

THE EFFECT OF POSTERIOR LOBE EXTRACT, ADRENALIN, AND PILO-
CARPINE ON THE RESPONSE OF THE THYROID GLAND TO THE THYREO-
ACTIVATOR HORMONE OF THE ANTERIOR LOBE OF THE HYPOPHYSIS

by

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A C K N O W L E D G M E N T

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I - INTRODUCTION.

Ever since our realization of the significance of the endocrine glands, biologists have been concerned with the very vital problem of investigating the mechanisms which control the activity of these organs. Numerous studies have been made on the role played by the environment, dietary factors, drugs, the nervous system, and even the endocrine glands themselves in the function of the various units of this complex system. These efforts have revealed to us many interesting endocrine inter-relationships, one of which is that existing between the anterior lobe of the hypophysis and the thyroid gland.

The stimulating effect of the anterior lobe of the hypophysis on the thyroid gland has been worked out in such great detail during the past decade that it stands to-day as an undeniable biological fact. Having ascertained one of the factors which control thyroid secretion, attention was then directed from that immediate problem to one directly related to it. Is it possible to alter the normal response of the thyroid gland

to the thyreoactivator principle of the anterior lobe of the hypophysis; and if so, by what agents? In the experiments to be described the effects of three substances, an extract of the posterior lobe of the hypophysis, adrenalin, and pilocarpine, on the response of the thyroid gland to the thyreoactivator hormone have been studied.

Before describing my own experiments, I will review some of the work done by previous investigators on the thyroid - anterior lobe relationship. The data pertaining to the effect of posterior lobe, adrenalin, and pilocarpine on the thyroid gland, as well as the bearing that this knowledge has to my findings will be discussed along with the experiments.

II - THE HISTORY OF OUR KNOWLEDGE OF THE THYROID - ANTERIOR LOBE RELATIONSHIP.

The stimulating effect of the anterior lobe on the thyroid gland can be studied by either anatomical, physiological, or chemical methods. Anatomically an activated thyroid shows an increase in size. Histologically a hyperactive thyroid reveals collapsed follicles, a scarcity of colloid, vacuoles in the cells and colloid, high columnar cells, wide interfollicular blood and lymph spaces, and an increase in the number of mitotic figures.

Physiologically an activated thyroid gland accelerates amphibian metamorphosis, and elevates metabolism. Chemically the iodine content of an active thyroid gland is decreased. Accompanying this there is an increased blood iodine.

Prior to our knowledge of the above-mentioned criteria of thyroid activity efforts to study the effect of the anterior lobe on thyroid activity were not very successful. Adler (1914) was the first one to obtain significant evidence of an influence of the anterior lobe on the thyroid. He operated on 1200 tadpoles, and tried to destroy the hypophysis by means of a cautery. At the end of his experiment only 3 of the completely hypophysectomized animals survived. These animals had atrophic thyroids, and they failed to metamorphose. Adler therefore concluded that the atrophy of the thyroid was the result of the removal of the hypophysis. Since Gudernatsch (1913-14) had shown that the

thyroid causes amphibian metamorphosis, Adler referred the failure of his hypophysectomized tadpoles to metamorphose to the atrophic state of the thyroids of these animals.

Though Adler had successful experiments in only 3 animals, his observation was a correct one, for it was soon confirmed by a number of investigators, Allen (1917)(1927), Smith and Smith (1922)(1923), and Smith (1929-30) in amphibians, and Smith (1927), Foster and Smith (1926), Richter and Wislocki (1930), and Anderson and Collip (1934) in mammals.

The atrophy of the thyroid following hypophysectomy is due to removal of the anterior lobe, for Smith and Smith (1922), Smith (1929-30), and Allen (1927) were able to repair the atrophic thyroids of hypophysectomized amphibian larvae by means of anterior lobe extracts. Neither the posterior nor intermediate lobes had similar effects. Smith (1927) was able to repair the thyroids of hypophysectomized rats with transplants of fresh rat pituitaries. He did not obtain any definite thyroid restoration with fresh bovine anterior lobe fluid. Mixtures with posterior lobe gave no better results. An alkaline extract of the anterior lobe also proved to be unsuccessful in the hands of Richter and Wislocki (1930).

While the role of the anterior lobe in maintaining the thyroid in its normal state was definitely established by Adler (1914) and his successors, it was not until after a number of vain efforts had been made by various investigators that evidence was obtained to show that the normal thyroid gland could be stimulated by the anterior lobe. Renon and Delille (1908), and

Hallion and Alquier (1908) were among the earlier investigators to study the effect of hypophysis administration on the thyroid gland. Hallion and Alquier fed dried powder of the entire hypophysis to rabbits. Since we now know that the thyreoactivator hormone is ineffective by mouth, it is easy to explain the inconsistent effects observed in the thyroid by these authors. They were undoubtedly due to factors other than the pituitary diet. Renon and Delille (1908) administered intraperitoneal injections of total hypophysis. The thyroids of these animals were found to have small follicles containing little colloid, and they were made up of high cells. While this is the picture of an activated thyroid, Renon and Delille were evidently unaware of it. They obtained similar results with posterior lobe which we now know to be ineffective. With anterior lobe extract they produced what they believed to be a hyperactive thyroid, but from their description it is evident that they were dealing with an inactive gland; for the cells were flat, the colloid abundant, and the follicles large. It is quite obvious that what was lacking at that time were the histological criteria of thyroid activity.

Hoskins (1916) could show no consistent changes in the size of the thyroids of rats on a pituitary diet. Pal (1916) injected an extract of the hypophysis and observed that the thyroid was markedly increased in size. He referred the latter to colloid accumulation, and concluded therefore that hypophysis extract stimulates the thyroid cells to function, but at the same time inhibits the passage of the thyroid secretion into the blood stream. That there might be two thyrotropic hormones in the

anterior lobe, one for the secretory and storage phase, and the other for colloid release was recently indicated by Blount (1936).

Allen (1920-21) was able to increase the size of the thyroids in pituitaryless tadpoles to above that of the normal animal by means of anterior lobe. While he asserted that the anterior lobe accelerated metamorphosis through the thyroid, he was not certain of his stand, for he concluded that it was highly probable that the pars tuberalis plays a role. Contrary to the trend of the evidence at that time, Smith (1923) concluded on the basis of metamorphosis experiments in Colorado axolotls, that anterior lobe extract is opposed to the activity of the thyroid gland. Loeb and Kaplan (1924), and McCordock (1928) came to a similar conclusion, for they found that feeding of Armour's anterior lobe tablets prevents compensatory thyroid hypertrophy in guinea pigs which had previously been partially thyroidectomized.

It was at this stage, however, that Uhlenhuth and Schwartzbach (1926)(1927) reported on a series of experiments that definitely settled the issue, and served as the basis for future investigations. By means of injections into *Amblystoma tigrinum* larvae of Armour's anterior lobe powder, not only was metamorphosis accelerated, but the thyroid was also stimulated. The thyroids of these salamanders showed changes which Uhlenhuth (1927) had designated as indicative of hyperactivity. These changes are, 1) increase in the cell height, 2) massing of secretion granules at the apical ends of the cells, 3) the development of secretion vacuoles in the cells. The Mexican axolotl, a normally neotonus salamander, responded similarly. Uhlenhuth and Schwartzbach

(1928) were able to confirm the effects of Armour's powder by means of an extract prepared from dried cattle anterior lobes. They concluded "that genuine anterior lobe extract has the same action on the thyroid as Armour's commercial product, that a fully potent extract can be obtained not only from dried, but from fresh glands; that anterior lobe contains a substance which alone among other substances tested and quite contrary to thyroid substance and substances stimulating the autonomic nerves, forces the inactive thyroid gland to function; and that in amphibians, metamorphosis is caused by this principle only indirectly, through the function of the thyroid gland." That both Armour's preparation of anterior lobe, as well as that made by the authors themselves are ineffective by mouth was also demonstrated by Uhlenhuth and Schwartzbach (1928)..

Ingram (1928) was able to confirm the findings of Uhlenhuth and Schwartzbach by means of anterior lobe transplants into rana clamata. Loeb and Bassett (1929) however, while they admitted that acid and alkaline preparations of the anterior lobe stimulate the thyroid, still maintained that Armour's anterior lobe tablets inhibit the thyroid when given orally. Loeb and Siebert (1930) again reported that the feeding of Armour's tablets prevented the usual compensatory hypertrophy which followed partial thyroidectomy. Loeb's own pituitary preparations did not inhibit compensatory hypertrophy, however, when fed to guinea pigs.

The inhibitory effects reported by Loeb and his coworkers by oral administration of Armour's anterior lobe tablets are explainable on the basis of the iodized proteins present in these tablets.

According to Smith (1926) the iodine content of the latter exceeded that of other anterior lobe preparations by 120 times. Though Swingle and Martin (1926) believed that iodine could stimulate the thyroid, Seecof (1925) was able to involute the guinea pig thyroid with iodine. With small doses of iodine Uhlenhuth (1929) was able to cause an increase in the activity of the salamander thyroid, but if iodine administration was continued sufficiently long degenerative changes resulted. Large doses of iodine produced similar effects which were more marked, however. Uhlenhuth and Winter (1929) obtained almost complete destruction of the thyroid as the result of intraperitoneal implantation of iodine crystals. Severinghaus (1933) was also able to cause thyroid involution by administering iodine.

With most of the obstacles removed, rapid strides were made in the further study of the response of the thyroid gland to the anterior lobe. Aron (1930)(1930a) activated the thyroids of the guinea pig, dog, and cat by means of an anterior lobe extract; Grant (1930) stimulated the thyroid of necturus with anterior lobe implants; and Verzar and Wahl (1931) stimulated the guinea pig thyroid, as did Loeb and Bassett (1930), and Loeb and Friedman (1931).

At this time Schockaert (1931)(1932) showed that the duck thyroid also responded to the activating effects of the anterior lobe. Among the investigators who further contributed to the support of this problem are Crew and Wiesner (1930), Severinghaus (1933), Figge and Uhlenhuth (1933), Anderson and Collip (1933) (1934), Eitel and Loeser (1934), Kippen (1934), Kippen and Loeb

(1935), Friedman and Loeb (1934), Hertz and Kranes (1934), Friedgood (1934). Uhlenhuth (1934), Schneider (1934), Max, Schmeckebier and Loeb (1935), and Uhlenhuth, Thompson and Schenthal (1936). Various important histological and physiological details have been pointed out by the abovementioned investigators. Because of space limitations only some of the more representative contributions will be mentioned.

It was possible to show by chemical methods that the thyroid gland is activated by anterior lobe. Grab (1932), and Closs, Loeb and Mackay (1932) produced a decrease in the iodine of the thyroid, and an increase in blood iodine as the result of anterior lobe extract injections. Schockaert and Foster (1932) confirmed the decrease in thyroid iodine in the duck; as did Loeser (1932) in the dog. Schneider (1934) obtained an increase in blood iodine by means of the thyrotropic hormone.

The stimulating effect of anterior lobe on the thyroid was also established by metabolism studies. Since the classical studies of Magnus - Levy (1895) it is a well known fact that the thyroid gland is capable of increasing metabolism. If it is correct that anterior lobe activates the thyroid, it should also elevate metabolism through the latter. That anterior lobe does elevate metabolism was shown by Schwartzbach and Uhlenhuth (1929) (1936), and Thompson and Uhlenhuth (1936) in the salamander. Artundo and Solari (1933-4) in the toad, and Drexler and Issekutz (1935) in the fish, could not obtain a rise in metabolism with anterior lobe, however.

In the mammal metabolism was elevated by means of anterior lobe by Siebert and Smith (1930), Verzar and Wahl (1931), Artundo and Solari (1933-4), Anderson and Collip (1934) and Friedgood (1934). This rise was found to be absent in thyroidectomized animals by Siebert and Smith (1930), and Verzar and Wahl (1931).

III - THE EFFECT OF POSTERIOR (AND INTERMEDIATE*) LOBE ON THE RESPONSE OF THYROID GLAND TO ANTERIOR LOBE.

From the data already cited it is quite evident that the stimulating effect of anterior lobe on the thyroid is a specific action, for it is not shared in by either the intermediate or posterior lobes. While studies have been made on the effect of the posterior lobe on thyroid activity, most of these have given negative results. The findings of Spaul (1930), however, have indicated that the posterior lobe has an inhibiting effect on the response of the thyroid to anterior lobe.

The non-essential nature of the posterior and intermediate lobes in maintaining thyroid activity has been pointed out by Smith and Smith (1922). They found that the thyroid atrophy that follows hypophysectomy was not benefited by injections of either posterior or intermediate lobe. At that time, Smith and Smith (1922a) believed that metamorphosis was inhibited by anterior lobe injections. They found that their animals became black after repeated injections. The presence of the chromatophore hormone would indicate that the extract employed was contaminated with some intermediate lobe. It can hardly be maintained however that the presence of intermediate lobe played any role in the inhibition of metamorphosis reported. Confirmatory evidence has failed to appear. Also the negative findings of Smith and Smith

*While the posterior lobe alone will be referred to in the experiments to follow, it must be remembered that in reality the intermediate lobe is also included, for these two lobes of the pitui-

(1922) on the effect of intermediate lobe on the atrophied thyroid is contradictory rather than confirmatory. Concerning the posterior lobe, Smith (1926) states definitely that it is ineffective as a metamorphic retardent.

In the rat, Smith (1927) found that the restorative effects of the anterior lobe on the atrophy that results in the thyroid after hypophysectomy, are not augmented by an admixture of posterior lobe. Allen (1920-1)(1927) similarly found that the posterior lobe, as well as the intermediate lobe have no effect on the thyroids of hypophysectomized amphibia. Riddle (1931) tested the effect of pitocin and pitressin on the thyroid gland, and found that these two posterior lobe principles had no effect on the weight of the thyroid.

In contrast to the negative results cited above, Renon and Delille (1908) reported that posterior lobe extracts produced a hyperactive state in the thyroid. As has already been pointed out, these authors were not in the position to interpret their results properly, for they lacked a clear understanding of the histological criteria of thyroid activity.

Of greater significance are the more recent findings of Spaul (1930) who reported that the posterior lobe has an inhibiting effect on metamorphosis. Animals given posterior lobe together with either thyroid or anterior lobe, showed signs of metamorphosis earlier than those given thyroid or anterior lobe alone. But as the experiment progressed the rate of metamorphosis in those animals receiving posterior lobe slowed, and eventually

those receiving thyroid or anterior lobe alone became adult first.

The effect of posterior lobe extract on amphibian metamorphosis, and on the metamorphosis accelerating effect of anterior lobe extract.

Because of the discrepancies existing between the results of an earlier experiment (unpublished) carried out by Dr. Uhlenhuth, and those of Spaul, the following experiments were executed on larvae of *Amblystoma tigrinum* to check the effect of posterior lobe extract on the rate of metamorphosis.

Material and method: The animals used in this experiment were collected in Colorado, and shipped to Baltimore. They arrived in 2 batches on August 8, 1932, and November 27, 1932 respectively. Soon after arrival they were placed in dishes of uniform size and kept in a dark ice box at 6-10°C in tap water. No food was given. On January 10, 1933 they were removed from the ice box and kept at room temperature (12-20°C) in large glass bowls. They were fed liver.

Thirty-five larval salamanders were divided into 7 groups of 5 animals each. Group a was injected with neutralized acid Ringer's solution, group b with neutralized acid extract of anterior lobe, group c with neutralized acid extract of posterior lobe, group d with neutralized alkaline extract of posterior lobe, group e with neutralized acid extract of anterior lobe and neutralized acid extract of posterior lobe, group f with neutralized

acid extract of anterior lobe and neutralized alkaline extract of posterior lobe, and group g, which was kept on a starvation diet for a short period, but was fed later on, received injections of neutralized acid extract of anterior lobe.

The solutions and extracts used were as follows. A 30% amphibian Ringer's solution was acidified by adding sufficient glacial acetic acid to make a 0.5% acid solution. This served to make up all acid extracts, and for the Ringer controls. Neutralization was carried out with N/5 NaOH by means of litmus. Enough hydroxide was added to bring the acid extracts almost to the neutral point. Complete neutralization was avoided.

The alkaline extracts were made with 30% amphibian Ringers and sufficient NaOH to make a 0.01 N solution. Neutralization was carried out with 0.05 N. Hcl by means of litmus.

The anterior and posterior lobes were collected by the author at the slaughter house. The glands were removed from the skulls usually about 40 minutes after the cattle had been killed, and the anterior and posterior lobes were immediately separated. On returning to the laboratory, the two lobes were cut up separately into small pieces, and dried in the breeze of an electric fan over night. In the morning the dried tissue was placed in a dessicator over calcium chloride and left for at least one week in the ice box. Then the glands were ground in a mortar, and kept in the ice box over calcium chloride as a fine powder.

In preparing the extracts, a quantity of powder is weighed.

as required, and mixed with a predetermined volume of extracting fluid, so that the end result will give the desired concentration of extractable material. The mixture of fluid and powder is heated over a small flame to the boiling point, and filtered immediately to remove coagulable proteins. The filtrate when neutralized makes up the desired concentration.

The extracts were prepared at first so that 0.25 cc. of the final product contained the extractable material of 50 mg. of powder, either anterior or posterior lobe. For the animals which received combined injections, 0.25 cc. of anterior lobe extract was mixed with 0.25 cc. of posterior lobe extract to make a total fluid volume of 0.5 cc. So as to make the fluid volume equal throughout, the extracts for those animals which received either anterior lobe or posterior lobe alone, were mixed with equal quantities of Ringer's solution. Thus each animal received 0.5 cc. of fluid. After the eighth injection the dose was doubled, so that each animal received the equivalent of 100 mg. of powder, instead of 50 mg. The injections were carried out under chloretone anesthesia by the intraperitoneal route every other day, excepting Sunday.

Results.

Neutralized acid Ringer injections (group a).

It is evident from table 1 that the intraperitoneal injection of neutralized acid Ringer's solution has no specific effect on the rate of metamorphosis in *Amblystoma tigrinum* larvae. Of the five animals, two, a2 and a4, metamorphosed rather rapidly.

They began to shed their skin after 14 and 19 injections, and their gills were reduced after 17 and 23 injections respectively. The early metamorphosis in these two animals resembles the results obtained in the anterior lobe injected animals in group b. If, however, the other 3 animals in group a are considered, it becomes evident that the changes in a2 and a4 must be referred to spontaneous metamorphosis. Animal a3 began to shed skin 2 days after the 35th, or last injection, which was 80 days after the beginning of the experiment. This animal's gills were reduced 19 days after the last injection, showing that the injections had little to do with the metamorphic process going on. Still more convincing are a1 and a5. Animal a1 died in a larval state 125 days after the last injection, and a5 died while still larval, 35 days after the last injection. The results therefore indicate that injections of Ringer's solution have no specific effect on the rate of amphibian metamorphosis.

Neutralized acid anterior lobe extract injections (group b).

The results in this series are quite different from those in group a. In contrast to the great intervals of time existing between the metamorphosis of the individual animals in group a, all of the 8 larvae which received injections of anterior lobe extract metamorphosed as one block. They shed skin within 22 to 38 days, an average of 28; and within 10 to 16 injections, an average of 12. Compare this with the 94+ days, and 28+ injections of group a. In group b, the gills were reduced in 30 to 50 days, an average of 38; and 14-22 injections, an average of 17.

Compare this with the average of 100+ days and 29+ injections respectively for group a. These results agree with the data already quoted on the ability of the anterior lobe of the hypophysis to accelerate amphibian metamorphosis. The accelerating effect is through the thyroid gland.

Neutralized acid posterior lobe extract injections (group c).

The animals in this group compare very favorably with those injected with Ringer's solution (group a). There is a great amount of variation in the time of metamorphosis of the individual animals. Animal c1 was still larval 125 days after the 35th, or last injection. Animal c2 shed skin after 24 injections, while c3, c4, and c5 metamorphosed at various times after the last injection, as is evident in table 1. The averages are slightly, but insignificantly higher than those of the controls in group a. Neither acceleration nor inhibition of metamorphosis was observed.

Neutralized alkaline posterior lobe extract injections (group d).

As is evident in table 1A, this group compares very favorably with the animals in groups a and c. The averages, as indicated by the number of injections, are lower than in group a. This is due to the fact that two of the animals d4 and d5, metamorphosed as rapidly as did the anterior lobe animals. Yet the fact that the others in this group continued to remain larval for some time indicates that this rapid metamorphosis was spontaneous. For when there is a metamorphosis accelerating factor present individual variability is wiped out, as in group b. The absence

of a metamorphosis inhibitory factor in posterior lobe is pointed out very strikingly in this series by the early metamorphosis of animals d4 and d5. It is equally evident that posterior lobe does not accelerate metamorphosis.

Neutralized acid anterior lobe and neutralized acid posterior lobe injections (group e).

That the accelerating effect of anterior lobe extract is not inhibited by an acid posterior lobe extract is evident from table 1A. The animals all responded as one group, as in group b. Animal e2 died after the 12th injection in a far advanced stage of metamorphosis. It had marked exophthalmos, the body and tail fins were gone, and the gills were short. Animal e5 died after 14 injections with marked exophthalmos, no body or ventral tail fin, with half the dorsal tail fin gone, and short gills. These two animals compared very favorably with the other 3 in the group. If the results in the individual animals, or their averages, in group e are compared with those of group b, it becomes evident that there are no signs of inhibition or accentuation of the acceleration due to anterior lobe.

Neutralized acid anterior lobe and neutralized alkaline posterior lobe injections (group f).

The three surviving animals in this group go very well with those of group e, showing that the alkaline posterior lobe extract has no effect on the metamorphic action of anterior lobe as in the case of the acid extract. While the averages are slightly

lower than those for group b, the individual animals fit in with those injected with the anterior lobe extract alone. Two of the animals, f2 and f3, died after the third injection, and are therefore not included in table 1A.

Discussion.

Neither spontaneous, nor anterior lobe induced metamorphosis is affected by either acid or basic extracts of the posterior lobe. The posterior lobe extracts used produced metamorphic signs before the latter appeared in the Ringer injected controls; but not before they appeared in the anterior lobe injected animals as maintained by Spaul (1930). Posterior lobe injected animals showed signs of beginning metamorphosis as early as the 9th and 10th injections in some instances. These changes (exophthalmos and diminution of gills and fins) which appeared earlier than in the Ringer injected controls (13 injections) progressed slowly, however, and some of the posterior lobe animals remained in a semi-metamorphic state for months (c1 and d2).

In the course of the experiment it was observed that a number of the animals which received posterior lobe injections developed oedema which was very severe in some cases. This response was present in animals c3, d5, e4, f1, and f2. It occurred between the first and third injections, and then disappeared.

Summary.

- 1). The anterior lobe of the hypophysis accelerates amphibian metamorphosis when compared with controls.

2). Acid and basic posterior lobe extracts do not inhibit or accelerate either spontaneous or anterior lobe induced metamorphosis.

IV - THE EFFECT OF ADRENALIN ON THE RESPONSE OF THE THYROID GLAND TO ANTERIOR LOBE.

Before presenting the experiments on the effect of adrenalin on the response of the thyroid gland to the thyreo-activator hormone it is important to become acquainted with what is already known about the interrelationships existing between these three glands. In connection with the present problem it is indispensable to know whether the adrenal medulla has a direct effect on the thyroid gland. If the adrenal medulla does affect the thyroid directly, then we are faced with the following question. Is the augmentation by adrenalin of the response of the thyroid to the thyreoactivator hormone merely one of a summation of effects, or is it really a case of true accentuation of the thyroid stimulating power of anterior lobe? Before describing the experiments, some of the earlier work on the thyroid - adrenal medulla relationship will be discussed.

Falta, Eppinger and Rudinger (1908), and Falta, et al (1909) had the thyroid and adrenal glands classified as mutually stimulating organs.

Cannon and Catell (1916) found that adrenalin has a stimulating effect on the thyroid gland. Levy (1916), one of Cannon's students, confirmed the findings of Cannon and Cattell. Black, Hupper and Rogers (1922), however, found that adrenalin had no

effect on the iodine content of the thyroid gland; and Carlson, Hektoen and Schulhof (1924-5), and Hektoen, Carlson and Schulhof (1927) could not obtain any specific effect on thyreoglobulin output from the thyroid with adrenalin. Crawford and Hartley (1925) also obtained negative results when they studied the effect of adrenalin on the histological picture of the thyroid, as did Kippen (1934) and Uhlenhuth and Schwartzbach (1927). The latter also observed that adrenalin had no effect on amphibian metamorphosis.

The adrenal medulla and the hypophysis.

Before discussing the relationship between the adrenal medulla and hypophysis it seems expedient to point out that there is no dearth of evidence supporting a specific relationship between the anterior lobe of the hypophysis and the adrenal cortex. If a relationship between the adrenal medulla and the anterior lobe does exist, it is still hidden by obscurity.

Elliott (1912) found that pituitary extract had no effect on the amount of adrenalin in the adrenal gland. It is not indicated whether the whole gland or only one of the lobes was the basis of the extract used. It probably was the whole gland. Kepinow (1912), however, reported that whole pituitary extract was capable of augmenting the effect of adrenalin. In his experiments the threshold for adrenalin was lowered considerably by pituitary extract. This relationship was not found to work in the opposite direction, however, for adrenalin did not affect the threshold for pituitary extract. It is doubtful that the

effect observed by Kepinow was an anterior lobe phenomenon; for he used the blood pressure response as an index of both adrenalin and pituitary activity. That the vasopressor activity of the hypophysis is limited to the posterior lobe is now universally accepted. Pal (1916) was of the opinion that there was a definite antagonism between adrenalin and hypophysis extract. And to add further to the confusion, Lieb and Hyman (1922-3) found that hypophysis extract had no effect on the action of adrenalin.

More recently Emery and Atwell (1933) demonstrated that whole pituitary extract caused an increase in the weight of the adrenal gland. This increase involved both the cortex and medulla. The cortex however showed the more marked response.

It can hardly be claimed that any direct relationship between the adrenal medulla and the hypophysis, either anterior or posterior lobe, has been definitely established. A new approach to the adrenal medulla - thyroid - anterior lobe relationship has recently been attempted. Experiments were designed to study the effect of adrenalin on the response of the thyroid gland to the thyreo-activator hormone. Uhlenhuth, Van Slyke and Mech (1934) found that adrenalin definitely accentuates the ability of the thyreo-activator hormone to accelerate amphibian metamorphosis. That this accentuation involved a more active state of the thyroid gland was further substantiated by Uhlenhuth and Thompson (1934-5). Kippen (1934) reported however, that neither ergotamine, adrenalin, atropine, or calcium had any effect on the response of the thyroid gland to an anterior lobe extract. Particular attention was paid

to the effects on mitotic proliferation. That this criterion of thyroid activity is not the best one for the purpose shall be pointed out subsequently.

The positive results obtained by Uhlenhuth and his associates in salamanders with regard to the effect of adrenalin on the response of the thyroid gland to the anterior lobe opened a new path for the study of endocrine interrelationships. As a further step in the investigation of this problem it was undertaken to find out if a similar response is obtainable in mammals. This work will be reported now.

Material and method:

Forty-eight guinea pigs, weighing between 100 and 175 grams, were divided into 4 groups. One group served as uninjected controls, a second was injected with anterior lobe extract, a third was injected with adrenalin, and a fourth group was injected with anterior lobe and adrenalin. One or two animals of each of the four groups were sacrificed after each of 11 injections. Wherever possible, as was the case in most instances, animals receiving a comparable number of injections were litter mates. In the appended tables animals indicated by similar series (Roman) numbers are of the same litter.

The anterior lobe extract was made up exactly as described in the posterior lobe experiment. Each animal received 0.5 cc. of extract which contained the extractable material of 25 mg. of anterior lobe powder. The injections were given intraperitoneally every morning. The dose was decided upon after a preliminary

series of experiments. It was found that the response to 25 mg. of anterior lobe powder was not too great, so that it would not be impossible to augment it with adrenalin if adrenalin were capable of producing such an effect. A dose that would elicit a maximum response would be impractical as it would conceal the adrenalin effect, if such an effect exists.

The adrenalin used was Parke Davis and Co. adrenalin chloride, 1-1000. After a preliminary experiment to test toxicity it was decided to use 0.025 mg. per injection, as this dose elicited a typical physiological response which showed no after effects. It was considerably below the toxic dose which varied between 0.3 and 0.5 mg. of adrenalin. A single dose of 0.5 mg. is not necessarily fatal. It does have severe effects for several hours, however. Because of the short duration of the effect of adrenalin, two intraperitoneal injections were given daily. The first was given immediately after the anterior lobe extract injection, and the second at about 6 p.m.

All animals were sacrificed by means of a blow on the head on the day following the last injection. The thyroids were dissected out with the aid of a binocular dissecting microscope, and one was fixed in Bouin's and the other in Champy.

After embedding and sectioning, the tissue that was fixed in Bouin's was stained by the Mallory Heidenhain technique. A section was then chosen from the middle of each gland, and the degree of activity was estimated by measuring an appropriate number of cells with an ocular micrometer. The average of the measurements for each gland was taken as the index of activity

for that thyroid, as in an active thyroid the cells are columnar, while in an inactive thyroid the cells are cuboidal, and sometimes even squamous in shape.

Since the degree of activity of an activated guinea pig thyroid is not uniform throughout the gland, it is essential to measure cells in comparable areas of the various thyroids in order to obtain dependable results. If one studies a cross-section through the middle of an activated guinea pig thyroid, it becomes evident that there are three distinct zones, each showing a specific degree of activity. These three zones, as illustrated in fig. 1, make up the periphery of the section, (fig. 1a), the intermediate area of the section (fig. 1b), and the central part of the section (fig. 1c) respectively. While in an inactive gland the cells of the peripheral follicles are low (fig. 3a), those of an activated gland are high (fig. 2a). The follicles directly underneath the peripheral zone make up the intermediate zone (fig. 1b). The cells in this zone are as low in an active gland, (fig. 2b), as in an inactive gland (fig. 3b). The central zone (fig. 1c) of an active gland is made up of relatively high cells (fig. 4), while in an inactive gland they are low.

While this pattern of cellular activity has been found to be consistent in all of the glands studied it does not agree with the report of Friedgood (1934) and Greep (1935) who found that the cells in the central portion of the gland are usually higher than those in the periphery.

In this series of thyroids 250 cells were measured in the peripheral zone of each gland and the average taken as an index

of the degree of thyroid activity. In addition 250 cells were measured from the combined intermediate and central zones, and the averages for the individual glands compared with each other. The latter measurements will be referred to as the central cell measurements, in contrast to the peripheral cell measurements. It is significant that both sets of measurements gave the same results, the only difference being that the peripheral cell measurements were consistently higher.

In addition mitotic figure counts were made in part of this series. Every tenth one of the first 100 sections of each gland examined was studied for this purpose.

Results.

Superficial examination of the sections of the various thyroids was sufficient to differentiate the active glands from the inactive ones. In order to determine the finer degrees of thyroid activity, a more quantitative technique, as described above, was required.

Uninjected control animals.

The average height of the peripheral cells of the uninjected controls ranges from 6.10 microns to 8.22 microns as is evident in table 2. Animal DCXXX111-1, which had an average of 11.85 microns, is the only one of 40 controls that gave such a high reading. It is therefore proper to exclude this animal from the experiment. If to the 8 animals in this series are added the 9 uninjected animals of the pilocarpine series (table 4),

we have 17 animals with average cell height readings ranging between 5.99 and 8.37 microns. These 17 animals give a total average of 7.22 microns, with a probable error of ± 0.412 microns.

As will be pointed out, adrenalin and pilocarpine have no effect on the thyroid gland when injected by themselves. If the 11 adrenalin, and the 11 pilocarpine animals are added to the 17 uninjected controls, we have 39 animals with a total average of 7.37 ± 0.532 microns.

Central cell measurements were made only for the adrenalin series. The uninjected controls range between 5.29 and 7.34 microns, as recorded in table 2. The average for these animals is 6.17 microns. If the measurements for the adrenalin injected series are added, we get an average of 6.20 microns for 20 animals.

Mitotic figure counts were made in 6 uninjected controls. Every tenth section of the first 100 sections of each gland was examined for mitotic figures. None was found in 3 glands, 1 in 1 gland, and 3 each in 2 glands (table 3).

Adrenalin injected animals.

It has already been mentioned that adrenalin was found to have no specific effect on the height of the thyroid cell. The average peripheral cell measurements of the 11 animals in this group range from 5.55 to 8.72 microns (table 2). These compare well with the control measurements of 5.99 to 8.37. While the number of injections ranges from 1 to 22 in the respective animals, there was no correlation between cell height and the amount of adrenalin administered.

The average central cell measurements ranged from 4.38 to 7.54 microns. They compare with those of the uninjected controls, namely: 5.29 to 6.73 microns. The total average for all of the adrenalin injected animals is 6.22 microns.

Mitotic figure counts were made in the glands of 9 animals that had received from 1 to 18 injections of adrenalin. They were found to be no different from the counts obtained in the uninjected controls (table 3).

Anterior lobe extract injected animals.

The results in this series are quite different from those in the preceding two series. Not only is there a marked increase in the height of both the peripheral and central cells, but there is also a definite response which can be correlated with the number of anterior lobe injections administered. While this is evident in table 2, it is much more obvious in fig. 5, and fig. 6. In fig. 5 the solid black line with the points indicated by diamonds, represents the uninjected controls. The line made of small dashes, with the points indicated by squares, represents the adrenalin injected animals. Neither one shows a specific trend. However, if the line made of long dashes with the points indicated by circles, is examined it is evident that the average cell height of the thyroids receiving anterior lobe extract injections is higher than in the other two cases mentioned. This increase in cell height reaches its peak at the 5th injection, and then gradually falls, to be down among the controls again by the 9th injection of anterior lobe extract.

In the case of the central cell measurements, the effect of the anterior lobe injections is not definitely established until the fifth injection (table 2)(fig. 6). From the fifth to the ninth injections the thyroid cells of the animals receiving anterior lobe injections are distinctly higher than in the uninjected controls and adrenalin injected animals. This is clearly pointed out in fig. 6. The peak is reached after the seventh injection in this instance. The failure to get an immediate response in the case of these measurements is undoubtedly due to the fact that the relatively inert cells of the intermediate zone were included. Had only the more active central cells been measured, the curves in fig. 6 would have resembled those in fig. 5 more closely. But even despite the inclusion of the inactive intermediate zone cells, the response due to anterior lobe is definite and clear cut here.

Mitotic figure counts were made in 11 glands. The number of injections ranges from 1 to 9. In table 3A it is very evident that there was a marked increase in mitotic proliferation when the counts are compared with those recorded in table 3. The peak of mitotic proliferation is reached at the third injection, when it was found to be 16 for the 10 sections counted. This agrees with the findings of Kippen and Loeb (1935) who observed that proliferative activity reached its height after 2 injections, irrespective of the dose of anterior lobe given.

Adrenalin and anterior lobe extract injected animals.

As in the case of the anterior lobe extract injected animals, the average cell height measurements of the adrenalin and anterior

lobe injected animals are much higher than in the uninjected controls and the adrenalin injected animals. Offhand any difference between the measurements of the thyroid cells of animals injected with adrenalin and anterior lobe and those of the animals receiving anterior lobe alone might not be very evident; but if fig. 5, in which the values for the peripheral cells are recorded, is examined, it becomes obvious that the adrenalin has had a distinct and consistent effect on the anterior lobe extract. From the 2nd to the 10th injections. the line of dots and dashes, with the points indicated by triangles, is above the line representing the anterior lobe animals. The parallelism of the two lines is extremely interesting. They both reach a peak at the 5th injection and then drop gradually. The adrenalin - anterior lobe line not only reaches a greater height; but its rise is also maintained for a greater length of time.

The central cells (table 2)(fig. 6) show a similar trend. From the third to the seventh injections the curve for the adrenalin and anterior lobe animals is higher than that for the animals receiving anterior lobe alone. While the duration of this increased response is not as great as in the case of the peripheral cells(fig. 5), the response of the central cells is very definite. It is important to remember that even despite the inclusion of the relatively inert cells of the intermediate zone in these measurements, the results obtained here duplicate those obtained from the peripheral cell measurements, as is evident when fig. 5 and fig. 6 are compared with one another.

While the mitotic figure count for the adrenalin and anterior lobe animals is greater than that for the uninjected controls and adrenalin animals, it is not as great as that for the anterior lobe animals. Interestingly enough the peak of 17 mitotic figures was again reached after the 3rd injection. The failure of the accentuating effect of adrenalin on the response of the thyroid gland to anterior lobe to manifest itself in the proliferative activity of the thyroid agrees with the findings of Kippen (1934). It is not surprising that mitotic proliferation has failed to give positive results in this case, for it has been quite definitely established that proliferative and secretory activity do not necessarily run hand in hand in the thyroid gland. Evidence in support of this concept has been provided by Williamson and Pearse (1923) for the human thyroid, and by Uhlenhuth (1927) for the salamander thyroid. In addition the recent findings of Kippen and Loeb (1935) also point out that proliferative and secretory activity do not parallel one another in the thyroid gland. "The fall in mitotic proliferative activity", they state, "takes place at a very much earlier date, and is much more precipitate than is the case of the other structural changes indicative of hyperactivity of the thyroid gland; the curves representing the latter are flatter than those representing the proliferative activity." Examination of figure 5 will reveal that the effect of adrenalin plus anterior lobe first becomes evident at the third injection, at which time the proliferative activity of the thyroid is at its peak according to the counts presented here. On the basis of Kippen and Loeb's (1935) report the mitotic

figure count is already on the decline in a stimulated thyroid after the third injection.

Discussion.

The results of the cell height measurement experiments described above for the guinea pig thyroid agree with the observations of Uhlenhuth, Van Slyke, and Mech (1934) that adrenalin is capable of accentuating the metamorphic action of the thyreoactivator hormone, as well as with the report of Uhlenhuth and Thompson (1934-5) that adrenalin augments the thyroid stimulating effect of anterior lobe extract in the salamander. The negative effects of adrenalin alone, as observed in the salamander by Uhlenhuth and his associates, and as confirmed here in the guinea pig fit in with the negative reports cited in an earlier part of this paper.

The negative experiments of Kippen (1934) do not contradict my findings in the guinea pig; for using Kippen's technique in the very same glands, I have also obtained negative results. The proliferative response of the thyroid gland to anterior lobe hormone is too shortlived, and not sensitive enough to show fine differences in thyroid activity, as the degree of variation is considerable.

Summary.

While adrenalin when injected alone, has no effect on the thyroid gland of the guinea pig as determined by cell height measurements, when injected along with anterior lobe extract it

accentuates the thyroid stimulating action of the latter.

V - THE EFFECT OF PILOCARPINE ON THE RESPONSE OF THE THYROID
GLAND TO ANTERIOR LOBE.

Because of the parasympathicomimetic action of pilocarpine a number of studies have been made on the effect of this drug on the thyroid gland. It was thought that such studies would cast some light on the innervation of the thyroid. Wyss (1889) observed that pilocarpine causes the thyroid to become turgescient and dark red. Microscopically the gland showed high vascularization and activity. The cells were more voluminous and the nuclei unclear. Wyss concluded that secretion occurred in various parts of the gland with varying intensity. That pilocarpine caused marked vasodilatation cannot be denied; but the evidence for secretory activity seems doubtful.

Andersson (1894) studied the effect of pilocarpine on the thyroids of cats and rabbits. He also found that the thyroids became distended and hyperemic. Histologically Andersson observed that there were changes that he believed were characteristic of stimulation. He described the active thyroid as having high cells the apices of which were distended with vacuoles. These vacuoles separated from the cells to form the chromophobe vacuoles in the colloid. It can hardly be doubted that Andersson interpreted his histological findings correctly, for the criteria of thyroid activity that he mentioned are now accepted by all investigators.

From the work of Wyss (1889) and Andersson (1894) one is led to the conclusion that pilocarpine stimulates the thyroid gland. Schmid (1896) however could notice no difference in the thyroids of uninjected and pilocarpine injected dogs and cats, and Briau (1897-8), and Wiener (1909) agreed with Schmid's conclusion. Among the more recent investigators, Carlson, Hektoen, and Schulhof (1924-5), and Hektoen, Carlson and Schulhof (1927) obtained negative results on the effect of pilocarpine on the output of thyreoglobulin by the thyroid of the dog. The negative findings of the majority of the investigators mentioned were confirmed by Crawford and Hartley (1925), Nakayama (1925), Cannon (1927), and Uhlenhuth and Schwartzbach (1927).

With regard to the relationship between pilocarpine and certain thyroid preparations, Oswald (1916) reported that iodothyreoglobulin augments a transitory increase in pulse rate which pilocarpine elicits, while Gessner (1928) found that the metamorphic effect of thyraden in tadpoles is inhibited by pilocarpine.

Recently Uhlenhuth, Van Slyke and Mech (1934) reported that pilocarpine is capable of accelerating the metamorphic response of larval amphibia to the thyreoactivator hormone of the anterior lobe of the hypophysis. Pilocarpine alone had no effect on metamorphosis, but when injected in conjunction with anterior lobe extract the response to the latter was accentuated considerably. That this accentuation involved an augmentation of thyroid activity was established by Uhlenhuth and Thompson (1934-5). Since pilocarpine, when added to anterior lobe, causes

the thyroid to respond to a greater degree than when anterior lobe is given alone, one would expect atropine to have an inhibitory effect. Kippen (1934), however, found that the effect of atropine was negative. From the data cited it is quite evident that it has not been established with certainty that pilocarpine when given alone has any specific effect on the thyroid gland. That this drug is capable of altering the response of the thyroid of the salamander to anterior lobe extract, however, has been pointed out by Uhlenhuth and his coworkers. It was of interest to determine whether this relationship also held for mammals. To investigate this problem a series of experiments was carried out in guinea pigs.

Material and method.

Fifty guinea pigs were divided into 4 groups which were injected with pilocarpine, anterior lobe, and anterior lobe plus pilocarpine respectively. One group was uninjected. The dose of anterior lobe was 25 mg., once daily; and that of pilocarpine 0.125 mg., twice daily. Pilocarpine hydrochloride was dissolved in sterile distilled water, so as to give 0.125 mg. per 0.5 cc. of solution.

As to toxicity, 2 mg. of pilocarpine was fatal. One mg. and 0.5 mg. caused violent reactions; but the animals were quite normal the next morning. A dose of 0.25 mg. gave a less violent reaction. The animal was quite normal 4 hours after the injection. A dose of 0.125 mg. caused a response which was still less violent.

Salivation and defecation occurred consistently. Recovery was complete in several hours.

The peripheral cells only were measured in this series. In the animals receiving 2, 3 and 4 injections of anterior lobe, as well as in those of the other 3 groups that were comparable, both glands were studied. The results in the two glands of each animal were comparable with only a slight degree of variation.

Results.

Uninjected controls.

The measurements obtained from the thyroid glands of these animals (table 4) compared very favorably with the uninjected controls of the adrenalin series (table 2). They were included in the calculation of the average cell height for the control animals along with the adrenalin and pilocarpine injected animals as presented in the discussion of the adrenalin series.

Pilocarpine injected animals.

As is evident in table 4, the thyroids of these animals were no different from those of the controls. The measurements were included in the calculation of the average cell height for the control animals as discussed in the uninjected control series of the adrenalin experiment.

Anterior lobe extract injected animals.

The results in this series are very similar to those of the anterior lobe series in the adrenalin experiment. There is a

definite increase in cell height that follows a consistent trend. This is illustrated in fig. 7. The increase in cell height rises gradually up to the 5th injection, after which it falls again. The peak after the 5th injection is the same as in the adrenalin experiment (fig. 5).

Pilocarpine and anterior lobe extract injected animals.

It has already been mentioned that Uhlenhuth, Van Slyke and Mech (1934), and Uhlenhuth and Thompson (1934-5) were able to accentuate the metamorphic and the thyroid response to anterior lobe extract by means of pilocarpine. The results obtained in the guinea pig are different in certain respects from those obtained in the salamander. As is evident in table 4, and in fig. 7, up to the 5th injection the response of the thyroid to anterior lobe extract is definitely inhibited by pilocarpine. Of the 2 animals given 5 injections of anterior lobe and 10 injections of pilocarpine, one had a thyroid with a high average cell height (9.22 microns), and the other had an average cell height of 6.22 microns. From the 6th to the 9th injections of anterior lobe, a time when the response to the thyreoactivator hormone has been consistently found to be regressing, the average cell height of the thyroids of 8 animals receiving pilocarpine in addition to anterior lobe was higher than in the animals receiving anterior lobe alone. A study of fig. 7 shows that early in the course of the experiment (1 to 5 injections) pilocarpine inhibits the thyroid stimulating effect of anterior lobe; while in the latter part of the experiment (6 to 9 injections) it accentuates the response of the thyroid.

Discussion.

While the results described are not in accordance with those obtained in the salamander, they fit in satisfactorily with our classification of adrenalin and pilocarpine as antagonistic drugs. The stimulating effect of pilocarpine with the greater number of injections coincides with the results in the salamander. When one takes into consideration the fact that the dose of pilocarpine given to the salamanders was considerably higher than that given to the guinea pigs, it becomes evident that large doses of pilocarpine may produce an effect opposite to that of small doses. For it is only after 10 injections of pilocarpine that the stimulating effect is evident in the guinea pig. The cumulative effects of small doses over a period of time can be compared to the immediate effect of large doses. To check this it would be essential to repeat the experiments, giving large doses of pilocarpine to guinea pigs, and small doses to salamanders. When the results of such experiments are available we will then be in a position to state whether the above explanation is an acceptable one.

Why should large doses of a parasympathomimetic drug like pilocarpine produce an effect similar to that of adrenalin which is the classical sympathicomimetic hormone? There are two possible explanations. The first involves the toxic effects of pilocarpine on the specific end-organs which react to it. With large doses of pilocarpine the parasympathetic system is overworked, with the result that it loses its normal tone. This disturbs the sensitive equilibrium between the sympathetic and

parasympathetic systems by removing the normal antagonistic effects of the latter on the sympathetic system. The result is that the sympathetic system is left in complete control, and pilocarpine therefore appears to be producing an effect similar to that of adrenalin. In reality the action of pilocarpine is the opposite of that of adrenalin, if the above explanation is a true one; for the adrenalin-like effect of pilocarpine is due to the removal of a mechanism which normally inhibits the sympathetic system. In the case of adrenalin, the reaction involves direct stimulation of the latter system.

That the state of irritability of each of the two divisions of the autonomic nervous system depends to a great degree on the antagonism exerted by one system on the other was pointed out by Cori (1921). It had been reported that during the summer the vagus of the frog loses its irritability to electrical stimulation. That the loss of irritability is due not to an actual loss of vagus tone, but to a heightened state of sympathetic activity which overshadows the vagus action is shown by the fact that ergotamine, which paralyzes the sympathetics, is capable of making a non-reactive vagus nerve become very irritable to stimulation. Physostigmine, which stimulates the vagus, is also capable of raising the response of the latter to electrical stimulation. The combined effects of both drugs raise vagus tone to an even higher level than does each of the drugs when given alone.

A second explanation for the adrenalin-like effect of pilocarpine is based on the existing evidence that pilocarpine is capable of stimulating the secretion of adrenalin. This

apparently paradoxical relationship is supported by Dale and Laidlow (1912-13), Edmunds (1923), Kure, Wada and Okinaka (1931), Tada (1933) and Feldberg, Minz and Tsudizimura (1934). That pilocarpine does not stimulate the secretion of adrenalin was found to be the case by Tscheboksaroff (1911), Elliott (1912), and Schkawera and Kusnetzow (1923). If pilocarpine does stimulate the adrenal medulla, it is easy to see how one can get a sympathicomimetic effect by the administration of pilocarpine. A check on this would involve a study of the effect of pilocarpine on the thyroid of adrenalectomized guinea pigs.

Summary.

While pilocarpine when injected alone, has no effect on the thyroid gland of the guinea pig as determined by cell height measurements, when injected along with anterior lobe extract it depresses the thyroid stimulating action of the latter up to the 5th injection, after which the effect reverses itself and the response of the thyroid is accentuated. Several probable causes for this reversal of effect are discussed.

FINAL SUMMARY.

1 - The literature pertaining to the effect of the anterior lobe on the thyroid gland has been reviewed in order to form a background for the experimental work.

2 - Posterior lobe extract was found to have no definite effect on either normal or anterior lobe induced metamorphosis.

3 - Adrenalin accentuates the response of the thyroid gland to anterior lobe extract, as determined by thyroid cell height measurements.

4 - Pilocarpine was found to inhibit the response of the thyroid to anterior lobe extract from the 1st to 5th injections of A.L. Following this inhibition, pilocarpine accentuates the response of the thyroid to the thyreoactivator hormone.

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TABLES.

TABLE 1 AND 1A

Amblystoma tigrinum larvae injected with anterior and posterior lobe extracts. The time required to induce metamorphosis is recorded for the individual animals in the different groups in terms of days and number of injections. The averages indicate the group responses. The criteria of metamorphosis chosen are the beginning of skin shedding, and the complete disappearance of the gills.

Ringer's Sol. Controls					Anterior Lobe					Acid Posterior Lobe				
Series No.	Skin Shed		Gills Reduced		Series No.	Skin Shed		Gills Reduced		Series No.	Skin Shed		Gills Reduced	
	No. of Days	No. of Inj.	No. of Days	No. of Inj.		No. of Days	No. of Inj.	No. of Days	No. of Inj.		No. of Days	No. of Inj.	No. of Days	No. of Inj.
a 1*	203	35	203	35	b 1	34	15	46	21	c 1*	203	35	203	35
a 2	32	14	36	17	b 2	38	16	50	22	c 2	55	24	62	27
a 3*	80	35	97	35	b 3	27	12	39	17	c 3*	117	35	119	35
a 4	42	19	53	23	b 4	26	12	39	17	c 4*	84	35	115	35
a 5*	113	35	113	35	b 5	22	10	32	14	c 5*	78	34	122	35
Aver	94+	28+	100+	29+	g 1	29	13	35	15	Aver	107+	31+	124+	33+
					g 4	24	11	32	14					
					g 5	22	10	32	14					
					Aver	28	12	38	17					

*See notes on next page.

TABLE 1

Alkaline Posterior Lobe					Anterior Lobe - Acid Posterior Lobe					Anterior Lobe - Alkaline Posterior Lobe				
Series No.	Skin Shed		Gills Reduced		Series No.	Skin Shed		Gills Reduced		Series No.	Skin Shed		Gills Reduced	
	No. of Days	No. of Inj.	No. of Days	No. of Inj.		No. of Days	No. of Inj.	No. of Days	No. of Inj.		No. of Days	No. of Inj.	No. of Days	No. of Inj.
d 1*	191	35	191	35	e 1	29	13	34	15	f 1	29	13	35	15
d 2*	203	35	203	35	e 2	22	10			f 4	24	11	32	14
d 4	34	15	39	17	e 3	19	8	27	12	f 5	22	10	32	14
d 5	25	11	34	15	e 4	19	8	30	13					
Aver	113+	24+	117+	25+	e 5	25	11			Aver	25	11	36	14
					Aver	23	10	30	13					

*See notes on next page.

TABLE 1a

Notes for Table 1 and 1A.

- *a 1 - Died 203 days after the beginning of the experiment, or 125 days after the 35th (last) injection. It was still larval.
- a 3 - Shed skin 2 days after the 35th injection. Reduced gills 19 days after the 35th injection.
- a 5 - Died 113 days after beginning of experiment, or 35 days after the 35th injection. It was still larval.
- c 1 - Still larval 203 days after the beginning of the experiment, or 125 days after the 35th injection.
- c 3 - Shed skin 40 days after 35th injection. Reduced gills 41 days after 35th injection.
- c 4 - Shed skin 6 days after 35th injection. Reduced gills 37 days after 35th injection.
- c 5 - Reduced gills 46 days after the 35th injection.
- d 1 - Died 191 days after the beginning of the experiment, or 113 days after the 35th injection. It was still larval.
- d 2 - Still larval 203 days after beginning of experiment, or 125 days after the 35th injection.

TABLE 2

Guinea pigs injected with adrenalin, anterior lobe extract, and a combination of anterior lobe and adrenalin. The response of the thyroids of these animals to various numbers of injections of these substances is indicated by average cell height measurements in microns. The animals represented by similar series (Roman) numbers are litter mates.

Uninjected Controls			Adrenalin Animals				Anterior Lobe Animals				Anterior Lobe -- Adrenalin Animals				
Series No.	Average Height of 250 Cells in Microns		Series No.	No. of Inj.	Average Height of 250 Cells in Microns		Series No.	No. of Inj.	Average Height of 250 Cells in Microns		Series No.	No. of Inj. Ant. Lobe	No. of Inj. Adren.	Average Height of 250 Cells in Microns	
	Peripheral Cells	Central Cells			Peripheral Cells	Central Cells			Peripheral Cells	Central Cells				Peripheral Cells	Central Cells
DCXI-1	6.24	5.86	DCXI-4	1	6.72	5.80	DCXI-2	1	8.47	6.87	DCXI-3	1	1	7.27	6.22
DCXIII-1	7.20	5.43	DCXII-1	3	6.44	6.15	DCXII-2	2	8.67	7.04	DCXII-3	2	3	7.67	7.27
DCXV-1	7.04	5.29	DCXIV-4	5	7.44	6.27	DCXIII-2	3	8.56	6.19	DCXII-4	2	3	9.24	6.77
DCXVI-6	7.36	6.67	DCXVI-2	8	8.72	6.47	DCXV-2	4	8.31	6.40	DCXIII-3	3	5	9.88	6.99
DCXVIII-1	6.64	5.57	DCXVI-5	10	6.62	5.55	DCXV-5	4	7.10	6.03	DCXV-3	4	8	8.67	6.86
DCXXI-1	6.10	6.64	DCXVII-2	12	7.85	5.82	DCXVI-3	5	10.40	7.51	DCXVI-4	5	10	10.72	8.87
DCXXXIII-1	11.85	6.73	DCXVIII-3	14	7.84	6.97	DCXVII-3	6	10.13	7.28	DCXVII-4	6	12	10.56	7.97
DCXXXIV-1	8.22	7.34	DCXIX-4	16	5.55	4.38	DCXVIII-1	7	9.60	8.00	DCXVIII-2	7	14	10.00	8.37
DCXXXV-1	6.97	6.01	DCXX-3	18	8.00	7.54	DCXIX-1	8	8.12	7.68	DCXIX-2	8	16	8.65	6.64
			DCXXXIII-5	20	8.58	6.41	DCXX-1	9	7.97	6.17	DCXX-2	9	18	9.53	7.00
			DCXXXIV-5	22	7.39	7.00	DCXXI-2	9	8.52	7.46	DCXXXIII-3	10	20	7.76	6.62
							DCXXXIII-2	10	8.29	6.86	DCXXXIII-4	10	20	8.39	7.26
							DCXXXIV-2	11	9.25	8.42	DCXXXIV-3	11	22	6.90	7.80
							DCXXXV-2	11	7.66	6.78	DCXXXIV-4	11	22	8.78	6.71

Table 2

TABLE 3 AND 3A.

Guinea pigs injected with adrenalin, anterior lobe extract, and a combination of adrenalin and anterior lobe extract. The response of the thyroids of these animals to various numbers of injections of these substances is indicated by the number of mitotic figures counted in every tenth one of the first 100 sections of each gland. The animals represented by similar series (Roman) number are litter mates.

Mr. Robert Crawford helped count mitotic figures in this experiment.

Uninjected Controls		Adrenalin		
Series No.	No. of Mitotic Figures	Series No.	No. of Inj.	No. of Mitotic Figures
DCXI-1	1	DCXI-4	1	2
DCXIII-1	0	DCXII-1	3	0
DCXV-1	0	DCXIV-4	5	1
DCXVI-6	3	DCXVI-2	8	0
DCXVII-1	0	DCXVI-5	10	0
DCXXI-1	3	DCXVII-1	12	1
		DCXVIII-3	14	2
		DCXIX-4	16	0
		DCXX-3	18	2

Table 3

Anterior Lobe			Anterior Lobe and Adrenalin			
Series No.	No. of Inj.	No. of Mitotic Figures	Series No.	No. of Inj.		No. of Mitotic Figures
				A.L.	Ad.	
DCXI-2	1	3	DCXI-3	1	1	1
DCXII-1	2	6	DCXII-3	2	3	1
DCXIII-2	3	16	DCXII-4	2	3	1
DCXV-2	4	2	DCXIII-3	3	5	17
DCXV-5	4	2	DCXV-3	4	8	4
DCXVI-3	5	7	DCXVI-4	5	10	1
DCXVII-3	6	4	DCXVII-4	6	12	2
DCXVIII-1	7	6	DCXVIII-2	7	14	1
DCXIX-1	8	2	DCXIX-2	8	16	4
DCXX-1	9	9	DCXX-2	9	18	1
DCXXI-2	9	7				

Table 3a

TABLE 4

Guinea pigs injected with pilocarpine, anterior lobe extract, and a combination of anterior lobe extract and pilocarpine. The response of the thyroids of these animals to various numbers of injections of these substances is indicated by average cell height measurements in microns. The animal represented by similar series (Roman) numbers are litter mates.

Uninjected Controls		Pilocarpin Animals		
Series No.	Average Height of 250 Cells in Microns	Series No.	No. of Inj.	Average Height of 250 Cells in Microns
	Peripheral Cells			Peripheral Cells
DCIX-1	7.82	DCX-3	3	7.70
DCXXII-1	8.19	DCXXII-5	4	7.89
DCXXIV-1	7.94	DCXXIII-4	6	7.67
DCXXV-1	6.83	DCXXIV-5	8	6.27
DCXXVI-1	5.99	DCXXV-5	10	6.86
DCXXVII-1	7.87	DCXXVI-5	12	7.57
DCXXIX-1	6.83	DCXXVII-5	14	7.90
DCXXX-1	7.15	DCXXVIII-4	16	7.28
DCXXXI-1	8.37	DCXXIX-5	18	7.90
		DCXXX-5	20	7.88
		DCXXXI-5	22	8.82

Anterior Lobe Animals			Anterior Lobe - Pilocarpin Animals			
Series No.	No. of Inj.	Average Height of 250 Cells in Microns	Series No.	No. of Inj. of A.L.	No. of Inj. of Pilo.	Average Height of 250 Cells in Microns
		Peripheral Cells				Peripheral Cells
DCX-1	1	7.92	DCX-2	1	3	7.06
DCXXII-2	2	8.97	DCXXII-3	2	4	7.95
DCXXIII-1	3	9.05	DCXXII-4	2	4	9.34
DCXXIV-2	4	9.11	DCXXIII-2	3	6	7.65
DCXXV-2	5	9.16	DCXXIII-3	3	6	7.91
DCXXVI-2	6	8.45	DCXXIV-3	4	8	6.58
DCXXVII-2	7	8.06	DCXXIV-4	4	8	7.82
DCXXVIII-1	8	7.96	DCXXV-3	5	10	9.22
DCXXIX-2	9	8.37	DCXXV-4	5	10	6.95
DCXXX-2	10	8.28	DCXXVI-3	6	12	10.08
DCXXXI-2	11	8.78	DCXXVI-4	6	12	9.75
			DCXXVII-3	7	14	9.46
			DCXXVII-4	7	14	8.55
			DCXXVIII-2	8	16	8.99
			DCXXVIII-3	8	16	8.35
			DCXXIX-3	9	18	8.70
			DCXXIX-4	9	18	9.53
			DCXXX-4	10	20	8.33
			DCXXXI-3	11	22	8.82

Table 4

FIGURES.

Fig. 1

A diagram of a cross section through the middle of an activated guinea pig thyroid to show the different zones of cellular activity.

A - Peripheral zone.

B - Intermediate zone.

C - Central zone.

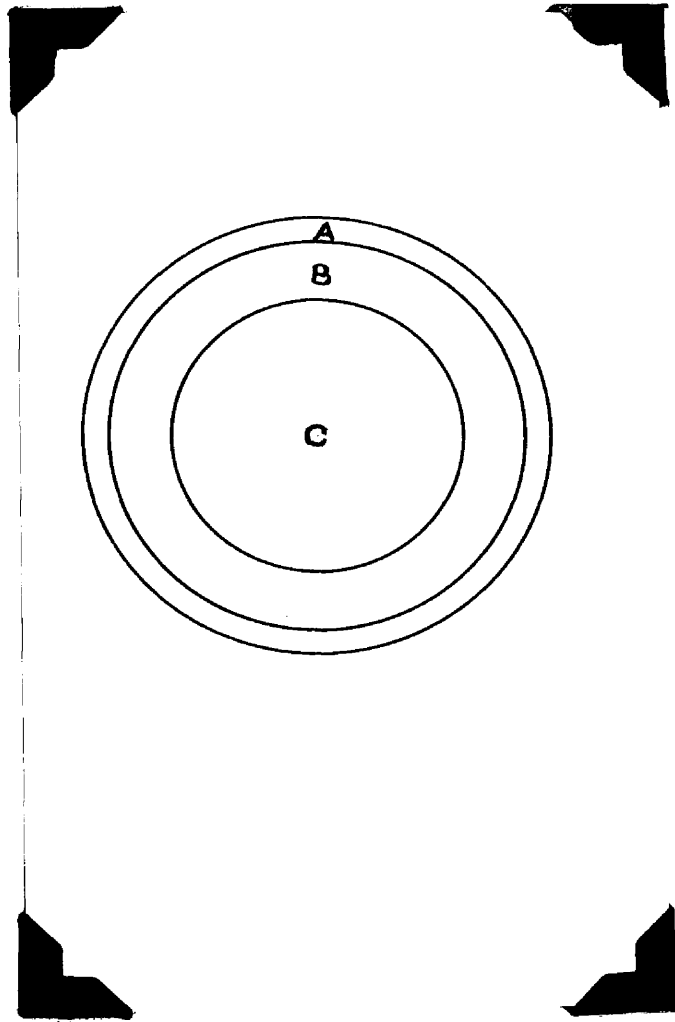


Fig. 1

Fig. 2

A photomicrograph of a portion of a cross-section through an activated guinea pig thyroid, DCXVI-3. The cells in the peripheral zone are much higher than those in the intermediate zone.

A - Peripheral zone.

B - Intermediate zone.

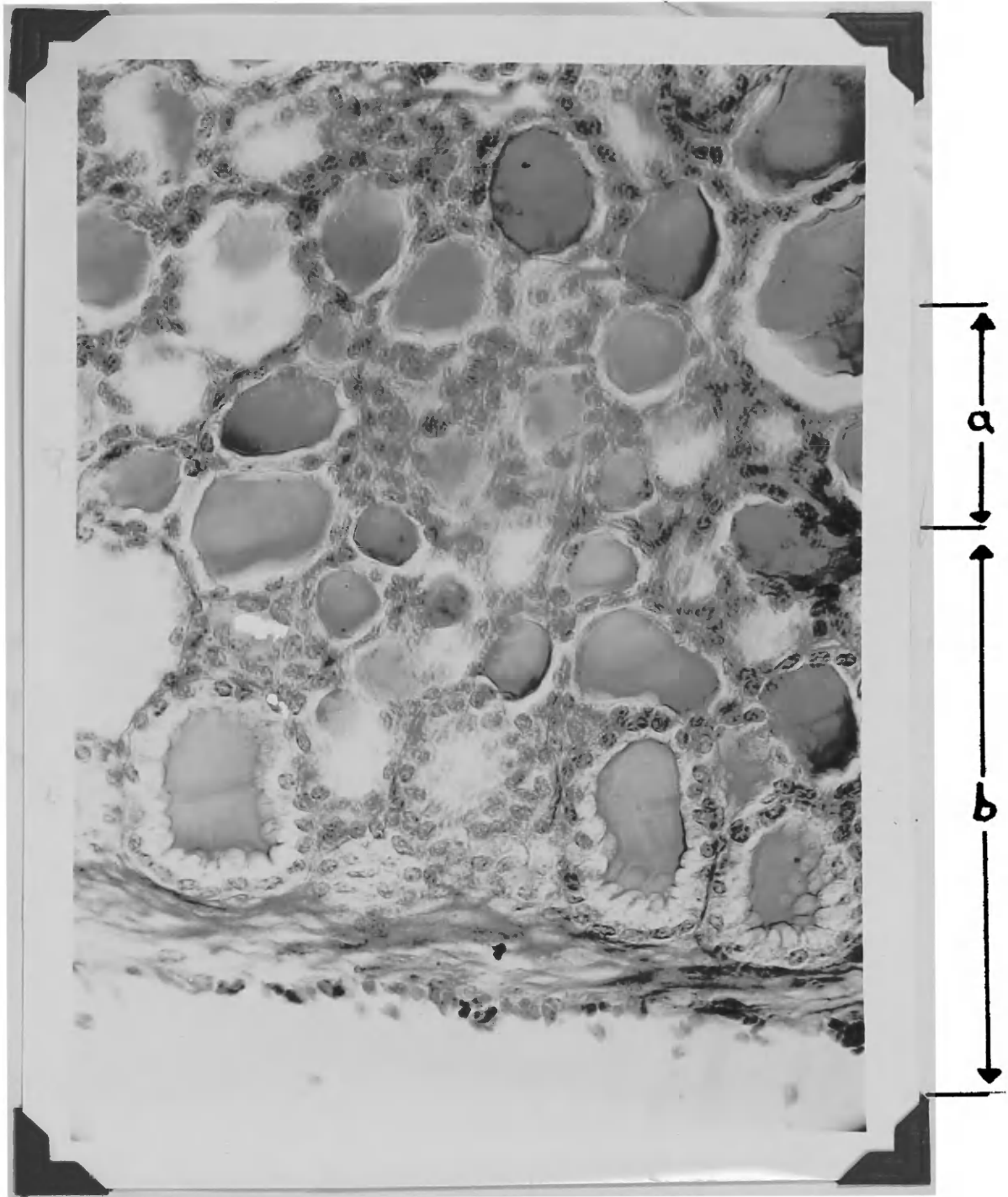


Fig. 2

Fig. 3

A photomicrograph of a portion of a cross section of an inactive guinea pig thyroid (DCXX1-1). The cells in the peripheral zone are only slightly higher than those of the intermediate zone.

A - Peripheral zone.

B - Intermediate zone.



Fig. 3

Fig. 4

A photomicrograph of a portion of the central zone of an activated guinea pig thyroid, DCXVI-3. The cells are much higher than those of the intermediate zone illustrated in Fig. 2B; but not quite as high as those of the peripheral zone, Fig. 2A.

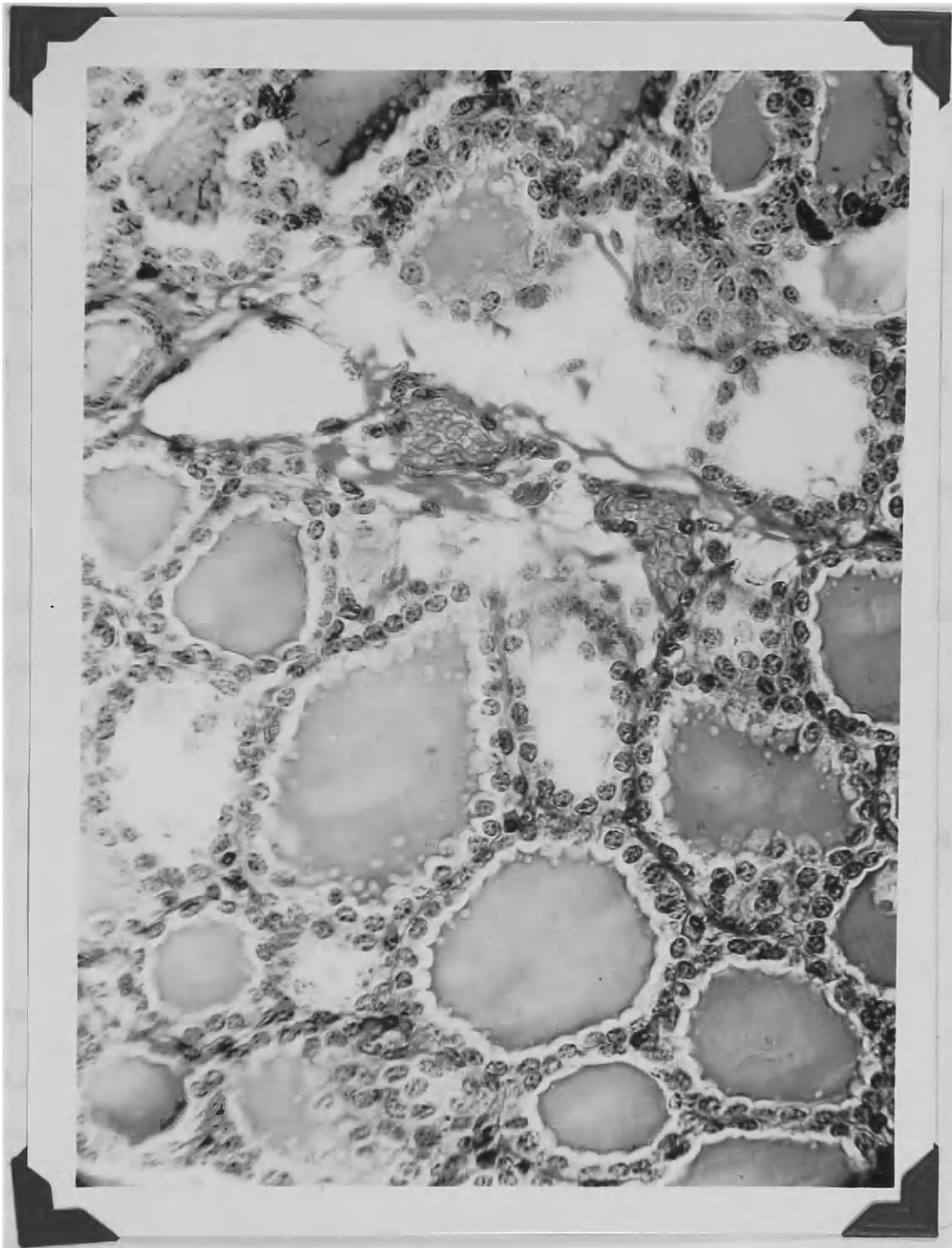


Fig. 4

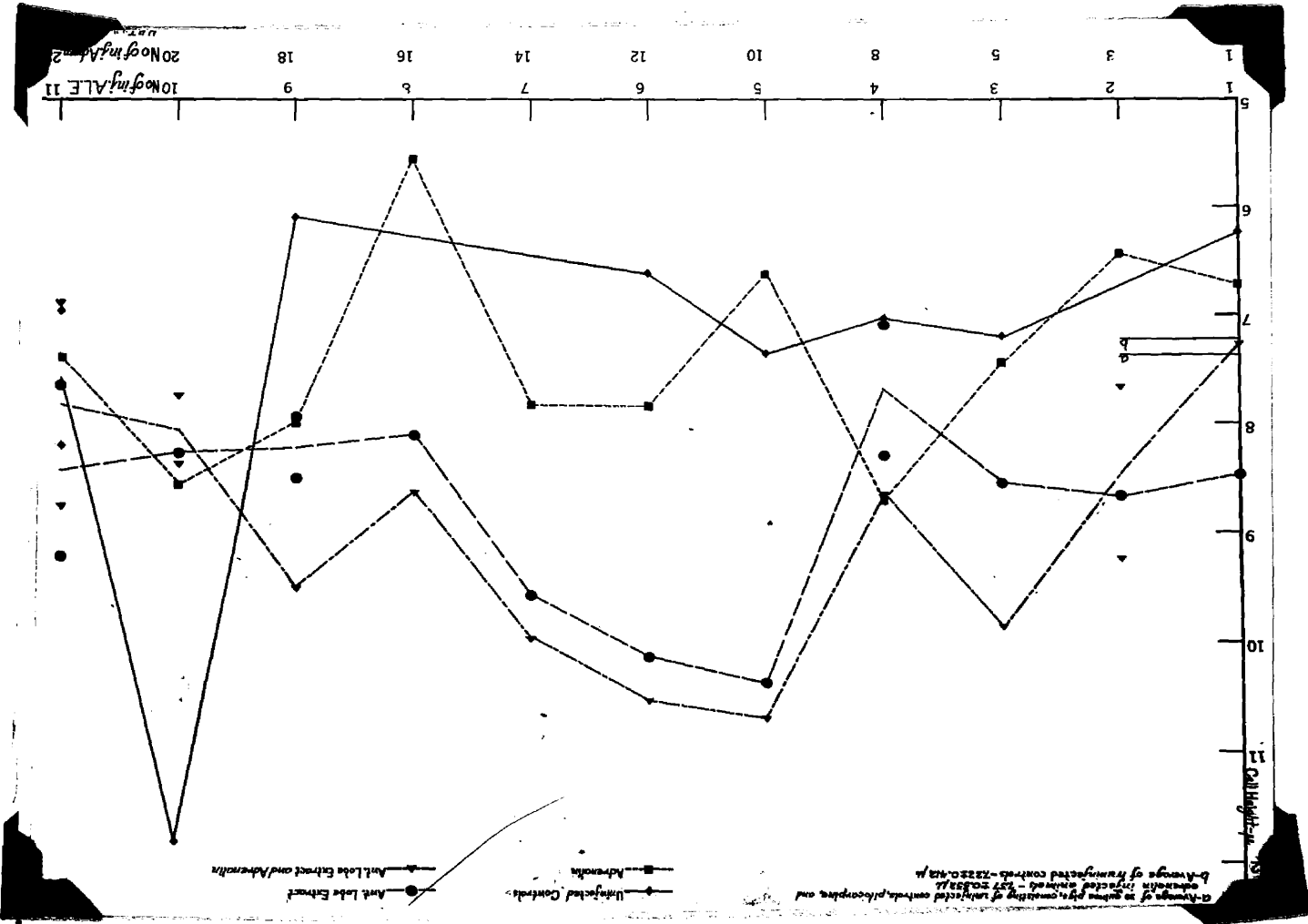
Fig. 5

This graph shows the response of the guinea pig thyroid, as indicated by peripheral cell height in microns (ordinate), to various numbers of injections (abscissa) of anterior lobe extract, adrenalin, and combinations of these two substances.

Uninjected controls - - - - - Solid black line and diamonds.
Adrenalin injected animals - - - - - Short dashes and squares.
Anterior lobe extract injected animals - - - - - Long dashes and circles.
Anterior lobe and adrenalin injected animals - - - - - Dots and dashes and triangles.

a - Average cell height of 39 guinea pigs, consisting of uninjected controls, pilocarpine, and adrenalin injected animals (7.37 ± 0.532 microns).

b - Average cell height of 17 uninjected animals (7.22 ± 0.412 microns).



10 No of mg ALE 11
20 No of mg Ad 2

Average of 20 gamma rays in counts per minute of uninjected controls, adrenalin, and antilobe extract injected animals - 257 counts per minute
Average of 20 gamma rays in counts per minute of uninjected controls - 7220.412 μ

Ant. Lobe Extract and Adrenalin
Ant. Lobe Extract
Uninjected Controls
Adrenalin

Fig. 6

This graph shows the response of the guinea pig thyroid as indicated by central cell measurements in microns (ordinate) to various numbers of injections (abscissa) of anterior lobe extract, adrenalin, and combinations of these two substances.

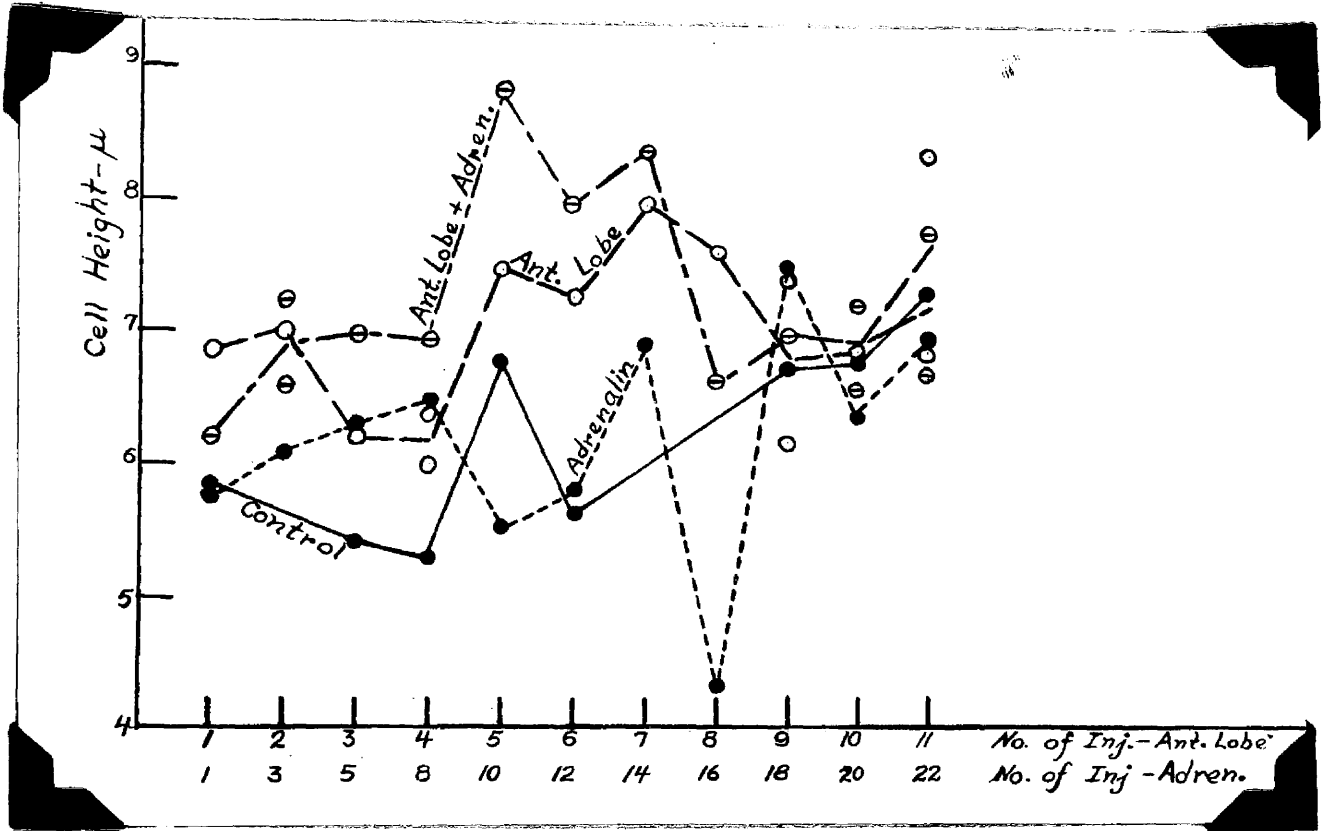


Fig. 6

Fig. 7

This graph shows the response of the guinea pig thyroid as indicated by peripheral cell height measurements in microns (ordinate) to various numbers of injections (abscissa) of anterior lobe extract, adrenalin, and combinations of these two substances.

Uninjected controls - - - - - Solid black line and diamonds.
Pilocarpine injected animals - - - - Short dashes and squares.
Anterior lobe extract injected animals - - - Long dashes and circles.
Anterior lobe and adrenalin injected animals - - - - - Dots and dashes and triangles.

- a - Average cell height of 39 guinea pigs, consisting of uninjected controls, pilocarpine, and adrenalin injected animals (7.37 ± 0.532 microns).
- b - Average cell height of 17 uninjected animals (7.22 ± 0.412 microns).

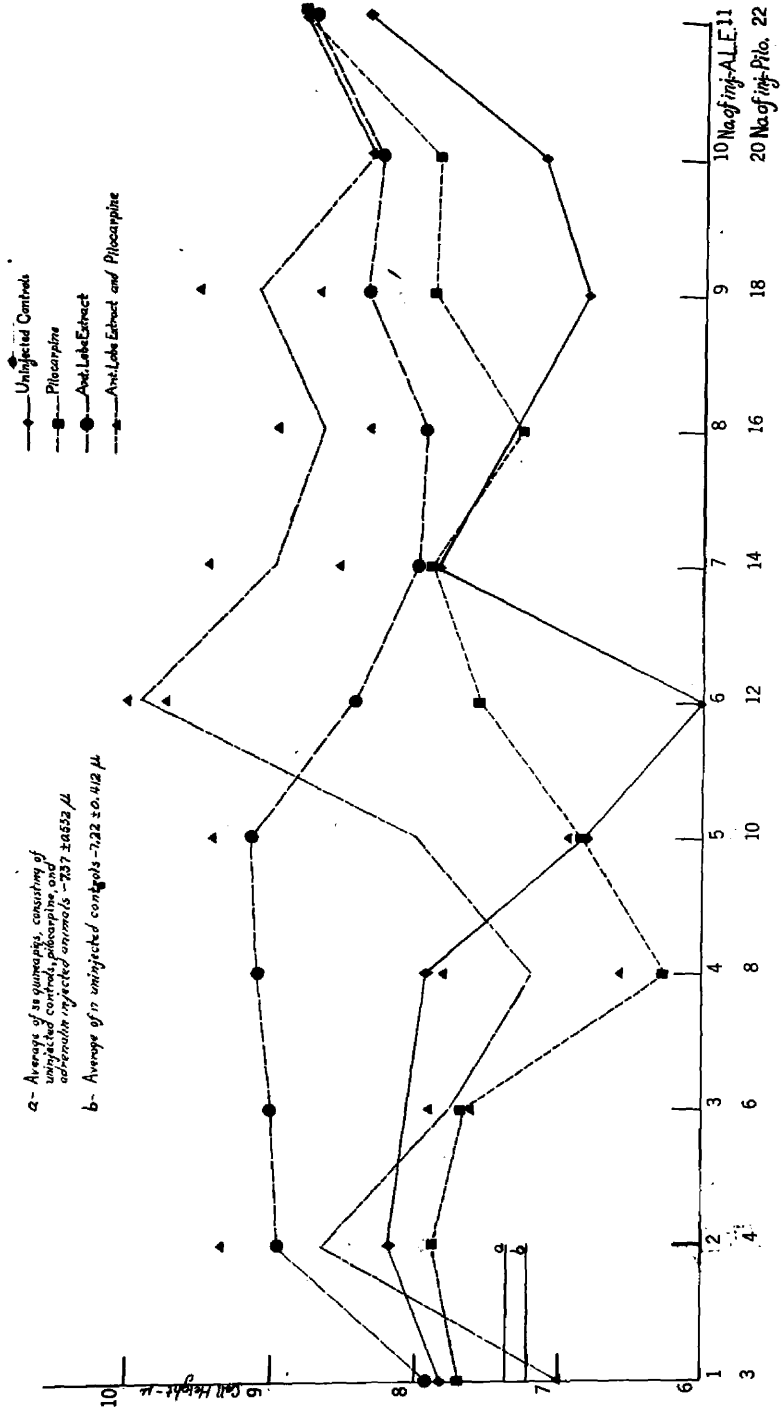


FIG. 7