A Study on the Functional Connection between Suprarenal Cortex and Testes.

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LITERATURE ON SUPRARENALS.

No attempt has been made to give the full literature, which has reached colossal dimensions. Only abstracts of the work which seemed to bear directly on this investigation have been given.

Interest in the function of the suprarenals was first roused in 1855 when Addison (2) described the disease that bears his name. He stated that the symptoms were associated with destructive disease of the suprarenal capsules.

In the following year a further advance was made in our knowledge of the suprarenals by the work of Brown Sequard(14) who removed the glands from dogs, cats, rabbits and guinea pigs with always the same result, death within a short period. Young animals survived the operation for a longer period than old ones. He also found that if only/

only one gland was removed, many animals survived, but when the second gland was removed even after a long interval, the animal died.

These experiments showed that the suprarenals were important organs and essential to life.

For a time, however, this was doubted because Harley (29) and others working on rats found that they can survive removal of the suprarenals. It was not known at that time, that their survival was due to the fact that this animal is exceptional in possessing in various parts of the abdomen small glandular structures which are composed of cells having the features and functions of the medulla or cortex of the suprarenal.

During the next twenty-four years a large number of observations were made chiefly by extirpation of the glands, but the significance of these glands was not yet/

yet understood. The conclusions of most of the observers, who had followed in Brown Sequard's footsteps, were that the death of the animal was due to operative procedure or injury to nerves.

In 1879 Pellacani (45) carried out experiments by injecting extracts of the gland substance. On injecting a water extract of six suprarenals of the ox into the vein of a rabbit, the animal died in two minutes, death being preceded by dilatation of the pupil of the eye, rapid respirations, general convulsions and marked peristalsis. Similar experiments were carried out on other animals and produced death within twenty-four hours. Pellacani and Foa (46) believed that death was due to the formation of coagula intra vitam. It should be noted that the dose given by these observers was an exceedingly large one.

E.Guarnceri/

D.Guarnceri and M.Zucco (24) confirmed the results of Pellacani and Foa and concluded that the toxic material obtained from the gland owed its activity to the presence of neurin. Other observers, notably Tizzoni (59), Alexais and Arnaud (5), did not believe in the existence of a toxic substance in the gland and regarded the toxic effect produced by injection as due to some chemical change occurring in the gland substance after removal from the body.

Up to this time no observations had been made upon the blood pressure. Oliver and Schafer (44) in 1894 gave the results of observations made by them with the aid of manometer, oncometer, and cardiometer. They were able to show that the gland extracts exert a potent influence upon the heart and blood vessels and to a less degree upon skeletal muscle. The results of/

of their experiments on the subcutaneous injections and extirpation of both glands in a number of animals of different kinds proved that a substance is produced in the suprarenals which maintains the tone of the muscular tissue particularly that of the vascular system and is essential to life in most animals. They concluded that this substance was a true internal secretion and they regarded its absence as the true cause of the symptoms which manifest themselves in animals deprived of their suprarenals and in the human subject when the suprarenals were involved in disease.

An important step forward was made when Swale Vincent (55) in 1898 showed that the toxic effect, like the physiological effects, was due to a substance contained in the medulla. As the investigations proceeded it was realised that this gland, the suprarenal, was really composed of two functionally separate parts, the/

the cortex and the medulla and after the discovery that the pressor substance came from the medulla, the attention of investigators was concentrated on the effects of administering extracts of this part of the gland in various ways. This work was greatly aided by the brilliant researches of Takamine (58) and Aldrich (4) who, independently, separated and prepared in a crystalline form the pressor substance of the medulla.

As the cortex did not seemingly secrete any substance that could be said to have an effect on the body generally, attention was turned to it in the hope of finding a possible function. Experiments along the lines adopted in the investigations on the secretion of the medulla (adrenin) were found to be unsatisfactory, chiefly owing to the difficulty of separating the two portions of the gland. It was only when investigations on the suprarenals of lower animals took place that some progress was made.

In/

In the anamnia the organ is represented by a number of small bodies, the amphibians in some respects occupy an intermediate position. In pisces and cyclostoma there are two distinct bodies consisting of masses of cells (Fig.1.). These bodies are homologous with the two constituents of the adrenals in higher vertebrates.

Before considering the work which has been carried out as to the possible function of the cortical cells it is interesting to refer to its development and structure.

Elliott and Armour (20) state that the large size of the human foetal suprarenal gland is due to a peculiar hypertrophy of the cortex which commences very early and continues until birth, this inner (next to the medulla) hypertrophied mass is richly supplied with blood vessels, but does not contain the fatty substance which is a characteristic/

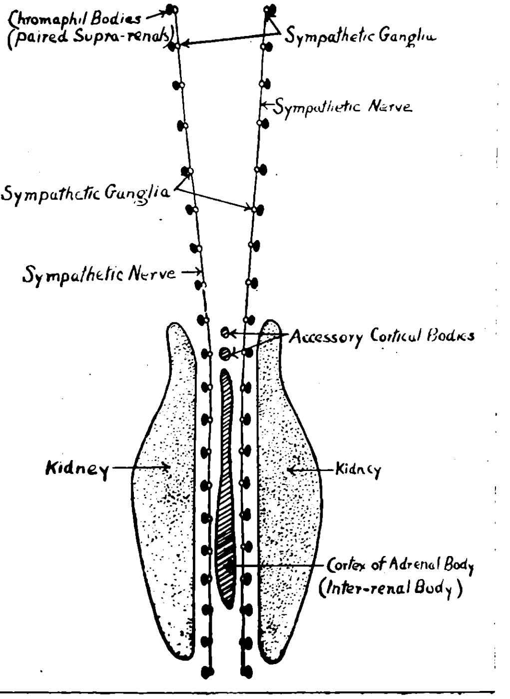

Fig.1. Diagram of the adrenal representive in Elasmobranch fishes.

characteristic product of the adult cortex. This foetal cortex degenerates immediately after birth, undergoing a fatty change and at the end of the first year all trace of it has disappeared. Enveloping this mass is a layer of small cells that early in foetal life assume the appearance of the adult cortical cells and store up fatty substance. This layer develops steadily and, alone, forms the adult cortex. So far as is known no other mammal shows such changes in the suprarenal.

The adult suprarenal glands (Fig.2.) in mammals lie in close anatomical relation to the kidney. In man and many mammals they consist of epithelium-like glandular substance enclosed in a connective tissue capsule.

The cortex is formed of polyhedral epithelium cells varying in diameter and massed together into columns incompletely separated by the fibrous trabeculae of the frame work. Three layers are recognised, the zona glomerulosa,/

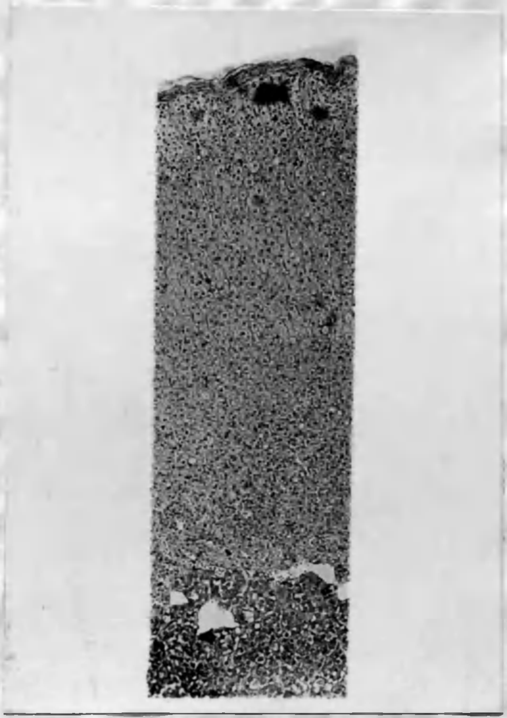

Fig. 2. Photograph of section of suprarenal showing cortex and medulla, from rat No. 5. x 60.

glomerulosa, or outer layer, is in some animals such as the dog and cat formed of long columnar cells arranged so as to form a kind of lumen, the zona fasciculata, the middle and broadest layer, consists of cells arranged in columns, the zona reticularis, which lies next the medulla has a more open arrangement of the cells, due to the plexus of sinus-like veins it contains.

When Abelous and Langlois (1) removed the medulla by cautery from frogs the animals died. They also noted the fact that after the destruction of one capsule, the frog remained in an apparently healthy condition but if the greater part of the remaining capsule was removed the animal died, but the interval was longer than after complete destruction. They observed also that summer frogs were better than winter frogs and that male frogs were more suitable for the experiment than female, presumably because symptoms developed quicker.

In/

In view of our present belief that the suprarenal cortex has a functional connection with the gonads, they came to the significant conclusion that the length of the survival was in inverse ratio to the metabolic changes going on in the body. The more rapid these changes, i.e., as in summer frogs, the more quickly did death supervene. The same authors found that by placing the suprarenals from a normal frog into the lymph sac of a frog from which the suprarenals had been removed, life was prolonged. Gourfein (26) carrying out similar experiments on pigeons found that if only a speck of one suprarenal cortex was left the birds lived from three to nine months. Pettit (47) carried out some experiments on eels to study the hypertrophy of one gland after removal of the other. The compensatory hypertrophy which occurred, he concluded, showed a secretory function of the adrenal./

adrenal. Pettit evidently looked upon this organ as the fundamental type of the suprarenal but the organ of the eel is composed entirely of cortical substance.

Poll (48) who repeated the experiments of Pettit on rats did not find any compensatory hypertrophy: this might be due to fifty per cent of rats having accessory suprarenals. Biedl (8) states that in cats, rabbits and dogs he succeeded in removing the cortex leaving the medulla intact. The animals died and he concluded that the cortex was essential to life. He also removed the cortical substance of Elasmobranches (9) and the animals died after showing the same symptoms as occur in mammals after complete extirpation.

Removal of the medulla was carried out by Wheeler (64) and in some cases the abdominal chromaphil body was removed. Although recognising that groups of sympathetic/

sympathetic chromaphil cells and cortical accessory bodies must have been left behind he concluded that the cortex was the essential part of the gland for the maintenance of life.

Attempts to advance our knowledge of the function of the cortex by transplanting experiments have not been very helpful. Canalis (16) grafted small portions of the adrenal into the kidney, but with the exception of one case in which he found cortical cells after fifteen days; the grafts became necrotic and were absorbed. Gourfein (26) transplanted one adrenal into the lymph sac of frogs and got similar results.

Graft experiments were carried out by Poll (49) who transplanted one adrenal into the dorsal muscles of the rat while in a second series of experiments the adrenal was placed under the skin of the back. He reports as follows:- The cells of the glomerulosa and outer part of the fasciculata changed/

changed to large polyhedral structures, sometimes containing pigment, while the cells of the inner part of the zona fasciculata and of the zona reticularis and medulla rapidly degenerated and were absorbed, and in the process of absorption the polyhedral mass formed from the outer cells of the cortex became changed to giant cells which disappear. During the third week a new mass of cells appeared resembling cortical cells. But the cell bodies were small; these masses fused to form layer masses. The small cortical like cells arranged themselves like those of the normal fasciculata. A change working from the centre took place, the small cells becoming fine reticular elements like cortical cells. The intramuscular were more successful transplantations than the subcutaneous, and old animals were unsuitable.

Hoskins (31) found that on feeding young animals with suprarenals the growth of the testes was stimulated.

Hewer (30) reports similar results but found that when the treatment was continued for some time, degeneration of the tubules occurred, the interstitial cells being also affected.

Histologists have been aware for a considerable time that the cortical cells of the suprarenal contain a fatty substance -'lipoids,'-but the true chemical nature of the lipoids is not yet known. Frankel (21) states that the different organs of the body contain different lipoids which are characteristic for each particular organ. The lipoids of various organs on the one hand and the lipoids of the same organ in different groups of animals vary both qualitatively and quantitatively.

In an investigation on the lipoid content of the suprarenal of the pig Biedl (10) found that the gland contained 25.39% of dry substance and that the dry substance consists of 61.25% protein and 38.88% lipoids and extractives./

extractives. As these percentages are for the whole gland, the cortex must contain a greater proportion of lipoids. Swale Vincent (56) has stated that on extraction of the adrenal body it does not yield any very special physiological principle, but that it contains numerous 'lipoid granules' - the so-called 'cortical granules' - and that it is not yet determined whether these lipoid granules are to be regarded as secretion products which are about to be poured into the blood stream.

The close connection between the gonads and the cortex suprarenalis or inter-renal tissue has been for long recognised. They are both developed from the mesothelium of the genital ridge. In structure the lipoid containing cells of the inter-renal tissue is indistinguishable from that of the interstitial cells of the testes and ovaries.

Hultgren and Anderson (33) were probably the first observers/

observers to suggest a functional connection. They found that castrated cats and rabbits resisted the effects of total extirpation of the suprarenals better than normal animals. Normal animals usually survived total extirpation at one operation for sixty-one hours while castrated animals lived for one hundred and twenty-one hours. When describing the symptoms that usually follow on total extirpation the authors state that there is a loss of weight even in unilateral operations, and that the loss of weight is less marked in castrated animals. These authors conclude that the adrenals have a varying functional significance in different classes of animals. Swale Vincent (56) in discussing the functions of the suprarenal cortex states that the evidence is now strongly in favour of the view that it has certain important functions in connection with the development and growth of the sex organs. He also says that there is a considerable amount of clinical evidence/

evidence that tumours of the adrenal cortex are frequently associated with sex abnormalities. The clinical evidence also favours the view that when cortical tumours occur in the female, an accentuation of male secondary sexual characteristics develop and simultaneously a hypoplastic condition of the internal generative organs supervenes. Additional evidence as to the connection between adrenal cortex and the sexual organs is furnished by the enlargement of the cortex during breeding and pregnancy.

Glynn (25) gives a very full account of the relationship of changes in the suprarenal to abnormal conditions of the gonads. Wooley (65) states that in some cases of precocious sexuality there is enlargement of the suprarenal, due presumably chiefly to the cortex.

Stelling (54) found enlargement of the adrenals in male rabbits during the breeding season. He also observed seasonal variations in the adrenals of frogs during the summer/

summer: the peripheral part of the cortex contained peculiar elements - summer cells - which atrophied when the sex glands began to enlarge. Aichel (3) noted that the adrenals were very large in animals with well developed sex organs or reproductive instincts. He also found that there was an increase in the size of the adrenals of birds and some amphibia during the breeding season. Guieysse (28) found considerable enlargement of the adrenals affecting especially the zona fasciculata in pregnant guinea pigs.

Gottschau (27) working on pregnant rabbits stated that the outer part of the cortex increased in thickness at the expense of the medulla and inner zone.

Schenke (53) found enlargement of the cortex after castration especially in the zona fasciculata and Cecca (17) stated that he found that the cortex and medulla enlarged after castration.

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Present Investigation.

A. Mole (63).

It therefore seemed desirable to ascertain if changes in inter-renal tissue go on concurrently with those in the testes. The mole was selected because of the well-marked oestrous cycle in this animal and because the previous work of Tandler and Gross (57) had dealt so fully with the changes in the testes and in their interstitial cells.

Methods.

The suprarenals and testes were taken from four to six newly killed animals each month. These were preserved in osmic acid or formalin for the investigation of the presence of lipoids, while others were preserved in picro-formalin for the study of the structure. For the presence of nerve endings all the known fixatives, various staining methods and modifications were made use of, but this part of the investigation proved negative.

Determination of lipoid content.

The suprarenals fixed in osmic acid were carried through what is known as the paraffin method, and were cut into sections by the rocking microtome. The sections were mounted in canada balsam after treatment to remove the paraffin wax.

The organs fixed in the formalin for staining with sudan III were removed from the fixative and left for twenty-four hours in a strong solution of dextrin, which was prepared by dissolving one part pure dextrin in two parts of water by boiling. They were cut with freezing microtome, washed in water and stained; only a short interval of time was allowed between the removal of the glands from the body and the mounting of the sections in Farrant's medium as it was found that sections which had been left in formalin for a few months failed to give any reaction with sudan.

The following table after Cramer and Gatenby (19) shows the histo-chemical reactions.

TABLE I.

	Sudan.	Double Refraction.	Osmic Acid.
True fats	+++	-	+++
Cholesterol	-	+	+
Cholesterolesters	+	+	+
Phosphatides	-	+	++
Cerebrosides	-	+	+
Phopho-cerebrosides	-	+	+
Cholesterol-fatty Acid Mixtures	+	+	++

It was necessary to imbed the testes in gelatin before cutting by the freezing method as it was found that as soon as the sections were put in water they broke up, the tubules separating from one another.

The sections fixed in picroformol were either stained with Haemalum and Eosin or by iron haematoxylin.

The maximum breadth and length of four sections taken from the middle of the suprarenal was measured by means of the eyepiece micrometer, the figures given in table (/) represent the mean of four sections. It will be seen that the/

the increase in breadth was relatively much greater than increase in length.

Cortex of Suprarenal.

	Length. mm.	Breadth. mm.		Length. mm.	Breadth. mm.
Jan.	2.75	0.30	July.	3.0	0.33
Feb.	3.0	0.38	Aug.	3.0	0.35
Mar.	3.5	0.41	Sept.	3.0	0.36
April	3.25	0.41	Oct.	2.75	0.36
May	3.2	0.38	Nov.	3.0	0.33
June	3.0	0.30			

On examining the series of sections stained with sudan it was found that those of the month of April showed the largest amount of lipoids. The sections of this month were then taken as a standard and the sections of the suprarenals from animals killed during each of the other months compared with them. The chart (3) indicates the changes in the lipoid content of the suprarenals throughout the year. The values are purely arbitrary. It is seen that during the month of April the lipoid content of the suprarenal is at its maximum. This/

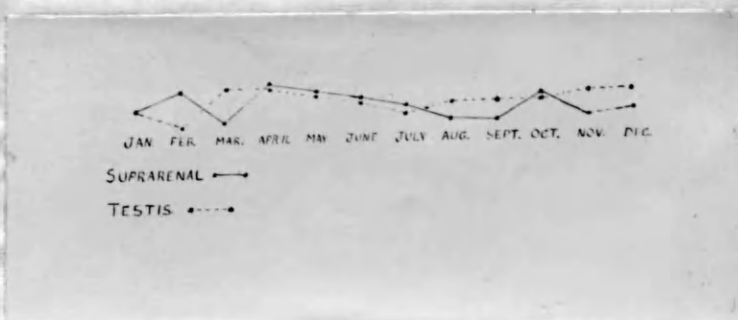

Fig. 3. Chart showing the changes in the lipoid content of the suprarenals, and the variations in size of testes throughout the year in the mole.

This is followed by a gradual decline until October, when there is observed a slight rise, again followed by a diminution until January. Thereafter the lipoid content is slightly increased until March when there is a marked decrease succeeded by the increase to the April maximum.

In order to correlate this lipoid cycle with the changes that take place in the testes during the oestrous cycle, serial sections of the testes were prepared from each of the above animals and stained with iron haematoxylin. On examination of these it was found that free spermatozoa were present in the tubules in the month of March; this agrees with Tandler and Gross' (57) observations. From April onwards the cells lining the tubules began to show signs of increasing degeneration accompanied by gradual disappearance of spermatozoa till October. During that month the tubules showed signs of a slight regeneration and this coincided with the increase in the amount of lipoids in the suprarenals. It seems

probable/

probable that the marked diminution of the lipoids in the month of March is due to a great demand made by the rapidly developing and active testes during the height of the rutting season. Millais (41) states that moles may have a second litter late in the year, which seems to indicate an original di-oestrous habit, suggested in our series by the slight regeneration of the tubules in October. Fig. (3).

It would seem as if a preparatory storage of lipoids in the suprarenals took place which was not called upon by the more or less suppressed activity of the testes in the second oestrous.

Sir Frederick Mott (42) states that one of the functions of the adrenal cortex is that of storing lipoids which pass into the blood and so keep constant the supply to the reproductive organs. These observations suggest that the interrenal cells play an ancillary part to the interstitial cells of the testes in the storage of lipoids which seem to be required in the production of spermatozoa.

Present Investigation (Contd.)

B. Rat. Structure.

Having arrived at such conclusions in an animal with a well-marked oestrous cycle, it was thought desirable to carry out a similar investigation on an animal which did not have a well-marked oestrous cycle.

The rat (Mus norvegicus albinus) was chosen because it develops rapidly to sexual maturity, which is reached at the age of three months. Moreover this animal does not seem to be of a seasonal type, but breeds at any season and continues sexually active for most of its life if the environment is favourable.

Observations on any structural changes and on the lipoid content of the suprarenals and testes were carried out. At least six animals were examined at the following ages, two weeks' embryo, birth, 2 weeks, 4 weeks, 12 weeks, 18 weeks, 26 weeks, 39 weeks and sterile old age. The method of examination was the same as that employed in the investigation on/

on the mole.

In the suprarenals of the embryo (Figs. 4 and 5.) the cells of the glomerulosa are small and faintly protoplasmic and packed closely together. In the fasciculata and reticularis the cells have large rounded nuclei and the protoplasm is plentiful and vacuolated. At birth the glomerulosa is little changed in appearance from the embryonic type; the cells forming the fasciculata are closer together giving a suggestion of the column formation; few of the cells show the vacuoles in the protoplasm. In the reticularis the cells are much more vacuolated than those of the fasciculata and between the cords of cells are large wide sinuses. At two weeks of age the cortex of the suprarenals has the normal appearance of an adult cortex; the cells in the deeper layer of the glomerulosa contain distinct round nuclei and are smaller than the other cells of this zone. By the fourth week the majority of the cortical cells

Fig.4. Suprarenal from two weeks embryo rat x 60.

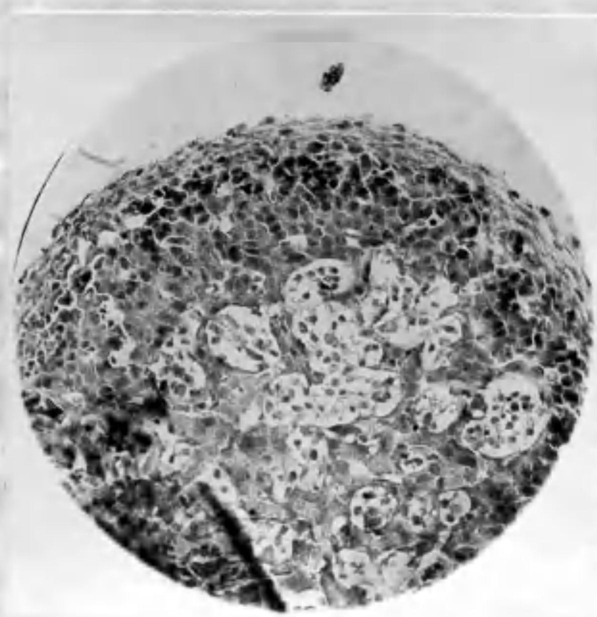

Fig.5. Suprarenal from same rat as fig.4 x 350.

are vacuolated, those of the glomerulosa entirely so.

By the twelfth week the cells which formed the ill-defined layer (Figs. 6 and 7) observed in animals of two weeks of age, between the glomerulosa and fasciculata are now quite distinctly shown as a definite layer; the cells are smaller and more rounded than even those of the fasciculata and the protoplasm is granular while that of the adjacent cells is vacuolated. As this layer is so distinct and as far as I can find has not been previously described, except by Jackson (34) who only refers to it as a lipoid free zone. Suprarenals from the dog and rabbit were examined and the layer can be shown there; it has in all likelihood been looked upon as the deeper layer of the glomerulosa. It cannot be a developmental layer or it should be most prominent during the embryonic stage; at that period it is not visible. It will be further referred to when discussing the lipoid content of the suprarenal cortex.

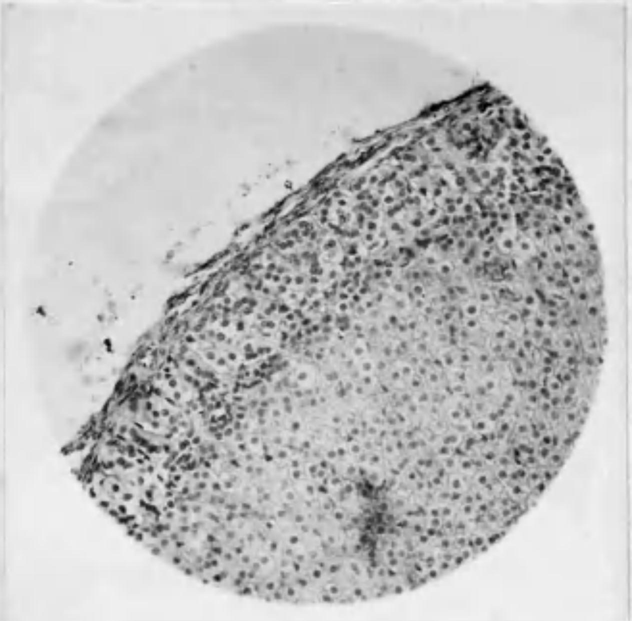

Fig.6. To show the granular cell layer between the glomerulosa and fasciculata in rat of 4 weeks. x 60.

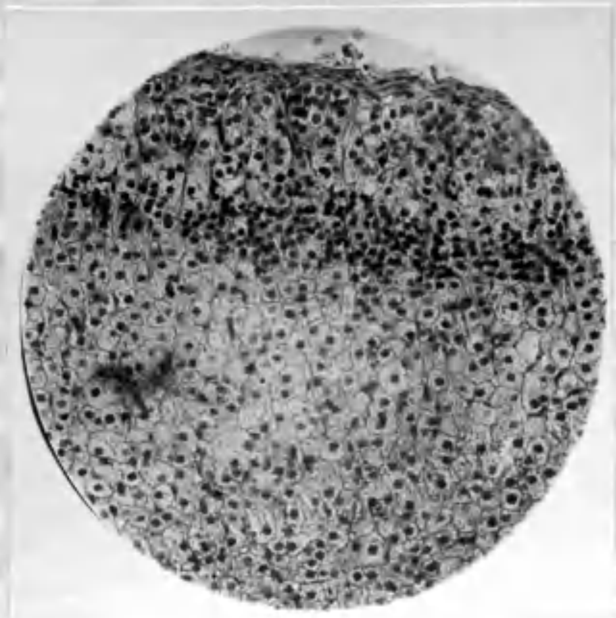

Fig.7. The same layer in suprarenal of rat of 18 weeks. x 60.

Lipoids.

An examination of the sections prepared to demonstrate the presence of lipoids, show that the lipoids are evenly distributed over the cortex in the embryo gland but by two weeks of age the lipoids of the glomerulosa are separated from the fasciculata by a layer of cells (Fig. 8) which corresponds to the zone of small cells referred to in the examination of the structure of the cortex. These cells are mostly lipoid free. In view of the fact that this layer is visible from the second week to sterile old age, Jackson's designation of it as a 'transition band' is not a happy one. By the fourth week the lipoids are as abundant in the glomerulosa as in the outer part of the fasciculata. Lipoids are also present in the inner part of the fasciculata and reticularis. The band between the glomerulosa and fasciculata is now practically clear of lipoids. During the twelfth week the glomerulosa shows a richer staining effect with sudan than other/

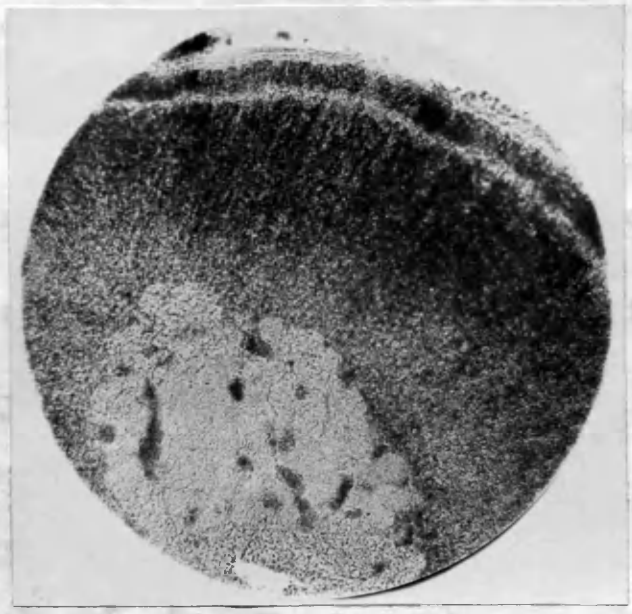

Fig.8. Section of Suprarenal stained with Sudan III to show the lipoid free layer x 60.

other parts of the cortex and this is the picture given throughout the rest of life. It may be noted that the rat is exceptional in this as it is usually found that the lipoids in the suprarenals are in most animals more numerous in the fasciculata than in other layers. There is no suggestion of a definite variation of lipoid in individual sections. Measurements of the organs were not carried out and hence an actual decrease or increase in lipoid content can not be wholly excluded.

Literature on Testes.

Having studied the lipoid content in the suprarenal attention has to be turned to the testes as the general belief is that if the lipoids have any influence on the gonads it is through the interstitial cells that they act.

Much work has been carried out on the testes to try to establish this view. Kudo (35) found that in guinea pigs, sheep and pigs, the interstitial cells resembled connective tissue cells and that they did not appear before sexual differences occurred.

In the guinea pig they are at first formed in very large amounts but later they are apparently fewer in number owing to the development of the seminal canals. They become fewest at the end of embryonic life and just before birth. In the early stages the cytoplasm is finely granular and in the later stages coarsely granular, fat granules and droplets are present from the earliest stages obtained and in adult animals there are many fat droplets in the cells. Rasmussen (40) and Mazette

(39)/

(39) also believe that the interstitial cells may be of connective tissue origin. In the horse Boinin and Ancel (12) state that in the foetus the gland is abundant, the tubules being well separated by the gland. Those cells which contain fatty substances are called zanthochrome interstitial cells. At birth intercanalicular spaces appear along with lymph nodules; the seminal tubules preserve their embryonic structure. At puberty, the spermatogenic stage, the young interstitial gland disappears and what the authors call the true gland forms. The tubules become arranged into zones, spermatogenesis commences in the centre. The interstitial cells disappear slowly during the establishment of spermatogenesis. The zanthochrome cells disappear first in the centre region of the testicle, persisting longer at the periphery. In the adult animals the interstitial gland forms large and vascular columns, the cells containing pigment granules. In the old animals spermatogenesis is slowed down, and a certain number of tubules are/

are in process of total atrophy and disappearance. The interstitial gland has diminished in volume due to atrophy of the interstitial cells.

Berberich and Jaffe (6) who have examined histologically one hundred human testicles discuss the reciprocal relationship between seminal canals and interstitial cells. They found Leydig cells present in the testicle at puberty, in some cases sparingly, but in others markedly increased. With regard to the fat in the Sertoli cells (the edge zone) which may also be laid down in and between the spermatogenic cells and may extend even into the lumen of the canal is regarded as physiological, and they have determined a definite reciprocal relationship between this fat and the fat content of the interstitial cells. Testicles from men of seventeen to fifty-six years of age are very resistant to acute illness, only in two of twenty-one cases was spermatogenesis affected, one with and one without affection of the interstitial cells. In ten other cases, almost intact testes in good condition,

very/

very marked interstitial cells rich in lipoid material, were found. In chronic illness of men (50 cases) there frequently appeared a diminution of sperm formation, including cases with atrophy of the canals, with a simultaneous marked appearance of Leydig cells; they also report cases with large groups of interstitial cells without any atrophy of the canals.

In old men it was found that in all cases of chronic and acute illness there was a destruction of spermatogenesis. In undescended testicles, spermatogenesis was not present and some of the canals were completely empty while others had two or three layers of epithelium.

The author considered that the marked increase of interstitial cells was a compensatory hypertrophy as happens in fibrosis of the testes and in alcohol degeneration.

Berberich and Jaffe could not agree with Berblinger(7) in considering that the interstitial cells exercised a trophic function towards the semen forming cells, since interstitial/

interstitial cells were found in embryonic life and in childhood when there was no spermatogenesis. They believed that there was an internal secretion of the interstitial cells, which was independent of the secretion of the seminal canals and stood in close relationship to other endocrine organs.

Sir Frederick Mott (42) in an investigation of the normal and morbid conditions of the testes from birth to old age, found that with the dawn and development of sexual desire there occurred two histological changes, - reappearance of interstitial cells in an active state and the accumulation in and around them of granules staining with Sudan 111, and this might be regarded as an indication of the presence of a phosphorised lipoid which was to serve as the raw material from which could be built up the nuclein necessary for active formative cell processes connected with spermatogenesis.

Frankenberger (22) when working on the lizard (viviparea) found/

found that the testicle in the month of July contained a germinal epithelium in the canals which was more or less in a resting stage or in the beginning of spermatogenesis, the canals being close together. In the interstitial spaces between many of the canals richly developed interstitial cells were found possessing granules blackening with osmic acid; those granules were to be found with considerable frequency in the canals. The author considered that a direct passage of the drops of fat took place into the interior of the canals.

In describing the male sexual characters in a case of ennuchoidism in a rabbit Lipschutz (37) stated that the interstitial cells in the mammalia formed an integral part of the internal secretory apparatus in the testicle and that the spermatogenic tissue could not alone be responsible for the secretory function.

Hausemann (32) in the marmot, Rasmussen (50) in the woodchuck/

woodchuck (ground hog), Friedman (23) and Mazette (39) in the frog, and Marshall (38) in the hedgehog found an increase in the number of the interstitial cells during active spermatogenesis. On the other hand Regaud (51), Tandler and Gross (57) and le Caillon (15) in the mole, and Champy (18) in the frog, found little or no increase of the interstitial cells during the period of active spermatogenesis but an increase as spermatogenesis declined (Figs. 9 and 10). Reeves (52) described the interstitial cells in the chick as being more numerous at three to five and a half months than later, and Boring and Pearl (13) found the interstitial cells only in the newly hatched chick.

With a view to elucidating the function of the interstitial cells a number of observations have been made upon operated animals. Myers (43) working on white rats, in which he had ligatured the vas deferens, found that the seminal tubules degenerated but that the interstitial cells were unaffected. Wagner/

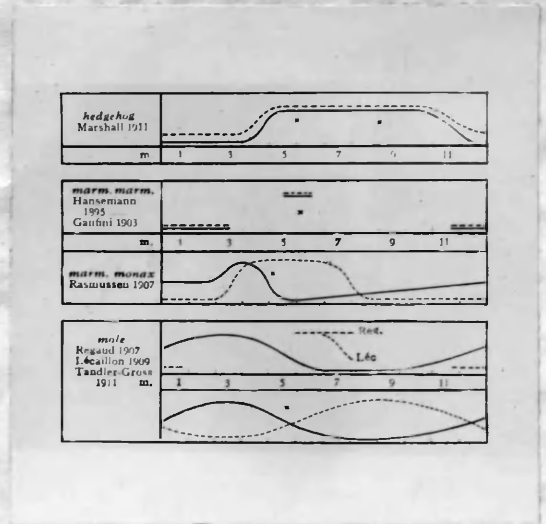

Fig.9. Cyclic development of generative and interstitial tissue of testicles of hibernating mannals. Plain line indicates generative tissue, dotted line interstitial tissue.

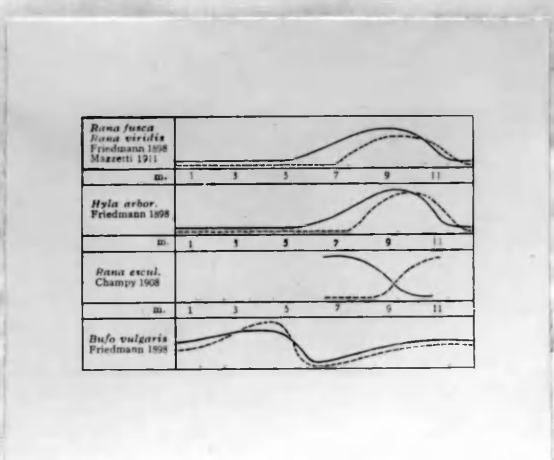

Fig.10. Cyclic development of generative and interstitial tissue of the testicle of amphibians. Plain line indicates generative tissue, dotted line interstitial tissue.

Wagner (60) reporting on the effects of unilateral castration in rabbits at the age of one and a half months, an age when the genatalia are still quite youthful in type, (puberty occurs at about four months) stated that three months after the operations the animals began to show differences. In one animal the penis had the post-pubertal form and in the the other animal as well as in the control it was still infantile. Two months later the penis of the normal animal was post-pubertal in form. On the remaining testicles being examined it was found that all three animals had approximately the same amount of testicular substance; spermatogenesis was everywhere in full go. The three animals had approximately the same number of Leydig cells. The testicles however showed differences in the mass of Leydig cells as a whole, this being dependent on the size of the individual cells, which was in turn determined by the size of the granules. The author considered that the differently sized Leydig cells and granules formed a developmental chain; he came to the

following/

following conclusion:- Neither the semen forming cells nor the sertoli cells alone are sufficient to account for the endocrinal function of the testicle. Since the Leydig cells possess the character of gland cells, it can only be they which are endocrinal. They are necessary for the post pubertal formation of the external genitals in the male rabbit, spermatogenesis can therefore proceed normally even if the Leydig cells are not completely developed. Lipschutz, Ottow, and Wagner (36) removed part of the testicle and sectioned the ductus epididymis in rabbits and found that in one case the testicle had an infantile appearance and in another contained brown stratified bodies similar to those described in the glandular spaces of the prostate and in hermophrodites. Somatically the operated animals presented the picture of totally castrated animals, evidence that neither the sertoli cells nor the young semen forming cells have the power to produce alone the masculinising hormone/

hormone. In discussing the results of partial castration Lipschutz (37) pointed out that in the small part of the testicle that remained a marked hypertrophy of the interstitial cells took place. Both the size and number of these seemed to be markedly increased. That this hypertrophy was not of a compensatory nature was shown by the following observations. It was found in all cases of partial castration, and small fragments of testicle with interstitial cells (of which the relative number was hardly increased and of which the absolute number was strongly diminished) were sufficient for a normal formation of the male sexual characters. Then if the blood vessel supply in the testicular fragments of the upper and lower poles were compared it was found that that fragment which had the best blood supply showed a stronger tendency to hypertrophy of the interstitial tissue: moreover the enormous hypertrophy of the interstitial cells of an upper pole fragment might occur even if the other testicle/

testicle was left uninjured in the body. Markèd interstitial cell hypertrophy could also result if both testicles were changed into "upper fragments" by cutting away a small part from the lower pole. From these observations Lipschutz concluded that the hypertrophy that occurred under various experimental conditions was not compensatory and had nothing to do with the internal secretion of the testicles in their relation to the organism as a whole. The hypertrophy was much more brought about by local conditions.

In view of the conflicting evidence advanced by various workers as to the presence and function of the interstitial cells of the testes I carried out an investigation on the avian testes to study the seasonal changes (62).

The work was at first carried out on sparrows but had to be abandoned as these birds are difficult to trap during the summer months. It was then decided that the green finch (Ligurinus chloris) would be the best bird to make use of as it only nested once in the year and was easily procurable.

Twelve freshly captured birds were got each month as it was considered that the results would be more reliable from them than from birds kept in captivity.

It was found that the testes varied in size with the time of the year. In early summer (May and June) as big as a whole pea and in early winter (November) no larger than a pin head. Serial sections were cut from the testes each month. All the sections were stained by Weigert's iron-haematoxylin method. The diameter of sections from the centre of each organ was measured by means of an eye-piece micrometer. The results obtained are given in Fig. 11. It will be noted that between January and June the rate of increase in size was much accelerated and between June and October a regressive change took place.

The changes which took place in the testes through the year are summarised in the table at the end of this section.

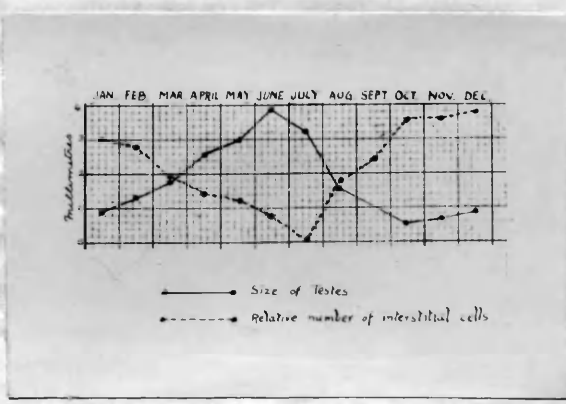

Fig.11. Showing variations in number of interstitial cells and change in size of testicles in the greenfinch throughout the year.

<u>October</u>. As this table shows the testes in October are in an embryonic state. The tunica albuginea is chiefly composed of cells with elongated nuclei, true fibrous tissue not being visible. The tubules showed no fibrous tissue in their coat except a thin band of fibres that act as the basement membrane of the intra-tubular cells. These are columnar in shape and very compact, the free borders touching one another so that no lumen is visible. There was no sign of cell division. In the interstitial tissue two types of cell were seen

1. Fibrous tissue cells.
2. Cells chiefly arranged round the tubules having a large rounded nucleus with numerous granules staining deeply and a cytoplasm which can be made out with difficulty (Fig. 12a).

<u>November</u>. In November there did not seem to be any change in the state of the tunica albuginea or interstitial tissue but some of the tubules now showed two rows of nuclei. In other tubules there was only one row, the/

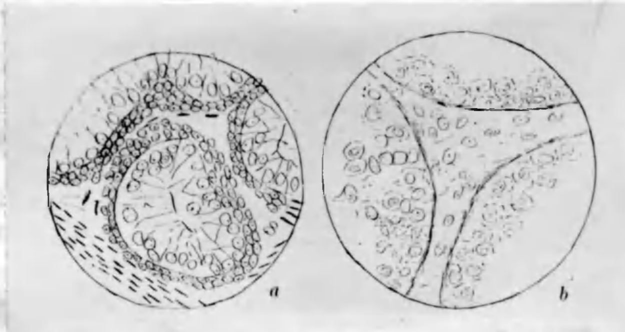

Fig.12a. October. Shows arrangement of interstitial cells around the tubules and the columnar form of the intra-tubular cells.
b. March. Shows the two types of interstitial cells. Camera lucida drawing, lens 4 m.m.

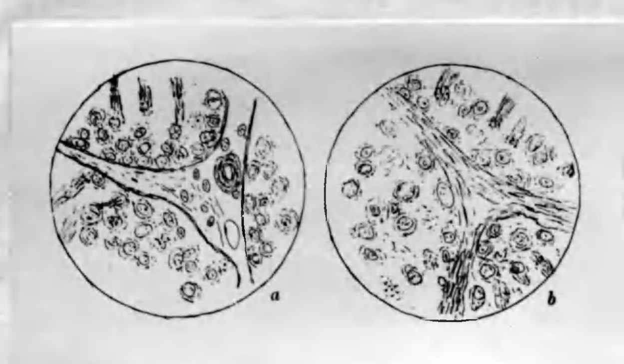

Fig.13a. May. Shows well formed blood vessels, few interstitial cells and karyokinetic figures in the intra-tubular cells.
b. July. Shows absence of interstitial cells and degenerative changes in the tubules. Camera lucida drawing, lens 4 m.m.

the nuclei being close together and oval in shape. In some tubules where the lumen was beginning to show, a faint cell outline could be seen.

<u>December</u>. In December the tunica albuginea became more fibrous but still showed a large number of cells which had round nuclei, the whole having the appearance of young fibrous tissue. The intra-tubular structure showed no marked change from the preceding month, but there was an increase in the number of interstitial cells (type 2). They now formed a thick coat of several layers around the tubules and also occupied the spaces between the tubules which were wide apart.

<u>January</u>. In January the tunica albuginea showed a slight advance towards true fibrous tissue, the outer half of the coat showed elongated nuclei and the inner half still retained the rounded form. In the tubules there/

there were now two irregular rows of nuclei for which no distinct characteristic cell body could be made out. The nuclei varied in size, some were very large. The whole picture was one of active cell division. The interstitial tissue showed some fibrous tissue, the interstitial cells with the granular nuclei were not so numerous and there were only two rows around the tubules. The cells varied in size but all seemed to belong to type 2.

<u>February</u>. By February the tunica albuginea had lost its cellular appearance and was now fibrous. The tubules had also got a distinct fibrous covering. The nuclei of the cells which surrounded the tubules had become elongated. The changes in the tunica albuginea and the coats of the tubules were simultaneous. The intra-tubular cells were now quite distinct from one another; most cells had/

had a very large nucleus with a prominent nucleolus. The interstitial tissue had become very vascular, but there was not much difference in the number of cells from that found in January.

March. In March the tubules were wider apart than in any previous month, and in the wide interstitial spaces thus formed two types of epithelioid cell were found.

1. The small cells of type 2 which were always present except in July, but they were not now so numerous as in previous months.
2. A cell with a large nucleus which showed no chromatin network, appeared for the first time.

The cells of the tubules showed very active division; some of them showed karyokinetic figures (Fig.12b).

April. In April there were very few interstitial cells. There were now three or four layers of intra-tubular cells arranged in an orderly way, and it was during this month that cells appeared that resembled Sertoli's cells. In the lumen of the tubules there were masses of spermatogonia and cells of various sizes.

May. In May there were still fewer interstitial cells to be seen and only those with a rounded granular nucleus were present, but well formed arteries and veins were now present in the interstitial tissue. The tubules were well developed and free spermatozoa were present (Fig.13a).

June. In June the interstitial cells were few and belong to type 2, and were found massed together in the angles formed by the tubules. The spermatozoa were now found to be arranged in bundles with their heads towards Sertoli's cells.

July. In July no interstitial cells were visible. The tubules were separated from one another only by a thin fibrous septum. The cells in the tubules had an indistinct outline and the nuclei had enlarged. Spermatozoa were still present in some tubules (Fig. 13b).

From/

From this month the retrogressive change was very rapid. The tunica albuginea which had been of a dense fibrous nature since February now showed a large number of nuclei of two types, the elongated form and the round one as in January. The fibrous coat of the tubules showed a similar change. The tubules decreased in size and interstitial spaces increased and by the end of August interstitial cells were again present in large amount. In the tubules the only cells present were columnar cells which were vacuolated and granular, the granules being confined to the upper two-thirds of the cells. In the lumen of the tubules there were some large round cells, but no sperms.

The source of these interstitial cells has still to be determined. In appearance they were markedly epithelioid, but the way in which they appear and disappear suggests that they must be modified fibrous tissue cells. On this subject further developmental work is required. These observations indicate/

indicate that in the bird the epithelioid interstitial cells of the testes showed their most marked development in the non-oestrous period and that as the spermatogenesis advanced they decreased in number. They accord then with the results of Tandler and Grosz in the mole.

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CHANGES IN AVIAN TESTES.

Parts of Testes.	January.	February.	March.	April.	May.	June.	July.
Tunica Albuginea.	Advance towards true fibrous tissue.	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous
Intra-tubular changes.	2 irregular rows of nuclei.	Cells quite distinct.	Active division taking place.	3 or 4 layers cells. sertoli cells.	Fully developed free spermatozoa.	Fully developed free spermatozoa.	Indistinct outline of cells. Nuclei enlarged.
Interstitial tissue.	Some fibrous tissue. Interstitial cells not numerous.	Some fibrous tissue. Not much change in number of interstitial cells.	Two types of interstitial cells.	A few interstitial cells.	Fewer interstitial cells.	Interstitial cells less in number (Type 2)	No interstitial cells seen.

Table (Contd.)

Parts of Testes.	August.	September.	October.	November.	December.
Tunica Albuginea.	Becoming cellular.	More cellular.	Cellular.	Cellular.	Fibrous with large number of cells.
Intra-tubular changes.	Retrogressive layer of columnar cells.	Layer of columnar cells.	Layer of columnar cells.	Some tubules show two rows of cells.	Some tubules show two rows of cells.
Inter-stitial tissue.	Large number of interstitial cells.	Large number of interstitial cells.	Interstitial cells very abundant around tubules.	No change from October.	Increase in interstitial cells.

Present Investigation.

The Rat Testes.

A. Structure.

On examining the testes of the rats it is found that in the two weeks' embryo (Fig.14) the seminal tubules are more distinct at the periphery and that they contain large rounded cells and between the tubules there is a large amount of fibrous tissue and nests or columns of interstitial cells which cannot be confused with the cells of the fibrous tissue which are present. At birth (Figs. 15 and 16) the tubules are more prominent and the spermatic cells are arranging themselves around the tube against the fibrous coat; the spermatogonia are quite distinct; the cells in the centre of the tubules have not got the well-marked nuclei, they form an amorphous mass. There is still a large amount of fibrous tissue and interstitial cells between the tubules which are separated from one another. By the fourth week (Figs.17 and 18) the tubules are well formed and packed closely together and although no spermatozoa are present/

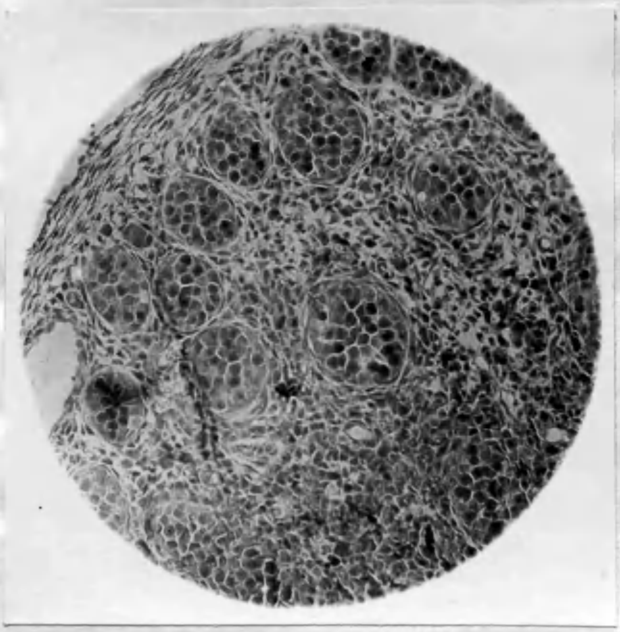

Fig.14. Section of testicle of 2 weeks' embryo rat to show arrangement of tubules at periphery and large interstitial spaces towards centre x 60.

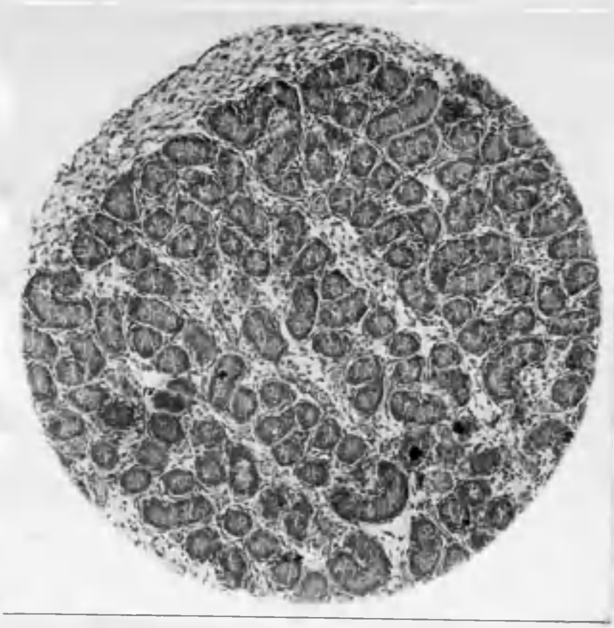

Fig.15. Section of testicle of newly born rat to show arrangement of tubules, x 60.

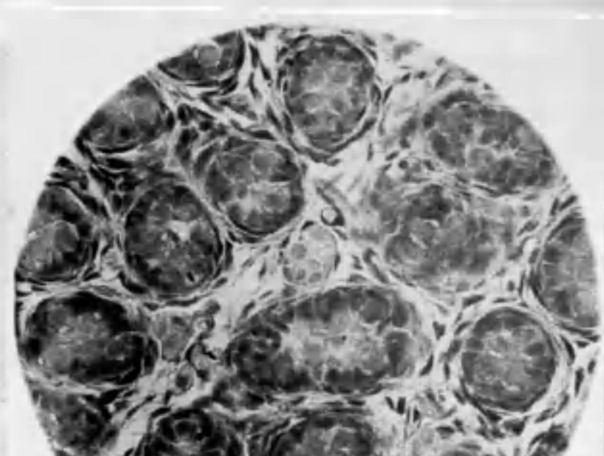

Fig.16. From same testicle as fig.15. Many interstitial cells are shown, x 350.

Fig.17. Section of testicle of 4 weeks' old rat showing tubules well formed and packed closely together, x 60.

Fig.18. Section of testicle of 4 weeks' old rat where tubules are artificially separated to show presence of interstitial cells, x 350.

present there is active spermatogenesis; the interstitial cells are now fewer in number and lie in some of the angles formed between the tubules; in some of the tubules there are two rows of regularly packed small cells with circular nuclei and in the lumen there are large angular cells with very large centrally placed nuclei. On examining sections from animals of twelve weeks of age (Fig.19) the tubules give the picture accepted as that of full spermatogenesis; the spermatozoa are present: the interstitial cells are still seen in some of the angles formed by the tubules. There is no change to be noted from now until sterile old age (Fig.20) when the tubules again show a separation but no increase in the number of interstitial cells. These wide spaces, Wagner (61) has suggested, are lymph spaces. They appear in the sections of this series at the fourth week and in sections from rats aged twelve weeks (Fig.21) contain a fluid and certainly have the appearance as noted by Wagner. But on examining the spaces in the testicles of sterile old rats they rather suggest artifacts/

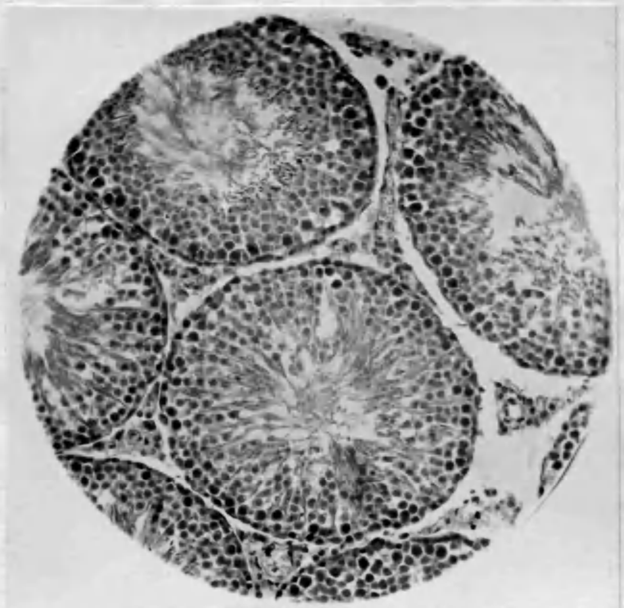

Fig.19. Section of testicle from rat of 12 weeks of age, showing fully developed tubules and presence of interstitial cells, x 350.

Fig.20. Section of testicle of old rat to show interstitial spaces and degeneration of tubules, x 60.

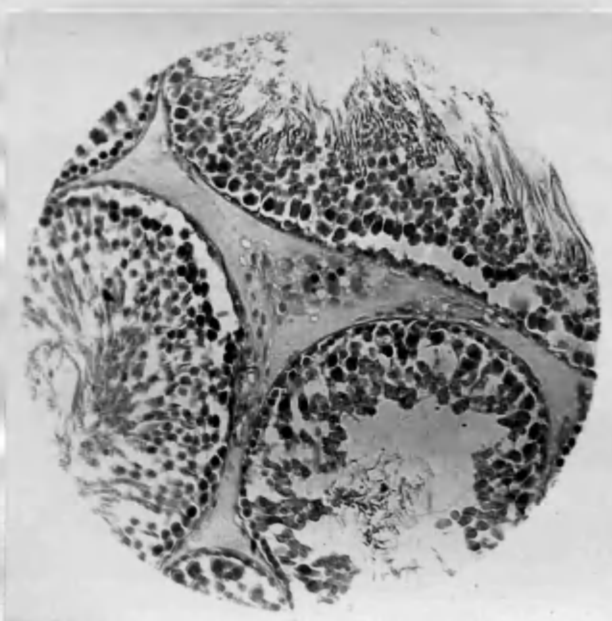

Fig.21. Shows the inter-tubular spaces filled with fluid, the lymph spaces of Wagner, x 350.

artifacts, as these spaces are not lined by any form of epithelial cells as we should expect. The separating of the tubules and the appearance of the large intervening spaces in the sterile rats may be due to the shrinkage of the tubules, the fibrous coat is puckered (Fig.20) and the cells in most of the tubules show signs of degeneration; the cells, and especially the nuclei, do not stain well. There are some spermatozoa present, but the intra-tubular cells are smaller than those of young animals. Some of the tubules only show small lining cells and a mass of amorphous matter. The animals (2) which were looked upon as sterile had grown very large and the front teeth had grown so long that they seemed to have lost their usefulness; the hair was rough. They had, according to the dealer, been kept for a long time with females but showed no interest in them; this conclusion was supported by observations in this laboratory.

B. Lipoids in testes.

In the embryo testes the interstitial cells which were mainly/

mainly to be found in the centre of the organ contained lipoids (Figs. 22 and 23). The reason for the interstitial cells being more numerous in the centre is probably due to the tubules lying closer together at the periphery. When the interstitial cells are seen near the margin they take the form of cords, whereas in the centre they are massed together having the appearance of a gland. Large blood vessels are present in the fibrous area at the centre of the organ. At two weeks of age the interstitial cells which are found in the angles formed by the tubules contain lipoids, but from this stage to old age no lipoids are found in the comparatively few interstitial cells seen. During the twelfth week lipoids are very prominent in some of the tubules; they are arranged inside the fibrous coat and between the cells, but not in the lumen as stated by Berberich and Jaffe (6) and others. In the early stage of spermatogenesis there is a large amount of lipoid among the spermatogonia, and as spermatogenesis advances the amount/

Fig.22. Section of testicle of 2 weeks embryo rat to show arrangement of interstitial cells containing lipoids, x 60.

Fig.23. The same as fig.22, coloured to show distribution of the lipoids, x 60.

amount becomes appreciably less until when free sperm are present in the tubules there are little or no lipoid present. This is well shown in Figs.24 and 25. There is a difference in the affinity for the Sudan stain between the lipoids in the interstitial cells and those in the tubules. While the lipoids in the interstitial cells stain a faint orange colour and are diffuse those in the tubules are in the form of globules and are deeply stained and refractile.

Fig.24. Section of testicle of rat of 12 weeks to show lipoids in tubules, x 350.

Fig.25. The same as fig.24 coloured to show distribution of the lipoids, x 350.

SUMMARY.

The result of a study of the changes occurring in the testes and suprarenals of the mole during complete oestrous cycle are consistent with the theory that the adrenals are necessary to development and proper functioning of the gonads.

Definite changes were found in the adrenal cortex during the oestrous cycle. The period of active spermatogenesis (March and April) coincided with maximum size (breadth) of cortex, and a slight regeneration of the tubules in the testes in October coincided with a much smaller increase in the breadth of the cortex during the same month.

The lipoid content of the cortex ran a parallel course with the breadth of the cortex with the exception of a well marked fall in March during the height of spermatogenesis.

In the rat on the other hand, which is sexually active throughout the year, as far as could be judged from an examination of sections, the lipoid content of the suprarenals was more/

more or less constant throughout the year.

A study of the development of the suprarenal of the rat from foetal life to old age showed that lipoids were evenly distributed throughout the cortex in the embryo, but after the age of two weeks and from then onwards a lipoid free zone was present between the fasciculata and glomerulosa.

In a study of the avian testes it was found that the interstitial cells were most abundant in the non-oestrous period and decreased as spermatogenesis advanced. This result corresponded with the findings of Tandler and Grosz in the mole.

A detailed study of the histological development of the rat testes from embryonic life to old age showed that interstitial cells were more abundant in early life and less abundant in sexual maturity and old age.

Lipoids were present in the interstitial cells of the embryo and up to two weeks of life, after which age they

were/

were absent. In this respect the rat differs from the human testes, where the onset of puberty according to Mott is accompanied by an increase in interstitial cells and the presence of lipoid in and around them.

From the twelfth week lipoids appeared between the cells lining the tubules of the testes, these lipoids were most abundant in the tubules where spermatogenesis was at an early stage. They were not identical with the lipoids of the interstitial cells.

The lipoids of the suprarenals and testes are identical; those of the interstitial cells have been regarded as material (1) suitable for building up the spermatogenic tissue or (2) for the preparation of an internal secretion to be poured into the blood stream.

If the lipoids are utilised in the formation of spermatogenic tissue, the cortical cells of the adrenals may be regarded as playing an ancillary part to the interstitial cells of the testes.

The abundant store of lipoid in the suprarenals of the mole suffered a definite depletion in March at the height of sexual activity, whereas in the rat no such change was detected.

This investigation was carried out in the Institute of Physiology under the direction of Professor Nöel Paton to whom I am indebted for much kindly advice and encouragement.

My thanks are due to Mr J. R. Bell for the care he has taken in preparing the photographs, also to Mr Tindall for the two coloured photographs.

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REFERENCES.

1. Abelous and Langlois, Arch. de Physiol. Vol.24, p.269. 1892.

2. Addison, cited Endocrinology and Metabolism, Vol.2. 1922.

3. Aichel, Munch. Med. Wock. Vol.36. p. 1228, 1900.

4. Aldrich, Amer. Jour. of Physiol. Vol.5., p.457. 1901.

5. Alexais and Arnaud, Marseille Med. Vol.28., p.94. 1891.

6. Berberich and Jaffe, Frankfort, Zeitschr. f. Path. Vol.27, p.395, 1922.

7. Berblinger, Verhand d. Deutsch. Pathol. 1921.

8. Biedl, Innere Sekretion, Vol.1. p.374, 1913.

9. Biedl, Innere Sekretion, Vol.2., p.61, 1913.

10. Biedl, Innere Sekretion, Vol.1. p.376, 1913.

11. Lipschütz, Deutsch. Med. Wochensch., Vol.48, p.320. 1922.

12. Böinen and Ancel. Arch. de Zoologie, Exper. et General, Vol.3. p. 393, 1905.

13. Boring and Pearl, Anat. Record. Vol.13, p.250. 1917.

14. Brown Sequard cited Endocrinology and Metabolism, Vol.2. 1922.

15. le Caillon. Compt. rend. soc. Biol. Vol.66., p.599. 1909.

16. Canalis, cited Endocrinology and Metabolism, Vol.2. 1922.

17. Cecca, Presse Med. Vol. 43, p.341, 1904.

18. Champy, Compt. rend. Soc. Biol. Vol.64, p.893, 1908.

19. Cramer and Gatesby, Microtomists Vade-Mecum, 8th Ed., 1921.

20. Elliot and Armour, Jour. Path. and Bacter. Vol.15, p.481. 1911.

21. Frankel, Biochem. Zeitschr. Vol.21, p.321. 1909.

22. Frankenberger, Anat. Anz. Vol.55, p.545. 1922.

23. Friedman, Arch. f. Mikr. Anat. Vol.52, p.856, 1898.

24. Gaurnceri and Zucco, Arch. ital de biol. Vol.10, p.325, 1880.

25. Glynn, Quar. Jour. Med. Vol.5., p.157, 1911.

26. Gourfein, cited Internal secretion, Swale Vincent, 3rd ed.

27. Gottschau, Arch. f. Gynaekol. Vol.70, p.445, 1903.

28. Guieysse, Jour. de l Anat. et de la Physiol. Vol.37, p.312, 1901.

29. Harley, cited Endocrinology and Metabolism, Vol.2. 1922.

30. Hewer, Brit. Med. Jour. Jan.28. 1922.

31. Hoskins, Arch. inter Med. Chicago, Vol.17, p.584, 1916.

32. Hansemann, Arch. f. path. Anat. u. Physiol. Vol.142, p.538, 1895.

33. Hultgren and Anderson, Skand. Arch. f. Physiol. Vol.9.p.73, 1899.

34. Jackson, Amer. Jour. Anat. Vol.25,p.221. 1919.

35. Kudo, Folia Anat. Japonica, Vol.1. p.125, 1922.

36. Lipschütz, Ottow and Wagner, Arch. f. Entwick d'organ, Vol.51. p.66.1922.

37. Lipschutz, Proc. Roy.Soc.,Vol.93.p.132.1922.

38. Marshall, Jour. of Physiol. Vol.43. p.247, 1911.

39. Mazzette, Anat. Anz. Vol.38.p.361. 1911.

40. Mazzette, Anat. Anz. Vol.38, p.361, 1911.

41. Millais, Mammals of Great Britain and Ireland, Vol.3. 1906.

42. Mott, Brit. Med. Jour. Nov. 22. p.655. 1919.

43. Myers, Anat. Record. Vol.10. p.228, 1916.

44. Oliver and Schäfer, Jour. of Physiol. Vol.18, p.230, 1895.

45. Pellacani, cited Innere Sekretion, Biedl. Vol.2. 1913.

46. Pellacani and Foa cited Innere Sekretion, Biedl, Vol.2. 1913.

47. Pettit, cited Endocrinology and Metabolism, Vol.2. 1922.

48. Poll, Arch. f. Mikro. Anat., Vol.54., p.440, 1899.

49. Poll, Zentralbl. f. Physiol. Vol.12, 1 & 10. 1898.

50. Rasmussen, Amer. Jour. of Anat., Vol.22, p.457. 1917.

51. Regand, Arch. d. Anat. Mikr. Vol.4, p.101. 1901.

52. Reeves, Anat. Record, Vol.9. p.383. 1915.

53. Schenke, Bietr. klein. Chirur. Vol.67. p.316. 1910.

54. Stelling, Arch. f. Mick. Anat. Vol.52, p.176. 1898.

55. Swale Vincent, Jour. of Physiol. Vol.22. p.111.1898.

56. Swale Vincent, Surgery, Gynecology and Obstetrics, p.294. 1917.

57. Tandler and Grosz, Arch. f. Entwr. der Organ, Vol.33. p.297. 1911-12.

58. Takamine, Amer. Jour. Pharm. Vol. 73. p.221. 1901.

59. Tezzoni. Beitr. z. path. Anat. u.z.Alleg. Path. Vol.6.p.3. 1889.

60. Wagner, Arch. f. Entwick. d. Organ. Vol.51.p.416. 1922.

61. Wagner, Internal Secretion of sex glands. Lipschütz.p.114.1924.

62. Watson, Jour. of Physiol. Vol.53. p.90. 1919.

63. Watson, Jour. of Physiol. Vol.58. p.240. 1923.

64. Wheeler, Trans. Roy.Soc. Canada, Vol.II. p.125. 1917.

65. Wooley, Amer. J.Med. Sc., Vol.125. p.33. 1903.

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