THE HISTORY OF OUR KNOWLEDGE
REGARDING THE FUNCTIONS
OF THE PITUITARY GLAND.

By John McWhan.

An Essay Submitted for
the Wellcome Prize in the History of Medicine.

May, 1931.
PREFACE.

The pituitary body has been made the subject of systematic and critical experiment only within the last forty years, while a large proportion of the most important work has been performed quite recently, or is still going forward. In these circumstances the writer has for the most part concerned himself only to display, in the order of their discovery, (which is hardly ever the logical order) the facts which form the basis of our present knowledge or speculation concerning the functions of the gland. He has rarely, therefore, attempted to reconstruct the interpretation which the writer of a paper might presumably have placed upon the experiments which he recorded; indeed such a reconstruction would in many instances be little short of impossible, so rigidly do modern physiological journals confine themselves to the facts.

In this province of physiology it is often very easy to demonstrate the existence of active substances in an organ, and extremely difficult to show that they play any part in the normal integration of the functions of the body. The writer has dealt only with these substances that appear to be true "hormones" in the sense of Bayliss and Starling.
CONTENTS.

I. The Nature and Relations of the Pituitary Gland. 1.

II. Early Speculations. 5.

III. The First Clinical Evidence. 20.

IV. The Contribution of Experimental Physiology. 27.

V. The History of Attempts to Destroy the Gland. 33.

VI. " " the Method of Experimental Lesion. 36.

VII. " " the Effects of Feeding with the Gland. 40.

" " Grafts. 43.

IX. " " Extracts. 45.

X. The Pituitary and the Female Reproductive Tract. 50.

(Note on the Relationship between the Pituitary and Ovulation in the Rabbit.) 85.

(Note on Menstruation.) 88.

XI. The Influence of the Pituitary on the Testis and Male Secondary Sexual Characters. 90.

XII. The Posterior Lobe and Pars Tuberalis. 100.

XIII. Pituitary and Vascular System. 106.

XIV. The Possible Function of the Pituitary in Parturition. 115.

XV. The Posterior Lobe and the Kidney. 122.

XVI. The Function of the Pituitary in Amphibian Ontogeny. 132.

XVII. Conclusion. 144.
CHAPTER I.

THE NATURE AND RELATIONS OF THE PITUITARY GLAND.
It will be convenient to open this account with a brief survey of the anatomy of the pituitary gland. Not only is a knowledge of this essential for the right understanding of the arguments upon which the older writers founded their theories, but, since the existence and gross structural features of the organ have been known since the earliest times, it is logical to describe them before one passes to the work of physiologists to whom they were already perfectly familiar. Fuller accounts will be found in Herring (1908), Sharpey-Schafer (1926) and De Beer (1928).

The pituitary gland is one of the most constant features of the vertebrate type of nervous organisation and is believed to be represented in at least some of the lower Chordates, such as Amphioxus (Hatschek's pit), and the Ascidians (dorsal tubercle).

Its structure in the higher forms may best be understood by a consideration of the manner in which it originates in the embryo. Some time before the fourth week (in man) the ectoderm in front of the buccopharyngeal membrane gives off a dorsal diverticulum (Rathke's pouch). As this pocket extends upwards it has been found experimentally to condition a thickening of the membranous floor of the diencephalon and to cause a portion
of this floor to sink downwards until it takes up a position between Rathke's pouch and the anterior end of the notochord. We may thus imagine one pocket extending upwards from the mouth, and a second sinking downwards just behind the first.

Shortly afterwards the anterior face of the nervous pouch fuses with the posterior wall of the pouch derived from the buccal ectoderm, and we have the pituitary body of the adult, as it were, in germ.

The cavity of the nervous pouch may persist as the Infundibular Recess; the cavity of the buccal pouch may persist as the cavity of the gland; while the nervous pouch itself remains with very little alteration except increase in bulk to form the Pars Nervosa of the adult.

Returning to the buccal part we find that its subsequent history is much more eventful, since the epithelium which lines its cavity undergoes a very considerable modification. This lining, to form the four walls of the cavity which it encloses. The posterior wall as we have seen adheres very closely to the anterior wall of the pars nervosa, and in this condition it persists to adult life when it forms a layer of epithelium not many cells thick, the Pars Intermedia. But the cells of the anterior wall multiply rapidly, extending forwards and laterally until they form
a solid mass which laps round the partes nervosa and intermedia, holding them "like cup and ball"; this mass is the Pars Anterior of the adult.

Before the process has been completed we may observe great mitotic activity among the cells of the lateral wall which rapidly form a pair of processes extending upwards and laterally on either side. Later these processes unite in the middle line in front of the stalk of the pars nervosa, and proliferate backwards on either side to fuse again posteriorly. They have thus formed a ring or collar round the tuber cinereum and infundibular stalk, so constituting a most important division of the adult gland, the Pars Tuberalis.

Reviewing this description we see that the organ is certainly not a morphological entity, for although it is entirely ectodermal one part is formed from neural ectoderm, the other from the ectoderm of the surface of the body. One might possibly expect that this difference in origin would be reflected by a corresponding difference in function, since the only secretory product known definitely to be formed from tissue whose anlage was nervous is adrenalin, and the outer layer of the body gives origin to many diverse substances of widely different chemical constitution.

In dissection one notices, as one
removes the brain, that a small lump of tissue apparently attached to its inferior surface has been torn away and left imbedded in a boney pocket, the Sella turcica of the Basisphenoid. One may be impressed with its close proximity to the cavernous bloodvessels, and, if the skull be split in the mid-sagittal plane, with its remarkable position, separated from the cavities of the nose by a little more than the spongy texture of the sphenoid bone.

Again if a section is made through the brain and if one half is placed in the corresponding half of the skull, it is easy to mark how the fourth ventricle and the aqueduct slope steeply upwards to join the cavities of the hemispheres, and to infer that the cerebrospinal fluid will tend to gravitate into the base of the third ventricle. It will be seen that the last cavity sends a prolongation downwards which appears to run into the pituitary body.

Although these facts appear to us to be quite irrelevant we shall see in the next section how powerfully they acted on the minds of Galen and those who followed him.


CHAPTER II.

EARLY SPECULATIONS.

For the first eighteen centuries during which its existence was recognised the pituitary body was never studied carefully and nothing whatever was known of its functions. This neglect it shared with most of the other members of the endocrine system, since the early investigators were irresistibly attracted to the larger and more conspicuous organs. Even today it is not difficult to understand how easy it must have been to ignore the existence of such a minute lump of tissue deeply embedded in the floor of the skull, and to devote the greater part of one's time to the study of the fascinating problems presented by the motions of the heart, or by the relationship of the nervous system to the contractions of voluntary muscle. Again, there was no adequate technique whereby the consequences of the extirpation of the pituitary might be explored, while the possibility of an endocrine function, as we understand the term, was as yet inconceivable.

In the absence of knowledge the gland was made the repository of these functions which were imagined on speculative grounds to be vitally important but which could hardly be ascribed to any organ whose physiology it was possible to investigate experimentally. It appeared quite reasonable to the contemporaries of Galen to
suppose that the fluid contained in the cavities of the central nervous system was in some way the foundation of consciousness, and of all nervous activity generally, and to identify it with those animal spirits which were believed to course down the nerves and to throw the muscles into contraction. Apparently the animal spirits were generated in the heart and carried in the blood-stream to the chorioid plexuses by which they were secreted into the ventricles of the brain.

As it was hardly likely that this fluid could maintain its purity unaltered, it was postulated that the dregs must in some way be separated out and removed from the body; and there remained only to find a suitable channel.

This channel was believed by Galen to run through the infundibular recess the pituitary gland and the body of the sphenoid. "Cavitas igitur", he wrote, "quae meatus has excipit in quam nonulli a figura, id est pelvim alii ab utilitate, id est infundibulum nominat". The function of the infundibulum is to act as a funnel, down which the effete fraction of the fluid pours on its way to the pituitary.

It was further supposed that when the metabolite had finally percolated through the sphenoid it was transformed into the "pituita" or mucous membrane of the nasal cavities.
This theory appears to have been considered as final until the Renaissance when many writers supposed that the pituitary might secrete the excretory matter into the blood, or that the fluid could flow out, "per omnia foramina hic in calvarii basi tum arteriis tum veis ipsis exculptis". (Vesalius N.D.).

The former theory is stated in very clear terms by Collins.

"The other Colatories by which Nature defaecates the Blood are the innumerable Glands interwoven with Arteries and Veins in the Plexus Choroëides, into which a large source of Blood is impelled by the Carotide and Vertebral Arteries, whose smaller Branches being inserted into the numerous Choroeidal Glands do transmit in Hydroptic Constitutions great quantities of Serous Blood where a Percolation is made of the more pure and alimentary parts of the Blood;

"And the watery remnants do force their way through the small insensible Meatus of the Membranese encircling the Glands into the cavities of the Ventricles and are thence derived to the Infundibulum and the Glandula Pituitaria".

He mentions at this point the doctrines of Wharton who favoured a slight modification of the
Galenic theory.

"The learned Dr. Wharton assigneth this use to it, (the pituitary gland) to entertain the serous Humidities of the Brain and to recover them into the Plexus Retiformis and Third Pair of Nerves, into the Tonsils and the Glands of the Eyes and Maxillary Glands".

Collins dismisses this as theory as being of a "far-fetched unnatural and preposterous notion", and goes on to give his own views:

"But I conceive another use may be assigned to the Glans Pituitaria with greater probability than the former, as being more agreeable to the structure and position of the Glans Pituitaria, and other parts of the brain relating to it, that the serous Humors should distil out of the substance of the Brain and Choroidal Glands and descend by the Infundibulum into the Glans Pituitaria, and thence be carried not into the Palate (as the antient Anatomists would have it) because this Gland hath no excretory vessel leading into the Cavity of the Nostrils and Mouth, but the neighbouring jugular Veins, returning the Blood in its circulation."

The objection such a view appears to have been the
Thus the serous humors found themselves once more in the blood-stream whence they could be excreted by the kidneys, or as was more commonly believed, by the wall of the alimentary canal.

In the writings of Lower we may observe the transition to our modern views on the secretion and fate of the cerebrospinal fluid. Boerhaave said in his lectures (published in 1740):

"Est unum ex emissariis quod ignotum primus in lucem suam constituit Lowerus. Nempe ex secundo ventriculo vapore coacti confluunt in tertium; ex tertio per infundibulum medullare descendunt in glandulam Pituitariam, et ibi resorbiti in venas et in corredeunt."

Thus as we have seen before the cerebrospinal fluid was secreted by the chorioid plexuses but absorbed by the pituitary. But Boerhaave continues: "Sed is exitus non unicus est, Ridleyus enim, qui vehemiter caret ne falleretur, demonstravit partem plexus choroidei fieri venis pellucidis valvulosis venis nempe vas vasis Lymphaticis quae in quartum finium exonerunt rosodium suum vaporem mox per laterales finus ad venas Jugulares et ad cor rediturum."

The importance of the pituitary body is becoming less and less since as it is realised that the functions postulated by the older writers may
be shared among other organs.

This passage was the last word that the Galenic physiology had to say on the functions of the pituitary body. We willingly recognise that the theories which it had propounded were fairly intelligent inductions from the anatomical facts, and, at least in the later period, were formulated with caution and sobriety. The fact that the organ was a gland being known, it was possible to suppose from its relations either that it abstracted principles from the blood and secreted them into the infundibulum or vice versa. The first of these is a common place statement in modern textbooks of physiology and it is not surprising that the older writers should have chosen the second since, without experimental or histological knowledge, both are equally tenable.

In the beginning of the next century it was Gall propounded the view that consciousness was not a function of the cerebrospinal fluid but of the brain itself, and Magendie showed that the circulation of the cerebrospinal fluid could be explained without postulating any secretory activity on the part of the pituitary. Thus the validity of two of the cardinal points in the Galenic physiology was destroyed and with the researches of Lavoisier on oxidation and
the growth of chemistry a scientific treatment of physiology had at last become possible.

It was in accordance with this new spirit that the majority of the textbooks of the early nineteenth century were written and accordingly one finds little mention of the functions of the pituitary gland. On the other hand the comparative anatomists of the period appear to be greatly interested in the organ and usually find no great difficulty in explaining its functions; we might therefore examine their teachings for a few lines.

Before the nineteenth century the biological sciences were obsessed with teleological doctrines. (In physiology this influence was due in great measure to Galen) It was supposed that every species of animal was designed to fill a particular ecological niche, and as a corollary that every organ in the body of an animal must subserve some essential function. Although today we may reject the argument we accept the conclusion; but the older writers were inclined simply to manufacture a the function to suit an organ about which they knew nothing, instead of inferring the function from the results of experiment. It is more than probable that this type of thought played a large part in retarding the progress of knowledge on the functions of certain of the
other organs. If the reader will turn to an account of Cullen's system of pathology, he will recognise the extremes to which this deductive and abstract tendency were sometimes carried. Once a system had been built up from theoretical considerations one proceeded to treat one's patients in its light and to discover functions for any organ that seemed to be of very little importance. Some of these functions were fantastic. Paley for example, a very well informed writer, tells us that Nature placed the spleen in the abdomen to fill up the interstices between the other organs; an author of one of the books in the Bridgewater series, says solemnly that the earth rotates on its axis at the speed it does, because man needs a certain amount of sleep.

Applied to the subject of this essay it will be understood how potent such arguments were in detracting the attention of investigators. If physiologists could accept Paley's theory of the spleen the Galenic view on the function of the pituitary would appear to be modest and sober in comparison.

At the end of the eighteenth century physiology and medicine shook off teleology and its naive explanations along with a great many of the other Galenic doctrines. Yet no experimental work appears to have been done on the pituitary for almost a century, in spite of a surgical
technique that was advancing rapidly. This neglect was undoubtedly due to many causes but the most important is most probably the theory of vestigeal organs.

According to John Goodsir the pituitary body of a mammal represented the mouth of an annelid. Nature having given a mouth to an annelid in order that it might keep itself alive placed a similar structure in a mammal simple for the sake of symmetry. This belief appears to us to be ludicrous, and by the end of the century it had transformed itself and appeared in a guise which at which few have smiled. Gaskell held that the pituitary body represented the uterus of the king-crab. This statement did not imply any personification of nature; it simply meant that man and the king-crab were descended from a common ancestor and that, although man had formed a different kind of uterus, the forces of heredity were too great to allow the old one to become entirely suppressed.

These arguments were nearly always taken to imply that the pituitary body was functionless. To show this I shall quote a passage from a celebrated work on the embryology of vertebrates published in 1834:

"The true nature of the pituitary body has not yet been made out. It is clearly
a rudimentary organ in existing craniate vertebrates, and its development indicates that when functional, it was probably a sense-organ opening into the mouth, its blind end reaching to the base of the brain. No similar organ has yet been made out in Amphioxus, but it seems possible to identify it with the small ciliated sac placed at the opening of the pharynx in Tumucata, \ldots . If this suggestion is correct the division of the body into three lobes in existing vertebrates must be regarded as a step towards a regressive metamorphosis.

Another possible view is to regard the pituitary body as a glandular structure which originally opened into the mouth in the lower chordates but which has in all existing forms ceased to be functional. "

There could be little encouragement to physiologists in these views and the majority of the textbooks of the nineteenth century reflect the blank indifference to the possible of the gland; some importance of the gland; some indeed never mention it.

Johannes Muller writes about the middle of the century says:
"The functions of the pituitary and the pineal gland are, we may say, entirely unknown. Greding it is true frequently found disease of the latter pituitary in mental affections; but in such cases disorganisations have been met with in all parts of the brain. Wenzel frequently observed disease of the pituitary in epileptic subjects."

These hints were destined to be neglected for another forty years and in 1869 Longet can only write; "La pathologie et l'anatomie comparée ne nous ont rien appris jusqu'à présent sur les functions du corps pituitaire."
<table>
<thead>
<tr>
<th>No.</th>
<th>Author</th>
<th>Title/Details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1908.</td>
</tr>
<tr>
<td>2</td>
<td>Sharpey-Schafer</td>
<td>The Endocrine Organs vol.2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>London 1926.</td>
</tr>
<tr>
<td>3</td>
<td>De Beer</td>
<td>The Comparative Anatomy, Histology and Development of the Pituitary Body.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Edinburgh, 1928</td>
</tr>
<tr>
<td>4</td>
<td>Galen</td>
<td>De Usu Partium, Lib. IX cap III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paris. MDXXXVII</td>
</tr>
<tr>
<td>5</td>
<td>Vesalius</td>
<td>De Humani Corporis Fabrica. Lib. VII Cap. XI. Basle. N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pp. 1023-1026. Savoy, MDCLXXXV.</td>
</tr>
<tr>
<td>7</td>
<td>Boerhaave</td>
<td>Praelectiones Academicae Vol. 2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gottingen MDCCXXX.</td>
</tr>
<tr>
<td>8</td>
<td>Muller</td>
<td>Elements of Physiology. vol.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>London 1840.</td>
</tr>
<tr>
<td>9</td>
<td>Balfour</td>
<td>A Textbook of Vertebrate Embryology.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>London 1881.</td>
</tr>
<tr>
<td>10</td>
<td>Gaskell</td>
<td>The Origin of the Vertebrates.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>London. 1908.</td>
</tr>
<tr>
<td>11</td>
<td>Longet</td>
<td>Traite de Physiologie.</td>
</tr>
</tbody>
</table>
CHAPTER III.

THE FIRST CLINICAL EVIDENCE.

The first definite evidence on the functions of the pituitary body appears to have come from a series of clinical observations which were made towards the end of last century, and, without doubt, the greater part of the credit for making them must be assigned to Pierre Marie.

Yet, writing in Edinburgh, one is bound to mention what was probably the first mention in history of an abnormal condition of the pituitary gland in conjunction with a definitely pathological configuration of the body; the more strongly since it was made by D. J. Cunningham, who subsequently occupied the chair of Anatomy in this university.

Considering Cunningham's account in the light of recent knowledge, it is not difficult to understand how the signs of Acromegaly were overlayed by others which flowed from an extensive and deep-seated derangement of the entire endocrine system, and one might lament the rarity in Scotland of the clearly defined cases which Marie was fortunate in being able to study.

When the patient was brought into the Infirmary, says Cunningham, he immediately attracted attention because of the hideousness of his appearance. The jaws and the teeth were
remarkably hypertrophied; the lips were swollen, while the tongue, enormously enlarged, filled the buccal cavity and protruded from the mouth. On post mortem examination, Cunningham found that the hypertrophy was shared in by the abdominal viscera, such as the spleen, liver, and kidneys, as well as by the heart, blood-vessels, and sympathetic nervous system. Later, when the cranium was opened, the condition of the pituitary body became apparent:

"At the base of the brain, an ovoid tumour, the size of a pigeon's egg, occupied the position of the pituitary body. At its widest part, the transverse diameter was 42mm., while its long diameter measured 30 mm. It was directly connected with the tuber cinereum by means of the infundibulum, which was more strongly developed than in the normal state. The pineal body was more than twice its normal size."

Unfortunately this was an isolated case and Cunningham could pursue his researches no further. The clinical work cannot therefore be said to have formed any definite conclusions concerning the functions of the gland before the publications
of Pierre Marie on the subject of Acromegaly.

This observer wrote a series of papers in the years from 1886 to 1890 which, in the first place, recognised the cooperation of a great number of complex symptoms to form a single disease, to which Marie gave the name of Acromegaly; and, in the second place showed that this disturbance was necessarily and inevitably, associated with some form of derangement of the pituitary body.

The most striking character of Acromegaly, and that, indeed, which gives the condition its name was described by Marie as being, "a remarkable non-congenital hypertrophy of the limbs and head"; symptoms which had been described quite fully by other observers more than a century previously. Among the other features which Marie recorded were, progressive blindness (due to the impingement of the enlarged gland on the optic nerves), an increase in the size of the larynx with a consequent deepening of the voice, hypertrophy of the heart, a diminution of sexual desire (or even impotence) in the male, and suppression of the menses in the female.

"Finally, among the lesions which seem to be constant in Acromegaly, must be mentioned hypertrophy of the
"pituitary body with enormous dilatation of the Sella turcica, persistence of the thymus, and finally, hypertrophy of the cord and ganglia of the sympathetic system."

(It is curious that, although both Cunningham and Marie were impressed by the enlargement of the sympathetic system, one finds little mention of this feature of the disease in the writings of later authors. It would be interesting to learn the condition of the sympathetic system in the rats of Evans and Simpson, for example.)

In his earlier papers Marie appeared to regard the hypertrophy of the pituitary gland as an effect rather than the cause of the disease, while, at a later period, he suggested that, in normal life, the organ serves to inhibit the development of the characters of Acromegaly. (Much as the ovary of the Brown Leghorn inhibits the development by the hen of a comb, and a male type of plumage.) When the sella became the seat of a tumour, which he supposed to be malignant in nature, the pituitary body was destroyed, and (as in the case of the Brown Leghorn after ovariotomy) the symptoms of the disease appeared.

This hypothesis was immediately disproved by the experimental work of Horsley,
Vassale and Sachi, Brown-Sequard, and others, who showed that the extirpation of the gland was followed, not by Acromegaly or increased growth but, usually, by death. Moreover, in 1900, Benda made a histological examination of the pituitary gland in cases of Acromegaly, showing that the tumours were adenomatous and formed, for the most part, of oxyphil cells. It was clear then that the symptoms of the disease were the result of hyperpituitarism, and that the gland played an important part in the regulation of skeletal growth.

Once this point had been reached the matter was taken up by the methods of experimental physiology, with very fruitful results. Although this work will be dealt with in succeeding chapters it might be as well to mention another type of pituitary disease, and one which may readily be induced in the laboratory.

In 1901, Frohlich described some of the first cases of what has come to be known as "Dystrophia Adiposo-genitalis", or simply, "Frohlich's Syndrome". The most remarkable characters of this condition are, as one might infer from the name, atrophy of the generative organs and a tendency towards adiposity. The fatty deposits appear to accumulate all over the body, but apparently have a predilection
for the buttocks and the thighs. Correlated with the state of the genital organs there is observed an imperfect development of the secondary sexual characters, and various disturbances of the reproductive function. It is said that the syndrome may be associated either with an enlarged or with a reduced stature. It appears probable that the tumours which caused these symptoms in Frohlich's patients were malignant, but for some years the relation of the pituitary to the condition of Dystrophia Adiposo-genitalis were doubtful.

Decisive evidence came from the experiments on the operative injury of the gland and from a remarkable case, described by Madelung in 1904, in which all the symptoms appeared in a girl of nine years of age, whose pituitary had been ablated by a bullet which lodged in the sella turcica. Dystrophia Adiposo-genitalis appeared therefore to be due to hypopituitarism.
<table>
<thead>
<tr>
<th></th>
<th>Author</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cunningham</td>
<td>Journ. Anat. and Phys. xii, 294, 1878.</td>
</tr>
<tr>
<td>2</td>
<td>Marie</td>
<td>Brain. xii. 1890.</td>
</tr>
<tr>
<td>3</td>
<td>Horsley</td>
<td>See the Harveian Oration 1931.</td>
</tr>
</tbody>
</table>
THE CONTRIBUTION OF EXPERIMENTAL PHYSIOLOGY

We have seen that the first knowledge of the functions of the pituitary was the result of a series of careful attempts to correlate the appearances presented by the gland on post-mortem examination with the symptoms which had previously been observed in the wards. As a means of establishing physiological knowledge this procedure may be criticised on several grounds. Cases of pituitary disease are not very common and, even then, are not always available for post-mortem examination, without which, (unless an operation has been performed) there can be little certainty that the gland is affected. Again, there is no guarantee that that the primary seat of disease does not reside in some other organ and that the enlargement of the pituitary is not an entirely secondary effect, a kind of compensatory hypertrophy. For example the affection of Myxoedema is commonly believed to be caused by a deficiency in the secretion of the thyroid gland, although the pituitary body may be abnormally large. It is also well to remember that different parts of the gland may be affected at different stages of the disease and that in any case it is unlikely that a disturbance will be confined entirely to one of its anatomical sub-divisions.

For these reasons and for many others the functions of the pituitary may be better investigated by different methods; this fact was early recognised and/
and we find that, even before 1890, attempts were being made to verify Marie's original hypothesis experimentally.

**METHODS**

It will be useful to give here a brief account of some of the principal methods by which experimenters have studied the pituitary gland, and to enumerate the conclusions which may legitimately be inferred from the results of each method.

In our investigations into the physiology of any organ we are attempting to answer two questions, namely, the possible functions of the organ and the methods by which it may perform them. In the case of the pituitary the former is not difficult to solve for it is only necessary to extirpate the gland, or to injure it, and subsequently to observe what functions of the body are modified by the operation.

This is (a) THE METHOD OF INJURY OR OF EXTIRPATION (EXPERIMENTAL HYPOPITUITARISM) and its results can be criticised only on one ground; the pituitary body is unfortunately in very close relations with the floor of the third ventricle and it is difficult to interfere with one of these structures without also affecting the other. Now while it was formerly believed that the latter was quite unimportant and "of simpler structure and with fewer nerve connections than almost any other part/
part of the brain", today there is very strong
evidence for believing that it contains ganglia
which direct the activities of a great part of the
autonomic nervous system. Camus has described two
nuclei in the tuber cinereum and states that injury
to one is followed by polyuria and injury to the
other by glycosuria. Other observers (e.g.
Sharpey-Schafer, Frank et al.) hold that these
effects are produced by the disturbance of the
pituitary gland and more particularly of its pars
tuberalis. On the other hand there seems to be
good reason to suppose that the kidney and other
organs work under both humoral and nervous control
and that their functions may therefore be affected by
injury either of the pituitary gland or of the
hypothalamus. Biedl, for example, believes that we
may yet have to recognise a humoral and a neural
type of Frohlich's syndrome. But however this may be it is impossible to neglect the hypothalamus. In a
recent paper Beattie, Brown and Long demonstrate
conclusively that the tonus of the cardio-accelerator
nerves is altered by stimulation or removal of this
part of the brain and their figures show how unlikely
it could have been that the pars tuberalis was
affected in any way.

(b) THE BUCCAL OR PARENTERAL ADMINISTRATION OF THE
GLAND

(EXPERIMENTAL HYPOPITUITARISM)

The autacoids in the gland may be introduced
into/
into the bodies of experimental animals by several methods among which we may notice

(a) BUCCAL ADMINISTRATION.

This method is the most convenient since it may be applied to the intact gland, there being no need to make extracts. Unfortunately the autacoids do not appear to resist the digestive fluids very well.

(b) INTRA-PERITONEAL INJECTION OF EXTRACTS.

The effects are slow to appear and may persist for a considerable time. The method is convenient in cases where it is difficult or impossible to free the extract from protein.

(c) INJECTION OF EXTRACTS SUB-CUTANEOUSLY OR INTRA-MUSCULARLY.

(d) IMPLANTS.

This method was apparently discovered by Zondeck and Ascheim only a few years ago but has since been employed by in many laboratories with very uniform results. A small shred of anterior lobe is implanted into the muscles of the hind limb or of the neck, and the wound closed. The graft does not "take" but undergoes a slow hydrolysis in the course of which any autacoids which it may contain are discharged into the surrounding fluids.

(e) GRAFTS.

A piece of the gland is implanted into some tissue of the host, care having been taken to promote hyperaemia. Under certain conditions the grafts may persist for a considerable time and may even retain their histological identity.
INTERPRETATION OF THESE EXPERIMENTS

This subject will be discussed later in more detail, but it may be said now that the modes in which the gland could conceivably influence the activities of the body are three in number:

(a) By reason of its nervous connections.

This possibility is eliminated when it has been shown that the effects of complete hypophysectomy may be abolished or at least mitigated by extracts, feeding or ectopic grafts.

(b) It might alter the composition of the blood which passes through its vessels.

This appears to be unlikely on a priori ground. However even the complete sufficiency of grafts does not render it impossible and it can be neglected only when we have shown that it is possible to maintain the health of a hypophysectomised animal by means of extracts or feeding. By that time there will remain only one possibility.

(c) It must secrete into the body fluids some autacoid or autacoids.

It will be observed that no matter how profoundly a function may be modified by the administration of pituitary the regulation of the function by the pituitary gland cannot be held to be proved until it has been shown that the function is also modified in the reverse direction by experimental hypopituitarism.
Notice that the rejection of the interpretation outlined in (a) involved also the rejection of the opinion that all the effects produced by ablation of the pituitary are really due to the incidental interference with the hypothalamus. It is however still legitimate to suppose that both tissues control the same functions but that in the absence of one the other may act vicariously.

In the succeeding pages the history of each of the classes of experiment which have been enumerated above will be described separately.
CHAPTER V.

THE HISTORY OF ATTEMPTS TO DESTROY THE GLAND

Apparently it was Sir Victor Horsley who first attempted to remove the pituitary gland from dogs, his experiments in 1886 being inspired by the work of Pierre Marie. Horsley was led to believe that extirpation unaccompanied by injury to the brain was not necessarily fatal. Many other workers essayed the operation in the next twenty years, with results which sometimes corroborated Horsley and sometimes not. If the animal died it was always possible to conclude that death was the result of operative shock while, if it survived, the gland might not have been completely removed. The first successful attempts appear to have been made by Faulesco whose monograph on the subject appeared in 1908. Of the earlier period Dott writes,

"Prior to this publication operations had been attempted on the pituitary in many species of animal and by diverse anatomical routes. In the frog a buccal and an intra-cranial method had been used. In the fowl a retropharyngeal approach was employed. In the rabbit an attempt to destroy the pituitary by a vertical transcerebral approach was made. In the dog and cat many observers availed themselves of the bucco-pharyngeal route, and in these animals intra-cranial operations by frontal and sphenoid-palatine avenues had also been performed. The gland itself was variously treated by total or partial removal, by gross contusion, by thermal and chemical cauterisation. A non-operative method by injection of a specific pituitary cyto-toxin had also been tried. Most of these experiments failed by reason of operative complications or of insufficiently accurate post-mortem examination. While they remained inconclusive, some of the accounts given obviously foreshadowed the more definite knowledge which later experimentation has yielded."
Paulesco commenced his studies by a careful investigation of the anatomy of the organ in various animals, finding that the dog was the most suitable mammal for the purpose; the canine pituitary body being less deeply embedded in the sphenoid bone and in the dura mater than is usually the case. The operation was performed intracranially, the brain being levered upwards by instruments inserted through large openings cut in either side of the skull. A similar technique was employed by Cushny and later by Dott, all three workers obtaining fairly uniform results. Death invariably followed rapidly; adult dogs succumbing after a few days, although young puppies were often found to remain alive for as long as a month. According to Cushny, the symptoms are these:—

"On the day after the operation the animal (adult dog) usually appears normal with fair appetite and no characteristic signs of loss of secretion/
secretion. Gradually it becomes lethargic, refuses food, and responds slowly or not at all to the voice. Later the respiration becomes slow, and the pulse both slow and feeble, the musculature limp, often with tremors and fibrillar twitching; the back is arched and the temperature subnormal; finally within forty-eight hours the animal becomes comatose and dies in this condition.

Dott believes that death is due to "a profound depression of the metabolism resulting in failure of the respiratory nervous mechanism". Several workers have studied the basal or rather "standard" metabolism of animals in this condition and their findings bear out this conclusion of Dott. Narbut (1900), Wolf and Sachs (1910) noticed a fall in the output of CO and were confirmed by Benedict and Homans in 1912.
CHAPTER VI

THE HISTORY OF THE METHOD OF EXPERIMENTAL LESION.

Little was learned from the experiments described in the previous section beyond the fact that the gland is essential for life. To ascertain what individual functions are maintained by the pituitary it is necessary to resort to another method of experimentation. If the gland is completely removed a series of functions, some of them vitally important, cease completely and instantaneously; if however, the gland is treated only in such a way as to diminish though not abolish its secretions, then it should be possible to keep the animal alive for some time and to discover the functions that are affected by careful investigation of the changes which are produced in the organ which subserve each of these functions. Many experiments of this nature have been attempted and much useful information has been won.

The first of these which we shall record here is that of Aschner in 1912. This observer, operating by the buccal route, tried to destroy the gland completely; however in every instance a small fragment was found to have been left behind.

The effects were remarkable; the animals grew much more slowly than the controls, their epiphyses did not ankylose and the milk teeth, juvenile fat and lanugo hair persisted. The thymus, thyroid and supra-renal cortex were notably enlarged while the reproductive organs remained infantile and the temperature/
temperature fell by as much as 1.5°C.

It was shown by Dandy and Goetsch that a large part of the blood supply to the pituitary of the dog is derived from the circle of Willis by way of small arteries which pass down the infundibulum. Severance of the stalk will therefore lead to complete or partial degeneration of the gland, and is by no means a difficult operation.

In 1917, Blair Bell cut the stalk in the dog with most interesting results. Aschner had previously declared the experiment to be equivalent to complete hypophysectomy but Bell was able to keep his animals alive for a considerable time, and observed that a perfectly typical Frohlich's syndrome was produced within two months of the operation. Besides the accumulation of fat, the animal (bitch) exhibited a complete atrophy of the mammary glands and the external genitalia. On section he found that one ovary which had been removed at the time of operation was perfectly normal but that, 128 days after the operation, the other had degenerated completely, and was represented by what was little more than a mass of connective tissue. (If the pituitary had not been damaged this ovary would have enlarged considerably, obeying the "Law of Follicular Constancy"). Moreover as one would expect from the condition of the ovary the remainder of the genital tract was markedly underdeveloped.

Similar results have now been obtained by many workers of whom we may mention Dott, who made some interesting/
observations on the subsequent condition of the skeletal system. "The average decrease in the epiphyscal activity due to anterior lobe deficiency was found to be 57%. Histological examination shows that in deficiency the cartilage undergoes a true degeneration. Its cells die and its matrix shrinks. At the ossifying junction the vascularity is decreased and the osteoblasts degenerated." Dott also observed most of the other symptoms.

In 1924 Smith and Graeser injected chromic acid into the pituitary body of rats and observed all the consequences of hypopituitarism. They state that no injury was done to the hypothalamus.

Two years later Smith described a method by which either lobe could be ablated in the same animal. The gland was approached through the nasal cavities and its dural sheath; one portion was dissected free from the remainder and sucked out by a cannula attached to an aspirator. According to Smith, injury to the tuber cinereum evokes a syndrome in which the thyroid and adrenal cortex are scarcely affected, so differing from the sequelae of hypophysectomy.

These last experiments will be referred to later when the relationship of the gland to the rodent ovary is being considered. But they are of far wider significance since the rat is one of the commonest and cheapest of laboratory animals.
THE PART OF THE GLAND CONCERNED IN THESE EFFECTS.

The pituitary gland is a complex of many structures, differing widely in their histology and development; it is reasonable to suppose that these structures must differ also in their functions; many attempts have therefore been made to effect a partial analysis by the removal of one subdivision of the gland. Dott has shown that removal of the posterior lobe is followed by no effects whatsoever. An animal so treated remained in normal health for over a year and actually gave birth to a litter. According to the same observer removal of the pars tuberalis is impossible.

All the effects which we have described appear therefore to be caused by injury to the anterior lobe or the pars tuberalis. We suspect from other experiments that the latter may be eliminated.

FUNCTIONS OF THE ANTERIOR LOBE.

It is legitimate to conclude from the results of these experiments that the anterior lobe (and pars tuberalis) are responsible for the maintenance of:-

1. Skeletal growth (in young animals)

2. The gonads, secondary and accessory sexual organs.

3. Normal temperature and metabolism.

4. The normal condition of the thyroid, thymus and adrenal cortex.

To confirm this knowledge it is necessary to investigate the effects of feeding, grafts and extracts.
CHAPTER VII.

EFFECTS OF FEEDING WITH THE GLAND

A. Whole Gland.

Several experiments of this nature had been made before the end of the first decade of the present century, most of them giving negative results. In 1912 Schafer investigated the effects of feeding pituitary, thyroid and ovary to young rats. Thyroid elicited a most remarkable rise in the rates of growth and of carbon-dioxide output but the other experiments led Schafer to conclude that "Ovary and pituitary have little or no effect on growth or metabolism". This result was confirmed by C.S. Smith in 1923 and by Evans and Long in 1921.

B. Separate Parts.

In 1916 Goetch showed that the feeding of anterior lobe to young rats stimulated growth of the body and of the reproductive tract while posterior lobe retarded both functions. Three years later Marinus instituted a series of very careful experiments on the feeding of different parts of the gland. He showed in the first place that ordinary preparations of anterior lobe were always contaminated by a small piece of the pars tuberalis; when this was removed there remained nothing but anterior lobe. Marinus neglected the possibility of the diffusion of substances from one lobe to another in the interval between the death of the animal and the isolation of the parts of the gland. The freeing of the pars tuberalis proved a more
more difficult problem and indeed Herring in one of his papers of 1908 had declared it to be impossible. Fortunately however this part possesses a tough fibrous consistency and when a cylinder had been cut from the neck of the body and stood upon a plate of glass it was not difficult to carry an incision down its surface parallel to the axis. It was then found that the pars tuberalis formed a membrane which could be stripped away from the surface of the infundibulum. It was then laid on the plate and cleaned with a sharp knife.

In these experiments the same weight of material was administered to each rat daily. The control substance was muscle.

After six weeks administration it was found that the animals fed with anterior lobe were heavier and longer than the controls, while those fed with pars tuberalis were lighter and shorter. This is an average; actually there was observed to be great individual variation.

Pars anterior increased the weight of the genital tract in either sex, while pars tuberalis had the reverse effect as may be seen in this table.

<table>
<thead>
<tr>
<th>Relative Weights of Genital Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pars ant.</td>
</tr>
<tr>
<td>168</td>
</tr>
<tr>
<td>140</td>
</tr>
</tbody>
</table>

Marinus was apparently ignorant of the vaginal smear method of diagnosing the stages of the reproductive cycle but he was able to show that the/
the premature enlargement of the ovaries was accompanied by the abnormally early induction of puberty, for, at the end of nine weeks, the animals fed with anterior lobe were obviously pregnant and their young were born a full fortnight before those of the controls.

In the same year Larson found that the feeding of anterior lobe tended to prolong the lives of hypophysectomised rats. He gives the following figures:

<table>
<thead>
<tr>
<th>Duration of Life from Operation onwards (in days)</th>
<th>Controls</th>
<th>Normal with ant. lobe</th>
<th>Operated with liver</th>
<th>Operated with ant. lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>193.5</td>
<td>196.9</td>
<td>93.5</td>
<td>174.8</td>
</tr>
</tbody>
</table>

In 1923 Dott fed large quantities of anterior lobe to puppies and kittens with positive results. It is necessary to emphasize the magnitude of his doses, .3 gm. of dry powdered anterior lobe per kilo per diem. (The entire gland in man weighs little more than half a gram) The average increase in the epiphyseal activity was 22%. Histological examination showed that the cartilage was stimulated to excessive growth, that its cells proliferated more rapidly and that its matrix was deeper than normal. "The ossifying junction is the site of intense ossification; the osteoblasts are unduly numerous and the cancellous spaces are closely crowded together. Radiological examination shows the wide cartilage characteristic of hyperpituitarism and that it tends to mature more rapidly than is normal and to ossify earlier." Dott found that
that although the rate was increased growth ceased unusually early so that gigantism was not produced. No effect was visible on examination of the thyroid.

It will be observed that enormous quantities of the gland are necessary to produce any effect. This would preclude extensive use of this method of administration in medicine. Most workers since Dott have used extracts or implants, much more economical methods. It is clear that a good proportion of the autacoids must be destroyed in the alimentary canal or else be unable to pass through its walls.

CHAPTER VIII

GRAFTS

a. Intact Animals.

No successful attempt has yet been made to induce hyperpituitarism by means of grafts. Indeed the endocrine organs form a "conservative system" which tends to neutralise any factors that would upset the delicate equilibrium that obtains between every member. Consider for example the response of the adrenals to the administration of insulin or the manner in which a small piece of ovarian tissue will in a few weeks regenerate as many Graffian follicles as existed in both ovaries before the operation. It is no wonder therefore that in the intact animals the all grafts of pituitary have undergone necrosis; Holstead has emphasized the fact that a grafted endocrine organ will only "take" when its presence may make good a "physiological deficit". Failure /
Failure has been reported by Schafer (1911), Clairmont and Ehrlich (1909) Crowe, Cushing and Homans (1910), together with many others. But it has since been shown to be possible to graft supernumerary ovaries or testicles into normal animals with every success; and the time is now ripe for a further attempt on the pituitary with the knowledge that has been gained in the interval.

b. Hypophysectomised Animals.

Cushing and his collaborators have removed the gland after extirpation to some other vascular situation such as muscle, bone-marrow, or brain. Eventually the transplant became disintegrated but in some cases life was prolonged for as much as a month. In some animals the cachexial symptoms were diminishing at the end of the 24th, 25th and 26th day, respectively, and microscopic examination of the transplant revealed the existence of apparently active anterior lobe cells.

These results demonstrate conclusively that the effects of complete hypophysectomy cannot be due entirely to lesions of the hypothalamus.

Further experiment is urgently required for if it were possible to maintain the life of animals with ectopic glands, the effects of injury to the hypothalamus could be studied without the possibility of accidental lesion of the pituitary.
In common with many of the other endocrine organs, the pituitary gland was first administered in the form of extracts by Brown-Sequard who found that the symptoms of operative hypopituitarism were somewhat mitigated by this means. In the intact animal, Schafer (1909) obtained very striking results. His extracts were made with saline from glands which had been smeared on glass plates and dissipated at a temperature of 40°C. At the beginning of the experiment the average weight of the rats was 44.25 gm. One group was submitted to daily injections of the extract. The animals in this group grew more slowly than the controls for the first 4 days, but at the end of the experiment the average weight of the treated animals was 160 gm., and of the controls 131 gm.

In 1916 Brailsford Robertson claimed to have purified a substance "Tethelin" which was present in alcoholic extracts of the anterior lobe, and which produced a curious effect on the growth of animals. If tethelin was administered to mice only so long as they were immature, the cessation of the injections was followed by a great increase in the size of the animals. It was supposed that during the period of administration the growth of the epithelial parts of the body was encouraged and that the growth of the connective tissue matrix was checked. When the injections ceased the/
the connective tissue was released from the inhibition, and when the results of its proliferation was added to the already highly developed cellular tissue (hitherto not noticeable) gigantic individuals were produced. (It will be remembered that Voronoff has suggested that the internal secretion of the testis may exert a similar differential influence on the two kinds of tissue). The effects of tethelin were not specific, being shared by cholesterol and by nervous tissue from which the cholesterol had not been extracted. The substance was also stated to increase the duration of life. Robertson's claims have not been confirmed by P.E. Smith or by Drummond and Canaan.

In 1921 Evans and Long injected solutions of the anterior lobe into female rats, and obtained an enormous acceleration in the rate of growth, resulting in part from accumulation of fat, but accompanied by an increase in weight of the skeleton, heart, lungs, liver, and ovaries; the last organs being twice as heavy as those of the controls, and formed for the most part of masses of enormously hypertrophied atretic corpora lutea. Oestrus was suppressed, the Graffian follicles were absent, and the uterus was only half the normal size.

(Their extracts were made from the anterior lobes of cattle; these were washed in 40% alcohol, immersed in Locke's solution and ground with sand. The mixture was/
was centrifuged, 1/2th of a c.c. of the filtrate being injected daily). Long and Evans give the following figures as the average values obtained from 38 treated animals and 38 controls:

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Wt. of Controls</th>
<th>Wt. of treated Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>19.02 gm.</td>
<td>20.2 gm.</td>
</tr>
<tr>
<td>75</td>
<td>183.5</td>
<td>227.8</td>
</tr>
</tbody>
</table>

Their findings were confirmed by Sisson and Broyles in the same year. It may be mentioned here that Long and Evans were able to diagnose the inhibition of oestrus produced by their extracts by means of the vaginal smear test which had been discovered some time previously by Stockard and Papanicolaou. It is principally because of the simplicity and convenience of this method that rodents have been so extensively employed for the researches which will be described in this and the next section. Evans and M.E. Simpson (1926) have investigated the effects of similar extracts on the male rat with equally striking results. The experiment was commenced with male rats of approximately the same size and all 21 days old. Injections were made daily, and at the end of 8 months the average experimental rat was heavier than its control by 160-250 gm. One rat attained a weight of 680 gm! The effect on the reproductive organs and the psycho-sexual behaviour was carefully examined. All the animals were fertile but there /
there appeared to be some reluctance to copulate, and on autopsy the testes were observed to be markedly smaller than normal.

The work described in the last section demonstrated that the pituitary was essential to normal growth; the significance of the other factor - the remainder of the body - was not considered, and it might have been supposed that the normal cessation of growth on the attainment of maturity was due, not to a diminution or cessation in the secretion of the pituitary, but to the failure of the other tissues to respond. This view is probably valid - within limits - but the experiments on the injection of extracts have revealed that other things being equal the maximum size of an animal is a function of the activity of its pituitary. If two rats from the same litter were taken, one of them partially hypophysectomised and the other treated with the extract, they might finally differ by more than five hundred grams, the difference being due entirely to variations in the concentration of the hormone in their blood. The potentialities of the new born soma dictate an upper and a lower limit for the size of the adult; within these limits the final size depends solely upon the functioning of the pituitary gland. The same relationship holds between the thyroid and the rest of the body but less strikingly, while it is more than probably that/
this gland is subject to the control of the pituitary. Incidentally, the word "control" must not be taken too literally; all that is implied is that after hypophysectomy the thyroid degenerates, and that in some cases its functions may be restored by the injection of extracts derived from the anterior lobe of the pituitary. Indeed it is more than probable that the pituitary activities of the pituitary itself are directed by a third gland and so on repeatedly. The endocrine system forms an organic unity; if the functions of one organ are interfered with experimentally, the immediate effect is a compensatory response on the part of all the others, and it is this response, and not the ablation of the original organ, or the administration of extracts made from it, which forms the immediate cause of any symptoms that may subsequently be observed. There remains to attempt an analysis of this train of intermediate causes, and in no instance has this been more boldly essayed than in the case of the relationship between the pituitary body and the female reproductive tract. Although the subject has not been studied for many years issues have already been disclosed of an almost unimaginable complexity. An outline of this work will be given in the next section.
THE PITUITARY AND THE FEMALE REPRODUCTIVE TRACT

a. Preliminary.

Because of their cheapness and the ease with which the condition of their genital organs may be deduced from an examination of the vaginal smear, small rodents such as rats and mice have been extensively employed in the researches which will be described in this section, and a knowledge of the physiology of reproduction in these animals is essential for a right understanding of the relationship between the pituitary and the female genital tract. Good accounts will be found in Parkes (1929) Donaldson (1924) and elsewhere. A short recapitulation of the principal features will be given below.

The female genital organs of rats and mice are characterised by cyclical changes of two entirely different types; the reproductive proper; and the oestrus, the occurrence of one type suppressing the other.

If a female is isolated it is found that between the periods of puberty and the climacteric the oestral cycles succeed one another, almost without intermission. Any one of these cycles/
cycles must embrace the stages of pro-oestrus, oestrus, met-oestrus and di-oestrus. Between the periods of di-oestrus and pro-oestrus several follicles in either ovary commence to enlarge, while the uterus, which in di-oestrus was constricted and anaemic, begins to be dilated with fluid, until, at the height of oestrus, it may have increased to more than twice its normal diameter, although the stroma and muscular constituents appear to be unaltered. Contemporaneously with these changes the vaginal epithelium heightens and undergoes cornification. During the period of oestrus the follicles burst and are discharged into the ovarian bursa which, like the uterus, is distended with fluid.

It is evident that the result of these changes is to prepare the animal for fertile coitus; the stratified squamous epithelium in the vagina facilitates the entrance of the penis, while the spermatozoa may easily ascend through the fluid in the uterus. During late oestrus moreover basal metabolism is heightened (Wiesner 1930), and mating reflexes appear to be intensified.

If the animal is kept in a separate cage it is found that the genital organs subsequently pass through the stages of met-oestrus and revert to their original condition.
A large number of experiments have shown that in the spayed animal these changes cease and the entire genital tract undergoes atrophy. If, however, the ovaries are merely transferred to the surface of the kidney or to some other region, and if the graft "takes" the oestrous cycles continue. It is probable, therefore that the ovary does not dominate the genital tract and the secondary sexual characters by virtue of its nervous connections, (although nerves have often been found to grow into such transplants) and a humoral mechanism of some kind is indicated. This hypothesis is confirmed by the well-substantiated fact that it is possible to induce a state of oestrus in oophorectomised animals by injection of extracts prepared from the ovaries. The active principle of these extracts has recently been prepared in crystalline form, and is known by various names, such as Oestrin, the Allen-Doisy hormone, the female sex-hormone, &c. Apparently this substance cannot be secreted entirely by the follicular epithelium (Although one of the most potent sources is the liquor liquor folliculi) since many observers have shown that the oestrous cycles may continue without abnormality when the germinal tissue or the epithelium have been destroyed by protracted exposure to X-Rays.
(Parkes et al. 1929).

From these experiments it is the custom to conclude that the oestrous cycles in the uterus and vagina are conditioned by the secretion in fluctuating quantities from the interstitial tissue or from the thecal cells of a number of substances, one of which is probably the Allen-Doisy hormone.

The rat attains to the condition of sexual maturity in the eleventh week of its extra-uterine existence and the mouse some four weeks earlier. The vagina of prepubertal animals is represented by a solid column of cells which become perforated by a lumen shortly before the advent of the initial period of oestrus. Premature opening of the vagina may be induced by injections of oestrin. The maturation of the animal appears therefore to be effected, not by any considerable lowering of the threshold of the organs concerned, but by the intensified secretion on the part of the ovary of the sex-hormone.

Although the cells of the membrana granulosa are invariably transformed into luteal tissue during the period which succeeds to oestrus and ovulation, it is commonly believed that the consequent corpora lutea remain functionless unless they have been activated at the beginning of their formation by stimulation of the cervix of the uterus possibly accompanied by the nidation of
the zygotes.
An experimental proof of the alteration in the properties of the uterine mucosa consequent upon successful impregnation was given by Leo Loeb in 1908 (34). If the endometrium of the uterus of the guinea-pig were incised during the period which followed copulation it quickly formed very characteristic deciduomata; about twelve days after the operation these nodules broke down and, in some cases, were discharged through the vagina to the exterior. Experiments with unfertilised animals gave only negative results.

The endometrium appears to acquire this property partly through stimulation of the cervix in coitus, and partly as a result of the nidation of the zygotes; it has been possible to analyse these factors.

If at the height of oestrus a mouse be mated with a vasectomised male, or if its vagina be titillated with a fine instrument the next oestrus is postponed for a considerable period, often as much as nine days. During the interval the uterine mucosa proliferates, the vagina mucifies, and the mammary glands develop.

Twelve days afterwards oestrus reappears and the usual prooestrous vaginal bleeding may become intensified as a result of the discharge through the orifice of the vagina of the products of the degeneration of the
hypertrophied uterine epithelium that we have mentioned above. This state reminds one both of menstuation and of the pseudo-pregant condition characteristic of these mammals whose oestrous cycle embodies a distinct luteal phase (dog, marsupial cat, cow, &c.). It would not be entirely unreasonable to compare this period of discharge to a birth and to call it a "pseudo-birth", since all that is wanting are the foetuses.

Pregnancy in the mouse is very similar except that it lasts for as long as 19 days. Parturition is followed by a single oestrous cycle, and the corpus luteum again becomes dominant, suppressing oestrus for another three weeks or so. During this period of lactation the corpus luteum is apparently activated by the stimulation of the mammae, since, if the young be removed immediately after birth the oestrous cycles continue without interruption.

This discussion may appear, on first sight to be irrelevant to the anterior lobe of the pituitary. Yet in every one of the phenomena that we have mentioned appears to be caused, in the last analysis by a functional change in this organ, the history of and the discovery of the relation between the


xx. 105. 1930.
pituitary and the reproductive function in the female should prove to be by far the most interesting chapter in this essay.

We shall consider at a later period the relationship between the ovary and pregnancy.
The Periodic Yield of Ova is Regulated by Extra-Ovarian Factors.

In the years from 1779 to 1783, John Hunter (35) studied the effects of unilateral ovariotomy on the fertility of pigs. Since the animals would be sterilized by the removal of both ovaries and since ovulation appears to occur with equal frequency (as judged by the number of corpora lutea or of ripe follicles) from either, it would be natural to expect the operation to be followed by a halving of the fertility. Hunter found that this was not true; in eight farrows the operated animal produced 76 young and the control only 87. A modern worker, accustomed to the use of Wistar rats, of guaranteed homozygosity, and to statistical methods would probably refuse to infer any conclusions from these experiments, since the animals were ordinary farm stock, and since John Hunter's means did not permit the purchase of and upkeep of large numbers. Hunter did not however share these scruples and considered himself to be justified in drawing the following prophetical conclusion:

"From this experiment it seems most probable that the ovaria ovaria are, from the beginning, destined to produce a fixed number of ova, and that the constitution has no power of giving to one ovarium the power of
propagating equal to both; but that the constitution has so far the power of influencing one ovarium as to make it produce its number in a less time than would probably have been the case if both ovaria had been preserved, is to be inferred from the experiment."

Although these conclusions of Hunter were later shown to be perfectly correct, it may be doubted whether they are a legitimate deduction from the experiment that has been described; recent work has shown that that the embryonic mortality of polytocos mammals like the pig is enormous, and that fertility is mainly determined not by the number of ova shed, (since these are always present in excess) but by the number of embryos which the uterus is able to gestate. (On the other hand it might be argued for Hunter that the nutritive conditions in the uterus are influenced by the number of functional corpora lutea and hence, indirectly, by the number of ova which were liberated in the preceding period of oestrus).

The most satisfactory way of gauging the activity of the ovary is to kill the animal when oestrus is indicated by the vaginal smear and count the number of ripe follicles. Ovarian regeneration has recently been investigated in this method by many workers, of whom we may notice Carmichael and Marshall (1908), Crew (1927), and Lipschutz (1928). It has been shown that if a small fragment of ovarian tissue be grafted into the body of a spayed adult, it is/
is found before very long to exhibit as many ripe follicles as were found in both ovaries of the host before the operation.

Provided that a minimal number of oocytes be present in the graft the number of ova shed at each ovulation will continue to remain constant until the number of oocytes remaining is less than the number which is usually shed; after a few periods of ovulation it will be found that the fragment is completely denuded of oocytes.

The number of ova shed must be controlled therefore not by the quantity of ovarian tissue but by some extra-ovarian factor or factors.

c. THE PHYSIOLOGICAL AGE OF THE OVARY IS DETERMINED BY EXTRA OVARIAN FACTORS.

In 1900 Foa found that when an ovary was removed from an infantile animal and grafted into the body of an adult female castrate, it underwent rapid development and presented the characteristic features of maturity long before the period at which its original owner would normally have arrived at puberty. On the other hand an adult ovary when transplanted to an infantile soma was observed to lose its histological characteristics; and moreover, failed to precipitate a precocious opening of the vagina, or any of the other signs of puberty.

Wiesner (1922) has extended these observations, and shown that the senile ovary is rejuvenated by being/
being grafted into a mature soma and vice versa.

Summarising these results

<table>
<thead>
<tr>
<th>Original state of ovary</th>
<th>state of host</th>
<th>Final state of ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infantile</td>
<td>Mature</td>
<td>Mature</td>
</tr>
<tr>
<td>Senile</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Mature</td>
<td>Senile</td>
<td>Senile</td>
</tr>
<tr>
<td>Infantile</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

The physiological age of any ovary is apparently the same as the age of the soma in which it happens to reside; it is controlled by extra-ovarian factors; a property it shares with the periodic yield of ova.

These extra-ovarian factors were demonstrated by Heape (1905) to control another type of ovarian activity, the degeneration of follicles in rabbits which have been allowed to persist in heat. Heape wrote, after a consideration of all the experiments which had been made previously and of his own, "The force which controls the activities of the whole generative system is not in the ovary". He further suggested the possibility that there might be some humoral mechanism such as the production by a somatic tissue of a gonadotrope autacoid or "generative ferment" for which he suggested the name, "Gonadin".

This hypothesis seemed very plausible since the possibility of a nervous control was (as Pflüger had suggested many years before) destroyed by the normal behaviour of ovaries which has been transplanted into various ectopic sites. It was also recommended by the results of the study of the condition of the ovaries in clinical or experimental Frohlich's syndrome/
syndrome and by some of the experiments in the oral administration of the pituitary gland; as well as by the observations on the hypertrophy of the pineal and adrenal cortex in various diseases of the reproductive system. It had also been ascertained that the thyroid and pituitary both enlarged during pregnancy.

Thus 16 years after Heape had published the humoral theory, it appeared as if many organs might compete for the distinction of secreting the generative ferment although the odds were certainly in favour of the pituitary. As we have said before it is almost certain that all the organs mentioned play some part in the regulation of the reproductive function; but the past ten years have contributed an enormous mass of evidence supporting the view that the last link in the cycle of humoral interactions, the final common path, as it were, resides in one of the endocrine functions of the pituitary body.

A summary of this work will be given in the next chapter.
THE PITUITARY AND PREGNANCY

a. Pituitary and Corpus Luteum.

It will be recollected that when Evans and Long (1921) injected their saline extract of the anterior lobe into the peritoneal cavities of female rats, oestrus was immediately suppressed, and that on autopsy the uterus was found to be abnormally small and the ovaries exhibited much luteal tissue. In experiments reported in 1924 Evans discovered that similar effects were produced by an extract prepared with decinormal NaOH, and made a careful histological study of the alterations in the ovaries.

He found that the immediate consequence of the injection was a swelling of the follicular epithelium, accompanied by a corresponding decrease in the volume of the antrum. This was followed by a series of atrophic changes in the ovum itself, such as fragmentation of the nucleus and a pronounced general shrinkage. The follicular epithelium continued to hypertrophy, until the antrum was entirely obliterated. No mitoses could be observed at any stage of this process, so presumably the increase in the volume of the epithelium must have been caused entirely by cytoplasmic growth in the individual cells. Although the corpora lutea atretica so formed were of large size and closely crowded together, they did not become confluent, but persisted as discrete bodies, sharply demarcated from each other, and from the surrounding epithelium.

Harvey Lecture 1924
Unboiled extracts prepared by saline or alkaline extraction are rich in protein and cause considerable irritation when introduced into the peritoneal cavity. These results were therefore criticised on the ground that the effect on the ovaries might have been due in part at least to irritation of the peritoneum in the neighbourhood of the ovary. In 1926 however Evans answered this objection by showing that the luteinisation and suppression of oestrus were no less obvious when the preparation was injected into the subcutaneous tissues.

In the discussion of the oestrus and reproductive cycles in rats and mice we said that corpora lutea were of two main types, one type apparently being functionless and the other competent to induce most of the conditions of pregnancy such as suppression of oestrus, mucification of the vagina, sensitization of the uterus, development of the mammary glands etc. It will have been apparent that the corpora lutea produced by alkaline and saline extracts of the anterior lobe most probably belong to the second type since they appear to inhibit oestrus, however it was necessary to ascertain whether they produced the other effects.

In 1926 Teel repeated the injections with this end in view and found that placentomata were readily produced by irritation of the endometrium once
once atretic corpora lutea had been formed by the method of Long and Evans, the effect ceasing on removal of the ovaries; he wrote

"Under the conditions described the daily injection of anterior hypophyseal fluid always brings about a typical decidual cell response to injury of the uterine mucous membrane in animals in which the ovaries are present".

"This response does not occur when the ovaries are absent".

These results were confirmed two years later by Brouha and in 1929 by Parkes.

In 1929 Parkes was able to show that deelderrata produced by Teel's method induced a development of the mammary glands.

Many of these observers noticed that the habits of the animals were altered by these experiments and even appeared to resemble the condition encountered during a normal pregnancy.

THE PITUITARY CONTROLS PREGNANCY THROUGH THE MEDIATION OF THE CORPUS LUTEUM.

It becomes evident that the conditions which characterise pregnancy are conditioned by a secretion of the corpus luteum. As early as 1903 Fraenkel had demonstrated that the presence of corpora lutea was necessary for the implantation of the ovum and for its subsequent nutrition; his findings have been confirmed by subsequent workers for many species of animals. It has even been possible to elicit some of the conditions of pregnancy by the
injection of extracts prepared from the Corpus Luteum. For example Loeb (1923) Kennedy (1925) Parkes and Bellerby (1927) and many others all claim to have been successful in inhibiting oestrus by this method.

It is well to remain sceptical however until it has been established that these extracts are not toxic, since previous investigators (Hermann and Stein 1916, 1920; Kohler 1924) obtained inhibition of oestrus by non specific organic irritants. The possibility of toxicity in Kennedy's work, for example, is evidently very great, since ovulation was suppressed for some months after the cessation of the injections. However, Weichert (1928), has produced placentomata by Loeb's method in castrated females which were treated with an extract of corpus luteum and Hissaw (1925) has described how extracts of this body were able to dissolve the public ligaments of the guinea pig and pocket-gopher. There can be little doubt that these effects were not due to the action of a specific autacoid.

Extracts of ovary must therefore produce the consequences of pregnancy in spayed animals, while in the absence of the corpus luteum none of these consequences appear. It seems reasonable to conclude that in the normal animal pregnancy is caused by some internal secretion of the corpus luteum which may either act directly on the genital tract or indirectly, by modifying the secretion of another...
another gland.

If the second alternative be true the intermediary gland cannot be the pancreas, the thyroid or some other part of the ovary, since the effects of pregnancy can be induced by extract of corpus luteum in the absence of these organs. Moreover it is unlikely to be the pituitary since a normal pregnancy may occur in the absence of the posterior lobe (Dott 1923) and since the changes of pregnancy are not produced in the absence of the ovary by any substance which we are at present able to extract from the anterior lobe. The supra-renal cortex might be investigated in this connection.

However although we are not able to disprove the second alternative the first is always accepted because of its simplicity.

The necessity of the pituitary for the activation of the corpus luteum has been proved by the experiments on injection and the fact that a partially or completely hypophysectomised animal has never been brought through a pregnancy. Moreover the second conclusion of Teel shows that the ovary is a necessary internunciary between the pituitary and the uterus; whether it is the only link we do not know and the simpler explanation is again assumed; the possible importance of the supra renal cortex might be mentioned again, for it is certain that the size of this gland is increased by injections of anterior lobe, and decreased by hypophysectomy (P.E.Smith 1916/
1916, for tadpoles) and that on the other hand tumours of the cortex are always associated with sexual precocity (See Sharpey-Schafer 1924 Vol.1 p. 165)

THE OESTROGENIC FUNCTION OF THE PITUITARY

Few observations in this field are more than four years old, and much of the most important work has been done quite recently. It was of course known more than twenty years ago that the entire female genital tract atrophies after lesions of the pituitary and consequently that this gland was necessary for ovulation and oestrus.

The first credit of finding positive evidence must be assigned to P.E. Smith who in 1926, having ascertained that hypophysectomy in the rat stops the oestrus cycle proceeded to investigate the effects of "implantations" of the anterior lobe. His results were spectacular:

"Daily homoplastic transplants of the pituitary from the adult given intramuscularly induce sexual maturity in the rat as early as the weaning date (22 days) thus transcending all normal variability. Animals which are but fourteen days of age when treatment is begun mature sexually in from eight to ten days; when treatment is begun at the weaning date they mature in from five to six days."

"The opposite response of the sex apparatus results from pituitary ablation before sexual maturity, the complete establishment of the vaginal canal being delayed and the genital system remaining underdeveloped."

"The anterior pituitary component of the transplant only is essential for the induction of premature sexual maturity, the posterior/
posterior lobe neither hindering or aiding in the response".

"No stimulus to the development of the uterus or vagina of the immature ovariectomised rat is given by these transplants".

"The invariable and rapid maturity which is induced by daily pituitary transplants, speaks against the view of those who maintain that hypophysectomy does not interfere with sexual development or function, and indicates an important hypophyseal-gonadal interrelationship".

These experiments are mentioned at this point because Smith seems to have been the first to make a systematic study of the consequences of intramuscular implantation. But he was primarily concerned with the restoration of growth in hypophysectomised rats, and resorted to this method only when negative results had been obtained by feeding as much as two bovine anterior lobes to each animal daily. The discovery of the reactions of the genital tract to these implantations appears to have been quite accidental.

These studies were extended in 1927 and in a paper which Smith and Engle published in that year, it was shown that the gonads were also affected; when fragments of the anterior lobe were implanted into the hind limbs of infantile animals the follicles ripened to such a degree that the ovary might be as much as 10 times the size of the control in the rat and 19 times in the mouse; in the adult superovulation with consequent superfoetation were elicited by similar treatment so that in one/
one uterine horn (the uterus of rodents is bicornuate) there were found 48 ova and in another animal there were counted 29 embryos (Engle 1927). If the grafts were administered over a considerable period it was possible to denude the ovary of oocytes.

In 1928 Engle was able to accelerate the process of compensatory hyperplasia in unilaterally oopherectomised animals, so partially explaining the results which John Hunter had obtained more than a century previously.

Before the publication of the later papers of the American authors (these experiments were carried out in the University of California). Zondeck and Ascheim working in the Gynaecological Department of Berlin University and apparently without knowledge of their results made a very extensive use of the method of implantation. They tell us that they wished to ascertain the relationship between the egg and the sex hormone in the human subject, and that the mouse was employed in the preliminary investigations simply because of its convenience, and also in view of the fact that the morphology of the cyclical changes in its ovary had been very thoroughly worked out by Sobotta. Unlike Smith their interests were primarily medical.

Zondek and Ascheim considered in the first place the possibility that the ovary might be controlled by the ovum itself. This could not be admitted since Schubert/
Schubert had destroyed the germinal tissue in mice by the simple expedient of grafting the ovaries into subcutaneous tissue, and exposing them to a Roentgen lamp. Nevertheless the characteristic changes of oestrus continued unaltered.

"Kein lebendes Ei und trotzdem Aufbau der Scheidenschleimhaut, kein lebendes Ei und trotzdem das reine Schollenstadium, kein lebendes Ei und trotzdem der typische Brunstvorgang, wobei Uterus und Scheid in charakterische Weise vergrosert sind".

An obvious conclusion was drawn, and the next possibility appeared to be the control of ovulation by the sex hormone. However the administration of a preparation of this substance to infantile mice was discovered to have no effect on the ovaries although the animal came into oestrus much earlier than was usual.

Moreover after mice had been fed for three weeks with salts of Thallium the changes in the uterus and vagina were entirely suppressed although the ovaries appeared to be normal.

"Im ovarium hingegen sehen wir grosse Follikel mit Cumulua oophorus und darin Eir mit Kernteilungsfiguren"

Into the muscles of animals in this condition shreds of various tissues were grafted. In most cases the results were negative but with the anterior lobe of the pituitary the condition was immediately repaired and the animals came into full oestrus within a hundred hours of the implantation. The...
experiments were then extended to infantile mice and
with results similar to those of Smith and Engle.

"Wir können bei infantilen bei, dem
Muttertier kaum entwachsenen (presumably
"weaned") Tieren reifende Eier auf dem wege
durch die Tube nachweisen".

(Parkes 1929 states that Zondek and Ascheim never
succeeded in inducing ovulation with their grafts).

The authors concluded that:

"Das Hormon des Hypophysen vordlappen ist,
wir gezeigt haben der Motor der Sexualfunktion.
Das Hypophsenvordlappenhormon mobilisiert erst
in den follicularen Zellen das Ovarial Hormon.
Das Vorderlappenhormon ist das Primare, das
Ovarialhormon das Sekundare. Das
vorderlappenhormon lost die Production des
Ovarialhormons aus, gleichzeitig bringt es das
Ei zu Reife".

"Hypophysenvordlappenhormon, Ei und Ovar-
ialhormon bilden aber eine Einheit im
funktion funktionellen Sinne. Sie dienen
gemeinsam der wichtigsten Funktion des
weiblichen Organismus, der Fortpflanzung".

Immediately after these experiments attempts
were made to discover the distribution of the
hypophyseal hormone in the human female. The
test objects were infantile mice into which were
grafted or injected fragments of human tissue or small
quantities of human body fluids. The substance was
found to be characteristic of pregnancy and was
found in,

1. the gravid decidua (in the first 4 months)
2. the gravid corpus luteum
3. the placenta (from the second month)
4. pregnancy serum (from the second month)
5. the blood of the umbilical vessels
6. the epithelium of the Fallopian tubes
   (after the second month)

7. the urine from the thirty fifth day after
   the last menstruation.

The diagnosis of pregnancy by the injection of
urine into infantile mice and subsequent examination
of the effects produced on the ovaries is now a
common operation in many laboratories. Particularly
accurate results have been obtained in Edinburgh
by Professor Crew.

The changes produced in the ovaries by this treatment
are very complex, and suggest the possibility that
more than one autacoid is present in the urine.
Apparently some follicles may ripen but without
dehiscence, while others are converted into
atretic corpora lutea; others again become engorged
with blood forming very characteristic "Blutpunke"

THE SEPARATE IDENTITY OF THE KYOGENIC AND
OESTROGENIC FACTORS.

Many workers believed at this time (1927–29) that
the hypophysis secreted only one gonadotrope hormone
which produced ovulation or the formation of atretic
corpora lutea according to its concentration in the
blood. It was pointed out that the methods of Evans
differed radically from those of Smith and of the
German workers. "The macerated suspensions (or
implants) are fresh and correspond only to minute
amounts, 50 to 20 mgms., of fresh tissue daily;
the sodium hydroxide extracts on the other hand,
made from ox-pituitaries may not be really fresh and
the daily amount injected corresponds to about one
gram of the original tissue."

In 1928 Evans and Simpson experimenting on the
antagonism between grafts and alkaline extracts
suggested that the growth principle of the pituitary
might also be responsible for the luteinisation;
ovulation and the secretion of the sex hormone being
incited by a different substance. A similar theory
was advanced about the same time by Zondek and
Ascheim. The former authors write:

"Precocious maturity have been secured by the
administration of various extracts of 8 hour
old bovine glands. The markedly different
results from these two procedures could have
been due to the freshness or staleness of the
glands used, the source of the glandular
material (rat or bovine) or the method of
implantation of the material. We can show
that the latter explanation is the correct one.
Both hormones reside in the anterior
hypophyseal tissue from either source, rat or
bovine and specific extractives can be used
for either. The growth hormone cannot easily
escape from the relatively large tissue
fragments used in the implantation method,
but it can readily do so when this tissue is
merely triturated, and extraction of rat
glands in a way identical with that employed
in the bovine growth extracts showed a similar
abundance of the growth hormone in the tiny
glandules, i.e. the rat glands. On the other
hand implanted bovine glands stimulate
precocious sexuality as do rat glands and but
little diminution in the hormone occurs with
six hours ageing after the death of the donor.
It has further been possible for us to secure
the maturity or sex-stimulating anterior
hypophyseal hormone by extracting this tissue
in ways different from that employed for
the growth hormone".
Smith and Simpson concluded that at least two hormones were secreted by the pars anterior, one being required for normal growth and the other for normal development of the gonads, thyroid and suprarenal cortex. Having found that the effects of either of these substances on the reproductive system could be neutralised by the synchronous administration of an equivalent dose of the other, they suggested "that the growth of the animal is usually accomplished before the attainment of sexual maturity may therefore plausibly be due to the early predominance of the growth hormone". This could hardly be true since the growing prepubertal female is not characterised by the presence of atretic corpora lutea in her ovaries. Further if one accepted the theory one would need to postulate that the pituitary is essential only to maintain that growth which takes place after the first ovulation, when luteal tissue appears for the first time; and this view has been refuted by experiments on the hypophysectomy of immature animals.

About the same time, Zondek and Ascheim, neglecting the significance of the effects of the alkaline extracts on growth, suggested that the pituitary generated at least two gonadotrope factors, Prolan A and Prolan B; the first bringing about the maturation and dehiscence of the follicle and the second the activation of the corpus luteum.
These workers were successful in effecting a partial separation of the two substances. Since both Prolan A and Prolan B appeared to be present in pregnancy urine, Zondek and Ascheim were able to explain in part the curious effect of injections of this fluid on the immature ovary.

Additional confirmation of the diharmonic theory has come from the work of Wiesner and Crew. In a paper published in 1930 they described the results of a series of experiments which proved conclusively the separate identity of the oestrogenic and kyogenic principles. They administered an alkaline extract in daily quantities of from .2 to .0012 cc., and found that in every case mucification of the vagina resulted, there being not the slightest evidence of any oestrogenic effect throughout the entire range of dosage. Moreover the continuous daily transplantation of comparatively large fragments of anterior lobe into the muscles of immature mice resulted invariably in cornification.

They also succeeded in preparing an extract the effects of which were entirely oestrogenic.
(This extract was prepared as follows. 35 grams of the anterior lobes of cattle were finely ground with sand and 50 cc. of 20% sulphosalicylic acid added. Having stood in a filter for 2 to 12 hours the mixture was diluted with 60 cc. of water, agitated and passed through a Buchner filter. After a final passage through a membrane filter and neutralisation with sodium bicarbonate the mixture filtrate was ready for injection.)

The method has been criticised by several authors, notably Parkes, on the ground that it is impossible to remove the precipitating agent; according to Wiesner however, the sodium salt of sulphosalicylic acid is non-toxic.

Wiesner and Crew described an interesting fact relating to the action of either of these extracts; the threshold of the ovary appears to be considerable higher than that of the vagina, so that under the influence of the alkaline extract mucification may be induced in the absence of luteinization; and, under the influence of implants, or injections of the acid extract, cornification without any perceptible change in the volume of the follicles.

In the same year Wiesner was able to prepare from the placenta by extraction with sulphosalicylic acid a fluid which appeared to
possess both oestrogenic and kyogenic properties according to the dosage. On boiling both properties disappeared in a very short time, but, since the oestrogenic principle appeared to be destroyed first, it was possible to effect a partial separation of the kyogenic substance. Since extracts of the placenta, however prepared, are without effect on growth, this experiment was understood to prove the untenability of the hypothesis advanced by Evans and Simpson. Yet, it is not as yet certain that the autacoids of the placenta are identical with those in the anterior lobe of the hypothesis, and Collip, for example, continuing Wiesner's work, has come to the conclusion that they are different substances.

Oestrogenic extracts from have now been prepared by many workers. Hill and Parkes, for example, extracted in the first place with sodium hydroxide neutralised with hydrochloric acid and then precipitated the proteins by the addition of alcoholic hydrochloric acid.

Terold Hisaw and Leonard have described the effects of an aqueous pyridine extract on the anterior lobe. A volume equal to 20 mg. of the original tissue was injected daily; rats of 20 to 24 days could be brought to maturity in three days. The vagina opened, the follicles ripened, and were
discharged their contents. 5 days after the injection's
were begun the ovaries were a mass of corpora lutea
and follicle. The extract was then fractionated,
and it was found possible to isolate two constituents,
one being soluble in water and almost entirely
oestrogenic, the other being insoluble in water,
and promoting the formation of corpora lutea.

The usual interpretation of the experiments
described in this section is both simple and
comprehensive. The periodicity of the female genital
tract might be due, 1.) to its possessing a refractory
period, the somatic stimulus being assumed to remain
constant, or (2.) to cyclic variations, quantitative
or qualitative, in the intensity of the stimulus.
The first of these may be discarded for many reasons.
by the injection of pituitary
In the first place it is possible to maintain an
animal in either oestrus or pseudopregnancy for
an unlimited duration. Even in very young animals
the threshold of response does not appear to
be unreasonably high.; witness the experiments of
Smith and Engle. Moreover the climateric appears
to be a property of the soma and not of the ovary,
since Zondek and Ascheim have succeeded in
"rejuvenating" the senile mouse by treatment with
anterior pituitary.

Today the second alternative is accepted universally
and we need only discuss the terms "stimulus" and
"qualitative". There seems to be every reason to
believe that the ovary is controlled by the alternate predominance of either of two autacoids, or, as is more likely, complexes of autacoids. Again, since in the virgin rodent these autacoids may be prepared only from one organ it is not unlikely that they are secreted by the pituitary alone.

As a preliminary and tentative hypothesis (the German word "Fiktion" might be better.) we might suppose that the orderly sequence of the various stages of the oestrous cycle might be conditioned by the secretion by the anterior lobe of fluctuating quantities of Rho 1. This view has indeed been endorsed by several prominent workers such as Parkes, Wiesner and Crew, and others.

A simple "experimentum crucis" is by no means difficult to devise. If the periodicity of the ovary is intrinsic, the introduction of ovaries into an intact female should lead to irregularities in the oestrous cycle (assuming that the ovaries of the host and those of the donor were "out of phase"). On the other hand, if the hypothalamic hypothesis outlined above is at all valid, the pituitary of the host should be capable of forcing the foreign ovaries to conform to its own rhythm, and the oestrous cycle should continue unaltered. Steinach Sand and others have shown long ago that the principle of "physiological deficiency" does not obtain in its full
Possible factors of the different trends.
wrigour when the organs grafted are gonads, so that these success of the experiment would not be prevented by this difficulty.

In 1930 Friedman and Nice attempted the experiments with startling results. Two additional ovaries were grafted into female albino rats; one being imbedded in the kidney, the other sutured to the abdominal dorsal abdominal wall. In 19 out of 22 cases the operation was entirely successful and the grafts persisted for a considerable time. In these 19 animals the oestraus cycles immediately became irregular and their length fell to about 2.2 days, a net decrease of 48%.

It has been known for several years that the ovarian cycle may be expedited by various means such as removing young corpora lutea or puncturing the developing follicles.

There is evidence therefore that the primary seat of the periodicity of the female genital tract does not reside in the anterior lobe and that what has been called the "Rho-Theory" is inadequate. This is the present position of our knowledge of one of the most interesting functions of the gland.
BIBLIOGRAPHY.

1. Sharpey-Shafer
   Livre Jubilaire de Ch. Richet.

2. Camus and Roussy
   Journ. de Physiol. xx. 509-535. 1922

3. E. Frank

4. See Abel.
   Harvey Lectures 1924.


6. See Sargent
   Harveyian Oration 1931.

7. Paulesoo
   L'Hypophyse de Cerveau.
   Paris 1908.

8. Cushing

9. Dott

10. Wolf and Sachs


12. Aschner
    Arch. f. d. ges. Physiol. cxlvi.

13. Dandy and Goetch

Smith and Graeser  Anat. Rec. xxvii 219. 1924

Smith  ibid. xxxiii 221 1926.


Larson  ibid.

Schafer  Croonian Lecture 1909.


Smith  Anat Rec. xi. 57. 1917.


Evans and Long  Anat Rec. xxi 62,19, 1921.


        London. 1837.


Wiesner  Dissert. Vienna 1922.


Pfluger  Uber die Bedeutung and Ursache der Menstruation. Berlin. 1865.


Teel  Am. Jour. Physiol. ibid.


Fraenkel  Arch. f. Gyn. lxviii 1903.


Parkes and Bellerby  Journ. Physiol. lxiv. 1927.


Kohler  Zeit. f. Gyn. iii. 1924.


<table>
<thead>
<tr>
<th>Page</th>
<th>Author(s)</th>
<th>Journal and Volume Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>Engle</td>
<td>Ibid. xxv 1927.</td>
</tr>
<tr>
<td>58</td>
<td>Zondek and Ascheim</td>
<td>Klin. Woch. n.s. vi i 1332</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vi 1333</td>
</tr>
<tr>
<td>60</td>
<td>Evans and Simpson</td>
<td>Journ. Amer. Med. Assoc. livi ixc 1337. 1928</td>
</tr>
<tr>
<td>61</td>
<td>Crew and Wiesner</td>
<td>Proc. Roy. Soc. Edin. 1 73. 1930</td>
</tr>
<tr>
<td>63</td>
<td>Hill and Parkes</td>
<td>Journ. Physiol. lxxi no1 1931.</td>
</tr>
<tr>
<td>64</td>
<td>Terold Hisaw and Leonard</td>
<td>Anat. Rec. iii1 2999 1931.</td>
</tr>
<tr>
<td>65</td>
<td>Friedman and Nice</td>
<td>Am. Journ Physiol. vc. 1930.</td>
</tr>
</tbody>
</table>
NOTE ON THE RELATIONSHIP BETWEEN THE PITUITARY AND OVULATION IN THE RABBIT.

In the course of the researches which we mentioned previously Heapé discovered that if a female rabbit were isolated about the beginning of oestrus it continued on heat until the ripe follicles had undergone involution. If on the other hand the animal were placed with a male it was found that the follicles continued to swell and that finally about ten hours after coitus it they burst liberating ova which rapidly shed their polar bodies and so came to maturity. This phenomenon is not peculiar to rabbits, being found for example in ferrets and guinea-pigs cats. Heapes observation has been abundantly confirmed and a year or two ago Hammond was able to watch the process of ovulation under a binocular microscope.

Apparently the stimulus for ovulation is the act of copulation itself since ovulation occurs equally readily with vasectomised bucks and may even be brought about by stimulation of the vagina with a glass rod (Hammond 1925).

The act of ovulation is apparently a reflex of some kind and the length of the period of latency suggests a humoral link. Zondek and Ascheim proved that the normal agency for the control of ovulation is a hormone secreted by the
anterior lobe of the pituitary and recently their experiments have been extended to the rabbit. In 1930 Fee and Parkes proved that under certain conditions hypophysectomy might inhibit the process of ovulation. The operation was performed in a very simple manner, the anterior part of the head being simply sawn off. It may be added that the possibility of unrecognised disturbances being introduced by this rather crude procedure was eliminated by the extensive use of controls.

If the operation were performed within one hour of coitus ovulation was inhibited, and the follicles gradually underwent atresia. On the other hand hypophysectomy later than one hour after after coitus was entirely without effect the follicles releasing their ova and the cells of the membrana granulosa forming an apparently normal corpus luteum.

This observation proves fairly conclusively that the anterior lobe intervenes in the arc between the vagina (or other receptor organ) and the ovary:

Vagina → Pituitary → Ovary.

The time taken to conduct the stimulus through the first part of the arc is relatively short, certainly less than an hour, while nine or ten hours appear to intervene between the
secretion of the gonadotrope factor in the pituitary and the consummation of the response by the cells of the follicle. Taken together with Robinson's work on the mechanism of ovulation in the ferret, this observation provides material for much further experiment upon the relationship between Prolan A and the secretory activity of the epithelial lining of the Graafian follicle.

In 1931 Hill and Parkes have shown that it is possible to induce ovulation in the hypophysectomised virgin rabbit by the injection of an extract of bovine anterior lobe (Details of the preparation of this extract were given in the last chapter.) They noticed however that the period of latency was somewhat longer than normal.

Parkes and his collaborators have also shown that local anaesthesia of the vagina does not hinder ovulation in the normal mated doe, and the lining of this passage cannot therefore form the receptor organ for the ovulation reflex.
Cycle in Pubes.

The Nature of the Menstrual Cycle.

[Graphs and charts with annotations indicating the nature of the menstrual cycle and associated changes.]
NOTE ON MENSTRUATION.

Since human physiology is not less interesting than the physiology of rodents and since its results are usually of great clinical importance, a discussion of the growth of knowledge on the relationship between the anterior lobe and menstruation would form an important part of this essay. None of the evidence however is unambiguous, and even the nature and significance of menstruation are still sub judice.

According to Marshall and to most modern writers the menstrual cycle is fundamentally diphasic and represents both the oestrous and pseudopregnancy cycles of lower mammals. Menstruation is believed be conditioned by the superimposition of prooestrus upon the breakdown of the decidua at the close of a period of pseudopregnancy, and may therefore be represented by the intersections of two sine curves which are slightly out of phase; Such a diagram is given on the opposite page, and shows very clearly the relation between the reproductive cycle in the woman and that in the rodent and dog.

To induce menstruation in a hypophysectomised Primate we must therefore supply both Prolan A and Prolan B in suitable quantities.

Dr. Wiesner at the meeting of the Physiological Society in Edinburgh last year gave a
microscopic demonstration of the results of such an experiment, and they appeared to be very successful. His extracts were prepared from the placenta by treatment with salicylsulphonic acid.

In 1930 Hartman Tirer and Geiling found that extracts of the anterior lobe caused bleeding in hypophysectomised female monkeys.

Collip using a modification of Wiesner's extract claims to have had considerable clinical success in the treatment of disorders of menstruation.

It is desirable that these experiments should be repeated with extracts containing only one of the gonadotrope principles.
CHAPTER XI.

THE INFLUENCE OF THE PITUITARY ON THE TESTIS AND THE MALE SECONDARY SEXUAL CHARACTERS.

Although the differences between the generative physiology in the males and females of a species are complex and numerous, most of them may, in the last analysis be ascribed to the fact that the nourishment of the young in uterine and early extra-uterine life devolves almost entirely upon the latter sex.

In the rat the female genital organs are characterised by the exhibition at regular intervals of two different types of cyclical change, one type apparently being designed to liberate the ova and to facilitate their fertilisation: the other set to facilitate the nidation of the zygote, to maintain the life of the embryo, and to feed the young animal for the first few weeks of its life. It would appear that each of these modes of periodic activity was regulated by a particular set of hormones, or of hormone-complexes; the sexual type by Prolan A and Cestrin, the second or reproductive type by Prolan B and Kythin.

In the male the reproductive cycle has been entirely suppressed, and therefore the hormones in charge of his reproductive apparatus are concerned only with the regulation of the production of spermatozoa and with their intro-
Relationship Between Male and Female Blood Concentration

In a Raising, Polycytemia Advice.

System = Concentration of Male Blood in the Venous

Bovine = Concentration of Female Blood in the Arteries

AB = Arterial Blood
CD = Blood
EF = Venous Blood
duction into the body of the female. The biological duty of the male has been discharged once his spermatozoa are safely lodged in the uterus of his mate, to whom alone is entrusted the subsequent care of the zygotes which they assist in forming.

The cyclical changes in the genital organs of the male correspond to these changes in the female that characterize the oestrous cycle. The cycle in the male is therefore monophasic and simple.

One of the most fascinating problems presented by the organs in the female is their remarkable periodicity, changes following one another with the same regularity as the heart beats systole and diastole of the heart. In the male, on the other hand, periodicity is less marked, and may be entirely suppressed; although a rutting season is probably a primitive mammalian feature it is often absent especially in domesticated species which generally find their way into the laboratory. Again, even when it does occur, the rutting season is synchronous with the entire breeding season, and may therefore include and overlap a multitude of the dioestrous cycles exhibited by the female.

These considerations would lead one to suppose that the relationship between the pituitary
and the testicle would be much less complex than the corresponding relations in the other sex and therefore that their physiology would be much more advanced. This however is not true and compared with the volume of litterature from which the author was able to select the experiments described in the previous chapter, the work on the connection between the pituitary and the male sexual characters appears to be scanty and inadequate.

Yet the problems presented by the male animal are interesting enough and their solution would undoubtedly be of great benifit to practical medicine. It certainly seems desirable that they should be investigated with the care that Parkes and his collaborators for example, have devoted to the study of the reproductive function in the female.

Clinical evidence on the subject accumulated early and probably still forms the greater part of our knowledge. It will be remembered that Pierre Marie described impotence as one of the characteristic features of Acromegaly, and that the male genital organs are underdeveloped in the condition of Dystrophia Adiposo-genitalis.
Experimental Work.

Successful reproduction is brought about through the delicate balance of two factors, the genotypic constitution of the gonad and the somatic environment in which the first factor is allowed to realise its potentialities. We may ask therefore, whether the male and female differ, either because of the complex of hormones in their bodies or (and more particularly these secreted by the anterior lobe of the pituitary) or because of their possession of a gonad which reacts in a different way in either sex to an identical or similar humoral constitution.

If an ovary were found to continue its normal functions in a male soma we would have good reason to believe that the anterior lobe of the pituitary was functionally equivalent in both sexes.

If on the other hand the ovary were found to degenerate, to form cysts, or persistent atretic corpora lutea, there would be reason to believe that one of the principal factors in determining the phenotypic sex of an animal was the functional pattern of the anterior lobe of its pituitary; in the second case the male anterior lobe would secrete only Prolan A and in the third case only Prolan B.

Experiments in heterosexual transplantation are gave us our first positive evidence on the function of the anterior pituitary
in the male and it would therefore be both interesting
and relevant to describe them in some detail.
Unfortunately however the results which they yield
are by no means unequivocal. The few experiments
that I shall describe are mainly taken from
Lipscutz and to his book I would refer the reader
for further information.

In 1912 Steinach grafted into young
male castrated rats and guineapigs immature ovaries
without ripened follicles and observed all the
changes characteristic of the normal ovary up to eh
the formation of corpora lutea. This condition last-
ed only a few months; the older the graft the
smaller was the number of follicles attaining ripe-
ness.

Athias (1915-6) made similar
experiments with adult ovaries, finding even eight
and a half months after transplantation besides
atretic follicles a great number of primary
follicles.

Steinach (1913) Sand (1918) and
others have shown that it is possible to suppress
the male secondary sexual characters and correspondi-
ingly to promote the development of the female
by the implantation of ovaries into castrated males.
A short time after the operation the teats and
mammary glands hypertrophy the female reflexes
develop and the body takes on a characteristically
female configuration.

On the other hand Steinach (1913) Sand (1918) Moore (1919) and others have transplanted testicles into the bodies of castrated females, finding that the teats and mammae undergo regression, while the clitoris enlarges and is modified to form an organ somewhat resembling a penis; and the outline of the animal comes to resemble that of an ordinary male.

Moore (1921) has described the histology of such a transplant. There was little or no hypertrophy of the interstitial cells in one case, the germinal epithelium being degenerate but not excessively so. In an other case the germinal epithelium had degenerated completely only a single layer of cells being present in the tubules, which were widely separated by large threads of interstitial tissue consisting of well-stained cells of Leydig.

Thus while the hormones from the anterior pituitary of one sex do not appear to be sex-specific, since they are competent to maintain the endocrine functions of a foreign gonad, they are obviously not identical in either sex since transplants do not maintain completely their histological differentiation. It is necessary therefore to isolate the two gondotrope hormones of the female and to study their effects
together or separately on the testis.

In 1913 Cushing and Goetsch stated that the male genital tract was hypertrophied after prolonged feeding with anterior lobe.

Ten years later Dott found that in some cases removal of the anterior lobe in dogs was followed by degeneration of the testis and of the organs under its control.

Evans and Simpson in 1926 found that the protracted administration of alkaline extracts to male rats was followed by reluctance to copulate and by a diminution in the size of the testicles. In considering these results it must be remembered that alkaline extracts are very probably toxic by reason of their high concentrations of foreign protein which they contain.

Steinach and Kuhn (1928) have shown that in the case of the immature male it is possible to induce precocious sexual maturity by administration of anterior pituitary. This maturity however endures only so long as the administration is continued. These writers deduced the degree of sexual maturity from the development of the seminal vesicles and prostate.

Brouha and Simonnet (1929) were able to stimulate the genital tracts of rats and mice by the daily intramuscular implantation of small
fragments of anterior lobe. The same effect was obtained by the injection of urine derived from a woman in the early stages of pregnancy. They confirmed the results of Evans and Simpson with alkaline extracts.

In 1931 Wiesner was able to restore the spermatogenic function to the tubules of senile rats by continued administration of the extract which he had prepared from the placenta by decoction with sulphasalicylic acid.

We have not as yet sufficient data to form any conclusions on the nature of the hormone which maintains the function of the testicle. Possibly both Prolan A and Prolan B are concerned but in the light of the experiments of Brouha and Simmonnet it is not impossible that the former is very much more important. We have described above how the reproductive cycle in the male appears to be homologous with the oestrous cycle in the female, and it would be fitting that both should be performed under the influence of the same hormone. In the present state of knowledge however such a statement would be unscientific, and many years of experimentation will be required before we can form an adequate and plausible conception of the relationship between the anterior lobe and the functions and structures which characterise the male. The great need appears to be for a simple and accurate method of estimating
the intensity and course of the reproductive function in the male. Several have now been described, such as the state of the accessory glands the vitality of spermatozoa in an isolated epididymis, and the electric excitability of the seminal vesicles, together of course with the method of the examination of sections of the testis. Most of these methods however involve the death of the animal. Something is needed which is analogous to the vaginal smear tests in the female.

Bibliography.

1. Heape
   Proc. Roy. Soc. B.
   lxxvii. 1905.

2. Hammond
   Reproduction in the Rabbit
   Edinburgh. 1925.

3. Fee and Parkes
   Journ. Physiol.
   lxx. 1930.

4. Marshall
   The Physiology of Reproduction.
   London. 1922. P. 74, et seq..

5. Hartmann, Tirer and Geiling
   Anat. Rec.
   iii 1931.

6. Lipschutz
   The Internal Secretions of
   the Sex Glands. Lond
   Cambridge. 1914.

7. Cushing and Goetsch
   op. cit..

8. Evans and Simpson
   op. cit.

9. Steinach and Kuhn

10. Brouha and Simmonet
    Press. Med.

11. Wiesner
    xxxvii 229. 1930.
CHAPTER XII.

THE POSTERIOR LOBE AND PARS TUBERALIS.

We learned in the previous section that, even as long as thirty years ago, it was known with certainty that lesions of the pituitary gland are usually accompanied by a great diminution in the intensity of various functions of the body, such as growth, metabolism, and reproduction. In the last ten years it has become possible to augment the intensity of these functions in the intact animal by the injection of extracts prepared by suitable methods from the anterior lobe, and it is found, indeed, that these functions are scarcely affected by the administration of any other division of the gland.

There is every reason to suppose that, in a few years' time we shall be able to maintain a normal life in completely hypophsectomised animals, simply by the regular injection of material derived from the anterior lobe.

Such an experiment might be considered by some to prove conclusively that all the functions of the pituitary gland were performed solely by its anterior lobe, that is. Such an inference would appear to be confirmed by the work of Dott who excised the posterior lobe (confirming the completeness of removal by microscopic sections) and found that the animal appeared to be unaffected by the operation.
At this point, if one knew nothing of the facts, it would appear to be reasonable to believe that the pars nervosa was functionless, and consequently, that extracts prepared from this region of the gland would prove to be quite inactive. Yet it seemed to the earlier workers as if the pars nervosa were enormously the most important division of the gland containing as it did autacoids which modified profoundly a great number of the most diverse functions and influenced the activities of such organs, and systems of organs, as the kidney, mammary glands, alimentary canal, uterus, heart, and blood-vessels.

Today it is not entirely unnatural to argue that the presence of these autacoids may be purely fortuitous and completely devoid of physiological significance. A certain number of authors maintain this position at the present time, and it must be admitted, not wholly without justification. The view probably represents a reaction from the extreme teleology of the earlier workers, some of whom supposed that the depressor principle of the posterior lobe was secreted by an animal to lower its blood pressure.

Yet the position of the older writers was probably nearer the truth than that maintained for example by Hogben, and one must be surprised at the results of the complete extirpation.
of the posterior lobe.

A possible explanation of these results, however will be shown by a consideration of these nature of these functions which the posterior lobe might be expected, from a survey of the effects of extracts to influence. We find that every one of these functions may also be modified by many other factors such as the sympathetic and parasympathetic nervous systems, and the autacoids in many various other glands. And this multiplicity of control must be expected to come into action when the posterior lobe is ablated.

Thus although in the normal animal the primary factor in the causation of parturition may well be oxytocin, this substance cannot be considered to be the only agent, since the uterus at least when gravid, responds by contraction to stimulation of its sympathetic nerves, to partial asphyxia, and to sufficiently high concentrations of any of the sympathomimetic bases, one of which, adrenalin, has been proved to be present in normal blood.

A similar statement would apply with scarcely less force to the musculature of the vascular system, and in a less degree, to the kidney. Any functions which the posterior lobe may possess are evidently shared between it and
the nervous system and the other endocrine glands.

One positive effect has been obtained by ablation of the posterior lobe—the contraction of the melanophores and the expansion of the xantholeucophores in amphibia. In this trivial instance the endocrine function of the lobe may be considered as proved, since the normal chromatic condition may be restored by the injection of an extract of the extirpated part. On analysing this result however we find that the pituitary and the suprrenals are the only organs which appear to affect the pigment-cells while, at least in amphibians there is good reason to believe that the latter play no part in the regulation of the normal coloration (Hogben 1924).

There is no reason to conclude from this experiment that because ablation of the posterior lobe does not impede efficient parturition in mammals oxtocin is not concerned with this function. The case of the bitch and the frog are not identical; the pigmentation of one is controlled by the posterior lobe alone, the contractions of the uterus in the other, by the pituitary posterior lobe (possibly) and by many other factors. In the latter case these other factors may be able to intensify their action in the absence of the posterior lobe and so to discharge its functions vicariously. If
the same function be subserved by the synergetic action of many organs there is no reason to suppose that every one of them is indispensable.

We conclude from this discussion that whatever the functions of the posterior lobe it is likely that in its absence they are undertaken vicariously by other tissues. The organs of the body conspire, as it were, to circumvent our experimental methods and we are left with only one opportunity of proving the endocrine function of the gland; we must demonstrate the presence in the blood or some other body-fluid of a substance identical with one of the autacoids found in extracts of the posterior lobe.

Two types of test may be employed, one being based on the chemical reactions of the autacoid and the other upon its physiological effect on the activity of some organ.

The first class is preferable but unfortunately no specific chemical tests for any of the autacoids of the gland are known, and even if they were known it is unlikely that they could be made sufficiently delicate to detect the minute quantities which are all that we may fairly expect to find in the blood.

The physiological tests are simple and sensitive but their specificity is usually open to doubt.
In the next section the possible influence of the pituitary on parturition, the vascular system and the secretion of urine will be discussed. It may be well to place on record the fact that in 1910, Ott and Scott found that the intravenous injection of pituitary extract into a lactating goat was followed by an increase in the amount of milk secreted. Since this effect has rarely been supposed to carry any functional significance it will not be further discussed.

Hogben The Pigmentary Effector System Edinburgh 1924.

PITUITARY AND VASCULAR SYSTEM.

The history of this function goes back as far as 1895 when Oliver and Schafer discovered that extracts of the whole gland exerted a most remarkable influence on the blood pressure causing a rise almost as great as that invoked by the autacoid in the suprarenal. Like the latter the substance in the pituitary appeared to act peripherally since the pressor effect persisted after section of the cord and ablation of the bulb, and since an extract when added to the fluid with which a frog was being perfused induced a decrease in the rate of perfusion.

The autacoid of the pituitary was however proved to differ from adrenalin since, when injected into the veins of an animal in which the central cardioinhibitory mechanism had been dislocated by atrophine or double vagotomy, the latter elicited an enormous increase in the vigour and rapidity of the heartbeat, while the former was almost without effect.

It is evident from some passages in Oliver and Schafer's paper that they were inclined to consider that the substance in the pituitary might exert a tonic influence on the arterioles and so play an important part in the maintenance of the blood pressure. Before the nineteenth century had ended this hypothesis was no longer tenable.

In 1898 Howell contributed two facts of capital/
capital importance; he showed that the pressor principle appeared only in the posterior lobe, and described the phenomenon of "Tachyphylaxis". If the initial injection were repeated after a short interval the blood pressure was found to remain unaltered or even to fall considerably; a short subsequent rise could be explained from the principle of "over-compensation".

Thus if the posterior lobe were to secrete into the blood stream the materials present in a saline extract, ablation would be followed by a rise in the blood pressure, and this would apply with equal force to almost any other organ in the body, since a saline extract of almost any tissue will depress the blood pressure.

These observations were confirmed and extended in the succeeding year by Schafer and Swale Vincent. If the posterior lobe were dissipated and extracted with absolute alcohol, the filtrate, after evaporation of the alcohol and extract of the residue with saline, was ascertained to cause invariably a decline of the blood pressure on intravenous injection. Moreover, although a first injection of a saline extract of the alcohol-insoluble fraction raised the blood pressure, succeeding injections were entirely without effect. Thus a pressor and a depressor principle were present in the posterior lobe; and the latter was found to be very widely distributed through the tissues of the body.

From this point we may pass without any serious omission/
omission to the work of Sharpey-Schafer and Macdonald in 1926. These authors confirmed the experiments of 1898 and 1899 before proceeding to a careful and detailed study of the effects of either principle on the blood pressure in both the systemic and the pulmonary arteries.

With regard to the pressor principle they found that the rise in the systemic pressure consequent upon its injection was interrupted by a very characteristic notch, while if the suprarenal capsules had previously been deprived of their vascular connections the kymograph tracing rose smoothly to its zenith. This suggests that the pressor principle may be employed by an animal to increase its blood pressure in an emergency, since if the presence of high concentrations of the principle were unphysiological one would expect the consequent rise to be mitigated by the action of the various depressor reflexes and not assisted by the simultaneous secretion of adrenalin. Although an observation like this might serve to encourage investigators to attempt to discover the presence of pitressin in the body fluids, by itself it proves nothing. The results of thirty years work on the effects of extracts on the posterior lobe have given us no reason to believe that any substance present in this part of the gland plays any part in the regulation of the blood pressure in the intact animal.
KROGH's Evidence.

In 1921 Pohle noticed that hyophysectomised frogs lost their characteristic dark colour and developed an intense cutaneous oedema. Rehberg working in Krogh's laboratory repeated these experiments on another species of frog. A few hours after the operation the capillaries began to expand, the state of expansion persisting for about a fortnight. Oedema did not develop, although the contraction of the melanophores was confirmed. If the anterior lobe alone was removed, the symptoms appeared, but the animal was normal within a week of the operation.

In mammals neither oedema nor dilation of the capillaries has ever been observed after injury to the anterior lobe; this suggests that the above experiments may be open to criticism.

In the first place cutaneous oedema appears rarely to have been observed by others who have worked with frogs. For example Rehberg, Hogben and others never seem to mention it, or at least ascribe their negative results to the fact that they worked with a different species of frog. This explanation appears to be most unlikely; if animals within the same genus differed completely in an important part of their physiology it would be necessary to study each species separately without there being for example any possibility of making clinical use of facts derived from such an animal as the dog.
Again the hypothalamus may have been injured. It must not be forgotten that in amphibians the lymph circulates almost as rapidly as the blood, so that it is possible to produce oedema by destruction of the contractile organs situated on either side of the urostyle. Since the contraction of these lymph-hearts is induced by periodic volleys of stimuli from the central nervous system, injury to the upper part of any tract involved in their control might suspend their activity and so bring about the oedema. The writer tried to investigate the probability of such a view by injuring the portion of the pituitary gland and the diencephalon which lies directly above the junction of the anterior and lateral processes of the parasphenoid using male toads just after the breeding season but with the clasp-reflex slightly developed or absent. It was found that the appearance of oedema was associated with restoration or intensification of the clasp reflex, and that in one case blanchening was produced without either of these symptoms. It would appear therefore that oedema only occurs when considerable injury has been done to the descending tracts in the diencephalon.

Considering the dilatation of the capillaries which Rehberg observed the evidence is much more satisfactory since although as far as the writer is aware no experiments have been made on the condition of the capillaries after hypophysectomy in mammals it is at least known that in this Class the tonus/

* See Lipschutz p.98
tonus of these vessels persists after their sympathetic nervous connections have been destroyed (Dale and Richards 1919); yet if a limb be completely denervated Burn (1922) showed that the dilator response to histamine was usually lost, and suggested that normal tonus might be due at least in part to the integrity of the posterior root fibres, a hypothesis which has become more plausible since the recent work of Ken Kure. These experiments of Rehberg's are therefore not conclusive.

In 1922 and the preceding years Krogh investigated the subject very carefully and with most interesting results.

In one of the first papers that he ever published Bayliss showed that after the abdominal aorta of a mammal had been clamped for a short time the removal of the ligature was followed by a profound decline in the flood pressure, an effect which was subsequently demonstrated to be caused largely by an increase in the diameter of the capillaries, possibly due to anoxaemia.

If the hind leg of a frog be perfused with venous blood a considerable portion of the blood is arterialised in its passage through the web of the foot, the capillaries of this region and the surrounding tissues possess therefore an independent source of oxygen. Yet in such a preparation Krogh found that the capillaries rapidly lost their tonicity/
tonicity. If in the intact animal the femoral artery were clamped the capillaries after a brief period of latency increased from a mean diameter of 5 to one of 20 micra. Five minutes after the restoration of the circulation the diameters were again normal.

From these experiments Krogh argued with some show of reason that normal blood contains a substance in the absence of which the tonicity of the capillaries is lost and that this substance is of low molecular weight and may very easily be destroyed by oxidation in stagnant arterial blood.

Krogh next attempted to discover the substance in normal blood.

When an isolated frog's leg was perfused with gum-Ringer the capillaries dilated; normal tonicity was restored by perfusion with ox-blood.

Moreover Krogh was able to separate by dialysis from ox-blood a substance which possesses the property of affecting dilated capillaries in very small concentrations.

Taken together with the results of hypophysectomy it seemed these experiments suggested the possibility that the unknown principle might be pituitrin; in confirmation of this it was found that the perfusion of the isolated frog-leg with Ringer containing from 1 in 50000 to one in a million parts of Parke Davis pituitrin was not accompanied by the usual increase in the diameter of the capillaries although arterioles and other/
other vessels were apparently not affected.

In every one of these experiments it was observed that the degree of pallor of the skin ran parallel to the dilatation of the capillaries.

These observations prove that pituitrin may maintain the normal tonus of the capillaries in an animal deprived among other things of its nervous system and suprarenals. But the work of Dale and Richards shows that adrenalin may replace pituitrin, while it is not inconceivable that the normal diameter might be maintained by the nervous system in the absence of either gland. Again, while experiment has shown that the degree of expansion of the melanophores is conditioned by the presence in the blood of small quantities of an autacoid derived from the pituitary, it should not be too hastily assumed that this autacoid also affects the capillaries. Herring has shown that the pressor principle is almost entirely confined to the pars nervosa, and Hogben that the melanaphore stimulant is most abundant in the pars intermedia. Moreover if a solution of pitressin be subjected to alkaline hydrolysis the pressor and melanophore effects disappear with unequal rapidity (Hogben 1930).

There is therefore some evidence that the tonicity of the capillaries is maintained at least in part by a secretion of the posterior lobe. None of it however is satisfactory and experiments should be/
be made with the method so successfully employed by Starling Verney and others. If a mammalian limb were perfused by a heart lung preparation and if the capillaries were found to remain expanded until a head had been introduced into the circulation, there might be some reason to accept Krogh's hypothesis: until this has been shown however it is necessary to withhold one's judgement.

Bibliography.


Schafer and Vincent Journ. Physiol. xxv. 1899.


Pohle Anatomy and Physiology of the Capillaries. New Haven 1922.

Pohle Pfluger's Archive. clxxxii 1920.


Burn Ibid. lvi 232. 1922.

NOTE.

In 1926 Florey and Carlton, (Proc. Roy. Soc. B. vol. 100 p. 30. observed the capillaries in a living cat, finding that they were contracted by the intra-venous injection of pituitrin and expanded by adrenalin.

This observation would appear to confirm Krogh's theory.
THE POSSIBLE FUNCTION OF THE PITUITARY IN PARTURITION.

It will be recollected that Sir James Simpson removed the spinal cords of sows from the first thoracic vertebra downwards and found that in those animals which survived the course of pregnancy was almost unaffected although the last foetus generally remained in the vagina.

In 1853 Brown Sequard had shown that contractions of the uterus were induced if carbon dioxide were allowed to accumulate in the blood.

In 1872 Oser and Sclechsiger stated that parturition can occur in animals after the division of the sympathetic nerves which pass to the uterus, but as Marshall remarks, "it is difficult to understand how this operation could have been made complete without interfering with the blood supply to that organ".

In 1867 Kehrer showed that an isolated uterus was capable of undergoing contractions if maintained at the temperature of the body and kept moist.

Thus long before anything was known concerning the functions of the pituitary there seemed to be good reason to suspect that the contractions of the uterus in normal circumstances and in parturition, were not entirely neurogenic.

In 1891 Spiegelberg put forward the hypothesis that parturition might normally be caused by the secretion by the foetus of autacoids into the maternal/
maternal blood.

The next advance was made in 1895 when Langley and Anderson showed that in most animals the uterus was excited by the administration of adrenalin.

In 1906 the first observation was made on the relationship between the pituitary and the uterus. Dale in that year found that when a very small quantity of an extract of the pituitary was added to the bath in which a cat's uterus was suspended the uterus immediately contracted violently. The effect was apparently exerted directly upon the muscle fibres, since it appeared with no less vigour in the presence of various derivatives of ergot.

In 1924 Marshall and Dixon published a description of some experiments which aimed at establishing the presence of oxytocin in the cerebro spinal fluid, and discovering the causes which led to its secretion.

Ovarian extracts were made from organs excised at various stages of the oestrus and reproductive cycles. These were injected into a dog and at intervals the dog's cerebro spinal fluid was tapped and assayed for oxytocin by the method which Dale and Laidlaw had described in 1912.

It was found that the addition of normal cerebro spinal fluid to the bath in which a virginal guinea pig uterus had been suspended produced no alteration in the tonicity of the uterine musculature. This/
This negative property of the fluid was unaffected by the injection via the femoral vein of extracts of such tissues as testis epididymis, pancreas etc.

Extracts of the ovary of the rabbit were then injected. (It is well to notice that these extracts had been boiled and that very large doses - about 20 cc. - were administered).

The effects from the ovary of an animal which had been pregnant for 8 days were negative; on the other hand the injection of extract from the ovary of an animal which was approaching the term of pregnancy was found, after a latent period of about thirty minutes to cause the appearance in the cerebro spinal fluid of easily detectable concentrations of the oxytocic principle.

The experiments were repeated with the ovaries of sows in various stages of oestrus; positive results being obtained in the stages of "coming on heat", "due on heat", and heat itself.

Two days after oestrus the results were still positive but with the lapse of another eight days all traces of the oxytocic principle had disappeared.

These results appear to indicate that the secretion of oxytocin is inhibited by the presence in the ovary of functional corpora lutea, and therefore indirectly, by the secretion by the pituitary of its kyogenic principle; on the other hand the oxytocin being present in the cerebro spinal fluid during the period of oestrus may conceivably/
conceivably be secreted under the influence of oestrin and hence indirectly of the oestrogenic principle of the anterior lobe. Thus in this aspect of its activity the posterior lobe may function under the complete domination of the anterior lobe.

Swale Vincent has criticised the findings of Marshall and Dixon on many grounds, some of which are rather inadequate. He says for example that since serum contains an oxytocic component, the effect of the uterus might have been produced by accidental contamination of the needle used for withdrawing the samples of cerebro spinal fluid, not realising that this possibility was no less strong in the case of the samples which proved to be inactive.

Dyke and Kraft however failed completely to confirm these results and until the experiment has been repeated it is as well to suspend one's judgement. It is necessary to emphasize the fact discovered by Cow that since the concentration of oxytocin is increased by extracts of the duodenum the effect is not specific for the ovary.

THE INFLUENCE OF THE PITUITARY ON THE THRESHOLD OF THE UTERUS TO OXYTOCIN.

One possible mechanism of parturition has been described; recent work has discovered others. In 1926 Knaus made an experimental investigation on the threshold of the uterus of the rabbit to oxytocin during the various stages of pregnancy. (In previous/
previous pages we have employed the word "oxytocin" simply as a convenient abbreviation for the "oxytocic principle of the pituitary". By this time however it had been possible to separate this from the pressor principle. Any possible effect of the hypertrophy of the myometrium was eliminated by the use of a sterilised horn containing no foetuses. He found that abortion could only be produced by oxytocin during the last few days of pregnancy. In 1927 the work was extended and it was shown that for the first eighteen days of pregnancy the uterus was almost insensitive to oxytocin; the threshold falling slowly until the normal time of parturition was almost at hand when the decrease was very rapid.

Several experiments have demonstrated the existence of a synergism between oestrin and oxytocin. Miura in 1926 showed that the minimum quantity of oxytocin necessary to produce abortion was greatly lowered by the previous injection of oestrin. Bourne and Burn two years later confirmed this result for the isolated uterus proving that the amplitude of the contraction caused by oxytocin was enormously increased if oestrin had previously been added to the bath.

Since the secretion of kythin and of oestrin are believed to be conditioned by the secretion of corresponding substances from the anterior lobe of/
A. Possible Factors in the Reduced Growth of Ponderosa

B. Effect of Height and Diameter on the Height of Ponderosa

Key:
- Ao = Age
- N = Number of Ponderosa

Legend:
- Line of Ao
- Line of N
- Line of Ponderosa

Note: The diagrams illustrate the relationship between age, number, and ponderosa growth.
of the pituitary the sensitivity of the uterus to oxytocin appears to be controlled in part by this gland.

Prolan A both stimulates the production of, and enhances the sensitivity of the uterus to, oxytocin; Prolan B reverses both effects.

According to this view parturition is due to an increase in the concentration of Prolan A and a decrease in the concentration of the other gonadotrope factor, and therefore to a functional change in the cells of the anterior lobe of the pituitary. If we accept the interpretation that current

Note. See a recent paper in the Quart. Journ. Expt. Physiol. by Robson for a confirmation of this view.
Bibliography.


5) Spiegelberg (Marshall p. 374.)


8) Marshall and Dixon ibid. lix. 1924.


10) Swale Vincent Physiol. Reviews. vii. 1927


13) Knaus. ibid. lxi. 1926.

14) Miura. Arch f. exp. Pharm. und Path. cxxiv. 1927

15) Bourne and Burn Lancet. ccxv 1928
THE POSTERIOR LOBE AND THE KIDNEY.

The best authenticated function of the posterior lobe is undoubtedly the influence which it has been proved to exert upon the activity of the secretory cells in the kidney. Six years ago a brilliant series of experiments, carried out in Starling's laboratory, placed the truth of this matter beyond all possibility of doubt.

The history of this division of the subject appears to date back to 1892, when Vassale and Sachi observed that the extirpation of the pituitary body was almost invariably followed by the secretion of enormous volumes of very dilute urine.

In 1901, Magnus and Scafer noticed that, although the intravenous injection of an extract of the posterior lobe produced contraction of most abdominal viscera, (such as the intestines and spleen) and of the limbs, the effect on the kidney was predominantly one of dilatation. Moreover this dilation was accompanied and followed by a clearly marked diuresis. Succeeding dilinjections produced a less smaller dilatation and a shorter diuresis.

The autacoid inducing these effects was confined to the posterior lobe and appeared to be insoluble in absolute alcohol, thus resembling the pressor principle.

In some cases the second injection
of a crude extract was followed by a drop in the blood-pressure so decided that, although the volume of the kidney was almost unaffected in spite of the dilation of its vessels; yet even then the diuresis made itself apparent.

Magnus and Schafer argued therefore that the autacoid acted directly upon the kidney cells, and this conclusion was confirmed by the fact that the diuresis persisted in the presence of atropine.

In 1909 Schafer obtained diuresis as the result of grafts of pituitary. Recent work has made it probable that these grafts were rather of the nature of implants, undergoing autolysis, and discharging the diuretic autacoid into the bloodstream.

The results of Magnus and Schafer have been confirmed by many workers such as H. Schafer and Herring (1908) Hoskins and Means (1913) King and Stoland (1913) and Stoland and Korb (1921). Moreover the greater number of these observers have noticed that the magnitude of the diuresis is extremely irregularly correlated with the concomitant alteration of the vascular conditions under which the kidney works, and have therefore confirmed the conclusion of Magnus and Schafer, that the autacoid acts directly upon the renal cells.
Knowlton and Silverman (1918) however studied the oxygen consumption of the kidney under pituitary diuresis, finding that it was never raised and often lowered. They concluded that the diuresis was brought about not by the action of the autacoid on the cells of the tubules but by indirectly by its effect on the general blood pressure and on the velocity of the blood in the renal vessels. The arguments upon which this argument was founded are almost certainly fallacious. The oxygen consumption of the kidney depends solely upon two factors, the volume of urine secreted in unit time, and the difference in osmotic pressure between the blood and the urine. Although more urine is secreted under the influence of extract of pituitary, this urine is considerably more dilute than it was in the period before the injection. One effect therefore may balance the other. On Cushny's theory one might suppose that the action of the extract was to suspend the activity of the cells of the tubules; thus the urine would come to resemble a dilute colloid-free glomerular transudate, and the oxygen consumption of the active part of the kidney, the cells lining the tubules, would fall.

X

Not long after the original experiments of Magnus and Schafer it was recognised that extract of posterior lobe only produced a diuresis when injected directly into a vein. If the material were injected subcutaneously, or intramuscularly, or given by the mouth, the flow of urine generally ceased, or almost ceased, for a considerable time. By 1913 indeed the gland was being employed in medicine for the control of diabetes insipidus.

Magnus and Schafer had noticed that an antidiuretic substance was present in the gland and could be extracted from it with absolute alcohol.

In 1914 Garnier and Schulmann injected into the subcutaneous tissues of rabbits aqueous extracts of the posterior lobe finding that the flow of urine was diminished for two days, in some cases falling as low as 10 cc. for the first twenty-four hours. On the third day however polyuria set in and the amount rose to as much as 500 cc.

Many experiments have been made on the effect of the extract on the diuresis resulting from the ingestion of large volumes of water. It should be mentioned that no matter how much water is absorbed by the system through the walls of the alimentary canal, hydramia is rarely produced. The water is usually absorbed by the tissues, and particularly by the liver as
as it diffuses into the blood. After a period of latency however, the tissues give up the water which is removed from the body by the kidneys and the sweat-glands.

The effect of pituitary on this diuresis has been carefully investigated by Craig. This observer confirmed the early work by showing that the water (really saline) was eliminated more quickly if an extract of posterior lobe were injected into the blood. On the other hand, with subcutaneous injection, the water might be retained in the tissues for as long as three hours. During the period of latency the volume of the blood (as measured by the percentage of haemoglobin) was never diminished, and usually increased; this suggests that the autacoid does not assist in the transference of fluid from the blood to the tissues, but has rather the reverse effect. One must therefore believe that the oligo-urea results from the direct action of the extract on the kidney.

In 1925 Starling and Verney introduced the technique of perfusing an isolated kidney by means of a heart-lung preparation. They found that, provided certain precautions were taken, (such as allowing the heart-lung preparation to function by itself for some time before the kidney was introduced; thus ensuring the elimination by
absorption of the toxic substances present in defibrinated blood) the flow of urine began within fifteen minutes and continued indefinitely. Although care was taken that the composition of the blood circulating in the system should not differ to widely from that to which the kidneys were accustomed, the urine was never normal. In the first place, it was secreted in enormous volume and chlorides were almost absent. Starling and Verney concluded that this phenomenon was due not to any injury to the kidney in the operative treatment incidental to the experiment, but rather to the absence from the defibrinated blood of certain autacoids essential to the normal functioning of the organ. Turning to the earlier experiments of Craig and others they decided to attempt to discover whether pituitrin might not be one of these substances.

When an extract of the posterior lobe was added to the circuit the amount of urine immediately fell and the concentration of chloride became almost normal.

This experiment is analogous to some of Krogh's work on the maintenance of the tonicity of the capillaries and is open to the same objections. In the same year however Verney prepared a similar HLK circulation and found a confirmed the earlier results obtained earlier in the same year. If now
the lower limbs of a dog were intercalated, the composition of the urine urine was unaffected. When however there was added to this, or to the original system, a head and neck, the composition of the urine was rapidly restored to normal. The pituitary body was now exposed and destroyed; the urine again increased in amount and the concentration of chlorides fell.

After a consideration of these experiments one would need to be a pretty hardy sceptic to doubt that the pituitary body was normally concerned in the regulation of the volume of the urine and of its chloride-content.

Similar observations were made for phosphates by Brull, Eicholtz, and Robison, and by Brull and Eicholtz.

The first group found that after removal of the pituitary body the kidneys of an animal lost their power of secreting the inorganic phosphate of the blood; although, if esters of phosphorus were injected into the circulation, they were immediately excreted in inorganic form. (It will be remembered that Robison had, two years previously, discovered the existence in the kidney of a phosphatase enzyme.)

Brull and Eicholtz a little later made a careful investigation of the first of these effects. Polyuria and a fall in phosphates and chlorides
could readily be induced by injury either to
the pituitary body or to the tuber cinereum. It
occurred after division of the renal nerves.
Drainage of the cerebrospinal fluid in the normal
animal was apparently without effect on the
quantity or composition of the urine. Phosphorus
and Chloride did not necessarily decline
simultaneously.

These observations suggest that the
influence of the tuber cinereum is not wholly nervous
since lesions in this region affect even the
denervated kidney, and that the material secreted
by the pituitary gland is carried away by media
other than the cerebrospinal fluid.

In 1930 Papa and Fielding described
in the human subject, the existence of a system
of veins running from all parts of the gland
into the tuber and hypothalamus. Injury to the

\[ \text{tuber cinereum may impede the absorption} \]
by the blood of the autacoids secreted by the
posterior lobe. If this suggestion could be
confirmed one of the longest and most acrimonious
disputes in the history of endocrinology might
be terminated.

It appears to be likely from recent
biochemical work that the diuretic and antidiuretic
effects of extracts of the gland are due to one
substance. Craig showed that the diuretic effect
persists after complete extraction with absolute
alcohol; in the purest preparations which have as yet been obtained the two effects appear to persist. It is more than possible that we have here an example of what has been called "Inverse Hormone Action". Adrenalin in smaller doses than are generally administered in physiological experiments is found to produce a fall in the blood pressure, while by the continued injection of very small quantities of insulin certain workers have been able to induce a considerable rise in the concentration of the blood-sugar.

There is no reason to suppose that there are ever present in the blood quantities of the autacoid sufficient to invoke diuresis, since lesions of the gland are hardly ever accompanied by oligouria. But when the same autacoid is introduced into the blood in concentrations higher than normal yet lower than would be sufficient to induce diuresis the flow of urine is hindered. This may be effected by intramuscular injections from which the principle is absorbed continuously yet in very small quantities.
<table>
<thead>
<tr>
<th></th>
<th>Name of Author(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Schafer and Herring</td>
<td>ibid. cxcix.</td>
</tr>
<tr>
<td>7</td>
<td>Stoland and Korb</td>
<td>ibid. lv.</td>
</tr>
<tr>
<td>8</td>
<td>Knowlton and Silverman</td>
<td>ibid. xlvii.</td>
</tr>
<tr>
<td>9</td>
<td>Garnier and Schulmann</td>
<td>C. R. Soc. Biol. lxxvii.</td>
</tr>
<tr>
<td>12</td>
<td>Brull Eicholtz and Robison.</td>
<td>ibid. xcix.</td>
</tr>
<tr>
<td>13</td>
<td>Brull and Eicholtz</td>
<td>ibid. xcix, 70.</td>
</tr>
</tbody>
</table>
CHAPTER XVI.

THE FUNCTION OF THE PITUITARY IN AMPHIBIAN ONTOGENY.

A. Anura.

The development of the pituitary body in amphibians was first adequately described by Gotte in 1875. A detailed account of the process would be irrelevant to this essay and it is sufficient to say that in this Class the buccal pouch is replaced by a solid cord of cells which extends upwards from the roof of the pharynx to the brain. When a tadpole of length 3.5-4 mm. is anaesthetized and placed under the dissecting microscope this rudiment is readily visible as a darker area between the stomodaeum and the developing fore-brain; in this position it is obviously very conveniently situated for surgical interference, and the study of the effects produced by its removal has led to a considerable increase in our knowledge of the functions of the gland in ontogeny.

In 1914 Adler, working on the tadpoles of Rana temporaria, attempted to destroy the hypophyseal anlage by means of a small cautery; he was successful only in 3 out of a total of 1200 animals. Two years later F. E. Smith and Bennet Allen were able to devise a much more satisfactory technique, finding that it was possible to cut out the strand of cells by means of very careful dissection with very fine instruments, an operation which was accompanied
only by a comparatively low mortality.

The results of these experiments were remarkably uniform. A short time after the operation the tadpole lost its characteristic black colouration and assumed a white almost metallic lustre. On microscopic examination of the integument it was observed that the melanophores in the epidermis had contracted and were almost spherical in shape, while, conversely, the deeper-lying xantholeucophores had extended all their processes.

On examination of the body some time after the operation the parathyroids, thyroids, and interrenal glands were seen to be underdeveloped; the thyroid having its vesicles distended with colloid.

These animals grew much more slowly than the controls, and, beyond an occasional slight protrusion of a posterior limb-bud they completely failed to metamorphose.

It must be mentioned here that at the time of these experiments it was already suspected that the pituitary was not the only gland concerned with metamorphoses, for Gudernatsch had shown as early as 1912 that the process was accelerated by the administration of thyroid to normal tadpoles; a few years later the thyroid was successfully extirpated without any untoward consequences but a decrease in the growth
rate and a complete failure to metamorphose.

There appeared to be good reason to suppose that the pituitary body might normally stimulate the thyroid to an increased activity, which was the direct cause of metamorphosis. According to this hypothesis it should be possible to induce metamorphosis in hypophysectomised tadpoles by the regular administration of anterior pituitary, or by means of grafts.

The effect of feeding was first investigated with the usual ambiguous results.

Gudernatsch (1912) working with intact tadpoles found that the time of their metamorphosis was not advanced appreciably by this treatment. P.E. Smith (1916) found that feeding the anterior lobe tended to increase the growth-rate in both intact and hypophysectomised animals. Hoskins and Hoskins (1920) obtained precocious metamorphosis by feeding preparations containing iodine but, later in the year Smith and Chaney showed that this effect was due principally to the iodine.

Experiments on feeding are most unsatisfactory since one is really testing the resistance of the autacoid to the enzymes in the alimentary canal and this is far better done in vitro. Further the nature of the intestinal juices varies from individual to individual, and in amphibians metamorphosis is accompanied by a reorganisation of the gut in preparation for
a carnivorous mode of life, which may render
digestion impossible for several days.

The effects of grafts was then investigated,
and Allen (1920) showed that autoplantation
(in some cases) was followed by metamorphosis. His
findings have subsequently been confirmed by many
workers.

The evidence from extracts is very
satisfactory, positive results having been obtained
by many workers, both in this country, and in America.
Smith (1920) found that the retardation of growth
caused by hypophsectomy might be abolished by
the injection at regular intervals of extracts
prepared from the anterior lobe. Two years later,
working with I. P. Smith he found the activity of
the thyroid (as deduced from histological appearances)
was increased by this treatment. In 1924 the same
observers discovered that metamorphosis might also
be induced by means of grafts.

In the same year Smith and Smith
showed that in mid-sagittal sections through
the bovine anterior lobe there could be distinguished
a core or central area composed chiefly of basophil
cells, surrounded by a cortex that was mainly
eosinophil in nature.

While extracts from the central
basophil area tended to delay metamorphosis and to
accelerate growth, extracts from the anterior lobe
possessed the reverse effect, metamorphosis being remarkable accelerated.

In 1924 the effects of extracts on the metamorphosis of hypophysectomised tadpoles were reported by Evans, using the method of alkaline or saline decoction which has been described in connection with the growth of mammals. Metamorphosis was immediately precipitated, and was accompanied by the hyperplasia of the thyroid, interrenal, and the other organs which are adversely affected by hypophysectomy.

These results have been confirmed by many writers, among whom we may notice Spaul (1923 and 1930) using extracts prepared with acetic acid. Spaul finds that the metamorphic principle is destroyed by both peptic and tryptic hydrolysis.

Since the extirpation of the pituitary inhibits metamorphosis and growth and since the loss may be remedied by extracts or implants of the gland, we must conclude that normally the activity of the pituitary secretes into the body-fluids, autacoids which are indispensable for growth and metamorphosis. It is inadvisable to say that the pituitary controls these functions since the presence of other glands is apparently necessary.

In reading accounts of experiments on tadpoles one should never forget that the human embryo possesses at one period of its history both a "tail" and gill-pouches, and therefore does
B. Pituitary and Thyroid in the Neotenous Larvae. The axolotls form a geographical race of the Mexican salamander, being distinguished by the fact that under normal circumstances they never undergo metamorphosis, but persist in the tadpole form until after the time of sexual maturity. The term "neotenus", it must be understood, is not strictly correct, since other animals within the same species metamorphose with great regularity; the axolotle indeed is really a pathological specimen, and suffers, as we shall see, from a hereditary endocrine deficiency.

A long time ago it was found that some animals in captivity in the Jardin des Plantes at Paris suddenly lost their dorsal fins and external gills, together with many of the other larval characteristics. It was noticed that the water in the tanks in which the animals were kept had been allowed to fall very low, and the naturalists of the period, considered that the metamorphosis was an adaption to a drier environment. When the matter was tested experimentally however it was found to be quite impossible to produce metamorphic symptoms other than the involution of the tail dorsal fin, and no further work was done for forty years.

In 1919 Julian Huxley read the report of Gudernatch on the reaction of tadpoles to
feeding with thyroid tablets, and decided to repeat the experiment with the axolotl. In every case metamorphosis was rapid and complete.

Shortly afterwdrs Uhlenhuth in America found that gigantism could readily be induced in Mexican salamanders which had already undergone metamorphosis, by feeding with bovine anterior lobe. In 1922 Hogben and Huxley obtained negative results either in respect of growth or of metamorphosis in axolotls. Either therefore the metamorphosi but in the next year Hogben found that the injection of extracts of commercial powdered anterior lobe brought about complete metamorphosis almost as rapidly as thyroid tablets. His results were afterwards confirmed by Spaul. Curiously enough, if the doses of pituitary were sufficiently massive metamorphosis will occur in the entire absence of the thyroid gland, and one might have expected that this organ would therefore be quite functionless and inactive, in axolotls.

This view was disproved by Swingle in 1923. Swingle showed that if a fragment of the thyroid of the axolotl were implanted in a hypophysectomised tadpole that tadpole rapidly underwent metamorphosis. Apparently therefore when the time of metamorphosis approaches (in these salamanders which do metamorphose) some agency must either sensitise the tissues to the action of the thyroid hormone
or cause the release of this hormone from the thyroid gland. Moreover it would be reasonable to suppose, as a first hypothesis, that this agency is some autacoid present in the anterior lobe of the pituitary.

In 1925 Greenwood, working in Edinburgh, compared the growth-rates of intact and of hypophysectomised axolots, finding that they were almost identical. Contrasting this results with the experiments of Smith on rats we find good reason to believe that the anterior lobe of axolots is at least as regards growth, functionless. Apparently the hypophysis of the axolotl endures one brief period of activity in which it incites the thyroid a precipitating metamorphosis; and then relapses into quiescence.

This view would appear to be confirmed by the work of Uhlenhuth and Schwartzbach who studied the histological changes in the thyroid of an animal undergoing metamorphosis under the influence of extracts of anterior lobe, finding that they differed but little from the changes in normal metamorphosis.

Yet, since metamorphosis may be induced in the absence of the thyroid it is evident that to some extent at least the pituitary must be able to replace this gland.
However in considering the possibility that the metamorphic factor in the anterior lobe is not predominantly thyreotropic one must bear in mind the fact that the thyroid might not have been removed completely or might have regenerated (All urodèles amphibians possess remarkable powers of regeneration.); on the other hand neither Hogben nor Spaul could find any traces of pes this tissue when the animal had been killed after metamorphosis.

Crew has lately written on an interesting corollary of these experiments. He found that both the kyogenic and the oestrogenic factors in the anterior lobe were destroyed by protracted boiling. Evans had made the same statement regarding the growth factor. Yet the metamorphic extracts of both Uhlenhuth and Spaul had been prepared by boiling the material with acetic acid for at least ten minutes, a treatment which would destroy any other autacoid which has yet been discovered in the anterior lobe.

The pituitary gland appears therefore to contain a specific metamorphic factor. What function this factor plays in the early development of mammalian ova we can as yet only speculate. It is well to remember however that at one stage in his life man possesses
both a "tail" and gill-pouches and may therefore be said with scarcely any exaggeration to metamorphose.
Bibliography.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Journal/Book</th>
<th>Edition/Volume</th>
<th>Year(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adler</td>
<td>Arch. f. Entwick.</td>
<td>xxxix,21</td>
<td>1914</td>
</tr>
<tr>
<td></td>
<td>Anat. Rec.</td>
<td>xv, 352</td>
<td></td>
</tr>
<tr>
<td>Smith</td>
<td>ibid. xi, 57.</td>
<td></td>
<td>1916</td>
</tr>
<tr>
<td>Gudernatsch</td>
<td>Arch. f. Entwick.</td>
<td>xxxv 457</td>
<td>1912</td>
</tr>
<tr>
<td>Hoskins and Hoskins</td>
<td>Endocrinology</td>
<td>iv,1</td>
<td>1923</td>
</tr>
<tr>
<td>Allen</td>
<td>Science</td>
<td>liii, 247</td>
<td>1920</td>
</tr>
<tr>
<td>Smith and Smith</td>
<td>Anat. Rec.</td>
<td>xxv,150</td>
<td>223 1923</td>
</tr>
<tr>
<td>Evans</td>
<td>Harvey Lectures</td>
<td></td>
<td>1924</td>
</tr>
<tr>
<td>Spaul</td>
<td>See next section.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huxley</td>
<td>Nature</td>
<td></td>
<td>1919</td>
</tr>
<tr>
<td>Uhlenhuth</td>
<td>Journ. Gen. Physiol.</td>
<td>iii,347</td>
<td>1920</td>
</tr>
<tr>
<td></td>
<td>ibid, iv,1922.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hogben and Huxley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hogben</td>
<td>ibid. lxxxxiv, 204,1923.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ibid. ii,33, 1924.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ibid. ii,427, 1925.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ibid. v,106, 1927</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ibid. v,212, 1928</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ibid. vii,49, 1930</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &amp; Howes</td>
<td>ibid. vii,154, 1930.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Swingle

Greenwood

Uhlenhuth & Schwartzbach
ibid, v, 1. 1927.

Crew & Wiesner
Conclusion.
It might be of interest if we were here to review briefly some of the most important lessons that may be learned from a study of the history of the gland.

Possibly the first of these is the extreme complexity of the endocrine system. One gland, for example, secretes into the blood-stream small quantities of a substance which modifies the activities of a second gland, which, in its turn, is able to influence yet another organ; moreover, it appears that the intermediary gland is able to influence the secretion of the hormone by which it is itself stimulated. Thus Crew and Mirskaya, in a recent paper, write:

"In the case of the normal immature mouse, experiment has shown that the physiological activity of the anterior pituitary invokes ovarian activity which leads to mating, and which, in turn provokes further pituitary activity, which incites further ovarian activity which exerts the stimulus to uterine development."

The endocrine system appears to form what has been called, "a closed system of interrelated factors," and its analysis will prove to be a most formidable task.
Claude Bernard was the first to recognise the physiological importance of the internal environment which the animal created from the products of its own metabolic processes; he showed how, in the course of time, "le milieu organique se spécifie et s'isole en quelque sorte de plus en plus du milieu ambiant"; and now from a study of the pituitary body and of organs like it, we may understand how, once the pH., osmotic tension, salt-balance &c., of the body-fluids had become approximately constant, it became possible for an animal to construct complex and accurate methods of integrating its functions by the use of infinitesimal quantities of specific chemical substances.

In Invertebrates where the means of maintaining even physical and chemical conditions in the body-fluids are primitive and unsuccessful, systems of endocrine organs rivalling in complexity those of the vertebrates have never been discovered, and it is noteworthy that the hormone which is most widely distributed in these animals appears to be adrenalin, which we now consider to be the basis of a certain type of muscular activity and not necessarily a true hormone at all.

We may notice for example that the secondary sexual characters of arthropods have been found by Meisenheimer and others to be quite
of their reproductive organs, and that C.M. Child has recently met with striking success in his attempts to explain the physiology of morphogenesis in Planaria on the basis of a simple kind of nervous conduction.

In these forms each cell must be regarded as differentiating under the direct control of its own genotype, and of the few relatively constant features of its environment (among which we may reckon the "metabolic gradient"). With the introduction of organs like the pituitary body the cell comes to be affected more and more by the fluid in its neighbourhood, and the number possible of phenotypes is strikingly increased since the body-fluids may, even in mammals, be modified experimentally, or by normal environmental factors. Consider the case of two genetically identical axolotls; the stimulus to metamorphosis is not evidently a genetic factor since either animal will metamorphose when exhibited to certain conditions. The same is true of milk-production in cows and of fertility in almost any animal.

One can easily realise that such an intricate and perfect system of humoral integration may well endow the mammalian organism with a degree of functional plasticity, which by enabling it to respond more rapidly and accurately
to the changes of a fluctuating environment, would heighten its biological efficiency and increase the chances of its survival. A lower and simpler animal might, in similar circumstances require to wait for many centuries before a suitable gene-mutation offered itself, and in the event of the environmental factor reverting to its original condition, would find itself at a considerable disadvantage.

We have now come to the end of our survey, and the reader might be advised, at this point to turn back and glance at the passage written exactly half a century ago in which Balfour declares that, although the pituitary body was probably employed by the early Chordates to increase the delicacy of their perceptions. It is more than likely that, if the present rate of progress continue, our own theories will, in fifty years time be considered almost as inadequate as Balfour's are today.
<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Edition</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Sharpey-Schafer</td>
<td>The Endocrine Organs vol 2.</td>
<td>London 1926.</td>
</tr>
<tr>
<td>c Blair Bell</td>
<td>The Pituitary.</td>
<td>London 1929.</td>
</tr>
<tr>
<td>f Parkes</td>
<td>The Internal Secretions of the Ovary.</td>
<td>London 1929.</td>
</tr>
<tr>
<td>g Lipschutz</td>
<td>The Internal Secretions of the Sex Glands.</td>
<td>Cambridge 1924.</td>
</tr>
<tr>
<td>h Hogben</td>
<td>The Pigmentary Effector System.</td>
<td>Edinburgh 1924.</td>
</tr>
<tr>
<td>i Marshall</td>
<td>The Comparative Physiology of Internal Secretion.</td>
<td>Cambridge 1928.</td>
</tr>
<tr>
<td>k Brambell</td>
<td>The Development of Sex in Vertebrates.</td>
<td>London 1930.</td>
</tr>
</tbody>
</table>