

EXTIRPATION OF THE THYMUS IN THE GUINEA PIG.

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PLATES 20 AND 21.

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HISTORICAL.

In 1845 Restelli attempted to determine the function of the thymus by extirpating the gland from dogs, sheep, and calves. Since then more than twenty-five investigators have performed many series of extirpation experiments and extended their operations to more than fourteen species of animals, without obtaining results of sufficient agreement to bring the question of the effects produced by thymus extirpation to a final settlement. Some have found that removal of the thymus is without effect (Fischl, Pappenheimer, Nordmann, and others), and inferred that the thymus has either no function in postfetal life (Fischl) or none that can be demonstrated. Others have seen changes of a transitory character develop, and consequently have thought that the thymus exercises a transitory function corresponding to its own life history. Most prominent among these is Basch, whose experiments on dogs have been the models for subsequent investigators. Still others (Abelous and Billard, Matti, and Klose and Vogt) have determined that removal of the thymus is followed by symptoms and pathological changes of a profound character, culminating in death; and they have drawn the conclusion that the thymus is essential to life and assign to it an importance comparable to that of the adrenal, parathyroids, and pancreas.

The animals that have been used for extirpation experiments are the dog, rabbit, rat, guinea pig, frog (also tadpole), cat, fowl, pigeon, goat, sheep, calf, pig, and monkey; but some species are better adapted for thymus extirpation experiments than others. Basch is the authority for the frequently quoted statement that herbivorous animals are poor subjects, because very early in these animals bone has a high calcium content, which acts in a protective manner against the influences that result from the cessation of thymus function. It is well known, however, that rickets occurs spontaneously in herbivorous animals (Jost and Koch), and numerous investigators have reported changes in the skeleton of the rabbit following thymectomy, and one (Klose) has obtained rachitic-like changes in goats. But no one has succeeded in producing alterations in the skeleton of the guinea pig by thymus extirpation—unless the slight retardation in growth, noted by Soli,

is to be regarded in that light. If Basch's observation is true, that deprivation of the thymus is less apt to be followed by bone changes in herbivora than in omnivora or carnivora, another explanation is that extirpation of the thymus in the former is almost invariably incomplete.

In order to make clear the anatomical relations of the thymus in the guinea pig which distinguish it from all other mammals and make complete removal impossible, it is first necessary briefly to trace the development of the thymus not only in the guinea pig but in other mammals.

Development of the Thymus in the Higher Mammals, with Especial Reference to Its Development in the Guinea Pig.

The higher mammals may be divided into three groups, according to the situation of the thymus (Hammar). In the first group the thymus is chiefly or entirely in the thorax; in the second, in both thorax and neck; and in the third, in the neck alone.

The thymus of most of the higher vertebrates is derived from the third pharyngeal pouch alone or from the third and fourth pouches, and is therefore purely entodermal in origin. The thymus of the swine (Zotterman, Badertscher) and of the mole (Schaffer and Rabl), on the other hand, takes origin not only from the third pharyngeal pouch but also from the ductus precervicalis medialis, a derivative of the ectoderm, and the thymus of the guinea pig also has a twofold origin from ectoderm and entoderm, as will be pointed out later.

The mammalian thymus appears very early as a cylindrical outgrowth in a ventral and mesial direction from the ventral diverticulum of the third pharyngeal pouch. Simultaneously with the appearance of this thymus anlage, called thymus III, there occurs a proliferation of the epithelium of the dorsal diverticulum and adjacent parts of the pouch which soon undergoes histological differentiation into the anlage of the larger of the two parathyroids, parathyroid III.

While thymus III and parathyroid III are forming from the ventral and dorsal diverticula of the pouch, the mesial portion, roughly that part of it between these structures and the pharynx, called the ductus pharyngobranchialis, narrows, atrophies, and disappears

altogether, so that thymus III and parathyroid III are freed from their connection with the pharynx, becoming independent structures. The thymus anlage elongates and is now termed the thymus cord.

In the species constituting the first group, of which man and the dog are representative, the caudal end of the thymus cord thickens and the cranial end becomes thinner. The entire cord then begins a migration in a caudal direction, its descent being associated with that of the great vessels and heart, and since the thick caudal portion moves at a more rapid rate than the thin cranial portion, the latter becomes drawn out into a thin strand. The thick caudal portion, continuing its migration, enters the thorax and, joining its fellow of the opposite side, forms the thoracic portion of the thymus. The thin strand representing the cranial portion of the cord atrophies and entirely disappears, except the lowermost part, which persists as a continuation of the thoracic portion into the base of the neck, forming the cervical process, or cornu of the completely developed gland. Parathyroid III, which remains connected with the upper end of the thymus cord, is carried caudally with the latter, but halts near the lower end of the thyroid anlage, assuming its permanent position near the lower pole of the thyroid.

In the second group of animals, which have both neck and breast thymus (the hoofed animals are examples), the caudal portion of the thymus cord enlarges and enters the thorax to form the thoracic thymus, exactly as has been described for the first group. But the remainder of the cord does not atrophy and disappear; indeed the cranial end of it hypertrophies, forming an enlargement, known as the head of the thymus, and in certain members of the group, at least (ox, horse, goat, and swine) moves in a cranial direction with the growth of the neck or, according to some embryologists, is caught in the loop of the hypoglossal nerve and drawn towards the cranium to a position opposite the bifurcation of the great vessels, well above its point of origin. Shortly before birth, or during the first year, the stem of the thymus, connecting cervical and thoracic portions, disappears, so that the two parts of the gland become separate.

In the guinea pig,¹ which forms the third group, the thymus anlage

¹ The development of the thymus in the guinea pig has been investigated by Maximow and Ruben. The account of its development is based on the work of Ruben.

develops from the whole of the third pharyngeal pouch with the exception of those parts of it which form parathyroid III and the neck. When set free together with parathyroid III from its connection with the pharynx through atrophy of the neck of the pouch, it does not elongate as in those animals destined to have a thoracic thymus, but assuming a rounded or oval form remains essentially in its original position. According to Ruben, the loop of the hypoglossal nerve cuts in between parathyroid III and the thymus mass, separating the former from the latter without carrying the latter upwards. A portion of the ectodermal precervical vesicle early becomes incorporated.

In certain species of animals a thymus anlage, first described by Groschuff, develops also at the fourth pharyngeal pouch together with the parathyroid of that pouch, and is known from its origin as thymus IV, or thymus metamere IV. When the neck of the fourth pharyngeal pouch disappears, thymus IV and parathyroid IV in their turn migrate, but in a mesial direction, to the lateral portion of the thyroid anlage, where they take their permanent position together. In several species parathyroid IV regularly becomes incorporated in the substance of the thyroid, and thymus IV also, if present. Thymus IV has been found in the cat, ox, sheep, horse, goat, rat, rabbit, bat, and more rarely in man and the dog (Groschuff, Kohn). In the guinea pig, however, it does not develop, and even parathyroid IV is frequently rudimentary or present only on one side, or is even absent altogether (Ruben).

Thus the thymus in the guinea pig, unlike the thymus in other mammals, remains a purely cervical organ² (Ruben, Maximow) and does not possess the accessory lobe derived from the fourth pharyngeal pouch so frequently seen in other species. It would seem, therefore, as if the guinea pig should be especially adapted for complete extirpation of the thymus. That this is not the case, however, will be shown later.

² Though the thymus of the mole has an apparent resemblance to that of the guinea pig in that it also is limited to the neck, the actual development in the two animals is different, because a thoracic thymus actually forms in the mole and subsequently atrophies, while in the guinea pig none ever forms.

Results of Previous Investigators Following Thymus Extirpation in the Guinea Pig.

At least four investigators have used the guinea pig for the study of the effects of thymectomy. Basch states that he has performed thymectomy experiments on guinea pigs, but he has left no record of them so far as can be found.

Vincent, in 1903, removed the thymus from a series of guinea pigs aged from 10 days to 1 month, but was unable to discover any effect on growth or any change in the blood.

Paton and Goodall, in the following year, performed extirpation experiments on the guinea pig. They found that deprivation of the thymus was followed by a decrease in the number of lymphocytes for about 2 months without coincident alteration in the other elements of the blood, but they observed no disturbance in growth and no variation of any kind from the normal.

In 1905, Paton published a new set of thymectomy experiments on guinea pigs, in which special study was made of the effects produced on the genital organs. His experimental material comprised twenty-four guinea pigs, 1 day to 1½ months old at the time thymectomy was performed, and killed at varying periods after operation. The control animals were selected from the same litters when possible, but when this was impossible (and he does not indicate how often this was) they were matched with the operative animals according to weight. Inasmuch as he had no record of the dates of birth of his animals he was compelled to estimate their ages from their weights. Since he found that sexual maturity in the guinea pig occurs at about the 3rd month, when the animal has reached a weight of approximately 300 gm., he assumed in his experiments that animals under 300 gm. were under 3 months of age and sexually immature, while animals over 300 gm. were over 3 months of age and sexually mature. The first group of six guinea pigs (there were six controls) weighed between 75 and 145 gm. at the time of operation and were allowed to live from 2 to 6 weeks; they were killed at such times as to make the final weight of all members of the group lie between 100 and 200 gm. The average postmortem weight of the operative animals was 165 gm., of the controls 152 gm.; of the testes and epididymes of the operative animals, 0.23 gm.; of the testes and epididymes of the controls, 0.18 gm. The second group of sixteen animals (there were fourteen controls) weighed from 84 to 280 gm. when the thymus was removed, and were killed at the end of 4 weeks—in two instances 8 weeks—when the weights of all were between 200 and 300 gm. The average final weight of the operative animals of this group slightly exceeded that of the controls, but the average weight of the testes and epididymes of the thymectomized animals was 0.88 gm., while that of the controls was but 0.60 gm. The two groups just mentioned comprised the immature material of Paton's experiments. The third group, composed of but two operative animals (there were three controls), represented his entire mature material. One of the two, weighing 250 gm. at the operation, was killed at the end of 3 weeks,

weighing 310 gm. The other, weighing 280 gm. when operated upon, was allowed to live only 1 week. The testes, with epididymes attached, of these two animals weighed almost the same (1.24 gm.) as those of the three controls (1.33 gm.). Paton was unable to show that the loss of the thymus affected in any way the time of conception of female guinea pigs.

Though it seems questionable whether Paton could draw definite conclusions from his experiments, except perhaps such general ones as that removal of the thymus in the guinea pig is compatible with life, or that it does not produce any very gross changes, he drew the specific conclusion that the thymus inhibits the growth of the testes in immature but not in mature animals; and since Calzolari, Henderson, and others had already established the fact that the thymus continues to persist uninvolved in animals castrated before maturity, he put forward the theory that prior to the period of sexual maturity thymus and sex glands act antagonistically, each exerting an inhibitory influence on the other.

In the same year (1905) and again in 1909 Soli performed thymectomy experiments on guinea pigs. The major part of his work, however, was done on fowls, in which he discovered that extirpation of the thymus resulted in well marked retardation in growth and development of the testes. He obtained similar results in the majority of fifteen guinea pigs, although they were much less marked than in the case of the fowls. Although Soli's results in guinea pigs appear to contradict the findings of Paton, they do so only to a limited extent, for all Soli's guinea pigs with the exception of two weighed more than 300 gm. when killed (Paton). (The maximum weight for immature animals in Paton's experiments was 300 gm.). The testes of Soli's two thymectomized guinea pigs, which weighed under 300 gm., were, however, slightly lighter than those of the controls.

In 1911 Paton returned to the problem of the relationship of thymic and testicular functions. Was it not possible that each organ exercised a primary, independent, stimulating influence on growth rather than an inhibitory influence on the other, as according to the theory he had previously advanced? To put this new hypothesis to the test, he first determined in a series of four guinea pigs and controls that castration alone before sexual maturity, *i.e.*, in animals under 300 gm., did not affect the weight. He next found by combined extirpation of both thymus and testes in a series of sixteen guinea pigs under 300 gm., that retardation in the growth of the animal measured in terms of weight did occur. Since his previous experimental work had shown that loss of the thymus alone had no influence on growth, he regarded the chain of experimental evidence necessary for the new hypothesis as complete. His final conception of the relationship of the two organs to each other was, therefore, that each stimulated growth in the immature animal independently of the other and consequently that the hypertrophy of the testes, when the thymus was removed, and the continued persistence of the thymus when the testes were removed, in sexually immature animals were compensatory in nature. On performing similar experiments with female guinea pigs he was unable to produce changes corresponding to those in the male.

It should be added that Paton used controls from "the same batches" of animals, and again that he had recourse to rough estimations of the ages from the weights.

Halnan and Marshall are the most recent investigators to attack the thymus problem in the guinea pig. They found that its removal in young guinea pigs, most of which weighed about 150 gm. did not affect growth, and that castration led to an arrested atrophy and subsequent hypertrophy of the thymus, and, more important, as bearing on Paton's work, that simultaneous removal of the testes and thymus did not affect the growth of immature animals, and thymectomy was not followed by hypertrophy of the testes. Thus they entirely failed to confirm the results of Paton. Their paper concludes with a note from Yule showing by methods of statistical analysis that the differences which Paton found in the testes of thymectomized and control guinea pigs were not greater than could be explained on the basis of chance variation.

Paton and Goodall remark: "The chief obstacle to the investigation of the functions of the thymus is the difficulty of its removal on account of the situation of part of it, in most animals, in the thorax. We were fortunate enough to begin our investigations upon guinea-pigs and we found that in these animals the structure is entirely cervical." Halnan and Marshall speak of thymectomy in the guinea pig as a simple operation. Klose and Vogt, on the other hand, describe thymectomy in the guinea pig as a dangerous operation which must be done in two stages and frequently causes death from hemorrhage. The writer selected the guinea pig for his experiments.

EXPERIMENTAL.

The operation for the removal of the two main lobes of the thymus in the guinea pig is simple, owing to their superficial position, for they lie one on either side of the median line of the neck, just beneath the platysma and deep fascia about midway between the tip of the lower jaw and the upper end of the thorax, a little in front of, but almost opposite the angle of the jaw. A median incision about 0.5 cm. long, with its middle point a little anterior to the angle of the jaw, is carried to the deep fascia, and when the edges of the wound are pulled apart the two flat, oval-shaped, granular-looking lobes of the thymus can be plainly seen shining through, having a paler color than the pink salivary glands which lie near them. Each lobe in the well nourished new-born guinea pig weighing 100 gm. measures from 10 to 15 by 7 to 10 mm. The deep fascia is then incised and each lobe in turn lifted up and freed from the salivary glands by blunt dissection. As these latter are pushed away a sheet of transparent fascia

is exposed, connecting the thymus with the neck, and in this sheet of fascia two blood vessels, larger than the rest, can be seen, one entering the lobe a little behind the anterior pole, the other a short distance in front of the posterior pole. In the earlier experiments these vessels were carefully tied off, but in the later experiments they were burned through, together with the fascial sheet, by means of a hot platinum wire. At this point in the operation great care is necessary not to leave accessory lobes of thymus, which become visible only as the main lobes are lifted up, lying near but unattached to the main lobe or close to the salivary gland, in the fascial sheet mentioned. If the main lobe of the thymus is removed first with the expectation of excising the accessory lobes later, the latter are found to fall back into the cavity of the wound the moment tension is released, and are lost. It is necessary therefore to cut or burn through the fascial sheet as far away from the main lobe as possible in order to secure the accessory lobes with the main lobes, and often to remove also fairly large parts of the salivary glands, if the latter cannot be easily separated. Frequently as many as two accessory lobes are present on each side, not larger than pin heads.

The Discovery, by Serial Section, of Accessory Lobes of the Thymus in Close Relation to the Parathyroids.

The operations were conducted under ether anesthesia. Little preparation aside from shaving the operative field and cleansing with alcohol was necessary, for the animals never became infected. As soon as they had recovered from the anesthesia they appeared to be well. A number of the guinea pigs died a short time after the operation but not as the result of it, for the mortality was equally high among the controls.

The animals were kept under observation for variable lengths of time, and were then killed. At autopsy the tissues of the neck of all the thymectomized animals were taken out *en masse* to be studied in serial section for thymus rests. As the block of tissue was removed from each animal it was turned over on its under surface, and first the thyroid, then the larynx and trachea, were dissected away, the former for finer histological study than would otherwise have been possible, the latter to facilitate serial section cutting. Great care

was taken to carry away with the trachea and thyroid as little other tissue as possible.

When the serial sections from the cervical tissues of nine of the thymectomized guinea pigs were studied, it was found that thymus rests near the parathyroid were present in six of them; in the remaining three no thymus tissue could be discovered. In these three thymus-free animals, however, no parathyroid tissue could be found, and inasmuch as the only conceivable explanation for the absence of the parathyroids was that they had been lost when the thyroids and trachea were removed, the possibility became apparent that accessory lobes of thymus lying near the parathyroid might also have been there and been lost with the latter. In the cervical tissues of two of the animals which had thymus rests near the parathyroid, accessory thymus tissue at some distance from the parathyroids was also found.

Since it seemed scarcely worth while to investigate the mutilated cervical tissues of the remaining thymectomized guinea pigs in order to throw further light on the occurrence of accessory lobes of thymus tissue, the intact cervical tissues, including trachea and thyroid, of five additional guinea pigs were examined in serial section. Of these five guinea pigs, four came from the same litter, and of the four, two had been freshly thymectomized. In all five animals accessory lobes were found in proximity to the parathyroids, and in three of them additional accessory lobes not connected with the parathyroids and too far removed from the main body of the thymus to have been reached at operation.

Although the number of guinea pigs examined is too small to permit any general conclusions, it seems probable that accessory lobes of thymus occurring in relation to the parathyroids are usual in the guinea pig and that other accessory lobes too remote from the main lobes of the thymus for removal with the latter are not uncommon.

The Relations and Characters of the Accessory Lobes of the Thymus Found in the Guinea Pig after Supposed Complete Thymectomy.

The accessory thymus tissue which was found in relation to the parathyroids was in the form of lobes having well defined cortex and medulla, and in short a histological appearance identical with that of the main lobes. In some instances only a single lobe could be found

in relation to a parathyroid, but in other instances there appeared to be several separate lobes clustered around it; though when certain sections seemed to indicate the presence of several separate masses of thymus, the study of the other sections sometimes showed that in reality a single lobulated mass of thymus tissue existed. In some of the animals the accessory lobe of the thymus, or the largest of them if several existed, was two to three times the size of the parathyroid, but in a number of the animals the accessory thymus lobe was about the same size as the parathyroid or even smaller. In at least half the animals thymus tissue and parathyroid were fused together, or at least one passed directly into the other. In one animal the parathyroid on the right side was drawn out into a thin strand which was much longer than the thyroid and extended considerably in front of it; it terminated in a small thymus lobe, so shaped that it would have been regarded as the continuation of the parathyroid but for its different structure (Fig. 2). In sections from another animal, thymus and thyroid tissue were so intermingled at one point that there was great difficulty in distinguishing them, while at another point in the sections an island of thymus surrounded by parathyroid tissue could be seen (Figs. 3 and 4). In the animals in which thymus tissue did not actually join parathyroid, it was situated close to it, never much farther away than the thickness of the parathyroid.

Of course in the mutilated cervical tissues of the six thymectomized animals first studied, it was not possible to determine more than the size and relations of the accessory lobes with reference to the accompanying parathyroid, but it was possible to obtain additional and more precise information from the study of the intact cervical tissues of the five guinea pigs killed for that purpose. In them it was found that the accessory lobes of thymus occurred on both sides in only three animals, though they occurred on one side in all five, as already stated. It was also clear that the parathyroid which the accessory lobes accompanied was the one from the third pouch. The identity of this parathyroid could be determined (1) by its larger size, and (2) by its position with reference to the thyroid, for in all five animals it lay on the outer side of the thyroid, in three instances separated some distance from the latter. In most of the animals it was in proximity to a comparatively large blood vessel, supposed to be a thy-

roid artery. In one animal the accessory lobe lay on one side of this vessel, the parathyroid on the other. On the other hand, parathyroid IV, which was much smaller than parathyroid III, lay close to the thyroid on its inner surface in all but one instance, and in three animals was partially embedded in its substance. It was absent on one side in three animals, and so small as to be barely visible in one. Accessory lobes of thymus were not found in association with this parathyroid (Fig. 1).

In this connection it is worth noting that no constant relationship in the positions of the two parathyroids in the cranial caudal direction existed. In some of the guinea pigs parathyroid III was anterior, in others posterior to parathyroid IV. In one animal parathyroid IV was in front of parathyroid III on one side and behind it on the other. The commonest arrangement seemed to be for parathyroid III to lie near the anterior end of the thyroid and parathyroid IV near the middle or posterior part. In the mammals in which the thymus migrates caudally, it will be remembered that parathyroid III is carried caudally with the thymus so that it comes to be the more caudally situated of the two parathyroids. The absence of this relationship in the guinea pig is easily explained by the failure of the thymus to migrate in that animal.

In addition to the accessory lobes already described as occurring in intimate relationship with the parathyroids, accessory lobes were found in two of the animals at some distance from the parathyroid in the neighborhood of the main thymus lobes, but they were probably too far separated from them to have been removed at operation.

The existence of accessory lobes of thymus derived from the third pharyngeal pouch lying in close relation to the parathyroids, has never before been described in the guinea pig, as far as the writer is aware, but their occurrence in other species has been known for some time. They are usually present in the rabbit, cat, and rat (Kohn), and have been found in the dog and in man (Ammann, Kürsteiner). The study of the development of the thymus makes their presence and also their close connection with parathyroid III easy to understand, for, as already pointed out, thymus and parathyroid take origin coincidentally in the closest proximity from the third pharyngeal pouch, are freed at the same moment by the atrophy of the neck of the pouch, and, if

migration occurs, migrate with each other. To explain the presence, therefore, of an accessory lobe of thymus in close connection with the parathyroid it is necessary merely to suppose that a part of the original thymus anlage adjacent to parathyroid III adhered to the latter at the time of their separation. If the theory of Ruben is correct that in the guinea pig parathyroid III is separated from the thymus mass through the agency of the loop of the hypoglossal nerve in its passage upward, it would be necessary simply to assume that the nerve had cut off a small portion of the main mass of the thymus with the parathyroid instead of accurately dividing the one from the other.

The practical significance of the discovery of these minute accessory lobes of thymus in such intimate relation to the parathyroids is that the thymus can only rarely be completely extirpated in the guinea pig. Almost all, if not all, thymectomies in this animal have been, therefore, partial thymectomies, and the positive experimental findings of Soli and Paton must be interpreted in that light.

The Value of Incomplete Extirpation of the Thymus as a Means of Determining Thymus Function.

The question naturally suggests itself whether incomplete extirpations of the thymus have any value as a means for determining thymus function. Since it is well established that reduction of certain organs of internal secretion, for instance the adrenal, thyroid, and parathyroid, to minute rests causes well marked signs of insufficiency, it might reasonably be supposed that sudden reduction of the thymus to a small rest in a newly born animal would make itself known, if it plays the important part in metabolism attributed to it by Matti and Klose. And the literature contains considerable evidence that this is the case. Some of Basch's³ extirpations in dogs seem to have been incomplete. Fulci obtained marked rachitic changes in a rabbit by means of thymectomy, although a thymus rest almost as large as the normal gland was found at autopsy. Flesch discovered that incompletely thymectomized rats died in a manner indistinguishable from the completely thymectomized animals, with but a single excep-

³ See Basch, K., *Wien. klin. Woch.*, 1903, xvi, 893.

tion. Though Klose and Vogt criticize the validity of a large part of the negative experiments of others by assuming that the thymectomies were incomplete, they accept the experimental work of those who have obtained results similar to their own, passing over the fact that the larger part of these thymectomies would be shown to be incomplete if subjected to the analysis which they applied to the other experiments. But recently Klose has reported rachitic changes in swine and goats induced by thymectomy, although complete thymectomy is impossible in both species, as he admits. To the group of investigators who have obtained positive results by means of partial thymectomy may now be added the names of Paton and Soli.

Doubtless many of the extirpations of the thymus which have been regarded as complete and reported as such in the literature in reality were partial extirpations, for it is impossible to be certain at the time of operation that a branching, friable organ like the thymus of the larger animals that have been used for extirpation experiments has been completely dissected out of the thorax and neck, especially considering the possibility of the accessory lobes. Likewise the failure to discover thymus rests at autopsy by inspection of the neck and thorax or microscopical section of bits of thymus tissue falls far short of proof that no thymus rests were actually there. The painstaking researches of Grosser and Betke have shown that in children accessory thymus lobes occur in the neck with a frequency never before suspected. If the thoracic and cervical tissues of larger animals that have been thymectomized, such as the dog and rabbit, could be studied in serial section, as was done by Pappenheimer in the rat, and by the writer in the guinea pig, it might be shown that complete thymectomy was rarely accomplished. Klose's statement that the most minute thymus rest left at operation regenerates until it reaches a size approximating that of the intact gland is not true. The literature seems to furnish, therefore, numerous instances of the effectiveness of partial thymectomy, and particularly, when the positive results of the partial thymectomies in guinea pigs of Paton and Soli are considered, justifies the publication of the following partial thymectomy experiments. But the actual problem is not whether partial thymectomy can produce results identical with complete thymectomy, but whether thymectomy, partial or complete, actually causes the changes which have been ascribed to it.

Results of Thymus Extirpation.

Fifty-five guinea pigs were thymectomized in the manner described, on the 1st, 2nd, 3rd, or 4th days of life, and in all but two instances were controlled from the same litter and sex. But since the mortality among such young animals was high, from trampling, inanition, and other causes, a large number of the experiments were interrupted at the outset. If the control died at the beginning of an experiment a guinea pig differing only a day or two in age was supplied in its place from another litter and the experiment continued. Later two epidemics of dysentery were encountered, which caused a number of fatalities and still further reduced the material, leaving eighteen thymectomized and sixteen control guinea pigs, which form the basis of this report. Twelve of the eighteen thymectomized animals had their original controls, and form, therefore, a complete series for the study of the effects of thymectomy from all standpoints. The remaining six thymectomized and four control animals lost their mates too late in the course of the experiments for anything to be gained by pairing them with each other or with fresh animals from other litters. Accordingly the six operative animals, with the four normal animals, are grouped separately as imperfect experiments which have value for the histological study of the organs but not for the study of the effects of thymectomy on growth or development.

All the animals were confined, in pairs, in wire cages about $1\frac{1}{2}$ feet long by 1 foot broad in a well lighted room, and were fed a diet of greens and oats each once daily. They were inspected frequently and systematically examined at the weighing times, which appear on the weight charts, for the various changes which have been reported in animals deprived of the thymus. One operative animal and two controls died spontaneously at the conclusion of the experiment, but the others were killed with ether. The bodies of all were autopsied at once, the stomach and intestines with their contents removed and weighed separately in order to determine the actual body weights of the animals (minus the weight of the food), and the weights of the different organs were taken. The tissues were preserved in formalin and the sections stained with hematoxylin and eosin. The growth of the thymectomized animals was normal.

The weight curves of the twelve operative animals and their controls were compared in pairs, but without demonstrating any preponderant variation at any stage of development, although there was considerable variation in the weight of the pairs of animals considered separately. In some unpublished experiments by Auchincloss and the writer guinea pigs were obtained prematurely by Cæsarean sections and thymectomized at once without causing any alteration in growth or development. The series of weights in two of these experiments are appended to the table of weights of the animals of the writer's own experiments (Table I).

The strength and activity of the operative animals also appeared to be normal. As the two guinea pigs constituting each experiment were caged together, it was possible to form some idea of their relative strength by observing which drove about the other. In some instances it was the control, in others the operative animal that had the mastery.

The evidence of sexual maturity appeared to be unaffected by thymectomy. This observation was made in the experiments of Auchincloss and the writer already referred to, in which it was found that the testes of thymectomized and control males descended at about the same time and that several pairs of males and females thymectomized soon after birth, caged separately, and allowed to breed in competition with control pairs of intact males and females of the same age, produced young at about the same age.

No trophic disturbances of the hair or abnormality in the growth of the teeth were noted in the operative animals.

At autopsy the fat appeared to be equally distributed in both sets of animals, and the viscera of the thymectomized animals did not differ in their gross appearance from the viscera of the controls. The weights of the animals and of their organs are given in Table II. Although there is exhibited the greatest individual variation in the weights of the organs, there seem to be no constant differences in the two groups. The most interesting feature brought out in the table is the relatively large weight of the gastric and intestinal contents in the guinea pig. It will be noted that the excised stomach and intestines and contents of the guinea pigs—they were killed at various times of the day—weighed between 38 and 160 gm., with an average

TABLE I.
Weight of Thymectomized and Control Guinea Pigs.

No. and sex.	Age at operation.		Killed or died.	Record of weights in gm.									
	days	mos. days		1915	1915	1915	1915	1915	1915	1915	1915		
1* ♀	1	2	Died.	Mar. 2	Mar. 9	Mar. 23	Apr. 6	Apr. 27	May 11	May 17			
1C ♀	1	2	"	50	90	140	190	220	210	190			
2* ♀	1	2	Killed.	Mar. 2	Mar. 9	Mar. 23	Apr. 6	Apr. 27	May 11	May 17			
2C ♀	1	2	Died.	60	120	165	150	200	290	266			
3* ♀	4	6	Killed.	May 14	July 8	Aug. 11	Sept. 4	Oct. 20	Dec. 4				
3C ♀	4	6	"	158	270	540	480	560	560				
4* ♀	3	7	"	Mar. 28	Apr. 5	June 6	July 8	Aug. 11	Sept. 4	Oct. 20	Nov. 20		
4C ♀	3	7	"	121	132	320	490	540	525	580	520		
5* ♀	3	6	"	May 14	June 6	July 8	Aug. 11	Sept. 4	Oct. 20	Nov. 20			
5C ♀	3	6	"	115	129	370	460	550	505	695			
6 ♀	3	6	"	120	190	265	380	290	375	385			
6C ♂	3	6	"	122	195	285	400	340	445	450			
	3	6	"	May 14	June 6	July 8	Aug. 11	Sept. 4	Oct. 20	Dec. 4			
	3	6	"	132	215	360	435	345	485	552			
	3	6	"	152	235	370	510	455	585	607			

TABLE II.
Body Weight and Weight of Organs in Thymectomized and Control Guinea Pigs.

No. and sex.	Age.		Killed or died.	Body weight. gm.	Weight of gastro-intestinal tract with contents. gm.	Body weight without gastro-intestinal tract and contents. gm.	Weight of organs.								
	mos.	days					Liver. gm.	Kidneys. gm.	Heart. gm.	Pancreas. gm.	Testes. gm.	Spleen. gm.	Adrenals. gm.	Thyroids. gm.	Thymus. gm.
1* ♀	2	15	Died.	190	46	144	7.5	2.1	0.95	0.65	0.34	0.25	0.01		
1 C ♀	2	15	"	140	38	102	5.8	1.7	0.5	0.57	0.6	0.2	0.05		0.16
2* ♀	2	15	Killed.	265	85	180	13.5	1.4	0.8	1.05	0.32	0.25	0.07		
2 C ♀	2	15	Died.	140	38	102	5.8	1.7	0.5	0.57	0.6	0.25	0.05		0.16
3* ♀	6	20	Killed.	560	141	419	21.5	3.4	1.8	1.6	0.67	0.32	0.1		
3 C ♀	6	20	"	500	113	387	25.3	4.2	2.23	1.7	0.79	0.36	0.05		0.3
4* ♀	7	25	"	520	136	384	38.0	5.52	2.32	1.67	0.92	0.33	0.065		
4 C ♀	7	25	"	530	160	370	48.1	5.0	1.8	1.2	0.92	0.41	0.038		0.2
5* ♀	6	7	"	385	84	301	26.0	3.67	1.58	1.2	0.42	0.33	0.03		
5 C ♀	6	7	"	450	115	335	21.0	3.7	1.9	1.5	0.46	0.21	0.025		
6 ♀	6	19	"	552	126	426	37.5	6.8	1.9	1.77	0.82	0.4	0.1		0.21
6 C ♂	6	19	"	607	127	480	34.6	4.9	2.3	1.89	1.66	0.62	0.28	0.11	
7* ♀	6	25	"	440	134	306	19.5	3.15	1.1	1.0	0.55	0.26	0.01		
7 C ♀	6	25	"	380	119	261	17.0	3.0	1.05	1.15	0.43	0.3	0.03		0.13
8 ♀	6	8	"	430	121	309	26.2	3.7	2.0	1.7	0.7	0.45	0.05		
8 C ♀	6	8	"	375	85	290	24.0	4.0	1.7	1.5	0.6	0.37	0.05		0.35
9* ♀	6	4	"	410	104	306	31.5	4.1	1.48	1.38	0.45	0.35	0.1		0.2
9 C ♀	6	4	"	310	83	227	28.0	3.2	1.45	0.8	0.4	0.35	0.1		
10* ♂	3	20	"	335	95	240	13.7	2.7	1.0	1.05	0.45	0.3	0.01		
10 C ♂	3	20	"	355	97	258	15.5	3.3	0.8	1.2	0.3	0.3	0.05		0.2
11* ♀	3	17	"	425	87	338	12.7	3.1	1.21	1.21	0.31	0.21	0.08		

11	C ♀	3	17	Killed.	460	101	359	13.4	3.61	1.36	1.05	0.56	0.25	0.06	0.55
12*	♀	3	14	"	310	67	243	12.61	2.81	0.85	1.11	0.35	0.2	0.07	
12	C ♀	3	14	"	335	95	240	13.4	3.11	1.1	1.1	0.36	0.2	0.05	0.4
13†	N ♀	3	8	Killed.	410	90	320	10.4	2.55	1.15	1.2	0.5	0.3	0.05	0.2
14	♂	3	13	"	230	58	172	9.0	2.01	1.0	0.81	1.21	0.15	0.01	
15	N ♂	3	19	"	370	132	238	14.0	3.0	1.05	1.15	0.31	0.21	0.05	0.16
16	♀	5	10	"	560	105	455	19.8	4.3	1.4	1.3	0.85	0.4	0.1	
17	N ♀	6	14	"	455	94	361	36.5	4.1	1.5	1.6	0.62	0.32	0.12	0.2
18	♂	7	4	"	475	97	378	13.5	4.01	1.15	1.21	0.45	0.31	0.1	
19	♀	8	13	"	512	127	385	33.7	4.2	2.13	1.55	0.83	0.4	0.12	
20	♂	11		"	525	152	373	26.46	4.82	2.17	1.52	0.62	0.35	0.06	
21	♂	11	9	"	605	126	479	39.4	5.16	2.5	1.25	0.66	0.55	0.15	
22	N ♂	11	22	"	595	131	464	28.5	4.4	2.4	1.8	0.5	0.27	0.04	0.6

* Both animals were from the same litter.

† This group of six thymectomized and four normal animals contains single survivors of experiments. The thymectomized members of the group have been used for the study of the effects of the removal of the thymus on the organs, and, for this purpose, the normal animals may be regarded as controls.

of 103 gm., amounting in most cases to about one-fourth of the total weight of the animal. If the weights of the gastro-intestinal tracts and actual body weights (body weight minus weight of gastro-intestinal tract) are compared, it will be seen that the living healthy guinea pig, fed in the usual way, is composed of 2 to 5 parts of body and 1 part of food.⁴

No histological changes, which were constant, could be found in any of the organs of internal secretion or in the bones. There were no signs of rickets. The femora and ribs cut before decalcification with the same stony resistance as the bones of the controls, and on microscopical examination showed no osteoid zone bordering the trabeculae or irregularity in the growth of the cartilage. The thyroids displayed marked individual differences, as has been noted in other animals by different observers, but no variation common to one set of guinea pigs. The adrenals presented no constant differences such as have been described by Matti in the dog and also by Klose. Measurements of the cortex and medulla of the adrenals of the pairs of animals showed how variable the thickness of the medulla is in the guinea pig. Where the adrenal is flat and broad, the medulla is scarcely more than a line in thickness; where the adrenal has a more rounded shape, the medulla may have a diameter of 3 mm. The cortex measured uniformly about 1 mm. in thickness. Histologically the adrenals, hypophyses, and ovaries, and the testes of the few males in the experiments, were normal.

Although the negative experiments just described furnish no evidence for or against Paton's conclusion that extirpation of the thymus in immature guinea pigs causes the testes to hypertrophy, yet there are reasons why Paton's work should not be accepted on its face value. His problem concerned growth, and in the investigation of a problem of that nature the cardinal principle is to plan the experiments so as to insure absolute parallelism between each pair of experimental and control animals, and the value of the results obtained depends largely on the strictness with which this principle has been carried out. But Paton chose his controls largely from the same batches of animals and guessed at the ages from the weights, though exact correspondence

⁴The error of estimating the age of the guinea pig from the weight should be considered.

in age between each pair of animals was of vital importance to the success of his experiments. And, finally, he made no allowance for the weight of the gastric and intestinal contents in his determinations of the final weights of his animals. These considerations do not prove Paton's results wrong, but they show that his methods were inaccurate and make corroboration of his work necessary. Halnan and Marshall have repeated Paton's work and failed to corroborate it. The changes which Soli found in the testes of guinea pigs after thymectomy were so slight that his experiments in that animal may be considered negative. In the light of these facts it seems proper to regard the thymectomy experiments on guinea pigs of all investigators up to the present time negative.

Whether the failure to obtain changes in the guinea pig by means of thymectomy indicates that the thymus has no function in that animal gross enough to be brought out in that way, or whether it signifies that the reduction of the thymus to the accessory lobes is insufficient to excite signs of thymus insufficiency in the guinea pig is a question which cannot be answered in the present state of our knowledge; and to speculate in regard to it is idle, until the principle that thymus function can be demonstrated by thymus extirpation has been firmly established in other animals in which complete or more nearly complete removal of the thymus is possible.

CONCLUSIONS.

1. Accessory lobes of thymus, derived from the third pharyngeal pouch, occurring in close association with the parathyroids from the third pouch, were found in serial section of the cervical tissues of eleven out of fourteen guinea pigs, and probably would have been found in all fourteen but for a technical error.

2. It is probable, therefore, that accessory lobes of thymus having this situation and origin are usually, if not always, present in the guinea pig.

3. Additional accessory lobes of thymus belonging to, but at some distance from the main lobe were also present in several of the animals.

4. The discovery of these accessory lobes makes it certain that the guinea pig is unsuitable material for complete thymectomy, and prob-

ably complete extirpation of the thymus in this animal is rarely, if ever accomplished.

5. The extirpation experiments of previous investigators in the guinea pig must now be regarded as partial extirpations, and their results interpreted in that light.

6. Extirpation of the thymus in the guinea pig produced no changes in the writer's experiments.

7. The study of the serial sections of the cervical tissues of the guinea pig indicates that Ruben's statements regarding the parathyroid derived from the fourth pharyngeal pouch in the guinea pig are correct,—that it is much smaller than parathyroid III, may be rudimentary, and is sometimes absent at least on one side.

8. No accessory lobe of thymus was found accompanying the parathyroid from the fourth pouch, a finding also bearing out Ruben's statement that no thymus anlage springs from the fourth pouch in the guinea pig.

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EXPLANATION OF PLATES.

PLATE 20.

FIG. 1. The right thyroid is shown with the parathyroid derived from the fourth pharyngeal pouch imbedded in its substance, and the parathyroid derived from the third pouch accompanied by an accessory lobe of thymus, lying on its outer border. *a*, accessory lobe of thymus; *b*, parathyroid from the third pouch; *c*, right thyroid; *d*, parathyroid derived from the fourth pharyngeal pouch.

FIG. 2. The parathyroid derived from the third pharyngeal pouch appears as a thin strand, which terminates anteriorly in an accessory lobe of the thymus also derived from the third pouch. The strand of parathyroid tissue measures two and one-half times the length of the accessory thymus lobe; less than half of it is included in the photograph. The thyroid does not appear as it lies too far posteriorly. *a*, small accessory lobe of thymus; *b*, larger accessory lobe; *c*, parathyroid continuous with thymus lobe; *d*, salivary glands; *e*, cartilage of trachea.

PLATE 21.

FIG. 3. Accessory lobes of thymus are shown clustering around the parathyroid from the third pouch. Two separate masses of parathyroid are shown which, in serial section, could be seen to be continuous. In the larger of the two masses a small island of thymus can be seen. A study of the other sections shows it

not to be an island, but an arm of thymus tissue thrust into the parathyroid. In the smaller of the two masses of the parathyroid there is thymus tissue and also a duct-like space, lined with cylindrical epithelium. *a*, accessory lobes of thymus derived from the third pouch; *b*, parathyroid from the third pouch; *c*, duct-like space lined with cylindrical epithelium.

FIG. 4. The same parathyroid and group of accessory thymus lobes as shown in Fig. 3, but at a different level. *a*, thymus from the third pouch; *b*, parathyroid from the third pouch; *c*, duct-like space lined with cylindrical epithelium.

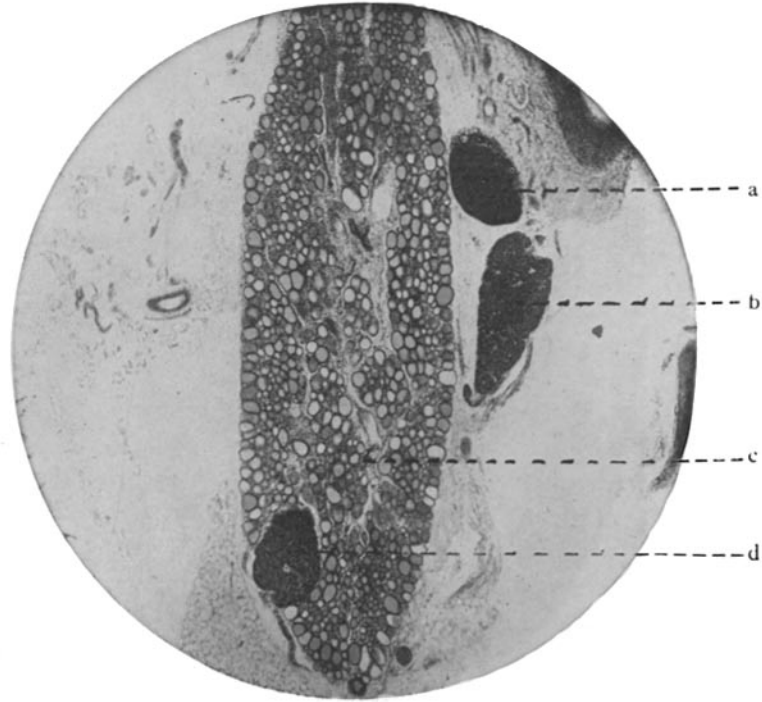


FIG. 1.

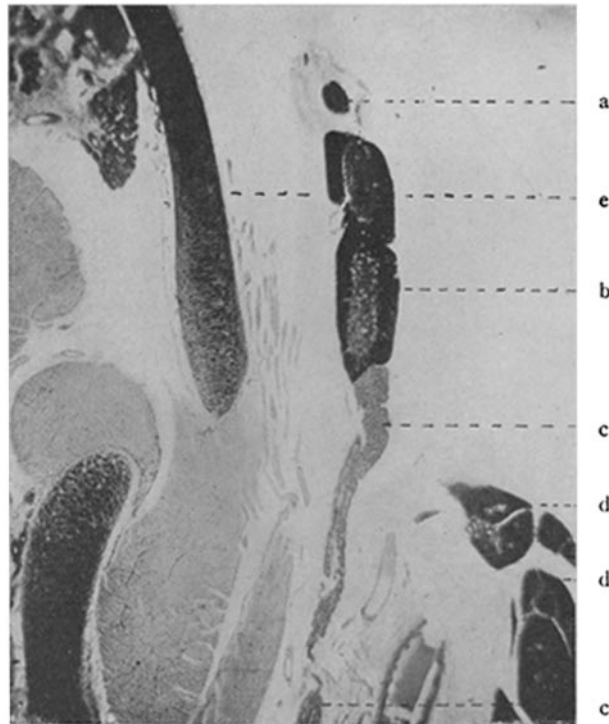


FIG. 2.

(Park: Extirpation of the Thymus in the Guinea Pig.)

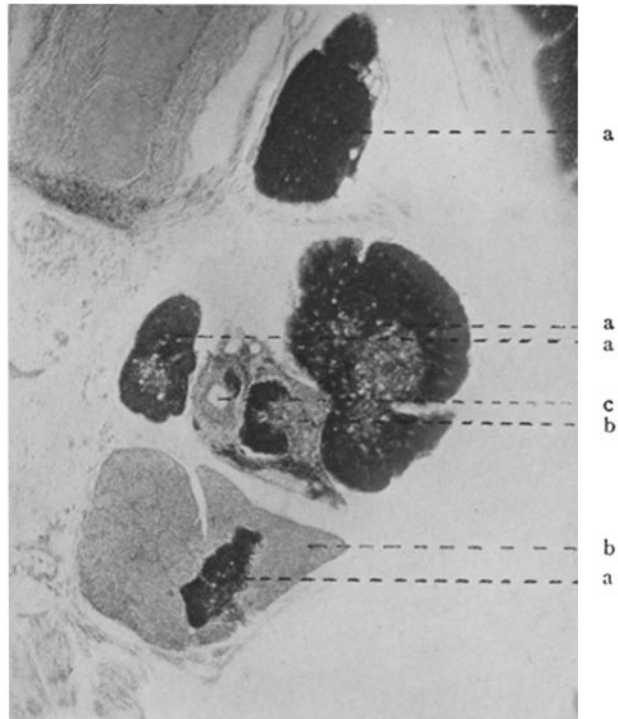


FIG. 3.

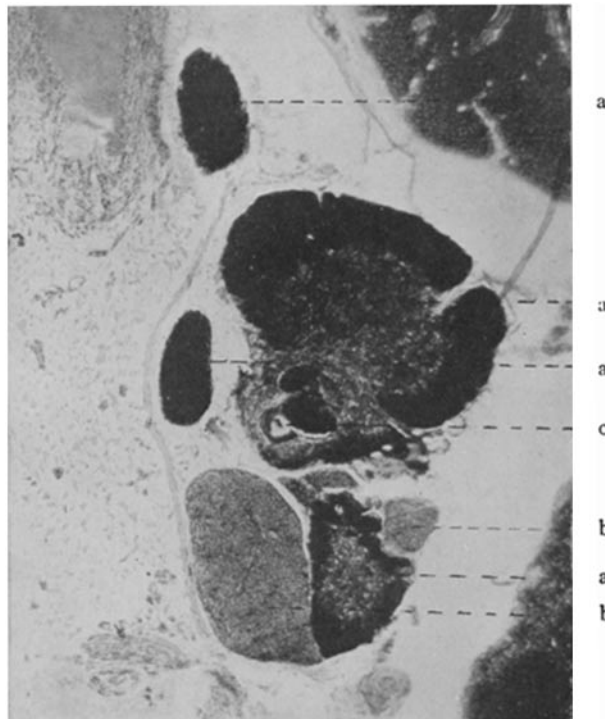


FIG. 4.

(Park: Extirpation of the Thymus in the Guinea Pig.)