The Structure, Developmental Course
and Some Aspects of the Functional Significance
of the Adrenal Gland of Sheep

Thesis for the Degree of Doctor of Philosophy

by

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Introduction

The relationships between the anatomy and physiology of the adrenal gland are so intimate that a great interest or striking result in either field always stimulates and awakens research in the other. This is natural, because the problems of function and structure are so closely bound up with one another that it is difficult or even impossible to separate or draw definite and distinct lines between the two. Indeed, it may be said so far as any critical investigation is concerned, that they are mutually dependent. Thus, since the earliest days when OLIVER and SCHAPER (1895) observed that aqueous extracts of the medullary portions of the glands contained a substance which exercised a distinct action on the heart and blood vessels, and FLINT (1899) presented his praiseworthy tome on the organogenesis and angiogenesis of the adrenal bodies of Dog, especial attention has been paid to ductless glands in general, with study of the adrenals coming well to the fore.

The gland investing the anterior pole of either kidney in the human (glandula reni incumbens) was first described by EUSTACHIUS (1564), the great anatomist of the Renaissance, and it was commonly believed that these organs were mentioned in the Bible. This notion however was an erroneous one arising, as BLANCHARD (1882) pointed out, from incorrect translation of the Vulgate.
From the onset, it was apparent that the relationship of these suprarenal bodies to the kidneys was merely one of juxtaposition. Certain early writers supposed that they were renal structures and named them "suocenturiate kidneys". BARTHOLIN (1666) was the first to take note of the gland medulla. He described it as a cavity containing a "black humour" and he published a remarkable delineation in which the adrenal resembles a cocoanut cut in transverse section, with the "lid" lifted. In accordance with this conception, he named the structures "atrabiliary capsules", and the name capsule is applied to them to this day.

DIEMERBROECK (1672) observed that "the glands are found at a place where there is a plexus of nerves, to which they are firmly united". Unquestionably, he was referring to the sympathetic coeliac plexus. Diemerbroeck also believed that in these capsules, a certain "juice" was removed from the plexus of nerves about them, "useless indeed to the nervous system, but which, flowing thence into the veins, may serve some useful purpose." Diemerbroeck concluded by hoping that physicians, through autopsies, would uncover "what diseases these glands give rise."

In 1855, Joseph Addison described the disease of the adrenal cortex which has brought his name immortality, but even such early observers as WOLFF (1759) recognised that the adrenal organs of vertebrate animals consisted of two functionally and embryologically distinct parts and that these two parts are separate in the lower vertebrates.
The interrenal gland of the Elasmobranchs is, in fact, a simple unpaired organ lying in the median plane of the caudal region between the kidneys. This interrenal organ corresponds to the cortex of the Eutherian adrenal.

The chromaffin adrenal tissue is scattered in the form of small paraganglia throughout the trunk of Elasmobranchs. In Tetrapoda, the interrenal substance becomes united with a considerable part of the suprarenal tissue, but only in mammalian adrenals does the characteristic arrangement of cortex and medulla become observable and, whilst no appreciable difference has been noted to date in the medulla, the cortex varies greatly in structure from species to species.

Until the time of Mörs (1864) and Joesten (1864), there were only two views on the structure of the suprarenal cortex. These were proposed respectively by Eckert (1846) and Von Kolliker (1854). Eckert thought that the cortical substance was made up of glandular tubules surrounded by a homogeneous, structureless membrane which embraced cells and fat globules, while von Kolliker, unable to demonstrate the membra propria of Eckert, held that connective tissue processes or laminae derived from the capsule divided the cortex into spaces which he called "cortical cylinders". Included in these spaces were the cells and fatty granules. Von Kolliker also described in the inner layers of the cortex, small, round or oval Blasen, composed of cells surrounded by a sheath or membrane. On the other hand
HARLEY (1856) considered that the cortex was made up neither of tubules nor cylinders, but of columns of cells which were closely packed together and surrounded by connective tissue. Moreover, a point lending weight to the arguments of this investigator was that he was the first in the field to study stained sections of the adrenal gland. He used carmine as his dye and gave a detailed description of his technique.

In 1864, Moers and Joesten gave up the older ideas of Ecker and Von Kolliker and stated that the cells of the cortex were embedded in a fine meshwork of connective tissue. Joesten separated the cortex into two layers made up of Kapseln which were elliptical in shape and had their long axes running perpendicularly to the fibrous capsule investing the gland. According to this investigator, the cells of the cortex possessed no membrane at all, and each cell was embedded in a space in the connective tissue framework. Moreover, Moers also failed to find tubules with a homogeneous limiting membrane in the cortex and, like Joesten, believed that the cells rested only in a connective tissue matrix. HENLE (1865), reverting to the view of Ecker, described in the cortex columns and tubules, the former identical with that part of the cortical substance designated later by ARNOLD (1866) as the Zona Glomerulosa, and the latter provided with membranae propriae probably corresponding to Arnold's two inner cortical zones, Zona Fasiculata and Zona Reticularis.
The generally accepted nomenclature of the cortex was, as mentioned above, suggested by Arnold, who first divided the region into three layers and named them from the arrangement of the blood vessels and connective tissue. Many investigators, including Joesten, Kolliker, Ecker and Boll (1868) while agreeing with Arnold's nomenclature, described only two layers in the cortex; others, Von Brunn (1872) and Gottschau (1883) found in some animals two, and in others, three zones. Flint (1900), notwithstanding the great variation in the cortices of different animals, believed that the cortical substance was always divided into three more or less distinct zones.

According to Grandry (1867), the cortex was made up of "cylinders" and "closed vesicles", which were limited by prolongations of the fibrous membrane of the blood vessels. The vesicles were small, so he observed, and lying external to the cylinders. They corresponded probably to Arnold's outer cortical zone, while the cylinders, which had a parallel course between the capsule and the medulla, were identical with the cell columns of the Zona Fasiculata. Grandry stated that these cylinders possessed no membrane in the adrenal of the cow, but he believed one to be present in the glands of Dog and Cat.

EBERTH (1872) divided the cortex into two layers, an outer of horse-shoe shaped structures of columnar cells (the so-called Zona Arcuata of present day literature), and an inner of polygonal cells arranged as von Kolliker
described, in cylindrical spaces limited by fibrous lamellae derived from the capsule. In general, von Brunn agreed with the description of Arnold, but hazarded that the cells of the Glomerulosa were not columnar but spindle-shaped, and were provided at each end with long, filamentous processes which passed and intertwined with the fibrils of the adjacent processes of connective tissue which he considered were derived from the capsule.

The effort of CREIGHTON (1886), to show a homology between the suprarenals and corpora lutea, which he designated as "obsolescent Graafian follicles", is interesting purely from an historical point of view. His observations, if inaccurate, were however intelligent, for there is a strong resemblance between the structure of a corpus luteum of pregnancy and the adrenal cortex of some animals, especially Sheep (see Plate I). Likewise, Creighton's attempt to show that the true cortex of the adrenal was composed of the columnar cell groups of the Zona Glomerulosa alone, and that the Zona Fasiculata and Zone Reticularis belonged to the medullary portion of the gland, has sunk into the mists of academic antiquity.

Up to the turn of the century, there was then three main views as to the structure of the adrenal cortex. Some investigators held that it was composed of glandular tubules complete with basement membrane, while others considered that the cortex consisted of cylindrical spaces filled with cells and limited by fibrous processes from the capsule. A third set of workers believed that there was
a fine network of fibrils in the cortex containing in its meshes the cells which were given a more or less columnar arrangement by fibrous septae or trabeculae from the capsule. These views were accepted in part by contemporary observers such as DAGOBERT (1885) and QUAIN (1896). They were combined and modified by many later authors, especially MULON (1903), GRAHAM (1916), ZWEMER, WOTTON and NORKUS (1938), BENNET (1940) and SARASON (1943), through whose efforts the morphology of the adrenal cortex as we know it today has been established.

The peculiar reaction of adrenal medullary cells to some of the salts of chromic acid was first pointed out by MOERS (1864) and HENLE (1865), who found that when the adrenal gland was fixed in some reagent that contained the salts of chromic acid such as Miller's fluid, the cells of the medulla took on a deep brown coloration which was often sufficient to obscure the cell nuclei entirely. At that time, the morphology of the adrenal medulla had been fairly thoroughly investigated by workers such as ARNOLD (1886), GRANDRY (1867) and EBERTH (1872). They stated that the adrenal medulla was composed of closed vesicles of variable shapes surrounded by a basement membrane and containing irregularly polygonal cells surrounded by a fibrous stroma. It was left to VON BRUNN (1872) and FLINT (1900) to describe groups of cells embedded in a fine reticulum surrounded by thicker strands of connective tissue.

Interesting views on the derivation of the medulla
were held by GOTTSCHAU (1883). He considered that the medulla did not arise from a separate anlage from the cortex, but consisted merely of a transformed portion of the inner cortical layer. Gottschau saw medullary cells projecting into the lumen of the central vein in some of his preparations, and by exerting a slight pressure on the glands, he was able to obtain a drop of blood from the vein which contained the protoplasmic masses. This he explained by supposing that the germinal centre of the adrenal was at the periphery of the cortex and that new cells were continually being formed there which passed down to the medulla, where they underwent a transformation to be finally secreted through the suprarenal veins as amorphous, protoplasmic masses. Accordingly, he suggested the following revised nomenclature for the adrenal: Zona Bulbosa, equivalent to the Glomerulosa, where there was a continual new formation of cells; Zona Germinativa, situated just beneath the Bulbosa, where the cells attained their full development; Zona Fasiculata, where the cells were arranged in columns, and Zona Consumptiva, which corresponded to the medulla where the cells were transformed before being ejected via the suprarenal veins. In this postulate, Gottschau was a lot nearer the truth than he probably suspected. (See Section 3).

Inevitably however, there were authors such as FAUNDELER (1892) who disagreed with Gottschau's views, and when one considers how observers like DOSTOYEVSKY (1886),
RAHL (1891) and MUHLMANN (1896) propounded their own theories as to adrenal evolution and morphology, it is hardly surprising that the research worker, when considering a project in the massive field of adrenal histrionics, finds himself on the threshold of a bewildering world where conflicting, even antagonistic tenets are the byword.

Indeed, in conning the abundance of literature on the adrenals, it becomes more and more patent that while much work has been carried out in regard to these interesting organs, comparatively little is known of their more intimate structure and architecture, especially in the field of Veterinary Science, where adrenal upsets are more common and of more economic importance than is perhaps realised.

McGIRR (1954), in a paper addressed to the Eighth British Veterinary Association Congress, discussed recent advances in knowledge relating to the functions of the adrenal gland. The underlying theme of his observations was that in the present state of knowledge, contributions of adreno-cortico physiology should act as a stimulus to new exploration in the adrenal field. He cited the disappointing results obtained in the treatment of Ovine pregnancy toxaemia by ACTH (Adreno-cortico-trophic-hormone), and entered a plea for more veterinary work in the whole field of endocrinology.

To date, little detailed work appears to have been carried out on the adrenals of Sheep. CHAUVEAU (1891), in his Comparative Anatomy of the Domesticated Animals,
merely mentions that "the suprarenal capsules are discoid in the sheep". CLARKSON (1896), in his textbook of Histology compares the adrenals of Cat, Dog and Sheep. Whilst his work includes two plates of Sheep adrenals, his description of the glands in this animal is extremely superficial. VAUGHAN (1892) stated that "the suprarenal capsules are larger in the foetus than in the adult and are said to be replaced if removed".

In his study of the lipoidal content of the adrenal glands of various animals, HEUMER (1914) observed that "1.2 per cent of the dried substance of the suprarenal of sheep is free cholesterol, 0.2 per cent is cholesterol esters, and free fatty acids form 1.3 per cent of the total content". His study did not encompass the anatomy or the histology of the gland however.

During their observations on birefringence in the adrenal, YOFFEY and BAXTER (1947) worked on the glands of rat and cat, and one specimen each of the suprarenal of horse, man, chimpanzee and sheep. The human suprarenal was obtained from a man who died of acute coronary thrombosis but Yoffey and Baxter fail to mention from where the Sheep glands were obtained. In their discussion, they dismiss birefringence in the adrenal of Sheep by stating that crystals are not plentiful nor, as a rule, do they appear as readily in unfixed specimens as in rat and cat.

SISSON and GROSSMAN (1953) give brief comparative details of the anatomy of the Sheep adrenal, and MAY (1955)
devotes a paragraph to the glands in his Anatomy of the Sheep.

TRAUTMANN and FIEBIGER (1952) give scant mention to the adrenals of Sheep in their Fundamentals of the Histology of Domestic Animals, and several authors have included Sheep in a general survey of the glands of all the animals, such as ELIAS (1945), who has furnished us with a general comparative histology of Ox and Sheep.

NIGANDER (1952) included Sheep in his survey of histological and histochemical studies on the adrenal cortex of the domestic animals, yet the conflicting reports in these few works, and the myriad postulates in the literature concerning adrenal glands in general, render even more desirable a constant investigation of the organs from every possible approach.

Accordingly, at the suggestion of Mr. T. Graham, T.D., F.R.C.V.S., F.R.S.E., Head of the Department of Veterinary Anatomy, Edinburgh University, this present research was undertaken, its object being to study both the macroscopic and microscopic structure, and the embryological development of the adrenal gland of Sheep, in an attempt to throw some light on the function, and the functional disorders of the gland in this animal.
Material and Survey of Methods

This work was carried out in the Anatomy Department, Royal (Dick) School of Veterinary Studies, and for the most part, half-bred Sheep were used, the source of supply being animals brought in for slaughter at the City Abattoir, Edinburgh. Occasional pure-bred animals were met with, chiefly Scotch Blackface, Soays and Cheviots, and the adrenals from one Merino Sheep which had been sent in for post-mortem in the Pathology Department, Royal (Dick) School of Veterinary Studies, were examined. Unless otherwise stated though, the figures and text in the various sections of the work refer to findings in half-bred animals.

The age, sex and condition of Sheep used were ascertained as well as was possible in all cases, and the approximate total of animals examined is tabulated below.

Table I - Number of Sheep Used

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th>Young Animals</th>
<th>Neo-Natal</th>
<th>Postumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrates</td>
<td>4</td>
<td>84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rams</td>
<td>15</td>
<td>17</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>Ewes</td>
<td>18</td>
<td>26</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

* The heading 'Young Animals' refers to lambs born the year previous to slaughter.
The Sheep being shot, bled and eviscerated, the adrenals were recovered from carcases classified as being 'dead weight'; that is, carcases without thoracic or abdominal viscera but with kidneys and adrenals still in situ. Glands from condemned carcases were rejected, as were those from animals which evidenced abnormalities of any kind, whether the carcase was in whole or in part, passed as fit for human consumption by the Local Meat Inspector.

The adrenals being taken from animals which had been slaughtered meant that the glands were not available until 15 - 30 minutes after death of the animal, and that they contained but little blood. This was advantageous when there was any time lapse between removal of the glands and fixation, for it was observed, that in glands which had not been completely drained of blood, the onset of post-mortem degeneration was much more rapid than in glands which had been bled. In the case of animals not obtained through the Abattoir, they were dissected immediately after death and their adrenals fixed or frozen within 3 - 10 minutes of death.

It was found that, after removal of the abdominal viscera, the most convenient method of dissecting out the adrenals from perirenal fat was to have the carcases suspended as is customary in Abattoirs, by a splint of wood inserted through the Achilles Tendon of either side. Carcasses were weighed by means of the Abattoir scales and,
where purely macroscopic observations were to be made, dissected-out glands were placed in normal saline till opportunity arose for measurement by calipers, weighing by laboratory balance and displacement determination. In all such cases, the glands were stripped of surrounding connective tissue, as far as was possible to the capsule, before readings were taken.

For Histological work, pieces were carefully cut from one or both glands and treated accordingly to the different techniques used (see below). Those parts of the glands from large or aged animals where trabeculae or accessory nodules could be seen to complicate the structure were avoided as a rule, in order to facilitate the comparative study of sections from the different pieces. In the case of very small adrenals, the glands were fixed in toto, a small incision being made in the capsule to allow for good penetration.

A. Preparations for Dissection.

Hoerr (1931) experienced difficulty in obtaining a satisfactory number of successful perfusions of guinea-pig adrenals by the injection of embalming fluid through the whole carcase. Experience in the fixation of routine dissection specimens has proved the value of perfusion in the larger animals however, giving a high percentage of apparently complete fixation of adrenals in animals such as Horse, Ox and Dog. Accordingly, fixation of both
carcass and adrenals of Soay and Soay-Moufflon cross animals which were obtained for laboratory examination was for the most part by gravity-feed perfusion. The animals were killed by injecting half an ounce of chloral into the thoracic cavity and, when comatose, by severing either of the carotid arteries. Perfusion of the carcass was then carried out via the carotid artery, using a 10 per cent solution of formal, which proved to be excellent for the instantaneous preservation of finest cellular detail in all parts of the body. At one point, the addition of glycerine and carbolic acid to the formal was attempted to retain the natural colour of, and increase the longevity of the preserved specimens, but this was discarded because of the unavoidable growth of symbiotes on the carcass.

For convenience, dissections were in most cases, carried out on blocks of tissue which had been removed from the preserved carcass. These blocks contained the adrenals, kidneys, lumbar portions of the diaphragm, and surrounding tissues including portions of the aorta and vena cava. The blood and nerve supply of the adrenals was investigated in six Sheep by dissection, using a dissection microscope and a hand lens. Various sections of the nerves supplying the gland were taken at this point and treated with osmium tetroxide in order to determine whether or not the fibres were myelinated.

B. Injection Techniques

In order to supplement the observations made during
dissection, several injection techniques were used, including the perfusion of the aorta by a suspension of barium sulphate followed by radiography, and the manufacture of corrosion casts using Vinylite. NARAT, LOCK and NARAT (1936) were among the first workers to use Vinylite resin solution and it has distinct advantages over the older colloidin methods, the final cast being much less brittle and much cheaper to prepare than one made from cellulose nitrate.

Vinylite resin solution solidifies by solvent evaporation, this process being speeded up by the presence of water. The resulting cast is virtually unaffected by the action of concentrated hydrochloric acid and, whilst some contraction is inevitable, Vinylite forms an admirable injection mass with which to trace out the smaller arterial and venous trees. The materials used in the preparation of casts were acetone and Vinylite resin VNHH as supplied by Bakelite Limited, 187 Broad Street, Birmingham. A varying number of solutions were used, from 1 per cent to 8.5 per cent, according to the amount of penetration required. Vinylite was also found to be a suitable medium for introducing fine pigment into vessels and the solutions were coloured with Monolite Fast Scarlet Fast R.M.V.S. and Monastral Fast Blue Paste B.V.S., manufactured by the Imperial Chemical Industries.

Apparatus required for injection. The equipment necessary to carry out the perfusion procedures detailed
below was as follows:—

(1) An air pump and pressure chamber (Edwards Type IV compressor and vacuum pump).
(2) A mercury manometer.
(3) A pressure bottle.
(4) A water bath in which to immerse the pressure bottle.
(5) A deep tray on which the carcase was placed and bathed in warm water.
(6) Rubber tubing.
(7) Glass cannulas.
(8) Ligatures and Spencer Wells forceps.

Injection procedure. The abdominal aorta and the caudal vena cava were located in the carcase of the animal to be injected. The coeliac axis was ligatured off, as was the aorta caudal to the origin of the renal arteries, and the renal arteries just medial to the renal hilus. A glass cannula was introduced into the aorta at a point just cranial to the coeliac axis, and tied in position. The adrenal arterial system was then washed out with warm saline before the blood was able to clot. Washing was continued until the outflow from the venous side was tinged with blood. The saline was followed up by injection of acetone to prevent premature hardening of the Vinylite solution. Injection of coloured Vinylite followed and when perfusion was complete, the carcase was left for 24 hours in a bath of cold water to facilitate hardening of the injection mass. For maceration purposes, a block of tissue containing the
adrenals, portions of the aorta and vena cava, and the renal arteries was removed from the carcase and immersed in a bath of concentrated hydrochloric acid, where it remained until maceration was complete. The final cast was cleansed with a fine jet of water, the whole process taking approximately five days.

In no instance were perfect casts of the entire adrenal capillary bed obtained, but the imperfect casts were of great value in obtaining a three-dimensional picture of the vasculature of the organ.

Casting of the venous system of the adrenals was carried out in similar fashion to that of the arteries, in this case the injecting cannula being inserted into the vena cava caudal to the origin of the renal veins, and the renal veins and caudal vena cava being tied off, the ligature on the latter being placed just cranial to where the right adrenal was adherent to the Tunica adventitia.

A similar technique was also applied for the introduction of barium sulphate into the adrenal arterial system for purposes of radiography. The preparation used in this method was Fotogel, which was supplied by Evans Medical Supplies, Limited, London. No maceration was necessary in this case and the adrenals were dissected down to their capsules and photographed by X-ray. Owing to the complexity of pattern of the resulting arteriographs however (Plate II), it was extremely difficult to correlate them with the vascular tree as seen histologically and from
Vinylite casting, and as there appeared to be no obvious aberrations from the latter, the method was discontinued.

For histological demonstration of blood vessels in the adrenal, a warm solution of carmine-gelatine was introduced into the aorta, as in the Vinylite method above. A 200 cc. syringe was used in this case however, in preference to the vacuum pump, and when injection was complete, the glands were removed and fixed in 10 per cent formal for later processing and sectioning in series.

C. Preparations for Histological Examination.

Whilst the more specialised histological techniques will be dealt with under the relevant headings, the following summary is intended to give an overall picture of the fixation, processing and methods of staining adrenal sections as applied in this work.

For most of the detailed histological examinations of the adrenal, paraffin blocks were made from portions of the gland. Use was also made where necessary, of a freezing microtome. The adrenal being a gland in which post-mortem changes take place relatively quickly, blocks were made from tissues which had been placed in fixative within the least possible time after destruction of the animal. The optimum size of block for optimum fixation was found to be in the region of 5 mm. square, and in every case an effort was made to block the central portion of the glands.

Fixatives. Owing to the critical nature of adrenal fixation, it was necessary initially to experiment
with various fluids to determine which was the most efficient for general purposes. Gibson's, Flemming's and Telyesniczky's fluids, and HEIDENHAIN'S (1909) trichloracetic sublimate fixative were amongst those tested. The following fluids however, yielded the best results and accordingly were used throughout the pursuance of the work.

1. 10 per cent formol.
2. Susa.
3. Helley's fluid (Zenker-formol).
5. Orth's fluid.
6. Carnoy's fluid.

Despite a modicum of hardening of the tissue, Susa, of all the above fluids, was found to give the best fixation of adrenal blocks. Orth's fluid was used initially because of its efficacy in producing a recognizable chromaffin reaction in the adrenal medulla. It was found though that the fluid tended to cause considerable contraction of the gland parenchyma and latterly, Helley's fluid was used, it producing the typical brown coloration of the medulla equally as well as Orth's fluid, but without the same shrinkage.

When it was desired to demonstrate nerve trunks in the adrenal, 10 per cent formol, with the requisite amount of pyridine added, was used as a fixative, and for embryonic work, Carnoy's fluid and Bouin's fluid were found to give excellent results, the tendency of the latter fixative to decalcify tissue being convenient when it came to sectioning.
blocks of whole embryos.

After fixation and dehydration through alcohols in the usual manner, the glands were embedded, in some instances by Peterfi's double-embedding process in methyl-benzoate-celluloidin-paraffin. For double-embedding, the glands were fixed and processed in the normal way and then placed for 24 hours in a bath containing a 1 per cent solution of celluloidin in methyl benzoate. During the 24 hours, two or three changes of celluloidin solution were deemed necessary, according to the size of block. In the next stage, the blocks were immersed in a benzene bath and at the end of a further 24 hours, embedded in paraffin wax as for ordinary sections, the complete method being recommended by CARLETON and DRURY (1957).

After embedding, blocks were sectioned and several slides made from the centre portions of each gland. Serial sections were found to be very necessary in this work, especially in regard to Sections 3 and 4, otherwise single sections were cut at thicknesses varying from 3 to 10 μm.

During sectioning, it was found quite frequently that if the adrenal had been fixed whilst engorged with blood, the capsule was quite liable to separate off from the parenchyma under the microtome knife instead of yielding coherent sections. This exigency was overcome by painting the surface of the block before each section was cut, with a 0.5 per cent solution of celluloidin in alcohol, after
LILLY'S (1954) technique, and thereafter floating off in 70 per cent alcohol.

Whilst the loosening of sections during the Bodian activated protargol technique will be dealt with in the relevant paragraph, it might be mentioned at this point that prior to using silver impregnation techniques, sections were, as a rule, treated to prevent "lifting" under the action of ammonia, by the method put forward by Bolles-Lee and amended by GATTENBY and BEAMS (1950). After dewaxing the sections were rinsed in alcohol and then immersed for five minutes in equal parts of alcohol, ether and a 1 per cent solution of celluloidin. The celluloidin was removed after staining was complete by rinsing first with methylated spirits and then dissolving in equal parts of ether and alcohol. Subsequent treatment of the sections was as normal, the slides being cleared in xylol and then mounted in Canada balsam.

Stains. Amongst such stains as Eosin-methylene-blue, Kull's Copper-carmine and Picro-Mallory, the routine morphological stains listed below were used initially on sections taken from the same gland, in order to test their efficiency, and whilst a few idiosyncrasies were met with later on, their general utility for adrenal tissue was, on the whole, found to be quite satisfactory. Haematoxylin and eosin, whilst being the most convenient stains for early examination of sections, were found to be of more use in serial section work; the stain judged to be most
satisfactory for routine work was Masson's Trichrome stain, this being particularly effective on formal-dichromate material.

Throughout the work then, use was made of the following staining methods:

1. Haematoxylin and Eosin for serial sections.
2. Masson's Trichrome as a routine stain and to demonstrate muscle tissue.
4. Verhoeff's elastic tissue stain and MOLLER'S (1938) Orcein technique to demonstrate the presence of elastic fibres.
5. A modified method of Foot's silver impregnation technique for reticulum fibres.
6. Heidenhain's iron alum haematoxylin for cellular structure.
7. Sudan IV and Sudan Black B to demonstrate fat in frozen sections.
8. Osmium tetroxide technique for the detection of myelin in nerve trunks.
9. Babc's Aniline-safranin technique to demonstrate mitotic figures, after COWEN (1948), and the Feulgen reaction for thymonucleic acid.
10. a) Weigert's silver impregnation technique,
b) Bodian's activated protargol technique,
c) Ramson's pyridine technique,
d) Weigart-Pal modified haematoxylin technique,
c) Bielschowsky's, and an original, modified method of Bielschowsky's, silver impregnation technique for neurofibrils and nerve endings.

Much trial and error was necessary in connection with the demonstration of nervous elements within the adrenal, before results were obtained that were of sufficient merit to be recorded. Nerve fibres in both cortex and medulla showed clearly when impregnated by the techniques of Bielschowsky and Weddell, but Ranson's (1914) pyridine silver method was not found to be particularly effective. The pre-staining of tissue in bulk by Bielschowsky's and Ranson's techniques also proved to be disappointing, and it was observed that, whilst Susa appeared to be the best fixative for Bodian's method, results even then were inconclusive. This was partially due to the repeated loosening of the paraffin sections during their prolonged immersion in the protargol bath. The rinse which preceded the reduction of the sections in sodium sulphite and hydroquinone was acidified by a dilution of 0.5 cc. of 2 per cent acetic acid in 50 cc. of distilled water as Davies and Fingerson (1949) recommend to prevent the lifting of heterogeneous sections such as from the kidney. This however proved to be only about 30 per cent successful in the case of the adrenal, and because of this, and the fact that with the protargol technique it was only on rare occasions that the reticular network of the gland was not impregnated, Bodian's method was eventually discarded.
It might well be that the relative compactness of the reticular framework of the adrenal influences the affinity of the fibres for silver, but Bodian's method was by no means unique in regard to their impregnation. With the Bielschowsky technique especially, great difficulty was experienced initially in distinguishing reticular fibres from neurofibrils after staining was complete. This was so even after much experimentation with the times of immersion of sections in both the silver and reduction baths. Accordingly, an original modification involving the intentional over-staining of sections in ammoniacal silver nitrate and 'back-differentiating' with iodine was evolved. The technique, using both frozen and paraffin sections, was as follows:

(1) 10 per cent silver nitrate . . . . . . . . . 1 hour.
(2) Rinse with distilled water . . . . . . . 5 minutes.
(3) Ammoniacal silver nitrate bath . . . . 20 minutes.
(4) Thorough wash with distilled water.
(5) 4 per cent neutral formal reduction bath . . . . . . . . . . . . . . . . . . . . . . 15 minutes.
(6) Thorough wash with distilled water . . 15 minutes.
(7) Differentiate with iodine until the medulla is almost colourless to the naked eye.
(8) Thorough wash in distilled water.
(9) Tone in 0.2 per cent gold chloride . . 5 minutes.
(10) Fix in sodium thiosulphate bath . . 5 minutes.
(11) Thorough wash with distilled water . . 5 minutes.
(12) Mount in glycerine jelly or dehydrate and mount in Canada balsam.

After Stage 5, the sections are dark blue-grey in colour, and under the microscope both reticular and nerve fibres can be seen to be deeply impregnated. The action of the iodine is to remove the excess reduced silver from both the background and the reticulum. Repeated tests over a period of months with adrenals taken from Soays, Soay-Moufflon crosses and one Scotch Blackface ram, have proved the efficacy of the technique, giving maximum decolorisation of reticular fibres without affecting the neurofibrils at all. It should be pointed out that fixation of the sections with hypo after toning in gold chloride is not essential. The author considers it preferable though, for the thiosulphate appears to increase the longevity of mounted specimens.

Plate III shows a microphotograph of an unmodified Bielschowsky frozen section which illustrates well the impregnation of reticular fibres. A frozen section taken from the same gland but intentionally over-stained and treated with iodine and hypo, can be seen in Plate IV. Neurofibrils are obvious both in the cortex and medulla here but there is little evidence of impregnation of the reticular fibres.

Vital Stains. In an effort to demonstrate the littoral cells which are stated by MAXIMOW and BLOOM (1948) to line the sinusoids of the adrenal cortex, vital staining
using lithium carmine was performed on two Soay Sheep, with the co-operation of Mr. H. Scott McTaggart, B.A., B.Sc., M.R.C.V.S.

Lithium carmine has been employed in many classical experiments as a vital stain by such workers as ASCHOFF and KIXONO (1913) and SUZUKI (1912). 2.5 G of Carmine rubrum optimum were suspended in 50 cc. of cold, saturated, aqueous lithium carbonate and then boiled for 30 minutes in a water-bath. The suspension was then filtered hot, allowed to cool, and then filtered cold. After sterilising in an autoclave, 5 cc. per pound body weight were injected on five successive days into the jugular or saphenous vein, whichever was the more convenient to locate in the animal. On the sixth day the animals were killed and the adrenals removed, one pair being fixed in Helley's fluid, the other in 10 per cent formalin. Sections were studied without further staining, or after treating with a weak solution of picric acid, a 0.25 per cent solution of Methylene blue, or 0.1 per cent solution of Light Green.

D. The Demonstration of Birefringence in the Adrenal Cortex.

At the start of experimentation, very inconclusive results were obtained with regard to the detection of cholesterol esters in the cortex of Sheep adrenal. Frozen sections of formol-fixed glands which had been immersed in fixative at room temperature for one week, were mounted unstained in glycerol-gelatine and examined in polarised light. The birefringent crystals of cholesterol esters
when treated thus are said to show brightly against a dark background. Whilst some evidence of fibrillar birefringence was noted in the capsule, there was no obvious crystallization in the cortex. Accordingly, the technique of Leulier and Revol (1930) was attempted, glands being immersed in alcoholic-digitonin solution after formal fixation. Leulier and Revol state that the resulting cholesterol-digitonin complex forms birefringent crystals which are admirably evident under polarised light but, whilst varying strengths of digitonin, from 1 to 10 per cent, in varying percentages of alcohol, were used, the results obtained were as disappointing as when the glands were examined without previous treatment with digitonin.

From the lack of observations obtained with Leulier and Revol's technique, it was thought that the presence of alcohol, in however small a percentage, might remove some of the adrenocortical lipoids. Accordingly, an aqueous solution of digitonin was prepared after BENNET'S (1940) method. One gram of digitonin was dissolved in a litre of distilled water and the solution warmed to 60 - 70 degrees C. for a period of one hour. The solution was then cooled and allowed to stand in a refrigerator overnight, after which it was filtered and evaporated in a water-bath to 400 cc. giving a 0.25 per cent solution of digitonin in water.

The adrenals were removed from a freshly killed Sheep and fixed in a 10 per cent formol bath for 24 hours at
37 degrees C. The glands were then washed several times during the following 48 hours, care being taken to keep the temperature constant at 37 degrees C. Immersion for five days in a warm aqueous solution of digitonin followed, after which the glands were again washed thoroughly many times with warm distilled water. They were then transferred to a freezing microtome and sectioned at 25 μm. The sections were then mounted in syrup of laevulose and studied with polarised light.

Latterly, two techniques, suggested by XOFFEY and BAXTER (1947), which obviate the need for digitonin and which yield equally rewarding results, were followed.

a) Glands were fixed in 10 per cent neutral formal saline, then sectioned at 10 - 50 μm on a freezing microtome. The sections were then mounted in either syrup of laevulose or ZWEMER’S (1933) Glychrogel, the formula of which is as follows:

Glycerine ........ 20 cc.
Granulated gelatine .... 3 G.
Chrome-alum ........ 0.2 G.
Distilled water ....... 80 cc.

b) Glands were embedded in gelatine subsequent to fixation in 10 per cent formalin, and then sectioned on a freezing microtome.

Encouraging results were obtained by both methods, the sections being examined in polarised light in the unstained condition.
E. The Preparation of Embryonic and Foetal Material.

A sheep embryo of 10 mm. Crown-Rump length is probably one of the most instructive single stages of the later development of this animal. The majority of important organs are represented and yet the embryo is not so complex as to cause confusion. Moreover, the orientation when blocking of embryos younger than 10 mm. can often be exacting. Accordingly, study of the developmental course of the adrenal in Sheep was commenced by the microdissection and serial sectioning of embryos of upgraded ages from 10 mm.

Whilst gravid uteri were obtained from the Abattoir from approximately January to April, as might be expected embryos were met with accidentally in a great number of instances. This is to say that often a ewe was found to be pregnant only after destruction. In such cases, the uterus was removed as speedily as possible, its placental membranes divided and the embryo or foetus (sometimes more than one) removed, and fixed in either 10 per cent formalin, Bouin's fluid or Carnoy's (Van Gehuoten's embryonic mixture) fluid.

In each instance, the umbilical cord was severed as close to the abdominal wall as possible and the wall itself slit to allow penetration of the fixative.

Use was also made of a number of Sheep embryos of varying ages, which had been collected by the late Professor Bradley, former Principal of the Royal (Dick) Veterinary
College. These specimens had been preserved in formalin and, whilst there was considerable degeneration in some, others were found to be remarkably well-preserved.

**Measurement and ageing of specimens.** It was initially intended to take all embryonic measurements before the material was immersed in fixative. As mentioned above however, a large number of the embryos examined were already fixed, so in order to keep the ratios as constant as possible, freshly obtained specimens were calibrated after fixation.

It was found to be extremely difficult to establish the embryonic age of specimens with any great accuracy. Whilst the flexion of the head in Sheep embryos of three weeks and older might possibly have rendered expedient the greater accuracy of measuring the Greatest-Length or Neck-Rump distance, it was decided to use the Crown-Rump length (C-R) or Sitting Height in all cases, in order to bring the work into line with the majority of embryological literature. The Crown-Rump length was measured using damp nylon thread or calipers, but it should be pointed out that being taken after fixation and being thus subject to the inconstant shrinkage of the specimen, this length is only approximate. It was considered sufficient however to give an indication of the developmental time-table of the embryonic adrenals.

The exact age determination of Sheep foetuses can be equally as problematical as ageing embryos. The nature of the investigation into foetal adrenals did not warrant
close examination of this problem however, and only palpable bodily changes were used as a rough estimate of foetal age. These changes, correlated with approximate age in weeks and approximate C-R length in millimetres, are illustrated in Table II.

**Processing.** For the examination of larger embryos and also of foetuses, it was found convenient to dehydrate and block a 'mid-cut' of the specimen. Specimens were decapitated at a point just behind the caudal angle of the scapula, and the hinder portion removed by severing along a line taken perpendicularly from the patellae to the sacrum.

In order to minimise shrinkage of material as much as possible during processing, all specimens were carefully dehydrated through upgraded alcohols and their direct transference from clearing medium to pure molten wax avoided to prevent the tissues from becoming brittle.

Whilst much can be said for the double-embedding of embryos, especially in regard to serial sectioning, single-embedding was used throughout because of the inevitable difficulties of obtaining complete adhesion to the slide of double-embedded material.

Work with larger foetuses, term-foetuses and neo-natal animals entailed dissection and the removal of the developing adrenals. In the latter instance, the glands were fixed in either 10 per cent formal, Helley's fluid or Susa before being sectioned and stained.

To demonstrate adrenal growth, a concatenation of
embryos of upgrading ages were stained with haematoxylin and eosin and sectioned in series, the sections being made sagittally, horizontally and transversely. For the most part, every fifth section was mounted, except when the actual adrenal region was reached, when every section was mounted.

**F. Experimental Injection of Testosterone.**

KORENCHENSKY and HALL (1938) found that there was an alteration in the suprarenal cortex of female mice after gonadectomy. PANKES (1945), in his extensive review on the subject, states that 'the functional inter-relationship between the adrenal cortex and the gonads is a well-established fact'. LEHRBAN (1952) noted that structural variations occurred in the Zona Reticularis of the adrenal cortex of the cat which could be related to the sexual state of the animal.

With a view to determining whether any similar relationship existed in Sheep, four Soay animals of approximately six months of age were castrated. On recovery from the operation, three of the animals were given intramuscular injections of Testosterone Propionate B.P., as supplied by British Schering, Limited, London. The remaining Sheep was used as a control.

The three experimental animals were given 200 mg., 400 mg. and 600 mg. of testosterone respectively, as follows:

**Table III**/
Table III - Intramuscular Injection of Testosterone Propionate

<table>
<thead>
<tr>
<th>DAYS</th>
<th>SHEEP 1</th>
<th>SHEEP 2</th>
<th>SHEEP 3</th>
<th>SHEEP 4 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>death</td>
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<td>-</td>
</tr>
<tr>
<td>5</td>
<td>death</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>death</td>
<td>death</td>
<td>death</td>
<td></td>
</tr>
</tbody>
</table>

X represents a single dose of 200 mg. Testosterone Propionate.

Each of the experimental animals was destroyed 72 hours after its last injection of Testosterone. The control animal was killed at the same time as Sheep Number 3.

Both adrenals were removed from each animal immediately after death, and fixed for 3 days in Baker's formal-calcium, (BAKER, 1946). Paraffin sections were cut at 7 μm and taken longitudinally through the mid-line of each gland. Since the Zona Reticularis of Sheep adrenal demonstrates a pronounced acidophilia, Anan stain was used throughout.

**Determination of F/R ratio.** The relative
proportions of the Zona Fasiculata and Zona Reticularis in each adrenal were determined by the use of a micrometer eye-piece. The width of the zones was expressed as the number of divisions of the micrometer scale and from this the F/R ratio, that is, the ratio of the width of the Zona Fasiculata to the Zona Reticularis, deduced for each gland. This provided a convenient measure for comparing the increase in the Zona Reticularis in the three injected animals. The degree of shrinkage incurred during fixation was considered to be constant and was not calculated.
Macroscopic Anatomy of the Adrenal of Sheep

The "suprarenals" of Sheep are small, round, sometimes reniform bodies found in close contiguity to the anterior pole of either kidney, but not, as in the human subject, forming a "cap" fitted over it. Because of this, the term "suprarenal" as applied to Sheep and indeed the other domestic animals, is misleading. Throughout this work, the gland will be referred to as the Adrenal Gland.

I. Shape

The shapes of the adrenal glands of Sheep were found to be consistently different and definite enough for them to be recognised as either from the right or the left side. Six pairs of glands are shown in Plate V. Those at the top of the photograph represent the shapes most commonly met with in the left gland, this appearing with a consistent cleft in the lateral border. Six typical right glands appear below in Plate V, epitomising SISSON and GROSSMAN'S (1953) classical "bean-shape".

II. Weight and size

Adrenal glands in general are notoriously susceptible to changes in weight and size as well as in shape, brought about by sexual activity, disease processes and nutritional factors. A gross deviation from the normal measurements given in Table IV can be seen in Plates VI and VIIa, which are photographs of the left adrenal of a nine month old
ew lamb whose carcase was condemned at the Abattoir because of emaciation.

As mentioned in the Section on Material and Survey of Methods, the Sheep used in pursuance of this work were obtained for the most part at the City Abattoir, Edinburgh. Whilst casualty animals of most ages were met with from time to time, the average age of animal available for study was approximately nine months. The appended figures (Table IV) were tabulated from animals between the approximate ages of seven and eleven months. They give an indication of the weights, measurements and displacements of the adrenal glands of animals of the approximate age and class on which this work has been based.

III. A Correlation between adrenal weight and sex

There is much evidence that points toward an intimate relationship between the adrenals and the various phases of reproduction in rodents but as far as the writer has been able to determine, such a relationship has not been reported for Sheep. There are reports of changes in the weights of the adrenals with relation to sex and phases of the oestrus cycle in rats, guinea-pigs, rabbits, pigeons and moles, and ELLIOTT and TUCKETT (1906) noted that the suprarenals of the rat, cat, guinea-pig and rabbit develop equally in each sex until the time of reproduction. Growth after early youth, these workers observed, was of cortical tissue alone and after the animals were mature, the glands of the female were larger than those of the male.
The increase in the size of the gland in the female was apparently of medulla as well as of cortex. The right gland was almost invariably heavier than the left in the several different animals studied. Weights of the suprarenals from seven dogs were also given. Four of these were listed as young animals but the maturity of the other three was not stated.

ROGOFF and STEWART (1927) reported the weights of the adrenals of several dogs in connection with some work on the survival period after adrenalectomy during pregnancy. They did not however, study the relation of the weights of the glands to the different sexes. PIANESE (1929) studied the changes in the adrenals of three castrated male and the same number of spayed female dogs. The weights of these animals as well as those from three normal controls from each sex were listed, but Pianese did not give the ages, breeds or weights of his animals. He was only interested in the changes that occurred after removal of the gonads.

HATAI (1914) noted that, in the rat, the weight of the female adrenal was greater than that of the male. A sexual difference became clearly marked in the rats at forty days of age. The difference became greater as the body weight increased. JACKSON (1913) also states that the suprarenals of female rats increase in weight with increase in age, and he found that the absolute weight of the suprarenals was considerably larger in females although the body weight was much less.

RIDDLE (1923) studied the weights of the suprarenals
of four kinds of doves and pigeons at various periods in the oestrus cycle. He found that the most marked increase in the weight of the glands was at the time of ovulation.

Koimer (1918) showed that there is an enlargement of the suprarenals of the mole in both sexes during the breeding season, and stated that there was an enlargement in the glands of both pregnant and non-pregnant animals during the rutting season.

A study of the literature suggested probable variations in the weights of the adrenals of sheep with relation to sex and castration. Owing to difficulties experienced in obtaining specimens in the different stages of the sexual cycle, and the lack of sexual history of the animals readily available, it was, unfortunately, impossible to follow up the effects of oestrus and pregnancy on adrenal weight. It was decided therefore to limit study to the relationship of adrenal weight to carcass weight (dead weight), and make comparisons in the following three classes of animals:

i) Castrate lambs (Wedders).

ii) Ewes in anoestrus.

iii) Rams out with the rutting season.

To this end, a total of 109 animals, consisting of 71 wedders, 30 ewes and 8 rams were weighed at the Abattoir after slaughter and evisceration, and the combined weights of the right and left adrenal glands determined by laboratory balance. The variations observed in these weights are shown in Tables V, VI and VII.
The regression of gland weight on body weight for the three classes of animals was studied. Linear regression lines failed to give a satisfactory fit to the data, especially with regard to that for ewes, so it was necessary to calculate the orthogonal polynomials of the second degree (See SNEDECOR, page 452). Where $y$ represents gland weight and $x$ body weight, these were found to be:

a) Castrates:  
$$y = 0.4373x - 0.002122x^2 - 14.59$$

b) Ewes:  
$$y = 0.5846x - 0.00364x^2 - 16.28$$

c) Rams:  
$$y = 0.1662x - 0.00389x^2 - 3.71$$

The plots of these together with the actual data are shown in Graphs I, II and III. From the Graphs it will be noted that for ewes, the gland weight per unit of body weight is considerably greater than that for rams which in turn is greater than that for castrates. The response of gland weight to increasing body weight is much nearer linear with rams and castrates than is the case with ewes, so that the sex differences of gland weight, for body weights of approximately 60 pounds, are much greater than those where the body weights are around the 80 pound mark.

The mean adrenal weights adjusted for differences in body weight in the three groups of animals for an overall average body weight of 66.80 pounds (see Appendix) are as follows:

a) 5.026, for castrates,
b) 5.50G for rams,

c) 6.21G for ewes.

These may be compared with the unadjusted means of:

da) 5.09G for castrates,

b) 6.36G for rams,

c) 5.82G for ewes.

Analysis of covariance (see SNEDECOR, page 395; also Appendix) shows that the difference of the adjusted means is highly significant and that in all three classes of animals the variations in slopes of the linear regressions used in the covariance analysis are also significantly different. The actual elevation of the regression lines has little meaning unless the lines are parallel except within the range of weights used. The analysis of covariance does however confirm that the differences in slope and elevation mentioned previously may be regarded as statistically highly significant.

Thus it can be said that the fitting of orthogonal polynomials and the analysis of the covariance for the series of adrenal weights and body weights shown in Tables V, VI and VII, indicate that the weight of the female gland (per unit of body weight) is consistently higher than that of the uncastrated male gland, which in
turn is heavier than the castrated male gland. The difference in weight between coeval female and male glands is most marked in animals of approximately 60 pounds body weight. Thereafter there is a gradual decrease in glandular weight difference as body weight approaches 80 pounds.

IV. Position of adrenal glands

The right gland was placed between the medial surface of the anterior pole of the right kidney and the lateral border of the caudal vena cava. Its three borders were ventral, dorsal and lateral. The lateral was blunt and rounded, the ventral and dorsal borders were more sharply defined. The three surfaces were considered as being dorsal, ventral and medial. The ventral was smooth and convex and its anterior half was buried in perirenal fatty tissue.

(Continued overleaf)
fatty tissue. The dorsal surface was slightly convex and separated from the right crus of the diaphragm by a small amount of adipose tissue. The medial surface was narrow, straight and smooth, and at this point, the capsule of the gland was found to be intimately blended with the outermost layers of the caval vein.

The relationships and the surfaces of the left gland were different from those of the right. On an average, the left gland was 15 mm. medial to the upper pole of the left kidney. It was separated from the caudal vena cava and the cranial mesenteric artery by roughly 3 mm. of fatty tissue. The anterior half of the gland was broad and thinner in cross-section than the posterior half which was almost oval. The two surfaces of the gland were dorsal and ventral. The ventral surface was smooth and convex. Its anterior half was covered by peritoneum, while its posterior half was hidden by adipose tissue. The anterior portion of the dorsal surface was found to be irregularly concave, the posterior half, slightly convex. The two borders were medial and lateral, the two poles of the gland, cranial and caudal. It was from the caudal pole of the left adrenal that the adrenal veins were seen to emerge in cases where the capsule of the gland was not in complete contact with the left renal vein (see para. V).

V. **Vascularisation of the adrenals**

The vascularisation of the adrenal gland in the rabbit, rat and cat (HARRISON, 1951), although showing fundamental similarities to that in man (GERARD, 1913; ANSON, CAULDWEL,
PICK and BEATON, 1947), has a much simpler pattern. For example, although the rabbit adrenal is vascularised from the aorta, adrenolumbar and renal arteries, there are only about ten arteries supplying the periphery of the gland in contrast to the fifty or more vessels which supply the human suprarenal. Individual arteries are end-arteries to the adrenal cortex of rabbit, rat and cat (HARRISON, 1951), but because of the profusion of vessels supplying the human gland it is possible that this relationship may not hold for the adrenals of primates and also Sheep. It was thus considered desirable in this study, before looking at the vascular pattern histologically, to determine the number and the source of the arteries supplying the adrenal of Sheep, together with the venous and lymphatic drainage.

A. Arteries. Both adrenal glands of Sheep receive a large number of small arteries which form a rich network of vessels around the periphery of the glands. The two adrenals differ, however, in the number of arteries supplying them and the sources of origin of these arteries (Figs. I, II and III). On the left side the pattern is quite simple, the gland being supplied from three sources:

1) a trunk arising from the coeliac axis. This supplies two or three arteries to the antero-medial aspect of the gland and then divides into a phrenico-abdominal branch and an adreno-lumbar artery (Fig. I). The adreno-lumbar artery then gives off a varying number of tributaries, in so far as has been seen, not more than four, to the anterior and posterior aspects of the adrenal, a continuation
of one of these arteries passing on to the antero-dorsal pole of the left kidney as a renal capsular artery. A further renal capsular artery from the adreno-lumbar trunk was found to supply the ventral aspect of the kidney, finally anastomosing with the termination of vessels arising from the aorta and renal artery direct.

ii) an artery springing from the aorta direct. This vessel appears to furnish in the region of two arterioles to the medial aspect of the adrenal, before passing on to the antero-ventral aspect of the renal capsule to anastomose with the renal capsular vessels arising, as mentioned above, from the adreno-lumbar and renal trunks.

iii) approximately four arteries arising from the left renal artery, either independently or from a common trunk. These supply the ventro-medial region of the gland.

The picture of the vascularisation of the right adrenal is complicated by the fact that there is a well-marked dorsal or superior, and a ventral or inferior supply of arteries to the gland. The ventral supply seems to be derived from two parent vessels. One, arising from the aorta at the level of the right renal artery, passes posteriorly to the vena cava and follows a relatively long course, at first anterior to the right renal vessels, and then in close contact with the right border of the caval vein (Fig. II). It ends by arching over the cranial pole of the gland, anterior to where the gland is attached to the vena cava. This vessel furnishes in the neighbourhood of a dozen arterioles to the medial and dorsal aspect of the
gland. At its lower end, it gives of an ureteric artery and an arched vessel which, lying anterior to the right renal artery, provides a single adrenal twig before finally arching on to the antero-ventral aspect of the renal capsule to anastomose there with other renal capsular vessels.

The other vessel composing the ventral or inferior arterial supply to the right adrenal arises from the dorsal aspect of the right renal artery at the hilus of the kidney and furnishes four or five arterioles to the lower aspect of the adrenal (see Fig. II).

The dorsal or superior supply to the right adrenal is provided from an arterial stem (X in Figs. II and III), which arises from the angle between the aorta and the right renal artery to run anteriorly to the renal vessels before dividing into two or three main branches (Fig. III). The most dorsal of these branches passes superior to the medial pole of the gland, furnishes two adrenal vessels and continues as phrenico-abdominal and lumbar arteries. This vessel corresponds to the adreno-lumbar trunk of the left side. The middle of the three vessels ends by giving five or six arteries to the adrenal, whilst the most ventral vessel provides two renal capsular branches as well as several small adrenal twigs.

A variation was noted in the blood supply to the right adrenal in which the ventral arterial supply was provided from only one source, that arising from the middle of the right renal artery (Fig. IV). In this case, the ascending portion of the artery gave off fewer twigs to the dorso-
medial aspect of the gland. Branches to the ventro-lateral region of the adrenal, and to the ureter and renal capsule remained more or less as before.

The dorsal (superior) arterial supply, again arising in the angle between the aorta and the right renal artery (X in Fig. IV), also showed a different pattern from that described above. The most dorsal of the three stems arose independently from the right renal artery (Fig. V), and furnished a more profuse supply of vessels to the dorso-medial aspect of the gland, probably to compensate for the poorer vascularization of this region of the gland from the ascending artery of the ventral supply.

B. Veins Both adrenal glands of Sheep have an extremely proficient venous drainage, in common with rabbit, rat, cat and man (Fig. VI). In contrast to the arterial supply, the drainage of the glands is much more constant, and only two variations in the efferent veins of the left side were encountered:

i) The left adrenal gland may frequently lie in close contact with the left renal vein and, on occasions, the latter may even appear to tunnel through a portion of the gland parenchyma (see para. IV). In this case, the central medullary vein of the gland opened directly through several small apertures into the renal vein.

ii) The left renal vein may not come into contact at all with the adrenal but bypass the gland by several millimetres, through retroperitoneal tissue. In this case, two to four slender veins drain the gland, bridging the gap
between the adrenal and the renal vein (see Fig. VI).

In the large number of sheep examined, the venous drainage of the right adrenal was found to be completely constant. As pointed out earlier, the capsule of the gland is intimately blended with the Tunica adventitia of the caval vena cava. The central medullary vein opens on the medial aspect of the gland, directly into the lumen of the caval vein as did the left gland as cited in variation i) above, either by a single large aperture or a maximum of four small ones. It was observed that there was a flap of endothelial tissue guarding the exit of the central medullary vein in all of the right glands examined (Fig. VII). In Section Two of this work it will be shown histologically that this endothelial flap is continuous on one side with the endothelium of the central vein, and on the other with the Tunica intima of the vena cava, while a core of plain muscle separates the two endothelial layers, springing from the Tunica media of the parent vein. The function of this flap, mention of which the writer has been unable to uncover in any of the literature (see Section Two), is most probably to act as a brake on the aspiration of the gland by the flow of blood in the larger vena cava (Fig. VIII). Plate VII illustrates both this valvular apparatus and the several apertures in the caval wall, leading from the central medullary vein of the right adrenal. In none of the dissections was a similar feature seen in regard to the left central medullary vein when drainage of the left gland was achieved by the method cited in variation i)
above. This is a point which could be regarded as lending weight to the argument concerning the function of the right adrenal valve, for the flow of blood in the left renal vein is obviously not as great as that in the vena cava and thus, logically, its aspiratory powers will be less.

The calibre of the central medullary vein of either gland is worthy of note at this stage. According to NEUMANN (1911), the blood supply of the suprarenal is more abundant than that of any other organ in the body. This worker puts it as high as 6 - 7 ccs. per gram/minute, with a hydrostatic pressure of 130 mm. Hg. This is even higher than that of the thyroid, which comes next with 5 ccs. per gram/minute. In such a highly specialised endocrine organ as the adrenal, an adequately high blood level is to be expected, also an extremely proficient venous drainage.

The central medullary veins of the adrenal of Sheep are, in fact, of enormous size for the amount of glandular tissue present. Because of this, little imagination is required to visualise the facility with which adrenal hormones reach the systemic blood. Plate VIII shows the right central medullary vein as seen from the vena cava. The endothelial valve has been removed to illustrate the calibre of the vessel to best advantage. Plate IX illustrates the central medullary vein of the left adrenal in longitudinal section. The gland in this case drained directly into the left renal vein, without interposition of adrenal veins.

In birds and reptiles, the suprarensals have a venous blood supply as well as an arterial, blood being conveyed
to each gland by a "portal" vein which is usually formed by
the junction of two or three intercostal veins. Whilst
no homologous condition exists in Sheep, mention might be
given here to the observations of KUTSCHERA-AICHBERGEN
(1922). This worker described hitherto unnoticed
anastomoses between capsular veins of the suprarenal and the
branches of the hepatic portal vein in the human. A care¬
ful search of several dissections failed to reveal any such
anastomoses in Sheep where indeed, any anastomoses that do
occur are found in periglandular connective tissue (see
Section Two). This observation falls into line with that
of BENNET and KILHAM (1940), who failed to find any portal
anastomoses in injected specimens of Cat.

C. Lymphatics  Literature concerning the
lymphatic channels of the adrenal is very scant. STILLING
(1887) described a superficial plexus of vessels in the
gland capsule, and found a perivascular plexus about the
main trunk of the venous tree, but his observations did not
encompass the macroscopic lymphatic drainage of the gland.

SHARPEY-SCHÄFER (1924) stated that in the human adrenal
there is a subcapsular network of lymph vessels which
communicates with the glands along the aorta and also with
the glands in the posterior mediastinum. SISSON and
GROSSMAN (1953) however, and MAY (1955) agree that it is
only the renal lymph glands in Sheep which receive the
efferent adrenal vessels, and injection by the method given
below confirmed their statements.

The renal lymph glands in Sheep are found at the hilus
of each kidney. There may be one or even a pair present at either hilus, amidst a profusion of haemal glands from which, happily, they can readily be distinguished by their colour. The term 'renal lymph gland' is purely one of convention, for the organs in question are in reality members of the lumbar chain of lymph glands which is situated along the abdominal aorta, posterior vena cava and, in some cases, between the transverse processes of the vertebrae. The renal lymph glands drain either into the lumbar lymphatic trunk which is formed by the efferent vessels of the iliac lymph glands, or into the cisterna chyli at a point just caudal to the aortic diaphragmatic hiatus.

In their textbook of Histology, MAXIMOW and BLOOM (1948) state that in some animals the thyroid gland drains directly into the thoracic or right lymphatic ducts before they enter the jugular or subclavian veins, and recent workers such as JONES (1957) suggest that the hormonal output of the adrenal glands may be carried by the lymphatics in addition to the normal vascular pathways. Discussion of the merits or otherwise of these postulates is outwith the scope of this work, but with a view to determining whether the efferent lymphatics in Sheep offered a new and rapid escape route for adrenal hormones by bypassing the renal lymph glands and draining directly into the lumbar trunk or the cisterna chyli, an injection mass was prepared as follows:

1. Oil Colour Prussian Blue ...... 1G.
2. Turpentine .................. 2cc.
3. Ether ........................ 5cc.
Owing to its volatile nature, it was found to be expedient to prepare only small quantities of the injection mass at a time, and those just prior to use. Experimental injections were made on the carcases of several dogs and one calf to test the efficacy of the injection mass and when this was found to be satisfactory, two Soay-Moufflon cross Sheep were killed by intra-pleural injection of chloral hydrate. As soon after death as possible, the adrenals were exposed and a quantity of the injecting fluid introduced by hypodermic needle into the parenchyma of the glands. Within half an hour or so, traces of Prussian blue appeared in the renal lymph nodes and the periglandular connective tissue and though a careful search was made in both cases, there was no trace of colouring matter in either the lumbar trunk or the cisterna chyli.

This observation that the lymphatics of both adrenals of Sheep apparently drain only into the renal lymph nodes agrees, as mentioned, with that of Sisson and Grossman (1953) and May (1955). A supplementary observation might be mentioned at this point to the effect that whilst the medullary blood vessels seen in paraffin sections of glands which had been fixed in fluids containing chromic acid or one of its salts, often evidenced the brown coloration of the chromaffin reaction, the reaction was never seen in any lymphatics which happened to be present in the same region.

VI. Innervation of the adrenals

There are many descriptions of the innervation of the adrenal glands of a varying number of mammals from the
rabbit and guinea-pig to the human. The exact origin and distribution of the nerve fibres which supply the glands in Sheep however, does not appear to have been investigated in any great detail, at least as far as the writer has been able to determine.

Amongst the earlier workers was DOGIEL (1894), while later work on adrenal innervation has been reported by ALPERT (1931), PINES and NAROWTSCHATOWA (1931), STOKR (1935), HOLLINSHEAD (1936) and others. Minor differences of detail are to be found in the observations of these writers concerning the distribution of adrenal nerve fibres after arrival at the gland. It is the intention however, to deal with these in Section Two, and confine the present observations to the macroscopic innervation of the glands.

MITCHELL (1953) states that, relative to their size, the suprarenals in the human have a more profuse innervation than any other visera. Cursory inspection of dissected material indicates that this might well be so in Sheep, for in the connective tissue adjacent to the adrenals of this animal there are many bundles of nerves which, by the osmic acid technique, can be shown to contain both myelinate and amyelinate fibres. Whilst a few of these nerve bundles are obviously not concerned with adrenal innervation, some being directed to the renal plexuses for instance, they are, almost without exception, derived from the sympathetic division of the autonomic nervous system, either directly through the splanchnic nerves, or indirectly through the bilateral coelsico-mesenteric ganglion.
a) **Splanchnic nerves**  
In dissected specimens, the greater and lesser splanchnic nerves can be identified as they bend round the dorsal surface of the diaphragm, lateral to the crura.

i) **Greater splanchnic:** The greater splanchnic nerve arises from the thoracic part of the sympathetic trunk, in the region of the sixth to the thirteenth thoracic ganglia. It enters the abdomen between the lumbar part of the diaphragm and the psoas minor muscle, sends a branch to the adrenal, then continues to the homolateral coeliacomesenteric ganglion.

ii) **Lesser splanchnic:** The lesser splanchnic nerve arises from the sympathetic chain immediately caudal to the first lumbar ganglion. It consistently bifurcates a few millimetres after its origin and, as is the case with the greater splanchnic nerve, the two lesser splanchnic branches make their appearance after passing between the lumbar part of the diaphragm and the psoas minor muscle. Immediately on emergence, several filaments are given off which supply the adrenal direct, the two trunks then continuing to the coeliacomesenteric ganglion of the same side.

The origin of the left greater and lesser splanchnic nerves, and their termination in the left coeliacomesenteric ganglion are shown in Fig. IX.

b) **Coeliacomesenteric ganglia**  
The coeliacomesenteric ganglia, which theoretically can be divided in Sheep into a bilateral coeliac ganglion and a unilateral cranial mesenteric ganglion, are paired, elongated structures
about 50 mm. long and 10 mm. wide. They are placed medial to both right and left adrenal glands. The left ganglion lies on the celiac axis about 10 mm. from its origin. Its long axis is directed cranially and ventrally. The right ganglion lies partially on the medial wall of the caudal vena cava and partially on the base of the celiac axis, and, as with the left ganglion, it is inclined in a ventro-cranial direction. Both ganglia are intimately interconnected by a delicate plexus of filaments whilst numerous efferent filaments pass outward from the ganglia to the homolateral kidney and adrenal. Both right and left ganglia receive fibres from the greater and lesser splanchnic nerves of the same side.

The nerves which approach the adrenal capsule through periglandular fat and connective tissue can be shown by staining with osmium tetroxide to be, with very few exceptions, myelinated, and, as is shown in Section Two, these pre-ganglionic sympathetic fibres can, by serial section, be traced thus myelinated through the capsule and cortex to their endings around the chromaffin cells which can be considered as homologous with post-ganglionic neurons. Similarly, the few post-ganglionic fibres present in the periglandular region can be followed through the capsule to their terminations around the adrenal blood vessels.

Thus, if the majority of nerve fibres entering the adrenal are pre-ganglionic, and only a few are post-ganglionic in character, the question of the origin of both
these types of fibres immediately arises, for the adrenal filaments springing directly from the greater and lesser splanchnic nerves do not in any way account for the number of myelinated fibres that can be seen in silver impregnated sections of the gland. Conversely, the number of anyelinated fibres to be observed in the adrenal are far less than can be related with the post-ganglionic fibres which approach the gland from the coeliaco-mesenteric ganglia. It can be seen from Fig. IXa which is a diagramatic representation of the nerve supply of the left adrenal, that, as already remarked, pre-ganglionic fibres are given off to the gland from both splanchnic nerves, and apparently post-ganglionic fibres reach the gland from the left coeliaco-mesenteric ganglion. Regarding the latter, the term "apparently post-ganglionic" is used intentionally, for it follows logically that if there are fewer anyelinated fibres within the adrenal parenchyma than there are efferent coeliaco-mesenteric filaments approaching the gland, some of the splanchnic fibres which help in the make-up of the coeliaco-mesenteric ganglion (see Fig. IX) must traverse the ganglion without synapsing. Similarly, it follows that post-ganglionic fibres which supply the adrenal vessels within the gland parenchyma will be those fibres which have behaved in the more usual manner by synapsing within the ganglion.

Fig. X illustrates these two points schematically. For the sake of clarity, the coeliaco-mesenteric ganglion has been divided into a bilateral coeliac ganglion and a
unilateral cranial mesenteric ganglion all of which are intimately connected with each other. Pre-ganglionic filaments are received by the adrenal in two manners:

a) directly from both the greater and lesser splanchnic nerves,

b) indirectly from splanchnic fibres which fail to synapse in the ganglia.

Post-ganglionic adrenal fibres arise from splanchnic fibres which synapse in the usual manner in the ganglia.

**Discussion**

The adrenal glands of Sheep are paired structures lying in the region of, but not surmounting, the anterior pole of either kidney and for this reason, it is the author's opinion that they are more correctly referred to as "adrenal" rather than "suprarenal" glands.

Knowledge of the weights of organs is often of great help in estimating functional status and especially is this true of the endocrine organs in general. It was pointed out by Huxley (1932) that in post-puberal life, the adrenal does not grow isometrically with the body weight. As Jones (1957) indicates, the weights of the adrenal probably bear a more direct relationship to the surface area of the animal rather than to body weight. Be this as it may, Donaldson (1924) produced very useful reference tables for the albino and Norway rat which show the weight of the adrenal increasing regularly, pari passu with age, to a plateau. These tables appear to provide a rough general-
isation for eutherians in general, and the observations made in this Section on the weight of Sheep adrenals do not in any way contradict Donaldson's findings. The weights of female glands for instance, increase regularly with increase in body weight till there is a gradual levelling out at the 80 pound mark. This is equivalent to the plateau Donaldson recorded in the adrenals of rats, and if Sheep of body weights upward of 80 pounds had been available it is highly probable that this plateau, which is co-
etaneous with the start of the fully mature adult life of the animal, would have been much more obvious.

The weight of the adrenal gland, in addition to varying with age, also alters in accord with such physiological episodes as oestrus and pregnancy. Despite these temporary fluctuations however, it has been established that in some eutherians the relative and absolute weight of the adrenals in the female is greater than in the male. In the male mouse for instance, the adrenal weight is between 57 and 77 per cent of that of the co-eval female (Jones, 1948 and 1955), and likewise in the rat (Jackson, 1913; Hatai, 1914; Donaldson, 1924), and in men, where Swinyard (1940) found that the average volume of the white female adrenal exceeded that of the white male by 10.1 per cent, that of the negro female by 19.3 per cent and that of the negro male by 31.4 per cent. This sexual dimorphism which logically must depend to some extent on gonadal influence, occurs also in Sheep though apparently not in Dog, where Baker (1937 and 1938) found little difference between the adrenal weight of
the mature male dog and that of the female in anoestrus.

The absence of gonads, though not normally associated with a change in adrenal function, does result in a change in size of adrenal cortex, particularly in those animals which display post-puberal sexual dimorphism (JONES, 1957). Castration of the rat for instance, results in an increase of adrenal weight, tending toward that of the female. This same phenomenon can be observed in Sheep the increase in weight of the gland being seemingly dependent on the age of the animal at gonadectomy (see Section Five). Many of the histological changes in the gland, consequent to castration appear to turn on the Zona Reticularis for, as is shown in Section Five, the Reticularis increases in thickness in castrated animals. Gonadal hormones reverse this change, so that injection of testosterone in castrate Sheep results in a diminution of adrenocortical size or, as can be expressed another way, a bias from female glandular weight toward uncastrated male glandular weight.

The general plan of the blood and nerve supply of the adrenals of Sheep is similar to that in other eutherians. The glands receive branches from all the main arteries which pass near them and drain into the local major veins. The vascularisation of the right adrenal differs from that in Cat (BENNET and KILHAM, 1940) in being more complex than that of the left, and in having a distinct dorsal and a ventral arterial supply. This however agrees with the findings of GERSH and GROLLMAN (1941) in the rat, and of ANSON, CAULDWELL, PICK and BEATON (1947) in man.
That the majority of nerve fibres supplying the adrenal are pre-ganglionic and can be traced thus pre-ganglionic to the medulla (see Section Two) seems to vindicate the hypothesis of MITCHELL (1953) and others that the chromaffin cells act as post-ganglionic sympathetic neurons. Post-ganglionic fibres arrive at the gland after synapsing in the coeliacomesenteric ganglion, but why some splanchnic fibres, especially when filaments are given off directly to the gland from both the greater and lesser splanchnic nerves, should traverse the coeliacomesenteric ganglion and emerge to approach the adrenal still with their myelin sheaths, is a question that remains open to surmise.

Summary

The macroscopic structure, blood and nerve supply, and lymphatic drainage of the adrenals of Sheep have been studied in this Section of the work. This has yielded the following observations:

1. Both glands are different and definite enough in shape as to be recognised as either from the right or the left side.

2. The weight of the female gland, both relative and absolute, is greater than that of the co-eval male, and that of the male greater than that of the castrate. The difference in weight of the male and female gland is most highly marked at a body weight of 60 pounds but diminishes at a body weight of 80 pounds.
3. The arterial supply of the adrenal is derived from the aorta, coeliac axis and renal arteries. The right gland has a more profuse vascularisation than the left being derived from dorsal and ventral arterial supplies. Variations occur in the arterial supply of the right gland consistently enough to be considered normal.

4. The right adrenal drains directly into the caudal vena cava through a valved opening. The left adrenal drains into the left renal vein either directly, or through two to four slender veins.

5. The efferent adrenal lymphatics drain directly into the renal lymph nodes.

6. Innervation of the adrenal is achieved by both pre- and post-ganglionic fibres. Pre-ganglionic fibres are derived directly from the greater and lesser splanchnic nerves and indirectly from the coeliaco-mesenteric ganglion. Post-ganglionic fibres are derived in toto from the coeliaco-mesenteric ganglion.
At the outset of any microscopical investigation of any adrenal gland, there are two major problems which immediately beset the observer. The first of these is that when an adrenal cortex is examined histologically, it is at once apparent that there is considerable variation in the appearance of sections taken from the glands of different animals belonging to the same species, and on occasions, there is even variation in sections taken from different portions of the same gland. As was remarked in Section One of this work, many of these variations can be correlated with the age and sexual condition of the animal in question, but in order to preclude any confusion, the appearance of the adrenal cortex of a typical uncastrated male Sheep (Soay-Moufflon cross) will be described here as its zones may be observed in sequence from the capsule to the medulla. Reference will, of course, be made to observations on the glands of other animals at the same time.

The other problem which arises during morphological study of the adrenal cortex is one of subdivision of the cortex into zones, for it is certain that, in the light of present day knowledge, ARNOLD'S (1886) division of the region into Zona Glomerulosa, Zona Fasculata and Zona Reticularis is totally inadequate.

Since the days of this investigator's Glomerulosa
(dim. of Lat. glomus, a ball), Fasciculata (dim. of Lat. fascia, a bundle), and Reticularis (dim. of Lat. rete, a net), STARKEL and WĘGRZYNOWSKI (1910) for instance, have described the presence of a "foetal" cortex in the adrenals of human foetuses and infants. Whilst these observers noted that this zone degenerates almost completely during the first year of life, a similar zone was found in the adrenal of Cat by DAVIES (1937), and in the adrenals of primates and the larger Felidae by HILL (1930 and 1937).

In 1926, HOWARD-MILLER described a peculiar "X-zone" in the adrenals of young mice. This "X-zone" disappeared during pubescence in males and in females at the time of first gestation. In 1937, the same author described the deepest layer of the cortex in young rats as the "juvenile cortex" and though it did not degenerate but 'changed' into the Zona Reticularis, he believed it to be similar to the "X-zone" in mice.

More recently, JONES (1949) found that "X-zone" is maintained by hypophyseal gonadotrophic hormones and subsequent workers such as REISS and HALKERSTONE (1950), NOVIKOFF (1951) and NICANDER (1951) have designated this as the "androgenic zone".

ROTTER (1940) gave a similar explanation of the behaviour of the "foetal" cortex in man, stating that the zone appears to be under the influence of the gonadotrophic hormones of the placenta and therefore quite logically degenerates after birth.

GROLLMAN (1936), BROSTER et al. (1938), BLACKMAN (1946),
STIEVE (1947) and others consider the adult Zona Reticularis to be genetically related to the "foetal" cortex and to produce sexual hormones, especially androgens.

In view of all this evidence therefore, and by virtue of the fact that during the preliminary stages of this work it was found that Arnold's three zones did not appear to be sufficient for a rational subdivision of the cortex in Sheep, the following classification was considered to be the most appropriate (cf. NICANDER, 1952):

- Zona Glomerulosa
- Zona Intermedia
- Zona Fasiculata (outer Fasiculata
  inner Fasiculata
- Zona Reticularis.

The intermediate zone as applied in this work corresponds to the "sudanophobic zone" (REISS et al., 1936), "transitional zone" (GREEP and DEANE, 1947), "zone of compression" (MITCHELL, 1948), or "boundary zone" of the rat adrenal cortex, as noted in the literature.

The designation "juxtamedullary zone" was applied both by GOORMAGHTIGH (1922) and NICANDER (1952) to a layer of cortical cells possessing other morphological characteristics than the cells of the Zona Reticularis, and situated within the latter zone in the horse, but no such cellular layer was observed in the adrenal cortex of Sheep.

**General considerations.**

In common with those of other Eutheria, the adrenals of Sheep are composite glands consisting both functionally and
structurally of two distinct parts, the cortex (interrenal tissue) and the medulla (chromaffin tissue). In the fresh state, the cortex appears light in colour in contrast to the darker tone of the medulla. This difference can be accentuated by immersing the gland in the following solutions:

i) Chromic acid or its salts, when the medulla takes on a brown coloration (Chromaffin or Phaeochromic reaction).
ii) Ferric chloride, when the medulla takes on a greenish tint (Vulpian reaction).
iii) Iodine, when the medulla becomes deep yellow in colour.

Whilst a case of bi-lobing of the left adrenal with associated absence of medullary tissue has been reported in Sheep by Stokes (1957), during the course of this work it was noted that the ratio of cortical tissue to medullary tissue seemed to vary in the different species of Sheep examined. Under the dissection microscope, the adrenal glands of a Merino Sheep for instance appeared to have a considerably higher proportion of medullary tissue than the glands of Soay or Blackface animals. Such was the scarcity of pure-bred material however, that observations on cortico-medullary ratios in Sheep could not be pursued, but Jones (1957) states that "the proportion of cortex to medulla is different in different species and, in a given species, the amount of cortex depends very much on the functional state of the gland". Jones based his observations on the findings of:-
a) DONALDSON (1928) who noted that in albino rats, the cortex and medulla weighed 30.3 mg. and 2.8 mg. respectively in males, and 47.1 mg. and 3.1 mg. respectively in females, and

b) BAKER (1937) who put forward that in Dog, 17.5 per cent of the whole adrenal is medullary tissue in the mature male and 18.6 per cent in the female in dioestrus.

As with cortico-medullary proportions, variations in the histological appearance of the cortices in different eutherians are stated by KOIMER (1918) and NICANDER (1952) to be merely alterations of a common ground plan. In Sheep, there is a varying predominance of the three major zones of the cortex and in pure-bred animals at least (see below), except in pathological conditions, they are always present. The Zona Fasiculata is the widest zone of the three main cortical divisions, the Zona Glomerulosa takes up a narrow concentric band beneath the capsule, while the Zona Reticularis, subject to much intraspecific variation and stress which will be touched on later, can be relatively wide, though never normally as wide as the Zona Fasiculata. 

*Glomerular variation* (see also Section Three).

The meshes of the cortical framework of the adrenals of the domestic animals contain, for the most part, cell cords or fasiculi. In the peripheral regions of the cortex these cords have a radial arrangement, appearing as the solid cellular columns which epitomise the Zona Fasiculata. As they approach the capsule, these fasiculi arch and link up
with one another, and accentuation of these fascicular arches gives rise to the Zona Arcuata which is characteristic of Horse (and also Dog). In Sheep and Ox, which lack this accentuation, the fasciculi turn parallel to the capsule and, on occasions, this arrangement simulates in section the round cell masses of the Zona Glomerulosa of the human adrenal.

This glomerular pattern is very distinct in pure-bred animals such as Cheviots, Soays, Blackface and Soay-Moufflon crosses (Plates X, XI and XII). It was also noticeable in several of the cross-bred Sheep which were obtained from the Abattoir, but in the majority of these animals, a most remarkable feature of the cortex was the indistinctness or even absence of a recognisable Zona Glomerulosa. In these latter animals, there was on occasions, a slight demarcation of a Zona Glomerulosa in certain regions of the gland, but in other regions, the cellular columns of the Zona Fasiculata appeared to run radially toward the medulla without any sign of subcapsular arching at all (Plates XIII and XIV).

Of the early workers on the adrenal, FLINT (1900) is possibly the only one who attempted a serious examination of the gland in Sheep. He wrote concerning the gland in this animal that "the Zona Glomerulosa shows very little differentiation from the remainder of the cortex". ELIAS (1945) agrees with Flint and goes a step further by stating that "in Sheep, the Zona Fasiculata begins immediately beneath the capsule". Neither of these workers made
reference to the breed of Sheep on which they made their studies, though.

That a definitive Zona Glomerulosa appears to be evident only in pure-bred Sheep was noted from material used in pursuance of this work, but the total number of animals examined in this respect, both pure and cross-bred, was too small to permit any definite postulation as to the cause of the phenomenon. It could be hazarded though that, just as GROLLMAN (1936), BROSTER et al. (1938), BLACKMAN (1946) and STEEVE (1947) etc. consider there to be a genetical link-up between the Zona Reticularis and the "foetal" cortex, so might there be a genetical relationship between the Zona Glomerulosa and some as yet undiscovered factor. If this were so, it may be that this relationship is upset by cross-mating of the different species.

Observations

I. The Capsule

The adrenal gland of Sheep is encompassed by a capsule of considerable and varying thickness. In common with those of most other glands in the body, the adrenal capsule has a basic matrix of typically-staining connective tissue in which is embedded other elements such as vessels, nerves, cells, etc. Whilst the make-up of the capsule is described in detail in Section Three of this work, making some repetition unavoidable, two main features will be dealt with here:

a) Cells As mentioned, the bulk of the adrenal capsule of Sheep is made up of connective tissue in which it
is possible to discern several different types of cell. Spindle-shaped, fibroblast-like cells with long processes are amongst the most numerous present. Round, macrophagelike cells can also be seen in the matrix, and in animals such as Blackface and Blackface crosses which are normally melanodermal, melanocytes or chromatophores can readily be distinguished in the inner capsular layers (Plate XV).

In certain areas of the capsule, especially at the inner border near the Zona Glomerulosa and also in the glandular septae, are cells which differ from those already described in that they are more rounded and have shorter processes while their cytoplasm is more evenly distributed around the nucleus. Whilst no definite nucleolus can be observed in these cells, the nuclei are round or oval, and contain several scattered chromatin bodies. Variations in size of these cells may be quite readily seen in a single section and when treated for lipoids or birefringence, positive results can be obtained from their cytoplasm (see Section Three).

In the majority of glands examined, these cells were, in certain regions of the capsule, aggregated into nodules of varying prominence, some of these nodules even having their own individual fibrous capsule (see Plates LV, LV and LVI). This feature of capsular nodulation is dealt with fully under Corticoadrenal Morphogenesis in Section Three of this work so it will suffice at this point to mention that, from the evidence presented above and in
Section Three, the adrenal capsule of Sheep appears to be bi-zoned, having an inner mesenchymal layer of the cell type just discussed, and an outer fibrous layer (see Plates XLIX, and XLIXa).

b) Fibres Apart from collagenous fibres, there are three main types of connective tissue fibre present in Sheep adrenal capsule. The preponderance of the different types of fibre varies tremendously, and the three types will be described in descending order of density.

i) Reticular fibres. The adrenal capsule is especially rich in reticular fibres (Plate XVI). The arrangement of these fibres, and the way in which they pass inward from the inner surface of the capsule as delicate, fibrous trabeculae to subdivide the epithelial parenchyma into groups or columns, is fairly complex and will be dealt with under the heading "The framework of the adrenal".

ii) Elastic fibres. Elastic or yellow fibres are the second most numerous type of connective tissue fibre present in the capsule of Sheep adrenal. Most modern histology textbooks, when discussing the adrenal capsule, dismiss the presence of yellow fibres by stating that there are but a few embedded in the reticular network. Indeed, when attempting to demonstrate elastic fibres in the capsule by the more usual techniques such as that of Verhoeff, where differentiation of the parenchyma is gauged by the depth of staining of the elastic tunics of any arteries that happen to be present, very few yellow fibres do in fact retain the stain; and those that do, do so only by virtue of their size.
If however the times of differentiation are altered, in effect to 'overstain' any elastin that may be present, very many more fibres become obvious which would otherwise have been decolorised during normal differentiation. Thus by varying the times in Verhoeff's and the Crocin technique, it was shown that, in addition to the scant "large" elastic fibres seen normally, there is an abundance of extremely fine fibres in the outermost layers of the adrenal capsule of Sheep (Plate XVII).

That these fibres play a part in the corticoadrenal migration of cells is most probable (see Section Three), and the fact that they are so fine as compared with the stouter fibres seen normally in the adrenal and in other organs, is quite tenable when one considers that, in general, yellow fibres are present in organs which require to have a certain amount of resilience, viz. the mammary gland, epiglottis, lung etc.

In these organs the amount of 'contractability' required is far in excess of that ever needed in the adrenal; also, in the case of the latter organ, the fine meshwork of yellow fibres would tend to distribute an even pressure over the whole surface of the gland. Both of these features can be linked up with the mechanics of nodular incorporation dealt with, as mentioned, in Section Three.

iii) Muscle fibres. When a section of Sheep adrenal is stained by Masson's trichrome or some such technique for the demonstration of muscle, a few plain muscle fibres are evident in the gland capsule. They are
however, so scant as to be hardly noticeable. The fact that they are present at all though, might well indicate a complementary relationship between them and the elastic network already discussed.

The framework of the adrenal

The framework or "skeleton" of the normal Sheep adrenal is composed entirely of argyrophil or reticular fibres. As mentioned, the capsule consists of a dense network of reticulum which merges with the collagenous fibres of the retroperitoneal connective tissue and, in the case of the right gland, with the Tunica adventitia of the caudal vena cava.

The reticulum of the capsule is continuous with the supporting framework of the gland parenchyma (see Plate XVI). When impregnated by Foot's modified silver technique, the fibres emphasise the general pattern of the cell columns and blood vessels in the cortex. Hence, a section of silver impregnated cortex outlines the traditional Glomerulosa, Fasiculata and Reticularis, instead of a zoning on the basis of cellular structure and function (Plate XVIII).

The reticular fibres enclose groups and columns of cells in the outer cortex. They clearly indicate that the columns of cells, or fasculi, in the outer Zona Fasiculata are continuous with the coils of the Glomerulosa and the cuboidal cells of the Zona Intermedia, as well as with the strands of the inner Fasiculata (see Plate XX). In the Zona Reticularis, each cell is enclosed individually in a basketwork of reticular fibres.
The fibres between the parenchymal cells are finest in the two outer zones. They become gradually more dense as one proceeds centripetally from the outer Fasiculata, until, in the inner Fasiculata and also the Zona Reticularis, the argyrophil fibres are denser and more numerous than anywhere else in the cortex.

In the medulla the converse holds true, for reticular fibres are the least numerous than anywhere else in the adrenal. The anastomosing cords and clusters of medullary cells are surrounded by individual reticular fibres which, when silver impregnated, can be seen to be at least as delicate as those in the outer layers of the cortex.

II. The Zona Glomerulosa

When a definitive Zona Glomerulosa is present in Sheep, as in a Soay-Moufflon ram the adrenal of which is being described at present, the glomerular cells can be seen to be aggregated into groups. These groups are of varying size and they are separated by capillaries and collagenous fibres, the latter of which show a certain amount of variation in coarseness. It was observed that the larger glomerular units seemed to be formed by the more coarse strands of connective tissue which entered the parenchyma from the capsule at greater intervals than the finer strands and, whilst occasional, apparently isolated cell balls which enclose a single capillary can be observed when a gland is sectioned horizontally (Fig. XI), in transverse section, most of these glomerular units can be readily seen to be continuous with the cortical fasiculi.
The cells making up the glomerular units in Sheep adrenal have a distinctly merocrine appearance. They are somewhat larger in size than the cells of the Zona Fasciculata and, even with moderate fixation, they can be seen to be pyramidal or prismoid in shape.

The cell nuclei vary in shape from oval to spherical with intermediate forms evident. They are large and, whilst also being basophilic in reaction, they are, on the whole, darker staining than the nuclei of the fascicular cells. One or two nucleoli are always present, as are a few chromatin masses (Plate XIX).

Glomerular cells in Sheep always appear to show a fair amount of cytoplasm and, after using routine techniques involving the use of fat solvents, this cytoplasm is often vacuolated. The distribution of cortical lipoids is dealt with in Section Three, and the presence of cholesterol under the heading "Birefringence in the adrenal" in Section Five, so it will suffice at this point to mention that this vacuolation is quite to be expected, for the Zona Glomerulosa in the adrenal of Sheep is heavily laden with fat (see Plates I and II).

When stained with haematoxylin and eosin, the cytoplasm between the vacuoles displays different degrees of eosinophilia, but this eosinophilia is never pronounced. After treatment with Masson's trichrome, the cytoplasm has a reddish, granular appearance, but in no case were the "azo-carmine" granules of NICANDER (1952) noted. According to this worker, large, spherical granules which have an
affinity for the azo-carmine of the Asian technique are present in the Zona Glomerulosa of Horse, Ox, Sheep and Pig. Even after treatment with VINE'S (1938) acid-fuscin-ponceau, and Einarson's galloycyanin chrome-alum (after ROSSIS, 1948) however, both of which techniques Nicol and recommends for the demonstration of "azo-carmine" granules, there was no evidence of their presence in any of the Sheep adrenals examined during this work.

The overall colour of the Zona Glomerulosa after routine staining then seems, quite logically, to depend to a certain degree on the amount of vacuolation in the glomerular cytoplasm. In most of the techniques used, mitochondria could be seen as short, stout rods, and when vacuoles were also present in the cytoplasm, these mitochondria appeared to be arranged in the interstices. This however, was not so in all of the glands examined for, after staining with Masson's trichrome for instance, mitochondria were sometimes completely absent. Whilst no evidence of a relationship between the two could be found, variations in vacuolation of the glomerular cytoplasm might well be one of the factors which influences the appearance of mitochondria, for MILLER and RIDDLE (1942) suggest that there is an inverse correlation between the two.

III. The Zona Intermedia

That a layer of cells whose morphological characteristics appear to be intermediate between those of the Zona Glomerulosa and the Zona Fasiculata exists, and which is located between these two zones, has been more or less
established in the cat by Bennet (1940), by Nicander (1952) in Horse, Ox, Rabbit and Guinea-pig, and in the rat by Jones (1957). This layer is generally referred to as the Intermediate Zone of the adrenal cortex.

In Sheep, the second principal zone seen as one passes inward from the capsule to the medulla, is made up of cells less rich in lipoid than those of the Zona Glomerulosa, but nonetheless showing as a bright red band in Sudan IV preparations, and a blue band in Sudan Black B sections. For the purposes of this work, this band or zone has been termed the "Outer Fasiculata" (cf. Bennet's "secretory zone"), being located as it is, in the outer half of the Zona Fasiculata proper.

Study under oil, of sections which have been treated for differential staining of connective tissue, reveals that, in Sheep, this outer fascicular zone is separated from the Zona Glomerulosa by an extremely narrow, extremely variable band of cells which are cuboidal in shape (Plate XX). These cuboidal cells appear to be the equivalent of the "presecretory zone" of Bennet, and the Zona Intermedia of other workers and, whilst use of the terms "presecretory" and "secretory" tend to prejudge the issue as to the function of the cells of these zones, by careful study it can be seen that the cells of this Zona Intermedia in Sheep bear a resemblance to their neighbours in the Zona Glomerulosa and Outer Fasiculata (see Figs. XII and XIII).

At the inner boundary of the Zona Glomerulosa, the high, prismoidal cells become quite abruptly cuboidal in
shape, and their nuclei become somewhat smaller and more basophilic than those in the Zona Glomerulosa. Whilst still retaining the staining characteristics of the glomerular cells (at least at the glomerular boundary), these cuboidal cells appear to be arranged in narrow, horizontally anastomosing cords, separated by sinusoids and connective tissue fibres which are often as coarse as those found in the Zona Glomerulosa.

The width of these cell cords is never great, and it varies much even in a single section of one adrenal. At its widest points however, the boundary between the cuboidal cells and the fasicular cells proper, is readily discernible by virtue of the fact that the boundary cells tend to become more lightly stained and thus more like true Zona Fasiculata cells.

IV. The Zona Fasiculata

In Sheep, the Zona Fasiculata as a whole can be distinguished by its relatively large, polyhedral cells which are arranged in narrow radial cords separated by capillaries and connective tissue. The nuclei of these cells are round in section, they are larger than those of the Zona Glomerulosa and they have one or two nucleoli. Above all, they appear paler when stained than glomerular nuclei (Plate XXI). The abundant cytoplasm of the cells of the outer region of the Zona Fasiculata contains many lipid droplets and, with ordinary techniques, the cells have the characteristic "foamy" appearance which has given rise to the terms "zona spongiosa" and "spongiocytes" often
applied to fasicular cells, especially in the French literature (qv. GUÉYSE, 1901).

The Zona Fasiculata can be indistinctly divided into two sub-zones in Sheep, the Outer Fasiculata and the Inner Fasiculata. For the purposes of this work, the margin of division between these two sub-zones was taken arbitrarily as a line separating the typical outer spongiocyte cells from the inner, much smaller fasicular cells. In very few cases was this observed to be a clear-cut concentric boundary though, for the Outer Fasiculata often dipped into the deeper layers of the Inner Fasiculata and, on occasions, even formed islets there.

i) The Outer Fasiculata  The cells in the Outer Fasiculata stain very lightly with the usual protoplasmic stains. They appear pale and granular. Very close study of well fixed and well mounted paraffin sections seemed to indicate that the granules present in this sub-zone were not in actual fact granules, but 'knots' in a very fine protoplasmic network. It is emphasized that these 'knots' were only apparent in sections which had had optimum fixation. Thus it could be concluded that in poorly fixed preparations, if this network were consistently present, it is broken up, giving the impression of much larger granule size.

ii) The Inner Fasiculata  Inward from the 'spongiocyte zone' of the Fasiculata just described, the cells gradually diminish in size. This could well be due to secretion losses or a condensation occurring in the
cytoplasm of these cells. As previously mentioned, in most cases this cytoplasmic difference was found to be very gradual and only in one or two sections was there a fairly definite line of demarcation between the large, more darkly staining outer fasicular cells and the smaller, inner fasicular cells.

The inner cells stain well with routine cytoplasmic dyes and, whilst they do not seem to contain as many visible lipid droplets as do their immediate predecessors, they frequently contain small basophilic granules which are quite obvious under haematoxylin. These granules seem to have reducing properties since they may be observed after treatment with silver nitrate. Those reducing silver nitrate have been associated by Bourne (1933) with the presence of Vitamin C.

V. The Zona Reticularis

The demarcation of the Zona Reticularis in Sheep is generally quite obvious, the zone first appearing where the radial rows of the Inner Fasiculata cease and, becoming disrupted, surround large blood sinuses. The cells of the Reticularis are the smallest of the whole cortex with the possible exception of those in the Zona Intermedia, and in general, they are characterised by their size and their more acidophilic cytoplasm.

Two types of cell can be distinguished in the Reticularis of Sheep, the so-called 'light' and 'dark' cells of Dostojewsky (1886) and Hoerr (1931). For the most part, the dark cells are smaller than the light cells. They
have excavated contours and there seems to be many mitochondria present in their cytoplasm. The cytoplasm of the dark cells stains intensely with the usual cytoplasmic dyes, and gives a rich black colour with iron haematoxylin for which reason they are often referred to in the literature as 'siderophil' cells.

The light cells are round in section and their nuclei are large, vesicular and lightly staining. The presence of these light and dark cells in paraffin sections of Sheep adrenal might be explained on the basis of lipid emulsification. In the light cells, the protein material which takes up the cytoplasmic dyes is thinly distributed between many lipid spheroids. On the other hand, when the fatty material is present in a few large-sized globules, the continuous phase is denser and gives the appearance of a darkly stained cell. The dark cells are usually seen in small groups which are packed between their lighter staining neighbours.

Whilst observers such as VACCAREZZA (1945 and 1946), and BACHMANN (1941 and 1954), have claimed that necrotic cells occur in the Zona Glomerulosa and Zona Fasciculata of the adrenal cortex of laboratory animals, no worker denies that senescent cells are always present in the Zona Reticularis.

Pyknotic nuclei and cellular debris can be observed near the reticulo-medullary junction in Sheep (Plate XXII). The fact that the phenomenon of pyknosis is present at all, can be correlated with continuity of cortico-adrenal cells
(see Section Three), and it is interesting to note at this point that, as DARLINGTON (1937) suggests, there appears to be a relationship between cellular degeneration in the Zona Reticularis as manifested by the presence of pycnotic nuclei, and what is generally termed 'adrenal stress'.

In Sheep, pycnosis seems to increase in amount according to the method in which the animal was killed. Thus it was observed that in Sheep which had undergone routine slaughter at the Abattoir with all its attendant 'stresses', the dead cell count of the Zona Reticularis was relatively higher than that in animals which had been put down efficiently in the laboratory with the minimum amount of 'adrenal demand' (Plates XXIII and XXIV).

In the Zona Reticularis in Sheep, macrophages are present in the interstitial spaces and on the endothelial walls. Fat near the medulla is, for the most part, limited to the macrophages and sinusoids and is not evident in the glandular cells of the Reticularis.

VI. The Medulla

Fixation of adrenal medullary tissue in general is fairly critical as medullary cells are very prone to shrinkage, especially with fixatives such as Susa. In well fixed preparations of Sheep adrenal however, the medulla can readily be distinguished from the cortex by its central position, smaller extent, irregular arrangement of cells and the presence of the central vein and its conspicuous tributaries. The boundary between cortex and medulla, though very irregular, with islets of cortical tissue appearing at times
within medullary tissue, can also be seen to be very sharp (Plate XXV).

A varying number of cell types can be distinguished in the medulla of Sheep adrenal, of which secretory cells form by far the greatest bulk. These secretory cells are not arranged in layers as are the cells of the cortex. Nor do they have any reaction to fat stains or digitonin. Nevertheless, they have a distinctive appearance. They are columnar in shape and are arranged in anastomosing cords and clusters so that the poles of the cells are in close contact with the blood sinusoids which abound in the region (Fig. XIV).

The cell nuclei stain feebly with haematoxylin. The cytoplasm is loaded with chromaffin granules the reactions of which to chronic acid, ferric chloride and iodine have been stated earlier (Plate XXVI).

In addition to chromaffin cells, there are evident in the medulla of Sheep adrenal, frequent single or grouped sympathetic ganglion cells whose axons seem to end round the chromaffin cells. It was also observed on occasions, that collections of small, round cells with deeply staining nuclei and very little cytoplasm were present. Although these cells bore great resemblance to the sympatho-chromaffin cells which are the forerunners of adult medullary cells (see Section Four), it was thought that they were in actual fact, lymphocytes, for large aggregations of lymphocytes, somewhat resembling the Peyer's patches of the small intestine, had been observed in earlier sections. Whether
or not the few lymphocytes seen normally are connected in any way with these aggregations is problematical, for the presence of such an abundance of lymphocytes in a section was taken to betoken some pathological condition and accordingly all such sections were discarded.

Another notable feature of Sheep adrenal medulla which by virtue of the complete lack of evidence as to its cause, might be considered to be pathological, was the appearance in several sections of large aggregations of adult eosinophils. These eosinophils were arranged around the central vein in clusters of varying density, and on occasions, these clusters were of such density as to obscure the parenchymal cells entirely (Plates XXVII, XXVIII and XXIX).

Eosinophils were found to be present in the medulla of approximately six per cent of all the adrenals examined. They occurred in the glands of animals of both sexes, including castrated males and, though an attempt was made by doing a differential blood count to find a correlation with the physical or the sexual condition of the animals concerned, none was uncovered.

As far as the writer has been able to determine, this phenomenon has been reported only by FLINT (1900) who writes: "In one instance I observed in the medulla of the sheep's adrenal a great number of eosinophilic leucocytes without any evidences of inflammation, and although many sections were cut, the observation was never repeated and the fact remains isolated and unexplained."
The relationship between chromaffin cells and the medullary blood vessels

Whilst the exact distribution of blood vessels in the adrenal of Sheep is dealt with under the heading "Microscopic Angiology of the Adrenal", it is convenient at this point to discuss the relationship or "polarity" between chromaffin cells and medullary vessels.

Among other endocrine glands, a polarity of the cells is obviously present in the thyroid, where one end of the cell presents to blood vessels and the other to the lumen of the follicle. A polarity based on cytological features has been noted in the hypophysis (DAWSON and FRIEDGOOD, 1937), and a similar polarity has been alleged in the parathyroid by COURRIER and HEISS (1922) and ROSCH (1934), which workers regard the cells of the parathyroid as possessing a "nutrient" and an "excretory" capillary. BENNET (1939), in a paper addressed to the American Association of Anatomists, stated that a similar polarity exists in the adrenal medulla of the cat, so, in face of this and the above evidence a series of carmine-gelatine injected sections of Sheep adrenal was prepared with a view to determining whether or not such a polarity exists between the chromaffin cells and medullary vessels in that animal.

With careful study of injected serial sections, it can be clearly seen that the veins of the medulla and their radicals, which are formed by the junction of capillaries from the cortex and the sinusoids of the Zona Reticularis,
penetrate every portion of the adrenal medulla. It is also equally evident that all parts of the medulla are nourished by means of capillaries which have their origin in an arterial plexus situated in the subcapsular region of the gland.

Injected preparations showed that these medullary capillaries and their branches take positions between the ramifications of the venous tree, and in stained injected specimens, where the cells were faintly tinged with haematoxylin or 0.5 per cent Light Green, it was apparent that the ramifications of the capillaries are, in general, separated from the veins by the length of a single medullary cell. In consequence of this, the chromaffin cells along the veins present, in general, one pole to the vein, while the other pole, the nuclear pole, of the cell abuts on a medullary capillary receiving fresh blood from the subcapsular arterial plexus. Thus the medullary cells can be considered to possess a true polarity with respect to the blood vessels, displaying a venous pole which, logically, is the predominantly secretory pole of the cell, and a capillary pole which is presumably nutrient.

This essential relationship between the cells and the blood vessels of the medulla can be readily seen in the medulla of routine paraffin embedded glands whenever the plane of section cutting a vein is favourable (Plate XXX, see also Fig. XV). The relationships are particularly evident when the vessel is cut longitudinally or transversely through a plane passing through or near, the maximum diameter
of the vein. In medullary cells cut in planes other than those parallel to the long axis of the cell, their polarity is less evident in the cells around the smallest terminal venous twigs.

From a careful study of many sections, it appears probable that a minority of the medullary cells fail to abut directly on the wall of a vein, whereas a larger fraction of the cells do not directly contact a capillary. But even these cells which do not impinge directly on a vein or on a capillary may show the typical polarity of the medullary cells in that one end can usually be shown to be close to a vein, whereas the other end will not be far from an arterial capillary.

**Microscopic Angiology of the Adrenal**

**A. Arterial System**

As was remarked in Section One of this work, the blood supply of the adrenal is of extreme significance, for the gland contends with the thyroid for recognition as the most highly vascular organ in the body.

The microscopical topography of the blood vessels in the adrenal was first described by FLINT (1900). Flint used the dog as his subject and such was the detail of his observations that, since 1900, most histology textbooks base the angiology of adrenal glands in general on his findings. Nor does it appear that they are wrong to do so either, for no great variation in the general principles of adrenal vascularisation put forward by Flint has been noted to date in other animals studied.
BENNET and KIHAM (1940) for instance, investigating the blood vessels of the adrenal of Cat, found only minor differences from Flint's picture of the vascularisation of the adrenals of Dog, and GERSH and GROLIMAN (1941) observed that the basis of the blood supply to the adrenals of the rat and mouse was similar to that in the same animal.

In Sheep, the adrenal arteries, as shown in Section One, are derived from three sources, the aorta, the coeliac axis and the renal arteries. These arteries (Figs. I to V) pass to the adrenal, where they abruptly penetrate the capsule and assume a position directly under the connective tissue of the capsule and outside the parenchymal cells of the gland proper (Plate XXXI). Study of serial sections which were made from carmine-gelatine injected glands showed that in Sheep these arteries ramify and anastomose freely under the capsule, forming an extensive subcapsular arterial plexus which invests the whole gland and whence capillaries are given off which supply the capsule and the cortex in the same manner which Flint described in Dog. At fairly regular intervals vessels also leave the subcapsular plexus at right angles to pass radially through the cortex to the medulla. These are the "arteriae medullae" of Flint, and their course through the cortex shows patently that they are independent of both cortical and capsular capillaries which, as mentioned, also spring from the subcapsular arterial plexus. Thus the vessels in Sheep adrenal can be seen to fall into three separate and distinct categories:

1) Capsular capillaries,
ii) Cortical capillaries,

iii) Medullary capillaries.

The arteries of the subcapsular plexus then branch repeatedly and form a multitude of fine twigs which give rise to three independent capillary networks whose destinations are the capsule, the cortex and the medulla.

1. Capsular Capillaries The capillary plexus which supplies the capsule of the adrenal lies in the same plane as does the arterial plexus. When injected it can be recognised as a rich and extensive anastomosing network of small vessels which is interwoven with the capsular connective tissue (Plate XXXII). Veins are present in the capsule, as can be seen in Plate XXXII, and these carry the blood from the capsular capillaries to one of the numerous connective tissue veins located in the neighbourhood of the adrenal.

2. Cortical Capillaries From the subcapsular arterial plexus where its meshes abut directly on the parenchyma of the glomerulosa, innumerable radial cortical capillaries are given off at right angles to pass centripetally into the substance of the cortex. These capillaries embrace the knots of cells in the Zona Glomerulosa, passing inward within the connective tissue trabeculae demarcating the glomeruli and the cortical fasciculi. At the Zona Fasiculata the capillaries assume a straighter course to run parallel with, and adjoining the individual cell columns (Plate XXXIII). There is a certain amount of branching and anastomosis within the fasiculata till, at the boundary
between the fasciculata and the Zona Reticularis, the capillaries widen out and the vascular pattern becomes as plexiform and intricate as the argyrophil network in the same region.

The venous return of the cortex commences within the Zona Reticularis as the capillaries drain into the sinusoidal network of the cortico-medullary junction. These sinusoids lead into the complex and highly-branched venous tree which extends throughout the medulla and ends in the central medullary veins.

3. Medullary Capillaries

The medulla of Sheep's adrenal receives a twofold blood supply, one consisting of venous blood from the cortex, and the other which has been conveyed directly to the medulla by means of radial medullary capillaries. The latter spring, as mentioned above, from the subcapsular arterial plexus, and they run directly to the medulla without branching. Plate XXXIV shows two such capillaries originating in the subcapsular region; Plate XXXV illustrates a single, unbranched capillary entering the medulla.

Once within the medullary parenchyma, the capillaries break up into an extensive bed of thin-walled vessels which eventually drain into the medullary veins. Thus, apart from that in the capsule, all the blood entering the adrenal gland traverses the medulla, a feature of some importance in consideration of the production of adrenaline and nor-adrenaline (COUPLAND, 1953; WEST, 1955).

In respect of the latter point of a double blood supply,
an engaging parallel can be drawn between the adrenal medulla and the liver. Hepatic parenchyma receives arterial blood from the hepatic artery and what might be termed "de-oxygenated" blood from the portal vein, both of which vessels finally terminate in the hepatic veins. The adrenal medulla receives its oxygenated blood from the medullary capillaries, venous blood arriving by way of the cortical capillaries. Drainage of both 'bloods' in this case is attained by the central medullary veins.

**Cortical capillary size.**

Variations in capillary size in the adrenal cortex are interesting in their reciprocal relationship to cell size and content. As shown in Fig. XII, corticoadrenal cells decrease in size as they progress centripetally toward the medulla. The small arteries in the subcapsular network break up at once into capillaries having only endothelial lining and a few reticular fibres for support. These capillaries perforate the gland in large numbers so that each cell in its individual fasciculus appears to have a capillary pole. In fixed, normal tissue the vessel lumen in the region of the Outer Fasiculata is almost obliterated by the 'bulging' of the spongocyto cells. Further inward, where the fasciculi are narrower and the cells smaller, the cortical capillaries increase in size; the endothelium is then in direct contact with the gland cell surface.

In the reticularis, the region of cell degeneration and disintegration, anastomoses between the capillaries or as can be termed now, sinusoids, are quite frequent, and the
cell arrangement appears in sections as a network. These reticular sinuses greatly resemble the sinuses of the liver, for they are lined with both endothelial cells and fixed macrophages. The latter are the so-called littoral cells of MAXIMOW and BLOOM (1948). They have the property of storing intravital lithium carmine, and in heavily stained animals their number appears to be greatly increased (Plate XXXVI).

B. Venous System

The venous drainage of the adrenal should be considered in two parts for there are two separate routes by which de-oxygenated blood escapes from the gland. The first concerns the capsule alone, the second, the cortex and the medulla.

1. Capsular Drainage

Capsular drainage is attained as mentioned, by capsular veins (see Plate XXXII), and these veins, together with the arteriovenous anastomoses which can be seen in the loose connective tissue surrounding the adrenals, provide channels whereby blood which has entered the capillaries destined to supply the capsule can escape without passing through the adrenal vascular bed.

2. Cortico-medullary Drainage

The venous drainage of the cortex commences as we have seen, in the sinuses of the Zona Reticularis, where de-oxygenated blood is collected from the preceding zones and transported over the cortico-medullary junction to the ramifications of the central medullary vein. Venous blood from the medullary capillaries also drains into the central vein, and thence, as shown in
Section One, into the caudal vena cava in the case of the right gland, the left renal vein in the case of the left gland.

**Structure of Medullary Veins**

The structure of the central medullary veins of adrenal glands in general has been the subject of much postulation in the literature, especially in regard to the presence or absence of plain muscle fibres in the vessel walls.

In 1906, FERGUSON stated that all efferent veins in all adrenals are characterised by having smooth muscle fibres in their walls. BARGMANN (1933) however refuted this by presenting a list of animals in the adrenals of which he was unable to demonstrate muscle at all. This worker, it may be observed, considered Sheep as being amongst those animals without a rich musculature in the medullary veins. TRAUTMANN and FIEBIGER (1952) agree with the latter and go a step further by stating that ruminants in general are lacking in adrenal medullary muscle.

Whilst this is contrary to the situation in the human adrenal, where FERGUSON (1906), KOLMER (1918) and BARGMANN (1933) have noted longitudinal plain muscle fibres in the walls of the central medullary vein and some of its larger tributaries, study of sections stained with Masson's trichrome shows that in Sheep there can be no doubt that medullary veins are completely devoid of muscle fibres of any kind.

**Structure of the Right Adrenal Valve**

Whilst muscle fibres may be absent from the medullary
veins of Sheep's adrenal, this is, as is well-known, not so in the case of the caudal vena cava, where longitudinal and circular plain muscle fibres are abundantly obvious. The presence of these fibres and especially the plane in which they run, is interesting in relation to the valvular apparatus which was noted in Section One of this work to be guarding the exit of the right central medullary vein into the vena cava, and which can be seen in horizontal section in Plate XXXVII.

Lying as it does along the direction of blood flow in the vena cava, this valve can be observed to consist of three distinct layers or tunicae. The innermost tunic, facing the lumen of the medullary vein, is constituted by a single layer of endothelial cells which are continuous with those of the wall of the vein. The outer tunic also consists of a single layer of endothelium which this time however, is a continuation of the Tunica intima of the vena cava. Both these endothelial tunics unite at the free border of the valve. The intermediate tunic of the valve is composed purely of plain muscle fibres embedded in a light connective tissue matrix (see Fig. VIII). These muscle fibres, whilst being in the same plane as those of the Tunica media of the vena cava, cannot strictly be considered as being continuous with them though, for they are arranged at right angles to the longitudinal fibres of the media. That is to say, they run with the circular caval fibres, transverse to the longitudinal caval fibres and parallel to the periphery of the valve. Plate XXXVIII
is a microphotograph of a sagittal section taken through the media of the valve. Whilst the majority of caval muscle evident in the section consists of transversely cut circular fibres, it can be seen that the fibres of the valve are arranged approximately parallel to the free border of the valve and at right angles to the small amount of longitudinal muscle present.

C. **Lymphatic System**

It can be said without much fear of contradiction that the adrenal cortex acts as a kind of blood filter, since, like the liver and spleen, the endothelium is phagocytic for lithium and other particulate matter such as trypan blue (COWDREY, 1938). As we have seen, this is particularly the case in the Zona Reticularis where the character of the blood, having nourished the cells of the fasciculata and glomerulosa and having removed the waste products, tends toward the venous side. A logical follow-up to this is that the demand for lymphatics to drain the tissue spaces of the cortex is but slight, and indeed, only a few lymph vessels can be seen in the vicinity of the cortical capillaries.

In the more venous medulla lymphatics can, however, be readily detected, even in routine paraffin sections. They form a network around the central medullary vein and its larger tributaries, eventually draining as remarked in Section One, into the renal lymph nodes.

During the course of this work, it was found that intraperitoneal injection of lithium carmine brought about a vast dilatation of the lymphatic vessels in the adrenal medulla. Whilst the relatively high toxicity of the
injection mass would undoubtedly be an exciting factor, the actual mechanism bringing about the phenomenon is not clear. It is sufficient however at present to remark that intraperitoneal injection of lithium was found to be an excellent method for demonstrating the lymphatic pattern in the adrenal (Plate XXXIX).

**Nervous System**

The manner in which the various parts of the adrenal gland receive their innervation is of importance in throwing light on their functional activity. Yet the exact distribution of the nerve fibres which supply the organ is, to date, not at all clear.

In much of the early literature, attention was focused chiefly on the presence of ganglion cells in the medulla. VircHov (1857), Holm (1866), Braun (1882), MitsuKuri (1882) and Renner (1914) described these ganglion cells as being separate and distinct from the medullary parenchyma, while Leydig (1857), Mayer (1872) and Elliott (1913) did not differentiate between ganglion cells and medullary cells, but considered the medulla as an essentially nervous organ acting as a nerve centre.

On the nerve fibres in the gland relatively little work has been done. Nagel (1836) was the first to describe their distribution, being followed later by Fusari (1891) and Hint (1930). In present day literature there is general agreement that the adrenal cortex lacks a nerve supply (Hollinshead, 1936; Swinyard, 1937; Bennett, 1940, and Creep and Deane, 1949) yet Fusari in his paper emphasized that there
was an abundance of nerve fibres in the cortex as well as in
the medulla. Hirt also described a network of fibres
surrounding the groups of cortical and medullary cells and,
in the case of the latter, penetrating between the cells.
A third and more recent worker, ALFERT (1931), studying
human necropsy material also reported the presence of
anastomosing nerve fibres in the zones of the cortex. As
mentioned, the balance of modern literature has swung in
favour of the adrenal cortex being devoid of nervous elements,
but the conflicting reports of the workers cited above are
valuable for they are an acute reflection of the difficulty
of differentiating between connective tissue and nerve fibres
in the adrenal by the usual silver impregnation techniques
(see Material and Survey of Methods).

The nerves which approach the adrenal through peri-
glandular fat and connective tissue are, as has been remarked
in Section One, almost without exception myelinated, and
many of them can be traced thus myelinated through the
capsule and cortex to the medulla. These nerve bundles
which are not seen to branch, vary greatly in size. They
can be found on all surfaces of the gland, but most
frequently they occur along the medial borders.

There are also to be observed in the periglandular
tissue, cells which are indistinguishable from the large
multipolar cells found in sympathetic ganglia. These
ganglion cells occur either singly or in small groups of
three or four, or even in large ganglia containing ten to
twenty cells or more (Plates XXXII and XL). The cells in
these large ganglia are separated by dense collections of
nerves, and a relatively thick fibrous capsule surrounds each ganglion. Nerve fibres enter the gland either directly or after the interposition of such ganglia along their course. In the capsule a few fibres branch to run in the connective tissue, and frequently along blood vessels, the walls of which they apparently supply. Larger bundles of fibres may run for some distance in the capsule before penetrating more deeply into the gland.

The mode of entrance of these fibres into the cortex is usually an oblique one, either with a blood vessel or independently, and it is obvious that the majority of such nerve bundles found in the adrenal cortex are destined for the medullary portion of the gland, since they run directly through the cortex in distinct bundles which branch only after reaching the medulla (Plates XLI and XLII).

In spite of careful search, no clear-cut example of innervation of the cortex was noted in any of the sheep adrenals examined during this work. Small groups or lone fibres were often traced from the capsule into the cortex. These frequently accompanied blood vessels and were soon lost upon their walls. Others ran independently through the cortex, often winding among the cortical cells. However, in no instance in which these fibres did not accompany blood vessels were they observed to branch before reaching the medulla, although in favourable serial sections such fibres were followed from the capsule to the chromaffin tissue. Here they were lost in the general nerve arborisations about this tissue.
As the nerve bundles reach the medulla they often branch immediately (see Plate XLII), breaking up into smaller trunks, but sometimes they may be traced practically intact for some distance. The various smaller nerve trunks branch and rejoin each other in a bewildering fashion, eventually forming a delicate plexus in the connective tissue around the medullary cell groups, with still more delicate fibrils present between the cells of a group. Indeed, an individual cell in such a group often gives the appearance of being entirely enmeshed by nerve fibres (Plate XLIII).

In addition to the fibres ramifying amongst the chromaffin cell groups, there are fibres which run in contact with the medullary blood vessels. Many of these may eventually leave the vessels to run amidst the chromaffin cells, but others are lost upon the vessels. In favourable sections, occasional nerve fibres were seen actually within the wall of the vessels, apparently concerned therefore with the innervation of the vessels in question.

The largest number of nerve cells counted in the medulla in the course of this investigation was nine. There were several sections in which only a single nerve cell was found (Plate XLIV), and others in which no cells were seen. When nerve cells are present in the medulla, they are found about the terminations of myelinated fibres and by the Bielachowsky technique, the neurofibrils may be seen to surround the ganglion cells and apparently pass in under the cell membrane to end in the cytoplasm.

**Nerve endings**

No nerve endings of any kind were noted in the cortex,
and whilst free nerve endings may occur amongst the medullary cells, it is difficult to be certain of this point. Apparent free endings were observed in several sections, but it was impossible to determine to what degree this appearance may have been due to variations of impregnation rather than to true ending of the fibres in question. In no case however, were definite intracellular fibrils observed.

Terminations of nerve fibres in the form of bouton-like swellings are prominent in the medulla. More than one of these swellings or bulbs may be present upon the same nerve fibre (Fig. XV), somewhat resembling small nerve cells. Closer inspection reveals that the bulbs are not nucleated however, but consist rather of a varicosity of neurofibrils encapsulated in much the same way as is the case in the end bulbs of Krause (Plate XIV and see Plate XLIII). ALPERT (1931) reports the finding of similar end bulbs in the medulla of human adrenals. Because of this and the above observations there can be little doubt that these swellings do represent nerve endings which, by virtue of their situation, could logically be said to be sensitive to changes in pressure.

Discussion

Most of the older literature on the histology of the adrenal cortex of domestic animals is included in a paper by GUNTHE (1906). This author designated the inner layers of the Zona Fasculata as Zona Reticularis, which he consequently considered the widest zone of the cortex in some species. The presence of a Zona Glomerulosa in Sheep adrenals was
denied. MILON (1903) described a "zone de transition" between the Zona Glomerulosa and the Zona Fasiculata of the adrenal of Dog, and also distinguished an Outer and an Inner Fasiculata. da COSTA (1913) found that ARNOLD'S (1886) three zones were distinct in Sheep adrenals, but HILL (1930) found no distinct glomerulosa in this animal. ELIAS (1948) stated that the Zona Glomerulosa is indistinct or absent in places in Sheep adrenal.

During the course of this study, a glomerular zone was found to be present without exception in the adrenals of pure-bred Sheep but lacking in a high percentage of cross-bred animals. Whilst not enough observations were made on this account to enable definite conclusions to be drawn, an obvious inference from the lack of a Zona Glomerulosa in poorly-bred Sheep is that some genetical relationship exists between this zone and some as yet undiscovered factor which is upset by the cross-mating of different breeds of Sheep.

For the purposes of this section of the work, the adrenal cortex of an adult male Sheep was divided into five zones, based on distinct cytological differences in the cells of each zone. This division of the cortex into glomerulosa, intermedia, outer fasiculata, inner fasiculata and reticularis has to be modified when applied to the adrenals of immature animals where a "foetal" cortex is present and where cortical zones are often not evident till quite late in post-natal life. Preliminary observations of a number of neo-natal animals indicated that it was advisable to divide the cortex of such animals into Arnold's
classical three zones. Nevertheless, whether division into three or five zones is in order, it is apparent, from study of adult Sheep, that a zoning which is referable to the secretory phases of cortical cells does not coincide exactly with the current divisions into a glomerulosa, fasiculata and reticularis.

In Sheep, the Zona Glomerulosa when present, was found to be different from the inner cortical layers as regards shape, size and staining properties of the cells, and a Zone Intermedia, consisting of a band of narrow cuboidal cells with small, darkly staining nuclei and which lay between the Zona Glomerulosa and the fasiculata, was observed to a greater or lesser degree in most glands studied. This zone is equivalent to MULON'S (1903) transition zone in the adrenal of Dog, and MITCHELL'S (1948) zone of compression observed in the cortex of the rat. The origin of this intermediate zone could not be determined but it seemed to be closely related to both the glomerulosa and the fasiculata. Further studies are required to determine the nature of this zone.

The columnar cells of the Zona Fasiculata arranged in radial rows, were observed to have nuclei which were round in section and had one or two nucleoli. The lipoidal pattern in the fasiculata enabled this zone to be divided into two sub-zones, the Inner and Outer Fasiculata. The lipides in the latter sub-zone give the cells their characteristic spongiocyte appearance and in most glands examined the distinction between the two sub-zones of the
cortex was more or less acute.

In all sections, the Zona Reticularis showed varying degrees of cellular degeneration which was manifested by karyolysis, karyoklasis and pyknosis. The adrenals of animals which had been shot and bled at the Abattoir showed a higher proportion of degenerating cells than Sheep which had been killed in the laboratory. This observation falls into line with that of DARLINGTON (1957) who suggests that there is a relationship between cellular degeneration in the Zona Reticularis and what he terms 'adrenal stress'.

The 'adrenal stress' created by reflex action when an animal is in the process of bleeding to death is self-evident. It is logical then to think that the adrenal, violently trying to combat the effects of continued hemorrhage with massive hormonal outpourings, must inevitably suffer some wear and tear which would be manifested in the normal zone of degeneration, the reticularis.

Such an argument of reflex adrenal stress being created when an animal is dying can be equally well applied to laboratory slaughtered Sheep. In these cases however, death supervened much earlier than in those animals killed in the Abattoir. Consequently the duration of the adrenal stress was not so prolonged and thus, wear and tear of adrenal parenchyma not so evident.

That the adrenal capsule should be bi-zoned is a first essential if the hypothesis of corticoadrenal cellular migration is to be entertained at all. In almost all of the glands examined, a definite layer composed of cells
other than those belonging to the connective tissue matrix, was noted in the capsule, in close approximation to the outermost merocrine zone of the gland. Further evidence of the presence of this cellular zone is presented in Section Three so it will suffice at this point to mention that a similar zoning has been observed in the adrenal capsule of the guinea-pig by Hoerr (1931), of the human by Gruenwald (1944) and of the horse by Elias (1948).

It is possible also to correlate the presence of numerous elastic fibres in the capsule with cortico-adrenal migration. These fibres, hitherto undescribed as far as the writer has been able to discover, are extremely plentiful and so fine as to escape definition with routine elastic tissue stains. When the times of differentiation in Verhoeff's technique are modified, in effect to vastly over-stain any normal elastin that may be present, these fine yellow fibres are not decolorised and are thus quite obvious. The fine meshwork which they form around the periphery of the gland will tend to distribute an even and gentle pressure over the whole surface of the gland, a pressure which logically will assist the inward migration of cortical cells (see Section Three).

The concept of polarity of cells in the adrenal medulla was first set forth by Hulgreen and Andersson (1899) and though these workers failed to present any evidence to back their statement, the significance of the chromaffin cells characteristically bordering on a venous radical at one pole of the cell, with the nuclear pole directed toward a
capillary, is of extreme interest. Undoubtedly it is a prime factor in attaining maximum secretory efficiency of the chromaffin cells.

The medullary veins contain blood which has passed through both cortical and medullary capillaries and is hence depleted of much of its oxygen and nutrient material, whereas the capillaries are well supplied with fresh arterial blood at a hydrostatic pressure higher than that in the veins. Thus it would be logical to assume that the capillary supplies the cell with most of its metabolic raw materials, and the nuclear pole of the cell directed toward the capillary could be regarded as the "nutrient" pole of the cell. Similarly, the venous pole would logically be that surface of the cell through which most of the secretion passes, and it could be termed the "secretory" pole.

As pointed out earlier, COURRIER and REISS (1922) regard the cells of the parathyroid as possessing a "nutrient" and an "secretory" capillary, but they fail to state whether the alleged two types of capillaries are distinct entities, whether they receive their blood from distinct types of vessels, or whether they contain blood in different states of oxygenation. Hence the cells of the adrenal medulla must be regarded as the only endocrine cells known to display a polarity with respect to two different types of blood vessels, and where the predominant nutrient function of a vessel at the nuclear pole of the cell and the secretory function of the vessel at the opposite pole can be inferred from injected preparations.
The basic features of the blood vessels of the adrenal of Sheep are similar to those in Dog as described by FLINT (1900), or in the human, as cited by von EHREN (1902). Three independent capillary networks arising from a large subcapsular arterial plexus were noted in carmine-gelatine injected serial sections. These capillary networks supply the capsule, the cortex and the medulla respectively, the last named capillaries travelling centripetally through the cortical tissue to branch only after reaching the parenchyma of the medulla.

Venous drainage of the capsule was noted to be accomplished by capsular veins and connective tissue arterio-venous anastomoses. Both cortical and medullary capillaries empty into the ramifications of the central medullary vein.

In Sheep, as in many other species (BARGMANN, 1933), the adrenal veins of the medulla have no demonstrable muscle fibres in their walls, but the central medullary vein of the right gland possesses, as far as the writer has been able to determine, a hitherto undescribed valvular apparatus. This consists of two layers of endothelium separated by a corium of transversely arranged plain muscle fibres. The valve is directed along the blood flow in the vena cava. Its inner endothelial lining is continuous with that of the central medullary vein while the outer is continuous with the Tunica intima of the vena cava. The function of the valve is most probably to prevent aspiration of the adrenal gland, certainly the medulla anyway, by the flow of blood.
in the larger parent vein. The fact that the corium of the valve is constituted by plain muscle fibres is quite tenable when one considers that the outpouring of adrenaline by the chromaffin cells will cause contraction of that muscle and accordingly a widening of the lumen of the central medullary vein which, logically, will facilitate the passage of medullary hormones into the bloodstream.

The lymphatic system of the adrenal of Sheep is similar in principle to that described in Dog by KUMITA (1909). The cortex has a few sparse vessels, the medulla an extremely profuse supply. Intraperitoneal administration of lithium carmine was observed to cause vast dilation of the lymphatics in the adrenal, especially those in the medulla. Whilst the mechanisms involved are not clear, intraperitoneal injections were used for routine demonstration of the lymph vessels.

The failure to demonstrate a cortical innervation in any of the Sheep adrenals examined is at variance with the results of DOGIEL (1894), PINES and NAROWITSCHATOWA (1931) and ALFERT (1931) but agrees with HOSHI'S (1927) findings in mammals, and those of HOSHI and KOLOSSOW (1930) on the homologous tissue of birds and reptiles. The only nerves noted in the cortex were radial bundles of fibres traversing the cortex to pass to the medulla which is innervated by preganglionic fibres. Whilst the exact mode of ending of the nerve fibres on the medullary cells is still disputed, end bulbs, apparently similar to those described by HOSHI and KOLOSSOW (1930), PINES and NAROWITSCHATOWA (1931) and
were frequently encountered in Sheep adrenal.

Summary

The adrenals of a Soay-Moufflon cross ram were made the basis for a study of the microscopical anatomy of the gland, and the observations reported in this section have yielded the following results:

1. Variations in the three main types of zones of the cortex were found consistently enough to demand enlargement of the descriptive terminology. The zoning found to be most useful was:

- Zona Glomerulosa
- Zona Intermedia

  Outer Fasiculata
  Zone Fasiculata
  Inner Fasiculata

- Zona Reticularis.

2. The ratio of cortex to medulla was found to vary in different animals.

3. Pure-bred Sheep were, in every case, observed to possess a definitive Zona Glomerulosa. Some cross-bred animals also evidenced a glomerular zone, but in the majority of these, the Zona Fasiculata was found to commence immediately beneath the capsule without interposition of a Zona Glomerulosa.

4. The capsule of the adrenal was found to be bi-zoned, having an inner cellular layer and an outer fibrous layer. Very many extremely fine elastic fibres were demonstrated in this latter layer by modifying the stages in Verhoeff’s and the Orcein techniques.
5. No evidence of the "azo-carmine" granules said by HICANDER (1952) to be present in the Zona Glomerulosa of the adrenal of Sheep, was noted.

6. A relationship between cellular degeneration in the Zona Reticularis as manifested by pycnosis, and adrenal demand, or "stress" was observed.

7. Approximately six per cent of the adrenals examined showed the presence of large aggregations of adult eosinophils in the medulla, the reason for the appearance of which could not be uncovered.

8. An apparent polarity of medullary cells to medullary capillaries and medullary veins was noted.

9. The arterial and venous systems of Sheep adrenal were observed to be basically similar to those described in Dog by FLINT (1900).

10. A hitherto undescribed valve present at the exit of the right central medullary vein into the caudal vena cava was found to consist of a double layer of endothelium with a corium of transversely arranged plain muscle fibres.

11. Intraperitoneal injection of lithium carmine was found to be an excellent method for the demonstration of lymphatic vessels in the adrenal by bringing about their distension.

12. The distribution of nerves in the adrenal of Sheep was found to be similar to that described by HENDER (1940) in Cat, and GREEF and DEANE (1949) in Dog. Nerve endings in the form of end bulbs similar to those demonstrated in the human adrenal medulla by ALPERT (1951) were observed.
Corticoadrenal Morphogenesis

The significance of the different kinds of cell that can be observed in the adrenal cortex of most of the higher vertebrates, and also the differences in cell morphology which have been observed and noted in the adrenal cortex of Sheep in Section Two of this work, must inevitably depend to some extent on one, or perhaps more than one, of the following tenets:

1) that the cells have different functions,
2) that the cells have an independent derivation,
3) that the cell types represent functional and diverse states of the same cell.

That the cells have different functions, that is to say, functions which are entirely divorced from one another, is questionable, but as regards their derivation, JONES (1957) states in his exhaustive survey of the adrenal cortex, that there is no doubt that all corticoadrenal cells are mesodermal in origin (see also Section Four).

This leaves the third hypothesis open to debate, and evidence favouring the continuity of cell type and 'expansion' of the adrenal from without inward, has been comprehensively summarised by GRUENWALD (1946). GOTTSCAU (1883), as mentioned in the Introduction, is customarily credited with being the first to suggest that the cell layers of the adrenal cortex appear to form in the outer region of the
cortex (qv. "Zona Bullosa"). From there, this observer stated, the corticoadrenal cells migrate centripetally to reach senescence and death in the most central layer of the gland or, as he named it, the "Zona Consumptiva".

Whilst the most obvious flaw in Gottschau's concept was that his so-called "Zona Consumptiva" was in reality the embryologically separate medulla, it was established by MULON in 1902, that in the guinea-pig, the senescent corticoadrenal cells of the Zona Reticularis are removed via the blood sinusoids which abound in this region. In the following year, MULON (1903) showed that these senescent cells are replenished by younger cells from the Zona Fasciculata and Zona Glomerulosa. Since then numerous authors have put this concept on a sound histological basis, BACHMANN (1937) for instance, presenting supportive evidence for Mulon's theory of inward migration of corticoadrenal cells based on the appearance of the reticulum in the various layers of the cortex.

If one bears in mind this centripetal cellular movement in the adrenal cortex then, and accepts that the reticularis is the graveyard of the adrenal, the question of replacement of dying cells immediately arises. During the years following the investigations of Mulon, it was the general opinion that this replacement was accomplished by normal cell division. In 1931, HOERR observed that the site of cell division or mitosis in the adrenals of guinea-pig was in the outer region of the Zona Fasciculata. BACHMANN (1939), working on human material, reported numerous mitotic figures
and transitional cells in a narrow zone (which he designated the "Germinal Zone"), lying between the capsule and the glomerulosa.

In the following year, BLUMENTHAL (1940) confirmed Hoerr's findings and stated that "the site of mitotic division in the adrenal cortex of the guinea-pig is confined to an area consisting of the inner portion of the Zona Glomerulosa and the outer portion of the Zona Fasiculata." At the same time, this observer gave mitotic indices of 1.3 to 4.6 for a longitudinal section of the adrenal in this animal. That is to say, the average number of mitoses in a section of the adrenal gland of the guinea-pig, 6 mu. thick, was found by Blumenthal to vary between 1.3 to 4.6.

Using Babe's Aniline-safranin, it was observed that the site of mitosis in the adrenal cortex of Sheep was approximately equivalent to that described by Hoerr and Blumenthal in the guinea-pig, that is, the inner glomerulosa and outer fasciculata (Fig. XVI).

Blumenthal, Hoerr and CANALIS (1887), who recorded 5 to 12 mitoses per section of the adrenal gland in normal guinea-pigs, arrived at their conclusions by counting the number of mitoses in a few longitudinal sections through the centre of the gland. The method adopted for this work has been to count 16 longitudinal sections through the middle portion of the adrenal with the aid of a Metz eye-piece, the sixteen mitotic indices thus obtained for each animal then being divided into four groups of four, in order to give a more accurate average result. A study of Tables VIII, IX, X and XI illustrates the considerable range of
variations in the number of mitoses that can occur in
different sections of the same gland, and also gives an
indication of the necessity of counting a large number of
sections in order to obtain accurate results. Because of
the fall in mitotic index which normally occurs with advance¬
ment of age, the four Sheep used for mitotic determination
were of an average age of ten months. In each case the
glands from these animals were fixed in acetic-sublimate
and longitudinal sections cut at a thickness of 4 μ. The
presence of dividing cells was demonstrated by the following
techniques:

a) Babe's Aniline-safranin, with 0.25 per cent Light
Green as a counterstain,
b) the thymonucleic acid test (Feulgen reaction).

Precautions being taken to space the sections in such a
way that the same mitotic figures were not recorded more
than once, the results obtained yielded a slightly higher
mitotic index than that for the guinea-pig, being approxim¬
ately 7.2. The figures from which this overall average
mitotic index was deduced are shown in Tables VIII, IX, X
and XI, and though, as mentioned, higher than the mitotic
index for the adrenal of the guinea-pig, the figure of
is nonetheless worthy of note, for it remained more or less
constant in all four animals, two of which had been given
5 cc. of Tinctura Colchici (B.P.) by intramuscular injection
twenty-four and twelve hours prior to slaughter. One of the
actions of colchicine is to arrest mitosis in the metaphase
and consequently, its use made calculation of the mitotic
index easier. It could be argued that the administration of the drug may itself have been a source of stress and thus have caused enhancement of mitotic activity before arrest, but this factor tends to be negated by the similar mitotic indices which were calculated for the two control animals.

A mitotic index of 7.2 for a longitudinal section of the adrenal of Sheep by no means accounts for the number of senescent reticular cells which can be seen in the same section, and that such a large discrepancy between cortico-adrenal procreation and degeneration existed in the cortex of the guinea-pig adrenal was quickly remarked by ZUMER, WOTTON and NORKUS (1938). These workers were in complete agreement that cortical cells formed in the outer layers of the adrenal, but went a step further and considered the capsule itself as being the germinative zone, construing the Zona Glomerulosa as being derived from undifferentiated mesenchymal cells embedded in the connective tissue of the capsule.

ELIAS (1948) gives evidence favouring this cellular 'metamorphosis' in the horse and dog, and transplantation experiments in laboratory animals appear to have established that, in general, the formation and maturation of new cells in the adrenal cortex takes place in the capsular region.

Thus, LUX, HIGGINS and MANN (1937), and HIGGINS and INGLE (1938), have noted that when adrenal tissue is transplanted into connective tissue of the host, regeneration takes place from cells in the capsular region, while TURNER
(1939) observed the same phenomenon in adrenals transplanted to the anterior chamber of the eye. This latter worker was even able to get a fairly normal cortex to form from homotransplants of the capsular region alone.

From this it follows, as Ivanov (1932), Zweiner, Wotton and Norkus (1938), and Turner (1939) point out, that if one observes the cells of the cortical fasciculi in sequence from the capsule to the medulla, one can visualise the changes which a single cell undergoes as it passes from layer to layer, from its formation and maturation, through its secretory phases, and on to its degeneration and death (see Fig. XII).

As mentioned in Section Two, certain cytological observations on corticoadrenal cells will be repeated here in order that they might be presented with findings on capsular morphogenesis, in the hope that together these might bear on the origin of, the life history and the removal of old corticoadrenal cells in Sheep.

The cell types found in a normal, adult adrenal capsule and Zona Glomerulosa of Sheep will be described in the order in which they may be observed when studying the gland from the outermost regions of the capsule toward the appearance of a recognizable Zona Fasciculata. It is emphasized that only adult glands are referred to in this Section of the work. Embryonic, foetal and early post-natal development of the adrenal cortex occurs in a way entirely different from the growth of the adrenal in later infancy and adulthood (see Section Four). Consequently, only the latter
phase of growth and methods of cell replacement in fully developed adrenal glands are considered.

The capsule of the Sheep adrenal is usually composed of spindle-shaped, fibroblast-like cells with long processes which are intimately associated with typically staining connective tissue. Extremely fine elastic fibres abound in the outermost regions of the capsule and reference to these will be made later. In addition, some rounder, macrophage-like cells are generally present, as are melanophores in Blackface and half-bred animals (see Plate XV).

In certain regions of the capsule however, especially at the inner border near the glomerulosa, and in the trabeculae penetrating into the gland, are cells which differ in having shorter processes and being more rounded, with their cytoplasm more evenly distributed around the nucleus. The latter is clear, oval and contains several scattered chromatin bodies, but no definite nucleolus. Different degrees of shortening and rounding of these cell bodies may be readily observed in a single section. With fat stains, and treatment for birefringent crystals, it can be seen that the rounder cells contain lipoid droplets, especially when aggregated in the nodules described below (Plate XLI). This evidence suggests that the adrenal capsule of Sheep is bi-zoned, having an outer fibrous layer and an inner mesenchymal cellular layer, and on occasions, these two layers are readily discernible (Plates XLVII and XLVIIa).

The subcapsular cells of the Zona Glomerulosa are distinctly glandular. Their nuclei are large and spherical,
with one or two well defined, deeply staining nucleoli. Under normal conditions, these cells are pyramidal or prismoid and show a fair amount of cytoplasm. They contain a considerably higher proportion of lipoid than do their sister cells of the Zona Fasiculata. Plate XLVIII shows a section stained with Sudan IV; Plate XLIX is a colour transparency of a Sudan Black B preparation. The sections were taken from different animals, and both illustrate well this glomerular distribution of lipoid which appears to be characteristic of Sheep despite the fact that this is quite contrary to the picture seen in other domestic animals where fascicular lipoid is far in abundance of that in the glomerulosa. A possible explanation for this phenomenon might have been uncovered if an attempt had been made to correlate the feeding habits of the animal in question with adrenocortical lipoid distribution, but such was outwith the bounds of this work.

The glomerular cells of Sheep adrenal cortex frequently show evidence of mitotic proliferation. This is a point that should be noted, for the frequent association of these cells in rounded or arched groups would seem to be inconsistent with the conception of continuity of corticoadrenal cells from the capsule to the medulla. This however, can be explained by the following observations. A growth arc, if a cortical fasciculus may be thus called, when cut longitudinally would show the merocrine column to be arched at the top and entirely independent of the capsule (Fig. XVII - C). These arched tops may sometimes be cut to lie
separately, giving the appearance of cell clumps which have incorrectly been given the name of glomeruli (Fig. XVII - B). When cut obliquely or transversely, these foci can appear as islands of cells surrounded by the connective tissue of the capsule (Fig. XVII - A). Such clumps or closely packed cells can be shown by sectioning in series to be the starting focus for cell columns which extend inward in the accepted manner toward the medulla (Fig. XVII - D), or, in aged animals, outward to join with or become accessory adrenal tissue as SPEED and MORRIS (1946) report to be the occurrence in the horse. Plate L illustrates two such cortical fasciculi within the capsule, one completely isolated, the other still retaining attachment to the cortex proper.

Whilst no exact reconstruction of the cortical fasciculi of the adrenal has been seen, it can be inferred from sections of the gland that in Sheep, continuous columns of cells stretch from the glomerulosa to the reticularis, and that these columns branch and anastomose somewhat and taper very gently like long, slender cones, with their apices directed towards the medulla. A large number of such cones can be regarded as being packed together much as are the ommatidia in the arthropodal eye, thus allowing for close packing of columns of cells in a mass roughly like a hollow sphere, with the small circumference near the corticomedullary junction, and the large circumference immediately beneath the capsule.

In addition to the foci or islands of cells mentioned above, which are continuous with the cortical fasciculi,
bodies of cells or nodules have been found within the capsule which can be seen by serial sectioning, to be entirely independent of the cortex. Plates LI - A, B, C, D and E, are photomicrographs made from the left adrenal of a half-bred ewe lamb. The gland was sectioned in series at a thickness of 7 µ, and stained with haematoxylin and eosin. Unfortunately, owing to lifting of the section and irregular staining, the quality of the latter two plates is not high. It was however, decided to include them as they give an indication of how the three nodules which appear in the first plates, "fade out", still without communication with the cortex.

The capsular nodules in question are solid, parenchymatous masses of cells and almost every adult gland examined showed their presence to a greater or lesser degree. The fact that the nodules did not project beyond the surface of the adrenal was taken as an arbitrary distinguishing feature between them and the hyperplastic type of nodule which, as mentioned above, commonly protrudes beyond the capsule in aged animals.

Two entirely different types of nodules have been noted. The first of these, also observed in Sheep by Elias (1948), gives the appearance of floating in a space filled with liquid. This space is lined by flat, endothelial-like cells, and the nodule itself has very much the appearance of the corona radiata around a mammalian oocyte, the peripheral cells seeming to be fringed (Plates LII and LIII, and see Plate XLVIIa). In his paper, Elias considers these
"floating nodules" as he calls them, as representing a second method whereby cortico-adrenal cells are replaced from the capsule, but evidence will be presented in Section Four of this work to show that they are neither floating nor concerned with cortical replacement, but are in fact, vestiges of the foetal development of the adult cortex.

The other type of nodule frequently met with in the capsule of adult adrenals, is characterised by having its own individual capsule of dense fibrous tissue (Plates LIV and LV). An early stage of development of this type of nodule is seen in Plate LVI. A concentric arrangement of fibres and their associated fibroblasts around a small mass of metamorphosing mesenchymal cells indicates the primordium of an encapsulated nodule. The expression 'metamorphosing' is perhaps the most lucid that can be applied to these aggregations of capsular cells for, bearing in mind the evidence already presented, there is no other logical derivative site for such obvious merocrine cells other than the undifferentiated adrenal capsular cells; and that the nodular cells eventually do become merocrine as they mature, is well brought out in Plate LVII. This is a high power microphotograph of a section of the adrenal gland of a Blackface tup, taken through an encapsulated nodule as it lay in close proximity to the Zona Glomerulosa. From the plate, it is quite obvious that it is extremely hard to distinguish the glomerular cells from the nodular cells. The cells on the left of the photograph are in fact the nodular cells.
ELIAS (1946) stated in his studies on cellular replacement in the adrenal cortex that "there seems to be but one type of cellular replacement going on in any one adrenal at one time". This has been observed to be the case in adrenals examined during this work, but as pointed out earlier, Elias considered both floating and encapsulated nodules as being derivative of new cortical cells, and his statement was prompted by the fact that he was unable to find both types of nodule in the same gland. That the "floater" and the encapsulated type of nodule can and do occur in the same gland is shown in Plate LVIII. It should perhaps be pointed out at this stage though, to lend weight to the observations in Section Four regarding the origin of floating nodules, that in animals which were discovered to have "floaters" within their adrenal capsules, the nodules were never found to be of very great size as compared with the encapsulated type, neither were they ever noted to be in close approximation to the glomerulosa. Plates XLVIIIa, LII and LVIII give a very fair indication of the maximum size of floating nodule met with.

The incorporation of encapsulated nodules into the cortex.

Fig. XVIII illustrates schematically the incorporation of an encapsulated nodule into the Zona Glomerulosa. As such a nodule increases in size, it gradually develops a bulge toward the main body of the gland, sometimes even to the extent of slightly displacing the immediate glomeruli. With further proliferation of the encapsulated cells, the encapsulating fibres thin drastically at the point of
closest approximation to the glomerulosa until, coincidental with the development of a definite evagination into the glomerulosa, the limiting fibres rupture and allow a free flowing of the encapsulated cells into the cortex.

Plate LXIX shows a large, completely encapsulated nodule with, in the vicinity, an older nodule which is in the process of being incorporated into the cortex by the method described above. Plates LX, LXI and LXII illustrate different stages in this process of incorporation. The mechanisms involved in nodular incorporation.

a) Capsular resilience

Whilst plain muscle fibres in the adrenal capsule of Sheep are relatively scant, when the gland is treated by the slightly modified Verhoeff's technique mentioned in Section Two, an abundance of very fine elastic fibres immediately becomes obvious. From observations made during the last two years on the presence or absence of elastic fibres in other organs, it can be put forward that apart from the spleen, epiglottis, mammary gland and such features as blood vessels, the ligamentum mactae and ligamenta flava, the adrenal capsule has possibly the highest content of yellow fibres in the body (see Plate XVII).

The presence of these elastic fibres must necessarily influence the centripetal movement of capsular nodules by virtue of their resilience. If one examines the question though, it becomes apparent that the role played by these capsular fibres is hardly one of straightforward compression, for it is difficult to visualise the adrenal gland as being
subject to a constant capsular pressure throughout life. It is more logical to think of these yellow capsular fibres as normally being relaxed and yet with the ability to use their reserves of resilience in much the same way as a punching-ball bounds back after being struck. The formation of a nodule must initially cause some evagination of the capsule with associated tension of the capsular elastic fibres. A dialectal follow-up to this (hyperplastic nodules apart, where the continued proliferation of cells would stretch the fibres beyond the 'point of no return') is that a stage is reached when the fibres react like the punching-ball and bound back to 'punch' the nodular cells into the cortex.

b) Reticular vacuum.

Another feasible factor in the centripetal migration of nodular cells which should be taken into account, is the creation of a "vacuum" by the removal of dead cells by the sinusoids and macrophages in the Zona Reticularis. It has been remarked earlier that in a longitudinal section of the adrenal of Sheep, an average of 7.2 mitotic figures can be seen. Whilst no attempt has been made to enumerate the actual number of senescent or dead cells in the gland, a brief scrutiny of routine paraffin sections reveals a high proportion of pyknosis, karyolysis and cellular debris near the cortico-medullary junction. Removal of the owners of such senescent features must inevitably disturb the equilibrium of the other layers of the cortex, and this disturbance, with the elasticity of the capsule as a complementary factor,
would tend to set up a centripetal pole of attraction in the gland.

c) Alterations in medullary blood pressure.

A prominent feature of the adrenal medulla which has already been touched upon in Section Two, is the presence there of numerous and vast venous sinuses. The adrenal is a highly vascular organ and, being situated as it is in close proximity to the abdominal aorta, changes in systemic blood pressure must eventually be reflected in these venous sinuses. A rise in pressure, however slight, would almost certainly cause some dilatation of the sinus walls and an according increase in intraglandular pressure. The converse would be true for a fall in systemic pressure, the net result of this being a decrease in intraglandular pressure.

Whilst no literature on the subject of adrenal pulsation in the living animal has been uncovered, it could well be suggested that the adrenal gland contracts and expands in vivo in much the same way as does the spleen, the former however dependent on the influence of the systemic blood pressure. Thus it follows that a lowering of intraglandular pressure would logically assist the centripetal flow of cortical cells, while an increase in intraglandular pressure might well promote the expulsion of dead cells from the Zona Reticularis.

Appositional cellular growth in the adrenal.

It is evident from a paper by GRUENWALD and KONIKOV (1944), that, in addition to the nodular incorporation of
capsular cells into the adrenal cortex, the undifferentiated cells in the capsule can arrange themselves into groups or plates for what might be termed 'appositional growth'; in other words, the downward movement of broad cell masses and their final fusion with the cortical fasciculi.

It was pointed out in Section Two that in some of the Sheep examined, the indistinctness or even absence of a Zona Glomerulosa was a notable feature of the adrenal cortex (see Plates XIII and XIV). It was also remarked that whilst there might be some slight demarcation of a Zona Glomerulosa in certain regions, in others the Zona Fasiculata appears to take shape immediately beneath the capsule. (It should be emphasised that the term 'Zona Fasiculata' as used at this point refers purely to the morphological arrangement of the cellular columns, for it would be illogical to presume that certain animals, no matter how in-bred (qv. Section Two) are entirely deficient in cells with which to perform the normal functions of a normal glomerulosa.) The "loss" of a Zona Glomerulosa in cross-bred animals refers merely to the fact that the cortical fasciculi fail to arch and link up with one another as they reach the capsule.

Appositional cortical growth has only been observed in the glands of animals which did not present an obvious Zona Glomerulosa, and where appositional growth was apparent in a gland, no encapsulated nodules in any stage of development were noted.

Appositional cortical growth is much simpler to understand than the formation of nodules. It seems to be, in
effect, a simple, gradual, fluent transition of metamorphosed capsular cells into the Zona Fasiculata. The cells of the capsule enlarge very gradually and as gradually are transformed into cortical cells (Plate LXIII). It is emphasized however, that in this type of cellular reproduction, the gland wherein it appears to occur, has no sharp boundary between capsule and Zona Fasiculata at places where the replacement of cells occurs.

This method of cell replacement has been described by BACHMANN (1939) for the cat, and GRUENWALD (1942) postulates this same method of cell replacement in the human adrenal. GRUENWALD and KONIKOV (1944) indicate that the same occurs in the Rhesus monkey and go a step further by stating that "the great variability in the structure of the Zona Glomerulosa in mammals suggests that the number of mechanisms of junction between new and old cortical tissue may not be limited to nodular and appositional growth". No other type of cortical replacement other than by the encapsulated nodules described above and by appositional growth has been observed in Sheep however.

Discussion

Whilst several authors, notably CALMA and FOSTER (1943) and BAXTER (1946), have expressed scepticism as to the validity of the corticoadrenal cell migration theory on the basis that some animals such as the mouse possess little or no Zona Reticularis, and others such as Horse and Dog have an obviously demarcated Zona Glomerulosa, while there are
animals like the bat which have a poorly developed glomerulosa, in order that the hypothesis of corticoadrenal morphogenesis and migration be applied to Sheep, there are three primary questions for which answers must be found. The first of these, does the adrenal cortex require a continual supply of young cells? In other words, do corticoadrenal cells have a limited life span? The second question, equally as basic as the first, is, that if corticoadrenal cells do indeed have a life cycle and observations certainly indicate that in Sheep, the reticularis is the graveyard of the cortex, are new cells produced as a result of mitotic division? Apparently this is not so, for not enough dividing cells can be demonstrated either by Bae's Analine-safranin or the Feulgen reaction to account for the dead cell count in the Zona Reticularis. The third question is, that if mitosis does not replace the dying cells cell for cell, is there a logical site from which new cells could spring? The fact that a definite cellular layer is visible in the adrenal capsule, and that nodules of cortical-like cells are seen within capsular tissue which by serial section can be shown to be entirely independent of the cortex, is adequate evidence that there is indeed a logical derivative site for new corticoadrenal cells.

The mitotic factor is perhaps the most interesting of the three points made above, and the fact that mitosis is evident in Sheep adrenal cortex (apart from the mitotic figures which one would normally expect to see in the connective tissue of the trabeculae) is even more intriguing
in the light of the observations made in this Section on corticoadrenal morphogenesis and cellular replacement from the capsule.

The question which immediately springs to mind is that if mitotic figures are present in the cortex, and it was observed that in Sheep these average in the region of 7.2 for a longitudinal section of the gland, why then should not all cortical replacement be accomplished by mitotic division?

This could possibly be explained by the fact that, in respect to its merocrine talents, a mitotic cell is a temporarily non-functional cell. It is self-evident that, for a given number of cells, the adrenal cortex functions efficiently. MULON (1902) proved that dead cells in the cortex of guinea-pig adrenals are removed in the Zona Reticularis, and senescent cells have been observed in that zone in Sheep (see Plates XXII, XXIII and XXIV). MULON (1903) showed that dead reticular cells are replaced by cells from the fasiculata and glomerulosa, and if one accepts that corticoadrenal cells have a limited life cycle, then the number of mitoses required to replace the number of senescent or dead cells in the reticularis would necessarily be very high; high enough in fact, to upset the delicate balance of cortical efficiency by virtue of these cells being out of action when undergoing division.

An obvious parallel to this hypothesis is the cyclic destruction and production of red blood corpuscles every fifteen days or so, for the adrenal cortex appears to have a minimal number of cells with which to carry out its normal
functions, just as the body has a minimal number of red cells essential for life. In order to obviate going below this minimal by introducing the mitotic factor, senescent corticoadrenal cells are replaced from the capsule, the cycle being maintained throughout the adult life of the animal.

It was remarked at the start of this Section that the adrenal cortex of Sheep consists of a large number of tapering cones or columns packed closely together with the apices of the cones directed toward the medulla. Shrinkage of cells in the Inner Fasciculata accounts for part of the tapering of these columns as does the further shrinkage and loss of cells in the reticularis. Thus if one accepts that corticoadrenal cells are arranged in tapering columns stretching from the glomerulosa to the reticularis, and also bears in mind that observations in Section Two of this work indicate that cell death is rare outside the Zone Reticularis, it follows logically that each corticoadrenal cell gradually traverses the length of such a column, being pushed by the cell peripheral to it, and in turn pushing the one ahead of it, until it reaches the reticularis when the regularity of its progress is interrupted by the death of scattered cells in the column. Since each cell passes gradually through the successive zones then, and since cells in all portions of such a moving continuous column will be travelling at the same speed, it follows that the width of a given zone will, under such conditions, represent the relative length of time a cell requires to traverse it. This will hold true provided there is no loss or gain in numbers of cells in the
course of their passage through the columns and provided there is no change in the size of the cells. Consequently, should sufficient observations be made, it is quite rational to believe that deductions concerning the relative velocities with which corticoadrenal cells pass through the various zones of the columnar portions of the cortex, and hence of the relative length of time consumed by the passage of a cell through a given zone, might well be made at some future date.

Summary

Methods of cell replacement in the adrenal cortex of the adult Sheep have been studied in this Section of the work. Cellular replacement takes place for the most part by the morphogenesis of undifferentiated capsular cells as BACHMANN (1939), GRUENWALD (1942), and GRUENWALD and KONIKOV (1944) put forward in their respective papers for adrenal glands in general.

Findings with regard to the presence of the so-called "floating nodules" within the adrenal capsule are at variance with the observations of ELLAS (1945 and 1948) on two counts:

i) "floating nodules" and encapsulated nodules can be present in the same gland,

ii) "floating nodules" are not concerned with cortical cellular replacement but represent foetal remnants (see also Section Four).

It has been observed that the adrenal capsule of Sheep possesses two more or less distinct layers:
i) an outer fibrous layer containing amongst other connective tissue elements, an abundance of extremely fine elastic fibres which appear to influence the centripetal migration of corticoadrenal cells,

ii) an inner cellular layer of undifferentiated mesenchymal cells which have the ability to metamorphose into metoaromic glomerular cells.

Study of Sheep material indicates that cellular replacement in the adult cortex occurs in three fundamentally different ways:

i) mitosis to the tune of 7.2 dividing cells per longitudinal section of 4 mm. thickness, taking place in the outermost regions of the Zona Fasiculata,

ii) formation of encapsulated parenchymatous nodules of cortical tissue from mesenchymal cells within the glandular capsule,

iii) appositional growth by the formation of broad plates of cortical tissue within the capsule and their eventual inward migration and fusion with the cortex.
Developmental Anatomy of the Adrenal of Sheep

The study of the changes that occur in an organ during its developmental course naturally arouses curiosity as to the nature of any concomitant acquisition of function or changes of function which may occur. The possible function of the cells constituting the main mass of the so-called "foetal" cortex, which disappear during the first few months of life, for instance, offers an intriguing problem to the embryologist. The fact that these cells are present mainly during foetal life however, puts almost insuperable difficulties in the way of any investigation of their physiological significance, and it is perhaps by virtue of this and other similar problems that a great diversity of views concerning the histogenesis of the adrenal was held by many of the early investigators.

BALFOUR (1878) from a study of fish embryos, was one of the first to advance the opinion that the amniotic adrenal was derived from two separate anlagen, a mesoblastic and a nervous, the latter being furnished by the sympathetic ganglia situated along the course of the abdominal aorta. According to Balfour's view, the mature cortex was derived from mesodermal cells while the sympathetic ganglia contributed medullary tissue. This hypothesis received considerable support from contemporary workers such as BRAUN (1882), and applied to eutherians, twin development of the
adrenal is generally accepted today.

GOTTSCHAU (1883) however, as remarked earlier in this work, noted a small cluster of mesenchymal cells almost in contact with the caudal vena cava in pig embryos, and concluding that this anlage was derived from the mesoderm, went a step further by stating that the anlage represented a common primordium for both cortex and medulla.

JANOSIK (1883) on the other hand, while agreeing with Gottschau that the cortex and medulla were derived from the same source, noted that the mesothelium in the region of the caudal vena cava contributed cells to the mesoderm at that point where the adult adrenals are found in mammals and concluded from this that the adrenal anlage was derived from the peritoneum.

Novel views on adrenal histogenesis were recorded by HERTWIG (1897) and MINOT (1897). The former worker put forward an ingenious theory that the cortex was derived from the tubules of the Wolffian body, processes from which grew dorsally to envelop portions of the sympathetic ganglia which in turn gave rise to the medulla. The latter observer held that, in man, the adrenal anlage was laid down as a whole by the mesenchyme, the mesenchymal cells composing the anlage then undergoing differentiation into cortex and medulla.

It was left to the faithful FLINT (1900) who, in his exhaustive study of the blood vessels of the adrenal, devoted a chapter to the histogenesis of the gland as observed in pig embryos, to establish the basis of adrenal organogenesis
as we know it today. Flint did not undertake to determine the ultimate source of either the cortex or the medulla, but he stated that the cortical tissue is laid down first and showed that the medulla is developed from cells which migrate in from outside the primitive cortical anlage. He was able to trace these medullary cells in the form of small nodules which passed deeper into the cortex as the age of the embryo increased, until finally they reached their destination around the central vein. At the same time however, Flint expressed scepticism as to the derivation of these medullary cells from the sympathetic ganglia.

The seeds of adrenal histogenesis had been implanted and WEISEL (1901), studying adrenal development in pig embryos of 10 mm., 20 mm. 25 mm., and 30 mm., detected the cortical anlage first in 20 mm. embryos, in the shape of a projection on the medial surface of the Wolffian body. Ventral to this projection, this worker stated, the coelomic epithelium, while appearing to be highly developed, showed no differentiation from the epithelium of the coelomic cavity in general. This observation, Weisel thought, warranted the conclusion that the cortex is derived from coelomic mesothelium.

Hand in hand with his observations on cortical development, Weisel made a thorough study of the derivation of the medulla. He traced its origin back to the neural crest and described the passage of sympathetic cells in collections or, as he termed them, "rosettes", through the cortex to the vicinity of the central vein in exactly the fashion as
described by Flint the previous year.

The development of the human adrenal, which incidentally tells us much about the histogenesis of the gland in the lower vertebrates, was not studied in any great detail until after the turn of the century. Descriptions of the gland's origin vary in the literature of this time according to different methods of fixation, or to variations in measurement of the embryo's length arising from different degrees of embryonic flexure. However, proliferating cell nests in contact with the coelomic epithelium were found at the site of the future adrenal gland in 6 mm. human embryos by SOULIE (1903), at 6.5 mm. by ZUCKERKANDL (1912), at 7 mm. by KOGNO (1925), and at 8 mm. by POLITZER (1936). The invasion of the cortical anlage by sympatho-chromphil cells was noted in 25 mm. embryos by KEHNE and HEWER (1927) and also by subsequent workers such as FISHER (1938) and UOTILA (1940).

Since the observations of these investigators, it has come to be generally accepted that adrenocortical tissue is derived from the columnar epithelial cells which, differentiated from mesoderm, line the coelomic cavity, and, while WITSCHI (1951 and 1953) has put forward that the intermediate mesoderm is responsible for the formation of the cortex, it can be said without fear of contradiction that even if cortical cells do not differentiate from peritoneal epithelium itself, at least it is certain that they arise from mesenchymal cells in the vicinity of the mesothelium. It is equally as certain that adrenal medullary cells have their origin in the sympathetic ganglia of the neural crest.
As with study of the adult adrenal (see Section Two), the question of morphological terminology arises when the immature gland is considered histologically. It is well known that during embryonic, foetal and neo-natal life in man, the adrenal cortex consists of cells of two histologically distinct types. Keene and Hewer (1927) refer to the tissue composed of one of these cell types, which degenerates soon after birth, as the "foetal" cortex, and to the other group of cells, which persists in the adult adrenal, as the "true" cortex. As mentioned previously Davies (1937) noted the presence of a "foetal" cortex in the cat. Hill (1930 and 1937) reported the same feature in the adrenals of the larger Felidae and while Nicander (1952) has observed the presence of a "foetal" cortex in cattle, pigs, dogs, cats and rabbits, no reference to the presence of a "foetal" cortex in Sheep has been uncovered in any of the literature studied in pursuance of this work. Cell types similar to those observed in human embryos by various workers, and to those described by Nicander in the embryonic animals listed above, can readily be observed in Sheep embryos however, and in this Section, the term "adult" cortex has been substituted for Keene and Hewer's "true" cortex, for in the author's opinion the terms foetal and adult are preferable in the light of the development and the subsequent fate of the two types of cell.

There are several reports in the literature concerning the early appearance of the adult cortex in human embryos. Kohno (1925) for instance, found the progenitors of adult
cortical cells in the subcapsular region of a 14 mm. embryo. 
HABBAR (1925) noted the same in 16 mm. embryos and KETT (1925)
at 19.6 mm. While KEENE and HEBER (1927) suggest that in
humans the adult cortex does not arise from the foetal cortex
but rather has its own separate origin from an epithelial
cap which is applied to the ventral surface of the foetal
cortical mass, the number of embryos obtained at the proper
stage for a critical study was extremely limited and, as
Keene and Hewer themselves admit, not enough to warrant final
conclusions on the development of the adult cortex being
made. Accordingly, it is still generally assumed by most
authors that the cells of the permanent or adult cortex do
develop from those of the foetal cortex and no evidence to
the contrary was observed in the Sheep embryos examined for
this work.

The zonal terminology, customarily applied to descriptions of the adult adrenal, is not apposite when one con-
siders embryonic and early foetal glands since, as remarked
in Section Two, the distinct cortical zones do not make their
appearance until late foetal or early post-natal life.
Consequently, when reference is made in this Section to the
cortical anlage in various stages of embryos, unless other-
wise stated, the anlage of the foetal cortex is in fact
meant.

As mentioned in the Section on Material and Survey of
Methods, it was found convenient to commence study of
adrenal histogenesis with Sheep embryos of 10 mm. C-R length,
and to formulate from embryos and foetuses of upgraded
lengths and ages, a timetable for the appearance of the following features:

1. Formation of the foetal cortex,
2. Formation of the adult cortex,
3. Immigration of the cortical anlage by sympathetic elements and the formation of the medulla.

A concatenation of embryos and foetuses similar to the one on which the observations made in this Section have been based, is shown in Plate LXIV, together with indications of those ages of embryos which are significant by virtue of their being histogenic milestones in that the features noted above can be observed for the first time.

1. Formation of the foetal cortex

The cortical primordia appear early in development, and in embryos of approximately 10 mm. C-R length, they are characterised by the accelerated local proliferation of cells from the splanchnic mesoderm a few millimetres from the site of the genital ridge, on either side of the primary dorsal mesentery, medial to the cephalic pole of the mesonephros (Plate LXV). The relationships of right and left anlagen in a 14 mm. Sheep embryo are shown schematically in Fig. XIX.

In 12 mm. embryos, the cortical anlage can readily be distinguished from the surrounding mesenchymal cells (Plate LXVI). The nuclei of the cortical cells are large, vesicular, mostly spherical, and they show at least one nucleolus. Whilst the actual cellular outlines are not easily made out, the cells being closely packed together, the cytoplasm at
this stage has a darkly granular appearance. In contrast, the surrounding mesenchymal tissue is composed of typically appearing embryonic connective tissue, the cells being loosely packed, of a smaller size, and possessing smaller and varied shapes of nuclei than the primitive cortical cells. Even at this early stage, sympatho-chromophil cells have begun to migrate ventrally from the neural ectoderm, and it will be seen that this process continues right up to the 20th or the 21st week of gestation.

The coelomic epithelium is of the same columnar type as found in the Wolffian body. At that point where the cortical anlage is in contact with the mesothelium, the cells, as already remarked, appear to be in an active state of proliferation, being very closely packed and frequently showing evidence of mitotic division. As the anlage grows however (embryos of 13 and 14 mm.), the primitive cortical cells gradually lose contact with the mesothelium and become embedded in the adjacent mesenchyme till at approximately 16 mm., a distinct layer of mesenchymal cells can be observed lying between the glandular epithelium and the mesothelium, confirming that, like the primordia of the mouse (INABA, 1891), of the rat (PANKRATZ, 1931), and of the pig (PATTERN, 1948), the primordia of the adrenals of sheep form by the proliferation and differentiation of mesothelial cells, and their subsequent immigration into the neighbouring mesenchymal tissue.

Once the adrenal primordium has completely separated off from the mesothelium, it grows rapidly, displaying intense
mitotic activity, and reaching a considerable size in embryos of 18 to 20 mm. In 20 mm. embryos and above, capilliform blood vessels commence to invade the anlage, and sympathetic elements, though not as yet actually penetrating the primordium, can still be seen to be migrating ventrally en masse from the neural crest ganglia alongside the aorta, toward the foundation of what will eventually be the central medullary vein (Plate LXVII).

At the 30 to 40 mm. stage, more or less concomitantly with the differentiation of the non-glandular stomachs, the anlage of the foetal cortex is extremely well established and extremely vascular and, though joined, as discussed below, by medullary cells, capsular cells and the anlage of the adult cortex, it remains very obvious till its degeneration several months after parturition (see Plates LXXVI and LXXVII). Plate LXVIII is a microphotograph of a sagittal section of a 10 weeks old embryo, illustrating the marked vascularity of the cortical anlage and dorso-cranially, nodules of sympatho-chromaffin cells in the process of migration from the neural crest ganglia.

2. Formation of the adult cortex As has been pointed out, the development of the adrenal cortex in Sheep, like that of man (KEEN AND HAWK, 1927; UOTILA, 1940), consists of two fundamental stages, the derivation of a foetal cortex from mesothelial cells, and the overlapping formation of an adult cortex. In order that the appearance of these cortical anlagen, which can readily be observed together in embryos of 25 mm. and upward, be appreciated,
it is necessary to note the cytological differences between the cell types going toward their make-up.

The cells of the adult cortex are easily distinguishable from those of the foetal cortex in that they are smaller, and possess less prominent nuclei than the latter cell types which, as mentioned, have large, vesicular nuclei. The cytoplasm of the foetal cortical cells is granular and, at the time of first differentiation from splanchnic mesoderm, darkly staining, but more or less concomitantly with the appearance of the adult cortical anlage, the cytoplasm changes in character, becoming quite markedly acidophilic, which renders an acute discriminating feature from the cytoplasm of adult anlage cells, which is basophilic in reaction, and frequently vacuolated.

In embryos of 20 to 25 mm., that is to say in Sheep of approximately 5 weeks of age, the peripheral cells of the foetal cortical anlage can be observed to be undergoing further differentiation. This process consists of their arranging themselves into groups or nodules which, in the absence of a capsule, gradually form a complete ring of tissue around the original foetal cortical mass. This is the presumptive adult cortex and hand in hand with the continued differentiation of these cells, as mentioned, the affinity for acid dyes of the foetal cortical cytoplasm increases.

Thus, at approximately 25 to 30 mm., at which stage the adrenals may be recognised by the naked eye as resting on the upper poles of the kidneys, the main mass of the developing
adrenal consists of the rather large cells of the foetal cortex, characterised by their granular, acidophilic cytoplasm and large, pale nuclei, and a narrow rim of adult cortical tissue, the cells of which can be recognised by their size, their small, deeply stained nuclei, and their basophilic cytoplasm (Plate LVIX).

Apart from the characteristics of the adult cortical cells themselves, the arrangement of the actual anlage is representative enough to allow it to be recognised without difficulty. Small groups of basophilic cells in varying stages of isolation can be observed to surround the foetal anlage completely. These groups, as will be shown presently, are the focal points from which adult cortical fasciculi will spring in due course, and it can be seen that after first differentiation from the foetal anlage, the adult cell groups take the form of small horseshoes around a capilliform vessel. Whilst this is quite to be expected, adult cortical fasciculi being produced by the prolongation of the branches of the horseshoes, the structures present two remarkable features, in that the peripheral cells of the horseshoe groups appear to have a protoplasmic fringing, and that the whole cell group is surrounded by a delicate membrane formed by cells which seem endothelial in character. These features are well shown when, as frequently happens, the arch of a horseshoe is cut in transverse section. The arched top gives the appearance of a cell clump or node, whose periphery is fringed and which on first sight appears to be floating in a space filled with liquid, this space being lined by the
endothelioid membrane. Close study of such nodules and their surrounding membranes yields that they have exactly the same structure as the "floating nodules" described in the adult animal by ELIAS (1945 and 1948) and those remarked upon in Section Three of this work. Plate LXX is a microphotograph of a section of the adult cortex of a full-term foetal adrenal, illustrating the typical grouping of the cells. Plate LXI is a microphotograph at the same magnification of a section of the capsule of a fully mature adrenal showing a so-called "floating nodule" with its peripheral fringing and limiting membrane. The similarity between the two structures is highly significant, and these should be compared with Plates XLVIIa, LII and LIII.

The presence of a limiting membrane around these nodules and indeed the horseshoes of the adult cortical anlage, could be explained on the basis of the cells forming the membrane being rudimentary fibroblasts. The adult cortical anlage develops as mentioned, by centripetal growth of the cells in the branches of the horseshoes. At the same time, there must logically be some transverse expansion and when the fibroblastic membranes of adjoining horseshoes come into contact, it is quite tenable that they unite and with further growth, develop in thickness and in length to form the trabeculae which separate the cortical fasculi in the mature gland. Time would not permit of a full investigation of this point being made for this work though, but it is hoped to pursue a study of the function and fate of the membranes at some future date.
The fact that the arched tops of the horseshoes when cut to lie separately within a complete membrane, appear to be floating, is purely an illusion created by the routine processing of the tissue. As remarked earlier, one of the prominent features of early adult cortical cytoplasm is the presence of large vacuoles. As the nuclear pole of each cell is applied toward the capilliform vessel, these vacuoles naturally enough are found in that pole of the cell inclined toward the external membrane. Immersion in fat solvents dissolves the contents of the vacuoles which can occasionally fill the whole width of a cell, and the fibrils of stained cytoplasm remaining are often so slender as to be scarcely noticeable. At the same time, in the majority of cases what cytoplasm remains after evacuation of the vacuoles shrinks considerably during processing and thus gives the impression of a cytoplasmic fringe to the peripheral cells. Close study of favourable sections does however confirm that the cells of the horseshoes and also the nodules are in fact applied directly to the fibroblastic membrane, and to a certain extent this can be seen to be the case in the "floating nodule" shown in Plate LXXI.

At the time of the first differentiation of the adult cortical anlage, there is no sign as yet of the formation of a glandular capsule. Once the anlage has become established however, cells from the surrounding mesenchyme become organised around the periphery of the foetal adrenal body, and by the 150 mm. stage, a recognisable adrenal capsule has been formed (Plate LXXII). From this it can be stated that as
the adult cortical cell groups are formed before the capsule, it is possible that in some instances some of these groups may become isolated within the developing capsular tissue and thus, without an escape route to the cortex, have no alternative but to remain where they are to appear as "floating nodules" in the capsule of adult glands. Three features lend weight to this argument:

1) in the adult animal, "floating nodules" are invariably small and rarely show variation in size,

2) in the adult animal, "floating nodules" are few and far between as compared with encapsulated nodules,

3) whilst the cells of "floating nodules" are undoubtedly cortical in nature, their similarity to adult anlage cells in the foetus being marked, they are morphologically distinct from encapsulated nodular cells which, as shown in Section Three, become incorporated into the cortex. Supplementary to the first two observations, this indicates that "floating nodular" cells are not concerned at all with corticoadrenal cellular replacement in the adult but rather represent the primitive cortical cell type seen at the time of first differentiation of the adult cortical anlage in the foetus.

3. Formation of the medulla  It can be seen from Plates LXVI and LXVII which are microphotographs of a 12 and a 20 mm. embryo respectively that as early as five weeks, the cells destined to form the adrenal medulla are in the process of migrating ventrally from the neural ectoderm, and this migration continues right up to the end of gestation.
In the Plates, these sympato-chromaphil elements are represented by encapsulated masses of cells, which are similar to those seen in the sympathetic chains, situated between the aorta and the adrenal anlagen. These cellular masses, which were found bilaterally in 12 mm. embryos and upward, consist of cells which somewhat resemble lymphoid tissue in that they are relatively small in size, have darkly staining nuclei and show very little cytoplasm. The immediate goal of the cell groups is the central medullary vein around which the anlage of the medulla is laid down. Thus it can be seen in Plate LXVII that the sympato-chromaphil cells which are in the process of migrating from the neural ectoderm are, for the most part, bypassing the adrenal anlage on its medial aspect, and making toward the evagination in the wall of the cardinal vein which in this particular case will eventually form the right central medullary vein.

As the age of the embryo increases, the medullary anlage becomes well established around the central vein and its developing tributaries, and at 25 mm. bundles of cells can be seen to be breaking off from the main mass of sympathetic elements earlier than before to invade the cortical anlagen chiefly on, but not necessarily limited to, their medial aspects, and penetrate well into their substance. These invading cell masses break up to a certain extent after entering the primitive cortical tissue to make their way toward the medullary primordium either by infiltrating through the cortical anlagen on their own, or by invading the anlagen still in the groups in which they arrived from
the neural crest. In the latter case, the cell groups or nodules, which are equivalent to the "rosettes" of WEISSEL (1901) and KEENE and HEWER (1927), and which can be seen in Plates LXVII and LXVIII, still retain their connective tissue capsules which, logically, will go toward the make-up of the connective tissue septae of the future medulla.

The invasion of the cortical anlage of a 25 mm. embryo by sympatho-chromaffil elements can be seen in Plate LXIII.

During the following weeks of development, the concentration of sympathetic elements within the cortical anlage grows, till at the 150 mm. stage, that is to say approximately midway through gestation, the foetal adrenal body is represented by:

a) a definitive glandular capsule,
b) a peripheral rim of adult cortical tissue,
c) eosinophilic foetal cortical tissue,
d) a medullary anlage, which consists of large, thin-walled spaces filled with blood, around which are aggregated the small, round, darkly staining sympathetic cells which have infiltrated through the cortical tissue either singly, or in the groups described above (Plate LXXIV).

The chromaffin reaction can first be observed in the adrenals of foetuses of approximately fourteen weeks of age, the typical brown coloration being given by the larger migrating cell groups both inside and outside the gland. At a C-R length of 200 mm, which is roughly equivalent to an age of fourteen weeks, migration of ectodermal cells can still be appreciated, the cells penetrating the glandular
capsule which is now present, where they enter, often accompanied by blood vessels. From fourteen weeks onward, migration continues in diminishing degrees till at twenty to twenty-one weeks, the only chromophil elements to be observed outside the medullary anlage are occasional stragglers still progressing through the cortical anlagen.

The adrenal after parturition.

At full-term, the bulk of the adrenal consists of cortical tissue, this being made up of a subcapsular rim of enlarged, polygonal-shaped adult cortical cells whose cytoplasm shows the presence of many vacuoles, and a relatively wide expanse of foetal cortical tissue. This latter zone however, is beginning to show signs of degeneration which are typified by the foetal cell boundaries becoming very indefinite and the foetal cell nuclei growing very erratic in staining reaction, and it can be seen from sections of glands taken from lambs of one to two weeks of age that this degenerative process is progressive with age. The medulla is represented at full-term by a small, central zone of large blood sinuses and aggregated sympathetic cells (Plate LXXV).

Whilst neither time nor availability of material would permit of a critical study being made of the adrenals of Sheep from birth to six months which was the earliest age of animal in whose adrenal the characteristic zoning of the mature cortex was noted, it can be said conclusively that from birth to six weeks there is progressive degeneration of the foetal cortex with a corresponding increase in adult cortical tissue. Thus, when a section of neo-natal adrenal
is impregnated with silver, the reticulum pattern clearly indicates that at approximately one week, the adult cortical cells have already assumed the characteristic grouping of the Zona Glomerulosa which is seen in mature glands. This is well illustrated in Plate LXXVI and this microphotograph should be compared with the glomerular reticular pattern of adult glands as shown in Plates X and XII.

From the first week of life onwards, growth of the peripheral rim of adult cells is progressive and continuous, and at four weeks, the adult cells can be seen to be invading the outermost layers of the degenerating foetal cortex. At six weeks of age (the oldest ne-natal material available for this study), the breadth of the adult cortex has increased considerably, with an according decrease in the amount of foetal cortical tissue present. In Plate LXXVII, which is a microphotograph of a section of the adrenal from a six week old lamb, the increase of adult cortical tissue as compared with what can be observed in the full-term gland in Plate LXXV, is extremely well defined, for the cells are, as pointed out earlier, more basophilic than foetal cortical cells and thus show up as a darker peripheral zone around the lighter stained, eosinophilic foetal cortex.

**Discussion**

The evidence presented in this Section of the work in regard to the development of the adrenal gland in Sheep is in general agreement with the observations made by other workers on the histogenesis of the gland in other animals,
and also in the human. It is quite logical to suppose from this that the developmental course of adrenal glands in general, certainly those of Sutheria, follows a common ground plan, and the fact that the presence of a foetal cortex in Sheep has been hitherto unnoticed, can only be taken as a reflection of the lack of observation which the gland in this animal has suffered. It is self-evident that in animals which have varying lengths of gestation, the developmental timetable of the adrenals must vary accordingly, but regardless of that age of embryo in which the various histogenic milestones become obvious, this timetable can be summarised generally as follows:

1) formation of the foetal cortex,
2) peripheral formation of the adult cortex,
3) formation of the glandular capsule,
4) formation of the medullary primordium by the invasion of the cortical anlagen by sympatho-chromophil elements from the neural ectoderm, which process is coetaneous with the development of the above three features and which, in Sheep, was observed to occur in the glands of 12 mm. embryos right up to those of full-term foetuses.

That the cells of the adult cortex eventually give rise to the mature cortical zones has been noted in Cat by Davies (1937), in the larger Felidae by Hill (1930 and 1937), in the pig by Patten (1948), in the ox, pig, dog, cat and rabbit by Nicander (1952) and in the human by Keene and Hewer (1927) and Uotila (1940), and in Sheep, it has been shown that at birth, the adult cortex already consists of a
definitive Zona Glomerulosa. In the few weeks following
birth, there is progressive involution of the foetal cortex
as evidenced by pyecnosis and fatty degeneration and, at the
same time, a corresponding enlargement of the adult cortex.
Whilst it was not found possible to trace the development of
the adult cortex in lambs of over six weeks of age, in the
face of the increase in breadth of this zone which was noted
in lambs up to that age, and also in view of what has been
stated in the literature by various workers to occur in
other animals, it is highly probable that in sheep, the
adult cortical anlage does in fact eventually take on the
zonation characteristics of the mature gland.

Since the presence of a foetal cortex was first uncovered in the developing human adrenal, the zone has been
supposed by many workers to perform a variety of functions,
but as yet, none has been adequately demonstrated. Even to
the uninitiated however, it is more than feasible to suppose
that the aggregations of foetal cortical cells are associated with some function or other, and while GROBMAN (1936)
considers the role played by these cells to be androgenic in
character, and ALBRIGHT (1943) has shown that the foetal
cortex in the mouse is identical with the X zone in that
animal, because of the initial development of the foetal
cortex during early embryonic life and its subsequent
degeneration after birth, it is possibly more logical to
seek as ROTTER (1949) and BRUNER (1951) have done, whether
a relationship exists between the foetal cortex and the
placenta. It is an established fact that chorionic
gonadotrophin is at its maximum during the early stages of pregnancy at approximately that time when the first conglomeration of ccelomic epithelial cells which form the foetal cortex can be observed histologically. Towards term, the supply of chorionic gonadotrophin diminishes and this, if such a relationship does exist between the placenta and the foetal cortex, could well set the stage for the degeneration of that zone which would automatically follow after removal of the placenta at parturition. The fact that the foetal cortex can be seen to undergo progressive degeneration after birth falls in with this hypothesis, but it should be emphasized that the suggestion of a link-up between placenta and foetal cortex is only tentative, and no supportive evidence for its actually being the case was uncovered either during this work or in any of the literature studied for the work.

If one were to follow the hypothesis a step further though, an intriguing parallel could be drawn between the placenta and the foetal cortex on the one hand, and a corpus luteum of pregnancy and a Graafian follicle on the other. The presence of luteal hormone inhibits the further maturation of follicles in the ovary; degeneration of the yellow body releases this breaking mechanism and allows the inhibited follicles to carry on where they left off. Conversely, the presence in the body of placental hormone could act as a stimulus to foetal cortical growth while degeneration, or rather loss, of the placenta at birth would remove such a stimulus and thus permit involution to occur.
In view of the evidence presented in this Section and in Section Three on the structure and presence of "floating nodules", it seems to be beyond question that in the mature gland such features do in fact represent clumps of cells from the anlage of the adult cortex which have become isolated by the developing capsule. ELIAS (1948), when considering such nodules as being an alternative to cortico-adrenal cellular replacement by the encapsulated type of nodule, shows a microphotograph of a "floating nodule" within the adrenal capsule of one lamb. He does not state the age of the lamb and moreover, mentions that in the three lambs examined for his work, only one showed the presence of "floating nodules". Both these facts are significant in so far as the small number of animals studied by this observer goes, for as already pointed out, "floating nodules" are relatively sparse in adult animals, and in the limited number of older adult animals examined during this work, that is to say, Sheep of one year and above, they were even more rarely observed. Whilst no conclusive postulation can be made as to their presence or absence in animals of this age, it does tend to indicate that as Elias states, "floating nodules" do become incorporated into the cortex, but not as he believes by an "unknown force", and not as a routine method of cortical cellular replacement, but as the gradual, inevitable result of being under the influence of capsular resilience, alternating medullary blood pressures and negative pressures in the Zona Reticularis, as discussed in Section Three.
Summary

The developmental course of the adrenal has been studied in this Section in a series of young Sheep from embryos of 10 mm. C-R. length to lambs of six weeks of age. The following observations have been made.

1. During development, the adrenal of Sheep like that of the ox, pig, dog, cat, rabbit and human, possesses a foetal cortex. The foetal cortex differentiates from coelomic mesothelium in embryos of approximately 10 mm. C-R length and remains obvious till birth. Involution of the zone becomes apparent after birth and this is progressive up to an age of six weeks when a very much reduced zone can be observed.

2. Adult cortical tissue can be seen to surround the foetal cortical anlage in 30 mm. embryos. The arrangement of the cells is characteristic, their being formed in groups which appear to have a peripheral fringing and an external limiting membrane. The fringing of the cells is due to fixation artefacts and the cells of the membrane probably represent rudimentary fibroblasts. Contrary to the findings of ELIAS (1945 and 1948), the so-called "floating nodules" seen occasionally in mature glands are not concerned with corticoadrenal cellular replacement but represent isolated remnants of adult cortical anlage cells.

3. A definitive glandular capsule is present in the glands of 150 mm. embryos.

4. The medulla is formed by sympatho-chromophil cells
which migrate ventro-laterally from the sympathetic ganglia in nodules. Migration of the cells is apparent in 12 mm. embryos and continues till full-term.
SECTION FIVE

Some Aspects of the Functional Significance of the Adrenal of Sheep

I. Birefringence in the adrenal

To the histologist and physiologist the morphological appearance of a gland is not generally very enlightening as to the functional status of that gland, and especially can this be said of the adrenal cortex. For this reason, in an effort to gain more precise information regarding the activity of the adrenal as a whole, recourse has to be made to histochemical techniques, and variations in the amount of lipid present, and differences in the size of fat globules help nowadays to gauge adrenocortical activity.

In the adrenal cortex, fat is made up of a mixture of cholesterol and its esters, the palmitic, stearic and oleic esters of glycerol, and the actual steroid hormones themselves (Pearse, 1955). Whilst the most usual dyes for routine demonstration of lipid are the sudans and osmic acid, the extent of sudanophilia and osmophilia purporting to give an estimate of the functional status of the gland, certain other techniques have been evolved which depend on the fact that adrenocortical hormones possess a reactive ketone group in their make-up. Amongst such techniques is the 2, 4-dinitrophenylhydrazine reaction which, while not strictly being specific for adrenocortical steroids, was used extensively by Bennett (1940), and the plasmal reaction which is reputed by Albert and Leblond (1946) to give positive
results in the presence of the steroids of the adrenal.

The Liebermann-Burchardt reaction and its modification, the Schults reaction, yields a blue-green colour in the presence of cholesterol and its esters which are now considered by most observers to be the precursors of adrenocortical hormones for SAYERS and SAYERS (1948) have shown that, in the rat, variations in the total cholesterol content of the adrenal correspond directly to functional changes in the gland, and CAIN (1950) states that the distribution of the same substance helps to throw light on the functional state of the glands of other animals.

One of the most useful methods for demonstrating the presence of cholesterol in tissues is the WINTENHUS (1910) digitonin reaction, during which the monatsical alcohol is precipitated, and the resulting birefringent crystals can be examined with the aid of a polarising microscope. As mentioned in the Section on Material and Survey of Methods, this technique was used extensively by workers such as LEVULIER and REVOL (1930), and various adaptations of the technique by ZWEMER (1933) and WEAVER and NELSON (1943) which workers also claim that the quantitative and qualitative distribution of cholesterol in the adrenal as demonstrated by its birefringent properties, is intimately associated with the production of adrenocortical hormones.

The presence of such birefringent material in the adrenal cortex was first noted by KAISERLING and ORGEL (1902), but it was not till ALBRECHT and WELTMANN (1911), using frozen sections of formal fixed human glands, found
abundant spherical crystals in the cortices of these glands that the idea of the association of the crystals with cholesterol came about. LANDAU and MCNEE (1914), observed birefringent material in the adrenal cortex of rabbits and, bearing in mind the findings of Albrecht and WELTMANN, stated that they could establish a relationship between the amount of such material and the cholesterol content of the gland.

BENNET (1940), in addition to making use of the histochemical techniques previously mentioned, also noted birefringent crystals in the adrenal cortex of the cat, and concluded that these crystals were for the most part due to the presence of cholesterol. OXFORD and BAXTER (1947) found that, in the rat, the region of maximal crystal formation was the outer portion of the Zona Fasciculata, and also that this region coincided with that portion of the cortex which yielded most strongly positive results with the phenylhydrazine reaction. Accordingly, with a view to determining the areas of birefringence in the adrenal cortex of Sheep, and thus the possible distribution of cholesterol esters and associated adrenocortical hormones, the glands from four Sheep were fixed in either neutral formal saline or 10 per cent formal, sectioned at 25 µ, on a freezing microtome, and then mounted either in syrup of laevulose or ZWERGER'S (1935) glycerogel and studied by means of a petrographic microscope.

If a tissue under examination by this method is optically inactive, no light reaches the eye, and the tissue
appears either red, dark-grey or black according to the amount of rotation of the polariser. It is a fact however, that most tissues are optically active in part, so that in polarised light, the birefringent structures are brightly illuminated as compared with the darker background formed by the relatively optically inactive parts of the tissue. Thus when a section of the adrenal of Sheep is examined after processing by the techniques mentioned above and in the Section on Material and Survey of Methods, the cortex can be divided into four distinct zones:

1) The outer or subcapsular zone, comprising the Zona Glomerulosa, is relatively sparse in birefringent material as compared with the inner optically active zones of the cortex (Plate LXVIII). What optically active material is present within this outer zone however, consists of a fine, granular scattering of crystals which appear to have a uniform distribution throughout the whole of the Zona Glomerulosa. Under high magnification, the crystals can be seen to be contained both within the cytoplasm of the glomerular cells and in the lumen of glomerular capillaries and, as mentioned in Section Three, the fact that encapsulated nodular cells yield the same birefringent reaction as glomerular cells is convincing evidence that such nodules eventually replace senescent corticoadrenal cells (see Plate XLVI).

ii) The next zone that can be observed on progressing centripetally through the cortex is a relatively narrow and optically inactive area which lies between the Zona
Glomerulosa and the Outer Fasiculata. In all the glands examined for this Section of the work, the cells of this zone were consistently found to be completely devoid of birefringent material and close inspection yielded that this optically inactive zone corresponded directly to the sudanophobic zone of the adrenal cortex of the rat (REISS et al., 1936) etc., and to the Zona Intermedia as discussed in Section Two of this work. The presence of this zone is adequately demonstrated in Plate LXXIX, where it appears as a dark band between the dust-like birefringent particles of the Zona Glomerulosa and the large, irregular crystals of the Outer Fasiculata.

iii) The main optically active zone of the adrenal cortex of Sheep consists of all, or most of, the Zona Fasiculata. Whilst the optically inactive Zona Intermedia corresponds as pointed out in Section Two, to BENNET'S (1940) "pre-secretory zone" in the cortex of Cat, this region is for the most part equivalent to that worker's "secretory zone" in the same animal. The demarcation of Outer Fasiculata from Zona Intermedia is well shown in Plates LXXVIII and LXXIX and it can be seen from these and Plate LXXX that birefringence in the Zona Fasiculata as a whole is characterised by the presence of large, unevenly-shaped crystals which are scattered irregularly throughout the whole of the parenchyma. In addition to these large, highly birefringent globules, the cells (and occasionally the capillaries), of the Fasiculata can be seen to be packed with the same kind of small, intra-cytoplasmic particles of
birefringent material as were noted within the Zona Glomerulosa.

iv) The density and the relative size of the large crystals in the Zona Fasiculata are of importance when one considers the distribution of birefringent material in the fourth and final cortical zone which corresponds to the Zona Reticularis, for the change from the former zone to the latter is very gradual, and unless the cortico-medullary junction is used as a guide, the demarcation of the reticularis is frequently hard to determine under polarised light. It can however, be said that the Zona Reticularis is in general, poorer in birefringent material than the preceding zone, and what crystals can be observed are larger, more irregular, more scattered and much fewer in number than those in the Zona Fasiculata (Plate LXXXI). Occasionally, some of the birefringent crystals in this zone are so large as to fill an entire cell, but in such cases, no nucleus could be seen within the cells and thus these cells could logically be regarded as either being dead, or in the last stages of senescence.

There was no evidence at all of birefrigence in the medulla in any of the glands studied for this Section, and this, as already remarked, rendered an acute landmark for location of the Zona Reticularis with the petrographic microscope. This point is well illustrated in Plate LXXXII which is a colour transparency of the Zona Reticularis and the medulla of the gland of a Soay-Mouflon ewe, photographed under polarised light. The venous sinuses
of the medulla show up as dark areas and the large, irregular crystals of the reticularis are easily discernible.

That the main area of optical activity in the adrenal of Sheep is confined to the Zona Fasieulata, is in complete agreement with the findings of WEAVER and NEILSON (1943) in the rat adrenal, and YOFFEI and BAXTER (1947) in the same animal and, as the latter workers indicate that this same area is also strongly Schults- and phenylhydrazine-positive, it is quite logical to assume that the Zona Fasciulata as illustrated by the presence of birefringent crystals is, in Sheep, the most rich in definitive cholesterol of all the cortical zones.

The purpose of this Section of the work is to record observations on the distribution of birefringent material in the adrenal cortex of Sheep, and it is not intended to attempt a correlation between the distribution of cholesterol and its esters on the one hand, and the apportion of adrenocortical hormones on the other. However, it might be remarked that, in the face of the above evidence, it appears to be illogical to presume that, if a relationship between cholesterol and adrenocortical hormones does exist (and study of the literature certainly seems to suggest that it does), the majority of hormonal secretions are carried out by the Zona Fasciulata. It would rather be more tenable to suppose that the glomerulosa also contributes to the general hormonal output of the gland, but that its cholesterol is not always visible in birefringent form as it may be in solution in the high fatty content of the zone which, as
remarked in Section Three is an outstanding feature of the adrenal cortex of Sheep (see Plates XLVIII and XLIX).

Thus, considerable amounts of cholesterol could conceivably be masked by the presence of such a high proportion of glomerular lipoid and so not show evidence of birefringence just as OKÉY (1944) found that though the livers of guinea-pigs fed with large amounts of cholesterol might contain greatly increased amounts of both fatty acids and total cholesterol, all efforts to determine the localisation of the cholesterol and to distinguish it from other liver lipoids by means of the petrographic microscope were unsuccessful, possibly, as this worker states, "because the cholesterol was in solution in the fat, and the solution was itself not birefringent."

II. Consideration of the adrenal-gonad relationship

The existence of a functional inter-relationship between the adrenal cortex and the manifold processes of reproduction has been acknowledged by most workers since DEANESLY (1928) demonstrated that visible changes in the intracellular lipoid pattern in the rodent adrenal can be correlated with alterations in sexual activity. This worker also showed that the "X zone" of the mouse adrenal cortex undergoes distinctive degenerative changes which can be associated with puberty in the male and gestation in the female. Since 1928 many workers have investigated this problem of a link-up between the adrenals and the gonads and their observations have been comprehensively summarised by authors such as PARKES (1945), BURROWS (1949) and
Whilst both gonad and adrenal perform distinct physiological roles, the two glands possess a common denominator in that the adrenal cortex depends on pituitary adrenocortrophic for normal functioning, gonadal tissue on pituitary gonadotrophins. Whether or not a further interdependence exists between the glands by virtue of their common coelomic epithelial origin is still a point of debate in current literature but variations in staining reaction of cortical tissue which are attributable to sexual dimorphism have been recorded in the rat by KORENCHENSKY and HALL (1938), in the cat by BENNET (1940) and in normal, castrated and spayed cats by LOBMAN (1952). The last-named worker pointed out however, and further investigations by observers such as ZUCKERMAN (1953) have confirmed that such variations cannot be ascribed to a simple sex difference, for alterations in staining affinity and indeed, cortical morphology as a whole, turn primarily on the innermost zone of the cortex, the Zona Reticularis.

Both PARKES (1945) and ZUCKERMAN (1953) have established that in the female animal there is a close relationship between corticoadrenal tissue and the various phases of the oestrous and menstrual cycles and, as pointed out in Section One of this work, JONES (1957) in his symposial work on the adrenal cortex, states that gonadectomy results in an alteration of size of the adrenal cortex, the Zona Reticularis becoming thicker in the castrated male, the same zone declining in the spayed female. It could be surmised from
this that the Zona Reticularis acts as some kind of secondary sex organ, responding to changes in the sexual state of the animal in question in much the same way as does, for instance, the epithelium of the seminal vesicles. The fact that injection of gonadal hormones reverse these changes as recorded both by Lobb (1952) and Jones (1957), lends considerable weight to this hypothesis.

Accordingly, with a view to determining whether the experimental alteration of sex and also the subsequent injection of testosterone influenced in any way the Zona Reticularis of the Sheep adrenal, four adult male animals were castrated, three then being treated with upgraded doses of testosterone proprionate as shown in Table III, the remaining Sheep acting as a control.

The animals were killed 72 hours after their last injection and their adrenals fixed, sectioned and stained with Azan as stated in the Section on Material and Survey of Methods. The relative proportions of the different zones of the cortex of the four experimental animals and also six normal adult uncastrated male subjects were determined at their thickest and thinnest portions and thus the average width of the individual zones deduced. In order to give as accurate an overall picture as possible in the case of the experimental glands, the average zonal widths were deduced from a series of six sections cut from the mid-portion of each gland and compared with the readings obtained from the glands of the six uncastrated animals.
At the outset, the measurements of the zones were calculated as mentioned in the Section on Material and Survey of Methods, that is by use of a micrometer eye-piece, but latterly it was found to be more convenient to project the whole slide onto a screen and measure the width of the zones with a foot-rule. Thus, in the Tables below, the width of the zones in the different glands is expressed as the number of divisions of the foot-rule which they were seen to occupy under standard conditions of projection and magnification. From this the ratio of the width of the Zona Fasiculata to that of the Zona Reticularis was deduced for each gland, and the mean F/R ratio used as a gauge for comparison of the variation in the latter zone met with in the five different classes of animals used for this study.

Observations

With the Azan stain, the junction between fasicular and reticularis tissue is generally quite distinct, the cells of the latter zone being intensely acidophilic while the cells of the former zone possess a much paler staining cytoplasm. In the slides examined for this Section of the work, the acidophilic cells were comparatively small with dark staining nuclei and they had the common reticular arrangement of true Zona Reticularis cells so that in each case, the division between fasiculata and reticularis was taken as the junction between the pale staining cells and those with acidophilic cytoplasm.

The Sheep on which observations were based were divided into the following five classes according to their
sexual condition:

Class 1: Normal adult uncastrated males (Sheep A to F).

Class 2: Adult castrated control (Sheep G).

Class 3: Adult castrate with previous administration of 200 mg. testosterone propionate (Sheep H).

Class 4: Adult castrate with previous administration of 400 mg. testosterone propionate (Sheep I).

Class 5: Adult castrate with previous administration of 600 mg. testosterone propionate (Sheep J).

In all classes of Sheep, the width of the Zona Glomerulosa showed very little variation and as the small amount of variation in width of the Zona Fasiculata in the different animals was due largely to differences in adrenal size, this factor was adjudged to be normal and was accordingly disregarded. The width of the Zona Reticularis however, varied considerably in the individual classes, despite the fact that the F/R ratio was the same for both right and left glands in any one animal, and it can be seen from the following Tables that this ratio, that is, the width of the fasiculata to that of the reticularis, presents certain characteristics which can be correlated with the experimental sexual condition of the different classes of animals.
Table XII

Class 1 - Adult, non-castrated males

<table>
<thead>
<tr>
<th></th>
<th>Z.G.</th>
<th>Z.F.</th>
<th>Z.R.</th>
<th>F/R ratio</th>
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<tr>
<td>Sheep A</td>
<td>10</td>
<td>55</td>
<td>26</td>
<td>2.12/1</td>
</tr>
<tr>
<td>Sheep B</td>
<td>11</td>
<td>66</td>
<td>28</td>
<td>2.36/1</td>
</tr>
<tr>
<td>Sheep C</td>
<td>18</td>
<td>61</td>
<td>25</td>
<td>2.44/1</td>
</tr>
<tr>
<td>Sheep D</td>
<td>9</td>
<td>67</td>
<td>23</td>
<td>2.91/1</td>
</tr>
<tr>
<td>Sheep E</td>
<td>9</td>
<td>71</td>
<td>23</td>
<td>3.09/1</td>
</tr>
<tr>
<td>Sheep F</td>
<td>10</td>
<td>69</td>
<td>32</td>
<td>2.16/1</td>
</tr>
</tbody>
</table>

Mean F/R ratio for Class 1 Sheep = 2.51/1

Table XIII

Class 2 - Sheep G. Adult, castrated control

<table>
<thead>
<tr>
<th></th>
<th>Z.G.</th>
<th>Z.F.</th>
<th>Z.R.</th>
<th>F/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section I</td>
<td>12</td>
<td>49</td>
<td>45</td>
<td>1.09/1</td>
</tr>
<tr>
<td>Section II</td>
<td>12</td>
<td>41</td>
<td>39</td>
<td>1.05/1</td>
</tr>
<tr>
<td>Section III</td>
<td>11</td>
<td>46</td>
<td>45</td>
<td>1.02/1</td>
</tr>
<tr>
<td>Section IV</td>
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<td>47</td>
<td>46</td>
<td>1.02/1</td>
</tr>
<tr>
<td>Section V</td>
<td>12</td>
<td>46</td>
<td>45</td>
<td>1.02/1</td>
</tr>
<tr>
<td>Section VI</td>
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<td>46</td>
<td>44</td>
<td>1.05/1</td>
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</table>

Mean F/R ratio for Class 2 Sheep = 1.04/1
### Table XIV

Class 3 - Sheep II. Adult castrate injected with 200 mg. testosterone propionate.

<table>
<thead>
<tr>
<th>Section</th>
<th>Z.G.</th>
<th>Z.F.</th>
<th>Z.R.</th>
<th>F/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>24</td>
<td>11</td>
<td>2.18/1</td>
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<tr>
<td>II</td>
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<td>23</td>
<td>12</td>
<td>1.92/1</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>23</td>
<td>11</td>
<td>2.09/1</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>26</td>
<td>13</td>
<td>2.00/1</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>23</td>
<td>10</td>
<td>2.30/1</td>
</tr>
<tr>
<td>VI</td>
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<td>24</td>
<td>12</td>
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Mean F/R ratio for Class 3 Sheep - 2.08/1

### Table XV

Class 4 - Sheep I. Adult castrate injected with 400 mg. testosterone propionate.

<table>
<thead>
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<th>Z.R.</th>
<th>F/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>43</td>
<td>20</td>
<td>2.15/1</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>46</td>
<td>23</td>
<td>2.00/1</td>
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<tr>
<td>III</td>
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<td>46</td>
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<td>2.00/1</td>
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<td>9</td>
<td>46</td>
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<td>2.19/1</td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>48</td>
<td>23</td>
<td>2.09/1</td>
</tr>
<tr>
<td>VI</td>
<td>9</td>
<td>47</td>
<td>21</td>
<td>2.21/1</td>
</tr>
</tbody>
</table>

Mean F/R ratio for Class 4 Sheep - 2.11/1
Table XVI

Class 5 - Sheep J. Adult castrate injected with 600 mg. testosterone propionate.

<table>
<thead>
<tr>
<th>Section</th>
<th>Z.G.</th>
<th>Z.F.</th>
<th>Z.R.</th>
<th>F/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>33</td>
<td>16</td>
<td>2.06/1</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>31</td>
<td>14</td>
<td>2.21/1</td>
</tr>
<tr>
<td>III</td>
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<tr>
<td>IV</td>
<td>6</td>
<td>33</td>
<td>15</td>
<td>2.20/1</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>29</td>
<td>15</td>
<td>1.93/1</td>
</tr>
<tr>
<td>VI</td>
<td>6</td>
<td>34</td>
<td>15</td>
<td>2.27/1</td>
</tr>
</tbody>
</table>

Mean F/R ratio for Class 5 Sheep - 2.12/1

Zonal width is expressed in numbers of divisions of foot-rule.

Z.G. Zona Glomerulosa; Z.F. Zona Fasiculata;
Z.R. Zona Reticularis

Table XVII

Tabulation of the mean F/R Ratios

<table>
<thead>
<tr>
<th>Class</th>
<th>State of Animal</th>
<th>F/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-castrated adult male</td>
<td>2.51/1</td>
</tr>
<tr>
<td>2</td>
<td>Castrated adult male control</td>
<td>1.04/1</td>
</tr>
<tr>
<td>3</td>
<td>Castrate, 200 mg. testosterone</td>
<td>2.08/1</td>
</tr>
<tr>
<td>4</td>
<td>Castrate, 400 mg. testosterone</td>
<td>2.11/1</td>
</tr>
<tr>
<td>5</td>
<td>Castrate, 600 mg. testosterone</td>
<td>2.12/1</td>
</tr>
</tbody>
</table>
From the measurements recorded in the Tables above, it can be said that a Zona Reticularis is present in the adrenal cortex of male Sheep whether castrated or not, and that the zone undergoes structural alterations which, when put into ratio with a relatively constant factor such as the width of the Zona Fasiculata, can be seen to bear a direct relationship to the sexual condition of the animal in question.

That the Zona Reticularis can be directly correlated with gonadal activity in Sheep is in agreement with the findings of Nelson (1941) in the rat, and Lobban (1952) in the cat and also with what is said by Jones (1957) to be the general case in that group of animals (of which Sheep, as shown in Section One of this work, is a member) which normally display post-puberal adrenal sexual dimorphism.

The F/R ratio of the adrenal cortex of normal adult uncastrated male Sheep is 2.51/1. In other words, there is approximately 2½ times more fasicular tissue than there is reticular. The changes which can be observed in the Zona Reticularis of castrated animals, manifested by fluctuations in their F/R ratios can be summarized as follows: in the adult castrated animal, the Zona Reticularis shows a very marked degree of development so much so that calculation of the F/R ratio indicates that gonadectomy in male Sheep induces expansion of the zone to approximately the same width as the fasiculata.

Injection of testosterone in castrate Sheep brings about a decrease in width of the reticularis and it is
apparent from the F/R ratios calculated for the injected castrate animals that this decrease in width caused by the administration of the gonadotrophin is a progressive one. Thus, the F/R ratio for the animal which received 200 mg. of testosterone shows that the reticularis is approximately half the width of the fasiculata. The F/R ratios of the 400 mg. and 600 mg. animals indicate that the reticularis has decreased even more in width and, taking this progressive reduction in zonal depth a step further, it is quite reasonable to suppose that if the scope of the experiments had been widened, doses of testosterone higher than 600 mg. might well have yielded an F/R ratio of approximately 2½ to 1, which was the F/R ratio obtained for normal uncastrated male Sheep.

Discussion

I. Birefringence in the adrenal

Whether or not the quantitative and qualitative distribution of the birefringent material in the adrenal cortex of Sheep is closely associated with a) cholesterol, and b) the production of adrenocortical hormones, as is suggested for instance in the oat by BENNET (1940) and in the rat by WEAVER and NELSON (1943), cannot conclusively be stated to be the case from the observations made in this Section. A point which does lend weight to the hypothesis being applicable to Sheep however is the close association between the small birefringent particles which were readily seen within the cytoplasm of both glomerular and fasicular
cells and those within the lumen of the capillaries of these zones. The normal route by which adrenocortical hormones leave the gland is of course via the blood vessels, and taking this idea a step further, it could be surmised that such intra-cytoplasmic particles of birefringent material are precursors or perhaps even portions of cortical hormone which have already been discharged into these vessels. This is, of course, purely supposition, and no evidence to the effect that the birefringent material as a whole in the cortex of the adrenal of Sheep is linked with cortical hormones was noted other than this relationship between the birefringent particles within the glomerular and fascicular cytoplasm and those particles noted, as mentioned, in the capillaries of the two zones. Nevertheless, the idea does find some measure of support in HENNET'S (1940) observation that crystals of deoxycorticosterone acetate are birefringent when examined with polarised light.

When considering the formation of birefringent crystals in any adrenal cortex, there are several factors which must be taken into account, apart from the most obvious one of functional status of the gland in question. Of these, temperature, the effect of light, and the type of mounting medium used are perhaps the most important. In the case of the last named, the type of mounting medium undoubtedly influences the appearance of crystals, for if sections of glands mounted in syrup of laevulose are kept at room temperature in daylight, the crystals which can be seen at approximately 30 minutes after mounting gradually increase
in number until a point is reached when no more crystals appear. The obvious conclusion from this is that the crystals which appear after the first 30 minutes are derived from the mounting medium itself and it was because of this that during the experiments carried out for this work extreme care was taken to observe and photograph all necessary sections at approximately the same time after mounting. An example of a section which was mounted in syrup of laevulose and photographed 24 hours after mounting can be seen in Plate LXXXIII. The vast increase in birefringence is quite obvious when compared with Plate LXXVIII which was photographed 30 minutes after mounting. It will be noted too, that if left long enough even the capsule begins to show a certain fibrillar birefringence and the picture of the optically active Zona Glomerulosa is entirely different from that in the latter plate.

The possibility that the birefrigent properties of cholesterol can be hidden when the substance is in solution as OKEN (1944) mentioned in connection with his work on the livers of guinea-pigs could, as already pointed out, be applied to such cholesterol as may be present in the Zona Glomerulosa of Sheep. It appears to be quite possible though that cholesterol can also be present without being in fatty solution and without showing any optical activity for LEULIER and REVOL (1930) have recorded the total cholesterol content of the adrenal cortex of Sheep as being between 2.12 to 4.06 per cent, and of the medulla of the
same animal as 3.45 to 6.60 per cent. This high cholesterol content of the medulla of Sheep adrenal is extremely interesting from two points of view, the first, regarding the non-formation of birefrigent crystals within medullary parenchyma, and the second, the pronounced sudanophobia of this same region. Thus, if it can be assumed that cholesterol is present in the medulla of Sheep adrenal, and nothing has been uncovered either in the literature or during this work to contradict the findings of Leulier and Revol, such would lend weight to the argument of Okay that cholesterol when present in a tissue, need not always possess birefringent properties. At the same time however, it would tend to show that even if a high proportion of lipid can mask such birefringence that cholesterol in the same region may possess, as suggested by this worker, because of the lack of medullary lipid there must logically be another form of cholesterol which is normally optically inactive.

On the other hand, in face of this argument, it seems just as apt to suppose that the birefrigent crystals in the adrenal cortex are not formed by cholesterol itself but possibly by the breakdown of some optically inactive precursor which occurs in fresh adrenal tissue. YOFFEY and BAXTER (1947) agree with this suggestion, and in view of the fact that lipoproteins form approximately one-fourth of the blood plasma proteins as proved by GOREN (1946), the possibility that such an optically inactive precursor might be a lipoprotein and thus corticoadrenal hormones as a whole might enter the blood as lipoproteins could well be
entertained. To prove such a hypothesis though would of necessity require a high proportion of critical investigation of adrenal glands in all stages of physiological activity but, considering the problem dispassionately, it is not without the bounds of possibility that the actual form in which adrenocortical hormones are formed and are discharged from the gland, may be uncovered at some future date.

II. The adrenal-gonad relationship

The simplest interpretation which can be read into the observations recorded in this Section regarding adrenal-gonad relationship is that the Zona Reticularis in the adult Sheep responds directly to the influence of gonadotrophins whose presence or absence depends on the sexual condition of the animal in question. Expressed another way, this is to say simply that the Zona Reticularis of Sheep acts as a secondary sex organ.

Unfortunately though, the problem of adrenal-gonad relationship cannot be resolved so readily. There are many governing factors such as the effects of the anterior lobe and such physiological episodes as puberty and gestation which demand consideration. Because of this it is perhaps most straightforward to approach the problem in two ways, the first being an examination of the question of whether or not the adrenal cortex manufactures gonadotrophins on its own, and the second, whether or not there is any region in the adrenal cortex which can logically be regarded as having an influence on, or being influenced by, the sexual life of the animal.
That the Zona Reticularis is intimately bound up with the sexual life and condition of the animal has been shown indisputably to be the case in Sheep just as has been proved in other animals by workers such as HILL (1937), DAVIES (1937), LORRIS (1952) and JONES (1957). Whether the adrenal gland as a whole and particularly this 'sexual' zone of the cortex has the ability to perform its 'sexual' role by secreting sex hormones on its own, or it represents merely what might be termed a gonadotrophic receptive area has been the subject of much debate in the literature. Modern views have it however that the adrenal cortex does indeed have the capacity to secrete sex hormones and, taking this concept a step further, it is possible to put forward two (if not more) theoretical applications of the manner in which corticoadrenal gonadotrophins might be produced:

1) ACTH from the anterior lobe acting directly on the cortex to produce not only corticosteroids but also androgens and oestrogens,

   ii) gonadotrophins acting on the adrenal cortex to produce further gonadotrophins.

Taking the second hypothesis first, it is self-evident that gonadectomy will remove the prime source of gonadotrophins from the body. Whilst it is an established fact that in gonadectomised eutherians, gonadotrophins are stored in the anterior lobe of the pituitary (JONES, 1957), if the adrenal cortex (under the influence of the 'stored' hormones) were an additional, prolific source of sex hormones it would follow logically that in gonadectomised
animals these hormones would maintain amongst other features, the secondary sexual characteristics of the animal. This is, of course, not so. On the other hand, there is little doubt that the adrenal cortex if not able to produce gonadotrophins in bulk, is decidedly under their influence either directly from the gonads or indirectly from the pituitary. This is amply demonstrated by certain diseases in man, the Addisonian notably showing follicular atresia and a decline in secondary sexual characteristics in long-term cases.

Of all animals, probably the mouse presents the only unequivocal case where gonadotrophins have been shown to have a direct influence on the adrenal cortex. The cortex of the mouse adrenal possesses a juxtaglomerular zone which DEANESLY (1928) and other workers now recognise as the X zone. Whilst there is no record in any of the literature with regard to the X zone being secretory in nature, the homage which the region pays to gonadotrophic hormones in general is abundantly clear for, as previously mentioned, in the male mouse the X zone undergoes complete degeneration under the influence of the androgens first produced at puberty. The same phenomenon occurs in the female gland with the release of oestrogens at the time of first gestation. It has also been shown by JONES (1955) that if a male mouse is castrated after puberty such removal of the androgenic 'brake' evokes marked growth of a secondary X zone with the result that the adrenal cortex as a whole increases in size. Parenteral administration of gonadotrophins causes a diminution of this secondary X zone.
analogous to the reduction in width of the Zona reticularis in adult castrate cats after injection of testosterone as reported by LOBBAN (1952).

The question which necessarily arises from this is whether or not the X zone of the mouse cortex is comparable with the reticularis of the cat adrenal. HILL (1930) stated that during post-natal life the adrenal gland of the cat has no definitive Zona Reticularis. DAVIES (1937) however refuted this remark and described a well-marked reticularis centripetal to the fasciculata in the adult animal. Further observations by these workers (DAVIES, 1937; HILL, 1937) have also yielded, as mentioned earlier in this text, that during developmental life the adrenal of the cat possesses a foetal cortex. The X zone of the mouse adrenal is considered by some workers as being derived from foetal cortical tissue (JONES, 1957) and the suggestion by LOBBAN (1952) that as the cat also possesses a foetal cortex it might conceivably possess a true X zone, is quite tenable.

Similarly, as Sheep has been shown in Section Four to possess a foetal cortex it could be considered that this animal also displays a true X zone and one might even go a step further and suggest that such an X zone in this animal is identified with the adult Zona Reticularis being linked with, if not actually derived from, the foetal cortex, for while the X zone has its most typical representation in the adrenal cortex of the mouse, the zone in that animal demonstrates a potentiality in one species which may well be present in others.
It was not possible though to determine whether the decrease in F/R ratio in the castrated and castrated-injected sheep involved the reticularis in part or in toto. Neither has it proved possible to follow the degenerative processes which were observed in Section Four to be well established in the foetal cortex of lambs of six weeks of age. However, bearing in mind the evidence presented above regarding the influence of androgens on the X zone of both the pre- and post-puberal mouse adrenal and the influence of gonadectomy and subsequent injection of testosterone on the adult reticularis of both Cat and Sheep, one can surmise without being guilty of illogical presumption that if it were possible to make a critical study of the gradual disappearance of the foetal cortex and the appearance of the adult cortex, with especial regard to the development of the Zona Reticularis, the answer to the problems of adreno-gonadal relationship might well be uncovered.

**Summary**

The first part of this Section has been devoted to the demonstration of birefringence in the adrenal cortex of Sheep. The following observations have resulted:

1. Birefringent crystals are confined entirely to the cortex. No optical activity of any kind can be observed in the medulla.

2. Variations in optical activity in the cortex as manifested by the formation of birefringent crystals allow the region to be divided into four distinct zones as follows:
i) A subcapsular zone consisting in the main of the Zona Glomerulosa, which shows a scattering of fine granular crystals which have uniform distribution throughout the zone.

ii) A narrow and irregular zone which is optically inactive, consistently being devoid of birefringent material, and which is equivalent to the Zona Intermedia.

iii) A main optically active zone corresponding to the Zona Fasciculata. Birefringent crystals are present in the form of large unevenly-shaped globules and also the same type of small, intra-cytoplasmic particles seen in the outermost zone.

iv) An innermost zone corresponding to the Zona Reticularis which is relatively sparse in birefringent material. What crystals are present are very large, very irregular and very scattered.

3. In the optically active zone corresponding to the Zona Glomerulosa and Zona Fasciculata, dust-like particles of birefringent material are present within the zonal capillaries.

The second part of this Section which has been devoted to an investigation into the effects of gonadectomy and subsequent administration of testosterone propionate in adult male Sheep, has yielded the following observations:

1. The adrenal cortex of male Sheep whether castrated or not possesses a definitive Zona Reticularis.

2. Structural alterations in the width of the Zona
Reticularis have been demonstrated which can be correlated with the sexual condition of the Sheep.

3. The ratio of the width of the Zona Fasiculate to that of the Zona Reticularis in five different classes of animals is as follows:

i) 2.51/1 in non-castrated adult males.

ii) 1.04/1 in castrated adult male Sheep.

iii) 2.08/1 in castrate Sheep injected with 200 mg. testosterone.

iv) 2.11/1 in castrate Sheep injected with 400 mg. testosterone.

v) 2.12/1 in castrate Sheep injected with 600 mg. testosterone.
Appendix
(See SNEDECOR, Page 395)

Analysis of Covariance: Adrenal/Body Weight Data

<table>
<thead>
<tr>
<th>Line</th>
<th>Animal</th>
<th>f</th>
<th>Ex^2</th>
<th>Exy</th>
<th>Ey^2</th>
<th>Reg. Coef.</th>
<th>f Ex^2 - (Exy)^2 / Ex^2</th>
<th>Mean Square</th>
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<tr>
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<td>69 5.5408</td>
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<tr>
<td>2</td>
<td>Rams</td>
<td>7</td>
<td>561.87</td>
<td>63.32</td>
<td>7.2944</td>
<td>0.1126</td>
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<td>Ewes</td>
<td>29</td>
<td>3196.80</td>
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<td>0.1109</td>
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<td>4</td>
<td>Within</td>
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<td>108</td>
<td>9075.56</td>
<td>1108.73</td>
<td>177.7611</td>
<td></td>
<td>107 2160.19</td>
<td>20.1886</td>
</tr>
</tbody>
</table>

Adjusted Means

\[ \bar{y} - b(x - \bar{x}) \]

Castrates 5.09 - 0.1312(67.32 - 66.80) = 5.02
Rams 6.36 - 0.1312(73.37 - 66.80) = 5.50
Ewes 5.82 - 0.1312(63.80 - 66.80) = 6.21
As mentioned in the Introduction, the so-called suprarenal capsules were first recognised by Bartholomeus Eustachius Sanctoseverinatus, in the year 1564, and from many points of view, the manner in which knowledge of the adrenals has grown since then is highly intriguing. However, in this survey of the adrenal gland of Sheep, it is perhaps only apposite to note that in 1716, the Académie des Sciences of Bordeaux offered a prize for an answer to the question: "Quel est l'usage des glandes surrenales?" Many extraordinary theories were forthcoming in solution of the problem and finally this 18th century quiz was closed with the statement: "Le hasard fera peut-être quelque jour ce que tous les soins n'ont pu faire."

This chance was not long in coming, and as has been pointed out several times in the text of this work, the literature now available on the adrenal gland of eutherian mammals is enormous, and indeed, a brief survey of current journals indicates that it continues to grow constantly.

This work represents in the main, a general survey of the anatomical, histological and embryological constitution of the adrenal gland of Sheep, and while several new features have both been introduced and suggested, the general background and some of the current problems which are related to adrenal glands in general, have been slanted toward the gland in that animal for, whilst most of our
information on the adrenal body has been obtained from the rat, observations on the relatively wide range of mammalian glands available in the literature indicate that, in the field of comparative endocrinology, much can be learned that is of significance when a specific gland such as that of Sheep, which to date has been pretty well neglected by observers, is studied in any great detail.

The all-embracing nature of this study, from the embryological development of the gland to its intimate structure in the adult animal, has necessitated each relevant Section being discussed and summarised separately. Accordingly, this is intended to be what might be termed a 'synopsis of summaries', and only the main features uncovered during study of the gland will be set down in the order in which they occur in the text.

**Section One**

1. The gross anatomy of the adrenals of Sheep are similar to other eutherian glands with regard to topography and relationships, being in juxtaposition to the anterior poles of the kidneys, surrounded by adipose tissue and having their own characteristic shape.

2. For the average age of animal used in pursuance of this work, glandular length was found to approximate 24 mm., and breadth 10 mm. The average weight of both right and left glands was found to be in the region of 2.5 G. The relative and absolute weight of the adrenals in the ewe is greater than in the ram, and greater in the ram than in the castrate. That the glands do not grow isometrically with
body weight as applied to adrenal glands in general, has been shown to be the case in Sheep, where glandular weight increases with increase in body weight to approximately 30 pounds, whereafter fluctuations in adrenal weight are more likely to be dependent on such physiological episodes as oestrus, rutting and pregnancy rather than an increase in body weight.

3. The general topography of the blood supply to the adrenals of Sheep is more or less in accordance with that recorded for Dog by FLINT (1900). Major adrenal trunks are derived from the aorta, coeliac axis and renal arteries. The right gland has a more profuse vascularisation than the left, having distinct dorsal and ventral arterial stems which are subject to a small amount of variation in different individuals. A single adrenal vein, guarded by a hitherto undescribed valvular apparatus, drains constantly into the caudal vena cava in the case of the right gland. The left gland drains into the left renal vein either directly, or by a maximum of four slender veins. The efferent adrenal lymphatics drain directly into the paired renal lymph nodes which are present at the hilus of either kidney, and which strictly belong to the bilateral lumbar chain of lymph glands.

4. Innervation of the adrenal of Sheep is achieved by pre-ganglionic fibres which are derived from the following two sources:

1) directly from both greater and lesser splanchnic nerves,
ii) indirectly from the coeliac-mesenteric ganglion.

A fewer number of efferent post-ganglionic fibres can also be traced to the gland from the coeliac-mesenteric ganglion.

Section Two

5. Variations in the histological pattern of the adrenal cortex of Sheep are consistent enough to demand enlargement of the customary zonation. The zonation of NICANDER (1952) was found to be most apt and thus the cortex was subdivided as follows:

- Zona Glomerulosa
- Zona Intermedia
- Zona Fasciculata
  - Outer Fasciculata
  - Inner Fasciculata
- Zona Reticularis

6. The two types of tissue in the make-up of the adrenal of Sheep, that is, cortex and medulla, vary to a high degree; this variation depending to a certain extent on the functional status of the gland.

7. What pure-bred Sheep were available for study, were in every instance observed to possess a definitive Zona Glomerulosa. This was not so in the case of cross-bred animals, the majority of which failed to show the typical morphological groupings of glomerular cells. In these animals this was found to be due to the fact that the cortical fasciculi failed to arch and link up with one another immediately underneath the capsule.
8. In every case the adrenal capsule of Sheep was found to be comprised of two separate zones. The outermost zone consists of a network of connective tissue fibres, which, in addition to having a few large elastic fibres whose presence is generally accepted in modern literature, possesses a hitherto unobserved abundance of extremely fine elastic fibres which can only be demonstrated by modifying Verhoeff's and the Orcein technique. The inner capsular layer consists of undifferentiated mesenchymal cells quite distinct from ordinary connective tissue cells.

9. The so-called 'light' and 'dark' cells described in the Zona Reticularis of guinea-pig adrenals by Hoerr (1931), are present in the same region in Sheep and there appears to be a relationship in Sheep between adrenal stress and cellular degeneration in this zone as manifested by pyknosis, karyolysis and karyoklasia.

10. Approximately six per cent of the adrenals of Sheep of both sexes studied, including castrated males, revealed large aggregations of adult eosinophils in the medulla. Similar observations have been recorded for Sheep by Flint (1900) but no explanation can be offered as to the cause of the phenomenon.

11. The arterial system of the Sheep adrenal, like that of the dog (Flint, 1900) consists of three independent capillary networks as follows:

   i) capillaries to the capsule,

   ii) capillaries to the cortex,

   iii) capillaries to the medulla.
The three sets of capillaries spring from a subcapsular arterial plexus. Capsular capillaries drain either into capsular veins or adjacent connective tissue veins; cortical and medullary capillaries eventually anastomose to form the radicals of the venous tree which is a prominent feature of the medulla. The majority of medullary capillaries can be observed to be separated from the medullary venous sinuses by the length of a single chromaf-fin cell. The nuclear poles of such cells are inclined toward the capillaries, those poles containing chromaffin granules toward the venous sinuses. This has been taken to indicate that adrenal medullary cells in Sheep possess a nutrient and a secretory pole.

12. The valvular apparatus previously mentioned to be present at the exit of the right central medullary vein into the caudal vena cava consists of a double layer of endothelial cells with a corium of transversely arranged plain muscle fibres.

13. The lymphatic system of the Sheep adrenal can be admirably demonstrated by the intra-peritoneal injection of lithium carmine.

14. Owing to difficulties in distinguishing reticular from nerve fibres during routine silver impregnation of sections, a modification of the Bielschowsky technique was evolved which indicates that the picture of the innervation of the adrenal of Sheep agrees with that in other eutherian glands. The majority of nerves supplying the gland are myelinated, and destined for the medulla. What nerves can
be observed in the cortex are amylinate and appear to
terminate on the walls of the blood vessels of that region.
Nerve endings are present in the medulla similar in
structure to the end bulbs of Krause.

Section Three

15. Study of adult Sheep glands indicates that cortico-
adrenal cells have a definite life cycle, and that the
production of young cells occurs for the most part within
the glandular capsule. The inner layer of undifferentiated
mesenchymal cells mentioned in para. 8 have the ability to
metamorphose into merocrine glomerular cells and, under the
influence of a hitherto unnoticed abundance of fine elastic
fibres in the outermost layers of the capsule, migrate
centripetally to become incorporated into the cortex.

16. Cellular replacement in the adult cortex of Sheep
occurs in three fundamentally different ways:

   i) mitosis to the tune of 7.2 dividing cells
   per longitudinal section of 4 μm. thickness, taking place
   in the outermost regions of the Zona Fasiculata,

   ii) formation of encapsulated parenchymatous
   nodules of cortical tissue from mesenchymal cells within
   the glandular capsule,

   iii) appositional growth by the formation of broad
   plates of cortical tissue within the capsule and their
   eventual inward migration and fusion with the cortex.

17. Of the two types of nodule which can be present in
the capsule of the same gland, only the encapsulated nodule
is concerned with cellular replacement in the cortex.
Section Four

18. During its developmental course, the adrenal of Sheep, like that of the ox, pig, dog, cat, rabbit and human, possesses a so-called "foetal cortex". This foetal cortex differentiates from coelomic mesothelium in embryos of approximately 10 mm. C-R length and remains obvious till parturition. Involution of the zone becomes apparent after parturition and this is progressive up to an age of six weeks when a very much reduced zone can be observed.

19. The primordium of the adult cortex can be seen to surround the foetal cortical anlage in 30 mm. embryos. The arrangement of the adult primordial cells is characteristic, their being formed in groups which appear to have a peripheral fringing and an external limiting membrane. The fringing of the cells is due to fixation artefacts and the cells of the membrane probably represent rudimentary fibroblasts. The so-called "floating nodules" of ELIAS (1945 and 1948) which on occasions be seen in the capsule of mature glands, are not concerned with cortico-adrenal cellular replacement as are the encapsulated nodules mentioned in para. 17, but represent isolated remnants of adult cortical primordial cell groups.

20. A definitive glandular capsule is present around the developing adrenals in 150 mm. Sheep embryos.

21. The medulla of the Sheep adrenal, similar to the medulla of the glands of eutherians in general, is formed by sympato-chromaphil cells which migrate ventro-laterally in nodules or rosettes, from the sympathetic ganglia.
Migration of these cell groups is apparent in 12 mm. embryos and the process is continuous till term.

Section Five

22. When treated for the presence of birefringent material, optical activity in the adrenal of Sheep is invariably confined to the cortex.

23. Variations in optical activity in the cortex as manifested by the formation of birefringent crystals allow the region to be divided into four distinct zones:

i) A subcapsular zone corresponding to the Zona Glomerulosa. Birefringence is revealed in this zone by a fine scattering of dust-like particles both within the cytoplasm of the cells and in the zonal capillaries,

ii) An optically inactive zone corresponding to the Zona Intermedia,

iii) A wide, optically active zone corresponding to the Zona Fasiculata. Birefringent crystals in this zone take the form of large, unevenly-shaped globules with uniform distribution throughout the zone, and also the same type of small, intra-cytoplasmic particles seen in the outermost zone,

iv) An innermost zone corresponding to the Zona Reticularis which contains very large, very irregular birefringent crystals which have a sparse distribution throughout the zone.

24. A definitive Zona Reticularis is present in the adrenal cortex of male Sheep whether castrated or not.

25. Structural variations occur in the width of the
Zona Reticularis in adult male Sheep which can be correlated with the sexual state of the animal.

26. The ratio of the width of the Zona Fasiculata to that of the Zona Reticularis in uncastrated male Sheep is approximately $2\frac{1}{2}$ to 1. Castration causes a vast increase in width of the Zona Reticularis whereby this ratio is altered to approximately 1 to 1. Administration of testosterone to castrated animals brings about a progressive reversion of the latter ratio toward the former.
References


ECKER, J. (1846) Der feinere Bau der Nebennieren beim Menschen und der vier Wirbelthierklassen, Braunschweig.


ELLIOTT, T.R. (1913) Innervation of the adrenal glands. 


ROMEIS, B. (1943) Mikroskopische Technik, München, 399.


VINES, H.W.C. (1938) See BROSTER et al.


Plate I

A section of Sheep adrenal cortex. Haematoxylin and eosin. X 100.

Plate Ia

A section of Sheep's corpus luteum of pregnancy. Haematoxylin and eosin. X 100.
Plate II

Arterial system of the adrenal injected with Fotogel and photographed by X-ray. X 4.
Plate III

Adrenal capsule and Zona Glomerulosa. Normal
Bielschowsky silver impregnation for nerve fibres. X 900.
A. Nerve fibres
B. Reticular fibres

Plate IV

Adrenal capsule and Zona Glomerulosa. Modified
Bielschowsky silver impregnation for nerve fibres. X 900.
Note the lack of connective tissue fibres.

A. Nerve fibres
Table II - A Brief Reference Table of Ovine Foetal Development

<table>
<thead>
<tr>
<th>AGE in weeks</th>
<th>C-R in m.m.</th>
<th>SIZE in inches</th>
<th>Body Form</th>
<th>Stomach</th>
<th>Integument</th>
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<td>2</td>
<td>-</td>
<td>-</td>
<td>Fertilised ovum in utero</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1/3</td>
<td>Head and limbs discernible</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>1.25</td>
<td>First indications of hoofs</td>
<td>Stomach recognizable</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>88</td>
<td>3.5</td>
<td>-</td>
<td>Stomachs differentiating</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>152</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>Sinus hairs appear on lips and eyelids. Teats discernible in ♀</td>
</tr>
<tr>
<td>18</td>
<td>355</td>
<td>14</td>
<td>-</td>
<td>Stomachs obviously separate.</td>
<td>Eyelashes and bodily hairs developing.</td>
</tr>
<tr>
<td>21</td>
<td>457</td>
<td>18</td>
<td>Foetus full-term. Hoof's complete but pliable.</td>
<td>-</td>
<td>Body covered in hair.</td>
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Table IV - Normal Adrenal Weights and Measurements with Age and Body Weight

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<th>Age Mths.</th>
<th>Dead Weight lbs.</th>
<th>Left Breadth mm.</th>
<th>Left Length mm.</th>
<th>Right Breadth mm.</th>
<th>Right Length mm.</th>
<th>Weight Left gms.</th>
<th>Weight Right gms.</th>
<th>Displacement Left cm.</th>
<th>Displacement Right cm.</th>
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</table>
Plate VI

The lateral border of the left adrenal of a cross-bred ewe which was condemned for emaciation.

The scale is in millimetres.

Plate VIA

The dorsal surface of the above, illustrating the gross deviation from the measurements observed for normal glands.
### Table V - Adrenal Weight and Body Weight I

**Castrates**

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<th>Body Weight</th>
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### Table VII - Adrenal Weight and Body Weight III

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Graph I

The plot of adrenal weight and body weight in castrates. The small circles indicate the scatter obtained from the data shown in Table V.
Graph II

The plot of adrenal weight and body weight in ewes.
The small circles indicate the scatter obtained from the data shown in Table VI.
Graph III

The plot of adrenal weight and body weight in rams.
The small circles indicate the scatter obtained from the data shown in Table VII.
Figure 1

The arterial supply of the left adrenal of Sheep
as seen from the ventral aspect.

A. aorta.
A.L.T. adreno-lumbar trunk
C.A. coeliac axis
P.A. phrenico-abdominal artery
R.C.A. renal capsular artery.
The distribution of the ventral (inferior) arterial supply of the right adrenal of Sheep.

A. aorta
L.T. lumbar trunks
R.C.A. renal capsular artery
U ureter
U.A. ureteric artery
X. the point of origin of the dorsal (superior) supply.
The distribution of the dorsal (superior) arterial supply of the right adrenal of Sheep, arising from stem X in Fig. II.

A. aorta
L.T. lumbar trunk
P.A. phrenico-abdominal artery
R.A. renal artery
R.C.A. renal capsular artery
Figure XIV

A drawing of a variation met with in the ventral arterial supply of the right adrenal.

The abbreviations used are as in previous figures.
The dorsal (superior) arterial supply of the right adrenal in the variation whose ventral supply is shown in Fig. IV.
Figure VI

A diagram of the venous drainage of the adrenal glands of Sheep as seen from the ventral aspect.

A. aorta
A.V. adrenal veins
R.A. renal artery
R.V. renal vein
U. ureter
V.C. vena cava
R' right
L' left
Figure VII

A diagrammatic representation of the right adrenal of Sheep, illustrating the exit of the central medullary vein into the vena cava and the valvular apparatus partially occluding the exit.

Figure VIII

A diagrammatic illustration of the structure of the right adrenal valve. The arrow indicates the flow of blood in the vena cava; the dotted lines represent endothelial cells; the interrupted line in the corium of the valve represents transverse plain muscle fibres in the same plane as the longitudinal muscle of the caval wall.
Plate VII

Photograph of the exits of the right central medullary vein into the vena cava. X 5.

A. endothelial valve.
Plate VIII

The right central medullary vein as seen from the vena cava.  X 5.

The endothelial valve has been removed to illustrate the calibre of the vessel to best advantage.

Plate IX

A longitudinal section of the left adrenal showing showing the relative size of the central vein.  X 5.

The adrenal in this case drained directly into the left renal vein.
The formation of the left coeliac-mesenteric ganglion
by the splanchnic nerves.

A. aorta.
C.A. coeliac artery.
C.M.A. cranial mesenteric artery.
C.M.G. cranial mesenteric ganglion.
D. diaphragm.
G.S. greater splanchnic nerve.
L.C.G. left coeliac ganglion.
L.S. lesser splanchnic trunks.
O. oesophagus.
R.A. renal artery.
R.V. renal vein.
S.T. sympathetic trunk.
V.C. vena cava.
Figure IXa

A diagrammatic representation of the nerve supply to the left adrenal.

Ao. aorta.
Ad. left adrenal.
C.A. coeliac artery.
C.M.A. cranial mesenteric artery.
G.S. greater splanchnic nerve.
L.S. lesser splanchnic nerve.
S.T. sympathetic trunk.
Figure X

A diagram of the origin of pre- and post-ganglionic fibres to the adrenals.

For convenience, the bilateral coeliac-mesenteric ganglion has been divided into left coeliac ganglion (L.C.G.), right coeliac ganglion (R.C.G.) and cranial mesenteric ganglion (C.M.G.).

G.S. greater splanchnic nerve
L.A. left adrenal
L.S. lesser splanchnic nerve
R.A. right adrenal.
Plate X

Zona Glomerulosa in the adrenal of a Cheviot ewe.

Foot's modified reticulum stain.  X 250.
Plate XI

Definitive Zona Glomerulosa in the adrenal cortex
Plate XII

Glomeruli in the adrenal cortex of a Blackface castrate lamb. Foot's modified reticulum stain. X 600.
Section of the outer portion of the adrenal of a
cross-bred ewe lamb. Masson’s trichrome. X 500.

Note the indistinctness of the Zona Glomerulosa.
Plate XIV

Section of the outer portion of the adrenal of a cross-bred wether lamb illustrating the absence of definitive glomeruli.

Haematoxylin and eosin. X 500.
Plate XV

Melanocytes in adrenal capsule of Blaskface tup.

Haematoxylin and eosin. x 420.
Plate XVI

Reticular fibres in the adrenal capsule. Foot's modified reticulum stain. X 360.
Plate XVII

Elastic fibres in the capsule of Sheep adrenal.
Modified Verhoeff's. X 2800.
Plates XVIII

The reticular framework of the adrenal. Foot's modified reticulum stain. X 150.
Figure XI

Glomerular "balls" around a capillary, as seen in horizontal section.
Plate XIX

Cells of the Zona Glomerulosa. Periodic acid Schiff counterstained with tartrasine in Cellosolve. X 2700.

Note the vacuolated cytoplasm.
A camera lucida drawing of the morphology of corticoadrenal cells from their inception in the adrenal capsule to their degeneration in the Zona Reticularis. This diagram should be correlated with Section Two and the Section on "Corticoadrenal Morphogenesis".

C.C. capsule cell
N.C. nodular cell
G.C. glomerular cell
I.C. intermediary cell
O.F.C. spongiocyte of the Outer Fasiculata.
I.F.C. inner fasicular cell
R.C.1. early reticular cell
R.C.2. late reticular cell showing evidence of senescence
I.S. When a Zona Intermedia is not obvious in the adrenal cortex, glomerular cells can become transformed into what might be termed intermediate spongiocytes characterised by the presence of large quantities of lipoid in their cytoplasm.
Figure XIII

Schematic representation of the Zona Intermedia.

A. Zona Glomerulosa
B. Zona Intermedia
C. Outer Fasiculata composed of spongicocyte cells.
Plate XI

Zona Intermedia of adrenal cortex. Foot's modified reticulum stain. X 420.

A. Zona Glomerulosa.
B. Zona Intermedia.
C. Outer Fasiculata.
Plate XXI

Spongiocytes in the Zona Fasiculata. Periodic acid Schiff counterstained with tartrasine in Cellosolve. X 2700.
Plate XXII


Note pyknotic and karyoklastic nuclei.
Plate XXIII

Microphotograph of the Zona Reticularis of the adrenal of a Sheep which was killed in the laboratory with a minimum amount of "adrenal stress". Haematoxylin and eosin. X 2000.

Fewer senescent cells are present than in Plate XXIV which is a microphotograph of the Zona Reticularis of an Abattoir killed animal.
Plate XXIV

Microphotograph of the Zona Reticularis of the adrenal of a Sheep which was slaughtered in the Abattoir. Haematoxylin and eosin. X 2000.

The senescent cell count is higher than in the adrenals of animals killed in the laboratory with a minimum amount of "adrenal demand".
Plate XIV

Corticomedullary junction, showing islets of
cortical tissue within the medulla.  Picro-mallory.  X 300.
Figure XIV

Cortico-medullary junction. Note sinusoidal network in reticularis and distinct columnar appearance of chromaffin cells.
Plate XXVI

Medullary cells. Periodic acid Schiff, counterstained with tartrazine in Cellosolve. x 2700.

Note the chromaffin granules and the nuclear poles of the cells inclined away from the venous sinus.
Plate XXVII

Colour plate of eosinophils in the medulla of the adrenal of a half-bred ewe lamb. Haematoxylin and eosin. X 260.
Plate XXVIII

Eosinophils in the medulla of a half-bred wether lamb. Haematoxylin and eosin. X 260.

A. Chromaffin cells.

B. Eosinophils.
Plate XXX

Eosinophils in the medulla of a Blackface tap.

Masson's trichrome. X 260.

A Zona Reticularis.
B Medullary cells.
C Eosinophils.
Plate XXX

Polarity of chromaffin cells.  Kull's copper-carmine.

X 900.

A.  Capillary

B.  Venous sinus.

Note how the nuclear poles of the columnar chromaffin cells are inclined toward the capillary.
Plate XXX

Subcapsular arterial plexus. Carmine-gelatine injected slide lightly tinged with haematoxylin. X 110.
Plate XXXI

Capsular capillaries. Carmine-gelatine injected slide lightly tinged with haematoxylin. X 110.

A Capillaries
B Artery
C Veins
D Capsular ganglion.
Plates XXXII

Cortical capillary traversing Zona Fasculata and Reticularis. Carmine-gelatine injected slide lightly tinged with haematoxylin. X 200.

Note capillary branches supplying cortical parenchyma.
Plate XXXV

Two medullary capillaries originating in the subcapsular arterial plexus. Carmin-gelatine injected slide lightly tinged with haematoxylin. X 200.
Plate XXV

Medullary capillary crossing cortico-medullary junction.
Carmine-gelatine injected slide lightly tinged with haematoxylin.
X 200.

A  Littoral cell
B  Endothelial cell.
Plate XXXVII

Horizontal section of the right adrenal valve.
Haematoxylin and eosin. X 120.

Plate XXXVIII

Sagittal section of the right adrenal valve.
Haematoxylin and eosin. X 120.

A Transverse muscle fibres of valve.
B Circular fibres of vena cava.
Lymphatics in the adrenal medulla after intra-peritoneal injection of lithium carmine. Haematoxylin and eosin. X 150.
Plate XL

Large ganglion adjacent to adrenal capsule.

Haematoxylin and eosin.  X 200.

A  Myelinated nerves.
Flate XLI

Nerve trunks traversing the cortex en route for the medulla. Modified Bielschowsky silver impregnation. X 100.
Plate XLII

Nerve trunk entering the medulla. Modified Bielschowsky silver impregnation. X 500.
Plate XIII


A. Nerve ending.
Figure XV

Medullary cells and their relation to nerve endings, capillaries and venous sinuses.

A  Nerve endings.
B  Capillaries.
C  Venous sinuses.
Plate XLIV

Sympathetic ganglion cell at the medullary border.

Haematoxylin and eosin. X 900.
Plate XIV

Nerve ending in adrenal medulla. Modified Bielschowsky silver impregnation. X 900.
Table VIII - The mitotic count in the adrenal
of a 10-month old Soay ewe

| Group of four
longitudinal
sections | Average no. of
Mitoses/Section | Range of variation in
no. of Mitoses/Section |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>A</td>
<td>7.2</td>
<td>5 - 11</td>
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<tr>
<td>B</td>
<td>6.0</td>
<td>4 - 9</td>
</tr>
<tr>
<td>C</td>
<td>8.4</td>
<td>4 - 10</td>
</tr>
<tr>
<td>D</td>
<td>6.8</td>
<td>3 - 12</td>
</tr>
</tbody>
</table>

Average mitotic index per section 7.1
Table IX - The mitotic count in the adrenal of a 10-month old Soay-Moufflon ram

<table>
<thead>
<tr>
<th>Group of four longitudinal sections</th>
<th>Average no. of Mitoses/Section</th>
<th>Range of variation in no. of Mitoses/Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.0</td>
<td>7 - 12</td>
</tr>
<tr>
<td>B</td>
<td>4.4</td>
<td>1 - 9</td>
</tr>
<tr>
<td>C</td>
<td>9.8</td>
<td>6 - 16</td>
</tr>
<tr>
<td>D</td>
<td>10.0</td>
<td>5 - 16</td>
</tr>
</tbody>
</table>

Average mitotic index per section 8.3
Table X - The mitotic count in the adrenal of a 10-month old Soay-Mouflon ewe which had been given 10 cc. colchicine by intramuscular injection.

<table>
<thead>
<tr>
<th>Group of four longitudinal sections</th>
<th>Average no. of Mitoses/Section</th>
<th>Range of variation in no. of Mitoses/Section</th>
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<tr>
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<td>7.1</td>
<td>4 - 13</td>
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<tr>
<td>C</td>
<td>6.5</td>
<td>1 - 10</td>
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<tr>
<td>D</td>
<td>5.1</td>
<td>4 - 14</td>
</tr>
</tbody>
</table>

Average mitotic index per section 6.9
Table XI - The mitotic count in the adrenal of a 10-month old cross-bred castrate lamb which had been given 10 cc. colchicine by intramuscular injection

<table>
<thead>
<tr>
<th>Group of four longitudinal sections</th>
<th>Average no. of Mitoses/Section</th>
<th>Range of variation in no. of Mitoses/Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.4</td>
<td>4 - 19</td>
</tr>
<tr>
<td>B</td>
<td>6.2</td>
<td>2 - 10</td>
</tr>
<tr>
<td>C</td>
<td>7.5</td>
<td>2 - 14</td>
</tr>
<tr>
<td>D</td>
<td>6.9</td>
<td>4 - 11</td>
</tr>
</tbody>
</table>

Average mitotic index per section 6.5
Figure XVI

Camera lucida drawing of the distribution of mitotic figures in a longitudinal 4μm section of Sheep adrenal.

GL  Glomerulosa
FR  Fasciculata and reticularis
M  Medulla
V  Vein.
Plate XLVI.

Fresh frozen section of Sheep adrenal, mounted in ZweMer's Glycrogel, and photographed by polarised light immediately after cutting. X 150.

Note the birefringent crystals in the glomerulosa and the encapsulated nodule.
Plate XLVII

A typical section of the outer part of the adrenal of a cross-bred lamb. Masson's trichrome. X 375.

Note the cellular layer of the capsule and the indistinctness of a Zona Glomerulosa.
Plate XLVIII

Mesenchymal cells in the inner layer of the adrenal capsule of Sheep. Masson's trichrome. X 500.

Note the small "floating nodule" of cortical-like cells.
Plate LXVIII

Glomerular distribution of fat. Sudan IV. X 480.
Section of the adrenal of a half-bred castrate lamb treated for fat with Sudan Black B. X 240.

Note the melanocytes and the two encapsulated nodules within the glandular capsule showing the same staining reaction as the cells of the glomerulosa.
Figure XVII

A diagrammatic representation of growth arcs or fasiculi in the adrenal cortex.

A. Transversely cut fasiculus isolated within the capsule.

B. Arched top of fasiculus giving the impression of a glomerulus.

C. Fasiculus in longitudinal section.

D. Longitudinal section illustrating how a fasiculus can extend into the capsule.
Cortical fasciculi in longitudinal section within the capsule. Masson's trichrome. X 465.

A. Completely isolated fasciculus.

B. Fasciculus showing continuation with cortex.
The following Plates IIa, IIb, IIc, IID and IIE are microphotographs of the left adrenal of a half-bred ewe lamb, intended to illustrate how the capsular nodules of cortical tissue mentioned in Section Three are isolated within the glandular capsule and entirely independent of the cortex. The sections have all been stained with haematoxylin and eosin.

**Plate IIa**

Two well established encapsulated nodules (A and B) and in between them, the first indication of the start of a third (C). X 100.

**Plate IIb**

An enlarged view of the area between nodules A and B. The plate is reversed to the previous one, nodule B showing on the left. The presence of nodule C can be seen as a concentric arrangement of cells of other characteristics than those of the surrounding capsular connective tissue cells. X 600.
Plate LIIa

Nodules A and B are still present and the intermediate nodule C has become well established. X 100.

Plate LIIb

The same gland several sections later. Nodule A has all but disappeared without any communication with the cortex. Nodule B is still prominent. Nodule C is on the point of fading out. X 100.
Section through the gland showing how nodules A and B have disappeared and nodule C will disappear several microns further on. X 100.

Whilst a great portion of the overstaining evident in this section is due to lifting of tissue, it was often noted that when a section was taken very close to the start or to the finish of a nodule, the adrenal capsule was hyper-acidophilic at that region. This would probably be due to the aggregation of fibroblasts making up the capsule of the nodule.
Section of the adrenal cortex of a Blackface castrate showing a so-called "floating nodule". Masson's trichrome. X 500.

Higher power view of the above. X 900.

Note the fringing of the peripheral nodular cells which at the top of the nodule can be seen to be in contact with the limiting membrane.
Encapsulated nodule within the adrenal capsule of a cross-bred castrate lamb. Haematoxylin and eosin. X 500.
Plate LV

Colour transparency of an encapsulated nodule of cortical cells within the adrenal capsule of a half-bred castrate lamb. Azan. X 200.
Plate LVI

Early formation of an encapsulated nodule in the adrenal capsule of a half-bred castrate lamb. Masson's trichrome. X 1000.
Section of the adrenal of a Blackface ram taken through an encapsulated nodule as it lay in close proximity to the Zona Glomerulosa. Haematoxylin and eosin. X 900. The nodule is on the left of the photograph.
Encapsulated and "floating" nodule in the same gland. Haematoxylin and eosin. X 150.
Figure XVIII

Diagram of the mechanics of incorporation of an encapsulated nodule into the adrenal cortex.

(Solid black arches, Zona Glomerulosa; stippled, Outer Fasiculata).

I. Fully developed nodule in capsule.

II. Nodule grows elongated.

III. Nodular capsule bulges and becomes thinner at point of closest approximation. (Corresponds to Plate LXI).
Diagram of the mechanics of incorporation of a nodule into the adrenal cortex (cont'd.).

IV. Nodular capsule ruptures. Metamorphosed cells begin to flow into Zona Glomerulosa.

V. Greater part of nodular cells become incorporated.

VI. Incorporation all but complete. Two new glomeruli have been formed. The remaining cells are drawn into the Zona Fasiculata.
Plate LIX

Haematoxylin and eosin section of the adrenal of a Cheviot ewe. x 125.

A. Capsular cells being incorporated from a degenerating nodule.

B. Complete encapsulated nodule of young cortical tissue.
Plate LX


Plate LXXI

Mature ruptured encapsulated nodule in the adrenal capsule of a cross-bred castrate lamb showing point of incorporation of metamorphosed capsular cells into the cortex. Masson's trichrome. X 250.
A higher power view of the nodule seen in Plate IX. X 500.

Note the similarity between the nodular and glomerular cells.
Plate LXXII

Fluent transformation of cells of the capsule into cortical tissue. Hematoxylin and eosin. X 500.

Note large nerve trunk in capsule and the indistinctness of the Zona Glomerulosa.
Plate LXIV

Concatenation of young Sheep embryos similar to the one on which observations of the early development of the adrenals were carried out.

Reading from left to right Embryo 1 represents the 10 mm. stage in which the foetal cortical anlage is in the process of differentiating from coelomic mesothelium; Embryo 4 represents the 12 mm. stage when the anlage has become completely isolated from the mesothelium; Embryo 10 represents the 30 mm. stage when the adult cortical anlage makes its appearance.
Figure XIX

A schematic representation of the relationships of the right and left adrenal anlagen in a 14 mm. Sheep embryo.

Ad. Adrenal anlage.
Ac. Dorsal aorta.
G. Genital ridge.
M. Primary dorsal mesentery.
W. Wolffian body.
Plate LIX

The site of first differentiation of the adrenocortical primordium in a Sheep embryo of 10 mm. C-R length. Haematoxylin and eosin. X 75.

A. Mesothelial cells proliferating at a point cranial to the genital ridge.
Plate LXVI

Formation of the adrenal primordium in a 12 mm. embryo. Haematoxylin and eosin. X 75.

Ad  adrenal
Ao  aorta
G   gonad
W   Wolffian body.

Note the bundles of sympatho-chromophil cells dorsal to the aorta.
Plate LXVII

T.S. of a 20 mm. Sheep embryo showing the presence of capilliform vessels in the foetal cortical anlage and medially, bundles of sympatho-chromophil elements in the process of migrating ventrally to the diverticulum in the right cardinal which will eventually form the right central medullary vein. Haematoxylin and eosin. X 150.
Plate LIVIII

Sagittal section of a 40 mm. embryo showing the marked vascularity of the adrenal anlage and medially bundles of sympathetic elements in the process of migrating from the neural crest. Haematoxylin and eosin. X 150.
Plate LXIX

T.S. of the adrenal of a 30 mm. embryo showing the first signs of differentiation of the adult cortex.

Haematoxylin and eosin. X 200.
A high power plate of a full-term foetal adrenal
illustrating the characteristic morphology of adult cortical
tissue. Haematoxylin and eosin. X 470.

Note the vacuolated cytoplasm of the nodular cells which
helps to create the illusion of peripheral fringing.
A so-called "floating" nodule in the adrenal capsule of an adult Sheep stained with Masson's trichrome. The similarity between the nodule and the arrangement of adult cortical tissue in the foetus is striking. This Plate has the same magnification as Plate LXX and the two should be compared.
Plate LXXII

Mesenchymal cells in the process of becoming organised into a capsule around the adrenal anlage of a 150 mm. embryo. Masson's trichrome. X 400.
Plates LXXXIII

T.S. of the adrenal anlage of a 25 mm. embryo.

Haematoxylin and eosin. X 140.

Note the numerous capilliform vessels and the groups of sympatho-chromaffin cells traversing the anlage still with their connective tissue capsules.
Organisation of the medullary anlage in a 150 mm. embryo. Haematoxylin and eosin. X 300.

Note the blood sinuses and the dense lymphocyte-like sympatho-chromophil cells.
Section of full-term foetal adrenal. Haematoxylin and eosin. X 125.

Note the wide expanse of foetal cortex and the characteristic "floating" arrangement of the adult cortex.
The reticular pattern in the adrenal of a one week old lamb. Foot's modified silver impregnation technique. X 160.

The grouping of fibres in the outermost region of the cortex is characteristic of the glomerular reticular pattern seen in adult animals (see Plate X and XII).
Section of the adrenal of a six weeks old lamb.

Haematoxylin and eosin. X 180.

The increase in breadth of the adult cortex is quite apparent.
Plate LXXVIII

Frozen section of Sheep adrenal (formol fixed) photographed by polarised light within 30 minutes of mounting in syrup of laevulose. X 150.

Note the fine birefringent crystals in the Zona Glomerulosa and the optically inactive band which separates it from the distinctive luminescence of the spongiocyte zone.
Plate LXXIX

Frozen section of formol fixed Sheep adrenal mounted in syrup of laevulose and photographed by polarised light. X 150.

Note the zonal arrangement of crystals in the glomerulosa and fasiculata and separating them, the distinct optically inactive Zona Intermedia.
Plate LXXX

Frozen section through the Zona Fasiculata of Sheep adrenal after formol fixation. X 150. Coarse birefrigent crystals which are characteristic of this zone are very prominent against the background of faint, dust-like particles.
Section of Sheep adrenal fixed in formal-saline, sectioned at 25 μm on a freezing microtome and mounted in Zweier's Glychrogel. X 150.

The pronounced irregular luminescence of the Zona Reticularis is in contrast to the optically inactive medulla.
Fresh frozen section of the adrenal of a Soay-Moufflon ewe, 25 µm., mounted in syrup of laevulose and photographed in polarised light approximately 30 minutes after mounting. X 150.

The coarse, irregularly scattered birefringent crystals seen on the left of the plate are in the Zona Reticularis. The optically inactive medulla is on the right, the venous sinuses showing up as dark patches.
Plate LXXXIII

Frozen section of formol fixed Sheep adrenal photographed in polarised light 24 hours after mounting in syrup of laevulose. X 150.

The increase in birefringent crystalline material is striking when compared with Plate LXXVIII.