Thesis.

for the degree of M. D.

The Innervation of the Ovary

A Histological Study

by George Howie. (New Zealand).
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with box of slides.

Until within recent years our knowledge of the distribution of nerves within the ovary remained very deficient, and it was only after the introduction and application of more complete and more reliable methods of nerve-staining that our knowledge of this and allied subjects received any material advancement. A review of the literature treating of the innervation of the ovary will show the progress that has been made since the introduction of Golgi's method, and of Ehrlich's intra-vitam method of staining by means of methyl blue.

The first important reference to the nerve supply of the ovary was made in Frankenhäuser's (1) work on the nerves of the uterus published in Jena in the year. 1867.

In this work he describes the macroscopic anatomy of the ovarian and uterine nerves and according to his dissections and observations their arrangement is

as follows:-

The semilunar (coeliac) ganglia both give branches to the ovary and uterus, and nerve fibres pass from the renal ganglia and plexus to the ovary with the ovarian vein.

The superior mesenteric plexus also gives branches to the ovary and uterus, and two ganglia found in connection with the inferior mesenteric artery are to be regarded as entering into the distribution of nerves to the female genital organs.

The nerve fibres from these abdominal ganglia and plexuses do not pass directly to the genital organs but collect into two pairs of "spermatic" ganglia, or "genital ganglia." The right pair lie on the right side of the aorta in the cleft between it and the inferior vena cava. They receive branches of communication from the second and third lumbar sympathetic ganglia. This pair consists of a large upper ganglion and a small lower one. The upper ganglion lies with its long axis transverse, and it is separated from the lower one by a tributary of the

inferior vena cava. The genital branches of the renal and mesenteric ganglia and plexuses enter its upper surface. From its outer side nerves pass to the ovary, partly with the ovarian vein and its branches from the renal plexus, and partly with the ovarian artery and with branches from the lower ganglion.

The lower right "spermatic ganglion" lies with its axis longitudinal. It receives nerve fibres from the upper ganglion, and from the renal and superior mesenteric plexuses.

From its outer side nerves pass to the ovarian vessels forming a plexus around these vessels with nerve fibres from the upper ganglion.

The two left "spermatic ganglia" are of equal size and are very closely joined looking like one ganglion. They send nerve fibres across to join the right pair.

The ovary, then, derives its nerves from (α) the renal plexus, a considerable branch or several small ones passing to it with the ovarian vein, and (β) from the upper and lower spermatic

ganglia the fibres from these passing to the ovarian vessels, and forming a plexus around them.

The plexus around the ovarian vessels contains many ganglia and near the ovary it resolves itself into three main bundles of fibres

(1) An external bundle which passes to the outer end of the ovary and is distributed also to the outer border of the broad ligament and to the outer end of the Fallopian tube.

(2) A middle bundle which divides at a short distance from the outer border of the ovary into two chief divisions, one supplying the outer, the other the inner half of the ovary the branches entering the organ with the blood vessels

(3) An internal bundle, which runs with a part of the uterine venous plexus to the tube and fundus uteri. A branch from this bundle passes towards a point somewhat above and in front of the attachment of the ovarian ligament and there enters a ganglion from which nerve fibres are distributed to the broad ligament and to the Fallopian tube.

Such is the course taken by the various nerve fibres which pass to the ovary, according to Frankenhäuser's description. He does not mention the branches from the sacral nerves to the uterus as having any traceable connection with the ovary, and therefore it would seem that this organ derives its nervous supply entirely through the sympathetic nervous system.

Waldeyer(2) in his work on the Ovary and Ovum published in the year 1870 and in Stricker's "Handbuch (1871) draws attention to the great deficiency of knowledge at that time with regard to the distribution of nerves within the ovary. By treating sections of ovary with gold chloride he was able to show a few fine nerve fibres passing outwards from the hilus of the ovary to the periphery and he also succeeded in staining some delicate scantily medullated nerve fibres passing between the larger follicles. He was unable to demonstrate the endings of these nerves and expressed the opinion that in the

ovary all the nerves were destined for the supply of blood vessels and the non-striped muscle fibres which are described as forming part of the ovarian stroma

Investigations with gold Chloride were made with more success by Eischer (3) in 1876. This observer used in his researches the ovaries of the rabbit, sheep, and Cow. He employed the "gold chlorid-natrium" stain (Cerlach - Boll) after hardening in a 2% solution of Ammonium Chromate

In all observed cases the nerves were seen to enter the ovary at the hilus, and from the ligament of the ovary, and to accompany the blood vessels as finely medullated branches

Besides the non-medullated fibres which form a plexus round each of the blood vessels in the ovary, Eischer saw fine medullated nerve fibres branching dichotomously and taking a course parallel to but independent of the blood vessels and finally terminating with their branches by entering into the formation of the plexuses around the larger follicles.

His preparations showed a closely meshed

network of moderately thick nerve fibres in the condensed fibrous tissue ("theca folliculi") around the largest Graafian follicles, and from this network of nerves, a long-meshed network of finer more delicate nerve fibres, passing to the peripheral layer of the tunica granulosa. He described still another closer network of fine nerve fibres arising from this latter and surrounding the follicle in close apposition to the tunica granulosa.

Elischer found great difficulty in getting preparations which showed branches from this last mentioned network passing in between the cells of the tunica granulosa, but he did see ~~fibres~~ nerve fibres which appeared to pass into the follicle and to end in the nuclei of the cells of this tunic.

In the latter end of a communication on the development of the Human Ovary (1884) Meyer (4) touches upon the subject of Ovarian nerves. His method of investigation was as follows:-

The ovary of a young girl was placed as fresh as possible in a solution of .05% of Chromic acid after having been

bi-sected longitudinally. It was left in this Solution from one to six days and was then examined in a mixture of pyrogallic acid (one part) and glycerine (100 parts). Teased specimens gave the most definite results.

A few nerve fibres running through the stroma from the hilus were demonstrated but only one preparation from the follic-ular zone showed 11 nerve fibres with endings. One was a pale single con-toured fibre which showed fusiform varicosities in its course and broke up into a number of linear fibrils which ended some free, others in relation to cell nuclei of the ovarian stroma

Meyer did not find nerve fibres in relation to the Graafian follicles.

Nedeler (5) a Norwegian observer in treating the ovaries of sheep with gold Chloride was unable to find any trace of "follicle-nerves". He succeeded in staining many nerve fibres but believed them to be all associated with blood-ves-sels or muscle fibres.

In a communication on the nerves of the human ovary in 1890 Nedeler repeats his statement that the nerves of the ovary,

accompany the vessels and that no trace of so-called "follicle-nerves" exists.

The introduction of Golgi's method, and of Ehrlich's method of methyl blue staining, furnished histologists with a much more reliable and efficacious means of staining true nerve fibres and nerve-endings than any other then known, and as a result of ~~these two~~ the use of these two special methods we find a very interesting and instructive paper on the nerves and nerve endings in the ovary published by Riese in the Anatomischer Anzeiger for 1891.

Riese(6) describes the same general arrangement of the nerves in the ovary as Elischer viz., the nerve trunks entering at the hilus, breaking up into a large number of nerves which pass in a radiating manner through the substance of the organ to all parts of the periphery most of them as non-medullated nerve-fibres accompanying the blood vessels, a smaller number of them sparingly medullated and passing through the stroma of the ovary independently of the blood vessels. These nerves pass

to the zone of the smallest follicles, where they break up into a plexus in the meshes of which the smallest follicles lie. In their course they bend around the larger follicles which are furnished by them with a rich plexus from which according to Riese fibres pass in from the tunica fibrosa into the tunica granulosa, ending in relation to the cells of the latter layer. Whether similar nerve fibres occur between the cells of the smaller follicles could not be made out from want of clearness, but though the zone of the smaller follicles contained such a rich nerve plexus, Riese did not think that any nerve fibres penetrated between the cells of these follicles. From this plexus nerve fibres run outwards into the tunica albuginea of the ovary and some appeared to Riese to run to the ~~deep~~ surface epithelium and to end in relation to its deep surface.

In his methyl-blue preparations Riese saw a plexus of non medullated nerve fibres, situated in the tunicae fibrosae of the larger follicles, the nerve

fibres being, like the connective tissue fibres arranged concentrically around the follicles. From these nerve fibres, small branches appeared to run at various angles into the tunica granulosa. These fibres showed varicosities in their course which Rieze thought were natural, and for the purpose of filling up the intercellular space in which the nerve ran. Some of these nerve fibres appeared to Rieze to end in relation to the cells of the tunica granulosa, terminating in little end-knobs in apposition to the cells but not entering them.

In the stroma of the zone of the smallest follicles, Rieze saw fusiform cells resembling ganglion cells with nerve fibres coming into contact with them and dividing into processes.

Rieze saw the above appearances in his methyl blue preparations. He met with considerable success in subjecting the ovaries of cats and sheep to Cajal's modification of Golgi's method.

These preparations showed the same general arrangement of the nerves and their branches as the methyl blue spe-

-tions, and in a few cases he saw what appeared to be nerve fibres passing in between the cells of the tunica granulosa of the larger follicles.

He saw such fibres in some preparations obtained by treating the human ovary with Cajal's modification of Golgi's method.

The sheep's ovary gave very good results with Cajal's method in Riese's hands, and demonstrated very well the arrangement of the nerve fibres around the blood vessels, and in the zone of the smallest follicles.

In addition to the appearances already mentioned, Riese saw in the sheep's ovary, what he regarded as special nerve end-organs, situated among the innermost cells of the tunica granulosa. He observed pear-shaped bodies which stained black as the nerve fibres did, and which by their external contour reminded him of an elongated Krause's corpuscle of the human conjunctiva or an end-bulb of the sheep's conjunctiva. These bodies were seen to be more or less near the centre of the follicle and each was connected with the nerve

plexus of the Tunica fibrosa of the follicle by a fine, beaded nerve fibre running between the cells of the Tunica granulosa, and giving off in its course delicate side branches.

These structures resembling end organs did not all stain similarly; some stained a uniform deep black others remained more clear in their centres.

The fibre going to one of these bodies was thicker than the ordinary non-medullated nerve fibre, and began in a rounded or pyramidal swelling alongside a capillary of the Tunica propria.

The capillary blood vessels around the follicles were seen by Riese to be distinctly invested with a nerve plexus of non-medullated fibres, whatever may be their function.

Riese's general Conclusions are:-

(1) That nerves are very abundant in the ovarian stroma, the plexus of nerve fibres being especially rich in the zone of the smallest follicles.

(2) That fine non-medullated nerve fibres invest the capillaries, and that most of the nerves of the ovary end

are vaso-motor. A distinction must be drawn between the nerves for the muscle-fibres of vessel walls, and the nerves for capillaries, the former certainly being motor the latter partially or wholly sensory (3) that nerve endings occur in the larger follicles, in the tunica fibrosa and in the tunica granulosa. Endings are seen in the tunica fibrosa in the walls of the blood vessels.

Riese discusses the question as to whether the intra-epithelial nerve fibres of the tunica granulosa are related to capillaries, or destined solely for the innervation of the epithelial cells. In favour of the first supposition there is the fact that between the epithelial cells of the small follicles where no capillaries exist no nerve fibres are shown to be present

From Riese's observations on the sheep's ovary, and from the observations of others it is very questionable that capillaries run between the cells of the tunica granulosa

Riese is in favour of the opinion

that the nerve fibres in the tunica granulosa are indeed destined for the innervation of the Epithelium, and are not purely capillary nerves, but only accompany the capillaries in entering the follicle wall.

He goes on to discuss the function of such nerve fibres further, there being two points entertainable regarding them. They may be:-

(α) Trophic, and Secretory, for regulating the secretion of the liquor folliculi

(β) Sensory.

In favour of the former theory is the circumstance that intra-epithelial nerves have only been seen to exist in the larger follicles. It would be important to know if the nerves first grow in between the epithelial cells when the liquor folliculi begins to be secreted.

In favour of the ~~latter~~ second theory above mentioned it is difficult to bring forward proof. Riese thinks, however, that the peculiar end-organs observed in the Ovary of the sheep and their necessary significance,

render a denial of the existence of the sensory nerves within the follicle, impracticable.

In subsequent investigations, by using methyl blue, and fixing in Dogiel's mixture of Ammonium picrate and Boric acid solutions, Rose found that the nerve fibres of the "small-follicle" zone are not all non-medullated. They lose first the medullary sheath and then the sheath of Schwann the nuclei of which are easily seen.

Next we come to a short publication on the nerves of the ovary set forth in the "Zeitschrift für Geburtshülfe und Gynäkologie" for 1892 by Herff (7). He employed Cajal's modification of Golgi's method on human ovaries, obtained fresh from abdominal operations mostly from females between the ages of 22 and 42 years.

He found that the nerves entering at the hilus accompanied the blood vessels only for a relatively short distance, and then radiated outwards towards the periphery of the organ,

bending around the larger follicles in their course

He describes these fibres as breaking up into a plexus of fine nerve fibres arranged parallel to the surface of the ovary, and situated at the interface between the zone of the larger and the zone of the smaller follicles

From this plexus numerous fibres pass off into the latter zone and surround the smallest follicles. Some fibres pass still further into the tunica albuginea, and in some cases apparently pass to the deep surface of the germinal Epithelium.

Kerff observed that the nerves were most numerous in the most vascular parts, and he considered them chiefly vaso motor in function. He thought them almost all non-medullated and they formed a wide meshed plexus around the larger vessels, sending in branches to the tunica media. He obtained preparations which showed the terminations of the nerve fibres to occur near the nuclei of the muscle cells, the same nerve fibre supplying several cells. Entrance of a

nerve fibre into the nucleus or even into the body of a cell was not observed.

He frequently saw capillaries invested with nerve fibres.

He observed nerve-fibres coming into contact with the wall of a small follicle, and ~~ending~~ appearing to send fine branches into it between the epithelial cells. He saw what he took to be the ending of a nerve fibre in ~~the~~ relation to a cell of the epithelium of a follicle, but failed to find any accompanying capillary. He therefore thought that nerves had a distinct anatomical and physiological relation to the follicle.

Hertz describes the concentric arrangement of the nerve fibres around the larger follicles, and in a very few of his preparations he saw the appearance of nerve fibres entering the tunica granulosa. He could not find any special end organs, but he felt entitled to accept the existence of "follicle-nerves" in human ovaries.

The arrangement of the follicle epithelium in his preparations

favoured the idea that the cumulus proligerus was the place where the entrance of nerves into the follicle chiefly occurred, but in cases where the discus proligerus and ovum were retained in the section no nerve fibres were seen in the discus.

True corpora lutea were also found by Herff to be surrounded with nerves, which ran with blood vessels into the septa of the corpus, but not between the cells of the stratum granulosum.

False corpora lutea were seen with the appearance of nerve fibres running between the epithelial cells, but subsequent researches showed this relation to be only apparent.

In searching for ganglion cells in the ovarian stroma, Herff found numerous structures looking very like nerve cells with axis cylinders and processes. These were most common in three situations (1) at the deep surface of the zone of small follicles. (2) at the hilus and (3) in the angles of bifurcation of the larger arteries.

Kerff's general Conclusions are:-

(1) The ovarian stroma contains a large number of nerve fibres destined chiefly for the innervation of blood vessels, partly for the follicles.

(2) Nerve fibres reach the follicle Epithelium, which only come into re-Cation with the outer surface of the small follicles, but in the case of the larger ones they enter the membrana granulosa and end in it.

(3) Whether this entrance of nerves occurs mainly at the locus proliferus or not, is not at present decided

(4) Single nerve fibres go to the surface Epithelium, to the muscle cells of the hilus and of the middle coats of the blood vessels.

(5) The existence of ganglion cells in the ovary is not yet satisfactorily proved, but their presence, at any rate at the hilus is very probable.

The structures which Kerff mentions as resembling ganglion cells, were stained with Congo-dye and carmine in the same way as ganglion cells elsewhere, and he thought they probably were ganglion cells.

Since Retzius and Kerff published their papers Retzius (8) has taken up the subject and has embodied the results of his researches in a short paper published in Vol. V of "Biologische Untersuchungen" (1893). He subjected the ovaries of cats of different ages to Cajal's modification of Golgi's method and obtained his best preparations from kittens of three to six weeks old.

He describes the same general arrangement of the nerves as Riese and Kerff, viz:- the nerves entering at the hilus arranged in thinner or thicker bundles and passing through the central part of the ovary to all parts of the superficial zone, many fibres passing with the blood vessels and forming a plexus around them.

Retzius notes the scarcity of nerve fibres in the tunica albuginea which receives a few branches from the plexus occupying the zone of the smallest follicles. The nerve fibres in this zone appear to break up into fibrils which terminate as free running knobbed end branches. Here and there

end branches are seen in relation to a follicle wall but not entering the follicle. Only rarely was Retzius able to trace a fibre as far as the surface epithelium at the deep surface of which it ended

In relation to the larger follicles, Retzius describes the nerve fibres as winding around them, and sending branches into the follicle wall. But he did not see any branches passing into the tunica fibrosa and ending free in it. To him the nerve fibres appeared to end (1) at the outer surface of the follicle, (2) in relation to the blood-vessels, (3) in the connective tissue of the stroma between the follicles and (4) in tracts of peculiar looking parenchymatous cells which appeared alongside the follicles in the preparations. In these tracts of parenchymatous cells Retzius saw branching nerve fibres with free endings.

Retzius took great pains to find out nerve fibres in the tunica fibrosa of a follicle as described by Klischer Klein and Kerff but he did not succeed

He goes on to say that there are many

appearances which are very deceptive especially if the follicle under observation is not cut through the centre or is seen in partial surface view.

In preparations showing the centres of follicles, Retzius never saw nerve fibres entering the tunica granulosa, not even in the largest follicles.

If the follicle is not cut through the centre, in certain focussings, one can by projection get the appearance of nerve fibres running between the cells of the tunica granulosa.

Retzius also draws attention to the fact that many cells of the tunica granulosa are stained black by Cajal's modification of Golgi's method and they show various shapes with distinct outlines. He suggests that Rise mistook cells stained in this way for nerve end organs.

Retzius saw no nerve endings except branches running out to a free termination, and therefore no special end-organs. He did not see any nerves in the cumulus proligerus, and he considers that the entrance of nerves into the graafian follicle of the cat, if it ever

does occur, must be regarded as exceptional.

In the "Centralblatt für Gynäkologie" for March 1894 there appears a short abstract of a work by Gawronskey on the nerves of the female genital organs

I have not been able to procure Gawronskey's work for reading, but according to the abstract mentioned Mrs observed in a few cases trace a nerve fibre through the wall if a larger follicle into the tunica granulosa, the reading however not being demonstrated.

The preparations upon which this dissertation is based were obtained by subjecting the ovaries of cats ~~firstly~~ to Ehrlich's intra-vitam method of staining by injection of methyl blue; and to Ramon y Cajal's modification of Golgi's method.

Ehrlich's methyl-blue is a non-toxic, arsenic-free compound containing sulphur, this chemical constituent giving it a special affinity for living nerve tissues. It is only taken up by nerves and nerve cells while they retain their vitality and to give the reaction properly it is necessary that the nerve tissues should be alkaline and should be exposed to free oxygen.

There are three methods of using this staining reagent as follow:-

(1) Injection of .5% solution of the blue in normal salt solution into the jugular ~~a~~ other vein of the animal, allowing it to remain alive for between one and two hours, and then killing it and exposing the tissues to the atmosphere.

(2) Injection of a saturated (that is from 3% to 4%) solution of the blue in normal salt solution into the artery of the part to be investigated, the injection being performed as soon as possible after the death of the animal or while it is dying.

The animal should be bled during or previous to the injection. When the injection is completed the parts under observation are exposed freely to the oxygen of the atmosphere and kept moist.

(3) The third method is one much used by Dogiel. It consists in placing pieces of tissue immediately after excision into $\frac{1}{16}$% solution of the blue in normal salt solution on a slide. This method in Dogiel's hand has yielded most excellent results. It was tried by me on sections of a freshly excised cat's ovary, cut by means of the Ether freezing microtome, the ovary having been frozen in aqueous humour, but whether owing to the action of the freezing or of the ether vapour, to defective manipulation, or to a fault of the methyl-

blue, there was absolutely no reaction of nerves to the stain.

The first of the three methods mentioned, namely, injection of a .5% solution of the blue into the external jugular vein, I tried on rabbits, but quite without success. Whether due to the anaesthetic, or to the replacement of so much blood by blue solution, these animals are very easily killed, and it is apparently difficult to administer by this method a sufficient quantity to cause staining of the nerves without killing the animal.

All the methyl blue preparations therefore were obtained by the second of the methods mentioned namely, injection of a saturated solution of the methyl blue into the descending thoracic aorta so as to permeate the genital organs. At first it was tried on rabbits but without success, although the nerves of the broad ligament took up the stain.

It would seem that the ovary of the rabbit does not lend itself to investigation of the nerves by either Elec-

-lich's or Golgi's method for none of the continental observers as yet seem to have met with much success in working upon the ovary of this animal.

The only animal which gave me any results was the cat. The usual method of procedure was as follows:-

After the animal was put under the influence of Chloroform the abdomen was opened and the larger branches of the abdominal aorta rapidly tied, except the ovarian and internal iliac arteries, to insure injection of the genital organs, and to prevent waste of the methyl blue solution.

The thorax was then opened and blood was allowed to escape from the superior vena cava while the abdominal aorta was injected through a nozzle introduced into it from above the diaphragm. The methyl blue used was obtained from Merck of Darmstadt, and a saturated solution held about 3% of the blue.

From 20 to 30 cubic centimetres were injected and the ovaries were exposed to the atmosphere. At first they turned pale

As the solution diffused out of the blood vessels into the connective tissues. Then they rapidly became blue after exposure to the air, and after from twenty to thirty minutes exposure the ovaries were removed from the body, cut transversely into four or five pieces and placed in a moist chamber. The pieces of ovary were kept moist with aqueous humour and pieces were snipped off from time to time and examined microscopically to ascertain the time of deepest staining of the nerves, which was usually found to be from forty to sixty minutes after injection of the methyl blue.

When the nerves showed their deepest colouring the pieces of ovary were fixed as rapidly as possible, owing to the quick fading of the stain which constitutes the chief disadvantage of the method. It is therefore necessary to make frequent examination of pieces of the tissue in order to be able to fix the stain when it shows most plainly.

The method of fixing which is most recommended consists in placing the pieces of tissue into a saturated solution of Ammonium Picrate, and leaving them in this fluid for from twelve to twenty-four hours. This method has the disadvantage of causing the tissues to swell, and thus perhaps disarranging to a slight extent the exact relations of the nerves to the epithelial and other elements of the piece of tissue so fixed. Further this fluid has a powerful macerating action, and removes epithelium besides causing disintegration of connective tissues. It has still another disadvantage, in that it does not harden the tissues, but rather makes them softer.

It forms an insoluble compound with the methyl blue, and, after fixing, the courses of the nerve fibres are marked out by lines of a dark-blue finely granular precipitate.

To obviate the disadvantages of the saturated Ammonium picrate solution, Dogiel recommends the

addition to it of a small quantity of 1% solution of osmic acid, which prevents both the swelling and the macerating action of the fluid to some extent.

After being fixed in Ammonium picrate the pieces of tissue are then cut into sections by means of the ether-freezing microtome and the sections are mounted in an ~~aqueous~~ mixture of Equal parts of glycerine and water. In this mounting medium the stain of the nerve fibres will hold for some months, but my experience is that fading occurs sooner or later.

Another method of fixing which I found to answer temporarily very well, is to rapidly dry the preparation by waving it in dry warm air. Thus the tissue is kept exposed to the atmospheric oxygen. When dried it is mounted in inspissated Canada balsam. The preparation must be very thin in order that it may dry rapidly.

This method of fixing gave excellent results with the nerves of the broad

ligament and mesentery, but the stain was not retained by the nerve fibres for a longer time than two months. The advantage of this method is that the blue staining of the nerve fibres remains uniform and homogeneous, and is not converted into a granular precipitate such as is the case with the Ammonium Picrate.

It should be mentioned that in order to have the blood and tissues as alkaline as possible the animal was fed about two hours before the injection was made.

With a view to facilitate the development of the nerve staining I tried exposing the tissues, after injection and removal from the animal's body, to a mixture of about Equal parts of atmospheric air and Oxygen in a bell jar, but the pieces of tissue invariably bleached rather rapidly and became a uniform blue, the Connective Tissues as well as the nerves.

As will be seen from the pre-parations certain constituents of the organs besides the nerve fibres are

stained by the methyl-blue.
The central part of the smallest follicles invariably take up the stain. Most of the cells of the tunicae granulosae of the larger follicles, unstriped muscle fibres, and a considerable number of the cells of the ovarian stroma are susceptible to the stain. There seems to be special tracts of these connective tissue cells which take up the stain situated between and in the neighbourhood of the larger graafian follicles.
After fixing in Ammonium Picrate the parts of the ovary not stained by the blue, assume a light greenish-yellow colour. The surface epithelium is invariably removed by the macerating action of this ~~Ammonium picrate~~ fixing fluid.

The other method which was employed in my investigations was Ramon y Cajal's modification of Golgi's method.

Shortly this method consists in placing the tissues under investigation, in as fresh a condition as possible into a mixture composed of:-

Bichromate of Potash (3½% solution) 4 parts
Osmic acid solution (1%) 1 part.

The fluid with the tissues in it is kept in a warm Chamber. After a period varying from 2-3 to 8 days according to the character of the tissue and size of the pieces, the specimens are washed rapidly in distilled water and transferred to a .75% solution of nitrate of silver. In this they are left for two to four days, and at the end of that time sections are made by the hand or freezing microtome.

The nerve fibres are impregnated by a black deposit, and as there is no swelling of the tissues, the minuter details of the nerve fibres themselves, and of their relation to the other constituents of the ovary are much more

distinctly and accurately shown than in the preparations made by fixing Methyl-blue stained specimens in Ammonium Picrate

Certain other Elements of the ovary take on the black deposit occasionally. These are:-

(1) The capillary blood vessels, which are easily recognized and differentiated from the nerve fibres by their size, by the staining being of a considerably lighter shade when seen under the higher powers, and by their frequently containing red blood corpuscles.

(2) The surface Epithelium, which is rendered very indistinct by the deposit.

(3) Certain tracts of cells in the neighbourhood of and between the larger follicles.

(4) A few cells of the tunica fibrosa ovarii of the larger follicles, and the interstitial spaces between the cells

(5) Small round granules in the ovum itself, these appearing to be fat and yolk granules stained by the osmic acid.

The Methyl blue preparations show the general arrangement of the nerves in the ovary such as has been described by previous observers. In these preparations the staining of the nerve fibres has faded considerably, this being especially true of the deeper parts of the organ, where the staining was at first less thorough owing to want of such complete oxygenation as the more superficial parts received. The stain has held better in the zone of the smallest follicles, and in all the larger sections fading has occurred from the centre outwards.

The preparations show the general direction of the nerve fibres in the interior of the ovary to be radial, running outwards from the centre to the periphery, some with blood vessels, others between them. Those which run with the blood vessels form a plexus around them. Those which do not run with the blood vessels pursue a straighter course and give off side branches at acute angles.

These nerves and their branches bend around the larger follicles in their course, and in the region which separates the zone of the small follicles from the zone of the larger ones, these radially running fibres appear to break up and form a plexus the general arrangement of which is tangential, or, in a plane parallel to the surface of the organ. In the preparations the distinction between medullated and non-medullated fibres is not brought out, but the plexus just mentioned would appear to consist almost if not quite entirely of non-medullated fibres.

From this plexus, nerve fibres pass out at various angles into the zone of the smallest follicles, forming a plexus in this zone; the small follicles lying between the branches. This zone of the ovary as will be seen from the preparations is very richly supplied with nerves, and they here form a considerable proportion of the interstitial tissue between the follicles.

There appears to be a less distinct

plexus formation in that part of the ovary where the Tunica albugin-ea merges into the Zone of the smallest follicles. From this plexus fibres are seen to run outwards into the tunica albuginea towards the surface Epithelium, which in these preparations has been removed by the macerating action of the Ammonium Picrate.

The nerve fibres in these superficial parts of the ovary are very fine for the most part and show more or less numerous varicosities in their course. At their branchings they frequently show slight expansions, triangular in surface view, which are apparently homogenous with the rest of the nerve fibre, and which would seem to be too small for ganglionic cells.

Some of the nerve fibres in the Zone of the smallest follicles, and some also in the tunica albuginea appear to end in slight expansions of a flattened piriform shape. It is difficult to say whether these are true endings or not. The

nerve-fibres are varicose as previously mentioned and the apparent ending may be a varicosity beyond which the fibre is not stained, or it may be a bend in the fibre where it turns into another plane.

The ~~relation~~ exact relation of nerves and nerve endings to the large follicles is not well brought out in the methyl blue preparations owing to imperfect staining and fading. The expanding follicles in the deepest part of the "small-follicle" zone, however, are seen to be invested with fine nerve fibres, running in the connective tissue and coming into contact with the wall of the follicle.

No nerves are seen to penetrate the follicle-wall and enter the tunica framulosa, and the only terminations of nerve fibres to be seen are branches running out to a point in the interstitial connective tissue between the follicles.

The smallest follicles have a similar relation to the nerve fibres

amongst which they lie.

The characters of the nerve fibres and their more detailed relations to the follicles and blood vessels, are more distinctly shown in the sections made from the ovaries which were subjected to Golgi's method. They were cut by hand with an ordinary razor and are therefore necessarily thick, but they show the nerve fibres in their continuity better than thin sections would.

The pieces of ovary, after having been three or four days in the .75% Silver Nitrate solution, were cut into sections with a razor kept moist with alcohol, and the sections, after having been rapidly dehydrated with absolute alcohol and cleared up in xylol, were mounted in inspissated xylol balsam without a cover glass.

So far the nerve staining has shown no signs of fading.

In the sections made from the ovaries subjected to Golgi's method, only the nerves of the more superficial parts of the ovary are impregnated by the deposit.

These preparations confirm the results obtained by the employment of Ehrlich's methyl-blue injection method. The staining is much more complete in some spots than in others and these places show the zone of the smallest follicles to be richly supplied with nerves. There is a very noticeable scarcity of nerves running outward into the tunica albuginea from the plexus in the zone of the small follicles.

In these preparations there is no displacement of the relations of the different constituents of the organ, such as has been caused by the swelling of the methyl-blue preparations due to the ammonium picrate. The small follicles are seen to be arranged in groups separated by trabeculae

or septa of connective tissue the fibres of which run more or less at right angles to the surface of the ovary. A rich plexus of fine nerve fibres is seen in the part immediately internal to the zone of the small follicles and from this plexus fibres are seen to run outward chiefly in the septa of connective tissue above mentioned, some of them coursing between the small follicles as they lie in groups. In the deepest part of the tunica albuginea, these fibres which run outwards through the zone of the small follicles for the most part divide into branches and form a plexus the general arrangement of which is more or less parallel to the surface of the ovary. A few fibres are seen to run through the tunica albuginea outwards towards the surface Epithelium, some running out to an apparent ending others dividing in the outer part of the tunica albuginea into branches parallel to the surface Epithelial layer.

In these Golgi preparations, the nerve fibres of the zone of the small follicles, and indeed of the other regions of the ovary are distinctly seen to be arranged for the greater part around blood vessels, and it is manifest that these nerves chiefly belong to the vascular system of the organ.

In some cases the capillaries have become stained, or filled with the deposit, giving them under the high power a dark brown colour in parts of their length, and a plexus of nerve fibres, stained of a deeper colour, can be seen investing them. In some cases the stained part of the capillary ends abruptly while the investing plexus of nerve fibres is stained for some distance further on. In other instances the capillary has not taken up the stain, but its position and course are shown by a row of red blood corpuscles. In most cases there is nothing to show the actual presence of the capillary, but the arrangement of the nerve fibres

is plainly demonstrative of the fact that they are in relation to a capillary blood vessel.

The most usual arrangement seems to be that each capillary has a pair of nerve fibres running with it. Each nerve fibre shows a very wavy course in most of its length, and several varicosities or sessile swellings are seen on it. The two nerve fibres which belong to one capillary, appear to send here and there small side branches across to one another through which side branches they anastomose. In a few places they are seen to give off what are apparently short fine side-branches each ending in a rounded knob, which if it is a true ending will be in relation to the wall of the capillary. It is difficult to say whether these are true endings or not, it is possible that they may each only represent a side branch stained as far as its first vari-

varicosity but not farther.
They are however the only structures which seem to be nerve-endings in relation to these capillary nerves.

The nerve fibre is not diminished in thickness by giving off one of these side branches. It usually presents a slight expansion or varicosity at the point from which the side branch is given off.

The nerve fibres of the zone of the small follicles are, as previously suggested, chiefly in association with capillary blood vessels. Many however are apparently single, it may be that the associated nerve fibres of these single nes are not stained. They do not differ in any respect from the nerves of the capillary blood-vessels according to their appearance and course. They run, as previously mentioned, chiefly in the septa of connective tissue between the follicles where one would expect the capillaries to be most numer-

ous

A few nerve fibres are seen to run through the groups of follicles and to come into close apposition with their walls. In no case are they seen to give off branches which penetrate the follicle wall. Their side branches seem to be very scarce, but here and there they show small, lateral, sessile, rounded swellings, and occasionally a fine short branch thickening slightly into what appears to be an end-knob, just as is the case with the capillary nerves.

It is very difficult to decide whether the varicosities represent the actual natural structure of the nerve fibre, or whether they are products of manipulation. There is certainly no regularity in their distribution along the course of the nerve fibre. A considerable number of them would seem to be due to patchy, non-continuous staining of the nerve fibre. It is also possible that some of them may represent the commencement of a side-

branch which has not taken up the stain.

In some of the preparations nerve fibres may be seen running out towards the surface epithelium but their mode of ending is not satisfactorily demonstrated. In a few instances they may be traced as far as the black deposit which has obscured the epithelial cells in the sections.

It is shown in several of the preparations that the intercellular substance or spaces of the tunica fibrosa of a large follicle is capable of being impregnated by the deposit in Golgi's method.

Where the intercellular substance between two radially running rows of cells is impregnated a fine black wavy line is produced which might be mistaken for a nerve fibre running between the cells of the tunica fibrosa. An appearance of fine side branches is produced by the deposit extending between the cells sideways from the radially

running line, and varicosities appear opposite the ends of cells where the intercellular space is of large size.

Further, there seem to be larger intercellular spaces which have become filled with the deposit so as to appear as irregularly oval black bodies lying among the cells of the tunica granulosa. Such bodies are seen in several of the large follicles in the fetal preparations. Some of them correspond in shape and also in size to the bodies discovered by Riese in the tunica granulosa of the large follicles of the sheep's ovary, and described by him as special nerve-end organs. Riese is the only observer who has hitherto described such structures. It ought to be suggested that he has mistaken the impregnation of intercellular spaces for the staining of nerve fibres and nerve end-organs situated among the cells of the tunica granulosa of the larger follicles.

Some of these appearances show

an arrangement exactly such as is described by Kiss; namely:- a nerve fibre thicker than an ordinary non-medullated one, starting in a rounded or pyramidal expansion alongside a capillary in the wall of the follicle, passing between the cells of the tunica granulosa, to end in a dark oval structure situated more or less near the centre of the follicle, the nerve fibre giving off delicate side branches between the cells of the tunic in its course.

He also describes the oval body as sometimes running out into a long fine point at the pole opposite to the entrance of the nerve fibre.

The supposition that special sensory end-organs exist in the Graafian follicle is certainly rather hazardous. It is difficult to conceive the object of such bodies in such a transient structure as the Graafian follicle, and, unless they are for the purpose of receiving only some special occult sensory impressions, the sudden rupture of the follicle would occasion considerable nervous disturbance.

Observers are all agreed that the entrance of nerves into the tunica granulosa of a large follicle is rare, and not at all in proportion to the abundance of nerves around the follicle.

If there are no true "follicle nerves, that is, nerves piercing the wall of a ~~large~~ follicle and invading the tunica granulosa to end amongst its cells, the relation of the nerve fibres to the wall of a larger follicle can be accounted for on purely mechanical ground.

As a follicle enlarges it must produce a crowding together of the nerve fibres around it, just as it causes that condensation of the general connective tissues which constitutes its tunica fibrosa.

The nerve fibres are seen to be bent out of their course by the bulging of the follicle. It has been shown that there are nerve endings in the connective tissue which forms the ovarian stroma, and, as the follicle increases in size more and more

of these nerve endings must come to lie in relation to its wall.

The apposition, therefore, of nerve endings to the wall of an enlarging follicle may be adventitious, and not of special growth destined for the innervation of the follicle.

List of Specimens.

The Specimens mounted on the slides numbered 1 to 16 inclusive, are transverse sections of the ovaries of cats, prepared after injection through the abdominal aorta with a saturated (about. 3%) Solution of methyl blue. Those numbered from 1 to 8 formerly showed very well the general arrangent of the nerve fibres in their course from the central to the superficial parts of the ovary, but the stain of the nerve fibres has faded considerably towards the centres of the sections, especially in the thinner ones.

The slides Nos. 9. 10. and 12-16 inclusive show especially the arrangement of the nerve fibres in the Zone of the smallest follicle and the Tunica albuginea.

The surface Epithelium is absent as already explained, owing to the action of the Ammonium Picrate.

They are mounted in a mix-

-ture of Equal parts of glycerine and water.

The specimens mounted on the slides numbered 17 to 24 inclusive were prepared by subjecting the brains of full grown cats to Ramon y Cajal's modification of Golgi's method.

They are mounted in inspissated Xylol balsam without cover-glasses.

This figure represents a low power view of the arrangement of the nerve fibres in their course from the centre to the periphery of the ovary.

g. g. Small Graafian follicles of superficial zone.

g^1 g^2 g^3 follicles in different stages of expansion.

N. f. Nerve fibres.

B. V. Capillary blood vessel in zone of smallest follicles.

C. Connective tissue cells, arranged more or less in groups, which take up the methyl blue stain.

Fig. 1.

from slide No. 6. Oc 3 Obj. 3 (Leitz).

This figure represents a low power view of the superficial parts of the Ovary, and shows more especially nerve fibres passing from the zone of the smallest follicles outwards into the Tunica albuginea.

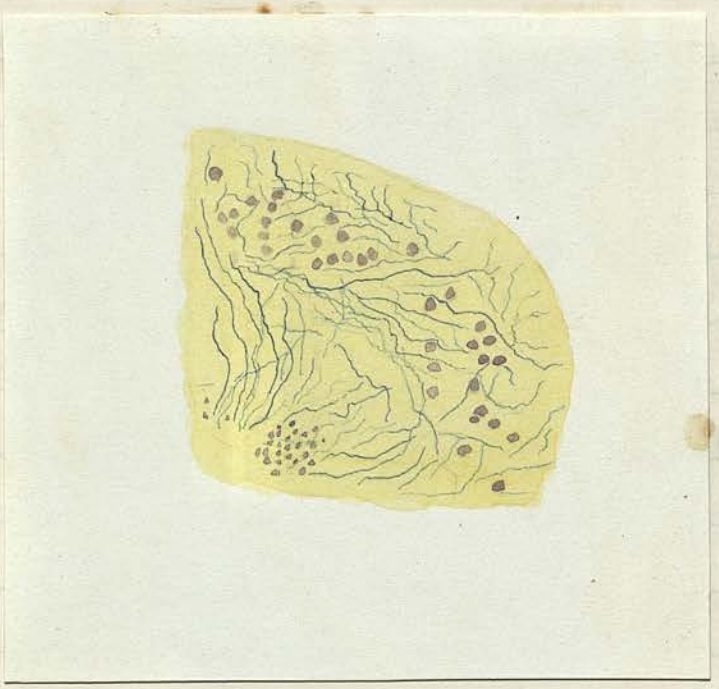

Fig. 2.
Inv. Slide No. 16 Oc 1 Obj. 3 (Leitz).

This figure represents a high power view of the arrangement of the nerve fibres in the zone of the smallest follicles. The central part of each of the smallest follicles has taken up the methyl blue stain

The thicker nerve fibres show triangular or pyramidal Expansions at their points of bifurcation

Fig. 3.
from Slide No. 16. Oc. 1 Obj. 7 (Leitz)

This figure represents a high power view of a follicle from the deepest part of the small follicle zone, with the nerve fibres around it.

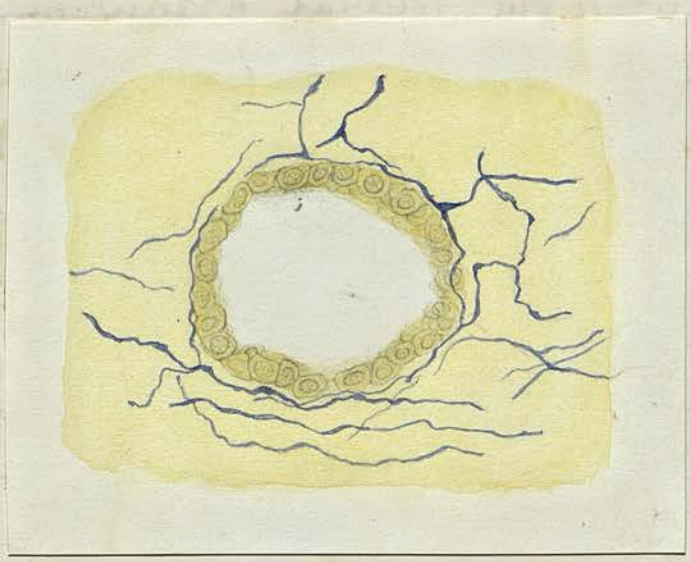

Fig. 4
from Slide No. 13. Oc. 3 Obj. 7 (Leitz.)

This figure represents a low power view of the general arrangement of the intrinsic nerves of the ovary, taken from one of the specimens subjected to Golgi's method.

g. follicles of small follicle zone.

n.f. nerve fibres.

b.v. Capillary blood vessel.

E. Surface Epithelium secured by deposit.

Fig. 5
from Slide No. 18. Oc. 3 Obj. 3 (Leitz)

This figure represents a high power view of the zone of the smallest follicles in a Golgi specimen.

G. Small fusiform follicle.

N.f. nerve fibres.

E. Surface Epithelium cleared by black deposit.

Exp. Expansion at bifurcation of a nerve fibre

N.E. apparent nerve ending near a small follicle.

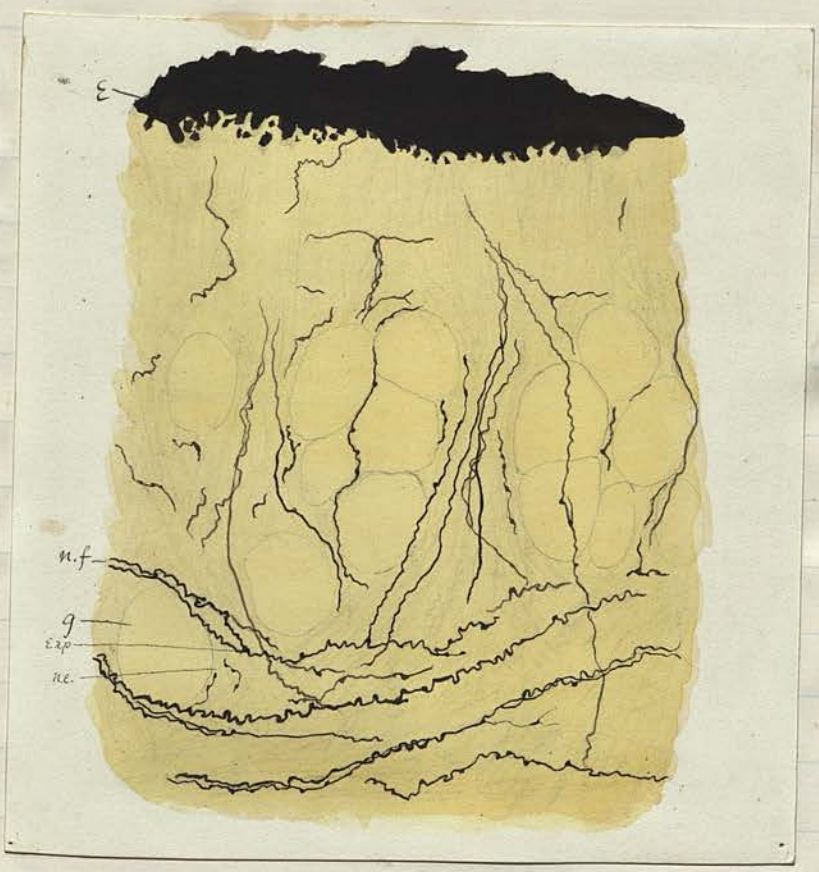

Fig. 6
from Slide No. 18 Oc. 1 Obj. 7 (Leitz)

This figure represents a high power view of the nerve fibres in relation to one of the large graafian follicles in a fetal specimen.

$g.^3$ - Cavity of follicle
N.f. Nerve fibres
B.v. Capillary blood vessel.

Fig. 7.
from Slide No. 17. Oc. 1 Obj. 7 (Zeiss)

This figure represents a high power view of the nerves around a capillary blood vessel in the deepest part of the zone of the smallest follicles.

b.v. capillary blood vessel, part of which is deeply impregnated by the deposit.

N.f. nerve fibre

N.E. apparent knob like nerve endings on branches of the nerve fibres.

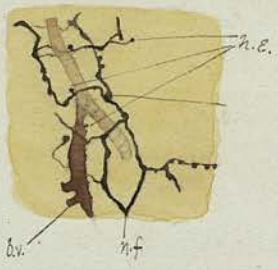

Fig. 8.
From Slide No 18. Oc 3. Obj. 7 (Leitz.)

This figure represents a high power view of a larger graafian follicle in which an intercellular space has been so impregnated as to simulate a nerve fibre and end-bulb in the tunica granulosa.

b. v. Capillary blood vessel.

Exp. Expansion alongside a capillary which appears to give rise to a nerve-fibre entering the tunica granulosa.

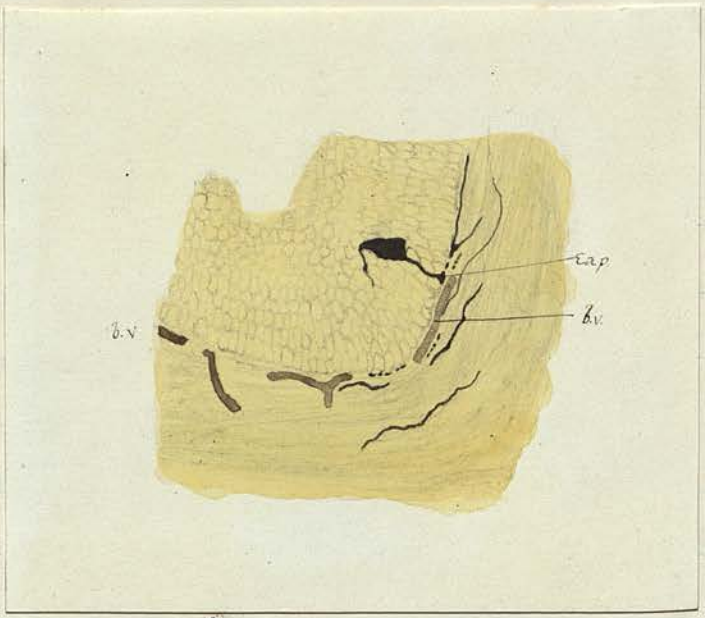

Fig. 9.
from Slide No. 19. Oc. 1 Obj. 7 (Leitz)

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March 17th 1894 (Jahrg. 18).

1 Mar 1895
2753

Northern Hospital.
Liverpool. April 29 1895

Dear Sir,

I am submitting for graduation as M.D. this year, a Thesis on "The Innervation of the Ovary" the first come of my work in accordance with the regulations of the Freeland Barbour Fellowship awarded to me on graduation as M.B. C.M. in August 1892. I enclose £1. for Matriculation and £5. 5- fee for the M.D. diploma as I shall not be able to visit Edinburgh myself for some weeks, and I should like to know by post if there are any other particulars necessary to be attended to.

I was 25 years of age in February this year, and since graduating I have successively acted as assistant to a practitioner in Kent from August 1892 till April 1893, worked in the Physiological Laboratory of Edinburgh University during the summer session of 1893, the winter session of 1893-4 and the summer session of 1894, and held the posts of assistant House Surgeon and House Physician of the Northern Hospital, Liverpool since August 1894. I still hold the post of House Physician to this hospital and it will be my address until the end of this year.

I am, Yours faithfully
George Yeoman

Professor J. R. Fraser