THE PART PLAYED BY THE HYPOTHALAMUS AND
PITUITARY GLAND IN THE CONTROL OF RENAL
EXCRETION.

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INTRODUCTION

The problem of the function and control of the kidney is one which has long occupied the minds of physiologists. The number of theories and the amount of experimental work, often conflicting, are testimony to the difficulties and complications involved in making any clear and positive definition of the working of the organ.

The reasons why this should be so are numerous. In the first place, the kidney is an organ with many vital duties to perform; the removal of waste products from the blood stream, the precise regulation of the electrolytic content of the blood, and the ultimate control of the water balance of the whole animal body, are three vital tasks which the kidney is called upon constantly to perform, and yet at any given moment of investigation, body conditions may demand that one or other of these functions is of primary importance. The kidney must then deal with that primary necessity in a sense at the expense, for the time being, of the other vital, but not so urgent, calls upon it.

Such circumstances do not make the interpretation of experimental results any easier, nor do they assist the formulation of a general theory of control.

And/
And in the second place, experimental work on the kidney has considerable technical difficulties. The organ is inaccessible in most species, and especially so in the warm-blooded animals, and in all is most sensitive to handling and to very minute changes in its environment. It is the kidney's duty to be sensitive to changes in the environment, and the extreme delicacy with which it can carry out its many functions depends on this very fact. Thus we find that much of the experimental work has to be done by indirect means, and the difficulty of interpretation of results thereby becomes the greater.

When, in addition, one considers the problems involved in the simultaneous study of the nervous structures at the base of the brain, the reasons why so much experimental work is conflicting, and so many results anomalous, become very easy to appreciate.

In reading over an account of the functions of the pituitary gland, one cannot but feel that, as yet, some basic and possibly fundamental facts have not been classified. This feeling is especially pronounced in considering the inter-relations of the various ductless glands. If the precise function of the kidney is rendered difficult of definition by complexity and experimental inaccessibility, that of the pituitary/
pituitary gland and of the hypothalamic area of the brain must be many times more so.

This essay consists of an attempt to assemble and to correlate the chief assured facts concerning these organs in their relationship. It may justifiably be said that few enough of the chief facts are indeed assured. Where a statement has been made regarding any function as relationship between the kidney and the pituitary body, or the associated hypothalamic structures, the experimental evidence of authority will, as far as possible, be summarised; and in many cases evidence will be presented from work in which the author has personally assisted. It is most noticeable in the literature of this field how many admirable pieces of experimental work have later been suspect because, at the time, some investigation was not carried out. Such supplementary investigation may well not have been contemplated as necessary at the time of experiment, and yet as the field of work grows, and degree of accuracy extends, much of the previous work has been realised to be debatable because some point was not examined at the time.

The scope and relative delicacy of the work of Dr. Mary Pickford, from whose unpublished experimental work all the following personal investigations are taken, have sought to leave no such possible loophole, at any rate in the state of present knowledge and experience of the field of work.
THE GENERAL PROBLEM OF KIDNEY OUTPUT AND CONTROL.

The methods whereby the kidney elaborates urine from the circulating blood have themselves been the object of much experimental work, and the problem is not yet to be regarded as conclusively settled. It is valuable to summarise and consider the main methods of experimental evidence that have shed the greatest light on the problem.

One method of direct investigation has been that carried out by Richards and his co-workers, who have developed such a remarkable technique of microdissection and of colorimetric micro-analysis that fluid actually withdrawn from the various functional parts of the renal unit has been analysed. This work has been of the very greatest value in establishing one of the important concepts, namely that the glomerulus and its capsule must be regarded as a mechanical ultra-filter for the pressure filtration of blood. Their technique is, unfortunately for our knowledge, not applicable to the mammalian kidney, which is more inaccessible and more sensitive to external environmental conditions than the frog's kidney on which the majority of their work was carried out.

The second, or indirect method, which has been largely employed in the investigation of mammalian renal excretion, depends on a mathematical concept introduced/
introduced by Möller, McIntosh and Van Slyke, in a paper published in 1928, which demonstrates a relationship between the same chemical substance in the bloodstream and in the urine being at that time elaborated. Their statement of the concept was published in respect of the substance urea only, but has been used to express the behaviour of numerous other chemicals naturally occurring or artificially supplied, and will be stated in general terms as follows.

Make simultaneous blood and urine analyses of a substance x. Then the quantity of x in one minute's flow of urine, divided by the quantity of x in each c.c. of blood gives a Value to which they gave the name of Renal Clearance, and which can be interpreted in either of two ways. The Renal Clearance may represent a virtual volume of blood which one minute's flow of urine could have completely freed of x. That this volume is virtual only is obvious, but the concept is of value. Alternatively, the Renal Clearance may be taken as representing the volume of blood necessary to furnish the amount of x which it took one minute to excrete.

The value of this concept of renal clearance lies in the following circumstances. No substance can have a higher renal clearance under any circumstances than /
than the glomerular blood flow, which is an absolute upper limit, in real life never nearly attained.

If several substances, non-toxic and relatively non-diffusible, are found to have the same clearance figures under the same circumstances, and that figure a high one, and no other substance can be found which in the same animal and under the same conditions has a higher clearance figure, a good measure of glomerular filtration has been achieved (Smith, 1937).

The last paragraph requires the addition that if another substance is found to have a very high clearance, and is suspected to have a higher value than glomerular filtration, it is probable that that substance is undergoing actual tubular excretion, and is being actively added to the urine in the tubules.

Smith (1937) has summarised the evidence resulting from many clearance investigations on many species of animal, and with numerous different chemicals, and puts forward the following conclusions regarding the method of urine production of the kidney.

1) That the action of the glomerular tuft and its capsule is that of a mechanical micro-filter only.

2) That large volumes of water, and of substances necessary for the organism at the time, are reabsorbed into the blood-stream from the renal tubules, thereby concentrating the dilute blood-filtrate into true urine/
3) That active excretion into the tubules can and does occur in certain circumstances.

4) That considerable species differences exist in behaviour, and results on a given animal do not necessarily prove true in an allied species.

Detailed consideration of the above facts will be taken up as the necessity arises, but they afford a working basis for consideration of the control of urine flow, and are represented in schematic form.

There are five main influences that can be brought into action in the body so as to alter the rate of urine output, and it is desirable that they be each considered briefly. Although this essay is concerned largely with two of the factors, which will be shown to be the two main factors, so much early experimental work has been confused and distorted by other, if minor, causes of variation, that all possible causes will be referred to in the later pages.

The possible media of variation of urine flow are, then, as follows:

1) The control of output from time to time by relatively simple physico-chemical changes in the circulating blood. In the simplest possible terms - that blood dilution should cause excess fluid excretion, and that excess of a filterable substance in the plasma should/
should cause that substance itself to be excreted at a high rate. At first sight, such a mechanism seems the reasonable way in which the kidney might work, and before the realisation of the facts concerning glomerular filtration, urine variation was assumed to be caused very largely on some principle of this kind. Modern knowledge of the osmotic gradients existing in the various functional parts of the nephron has shown that the human variations cannot be attributed to such a mechanism. The literature is involved, and the problems not easily stated in simple terms, but the few selected points of evidence may be of value.

Such a control cannot account for the known existence of the "threshold substance," which, up to a pre-set value, are reabsorbed in toto from the glomerular filtrate, and above that value are excreted in the resultant urine. In ideal man, glomerular filtration is about 120 c.c. per minute, and the range of urine flow is between 20 c.c. per minute and 0.5 c.c. per minute. Actual mensuration of the physico-chemical composition of healthy blood cannot allow of such varying figures by osmotic gradients alone. It is worth noting here that blood dilution to the point of haemolysis may occur without diuresis, owing to excess action of one of the other methods of kidney control.

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2) The urine output might be varied by a pressure mechanism acting on the glomerular filter; either a generalised rise of blood pressure, or a local glomerular rise of pressure by constriction of the efferent arterioles. A great deal of experimental work has conclusively shown that there is no consistent relationship between blood flow through the kidney and urine output from that kidney. A renal arterial blood pressure adequate to provide a head of pressure greater than that of the protein content of the blood is essential if filtrate is to escape from the glomerular capillaries, but above that the effects of blood pressure changes are inconstant - they differ, for example, in the normal and in the anaesthetised animal - and not great enough to account for the urine flows obtained.

Shannon (1936) and Chasis (1937) state that in the rabbit and in amphibia renal filtration variation has in fact a considerable effect on urine flow, but they deny that blood flow is the regulating mechanism in the dog and man. Blood flow variation is a combined function of increased pressure and the opening up of unused glomerular units, and is used as a term to cover mechanical circulating kidney changes. The results on amphibian preparations are open to a teleological explanation; those on the rabbit can only/
only be noted as an example of the species differences in kidney function, and it is well realised that the vasomotor arrangement of the rabbit is a peculiarly unstable one. The results on dog and man are borne out by many other workers, and it may safely be said that circulatory variations in the sense of blood flow cannot account for the range and nature of the urine variation.

3) The kidney possesses a fairly rich sympathetic nerve supply from the lower dorsal and upper lumbar segments of the chain. Several very clear cut experiments have disposed of the possibility of this being the regulating agency of urine output. Although a denervated kidney tends to show a relative diuresis, it is a small one, and attributable to vasomotor changes (Marshall and Crane, 1922). An implanted kidney, without possibility of nerve supply, works normally. Klisiecki et al (1933) show conclusively the essential parallelism between a normal and a denervated kidney in the unanaesthetised and healthy dog, and state as a result of many varied tests that the renal nerves play no part in water diuresis. The denervated kidney responds simultaneously and correspondingly with the normal under all conditions influencing urine flow variation.

4)
4) There remains only one further mechanism which has access to the renal tissue and that is a humoral one. As this essay is concerned with this hormonal control, the evidence and discussion will be dealt with immediately in detail. In order, however, that the list of control mechanisms may be related to real life, it is necessary to include one more, of a more indirect nature.

5) The possibility of control of the kidney output by higher nervous centres. That this occurs is very evident: to mention a few brief examples, the normal effect of sleep and of anaesthesia, the establishment of conditioned reflexes with regard to diuresis, and the effects of emotion upon urine output are sufficiently indicative that higher centres do have a profound effect upon the kidney. The thesis of this essay is that they do so by influencing the discharge of one or more hormones from the pituitary gland, and the question will now be reviewed in detail.
HISTORICAL SURVEY.

The earliest planned work on the problem of the possible hormonal action of pituitary extracts was carried out at the beginning of the century by Magnus, Schaefer and Herring. It is perhaps unfortunate that they should have hit precisely on one of the problems for which as yet very little explanation can be given. Employing an acute technique on anaesthetised animals, they reported that posterior lobe extracts had a diuretic effect. Anaesthesia is probably the most confusing single factor in renal investigation, and it appears in the light of recent work that while their experimental results were correct, their interpretation thereof was completely false. The question of the effect of anaesthesia and some conjecture as to its mode of action will be discussed in detail later, but it can be said here, subject to later proof, that anaesthesia modifies the specific effect of pituitary extract upon the kidney and allows the other substances in the gland extract to have side effects which do in fact result in a rather inconstant and temporary diuresis, due, as we now know, to vasomotor changes in the renal blood supply. The finding of these reliable and accurate workers that pituitary extract is diuretic in action confused the field of investigation for the next ten years.

In/
In 1913 Van der Velden and Farini, working independently on the clinical problem of Diabetes Insipidus, put forward clinical proof that extracts from the neural lobe of the hypophysis are anti-diuretic in action, and can successfully control the diuresis in that condition. This was an important discovery, and it is worth bearing in mind when experimental laboratory findings are being considered that since that time clinicians have been agreed that diabetes insipidus can be controlled in nearly every case by pituitary extract medication, and that its use in such cases has been going on constantly since that date. From this year onwards much experimental work began to appear on the causation of experimental diabetes insipidus, and by 1918 the main problems had been variously stated and denied.

Goldzieher, in 1913, and Simmonds, in 1914, put forward the theory that diabetes insipidus was a deficiency syndrome, the substance lacking being an anti-diuretic, a "diuresis-controlling" factor from the hypophysis. Their evidence was largely based on post-mortem reports of clinical cases where destruction of the hypophysis was found. But in the same years Camus and Roussy, on experimental grounds, maintained that lesions of the hypothalamic region of the brain carried out so as to avoid damaging the hypophysis/
hypophysis at all, resulted in diabetes insipidus. They further showed that total removal of the hypophysis does not affect the fluid exchange in animals observed from day to day. They concluded, with apparent reason from their results, that therefore the hypophysis had nothing to do with diabetes insipidus, and that the cause lay in the basal region of the brain. In 1918 a new complication was introduced by Van Hann who reported that diabetes insipidus develops only in the presence of pituitary pars anterior tissue, and on the basis of this finding he postulated the existence of a positively diuretic factor in the pars anterior.

Now it is the task of this essay to show as far as possible that all these three views are substantially correct. Each of them was backed up by subsequent workers, and each of them was in turn denied by other workers whose experimental results differed. The fact remains that until recently no single worker has been able to cover the ground and take every line of evidence into account in such a way that these views may be reconciled in one publication of experimental results. Nevertheless, they can be reconciled to a very considerable extent.
Diagram of Relations Between Mammalian Hypothalamus and Hypophysis.
ANATOMICAL DESCRIPTION

It is not proposed to go deeply into the developmental or comparative question. A diagrammatic representation of the region under consideration is given as the readiest way of appreciating the relations of the parts named and referred to later on. It may be taken as a scheme of the mammalian anatomy, and is valid for all the experimental animals to be referred to, including man (LeGros Clark, 1938). The supraoptico-hypophysial bundle of fibres was demonstrated as early as 1894 by Ramon y Cajal, and as a very definite structure in man by Purves and Greving in 1925. It is constant in all mammalia and has been worked out and described in great detail, along with its regional connexions and relations (Res. Publ. Ass. nerv. ment. Dis. 1940). The main anatomical feature which has not been represented in the diagram is the higher connexion of the hypothalamic structures with the cortex. This is not yet a matter of certainty. It is likely that an afferent bundle comes to the hypothalamus from the frontal lobes of the cerebral cortex and another from the hippocampus via the anterior fornix.

The hypophysis has two sources of nerve supply (LeGros Clark, 1938):

1) The supraoptico-hypophysial tract from the hypothalamic/
hypothalamic nuclei as shown, distributed to the pars posterior or pars nervosa.

2) A sympathetic nerve supply from the superior cervical ganglion distributed with the blood vessels to the portions of the hypophysis developed from the buccal outgrowth - pars anterior, pars tuberalis and pars intermedia.

Both the hypothalamus and the hypophysis possess a rich arterial blood supply.

**THE ANTI-DIURETIC HORMONE**

It is necessary to demonstrate beyond doubt that hormonal action does in fact affect the renal output before the question of its liberation and control comes under consideration. There exists a great body of facts which, taken together, prove the hormone's existence beyond a doubt. It has already been pointed out that none of the other possible renal control mechanisms have the scope and flexibility to account for the variations known to occur. The existence of a definite hormone can only be proved by evidence satisfying three main lines of approach, which are as follows:

1) Substitution therapy in an intact subject, whereby the administration of a hormone-containing extract/
extract can be shown to have the specific effect in physiological doses.

2) Withdrawal experiments in intact subjects, whereby some operative procedure is undertaken to prevent the natural liberation of the hormone, and after complete post-operative recovery, a specific effect is to be noted; such effect to be abolished by administration therapy as above.

3) Experimental evidence on isolated, perfused and implanted organs, where other sources of error may be completely excluded under controlled conditions. Evidence will be offered on all these three lines.

With regard to substitution therapy, ample evidence can be produced from the records of any large general hospital that the condition of diabetes insipidus can in most cases be controlled by administration of posterior pituitary extracts, either in the form of subcutaneous injection or as a powdered snuff. The administration of other substances, notably adrenaline, may produce a temporary inhibition of the urine flow, but does so inconstantly, and never with the time characteristics or extent that does pituitrin (Lhermitte, 1922). There are several important considerations with regard to the administration of pituitrin to normal animals. It has been shown that intravenous/
intravenous pituitrin inhibits urine flow, inversely proportionate to the water load in the body (Pickford, 1936). Therefore it is useless to give pituitrin to an animal and interpret the result as a weak or strong inhibition unless an accurate idea of the state of hydration of the animal is known. The effect of pituitrin will be considered, therefore, in the following standard condition, namely, its effect in inhibiting urine flow at the peak or plateau of a water diuresis, the animal having previously been hydrated. In other words, the effect of pituitrin on extra water, which it is in the animal's natural interest to get rid of as soon as possible. The term "water diuresis" will be held to refer to the excretion of this extra administered water. In nearly all of the appended graphs of diuresis curves the amount of urine passed (by catheter) over successive quarter-hour periods is plotted against time. Statistically, this gives only a rough approximation to true rate of urine flow, and the graphical points have been conventionally joined up by straight lines, although the alteration of rate of flow is of a logarithmic nature. However, as accurate interpolation readings of rate of flow are not in practice required, such curves do give a fair representation of time of onset of diuretic rise and of the maximum amount of urine/
FIG. I. The effect on water diuresis of posterior pituitary extract.
urine put out in unit time; the time unit being chosen in most cases as fifteen minutes.

Curve (1) represents the typical response of a well-hydrated dog to a dose of pituitrin, in this case the trade product "Infundin", given subcutaneously at the height of diuresis. If the anti-diuretic hormone does in fact exist normally in the blood stream, and diuresis is a function of its withdrawal, then administration of such hormone might be expected to have its most marked effect at the time when the blood hormone content would be lowest, namely at the peak of diuresis. One-thousandth of a standard international unit administered at that time produced the very definite effect shown. The recovery from the effect is fairly rapid, and diuresis resumes its course after the effect has worn off. As a very rough indication of the magnitude of the dose in relation to clinical practice, it may be said that, allowing for weight disparity, it is in the region of one two-thousandth of the dose used in clinical practice for oxytocic effect.

There are some other valuable facts to be got from administration experiments. Klisiecki et al.(1933) have shown that it does not produce its action by retarding water absorption from the intestinal tract. Newton and Smirk (1933) showed that it inhibits the excretion of water which has been administered intra- venously/
intravenously, in which case effects on gut do not come into the picture. Moreover, Klisiecki and his co-workers showed that if the kidneys of a dog are separately catheterised, after suitable operative measures and recovery, denervation of one of them does not in any way alter the response to pituitrin. The kidneys may not have an identical flow (they incidentally showed that normal kidneys commonly differ in flow one from the other) but their responses are essentially parallel. That is to say that pituitrin does not act through the nervous connexions to the kidney or at the nerve endings there.

One further line of evidence from administration technique: Gilman and Goodman (1936) find that the urine of dehydrated rats and dogs whose posterior pituitary glands are intact contains a substance which inhibits water diuresis in other hydrated animals. This bears out the hypothesis that an anti-diuretic hormone is responsible for water conservation. One would expect to find excess of the hormone (apparently sufficient for some to be excreted) in just such dehydrated animals where water conservation was an urgent need, and according to this work, inhibition of diuresis is actually produced by the excess of hormone, not only in themselves but in other hydrated specimens/
specimens.

Evidence from withdrawal experiments requires consideration under a heading of its own, as including the great body of work, much of it conflicting, done on experimental diabetes insipidus. The precise methods of production of diabetes insipidus are in debate, but the condition, once produced, is beyond doubt a withdrawal syndrome in which lack of an antidiuretic hormone produces characteristic permanent polyuria. The question of causation requires a section to itself.

Evidence from laboratory preparations is always liable to some objections in that the organ concerned is not under natural conditions, and therefore need not necessarily obey its normal laws. Nevertheless, there is a bulk of illuminating evidence of a positive nature on this problem. Starling and Verney showed as early as 1925 that the inhibition effects may be demonstrated on decerebrate preparations, on heart-lung-kidney preparations and even on pump-perfused kidneys completely isolated from the body. Subsequent work by Verney has brought out the following and significant experiments: firstly, the administration of pituitrin to a heart-lung-kidney caused reduced flow/
flow from the kidney; secondly, the insertion of an intact dog's head into the perfusion circuit also caused inhibition; thirdly, that the substitution of a dog's hind leg for the head did not produce inhibition; and finally, that the insertion of a perfused dog's head lacking the pituitary gland did not produce inhibition. No explanation will satisfy these facts other than that there is a hormone directly acting on the kidney, and that that hormone is elaborated only by the pituitary gland.

The site of action of the hormone in the kidney is much more difficult to determine. Burgess, Harvey and Marshall (1933) voice the generally accepted viewpoint that the anti-diuretic hormone stimulates the reabsorption of water in Henle's loop. Formal proof of this is not experimentally forthcoming, but as an assumption it covers all the known facts. There are one or two points worth considering.

One is that the degree of alteration of reabsorption necessary to produce urine changes is very small. It may be taken, from results obtained from maximum recorded glomerular filtration rates (see p.8), that out of the 120 c.c. which man can filter through his glomeruli per minute 100 c.c. must necessarily be reabsorbed, and only the reabsorption of the remaining 20 c.c. is facultative. Therefore the maximum possible limits of reabsorption are in the ratio of 100:120. Peters/
Peters (1935) points out that under average conditions in man a doubling of the daily urine output (which is a clinical polyuria) can be achieved with a 1.2% alteration in reabsorption. The second point is that, according to the workers first mentioned, fish and amphibia do not possess the loop of Henle and are not responsive to pituitrin. Fish and amphibia are not faced with the vital problem of water conservation. Mammals, for whom it is the fundamental need, are forced to elaborate a hypertonic urine in that they cannot afford the fluid for the isotonic type found in fish. But to elaborate a hypertonic urine means some special mechanism for causing water to be reabsorbed against an ever-increasing blood-urine osmotic gradient, and the stronger the excreted urine the more necessary and powerful such a mechanism must be. It fits the facts to say that this need for overcoming the osmotic gradients appears along with response to pituitrin. It also affords an explanation of why the hormone should be negative or inhibitory with respect to urine volume, for it is more easy to excrete much urine than little, and it is the reduction of volume that requires special mechanism.

DIABETES/
DIABETES INSIPIDUS - THE HYPOTHALAMUS

When experimental work began to be performed on animals with a view to producing the condition of diabetes insipidus, a considerable amount of data accumulated suggesting that the hypophysis was not primarily concerned, and that the true cause of the condition was to be found in damage or destruction to areas in the hypothalamus. The main evidence for this must be subjected to analysis and criticism before correlation between the various views can be attempted.

Camus and Roussy, in a series of papers published between 1913 and 1925, put forward quite definitely the three following propositions as a result of their experimental findings:

1) That removal of the hypophysis does not result in diabetes insipidus;

2) That in an animal where hypophysis has been removed, diabetes insipidus can be produced by subsequent injury to the hypothalamus;

3) That in a normal animal, diabetes insipidus can be produced by puncturing the hypothalamus and in no way injuring the hypophysis.

The work was confirmed by Houssay (1918), and by Bailey (1935) who denied that the neural division of the/
the hypophysis played any part in diabetes insipidus. Actually, in 1936, Houssay partially withdrew that view and said that polyuria in diabetes insipidus was due to deficient anti-diuretic posterior pituitary hormone.

More recent evidence on the same lines has been presented by Mahoney and Sheehan (1936). In a series of experiments on monkeys, they found that placing a silver clip on the infundibular stem did not produce diabetes insipidus; they deduce from this that interruption of the supraoptico-hypophysial tract does not produce the condition, in other words that denervation of the posterior pituitary has no effect. However, the same workers found that, in a series of 20 dogs, clipping the infundibular stem resulted in a polyuria lasting for several weeks. They reconciled these two series of opposing results by postulating a species difference between the monkey and the dog. A number of other workers, including Reichert and Dandy (1936) put forward similar but rather less definite results. Our view is that these results as far as they go are substantially correct, but that their interpretation is unsound and some of the data inadequate.

A number of workers have confirmed the observations of Camus and Roussy that hypophysectomy does not produce diabetes insipidus, and have made the assumption/
assumption on that basis that hypophysectomy does not make any difference to the water balance or behaviour of the animal towards fluid. We shall offer evidence that while removal of the pituitary gland does not produce any very gross alteration in water balance over the 24-hour period usually employed as index of polyuria, it nevertheless profoundly alters the behaviour of the excreting system when examined in finer detail.

To deal with the second point made by Camus and Roussy, that hypothalamic puncture in an hypophysectomised animal will produce diabetes insipidus: there are two matters of practical significance in connexion with all such results which may well be brought out now. True diabetes insipidus in the strict sense is a permanent condition of the animal concerned, and is subject only to minor variations with the animal's metabolism and general condition. A transient polyuria is quite a different matter, and is a common sequel to any operative procedure in the region of the hypophysis. Before diabetes insipidus can be truly claimed, it must be shown that polyuria persisted over a period of many weeks and preferably months. The other matter is this: that in common with so many of the organs of the body, the pars nervosa of the pituitary appears to have an enormous margin of safety, and a/
a very minute remaining part may supply adequate secretion to compensate for the loss of the major part. It follows that only complete and meticulous histological examination can guarantee a total hypophysectomy. And indeed, Fisher, Ingram and Ranson (1938) criticise Camus and Roussy's interpretation just on that basis, that neither their "diabetes insipidus" nor their "total hypophysectomy" is justified by their data. The results that they obtained can be explained rather readily if in fact the hypophysectomies were not complete, and a small portion of posterior pituitary left; in which case it is highly probable that puncture of the hypothalamic region might cause degeneration of the remaining fragment, consequent withdrawal of anti-diuretic hormone, and resultant polyuria. This will be discussed later.

Fisher, Ingram and Ranson state that in the case of Mahoney and Sheehan's monkeys there can be little doubt that some posterior pituitary was left undegenerated after the clip had been allowed to remain on for some time. Histological evidence from their own specimens indicates that denervation of the pars nervosa was incomplete, and that the median eminence, containing functional pars nervosa, was in any case above the level of the clip. Further, histological and phylogenetic considerations are very strongly against/
against species differentiation between monkey and dog, and it seems likely that anatomical differences in the angle and size of the dog's pituitary rendered the clips in their case more effective in causing pars nervosa denervation.

Several investigators have put forward the view that in hypophysectomised animals the hypothalamus itself may take over the duty of producing anti-diuretic substance, and thereby prevent the onset of polyuria (Sato, 1928; Trendelenburg, 1928). Their evidence has been firstly that hypothalamic extracts are richer in anti-diuretic principle when made from an hypophysectomised dog than when from a normal dog, and secondly that the cerebrospinal fluid of hypophysectomised dogs is rich in oxytocic posterior pituitary hormone. Neither of these claims has been confirmed by accurate workers and the second has frequently been denied. Scharfen (1940) believes that on purely anatomical grounds secretory cells do exist in the hypothalamic nuclei, but despite this finds no evidence of their being affected in various conditions of salt and water balance in rats, and agrees with Ranson and Magoun (1939) that it is very unlikely that such cells can take over the secretory functions of the pars nervosa.

One further view favouring the hypothalamic basis of diabetes insipidus requires to be considered, and that/
that is the argument that the fundamental physiological maladjustment is not the polyuria, but a desire for increased water intake, a polydipsia, produced by some higher centre stimulus resulting secondarily in polyuria. This view was put forward by Curtis (1924).

A good deal of evidence has now accumulated against the primacy of the polydipsia. Dryness of the mouth and throat, the presumable initial stimulus to drinking, has been shown not to affect thirst and volume of water intake (Steggerda, 1939). We have found in our own animals that polyuria comes on within a few hours of operation, when indeed the animal has not yet recovered from the anaesthesia, and while hesitating to make direct inferences from animals half-anaesthetised, no water intake is possible in these cases. Further, we find in several cases that urine outputs may considerably exceed intake, so that in fact the animal is being polyuric not on what it drinks but, at least partly, on its body water.

Summarising this section, it may be said that evidence that the hypothalamus alone is responsible for diabetes insipidus is not convincing, as no one series of experiments has so far taken every factor into account; that the hypothalamus is itself a potential source of anti-diuretic hormone appears unlikely upon good authority, and that the polyuria of diabetes insipidus is primary, and the polydipsia consequent upon it.
DIABETES INSIPIDUS - THE HYPOPHYSIS

The part played by the hypophysis in the control of renal excretion has been the subject of a great deal of controversy and experimental confusion. The problem with regard to the experimental evidence is that the gland consists of three functional parts, not readily separable one from the other, each capable of carrying out its normal function with a small fraction of remaining tissue. This implies firstly that extirpation experiments are technically difficult and secondly that the results of such work can only be accepted when accompanied by histological evidence of the presence or absence of a given tissue.

The first line of evidence that must be explained is the finding of numerous workers, that total hypophysectomy causes no change in twenty-four hour fluid exchange, or, if it does, merely a minor and transient polyuria in no way comparable with diabetes insipidus (Dott, 1923; Dandy, 1925; Smith, 1927; Koster and Geesink, 1929). Keller, Noble and Hamilton (1936) attempted denervation of the posterior lobe by section of the stalk and report no increased water exchange following on such section. Criticism of their work and of that of some of the earlier workers is given by Fisher, Ingram and Ranson (1938), who point out that/
that the section, while denervating the main bulk of the pars nervosa, left intact rostrally the median eminence tissue now known to be functional posterior lobe tissue. Now the conditions of the experimental animals in the cases of the earlier workers and of Keller are very different. In the one case, the animals lack both anterior and posterior tissue; in the latter case, after stalk section, according to Fisher, they lack nothing, being equipped with the intact anterior gland and enough functional posterior tissue to keep up the supply of anti-diuretic hormone. Nevertheless, there seems little doubt that in many of the earlier cases all functional pars nervosa tissue was genuinely removed, as Fisher, Ingram and Ranson point out. If such animals then lack entirely any source of anti-diuretic hormone, how can they remain anything other than grossly polyuric? These are the animals which have suffered total hypophysectomy.

On the other hand, the animals belonging to Keller, Noble and Hamilton retained a small amount of posterior lobe tissue, and the complete anterior lobe. Considering the problem from the other aspect, namely, histological accounts of animals which suffered from definite experimental diabetes insipidus, one fact does/
does seem well established. Kary (1924) and Kiyono (1925) first reported destruction of the supraoptico-hypophysial tract as such. The evidence that this is the chief factor in production of diabetes insipidus is summarised by Fisher, Ingram and Ranson (1938) who, in addition to subjecting other work to a critical analysis based on this assumption, bring forward a large series of experiments on eighty-five cats with varying degrees of diabetes insipidus. They show that nothing short of interruption of the supraoptico-hypophysial tract in the hypothalamus can cause complete degeneration of the posterior lobe, and emphasise the secretory powers of the median eminence and infundibular stem, commonly left intact in the so-called total post-lobectomy of other workers. The degree of polyuria which their animals exhibit permanently is well correlated with the degree of destruction of the tract. Furthermore, their histological evidence shows that both terminations of the tract degenerate, and complete section of it implies almost complete degeneration of supraoptico-filiform hypothalamic nuclei as well as of posterior pituitary tissue (see diagram p.15). Complete degeneration of the posterior lobe implies lack of anti-diuretic hormone and hence unchecked urine flow. They explain the non-appearance of/
of diabetes insipidus after total hypophysectomy by recalling and supporting Von Hann's theory of the diuretic activity of the anterior lobe, on which hypothesis diabetes insipidus occurs in the presence of anterior lobe tissue alone. Their conclusions with regard to interruption of the supraoptico-hypophysial tract are borne out by Farr, Hare and Phillips (1937), Rasmussen (1937) on rats, and by clinical post-mortem findings by Biggart (1937) and by other workers. Further, their explanations of earlier anomalous findings of other workers fit well into this hypothesis. Some original evidence confirming their results will be presented. Their account of the functional activity of the pars anterior is, however, far from complete, as they themselves admit, and some of the facts established with regard to it must be considered.

The pars anterior exercises a vital control over such general functions as metabolism and the activity of other ductless glands, and experimental work on it is correspondingly hard to interpret precisely. Administration of anterior pituitary extract might be expected to produce polyuria in animals. Evidence that this is so was given by Cushing as early as 1910, and reported by Teel and Cushing (1930), and Barnes, Regan/
Regan and Bueno (1933). Doses were relatively very large, and the action delayed for several days, both on beginning and stopping administration. White and Heinbecker (1937) report that beef pars anterior extracts contain a diuretic principle which acts only in the presence of the hormone of the thyroid gland. Transplantation results, checked histologically, do suggest that the anterior lobe (which is capable of persistence as a graft) has a definite diuretic action. None of the above results are altogether convincing. Richter (1934) in a large series of rats offers definite experimental evidence that presence of anterior lobe in absence of posterior is the cause of diabetes insipidus, and that the condition does not occur in the absence of anterior lobe. Pencharz, Hopper and Rynearson (1936) confirm this, and White and Heinbecker accept this view with reservations as to the mode of action of the pars anterior. Clinical evidence is available for it, and it is interesting to realise that four years before Von Hann's view of the diuretic anterior principle was put forward, Simmonds (1914) reported a case of diabetes insipidus diminished in severity as a tumour of the pars posterior encroached upon and gradually destroyed the pars anterior. Destruction of the anterior pituitary is known/
known as Simmond's disease to this day, and restriction of the fluid exchange has been reported in most of the instances of this rather rare condition (Marx, 1935; Silver, 1933; Curschmann, 1936).

At the same time the mode of action of the anterior lobe secretion is very hazily understood. Whether it acts by elaborating a specific diuretic hormone to form a counter-balancing mechanism to the anti-diuretic posterior lobe hormone, or whether by general depression of metabolism and activity it results in a lowered fluid exchange, remains unsettled. Fisher, Ingram and Ranson, and Richter favour the latter view; and the first-named workers believe that the general diuretic effect is produced partly through the thyroid gland and partly through the adrenal cortex. It is fairly well established that adrenal insufficiency gives rise to oliguria as one of its effects, and removal of the anterior pituitary as in total hypophysectomy does cause some degeneration of the adrenal cortex (Beaird and Swann, 1937). Another large field of work concerning the function of the pars anterior is involved in the discovery and discussion of the now recognised pituitary-gonad mechanism, whereby antagonism between these organs affords opportunity for the cyclical control of sex phenomena (Moore/
(Moore and Price, 1932; Van Dyke, 1936). It is not intended to consider this field here, but it has been demonstrated that administration of oestrin, by diminishing pars anterior activity, has an antidiuretic effect in diabetes insipidus. Such results emphasise the importance of the pars anterior in normal renal control.

Summarising this section, the importance of the pituitary body as a whole in renal control is stressed. The pars nervosa secretes a specific anti-diuretic hormone, whose removal results in unchecked urine flow; this profuse flow only occurring when the functional pars anterior is intact and exerting its effect by means as yet undetermined, but linked up clearly with the other vital glands such as the thyroid, adrenal cortex and the gonads.

THE HYPOTHALAMIC-PITUITARY COMPLEX AND THE PROBLEM OF HIGHER CORTICAL CONTROL.

So far the two systems have been considered in separate sections and as separate entities for experimental work, and the part that the actual mechanism plays in real life must be taken into account. We have seen that certain specific hypothalamic nuclei innervate/
innervate the pars nervosa of the pituitary gland via the supraoptico-hypophysial tract. Section of this tract produces degeneration of both its terminations, and a permanent polyuria. Section of the tract in anaesthetised animals produces a rapid effect (which will be discussed further) and it must be considered that the mechanism is in constant operation controlling the liberation of anti-diuretic hormone from the pars nervosa. Section of the tract in normal unanaesthetised animals has not hitherto been attempted, for obvious technical and mechanical reasons, although we have a feasible technique in preparation where results may be of value. Damage to the hypothalamic nuclei, as by needling, will naturally have the same effect in stopping the nervous impulses to the gland.

Before considering the mechanism in relation to environmental adaptability, there is one line of evidence of suggestive interest. Intravenous administration of acetylcholine results in a temporary inhibition of water diuresis in dogs. This inhibition, which occurs only in hydrated animals, is not due to direct action on the kidney, for it is abolished by hypophysectomy, and in the normal dog its course and time relations are similar to those resulting from pituitrin. It is concluded that acetylcholine acts as a stimulus to the liberation of anti-diuretic hormone/
hormones from the posterior pituitary (Pickford, 1939).

It appears probable that the hypothalamic-hypophysial complex is to be regarded generally as a further and rather complex example of the parasympathetic-sympathetic balance mechanism found in so many of the automatic functions of the body. The anterior pituitary is innervated by the superior cervical ganglion of the sympathetic chain and it seems that the parasympathetomimetic agent acetylcholine is a stimulus to posterior pituitary secretion; the two opposing glands being as it were effector organs of the autonomic nervous system. The hypothalamic nuclei on such a view appear to be one of the sites at which higher control can take effect on the automatic mechanism, and the cortex play its part in causing adaptation of renal output to environmental conditions appreciated through consciousness. There is some experimental evidence that this is indeed the case. In the first place, all workers on the urine secretion of normal unanaesthetised dogs find that the animals are very susceptible to extraneous influence. Dogs vary in their temperaments, but the diuretic response of all appears to settle down after the initial days when they have to become accustomed to the stand and the catheter. In some, the entry of a stranger into the room or of some minor procedure such as withdrawal of/
of blood from an ear vein will inhibit the normal course of a water diuresis. This is put on a formal experimental basis by Theobald and Verney (1935) and Rydin and Verney (1933), who show that inhibition of water diuresis can be brought on by emotion or exercise in dogs, and that the effect is not due to adrenaline liberation but has the time relations and renal effect of pituitrin administration. They also show that the inhibition is independent of innervation of the kidney. They put forward the view that during emotion or exercise the hypothalamic nuclei set up secretion of anti-diuretic hormone in the posterior pituitary gland, and thus bring about hormonal increase of tubular reabsorption. I can find no account of this having been repeated on dogs where supraoptico-hypophysial tract had been cut, or which had been hypophysectomised, though there is some evidence on rabbits that in such cases nervous stimuli will produce no inhibition (Haterius, 1939).

Normally, one presumes that the control is adjusted to respond unconsciously to very minute variations in the fluid and electrolyte content of the blood flowing through the hypothalamic area of the brain, and that these cortical or higher stimuli are superimposed on the normal control. Valuable support to this belief is afforded by a series of experiments on/
on anaesthetised dogs which we have carried out; as will be seen later, the results are difficult of general interpretation but do seem to show that some higher influence is at work in the normal animal and abolished by anaesthesia. The normal inhibition of urine flow during sleep cannot be due to lowered blood pressure, and it is maintained by Lhermitte (1932) that diabetes insipidus is reduced in intensity during sleep or anaesthesia. The possible anatomical pathway for such control has been mentioned (p.15), but the problem of the higher control remains largely unattempted as yet.
ORIGINAL INVESTIGATION

I. INTRODUCTION AND TECHNIQUE.

Personal investigation over the past nine months has not placed one in a position to make any large contribution to the solution of the problem, though a number of very suggestive facts have been proved. It is proposed to describe and discuss such experimental work as has been successfully carried out largely by means of graphical representation. This type of work lends itself to this rather than to a series of protocols which are difficult to compare one with the other. As the graphs have in some degree been simplified for clearness of presentation of the facts, a brief survey of technique is desirable for appreciation of them.

The work covers the examination of the diuretic responses of three dogs before and after operative measures on the hypothalamic-pituitary complex. At the same time renal clearances on these animals were measured for creatinine and for urea.

1) Twenty-four hour urine samples were obtained from a metabolism cage of standard type, and the diet was as reasonably constant as possible.

2) Each diuresis response was done at least twice, and in many cases three times. These curves do vary slightly from day to day in the same animal, and/
and the most typical or mean curve is that represented in the diagrams. In every case given here, the curve chosen very closely resembles the other similar ones, that is, no atypical or widely divergent curve has been used. In every case the animal has been hydrated at a standard time beforehand.

3) Creatinine clearances given are the mean of three clearances obtained during a water diuresis curve, after administration of 3-3.5 gm. creatinine. They have been corrected to surface area of the individual dog. Creatinine was estimated colorimetrically in serum and urine by making use of the Jaffé alkaline picrate reaction.

4) Urea clearances are similarly the means of three samples of serum and urine taken during a water curve. Urea was estimated by micro-titration direct into a Conway unit after the urease micro-diffusion method of Conway (1939).

5) General operative technique involved the access to the pituitary region by the trans-buccal route, under Nembutal anaesthesia. The manipulation was carried out by Dr. Pickford in each case, and details will be referred to later.

The first case, Dog 36, will be presented as a typical example of successful section of the supra-optico-hypophysial/
supraoptico-hypophysial tract, with analysis of the effects of that operation on diuresis response to water, saline, and concentrated salt solution. Dr. Pickford's unpublished work contains other cases verifying this particular one, which happens to be the one with which I was associated and at the same time a good demonstration case.

Two cases will be presented of dogs where attempted removal of the anterior pituitary has been the operation, and observations on them recorded. This is new work and cannot be put forward on a statistically sound basis as yet, but it is most suggestive.

Evidence from a series of 24 experiments on anaesthetised dogs will be summarised, and the effects of anaesthesia considered in relation to the problem as a whole. On account of the graphical presentation of the findings, the text will be split up into appropriate sections and will accompany the graph so that both may be considered together.

The original work was carried out in the Physiology Department, Edinburgh University, during the tenure of a Carnegie Research Scholarship 1940-41.
II. DOG 36. A CASE OF SECTION OF SUPRAOPTICO-HYPOPHYSIAL TRACTS.

As previously referred to (p.32), Fisher, Ingram and Ranson report degeneration of hypothalamic nuclei and of pars nervosa following tract lesions in a series of cats. This is a typical case of the same thing in one of Dr. Pickford's series of dogs, where the tracts were sectioned at open operation (Fisher employed the stereo-taxic instrument, which is not applicable to dogs). The sections were made when the animal was sacrificed one month after operation, at which time it was grossly polyuric. Similarly treated sections from a normal animal are given for comparison. The almost complete absence of nerve cell bodies from the filiform and supraoptic nuclei, and the shrunken cellular appearance of the posterior pituitary tissue are evidence of the degree of degeneration. The posterior pituitary tissue looks non-functional, and no normal tissue can be found on examination of the whole of the series from which this is merely one specimen section. Similarly, although only one slide can be reproduced of nuclear degeneration, serial sections of the whole region have been examined, and present identical degenerated appearances.

The/
Normal dog. Photograph X60 of Supraoptic nucleus of hypothalamus. Stain - Toluidine Blue. Note the density of the cell bodies and their compact grouping.

Dog 36. The region of the Supraoptic nucleus some time after section of the supraoptico-hypophysial tracts. Same magnification and stain as above. Note the complete disappearance of all nuclear nerve cells.
Normal dog. Photograph X60 of region of Filiform Nucleus of Hypothalamus, showing the edge of the third ventricle and the normal concentration of cell bodies in this nucleus.

Dog 36. The corresponding area of the hypothalamus in a dog whose supraoptico-hypophysial tracts were cut some time before. Only a few scattered cell bodies can be found in the region of the Filiform nuclei. X60 magnification; stain Toluidine Blue.
Normal dog. Photograph X400 of pars posterior tissue of pituitary gland. Stain Bodian method. Note the fibrils and size of the normal functional cells.

Dog 36. Photograph under the same conditions of the pars posterior tissue some time after section of the supraoptico-hypophysial tracts. Fibrils are entirely absent, and the normal cell pattern has been altered. The predominant cell type is a small oval or round dark-staining cell, rather shrunken. There are very few, if any, normal secretory cells to be found throughout the whole section of this gland.
FIG. II. Dog 36. The twenty-four hour urine output.

- Normal.
  Average Chloride/day 1.3gm.
  Average Spec.Gravity 1018

- After section of So-Hy. Tracts.
  Average Chloride/day 7.4gm.
  Average Spec.Gravity 1007

FIG. III. Dog 36. The creatinine clearances before operation and in the post-operative phases following section of the So-Hy. tracts.
The twenty-four hour urine output after tract section.

The characteristic features of the onset of diabetes insipidus after tract section are shown in Fig. II. In the first five or six days after operation, such dogs exhibit the stage referred to as transient polyuria, sometimes very great. This abruptly falls to an apparently normal level between the 7th and 10th days, which stage is called the normal interphase. In point of fact, the excretion arrangements of the animal in this interphase are far from normal; while the total 24-hour output is average, the dogs show an inability to excrete given water, which is frequently so extreme that administration of water produces symptoms of water intoxication in doses which the pre-operative dog would have got rid of in an hour or two. Following on this the true permanent polyuria sets in, and its extent appears to be dependent on the degree of destruction of the functional pars nervosa tissue. Reference to Fig. III shows the variation in creatinine clearances during these stages. We believe that these changes are significant, although enough evidence has not yet been obtained to warrant more than suggestion.

Creatinine clearance in the dog is at the level of glomerular filtration, and the substance is not reabsorbed/
reabsorbed in the tubules to any extent. Therefore it is probable that a polyuria with a high clearance is due primarily and obviously to increased glomerular filtration rather than to diminished tubular reabsorption of water. Such polyuria is not diabetes insipidus in the true sense, and it is suggested that the transient polyuria is not solely due to lack of anti-diuretic hormone. It is true that the nervous supply to the pars nervosa end-organ has been severed, but until evidence is brought out with regard to the absence of pre-formed anti-diuretic hormone in the pars nervosa (in other words, that the nerve supply is essential for the immediate formation of hormone from a precursor) one is justified in assuming that considerable stores of hormone will remain in the gland. The possibility that the transient polyuria with the high glomerular filtration is an anterior pituitary production will be discussed in connexion with the next two dogs.

The problem of the interphase is also linked up in some way with the anterior pituitary function, or so our results tend to show. The suggestion of some, that the interphase is just a period of general post-operative/
post-operative lowered vitality and metabolism, is not tenable on clinical grounds; these dogs do not show loss of appetite or any loss of vitality when subjected to the normal routine measures. During this inter-phase the dog cannot produce a water diuresis (though saline nearly always promotes increased flow, and it is our impression that it renders a dog in some discomfort from water retention more comfortable), and we have fairly regularly found a definite lowering of the glomerular filtration rate at this period. Such a condition is very much in line with that existing after removal of all, or a large part of the anterior pituitary gland. As far as we can judge, the blood pressure during the interphase is not lowered to any extent, and the glomerular filtration being low appears to be due to some alteration of the glomerulus itself. Evidence from the following cases also suggests this as a mechanism.

From the tenth or twelfth day the permanent polyuria stage persists indefinitely in a successful case, and there is little doubt that this is caused by the absence of reabsorptive activity in the tubules; that is, lack of the stimulus to reabsorption, which is the anti-diuretic posterior pituitary hormone. The degeneration of the posterior pituitary tissue has been demonstrated by micro-photographs. The behaviour of the dog towards water and salt diuresis will be described in the next section.
FIG. IV. Dog 36. The diuretic response to water and salt.
Pre-operative — Post-operative ——
The diuresis responses before and after tract section.

Fig. IV indicates the typical responses before and after operation to administration of water, saline, and 3% salt solution. All curves were obtained under the standard conditions already referred to. The post-operative curves all refer to the permanent polyuria phase - that is, to true diabetes insipidus.

First, the interesting point appears that a polyuric dog does not have more than the normal ability to produce diuresis; although the delay between ingestion and peak urine flow appears unaltered, the diabetic dog attains a similar or even lower maximum. Secondly, that in the diabetic dog, saline or 3% salt "brings out" water to a greater extent than in the same dog with normal pituitary arrangements. Yet the daily chloride excretion of this animal after the permanent polyuria had been attained averaged more than five times that in its normal condition (Fig. II). It seems as if the renal tubules, deprived of their reabsorptive stimulus from the anti-diuretic hormone, work at a relatively more constant level with regard to water (and therefore do not cease reabsorption to produce water excretion as in the normal withdrawal of hormone during diuresis), and depend far more upon the osmotic conditions in their/
their lumen. If the function of the pituitary anti-diuretic hormone is to produce a strongly hypertonic urine, one might expect the urine of this dog to be low both in chloride concentration and in specific gravity, which is in fact what is found. Diabetes insipidus, when subjected to these diuresis analyses, appears to be a degeneration from biological hormonal control to physico-chemical control of tubular reabsorption. Such an arrangement would naturally limit the peak water output in the absence of osmotic substances, and offers an explanation for the curves obtained.

Summary.

The effects of section of the supraoptico-hypophysial tracts are demonstrated histologically, the stages of development of diabetes insipidus discussed, and speculation made as to their mechanism. More detailed investigation of the diuretic capabilities of a diabetic dog than can be afforded by 24-hour urine charting is considered, and the physico-chemical possibilities briefly referred to.
FIG. V. Dog 37. The twenty-four hour urine output.

- Normal.

Average Chloride/day 5.3 gm.
Average Spec. Gravity 1022

--- After operation (see text)

Aver. Chloride/day 3.4 gm.
Aver. Spec. Gravity 1016

FIG. VI. Dog 37. The renal clearances before and after operation.

- creatinine.
- urea.
III. DOG 37. ATTEMPTED REMOVAL OF ANTERIOR PITUITARY.

In this dog, and in the succeeding example, an attempt to remove the anterior part of the pituitary was made. On account of the very restricted space for manipulation, the grave risk of breaking the delicate pituitary stem by rough handling, and the fact that the operation had only been practised twice before, removal was necessarily somewhat of a piece-meal proceeding. The experimental results will be presented but discussion postponed until the second similar case is considered.

The twenty-four hour urine output.

For the first five days after operation the dog showed a fairly gross polyuria. There was no definite interphase, and the daily output stabilised after a week to a very normal level (Fig. V). It should be mentioned that another dog, 35, (which has not been dealt with at length because it died a short time after operation) manifested very similar twenty-four hour urine characteristics in the early days after the same operation.

The creatinine and urea clearances.

During the initial polyuric phase, both clearances were/
were at a high level, indicating that glomerular filtration was high (creatinine) and that tubular reabsorption (urea) was low. Such a state of affairs results naturally in a very definite polyuria, which can be said in this case to have the double components indicated. From that time, both clearances show a significant fall, and at the end of a month from operation are at a value approximately two-thirds of their normal preoperative level (Fig. VI).
FIG. VII. Dog 37. The diuretic response to water and salt.
Pre-operative ——— Post-operative———
The diuresis responses to water and to salt.

The abnormal behaviour of this dog to ingested water has been most noticeable. Water curves have been taken at intervals since operation, and at no time has the peak flow been much over half the normal level. The curve instanced in the diagram (Fig. VII) is the typical post-perative response, and has been remarkably constant over nearly two months, tested at intervals, with a maximum urine flow round about 25 cc. per quarter-hour, which is extremely low for a large dog (22 kg.). The responses to saline have been less constant, and it is difficult to say anything significant in regard to them, except that a substantial but delayed diuresis appears to be the post-operative effect. With regard to 3½% saline (which is, incidentally, the strongest that a dog can be given without emetic results), this particular Dog 37 had a characteristic response to it when normal, in that the urine became very concentrated in appearance, very scanty in quantity and took a long time to return to resting level. The post-operative response has been a mild delayed diuresis very similar to that produced by saline.
saline. The drop from resting rate (which has been somewhat lower since operation) has never been evident.

As a result of this dog's definite limited capacity for getting rid of water it has been liable to a mild degree of water intoxication since operation. It will be recalled that this is a common feature of the interphase period of the dog with tract section. The symptoms have rarely been severe, as presumably a large dog has a considerable bulk of tissue fluid to act as buffer for body fluid dilution, but they have appeared.
FIG. VIII. Dog 33. The twenty-four hour urine output.

- Normal.  
  Average Chloride/day 2.6 gm.  
  Average Spec. Gravity 1018

--- After operation (see text)  
  Average Chloride/day 2.3 gm.  
  Average Spec. Gravity 1014

FIG. IX. Dog 33. The renal clearances before and after operation.
- creatinine.  
○ urea.
IV. DOG 38. ATTEMPTED REMOVAL OF ANTERIOR PITUITARY.

In this instance the operation was rather more cleanly carried out, largely as a result of experience, and we believe that all the pars anterior was removed and none of the posterior lobe damaged in the process. The results obtained have been, briefly, as follows:

Twenty-four hour urine output.

Fig. VIII indicates that at no time has the output been outside the normal limits. There has been no significant transient polyuria or interphase, and the specific gravity and chloride output are only slightly altered.

Creatinine and urea renal clearances.

The clearance figures for both substances have shown a tendency to fall progressively since operation and have not been up to normal level at any time when investigated, although the creatinine measurement on the 23rd day is nearly at normal level (Fig. IX). The measurements on the 11th day have given a result that indicates a higher urea clearance than creatinine. As is must be assumed that creatinine is at the level of glomerular filtration, the result is difficult to accept. At the same time, in justice to our methods, the/
the error of working necessary to produce these figures is well beyond the standard error of our other results, and the relative proportions of urea to creatinine are constant in both the samples from which the figure given is the mean. Tubular excretion of urea in the dog (which is the only fact apart from a fairly gross error in technique which can explain the results) is certainly not a normal function (Smith, 1937) but is a possibility, as behaviour of the nephron divisions towards urea is very uncertain and variable. We shall not stress the point beyond mentioning the possibility.

The/
FIG. X. Dog 38. The diuretic response to water and salt.

Pre-operative ———— Post-operative ————
The diuresis responses to water and to salt.

In this case again the significant feature has been the inability to attain normal diuresis after operation (Fig. X). Water intoxication symptoms have again been noticed on two or three occasions.

Saline responses parallel exactly those of Dog 37 in that a reasonable diuresis peak can be reached, and that it is delayed as compared with normal. This dog responded to strong salt solution with a mild delayed diuresis when normal and no significant variation has been charted since operation. Note that salt produced as good, and saline a better urine flow than water after operation.
V. DISCUSSION OF DOGS 37 and 38.

It is convenient to take the two cases together and consider them along with certain other facts. The behaviour of these dogs has been very much the same, except that it will be seen that Dog 37 had a transient polyuria stage characterised by a high clearance value both for urea and for creatinine. We were of the opinion at the time of operation that a part of this dog's posterior lobe had been removed in the attempt at isolated detachment of the anterior, and while it is possible that this may be the cause of the polyuria, it has been already pointed out that decrease of reabsorption, while causing polyuria, should not affect the creatinine filtration value. The simultaneous urea clearance was the highest recorded in this animal (the urine flow was not greater at the time of estimation than in the normal sample), and this does of course point to decrease of reabsorption as being a partial cause of the polyuria. The settled post-operative states of the two dogs are extremely similar; neither possesses the normal powers of diuresis with water, and both show a very similar response to saline. There is little doubt that this removal of the pars anterior has had the effect of preventing the upper levels of water diuresis from being/
being reached.

In order to get some notion of the mechanism involved in this, one must consider the renal clearance values. It is appreciated that on these cases alone results cannot be statistically valid, but merely suggestive. Rehberg (1926) quotes the normal creatinine clearance variation in dogs as ± 15%, although we have compensated for that source of variation to some extent by our system of taking a mean of three samples during the curve.

It is our impression, then, that both creatinine and urea clearances have shown a fall since the removal of the anterior pituitary in these two animals. Further, that fall is a more or less permanent one; at the present time, nearly three months after operation, the clearances of both dogs are still well below their normal level, and the poor response to water is still being obtained. Now the importance of this fact, if statistically demonstrated as valid, is that it implies some definite change in the functional mechanism of the kidney. Glomerular filtration is not altered by posterior pituitary extract, or in true diabetes insipidus, beyond the normal variation, and if it is altered in cases of anterior pituitary damage or removal, it indicates some mechanism operating/
FIG. XI. The Urea/Creatinine clearance ratio after attempted removal of the anterior pituitary gland in two dogs. (See text.)

- Dog 37.
- Dog 38.
operating upon filtration rate, possibly—and indeed probably—balanced against the recognised posterior pituitary-reabsorption mechanism. We have one or two subsidiary lines of evidence that this may be so. The low glomerular filtration rate accompanying the so-called normal interphase has been found in five dogs which have had tract section. We have had one dog whose records fall in very well with those of 37, and which died before long term post-operative conditions could be determined. Further, we have had one dog which after tract section showed a marked inadequacy for water diuresis and was found at post mortem to have a cyst of the anterior pituitary gland.

Fig. XI represents the urea/creatinine clearance ratio of each of these two dogs, and is an attempt at demonstration of variability of kidney function. The expression $1 - \text{urea/creatinine clearance}$ is an index of the extent of reabsorption taking part in the formation of the urine, and this is independent of the actual numerical value of the clearances or of the urine flow at the time. The degree of reabsorption of urea by itself means little enough, but taken in conjunction with the variable glomerular filtration figures and with the fact that these dogs had a normal 24-hour output, it indicates that there is some/
some balance between filtration and reabsorption which has been upset by the removal of the anterior pituitary. Reabsorption has varied with filtration in order to maintain a reasonably constant excretion. Constant urea/creatinine clearance ratio would imply urine output proportional to glomerular filtration, which we know has not been the case. It has been shown that posterior pituitary hormone is the stimulus to increased reabsorption, and that urine output can be controlled by a relatively very small percentage alteration in the degree of that function. However, these results seem to show that glomerular filtration is in some way the method, perhaps an accessory method, of producing acute diuresis, and that in the absence of the pars anterior the same degree of water diuresis does not seem to be possible, even with reabsorption at its obligatory minimum.

It is the fault of time that histological data cannot be given of Dogs 37 and 38 as yet. After criticising other work on the basis of lack of histological examination, and on failure to keep experimental animals long enough to pronounce a condition permanent, we feel that these dogs should not be sacrificed for the former until the latter is established. The findings may well require revision. It is/
is even possible that the pars anterior, which has the vitality to survive in grafts, may regenerate or hyper-secrete from remnants, though at the present time (nearly three months after operation) glomerular filtration is still low.

VI. EXPERIMENTS UNDER ANAESTHESIA.

A series of twenty-four acute experiments under conditions constant as far as possible has been undertaken after an observation of Dr. Pickford's that the normal effects of operation on tracts and pituitary gland were reversed under chloralose anaesthesia. The series has not been altogether satisfactory, and while the demonstrated effects are constant when they occur, they are not evident at all in some instances. We have as yet no explanation for this variability. The technique has been constant, and we speak only for chloralose anaesthesia, which has been used for the whole series.

One simple point does at least emerge: Peters (1935) puts forward a number of different workers as agreeing that pituitrin has no effect under anaesthesia. Presumably he refers to normal doses, as it will be recalled that the early workers found that posterior pituitary/
FIG. XII. Dog W24. Effect of post. pit. extract on anaesthetised dog. 

- Denervated kidney. 
- Normal kidney.

FIG. XIII. Dog W17. The effect on the anaesthetised of tract section previous to pituitary removal. Vertical scale 10x that of Fig. XIV. 

- Denervated kidney. 
- Normal kidney.

Figures represent Creatinine clearances of stages.

FIG. XIV. Dog W9. Effect on anaesthetised dog of Pituitary removal prior to tract section. Vertical scale 1/10 that of Fig. XIII.

- Denervated kidney. 
- Normal kidney.
pituitary extract was diuretic in anaesthetised animals, which it is in oxytocic doses. It is difficult to see why pituitary extract should fail to act in the anaesthetised animal when it works in the isolated perfused kidney; probably its effect is diminished by alteration in the situation of the water load of the body, which is known to affect the intact animal's response (Pickford, 1936); and anaesthesia is recognised as having an effect on water distribution.

Fig. XII shows quite definitely that the anti-diuretic hormone acts perfectly normally on a high urine flow in the anaesthetised dog. Further, one of the kidneys is denervated and yet follows faithfully the intact one's response.

With regard to the major experiments, we find that section of the supraoptico-hypophysial tracts in the preparation does not affect urine flow, nor does subsequent hypophysectomy (Fig. XIII). But if the order of procedure is reversed, hypophysectomy produces a polyuria which is terminated abruptly by section of the tracts. These are not blood pressure effects, which have been continually recorded during the experiments to avoid misinterpretation of results. (Fig. XIV, which represents the second type of experiment, has been inaccurately titled: "post-pituitary removed" should read "whole pituitary removed").
Is it possible to correlate these results with a conception of tonic cortical control of the hypothalamus? In the normal conscious dog, the impulses, if any, in the supraoptic tracts should be secretory in nature, and by liberation of more or less anti-diuretic hormone control excretion in that way. With anxiety or fright, we believe that the impulses increase and cause further liberation of hormone (and therefore lowered urine flow).

Tract section in the anaesthetised dog does not affect urine flow either way, and removal of the pituitary thereafter makes no difference. This appears to imply that under anaesthesia the tracts are "dead" and carry no impulses, so that their continuity is a matter of indifference. And the removal of the pituitary gland when its pars posterior innervation has been absent for some time might reasonably enough have no effect. That part of the experiment can be fitted in with the hypothesis fairly well.

Consider the second demonstration now (Fig. XIV). If we assume on the basis above that anaesthetised supraoptic tracts are "dead", and that secretion is inactive as a result in the posterior pituitary, why should its removal cause a polyuria? Note that this polyuria is very regularly and reliably obtained in our/
our series, and we are convinced that it is pituitary removal and nothing else that starts it. This seems to mean that a supply of anti-diuretic agent previously in action has been removed, and that in fact anaesthetised tracts are secretory, or what is equivalent, normal impulses are anti-secretory and diuresis-tending. Now subsequent section of the tracts, which can only operate upon such median eminence and stem tissue as may remain, terminates the polyuria abruptly. This is indicative of anti-diuretic hormone discharge; a secretion, in fact, on withdrawal of tract impulses, which have just been shown to be secretory in the anaesthetised animal! Reconciliation of the two aspects of the problem at present appears impossible, though there must be some combination of positive and negative impulses which will give an answer and allow the experimental evidence to fall into its right place. There is no doubt that anaesthesia reverses the effect of tract section, and of hypophysectomy, each done alone. We are quite convinced of that fact for a start. It is therefore likely that anaesthesia reverses the nature of the tract control by inhibiting (or exciting) impulses to the hypothalamus from the cortex.

Further, removal of the whole pituitary in the anaesthetised/
anaesthetised dog can only cause the polyuria it undoubtedly does by withdrawal of anti-diuretic hormone, as the positive possibility of pars anterior diuresis does not enter into this. Therefore the anaesthetised posterior pituitary, if we may describe it as such, is actively secretory. And active secretion of remnants after hypophysectomy appears to be caused by tract section. Therefore the supraoptic tract is anti-secretory under anaesthesia, and we are at once faced with a contradiction in that polyuria would be the regular result of anaesthesia, and oliguria the result of tract section in the presence of the posterior pituitary; which results in fact we do not find.

The problem is at present in just that anomalous position and only further work and perhaps further workers will produce a more logical account of the control; we can only put forward what we have found up to the present and leave the contradictions to be worked out. But it is worth repeating that the responses of an anaesthetised animal seem to be diametrically opposed to those of the intact conscious one, and that there is a valuable and important problem behind that fact.

SUMMARY/
SUMMARY AND CONCLUSION

The main established facts and the lines of approach that various workers have taken to the solution of the problem of renal control have been set out. The posterior pituitary hormone, with its stimulus to tubular reabsorption, has satisfied experimental and clinical investigation; and the linking of the hypothalamus to the posterior pituitary in a renal control complex is well established. It is possible that the supraoptico-hypophysial tract is nutritive only to the secretory pars nervosa cells, but it seems unlikely. There is little doubt that the anterior pituitary is an equally important agent in control; and on a slender basis of work not yet complete, it is suggested that the active agent is hormonal rather than metabolic, and that its action is upon the glomeruli. It may be that it increases filtration by calling into action a number of the glomeruli which we know to be resting in the normal kidney.

It seems possible to advance a tentative view of the whole problem as an autonomic system in balance between a sympathetic anterior pituitary hormone and a parasympathetic posterior pituitary one; between renal/
renal filtration and renal reabsorption as the ultimate mechanism of urine variation, controlled each by its autonomic agent. That emotion, sleep and anaesthesia profoundly affect the pituitary machinery makes a cortical control an established fact; but the details of that control have not yet been properly grasped by experiment.

The very delicate and complex problem of mammalian excretion, with its simultaneous need for conservation and rejection has built up an equally delicate and complex answer in this balanced and duplicated control, and one can only solve it piece-meal and slowly.

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REFERENCES


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