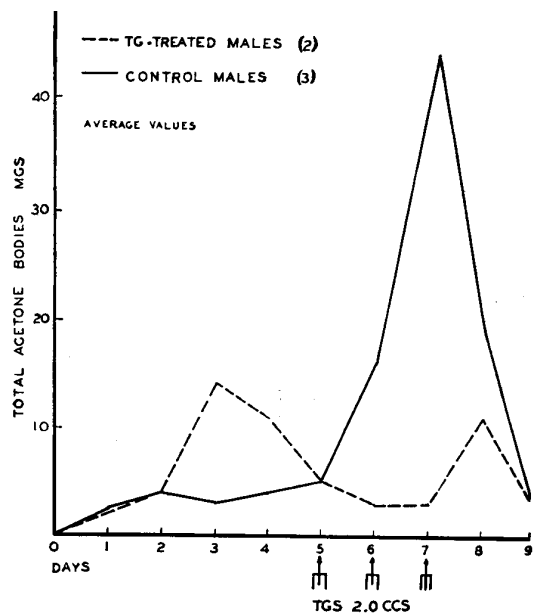


**Chart 15.** The metabolic effect in hypophysectomized rats treated (1) with thyretropic hormone; (2) thyretropic hormone + normal serum; (3) thyretropic hormone + antithyretropic serum.

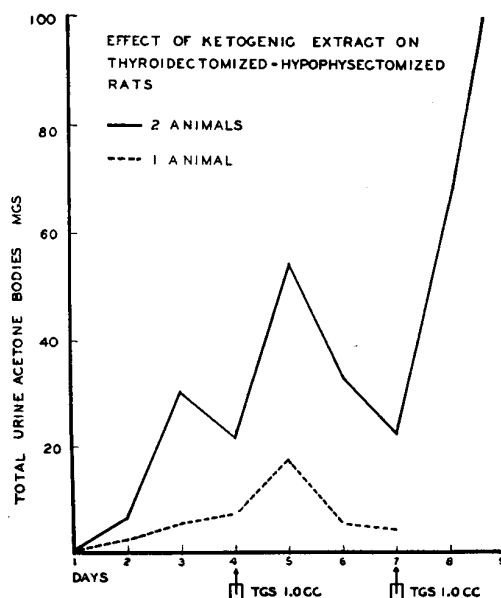


**Chart 16.** Showing absence of thyretropic principle in the pituitary of the normal rat treated for some weeks with the thyretropic extract. Upper curve (control) shows the effect of implantation of two normal pituitary glands and lower curve shows the non-effect of implantation of five pituitaries from chronic thyretropic hormone treated rats. Later the implantation of two normal glands caused a sharp rise in the metabolic rate as shown on the lower curve.

We have adopted as a working hypothesis the view that antihormones are present in the normal subject. If this is true then the production of some of those antihormone substances which we have reported can be looked upon as an increasing of the level of a substance or substances which are normally present. This broad principle is particularly attractive, since it may be used to explain such things as the great difference in the responsiveness of different species to hormones, as well as minor variations in responsiveness within a species. The extreme case is illustrated by the hypophysectomized animal, which shows not only a lack of thyretropic hormone, since the seat of its production has been destroyed, but a decrease in the antithyretropic substance, which is shown indirectly by the increased



**Chart 17.** Showing the ketogenic effect of thyreotropic extract T.G. in normal rats and the non-effect of similar treatment in normal animals treated for some weeks with the thyreotropic extract (Mr. Peter Black).



**Chart 18.** Showing that the ketogenic effect can be obtained in an otherwise untreated hypophysectomized-thyroidectomized animal by the administration of an anterior pituitary extract, T.G. (Mr. Peter Black).

sensitivity to the thyreotropic extract. It is possible, in view of this, that many apparent hypoglandular states may be due to an increase in antihormone level.

The detection of an antihormone in the blood is already possible in the case of the antithyreotropic substance.

One of the most important implications of this theory of "inverse response" is that the level of a hormone is related directly to the level of the corresponding antihormone. The demonstration of a hyperhormone state, associated with lowered responsiveness to injected hormone, implies the co-existence of a hyper-antihormone state. One might here draw an analogy between the highly buffered solution which is sluggish in regard to pH change on addition of small amounts of acid or alkali, and the hormone resistant animal which is, as it were, well buffered by a high level of hormone and antihormone substances in its system.

That the effect of a small dose of a hormone will be greater when the existing concentrations of hormone and antihormone circulating are small than when they are already large, will not be regarded as surprising. In many cases of physiological antagonism between drugs, it is known that the ratio between the concentrations of the two antagonists determines the physiological effect, whatever the actual amounts; this has been shown for the interaction of atropine and pilocarpine on salivary secretion (86), of atropine and acetylcholine on the frog ventricle (84, 87), of adrena-

line and ergotamine on the rabbit uterus (88) and elsewhere. The *ratio* will obviously be more disturbed by a given amount of hormone when the quantities of hormone and antihormone present are initially small.

The facts which have been proven with regard to the thyreotropic hormone suggest that the principle of *inverse responsiveness* may apply very generally. If it can be shown that somewhat similar conditions hold in the case of other hormones, then a general law—that of "*inverse response*"—may exist. There is already existent considerable support for this as a general basic view.

We have found that the growth hormone has no effect upon the rate of growth of young male rats and only a slight positive effect in the case of females (chart 1). Hypophysectomized animals, male or female, are exceedingly sensitive to the growth hormone. Immature female rodents are extremely sensitive to the various forms of oestrin. Thyroidectomized animals show an increased sensitivity to thyroid extract (93). Hypophysectomized animals are much more reactive to the diabetogenic substance than are normals. Other examples of apparent non-reactivity to hormones resulting from chronic treatment are to be found in the literature. Akimoto found that rabbits showed little or no increase in blood sugar after adrenalin administration provided they had been previously treated with adrenalin for 10-20

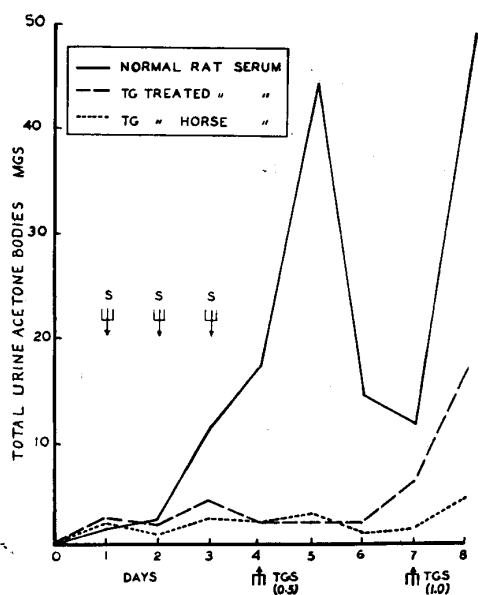


Chart 19. Showing anti-ketogenic effect of serum of the rat and horse treated with thyreotropic extract for some weeks as compared with the non-inhibitory effect of normal serum.

days. Zondek reported that mice treated with prolactin first showed great increase in ovarian weight but that after a certain time the weight of the ovary was normal in spite of continued injections of prolactin. Aron confirmed these observations in the guinea-pig, and further showed that the same loss of sensitivity developed after chronic treatment of the guinea-pig with pituitary gonadotropic extracts. Our own results on rats treated with A.P.L. or pituitary implants confirm these findings; and we were able to show also that the ovaries of such chronically treated animals not only return to normal weight but often to sub-normal size and show definite histological evidence of severe atrophy.

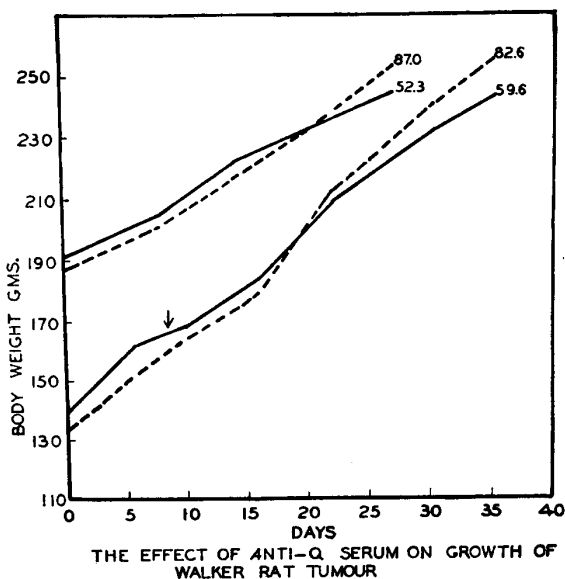
de Fremery, Luchs and Tausk (85) made the observation that by continuous administration of corpus lutein hormone one is unable to maintain the uterine mucosa of the rabbit in a progestational stage for more than approximately 17 days. After this the mucosa atrophies whether the hormone administration is continued or not. Hartman and Winter have observed recently that adrenalectomized animals treated with cortin over a long period may become non-responsive and show definite signs of cortical deficiency, although they are treated with large amounts of a potent cortical extract. Loeb and Friedman (1931) observed that guinea-pigs treated with thyreotropic anterior lobe extract lost their sensitivity after a period of 15–17 days. Aron (1932) obtained a similar result. In our own extensive experiments with the purified hormone on rats we have confirmed and

extended these observations to the point where the nature of the mechanism underlying such reactions has become manifested.

We have been able to demonstrate that rats become non-responsive to the gonadotropic effect of the anterior pituitary-like hormone of the placenta and also to the maturity factor present in fresh rat pituitary by repeated daily treatment with these substances. It is of special significance that in the non-responsive state, the gonad may become smaller than in the normal and show definite signs of atrophy. Ovaries that have reached this state as a result of chronic A.P.L. treatment still respond to the gonadotropic substance in fresh pituitary implants. Similarly ovaries that have become resistant to the gonadotropic hormone of implants still respond to the placental hormone. Furthermore, the blood serum of rats injected for many weeks with A.P.L. has been shown to inhibit the ovary-stimulating effect of A.P.L. in the immature animal. Also the blood serum of an A.P.L. chronically injected rabbit has been shown to inhibit A.P.L. in the immature rat.

Hypophysectomized rats which have been treated for some weeks with our purified growth extract have ceased to grow and indeed have actually shown a decline in the growth curve even though the injections of known potent extract have been continued (chart 2). A group of such growth hormone resistant animals were sacrificed and the blood serum of these was pooled. A suggestive result was obtained when this serum was used in conjunction with a standard growth extract. The test object, a recently hypophysectomized rat, did not show as great a growth response as did the control treated with growth extract plus normal serum. Since, for complete inhibition of the growth hormone action, a very definite amount of antihormone must be required in relation to the growth hormone used, a new method of testing for the antigrowth principle was devised. This consists in the continuous injection twice daily of a standard dose of Q extract. Antiserum is given in addition as soon as a definite growth effect is manifested. A positive effect consists in a slowing or the complete inhibition of the growth effect of the Q extract. In the course of a few days, when this has become established, normal serum is substituted for the antigrowth serum. Growth is then resumed as in the first period. Antiserum is again introduced until growth ceases or is greatly slowed and again the positive effect is restored by normal serum used in place of the antigrowth serum being tested.

Our antigrowth serum has been prepared by injecting a horse daily with the Q extract.

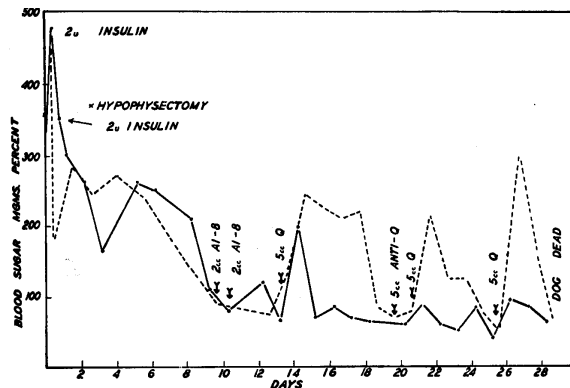


**Chart 20.** Growth curves of rats bearing the Walker tumour and treated with the anti-Q horse serum. Note that the average weight of the tumours in the case of the treated animals (broken line) was considerably less than in the controls (5 animals in each group) (Dr. C. S. McEuen).

Dr. C. S. McEuen has been studying the effects of various hormones on the Walker-rat tumour. Since the most positive type of inhibitory influence upon the growth of this tumour was shown to be manifested after hypophysectomy (McEuen and Thomson, 89) and since therefore there is evidence that the rapid growth of this tumour is related to a normal if not to a hyper-functioning anterior pituitary of the host, Dr. C. S. McEuen has carried out some preliminary experiments in which horse serum from the animal treated with Q extract has been injected into tumour bearing animals. The results of this preliminary test are highly suggestive (chart 20) and justify the further work along these lines which is being undertaken. The chief difficulty in the way of a practical application of the antigrowth principle is suggested by the failure of an antigrowth serum, which has been shown to be potent by the method outlined above, to affect appreciably the rate of growth of normal rats. This is due very probably to the great functional reserve which the normal pituitary must possess. Much more valuable information will be at hand when we have had an opportunity to complete a series of experiments in which the effects of continuous injections, for long periods, of antihormone principles of various types have been ascertained.

#### ANTI-DIABETOGENIC PRINCIPLE

As stated before, there is no positive evidence as yet as to whether the diabetogenic effect of

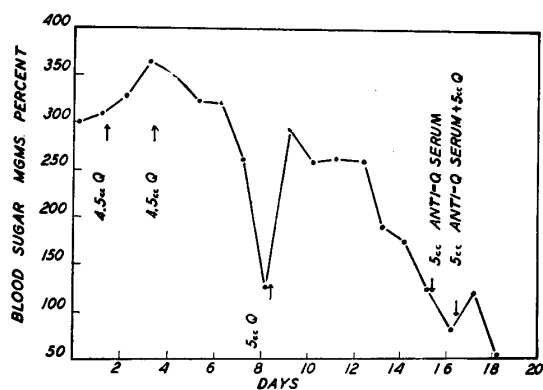


**Chart 21.** Dotted line—evening blood sugars. Solid line—morning blood sugars of a Houssay dog. Adrenotropic extract A.I., growth extract Q and anti-Q (horse serum) were administered. Shows suggestive evidence of a neutralizing of the diabetogenic effect of Q extract by the anti-Q horse serum.

anterior pituitary extracts is due to a specific principle or whether it is a side effect of one of the known pituitary hormones. Since our Q extract has been found to be diabetogenic, it was of interest to see if any anti-diabetogenic effect could be demonstrated in the serum of the horse chronically injected with Q extract. Due to the fact that normal animals are useless in this type of testing, we have confined our efforts to the Houssay dog. Here again there is great difficulty because the average Houssay animal has a blood sugar of 250 mg. and excretes large amounts of sugar. One animal which had been treated for some weeks with oestrogenic extracts of placenta and pregnancy urine prior to the Houssay operation of pancreatectomy and hypophysectomy did have a fasting blood sugar of less than 100 mg. This animal was treated with the Q extract and the anti-growth serum taken from hypophysectomized rats as indicated in the chart. The results are very suggestive of a neutralization of the diabetogenic action of the Q extract by the anti-Q serum. They are not conclusive, however, but they have encouraged us to extend the research along these same lines.

#### ANTI-KETOGENIC PRINCIPLE

Using what is no doubt altogether too artificial a test object, normal rats on a butter diet (Burn and Ling)—Mr. Peter Black has found that animals which have become non-responsive to the thyretropic hormone do not give a positive result by the Burn-Ling test for acetone body excretion. Also he has decreased the ketogenic effect of the thyretropic extract on normal animals by injecting them with antithyretropic



**Chart 22.** Houssay dog—morning blood sugars. Q extract, diabetogenic and anti-Q serum administered as shown on chart. Like no. 21, shows suggestive evidence of a neutralizing of the diabetogenic effect of Q extract by the anti-Q serum.

serum. These results, while not free of criticism, nevertheless suggest the interesting possibility that the diabetogenic effect (hyperglycaemia and glycosuria) and the ketogenic effect (increased excretion of acetone bodies; may be mediated through two entirely different mechanisms (charts 17, 18, 19).

### THE ANTI-ADRENOTROPIC SUBSTANCE

We have as yet no experimental evidence to submit which would establish the existence of such a principle, although we confidently believe that such exists.

### CLINICAL APPLICATIONS

Hormone therapy of any kind must be approached with much greater caution than has been the case in the past. While the results of our recent work suggest that many new dangers may attend the clinical use of glandular extracts, they also point the way to new applications. If the general principles established by the animal work are applicable to the human subject, then one must consider the possibility both of creating antihormones in an active way in the system of the patient, and of increasing the antihormone level by use of active extracts of the serum of horses or other animals which have been treated for long periods with the respective hormone.

I should like to point out, in bringing this discussion to a close, that some of the statements which I have made represent final conclusions which I trust will stand the test of time; some on the other hand are the expression of a point of view in keeping with the results of experiments made; and finally, some are purely speculative.

The part of the work of which I have spoken that has been carried out in the Biochemistry Department of McGill University has been due to the splendid cooperation of a relatively large group of workers to whom all credit must be given for such positive findings as have been made.

The members of this team are Drs. E. M. Anderson, C. Bachman, R. L. Kutz, C. S. McEuen, H. Mortimer, L. I. Pugsley, H. Selye and D. L. Thomson; Miss Margaret Hill; and Messrs. P. T. Black, O. Denstedt and T. McKeown.

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