THE INNERVATION AND ACTIONS OF THE NEUROHYPOPHYSIS; AN INVESTIGATION USING THE METHOD OF REMOTE-CONTROL STIMULATION*

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[Plates 15–17]

Remote-control stimulation has been applied to the hypothalamo-hypophysial region. Rabbits showed no ill-effects from the presence of the implanted unit for periods up to three years.

Stimulation of the neurohypophysis in the conscious rabbit has been shown to cause: (*a*) inhibition of a water diuresis; (*b*) increase in urinary chlorides; (*c*) no change in the level of the blood sugar; (*d*) marked increase in the activity of the oestrous and oestrogenized uterus, whilst little effect was observed on the activity of the anoestrous or pseudo-pregnant uterus, or on the organ under the influence of progesterone. The anti-diuretic, chloruretic and oxytocic effects could be duplicated by intravenous injection of posterior lobe extracts.

Stimulation caused a secretion which possessed less anti-diuretic than oxytocic activity, as compared with various posterior lobe extracts.

The ultimate aim of this research programme is to elucidate the control exerted by the hypothalamus and other parts of the nervous system over the pituitary gland, and thus indirectly over the endocrine system as a whole. The most direct attack on this problem would appear to be that involving some method of electrical stimulation of the pituitary gland and the different regions of the hypothalamus, whereby the positive effects of any secretory activity could be studied. Stimulation of the hypophysis or its nerve supply, however, is a task beset with difficulties. The neural lobe requires a method of stimulation which is applicable to the conscious animal, for many of its actions appear to be radically affected by anaesthesia, for example, the anti-diuretic, hyperglycaemic and pressor actions. The study of the secretory effects of the glandular lobe is likewise impossible if the routine methods of electrical stimulation are applied, for the control which this structure exerts over the other organs of the body is of such a chronic nature (viz. the control of body growth and the sex rhythm) that little change would be observable from acute stimulation of this gland. (The one noteworthy exception is the ovulatory mechanism of the rabbit, and this has already been made the subject of study by acute stimulation of the pituitary and tuber cinereum (Harris 1937).) Thus for studies involving electrical stimulation of the hypophysis, a method is required which may be used in the conscious animal and by which prolonged stimulation may be repeated for periods of weeks or months.

The method of remote-control stimulation is admirably suited to meet both these requirements. This method is a comparatively recent development in the field of neurophysiology. In order that it may be performed, a small secondary coil is buried in an animal, and from this coil electrodes are led to the tissues to be stimulated. After recovery from the

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preliminary operation, stimulation may be performed in the conscious animal by placing the part carrying the coil in an electromagnetic field. This method has been used previously in America for studies of the motor cortex and has been adapted in the present work for stimulation of the pituitary and hypothalamus with unexpected success. The results of stimulation of the neurohypophysis and its nerve supply have been chosen for preliminary study, as the activities of this part of the pituitary are more quickly observable and better suited for the development of a new technique.

The terminology used for the various divisions of the hypophysis is that proposed by Rioch, Wislocki & O'Leary (1940).

THE TECHNIQUE OF REMOTE-CONTROL STIMULATION

In an attempt to avoid the complications induced by the use of anaesthetics, two methods of stimulating the nervous system have been developed. Ewald (1898) appears to have been the first to use the method of implanted electrodes which was later developed by Talbert (1900), Lewandowsky (1903) and notably by Hess (1932), and others. This technique, whereby electrodes are fixed in some part of the nervous system and insulated leads carried through the skin to the exterior, lends itself admirably to acute experimental procedures. If, however, a chronic preparation is attempted, two difficulties may arise, either the leads break, or infection tracks down them to the deeper tissues. Thus, in the last ten years, the method of remote-control stimulation has been used when repeated stimuli over a long interval were required. The use of this method was first recorded by Loucks (1933, 1934) and independently by Chaffee & Light (1934 *a*, *b*, 1935).

Loucks's technique for stimulation of the motor cortex of dogs consisted of implanting a coil of enamelled wire, the coil being fixed to the skull with silver lugs and silver-plated machine bolts. The electrodes from the coil entered the cranial cavity through small trephine holes. The primary coil or 'field coil' was attached to the animal's head external to the scalp. The device used to produce the fluctuating current in the primary coil consisted essentially of a condenser bank, the discharge of which was 'fired' by means of a thyratron circuit.

The technique developed by Chaffee & Light differed in some respects from that of Loucks. They used monkeys, and in these animals buried a small secondary coil from which one or both of its terminals were led as electrodes to the excitable focus. Two forms of primary coils and stimulating currents were tried. The first, designed for short experiments in which the operator could maintain the primary parallel to the secondary coil, carried a fluctuating current produced by the rhythmic discharge of a condenser bank by a special mercury-pool tube. The second consisted of three primary coils set up at right angles to each other, a wooden box being contained inside the coils large enough to accommodate a monkey for several weeks. The discharge currents were commutated through each of these primaries successively, so that an animal in the cage could move about and yet receive a fairly uniform intensity of stimulation.

Variations of the above techniques have been used by Fender (1937, 1941), and Clark & Ward (1937). Fender used a triple circuit unit buried in the temporalis muscle of a dog, and the primary coil was wound around the cage containing the animal. Clark & Ward,

using cats, approached the motor cortex through the frontal sinus. Into the posterior wall of the sinus was screwed a threaded stainless steel plug, in the centre of which was a cork or rubber stopper pierced by a silver wire. The silver wire extended for about 1 mm. beyond the inner face of the stopper and so rested on or slightly pierced the cortex, whilst the inner edge of the plug was flush with the inner surface of the skull. A flat coil was then planted beneath the scalp, and one terminal soldered to the silver wire reaching the brain, the other to a flat disk of silver placed subcutaneously as the indifferent electrode. The primary coil was attached to a lighting circuit via a stepdown transformer capable of delivering potentials of 4, 7, 10 and 15 V from its secondary terminals.

Recently, Greig & Ritchie (1944) have described an implantable unit and stimulator, but so far have not reported any results obtained by its use.

THE TECHNIQUE DEVISED FOR THE STIMULATION OF THE HYPOPHYSIS AND HYPOTHALAMUS

The main difference between the technique described below and those of previous workers is due to the necessity of stimulating a deeply situated part of the nervous system, whereas the previous methods were designed for stimulation of the cerebral cortex. The hypothalamus and hypophysis are as far removed from the surface of the skull as any region of the nervous system, and, as stated by Cushing (1932), there is no other structure in the body so hidden and protected as the hypophysis. The stimulation of these structures by the remote-control method, however, succeeded beyond expectation. It was found that rabbits would live indefinitely and show no ill-effects, with a small secondary coil buried between the scalp and skull, and a stimulating electrode passing from this coil vertically downwards in the mid-line through the corpus callosum, hippocampal commissure and other median structures into some part of the hypothalamus or hypophysis. Female rabbits with this unit in place have lived normal, healthy lives for periods up to 3 years and have shown a normal capacity for reproducing and rearing their young.

(1) Structure of the buried unit (see figures 1 and 2)

The buried coil consisted of 1500 turns of fine, enamelled copper wire (s.w.g. 42). This was wound to form a flat, circular coil, 20 mm. external diameter, 6 mm. internal diameter and 4 mm. thick, which was then forced on to a spherical surface so that it adapted roughly to the form of the rabbit's skull. The whole coil was then soaked in bakelite varnish and baked at 100° C for 20 min.

The indifferent electrode consisted of a flat, oval plate of German silver ($10 \times 5 \times 0{\cdot}6$ mm.), silver-soldered to the outer turn of the coil. This electrode was eventually situated beneath the scalp over the frontal bones.

For the stimulating electrode, silver wire (s.w.g. 36) insulated with glass capillary tubing to within 0·5–1 mm. of its tip was first used. This metal tended, after several months *in situ*, to become blackened at its bared tip and to deposit black granules in the surrounding tissues (probably silver sulphide granules), so that in the later preparations, platinum wire was substituted. The electrode was carried on an ebonite bush (figure 2) shaped and flanged so as to plug firmly into the screw in the skull. The soldered junction between the copper wire

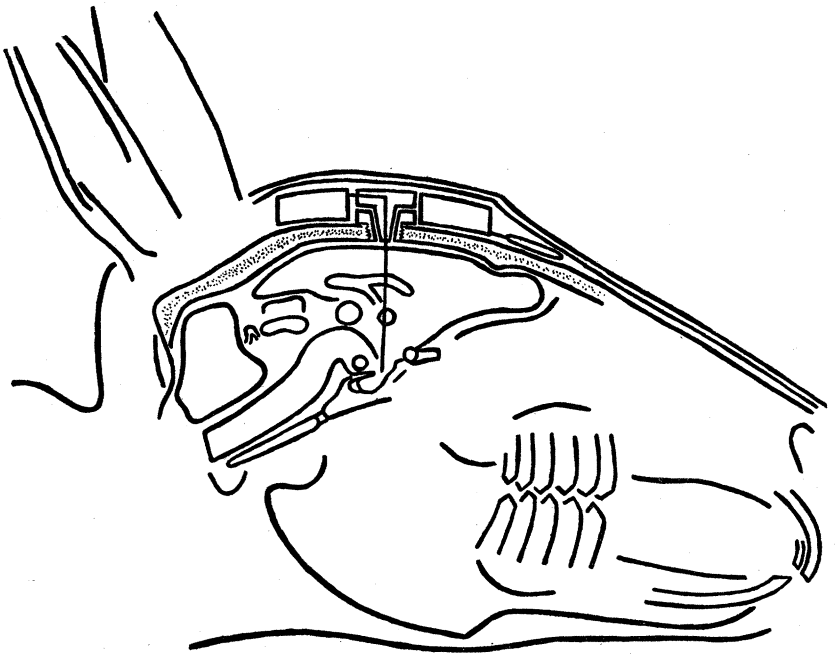

FIGURE 1. Diagram of a sagittal section through a rabbit's head with the buried unit *in situ*. The stimulating electrode, insulated to the tip, is shown descending through the corpus callosum and anterior commissure into the region of the tuber cinereum.

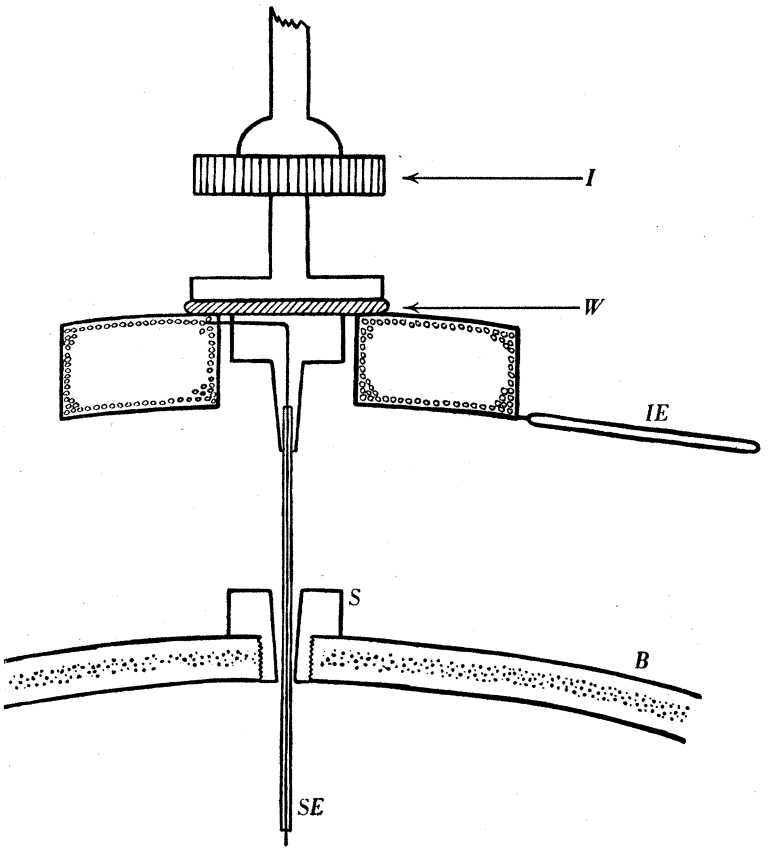

FIGURE 2. Sectional diagram to show the detailed construction of the implanted unit and the insertor. *B*, bone of skull; *I*, insertor; *I.E.* indifferent electrode; *S*, screw inserted in skull at bregma; *S.E.* stimulating electrode; *W*, wax for temporary attachment of the unit to the insertor.

of the coil and the platinum wire of the electrode was situated in a slot in the flange of the bush, and the glass insulation tubing was made to continue upwards and end in the spindle of the bush. All the connexions were painted with bakelite varnish and the whole structure rebaked. The length of the stimulating electrode varied with the region to be stimulated. For stimulation of the infundibular stem, it was made to measure 2·2 cm. from the tip to the under-surface of the flange of the bush, that is, equivalent to 2·0 cm. from the tip to the outer surface of the skull.

The screw inserted in the skull was made from 'non-toxic steel' obtained from Down Bros. Ltd., surgical instrument manufacturers. This metal was easily machined to suitable dimensions and then annealed. The majority of these screws were found to be very firmly held by the skull months or years after insertion, and only rarely did the bone show any marked inflammatory reaction to their presence.

(2) Method of inserting the secondary coil

The method of insertion at first offered some difficulty. In the preliminary animals, attempts were made to implant the unit by hand. This method was soon abandoned, as it was found impossible to avoid some slight side to side movement during the insertion, and it is obvious that in the region of the infundibular stem with its delicate nerve tracts and supply of fine vessels, this movement might cause severe damage. The method finally adopted made use of the stereotaxic instrument described previously (Harris 1937), an attachment with a universal joint being employed to secure the coil unit to the electrode carrier.

The complete operative technique was as follows. Healthy young adult rabbits, usually females, were given atropine sulphate gr. $\frac{1}{50}$ subcutaneously, and $2\frac{1}{4}$ c.c./kg. body weight of 2% chloralose in 10% urethane intravenously, half an hour before the operation began. Complete surgical anaesthesia was then induced with ether, the head was clamped in the stereotaxic instrument and orientated so that both the longitudinal and transverse tangents to the skull at bregma were horizontal. From this point onwards the level of anaesthesia remained satisfactory without the administration of further ether. A small trephine hole, bored vertically through the skull at bregma, gave access for rupture of the dura with a fine probe, any concurrent damage to the superior sagittal sinus causing only slight haemorrhage and no apparent ill-effects. A plug tap, of the same thread as the screw to be inserted, was then screwed in and out of the trephine hole. The insertion of the screw was performed by means of an attachment to the electrode carrier, so that the angle of the axis of the screw was finally related to the carrier rather than to the animal's skull. The coil unit attached to the carrier was manipulated so that the electrode tip was situated over the centre of the screw and racked down till the ebonite bush of the electrode plugged firmly home in the screw. The insertion of the screw in relation to the carrier, rather than to the rabbit's head, was found to be an important detail in the procedure, for although the electrode was slightly out of position in those animals in which the head had not been accurately orientated, it obviated the risk of any movement in the lateral or antero-posterior planes in the final plug home of the electrode. Three fine silk stays threaded through small trephine holes in the supra-orbital crests and the posterior end of the sagittal crest were tied over the coil to provide further support, and the whole covered with a thin layer of wax. No difficulty was ever encountered in approximating the skin edges, which

were carefully stitched with eversion sutures, before applying a dressing of celloidin. Full surgical asepsis was maintained throughout all operative procedures.

Healing occurred in the great majority of rabbits by first intention. Out of a total of thirty-seven rabbits, four died during the operation (three from over-anaesthesia, one from intraventricular haemorrhage), twenty-seven healed by first intention and six required secondary suture. Of the thirty-three rabbits that recovered from the operation, twenty-seven were suitable for experimentation and six were discarded. These figures include all the preliminary trials.

(3) The primary coil and stimulating circuit

After many attempts to provide a suitable a.c. by use of mechanical contact breakers, it was eventually found that a primary coil in series with a variable resistance connected with the commercial a.c. mains served the purpose simply and efficiently. The disadvantage of this method of stimulation, as pointed out by Loucks (1934), is that it entails the use of a primary coil too heavy to attach to the animal, but this was of little importance in these present experiments in which only short stimuli were required.

The primary coil in final use consisted of 2700 turns of thick enamelled copper wire (s.w.g. 20) wound on a former of paxolin tubing ($\frac{1}{2}$ in. diameter) containing an iron-wire core. The dimensions of the coil were 16 cm. long, 4·9 cm. external diameter and 1·3 cm. internal diameter. This was connected in series with a variable resistance (58–130 Ω) across the a.c. mains (200 V, 50 cyc./sec.), the root mean square value of the current passed varying from 1·2 amp. with 130 Ω in series, to 2·4 amp. with 58 Ω in series.

The voltage set up across the electrodes of the secondary coil, with the distances between the opposed surfaces of the primary and secondary coils set at 1·0 cm., was found to be 1·2 V, a value which is almost independent of the resistance across the electrodes. The form of current from the secondary coil is not an exact duplication of the sinusoidal wave form in the primary, the difference being due probably to magnification of the harmonics in the primary current.

(4) The effects of the presence of the coil

The animals were usually quiet and ate little for the first few days after operation. Subsequently, they quickly regained normal appetites and activities, and showed no effects from the presence of the coils of which they were apparently unconscious. In every way their behaviour was that of normal rabbits; their weight curves showed a gradual increase with advancing age, and their reproductive capacities were unimpaired (see figure 29, plate 15). The lack of any detrimental effects of the coil has been observed on one rabbit for over 3 years (see figures 26–28, plate 15), one rabbit for 2 years, seven rabbits for 1–2 years, eight rabbits for 6–12 months, ten rabbits for 3–6 months, and on six rabbits for less than 3 months.

All the animals had lateral radiographs of the skull and implanted unit taken immediately after operation, then at fortnightly and later at monthly intervals (see figures 26, 27, plate 15). By this means it was observed that some movement of the implanted unit occurred after a few months in a few of the earlier rabbits. There appeared to be two causes for this shifting of the coil. In one rabbit the indifferent plate electrode, which was placed posteriorly in the region of the lambdoid suture, became forced by nodding movements beneath the

edge of the coil and thus caused it to tilt. Movement in the other cases was due to the softening and accumulation beneath one edge of the coil of the paraffin wax with which the unit had been freely surrounded. This shifting was entirely eradicated in all the later cases by placing the indifferent electrode anteriorly over the frontal bones, and covering only the upper surface of the coil with a thin layer of wax. It is interesting that the few animals in which movement occurred showed no ill-effects from any damage caused by the displaced electrode.

On killing the animals that had the unit *in situ* for more than 1 month, it was found that the coil and indifferent electrode were enclosed in a very firm fibrous tissue capsule (see figure 31, plate 15). Rarely, small loculi of thick inspissated pus were found enclosed in side pockets of this capsule, but usually on dissection the coil and surrounding tissues were found to be quite clean. The screw in the skull was, in all except the few earlier trials, as firmly held as when first inserted. If the coil had been in place for more than 1 year, the screw was commonly found surrounded by a plate of new bone which had been fashioned in conformity to the under-surface of the coil, thus giving it further support (see figure 30, plate 15).

The histological procedure was as follows. The rabbits were killed with chloroform, the abdomen opened, and the abdominal aorta injected first with normal saline till clear fluid ran from the inferior vena cava, and then with 500 c.c. of a solution of 5% formaldehyde in $\frac{1}{2}$% acetic acid. The last 100 c.c. of the formaldehyde solution was injected with the inferior vena cava clamped. After half an hour the usual post-mortem examination was performed, the head with the coil still *in situ* was skinned and the lower jaw and orbital contents removed. The pituitary gland in its capsule was exposed from below by removal of the basisphenoid, and the specimen placed in the formaldehyde-acetic acid mixture for 1 week. At the end of this period, the coil and electrode were removed and the specimen dissected. If the stimulating electrode had been situated in the hypothalamus the brain was removed, trimmed to a suitable block, dehydrated and embedded in celloidin, serial sections cut at 30 μ and stained with toluidine blue. If the electrode tip had been situated in the hypophysis, a block of tissue, including the sella turcica, hypophysis and hypothalamus, was removed, dehydrated, decalcified in Jenkin's solution, embedded in celloidin, serially sectioned at 30–100 μ, and stained with haematoxylin and orange G, or haematoxylin and eosin. To clarify the histological picture in certain cases, selected sections were stained by the Weil and the Bodian methods.

The procedure of fixing the animal's head with the electrode *in situ* was of the greatest value, as the position of the electrode was easily visible as a small circular hole; and since the diameter of the bared wire at the tip of the electrode was less than the diameter of the insulating glass capillary tube, it was usually a simple matter to decide from the diameter of the hole in the sections the exact site of the stimulating tip (figures 32, 33, 35 and 36, plate 16).

The tissues around the electrode track showed surprisingly little reaction. The nervous tissue responded to the insulating glass tube with only a slight amount of gliosis, as shown by the presence of microscopically normal nerve cells up to the electrode site (figure 34, plate 16). In the earlier animals in which silver electrodes were used, a deposit of black granules intra- and extracellular in position around the tip of the electrode were seen. In the animals in which platinum electrodes were used, very little reaction was observed in the tissues surrounding the bare metal.

(5) The visible effects of stimulation

Conscious animals in which the tip of the stimulating electrode was situated in or near the pituitary stalk showed the following effects on applying a stimulus of gradually increasing intensity. First, elevation of the upper eyelids combined with rotation of the eyeballs was observed. On stronger stimulation, the elevation of the lids and rotary movements of the eyeballs was increased and an obvious enophthalmos occurred. Following this, the eyelids tended to close, thus obliterating the rima palpebrarum, the vibrissae quivered, the pupils constricted, the jaw muscles contracted, and finally extensor movements of the forelimbs could be obtained. The mechanisms underlying these signs are not entirely clear. The rotary movements of the eyeballs, the elevation of the upper lids and the enophthalmos are almost certainly due to spread of current to the third nerve which is in close proximity to the hypophysial stalk. The movements are abolished by sectioning this nerve. It is suggested that closure of the lids (abolished by sectioning the seventh nerve) and movement of the vibrissae (partly abolished by sectioning the seventh nerve) are mainly due to spread of current to the internal capsule, the anterior and medial fibres in this tract being chiefly affected. The contraction of the jaw muscles is possibly due to direct spread to the fifth nerve as it lies near the cavernous sinus, or again, spread to the internal capsule; the constriction of the pupils is probably due to direct stimulation of the autonomic fibres in the third nerve; and the extensor movements of the head and forelimbs to the further spread to the internal capsule. Stimuli strong enough to cause movements of the forelimbs were rarely applied and then only in animals under a sedative. Movements of the hindlimbs were never observed.

Under surgical anaesthesia, all these reactions tended to disappear. If stimulation was performed immediately after finishing the operation of implanting the coil, the above signs would be obtained in a mild degree, if at all, and would show a gradual increase in intensity during the following 4–6 hr. until recovery from the anaesthetic was complete.

The great majority of rabbits, in the unanaesthetized state, would sit perfectly still during the stimulation periods and show no sign of fear, pain or any emotional reaction, till a stimulus strong enough to cause extensor movements of the head was applied, when sharp, sudden, voluntary movements of the head would occur, followed if the animal was restrained, by struggling. In a small group of animals in which the stimulating tip was placed more anteriorly, that is, nearer the optic chiasma, signs indicative of an unpleasant subjective reaction occurred with a weaker stimulus, and in another group of control rabbits in which the electrode tip was in the dorsal hypothalamic area, near the anterior nucleus of the thalamus, the first visible sign on applying a stimulus was a jerk of the head. Apart from these few cases, the only need for restraint during stimulation was to prevent the animal sniffing its surroundings in the usual inquisitive fashion, and it was found that one hand of the operator gently holding the animal's neck was sufficient to keep it still, so that a constant stimulus could be applied.

(6) The standardization of the stimulus

The strength of the stimulus applied can be controlled and varied easily, either by varying the voltage applied across the primary coil, or the distance between the primary and secondary coils. The latter method was generally used. The distance between the two coils

was measured from the lower surface of the primary to the upper surface of the buried secondary, and will be referred to in future as the inter-coil distance (I.C.D.). The only difficulty in making this measurement lies in the estimation of the thickness of the soft tissues overlying the buried coil, and this measurement was made radiographically.

In the earlier animals, it was found that the efficiency of the buried unit began to decrease gradually after a period of about 8 months, so that finally a decreased I.C.D. or increased current in the primary coil was needed to give the same effect. This phenomenon appears to be due to several factors. First, corrosion of the buried silver wire at the stimulating tip with deposition of a layer of (probably) silver sulphide. The second factor was the detachment of the indifferent electrode from the coil. This occurred in a few of the earlier preparations in which the plate electrode had been joined to the outer turn of the coil with ordinary lead solder. And thirdly, the formation of scar tissue which, however negligible histologically, may play a small part in this loss of efficiency. It is hoped that these factors have been to a large extent overcome by (*a*) using platinum wire for the stimulating electrode and (*b*) fixing the indifferent electrode to the coil with silver solder. In the later preparations the efficiency of the unit has remained constant until the animals were killed, that is, for periods up to 10 months from the insertion of the coil.

To ensure standardization of the stimuli, the following procedure was adopted. The day after operation the animal was X-rayed, the thickness of the soft tissue measured and the preparation calibrated, the visible effects being noted at a series of different I.C.D. Assuming that similarly constructed buried units developed the same voltages across their electrodes when in a field of a given flux density, the above values served as a useful check and comparison for stimulation of different animals at later dates.

THE RESULTS OF STIMULATION OF THE NEUROHYPOPHYSIS

Remote-control stimulation of the supraoptico-hypophysial tract in the median eminence, infundibular stem and infundibular process has been performed, with control stimulations in the pars distalis and pars intermedia of the hypophysis, in various regions of the hypothalamus and in the region of the anterior nuclei of the thalamus.

The effect of stimuli in most of these areas has been noted on (*a*) water diuresis, (*b*) concentration of urinary chloride, (*c*) blood sugar, (*d*) uterine activity.

In the earlier experiments some of the results were obtained in animals under a sedative (small doses of paraldehyde, given by stomach tube, 1 c.c./kg. body weight, or 2% chloralose in 10% urethane, 2·5–3·5 c.c./kg. body weight injected intravenously), but in the later experiments all the effects were observed in the fully conscious animal.

It will be of assistance in describing the experimental procedures and results if the animals are first classified. They fall naturally into three groups on the bases of the position of the stimulating tip of the electrode and the response obtained by stimulation (see tables 1, 2).

TABLE 1

group A	control animals	electrode tip more than ½ mm. from the neurohypophysis or supraoptico-hypophysial tract
group B	animals giving submaximal responses on stimulation	electrode tip within ½ mm. of, but not in contact with, the neurohypophysis or supraoptico-hypophysial tract
group C	animals giving maximal responses on stimulation	electrode tip in, or in contact with the neurohypophysis or supraoptico-hypophysial tract

TABLE 2. POSITION OF ELECTRODE TIP IN THE THREE GROUPS OF ANIMALS
(see figures 32–43, plates 16 and 17)

Experiments on water diuresis were made in all the animals, on the urinary excretion of Cl in seven, on the blood sugar in five, and on uterine activity in thirteen of the animals

group A animals	position of electrode tip
2	in left ventromedian hypothalamic nucleus
3	near right paraventricular hypothalamic nucleus
4	near left paraventricular hypothalamic nucleus
8	near right paraventricular hypothalamic nucleus
10	near right paraventricular hypothalamic nucleus
11	in right margin of tuber cinereum
12	in right ventromedian hypothalamic nucleus
18	in pars distalis, $\frac{1}{2}$–1 mm. from infundibular stem
19	near right paraventricular nucleus
22	in dorsal hypothalamic area
23	between anteromedial thalamic nuclei
27	in pars distalis, $\frac{1}{2}$–1 mm. from infundibular stem
29	in right border of tuber cinereum
30	in posterior part of tuber cinereum, mid-line, 500–700 μ above upper surface of stalk
32	in right posterior region of tuber cinereum
group B animals	
25	in pars intermedia
26	in pars distalis, $\frac{1}{2}$ mm. from infundibular stem
28	in posterior region of tuber cinereum, $\frac{1}{2}$ mm. from infundibular stem
31	in pars distalis on right of infundibular stem, $\frac{1}{2}$ mm. distant
33	centrally in pars distalis less than $\frac{1}{2}$ mm. from infundibular stem
34	in posterior region of tuber cinereum, less than $\frac{1}{2}$ mm. from infundibular stem
group C animals	
13	in junction of infundibular stem with infundibular lobe
15	in infundibular lobe, slightly to left
17	in junction of median eminence and infundibular stem, slightly to right
20	in junction of median eminence and infundibular stem, mid-line
21	in contact with right side of infundibular stem
35	in contact with right side of infundibular stem

(1) THE NEUROHYPOPHYSIS AND WATER DIURESIS

The relationship between the nervous system and the control of the water balance of the body is a much discussed subject. The discovery by Magnus and Schafer (1901), and Schafer & Herring (1906), that extracts of the posterior lobe of the pituitary have a diuretic effect on anaesthetized animals led many workers to conclude that diabetes insipidus was caused by an irritative lesion of the hypophysis (Frank 1910). Von den Velden (1913) showed, however, that posterior lobe extract could exert a marked controlling effect clinically on the output of urine in patients suffering from diabetes insipidus, and Camus & Roussy (1913) demonstrated experimentally that a lesion of the hypothalamus was sufficient to produce this condition. In view of this and other work it was generally accepted that a destructive lesion was the true cause of the diabetes, and that the diuresis previously obtained by Schafer and others on injection of extracts was due to the anaesthetized state of the animals. The site of the lesion, whether hypothalamic or hypophysial, necessary to produce a polyuric condition was argued for many years; and the relationship of the pars distalis of the pituitary and the possible neurocrine secretion by the hypothalamic nuclei to the water balance of the body are still debatable points.

The concept advanced by Fisher, Ingram & Ranson (1938), after a series of classical experiments in which they produced diabetes insipidus in many cats and monkeys by placing small well-defined lesions in the supraoptico-hypophysial tracts, laid a firm foundation for the current views on this subject. They suggested that the supraoptico-hypophysial tract regulates the secretion of the anti-diuretic hormone by the neural division of the hypophysis, the term neural division being used so as to include the median eminence and infundibular stem, as well as the infundibular process. Under normal conditions, the anti-diuretic hormone and the diuretic processes of the body are balanced in their action on the kidney, and if a deficiency of the anti-diuretic factor occurs, the balance is upset and a polyuria results. The diuretic processes are to a large extent under the control of the pars glandularis of the hypophysis, probably acting indirectly through an effect on the general metabolism.

The work of Verney and his colleagues, starting with the well-known account by Starling & Verney (1925) on the excretory properties of the heart-lung-kidney preparation of the dog, has demonstrated very clearly the part played by the neurohypophysis in the regulation of urine output in the normal animal. Verney's views may be summarized as follows (see Verney 1936). Ingestion of water by an animal acts on the central nervous system, which in turn inhibits the secretory activity of the neurohypophysis, thus allowing the excretion of the excess water. When this is removed, the activity of the pituitary is resumed and the urine concentrated to the usual extent. Emotional stress, muscular exercise, pain or fear have been shown to exert an inhibitory influence over a water diuresis, the evidence being strongly in favour of the view that these factors act through the mediation of the nervous system and the neural lobe of the pituitary (see Rydin & Verney 1938; O'Connor & Verney 1942).

A great deal more work could be quoted to show the importance of the pituitary secretions in the regulation of the water balance; an excellent summary of all aspects, however, may be found in the monograph by Fisher *et al.* (1938). It may be stated that in spite of many discordant views, such as those of Molitor & Pick (1924, 1925 *a*, *b*, 1926), who emphasize the direct effect of a mid-brain centre on the tissue fluid, of Biggart (1935, 1936, 1937), who believes the pars tuberalis and centres in the tuber cinereum play an important part in the pituitary control, and of Vasquez-Lopez (1942), who draws the assumption from histological evidence that the neurohypophysis is a large chemo-receptor sensory organ, the evidence is much in favour of the views of Ranson and of Verney.

One striking though hardly surprising fact is that nearly all the experimental interference with the hypophysial region has tended towards the production of lesions with a consequent diabetic state, rather than the application of stimuli to investigate the possible inhibition of a water diuresis. The lack of interest in this latter mode of attack is due to the fact that the anaesthesia necessary for most methods of stimulation in itself inhibits diuresis. Haterius (1940) is one of the few workers who has used the method of stimulation, and the results he obtained are not altogether convincing. He studied the effects of pituitary stalk stimulation in anaesthetized rabbits. Out of several anaesthetics tried, a solution of chloralose and urethane given intravenously was found to exert the least depressing effect on a water diuresis. Stimulation was performed by clamping and orientating the animal's head in a stereotaxic instrument, trephining a small hole at bregma, and passing fine

electrodes into the region of the stalk. The anaesthesia and operative interference invariably inhibited the diuresis, and subsequent administrations of further water by stomach tube frequently resulted in death. Occasionally a good diuresis was obtained, and, in all, eight experimental and six control animals were available in which stimulation had been applied during an appropriate diuresis. Of the eight experimental animals, five showed a definite inhibition of urine flow, and three only a transient depression during the period of stimulation. In one of these three, post-mortem examination showed that the electrode points were situated 1–2 mm. lateral to the stalk; in the second the electrodes were perfectly placed, and there was no explanation for the lack of response; and in the third the brain was accidentally discarded before examination. Controls were performed in which the pituitary stalk was interrupted 2–4 days previously. In four rabbits with verifiably interrupted stalks, stimulation in the same region as in the experimental animals gave no inhibition of the diuresis. Haterius quotes a personal communication from Ingram who has obtained anti-diuretic responses in the cat by stimulation adjacent to the supraoptico-hypophysial tract.

Many workers, including Weed, Cushing & Jacobson (1913), Keeton & Becht (1916), Hanchett (1922), and Ingram & Barris (1935), have studied the effects of 'pituitary' stimulation on the urine flow of non-hydrated animals. They have all described a mild polyuria as resulting from the stimulation, but the position here is complicated by the anaesthesia and oft-resultant glycosuria, which in itself might have been associated with a slight polyuria.

Method

The method of performing the diuresis experiments was as follows. The rabbit with a coil unit *in situ*, starved from the previous day, was given 50 c.c. warm water/kg. body weight, by stomach tube, and an hour and a half later a second dose of 40 c.c./kg. by the same route. Urine samples were collected and measured every quarter of an hour, the bladder being emptied by manual expression. When the urine flow had increased to a level of 8–10 c.c./$\frac{1}{4}$ hr., usually within $\frac{1}{2}$–$\frac{3}{4}$ hr. of administering the second dose of water, the stimulation or injection was performed. In the earlier experiments, a sedative dose of some hypnotic was given, either 1 c.c. paraldehyde/kg. body weight by stomach tube with the second dose of water, or $2\frac{1}{2}$–$3\frac{1}{2}$ c.c./kg. body weight of 2% chloralose in 10% urethane solution, intravenously, at the time of the first dose of water. These doses were usually just sufficient to keep the animal lying quietly on its side, and had little inhibitory action on the diuresis. Since there was no obvious difference in the results of stimulation with or without the sedative, the later experiments were performed on fully conscious rabbits, and in the description given below all the results are grouped together.

Criteria of an anti-diuretic response

In deciding whether a decrease in urine flow should be accepted as a positive response, the following arbitrary standards were taken as characteristic of a true anti-diuretic effect.

(*a*) The urine flow at the time of stimulation must be rising or have attained a value of more than 8 c.c./$\frac{1}{4}$ hr.

(*b*) Although the first sample of urine following stimulation might or might not show a decrease, the second sample must show a marked decrease to a level of 3 c.c./$\frac{1}{4}$ hr. or less.

(*c*) The urine output must eventually rise again to a flow of at least 5 c.c./$\frac{1}{4}$ hr.

A marked anti-diuretic effect offered no difficulty in interpretation, for following stimulation the urine flow decreased to a value of about 0·5 c.c./$\frac{1}{4}$ hr., and remained at this level for 1 or 2 hr. before rising. A slight decrease in urine flow following stimulation was often of doubtful significance. In these cases the experiment was usually repeated and the relation of the size of response to the intensity of stimulation observed; if the fall had been a 'spontaneous' inhibition, no relationship was found, but if the fall was attributable to the stimulus, a slightly greater stimulus produced a greater inhibition of urine flow and vice versa. In this manner, the significance of the doubtful responses in which the urine flow fell to about 3 c.c./$\frac{1}{4}$ hr., was usually settled, not by a rigid adherence to the above arbitrary standards but by a repetition of the experiment on the *same* animal (see figure 3).

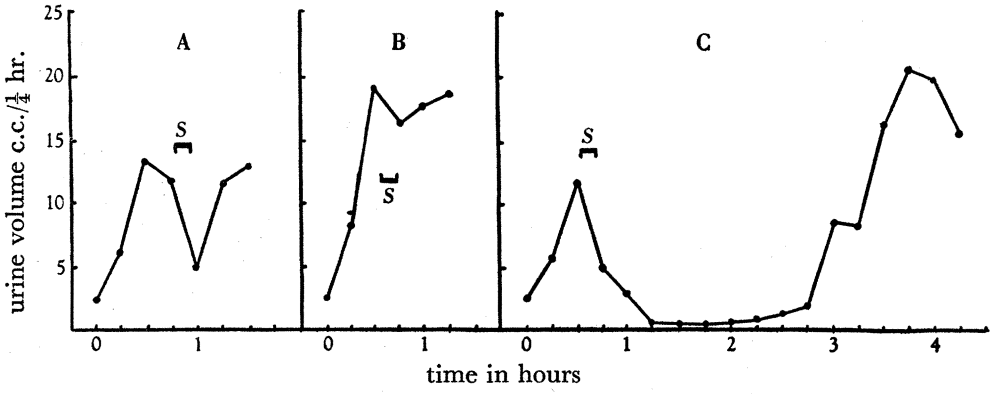

FIGURE 3. *A*, *B*. Diuresis curves, rabbit 18, 12 February 1943 (*A*) and 19 February 1943 (*B*). To show one method of distinguishing a questionable anti-diuretic effect (*A*) by repetition of same experiment. The stimuli applied during the course of the two experiments were of the same intensity (I.C.D. 1·75 cm.). *C*. Diuresis curve, demonstrating a well-marked anti-diuretic effect following stimulation of the infundibular lobe (rabbit 15, 14 August 1942).

It is well known from the work of Verney and his associates that emotional stress and exercise may inhibit a water diuresis in dogs, probably acting through a reflex stimulation of the neurohypophysis, and therefore the possibility arose that the handling of the rabbits necessary for the application of the stimulus, or some other factor apart from the stimulus itself, might be responsible for the anti-diuretic effects observed. That this is not so is shown clearly by the following facts: (i) the clear-cut difference in the results obtained in the control animals, group A, and the experimental animals, group C; (ii) the animals that responded to stimulation were subjected to control 'stimuli' in which the primary coil was held at right angles to the buried secondary coil. These control procedures involved similar degrees of disturbance to and handling of the rabbits, without the electrical stimulation, and in no case was an anti-diuretic effect observed; (iii) various procedures that effectively disturbed and frightened the animals (such as the passage of a stomach tube) only occasionally produced a mild anti-diuresis. Thus it may be assumed that the inhibition of urine flow observed to follow an appropriate stimulus was not due to any emotional stress accompanying the application of that stimulus.

Group A rabbits

This group includes fifteen control animals that had the stimulating tip of the electrode in the thalamus, hypothalamus or pituitary, though at a distance greater than $\frac{1}{2}$ mm. from the supraoptico-hypophysial tract or neurohypophysis. The exact site of the electrode tip was defined by histological examination (see table 2).

These fifteen rabbits received a total of 104 stimulations applied during water diuresis. As may be seen from table 3, ninety-four stimuli of sufficient strength (or greater) to produce a maximum anti-diuretic effect in the experimental animals in group C were without effect in these control animals. In ten cases, however, an anti-diuretic effect was observed, and of these ten, four on repetition with the same or stronger stimuli gave negative results, three were experiments on animals with the electrode tip in close proximity to the anterior nucleus of the thalamus in which stimulation produced signs of marked emotional stress, and the remaining three positive effects were seen in rabbits in which an excessively strong stimulus had been applied, sufficient to cause contraction of the muscles of the neck and forelimbs.

The results of an experiment which is typical of those in this group are shown in figure 4. Stimuli were applied at *S* over a period of 9 min.—1 min. on, 1 min. off, etc.; the I.C.D. was 1·15 cm., and the current in the primary coil 1·2 amp. Visible signs of stimulation were elevation of the upper eyelids, enophthalmos, marked closure of the lower lids and slight movement of the vibrissae; but so strong a stimulus, applied in this instance to the pars distalis, failed to produce an anti-diuretic effect.

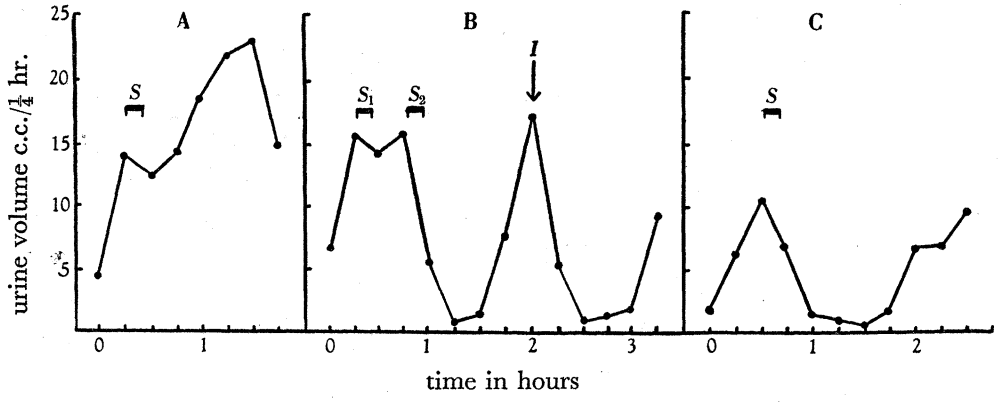

FIGURE 4. *A.* Diuresis curve, rabbit 27 (group A), 14 July 1943. No anti-diuretic effect seen to follow strong stimulation of pars distalis of pituitary (I.C.D. 1·15 cm.). *B.* Diuresis curve, rabbit 25 (group B), 21 June 1943. No anti-diuretic effect follows stimulation S_1 (I.C.D. 1·8 cm.), but on increasing the intensity of the stimulus, a moderate anti-diuresis occurs in response to stimulation S_2 (I.C.D. 1·4 cm.) slightly less in magnitude than the response to 2 mU 'pitressin', injected intravenously at *I*. *C.* Diuresis curve, rabbit 21 (group C), 5 January 1943. A marked anti-diuresis follows a weak stimulus (I.C.D. 2·8 cm.) to the infundibular stem.

Group B rabbits

This group includes six animals that had the stimulating tip of the electrode within $\frac{1}{2}$ mm. of the supraoptico-hypophysial tract or neurohypophysis. The exact sites of the electrode tip are given in table 2.

As may be seen from table 4, out of a total of sixty-three stimulations applied during water diureses in these six rabbits, thirty-nine were effective in producing an anti-

diuresis, whereas twenty-four were negative. These figures lack significance unless consideration is paid to the strength of the stimuli used, for all stimuli above a certain critical value of intensity were effective, and below this value ineffective, in inhibiting the urine flow. This critical value varied in the different animals and was most easily measured in terms of the I.C.D. (with a constant current of 1·2 amp. in the primary coil). The figures for the individual animals are given in table 4 and may be compared with the similar values given in table 5 for the rabbits in group C. These critical values of the intensity of stimulation, as given by the I.C.D.'s, are approximations to a slightly variable value. Walker (1939) found that the sensitivity of the anti-diuretic response of different rabbits to injections of posterior lobe extracts varies, as does the sensitivity of the same animal on different days. Thus, it is hardly surprising that the responses to stimulation vary slightly from day to day, since a response obtained by stimulation of the animal's own gland probably entails more variable factors than does the same effect obtained by intravenous injection of an extract.

The results of an experiment which is typical of those in this group are shown in figure 4. At S_1 stimuli were applied over a period of 9 min.—1 min. on, 1 min. off, etc.; the I.C.D. was 1·8 cm. and the current in the primary coil 1·2 amp. The visible signs of stimulation were slight elevation of the upper eyelids and slight enophthalmos on both sides. There is no appreciable change in the rate of urine flow. At S_2 stimuli were again applied, the I.C.D. having been reduced to 1·4 cm. The visible signs of stimulation were a well-marked elevation of the upper eyelids and enophthalmos on both sides. As a result of this stronger stimulus the urine flow is definitely inhibited, the response being slightly less than that to 2 mU 'pitressin' injected intravenously at *I*.

TABLE 3. THE EFFECTS OF MAXIMAL AND SUPRAMAXIMAL STIMULI ON WATER DIURESIS IN THE CONTROL RABBITS OF GROUP A

animal	total number of stimuli applied	negative responses	positive responses	remarks
2	3	2	1	one marked anti-diuretic effect; on repetition negative
3	8	8	0	—
4	1	1	0	—
8	3	3	0	—
10	4	2	2	very strong stimuli giving spread to the internal capsule with movements of neck and forelimbs
11	2	2	0	—
12	6	6	0	—
18	29	26	3	two with strong stimuli; on repetition, negative results. One with very strong stimuli, giving spread to internal capsule
19	18	18	0	—
22	6	5	1	very slight anti-diuretic response. Animal showed signs of disliking stimulus, viz. jerking movements of head
23	8	6	2	animal showed signs of disliking stimulus, viz. furious struggling
27	5	5	0	—
29	4	4	0	—
30	3	2	1	very slight anti-diuretic response; on repetition, negative
32	4	4	0	—
total 15	104	94	10	—

TABLE 4. THE RESULTS OBTAINED WITH STIMULI OF VARYING INTENSITIES IN RABBITS OF GROUP B

The proportion of negative and positive responses lack significance unless the strength of the stimuli is considered also. The critical value of the I.C.D. is a measure of the critical stimulus at which an anti-diuretic response is just obtained.

animal	total number of stimuli applied	negative responses	positive responses	critical value of the I.C.D. in cm.
25	15	9	6	1·4
26	27	8	19	1·5
28	6	1	5	1·7
31	5	1	4	1·5
33	6	4	2	1·6
34	4	1	3	1·9
total 6	63	24	39	

Group C rabbits

This group includes six animals that had the stimulating tip of the electrode in or in contact with, the supraoptico-hypophysial tract or neurohypophysis. The exact sites of the electrode tip as defined by histological examination are given in table 2.

As may be seen from table 5, the six rabbits in this group had a total of 158 stimulations applied during water diureses, of which 110 were effective in producing an anti-diuresis, and forty-eight ineffective. These figures again lack significance by themselves, for stimuli of less than the critical value would have produced probably 100% negative responses, and stimuli greater than the critical value, 100% positive responses. The critical I.C.D. were unfortunately not measured in the first two rabbits (13, 15), but the extent and duration of the anti-diuretic effect after stimulation in these two animals left no doubt as to the electrode site, which was verified histologically. In the other four animals the critical I.C.D. was in each case greater than 2·5 cm.; this means that a much weaker stimulus would produce an anti-diuretic response in these animals than in the group B rabbits, even though the latter had the stimulating tip of the electrode within $\frac{1}{2}$ mm. of the neurohypophysis.

Results which are typical of those obtained in this group are shown in figure 4. Stimuli were applied at *S* over a period of 9 min.—1 min. on, 1 min. off, etc.; the I.C.D. was 2·8 cm. and the current in the primary coil 1·2 amp. The visible signs of stimulation were slight elevation of the right upper eyelid, less of the left, and slight enophthalmos on the right side. This weak stimulus is followed, as the figure shows, by a marked anti-diuretic effect.

TABLE 5. THE RESULTS WITH STIMULI OF VARYING INTENSITIES IN RABBITS IN GROUP C

The proportion of negative and positive responses lack significance unless the strength of the stimuli is considered. The critical value of the I.C.D. is a measure of the critical stimulus at which an anti-diuretic response is just obtained.

animal	total number of stimuli applied	negative responses	positive responses	critical value of the I.C.D. in cm.
13	12	4	8	—
15	12	3	9	—
17	46	12	34	3·0–3·5
20	40	12	28	2·5–3·0
21	32	16	16	more than 2·8
35	16	1	15	more than 2·8
total 6	158	48	110	

It should be emphasized that the anti-diuretic effects of stimulation of the neurohypophysis were similar to those following intravenous injection of appropriate doses of posterior lobe extracts (see figure 4). The following extracts were used: 'pituitrin' and 'pitressin' (Parke, Davis and Co.), pituitary (posterior lobe) extract (Boots Pure Drug Co.), and extract of posterior lobe of the pituitary of rabbits.

The spread of the stimulus

Two salient facts emerge from these results. The method of stimulation used makes it possible to apply a precise and highly localized stimulus to the neurohypophysis, and the nerve fibres supplying this organ are relatively insensitive to electrical stimulation.

In group A were rabbits in which the stimulating tip of the electrode was situated at varying distances from the neurohypophysis and in which the anti-diuretic responses obtained on stimulation were few in number. That these responses were not due to direct stimulation of the neurohypophysis or its nerve supply is apparent from the evidence already given and from the fact that the number of positive responses obtained in the different animals was not related to the proximity of the electrode tip to the neurohypophysis or any known nerve supply of this organ. Thus, it is justifiable to assume that the few positive responses seen in this group were of a non-specific nature, and it follows that the current spread effective as a direct stimulus to the neurohypophysis was, with maximal or supramaximal stimuli, less than 1 mm. This point is well illustrated by reference to rabbits 18 and 27. These animals both had the electrode tip situated in the pars distalis of the pituitary at a distance of $\frac{1}{2}$–1 mm. from the neurohypophysis, and out of thirty-four strong stimuli applied only three were followed by an anti-diuresis. Of these three responses, two were not repeatable, and the one remaining positive response was obtained when a stimulus was applied which was sufficiently intense to spread to the internal capsule.

More complete evidence as to the current spread was obtained from the experiments on the rabbits in group B. In these animals the stimulating tip was within $\frac{1}{2}$ mm. of some part of the neurohypophysis, and it was found a simple matter to apply a stimulus with the I.C.D. greater than the critical value and so fail to obtain an anti-diuretic response, or alternatively, to decrease the I.C.D. and obtain a definite anti-diuresis. Thus it is possible to say that in these experiments, the effective current spread, so far as the fibres in the infundibular stem are concerned, is of the order of $\frac{1}{2}$ mm. with an I.C.D. of 1·4–1·9 cm., and that with the greater I.C.D., effective in producing marked anti-diuresis in the rabbits in group C, the current spread must be still less.

It is worth stressing the relative insensitivity of the fibres in the infundibular stem to the electrical stimuli used in this work, and probably to other types of stimuli. In no case was an anti-diuretic effect obtained unless a stimulus was applied strong enough to cause at least slight excitation of the somatic fibres in the oculomotor nerve, as shown by elevation of the upper eyelids. The third nerve in the rabbit lies about $1\frac{1}{2}$ mm. from the infundibular stem.

One other point of interest follows from the results already given. It was found that a weaker stimulus would suffice to produce an anti-diuresis when the electrode tip was in the infundibular stem rather than in the infundibular lobe, and when the tip was $\frac{1}{2}$ mm.

from the stalk rather than in the pars intermedia (less than ½ mm. from the infundibular lobe). Comparison might be drawn with the pulls exerted by a muscle when the stimulating electrode lies in the muscle on the one hand, and in its motor nerve on the other. In the one case, a direct stimulus is applied to a proportion only of the organ, and in the other case all, or the major part of the organ, is stimulated indirectly through its nerve supply.

The constancy and gradation of the anti-diuretic response

If, during a water diuresis, a given stimulus was repeated after the anti-diuretic effect of any previous stimulus had disappeared, the second response tended to be greater than the first (see figure 5). This fact has been observed in a series of fourteen experiments on three rabbits of group C. The first observations were made on rabbits under a sedative dose of chloralose and urethane, and it was thought that the increasing effect of similar stimuli was due to the decreasing level of anaesthesia as the experiments progressed. That this was not the whole explanation was shown by the fact that in five later experiments the same results were obtained without the use of any sedative. An alternative hypothesis is that the same stimulus had a greater anti-diuretic effect as the water load of the animal decreased (see Hart, D'Arcy & Verney 1934; Pickford 1936). However, in eleven experiments in which additional water was given by stomach tube between consecutive stimuli, and time allowed for absorption of this water, the response to the subsequent stimulus was diminished to the previous level in one case only.

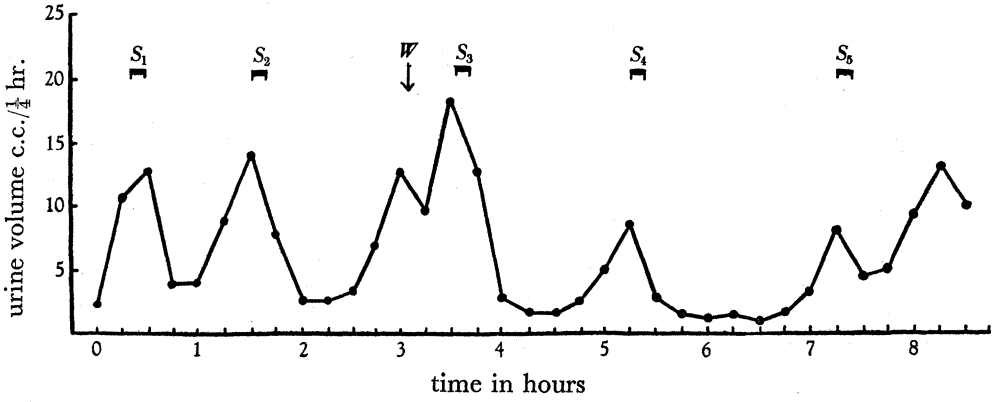

FIGURE 5. Diuresis curve, to show the effect of repeated stimuli rabbit (17), group C, 28 December 1942. Stimulations S_1, S_2 and S_3 of equal strength (I.C.D. 2·4 cm.); stimulation S_4 weaker than preceding (I.C.D. 2·8 cm.); and stimulation S_5 possibly below threshold value (I.C.D. 3·5 cm.). Note increasing effect of stimuli, in spite of 100 c.c. water administered by stomach tube at 3 hr. 5 min. (*W*).

Whatever the true explanation of the above phenomenon may be, it is clear that in comparing the effects of different stimuli, certain criteria must be observed as regards the water dosage and time interval between administration of water and stimulation. With these factors standardized the anti-diuretic responses to stimulations were remarkably constant from day to day and could be graded easily by varying the I.C.D. (see figure 6).

If, however, the responses to a given stimulus were compared over a period of months, they tended, in some rabbits, to show a slight decrease beginning about 8 months after insertion of the coil. Since the visible effects of stimulation in these animals decreased

pari passu with the anti-diuretic response, it is felt that the diminution in the response is almost certainly due to loss of efficiency of the implanted unit, rather than to any damage to the hypothalamus or hypophysis. It is perhaps noteworthy that two rabbits in which the stimulating electrode was made of platinum did not show this phenomenon over periods of 8 and 10 months respectively.

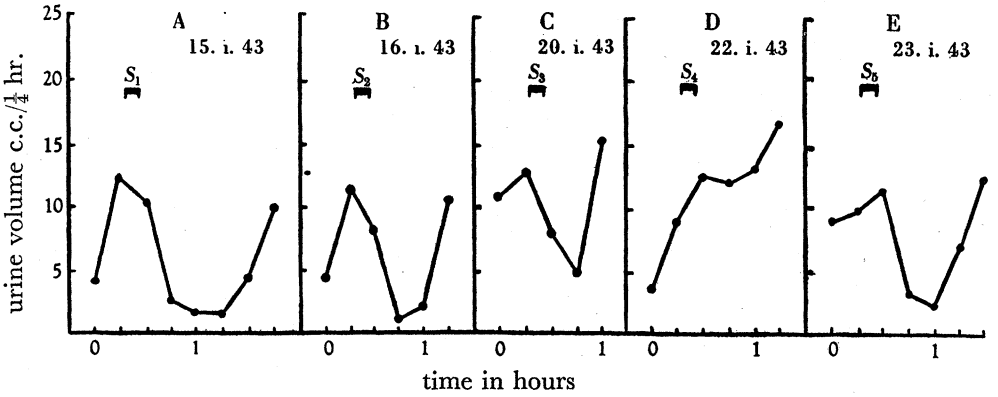

FIGURE 6. A series of diuresis curves, to show gradation of anti-diuretic responses to graded stimuli. Rabbit 17 (group C). *A*, 15 January 1943, stimulus S_1 (I.C.D. 2·5 cm.); *B*, 16 January 1943, stimulus S_2 (I.C.D. 3·0 cm.); *C*, 20 January 1943, stimulus S_3 (I.C.D. 3·5 cm.); *D*, 22 January 1943, stimulus S_4 (I.C.D. 4·0 cm.); *E*, 23 January 1943, stimulus S_5 (I.C.D. 3·0 cm.). These curves also demonstrate the constancy of response to stimuli of equal intensity—compare *B* and *E*.

The effect of sedative doses of anaesthetic on the anti-diuretic response

As stated previously, the preliminary experiments in three rabbits in group C were performed under sedative doses of chloralose and urethane. All the later experiments were performed on fully conscious animals. On comparing the results of stimulation applied with and without the use of the sedative, it is clear that a greater anti-diuretic response is obtained with a given stimulus if the animal is fully conscious, and it seems likely that this is due to a depressant action of the chloralose-urethane mixture on the nerve fibres supplying the neural lobe of the pituitary.

The exclusion of the adrenal medulla as participating in the anti-diuretic response

Since the experiments had been performed mainly on fully conscious rabbits in which some emotional stress is almost certainly evoked by the experimental procedures, the possibility that this stress participated in the anti-diuretic reactions had to be considered. This possibility was disproved by the control experiments and for other reasons given previously (see under 'Criteria of an anti-diuretic response').

The means whereby emotional stress may inhibit a water diuresis have been discussed by Rydin & Verney (1938), and these workers have shown that the reaction is not effected through the renal, suprarenal and splanchnic nerves, and that injection of adrenaline in the dog produces a very transitory anti-diuresis different from that produced by emotional stress or intravenous injection of post-pituitary extract.

It was felt, however, that it would be of interest to see what type of anti-diuresis would be produced in the rabbit under the present experimental conditions by intravenous

administration of adrenaline. For this purpose, a series of fourteen diuresis experiments were performed in seven normal rabbits under conditions similar to those of the previous experiments. On the development of a good diuresis following the second dose of water, 1 c.c. of 1/150,000 solution of 'adrenalin chloride' (Parke, Davis and Co.) was injected intravenously. This dose was sufficient to cause a marked temporary bradycardia, but in none of the fourteen cases was a definite anti-diuresis observed to follow the injection. This result might have been predicted from the observations of Rydin & Verney (1938), who showed in dogs that the anti-diuresis following the intravenous injection of a corresponding dose of adrenaline was of a fleeting nature and persisted for a few minutes only. This effect would tend to be masked by the collection of quarter-hourly urine samples.

The converse type of experiment was performed on rabbit 20, belonging to group C. This animal (which at the time had a vaginal fistula—to be described later) had the left adrenal gland removed, the right and left splanchnic nerves and the right lumbar sympathetic chain torn out. Thus the left adrenal was removed and the right gland denervated, according to the nerve supply of the adrenal described by Young (1939) and Maycock & Heslop (1939) in cats. The animal made a good recovery from the operation, and it was found that the effect of stimulation of the infundibular stem on a water diuresis differed in no way from the anti-diuretic effect obtained previous to the adrenal denervation. There still remains the possibility that the right adrenal may have received a few fibres from the left sympathetic chain (a contralateral innervation in the cat is said not to occur), but it is felt that at least a quantitative difference in the reaction would have appeared if the adrenal innervation had been of importance.

These results reinforce the belief that the secretion of adrenaline plays little or no part in the anti-diuresis obtained by and ascribed to stimulation of the neurohypophysis.

(2) The neurohypophysis and concentration of urinary chloride

Von den Velden (1913) first reported that the subcutaneous injection of posterior lobe extract in man inhibited a water diuresis and at the same time caused an increased concentration of urinary chloride. Following this work the effects of posterior lobe extracts on urinary salts were investigated by many workers, and it was found that in both the hydrated and non-hydrated animal one of the most typical actions of this extract was to increase the concentration of urinary chloride (Stehle & Bourne 1925; McIntyre & Sievers 1933; and many others). The chloride effect has more recently been used as evidence that an anti-diuresis produced by some experimental procedure has occurred through the mediation of the animal's own pituitary. Thus, Pickford (1939) showed that administration of acetylcholine inhibited a water diuresis in dogs and at the same time increased the concentration of urinary chloride, results which were shown to be dependent upon the integrity of the posterior lobe. These and other facts were produced as evidence that acetylcholine probably acted on some part of the nervous system stimulating the secretion of the hypophysis. Van Dyke (1936) stated in his monograph (p. 326) that the 'chloride-concentrating' effect of an extract may be studied to show its similarity or otherwise to the action of the vasopressor hormone.

It was thought that investigation of the effect of stimulation of the neurohypophysis on the concentration of urinary chloride might give additional evidence as to the site of action of the stimulus. The urinary chloride excretion was therefore estimated during water diuresis in seven rabbits by Whitehorn's method. (In some cases the animals were under sedative doses of the chloralose-urethane mixture; but this had no noticeable effect on the reaction.)

The following animals were used in these experiments: two rabbits belonging to group A (18, 19), two rabbits belonging to group B (25, 26), three rabbits belonging to group C (17, 20, 21).

The control rabbits (group A) had the stimulating tip of the electrode situated in the pars distalis of the pituitary (rabbit 18) and just posterior and superior to the right paraventricular nucleus (rabbit 19); and in five diuresis experiments, a total of thirteen strong stimuli were applied. No anti-diuresis or increase in urinary chloride (mg. Cl/100 c.c. urine) resulted from twelve of the stimuli, but in one case a definite transient rise of urinary chloride was obtained (rabbit 18) accompanied by a decrease in urine flow observed in the second urine sample following stimulation. The decrease in urine flow was insufficient to be labelled a definite anti-diuretic effect under the present criteria, but it seems likely that some slight activation of the animal's neurohypophysis had occurred, possibly induced by the emotional stress involved in the application of the stimulus. Both these animals showed the typical anti-diuresis and chloruresis in response to injections of posterior lobe extracts.

The two rabbits from group B had the stimulating tip of the electrode in the pars intermedia (rabbit 25) and in the pars distalis (rabbit 26). These animals had been noted to give submaximal anti-diuretic responses to strong stimuli, and the results obtained from study of the urinary chloride excretion were in complete uniformity with the previous observations. In the course of three water diureses, three strong stimuli were applied which caused a slight but definite rise in the concentration of urinary chloride, together with a definite anti-diuresis. The effects in one rabbit (25) were closely simulated by intravenous injection of 2 mU 'pitressin' (Parke, Davis and Co.) in 1 c.c. normal saline.

The three rabbits from group C (17, 20, 21) had the stimulating tip of the electrode in, or in contact with, some part of the neurohypophysis. Over a series of eight diuresis experiments, twelve stimuli of varying intensities were applied. In nine cases, anti-diuretic effects of varying degrees were obtained, and it was found that the intensity and duration of the chloride concentrating reaction, observed simultaneously, could be correlated with the intensity and duration of the anti-diuresis. In three cases the stimulus applied was too weak to produce an inhibitory action on the urine flow and failed also to affect the chloride concentration of the urine.

Results which are typical of those described in this section are illustrated in figure 7. In the animal to which this figure relates the electrode tip was situated in the supraoptico-hypophysial tracts at the junction of the median eminence and the infundibular stem. A very weak intermittent stimulus—1 min. on, 1 min. off, etc.—was given over the period *S*; the resultant anti-diuresis is accompanied by a large rise in the concentration of urinary chloride. The figure illustrates the further point that both these effects can be balanced by the intravenous injection of an appropriate dose of posterior lobe extract.

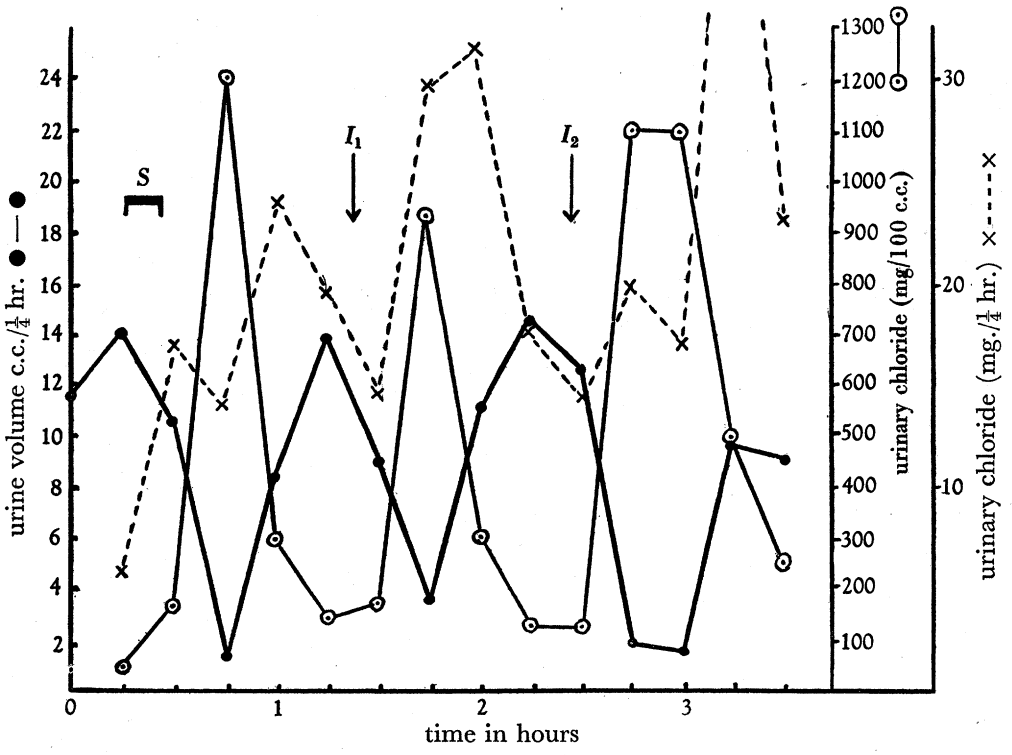

FIGURE 7. Curves showing urine and chloride excretion; rabbit 17 (group C), 12 December 1942. Note the similarity between the response to stimulation *S* of the infundibular stem and to intravenous injection of 'pitressin'. Injection I_1, 1 mU 'pitressin'; injection I_2, 2 mU 'pitressin'. The stimulation elicited a response intermediate between those obtained by 1 and 2 mU 'pitressin', as judged by the effects on both water and chloride excretion.

(3) THE NEUROHYPOPHYSIS, BLOOD SUGAR AND URINE FLOW IN THE NON-HYDRATED ANIMAL

Since the work of Borchardt (1908) it has been known that extracts of the posterior lobe of the pituitary will on injection produce a hyperglycaemia and glycosuria. Borchardt made his injections into rabbits and obtained the maximum rise in blood sugar 2–6 hr. later. Many workers have confirmed and extended Borchardt's observations and the general conclusion is that about 1 hr. after an injection of posterior lobe extract or its pressor fraction, a moderate hyperglycaemia develops, which is often followed by a fall in blood sugar, due probably to an increased secretion of insulin. The dose of posterior lobe extract necessary to give these effects is very large, and it is doubtful if the reaction is of physiological significance (Van Dyke 1936).

A different line of attack on this problem was started by Weed *et al.* (1913). These workers, who had noticed the frequent occurrence of hyperglycaemia following hypophysial lesions, showed that direct faradic stimulation of the hypophysis in cats and dogs produced a glycosuria, even after preliminary transection of the spinal cord and cervical sympathetic trunk. Similarly, stimulation of the superior cervical ganglion, by faradization or even by the manipulation necessary for its exposure, caused glycosuria in the cat, rabbit and dog. This reaction also occurred after section of the cervical sympathetic trunk, spinal cord and vagi, but was abolished by previous removal of the posterior lobe of the pituitary. They suggested the nerve supply of the posterior lobe of the hypophysis is derived from the

superior cervical ganglion, and that stimulation of any part of this mechanism will give a glycogenolysis through liberation of posterior lobe hormones. It should be noted that these experiments were performed with the animals under ether anaesthesia, though it was stated the amount of ether given was insufficient to cause a complicating glycosuria (p. 47). Keeton & Becht (1916) obtained essentially the same result on stimulation of the exposed pituitary glands in dogs under ether anaesthesia. They noted a rise in blood sugar to around 200 mg./100 c.c.; a rise which did not occur if the splanchnic nerves had been previously cut. Ingram & Barris (1935) showed that stimulation of the pars glandularis of the hypophysis occasionally produced a glycosuria often accompanied by a diuresis, although in many of their cats a glycosuria occurred soon after the start of the operation necessary for pituitary stimulation, and before the stimulation was begun. Their animals were anaesthetized with pentobarbital sodium 25 mg./kg. body weight.

The results of Weed *et al.* (1913) regarding the effect of stimulation of the cervical sympathetic system, have been confirmed by Shamoff (1916), Davis, Cleveland & Ingram (1935), and Hsieh (1938), and denied by Rabens & Lifschitz (1914), and Hill & Maycock (1939). All these workers performed their stimulations under anaesthesia, except Rabens & Lifschitz (1914), who in a preliminary operation in a cat, rabbit or dog, pulled the cervical sympathetic trunk out through a skin incision, allowed the animal to recover from the anaesthesia for 8–24 hr. and then applied the stimulus. They obtained no hyperglycaemia or glycosuria and criticize the results of previous workers as being due to the anaesthetic and associated partial asphyxia.

The experiments now to be described may be divided into two groups, the preliminary experiments in which the chloralose and urethane mixture was administered in sedative doses before stimulating and the later experiments in which the animals were fully conscious.

Method

In the preliminary experiments the technique was as follows. An animal, starved overnight, was removed from the cage early in the morning and placed on the experimental table. After the elapse of 1 or 2 hr. in order that the animal should become accustomed to its surroundings, a sample of venous blood was taken from the marginal vein of the ear, and $\frac{1}{2}$–1 hr. later, a second sample. An intravenous injection into the marginal vein of the opposite ear of $3\frac{1}{2}$ c.c./kg. body weight of 2% chloralose in 10% urethane solution was followed by the collection of blood samples hourly for 6–8 hr. If pituitary stimulation was to be performed, a strong stimulus was applied (at least 5 min. total stimulation, at intervals of 1 min. on and 1 min. off, etc.) 1–2 hr. after administration of the anaesthetic. The blood sugar was estimated by Harding's modification of the Schaffer-Hartmann method, as described by King, Haslewood & Delory (1937).

Results

In nine preliminary experiments, three rabbits (15, 17, 18)—with the electrode tip in the infundibular lobe, in the supraoptico-hypophysial tract at the junction of the median eminence and infundibular stem, and deeply in the pars distalis—were used. In four of these experiments, the effect of the anaesthetic alone, and in five the effect of the anaesthetic and pituitary stimulation was observed. In seven out of the nine cases the blood sugar

rose between 15 and 60 mg./100 c.c., but it should be stressed that the hyperglycaemia following administration of the anaesthetic alone was as great as that following pituitary stimulation. The hyperglycaemia was a slowly rising, long-lasting type, reaching a maximum some 2 or 3 hr. after giving the anaesthetic. In the other two cases, pituitary stimulation was applied, and the blood sugar showed not a rise but a slight fall. No difference was seen in the results obtained from the rabbit with the electrode tip in the pars distalis, and from the two with the tips in the neurohypophysis.

From these results it seemed possible that either the anaesthetic which had been shown to depress the anti-diuretic response to stimulation was masking a hyperglycaemia, or that a hyperglycaemia occurred which was missed by taking only hourly blood samples. Therefore, in the final experiments three rabbits (17, 20, 21) with the electrode tip in some part of the neurohypophysis had stimuli applied when in the fully conscious state, and blood samples taken every quarter of an hour for the hour following stimulation. Apart from these two differences the technique was the same as in the preliminary experiments. Out of fifteen experiments in which strong stimulation of the neurohypophysis was applied, only slight transient rises in the blood sugar were observed, rises of 3–45 mg./100 c.c. (average rise 19 mg./100 c.c.). These changes were usually observed only in the blood sample taken immediately following the stimulation; a time relationship therefore, unlike those recorded following injection of posterior lobe extract. It is thought likely that this transient hyperglycaemia is brought about by the action of adrenaline secreted under the influence of the slight emotional stress produced by the manipulations associated with the stimulation, the evidence being that control or 'mock stimuli' in which the primary coil was held at right angles to the buried secondary coil produced identically similar changes in the blood sugar (see figure 8).

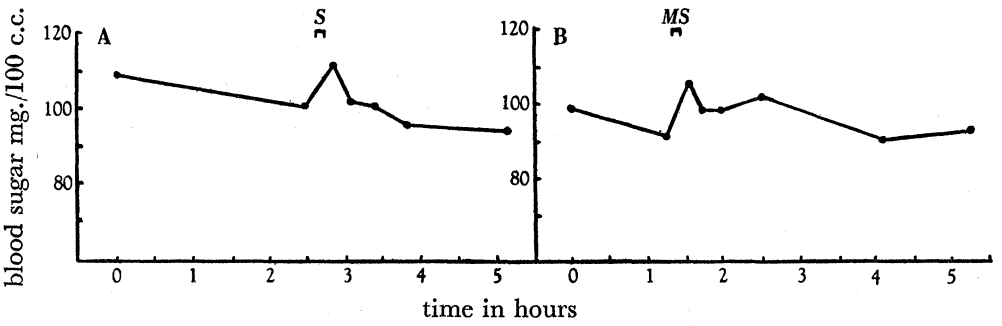

FIGURE 8. Blood-sugar curves on rabbit 20 (group C) showing the effects of *A*, strong stimulation *S* of the infundibular stem (I.C.D. 2·0 cm.), 8 April 1943; and of *B*, the control procedure of 'mock' stimulation *M.S.*, 12 June 1943. Note the lack of any significant difference on the blood sugar between the control and experimental procedures.

In twelve out of the above fifteen experiments in which stimulation was performed without anaesthesia, the urine was collected by manual expression every half-hour. Since the animals were not hydrated the urine flow was necessarily low, the average flow being (about) 1 c.c./$\frac{1}{2}$ hr. The method of collecting the urine by manual expression gives figures of limited accuracy at this level, and although no tests have been made, it is felt that variations of 10–20% in the rate of flow would probably have been overlooked. However, in ten of the twelve experiments, the urine flow remained apparently unaffected by the

stimulus; in the other two cases an increase of flow to 2·2 c.c./½ hr. (rabbit 17), and 6·8 c.c./½ hr. (rabbit 20) were seen. The significance of these two cases is rendered doubtful by the fact that out of four control experiments in which 'mock stimulation' was performed, a similar rise was observed in one case.

It is concluded that in the conscious rabbit, stimulation of the neurohypophysis does not cause a hyperglycaemia or a diuresis, and since the electrode tip in these rabbits was partly in the pars distalis (rabbits 17, 20, 21) and partly in the pars tuberalis (rabbit 17) also, it seems likely that acute stimulation of these regions also fails to cause a hyperglycaemia or diuresis. These results do not agree with those of previous observers, and it is felt that the difference may be accounted for satisfactorily by the fact that the earlier work was conducted on animals anaesthetized with ether (Weed *et al.* 1913; Keeton & Becht 1916) and pentobarbital sodium (Ingram & Barris 1935).

(4) The neurohypophysis and uterine activity

The first report on the oxytocic action of posterior lobe extracts was made by Dale (1909), who demonstrated that these extracts cause a powerful contraction of the uterine musculature. This work was quickly confirmed and extended, and at the present time there is much information to hand concerning the pharmacological effects of posterior lobe extracts on the uteri of many mammals, both *in vitro* and *in vivo*. The extent to which these reactions occur in the normal economy of the animal is a much-debated subject, and until a few years ago the evidence on this point was entirely presumptive. This question will be discussed again in the light of the experimental results given below; for the moment it will suffice to mention the results obtained by some American workers during the last decade.

Fisher, Magoun & Ranson (1938), during an investigation into the experimental production of diabetes insipidus by interruption of the supraoptico-hypophysial tract, noticed that seven cats which developed diabetes insipidus happened to be pregnant. Labour started at periods varying from 19 to 60 days post-operatively (in the cat, term is generally considered to be the sixty-third day), and it was seen that striking disturbances of parturition occurred in which four cats died in the middle of labour, two survived for some days, and only one remained alive and in good condition. The dystocia observed took the form of partial or total inability to deliver the young, and in the animals in which delivery finally occurred it was only after a very prolonged labour, which lasted in some cases over 2 days. Normal labour in the cat probably lasts only 2–3 hr. The conclusion drawn was that the neurohypophysis and its nerve supply probably form an essential part of the labour mechanism. Dey, Fisher & Ranson (1941) published a further report confirming their previous results. They found that slightly less than one-third of their pregnant guinea-pigs with hypothalamic lesions were able to deliver their young normally, and that about one-half of them had a definitely prolonged or delayed labour, or delivered their young dead. These experiments seem to offer strong evidence that the neurohypophysis forms an integral part of the labour mechanism, but since some animals in which complete denervation of the gland had been performed, delivered normally, the evidence cannot be considered wholly convincing.

What may be regarded as the converse type of experiment has been performed by Haterius & Ferguson (1938) and Ferguson (1941). They found that electrical stimulation

of the infundibular stalk of anaesthetized, post-partum rabbits, produced a marked increase in uterine activity. This response was not obtained if electrolytic lesions had been previously placed in the stalk, but was still obtained (in rabbits and cats) after crushing the animal's neck so that only the carotid arteries, jugular veins and a flap of skin were left as functional connexions between the head and trunk. These well-controlled experiments demonstrate clearly the release of the oxytocic factor on stimulation of the pituitary stalk.

Technique

In the present work the effect of stimulation of the neurohypophysis on the activity of the oestrous uterus has been observed in conscious rabbits. In order to record uterine movements in the rabbit some operative interference is necessary, since the length and shape of the vagina render the insertion of a balloon through the uterine cervix impossible in the intact animal. For this reason, vaginal transplants were prepared in animals with the buried coils *in situ*, by a modification of the operation described by Reynolds (1930*a*) and Reynolds & Friedman (1930*a*). Under chloralose-urethane anaesthesia supplemented by ether as necessary, the vagina was exposed by a mid-line abdominal incision and transected about 1 cm. distal to its point of attachment to the two uterine horns. The vulval end was closed by means of an inversion suture and the uterine end carried forward through an aperture torn in the anterior layer of the broad ligament, an aperture sufficiently large to avoid interference with the venous drainage of the vaginal stump and uterine cervices. The incised muscle coat of the abdominal wall was then sutured except at the caudal end where the vaginal stump was left protruding. This stump itself was then stitched firmly to the muscle wall so that the junction of the uterus and vagina fell in the same plane as the abdominal musculature. The most important step in the operation was to leave the sphincter-like opening in the muscle coat large enough to avoid constriction of the blood supply in the transplanted stump and small enough to avoid subsequent stretching with the development of a ventral hernia. (As a rough guide, the aperture should be of a size that will just not admit the tip of the little finger.) The skin flaps were then sutured except where a continuous eversion suture attached the cut vaginal edge to the skin edge. The final result was that the two uterine cervices were contained in a suprapubic vaginal pocket just below the level of the skin. At the conclusion of the operation, the transplant was commonly found to be slightly cyanosed, but within a few days the normal colour had reappeared.

The method of recording uterine activity was essentially the same as that of Reynolds (1930*a*) and consisted of strapping the fully conscious rabbit in the supine position on the experimental table, with an assistant holding its head so that the nose pointed vertically upwards. Rabbits are amenable to this treatment and will lie perfectly still for hours, unless frightened by a noise or some sudden tactile stimulus. With the animal in this position, a fine rubber balloon attached to the end of a no. 3 French rubber catheter was inserted a measured distance (5 cm.) into one horn of the uterus and the balloon inflated with water at a pressure of 25 cm. H_2O. The uterine pressure changes were recorded on a slow kymograph by water-air transmission to a modified bellows recorder.

In the preliminary experiments with this preparation, the uterine motility was found to vary greatly from animal to animal and in the same animal from day to day. The first few

recordings after recovery from the operation would usually show the uterine activity to be that of the oestrous state, and a marked increase in activity would occur in response to stimulation of the neurohypophysis. These reactions tended to disappear after a few days, and it seemed probable that the stimulation of the neurohypophysis was causing liberation of the luteinizing hormone from the adenohypophysis in amounts sufficient to abolish the oestrous condition. It is to be noted, however, that careful histological examination of many specimen follicles from the ovaries of these animals showed no signs of ovulation or lutealization, but complete serial sections necessary for the conclusive demonstration of the absence of luteal tissue were not made owing to lack of histological material. Attempts were made to circumvent this difficulty and produce a more constant oestrous state by subcutaneous injections of an extract of pregnant mare's serum a few days before an experiment was performed. The results of this procedure were unsatisfactory. It was found, however, that a good standard preparation could be obtained by removal of the ovaries when performing the vaginal transplant and implantation of a 50 mg. tablet of stilboestrol di-*n*-butyrate beneath the skin of the flank. Following this operation the animals became anoestrous for a few days and then as absorption of the stilboestrol tablet commenced, a well-developed state of oestrus supervened and remained practically constant for several months in spite of repeated pituitary stimulation. This preparation was found to be extremely satisfactory for the study of the effects of drugs and experimental procedures on uterine activity.

The normal uterine activity

The normal activity of the uterus as recorded with this technique has been well described by Reynolds in a series of papers (1930 *a*, *b*, 1931, 1932, 1933 *a*, *b*) and Reynolds & Friedman (1930 *a*, *b*) and fully summarized by Reynolds (1939). The results obtained in this present work substantiate many of the findings of Reynolds.

The anoestrous or spayed rabbit shows a quiescent uterus. The curves obtained are flat, show no rhythmic contractions, and in many cases are almost straight lines. The reactivity of this type of uterus to posterior lobe extracts is nil or minimal.

As the oestrous state develops, either through the activity of the animal's own ovaries or by the absorption of a subcutaneous stilboestrol pellet, spontaneous rhythmic contractions appear in the uterine tracings, and at the same time the uterus becomes sensitive to administration of posterior lobe extracts. In many rabbits spontaneous activity develops to a marked degree. The response of the oestrous uterus to posterior lobe extracts seem to vary from animal to animal, though it remained constant in the individual oestrogenized rabbits used in these experiments for several months. With small doses of the extract (10 mU) an increase in frequency of rhythmic contractions, followed usually by a more prolonged increase in amplitude, occurred. With larger doses (20–100 mU) the initial contraction following the injection tends to increase in size and to be maintained as a tetanus. These effects last a few minutes and are often followed by augmented rhythmic contractions. With larger doses still (100–500 mU) the initial contraction increases greatly in size and becomes tetanic. This is followed by a varying degree of inhibition which may reduce, though not abolish, the subsequent tetanus and otherwise augmented contractions. This inhibition varies in magnitude in different animals and is most easily observed after large doses of extract have been given. It is generally held to be due to the pressor factor

in whole extracts and is more marked after administration of 'pituitrin' than 'pitocin' (Parke, Davis and Co.). It is of interest that the inhibitory action of 'pituitrin' was more pronounced in the animals that had been artificially oestrogenized (after ovariectomy) than in those that were permitted to retain their ovaries, and since there is evidence (Byrom 1937, 1938) that oestrogens sensitize the blood vessels to the action of the pressor factor, it is possible that the depression of uterine activity following the initial contraction is due to vasospasm. The latent period of these responses following the intravenous administration of the drugs into the marginal vein of the ear is approximately 15 sec.

During pseudo-pregnancy and under the influence of progesterone, the general activity and reactivity of the uterus is the same as that of the anoestrous animal.

The effect of stimulation of the neurohypophysis on the uterine activity

Thirteen rabbits have been used to investigate the responses of the uterus to stimulation of the neurohypophysis. Eleven rabbits had coils implanted, and at a later date the operation of ovariectomy, vaginal transplantation and insertion of a stilboestrol tablet, was performed. The other two animals (rabbits 17, 21) were prepared in the same manner with the exception of the ovariectomy and the stilboestrol treatment.

All these animals had been investigated previously as to the effects of stimulation on a water diuresis and it is important to notice that rabbits in:

Group A (18, 19, 27, 32) showed no specific effects of the stimulation on either water diuresis or uterine motility.

Group B (26, 28, 31, 33, 34) showed submaximal effects on both water diuresis and uterine motility.

Group C (17, 20, 21, 35) showed maximal effects on both water diuresis and uterine motility.

The uterine responses to stimulation of the neurohypophysis will be described separately in the three groups of animals. Unless stated to the contrary, only the experiments performed when the uteri were showing the spontaneous contractions typical of oestrus are described below, since it is known that the quiescent anoestrous uterus is insensitive to posterior lobe extracts. It was routine in cases in which no effect or only slight effect on uterine motility was observed to follow stimulation, to give an intravenous injection of some posterior lobe extract, thus controlling the reactivity of the uterus. Only cases in which the uterus showed a good response to the injections are given. The posterior lobe extracts used were 'pituitrin', 'pitocin' (Parke, Davis and Co.), pituitary (posterior lobe) extract (Boots Co. Ltd.) and a $\frac{1}{4}\%$ acetic acid extract of the posterior lobe of rabbits' pituitary glands; the dosage used varied from 5 to 500 mU, injected intravenously; the vehicle in all cases being 1 c.c. of normal saline.

Group A rabbits

The four animals in this group had a total of thirty-two stimuli applied. The stimulating tip of the electrode in these four animals was situated, in one rabbit (19) near the right paraventricular nucleus, in another (32) just through the right posterior margin of the tuber cinereum and in the other two (18, 27) in the pars distalis of the pituitary.

In none of these experiments was any alteration of uterine motility produced by the application of a strong stimulus of 1 min. duration (see figure 9).

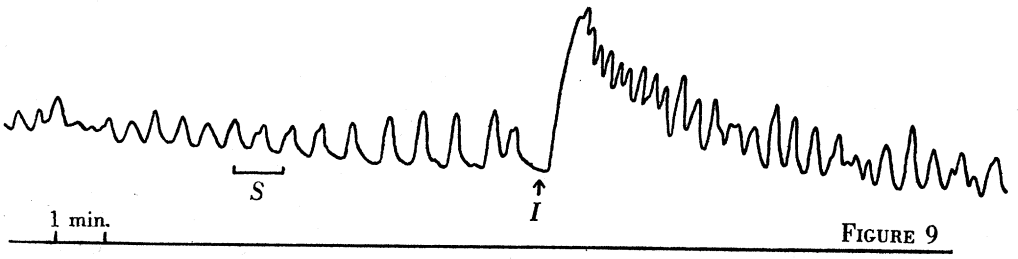

FIGURE 9

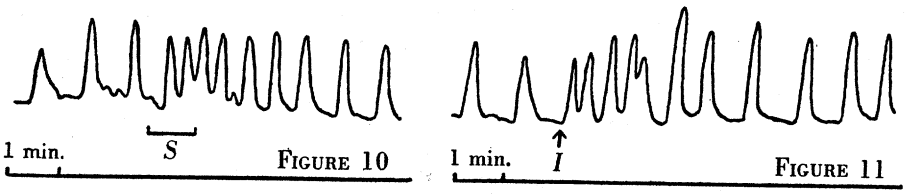

FIGURE 10

FIGURE 11

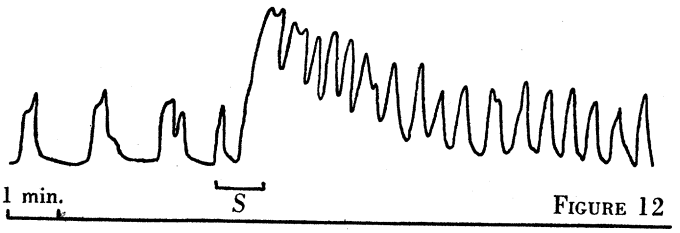

FIGURE 12

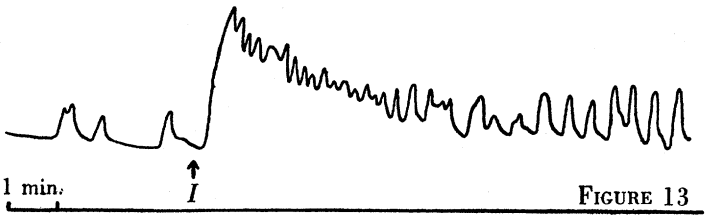

FIGURE 13

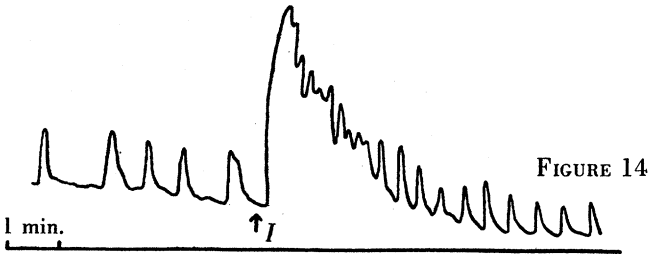

FIGURE 14

FIGURES 9–14. To illustrate the typical uterine response to stimulation in rabbits—groups A and B.

FIGURE 9. Uterine curve, rabbit 27 (group A), 21 April 1944. Note the negative effect of strong stimulation *S*, applied to the pars distalis of the pituitary, and compare with the effect of 100 mU 'pituitrin', *I*.

FIGURE 10. Uterine curve, rabbit 26 (group B), 21 December 1943. Moderate strength stimulation, *S*.

FIGURE 11. Uterine curve, rabbit 26 (group B), 22 December 1943. Intravenous injection 10 mU 'pituitrin', *I*.

FIGURE 12. Uterine curve, rabbit 26 (group B), 29 December 1943 (a.m.). Strong stimulation, *S*.

FIGURE 13. Uterine curve, rabbit 26 (group B), 29 December 1943 (p.m.). Intravenous injection 100 mU 'pituitrin', *I*.

FIGURE 14. Uterine curve, rabbit 26 (group B), 31 December 1943. Intravenous injection 500 mU 'pituitrin', *I*.

(Tracings of kymograph recordings $\times \frac{1}{2}$.)

Group B rabbits

The five animals in this group had a total of forty-six stimuli applied. The stimulating tip of the electrode in these animals was situated in three rabbits (26, 31, 33) in the pars distalis of the pituitary, and in two (28, 34) protruding partly through the posterior part of the tuber cinereum. In all five cases, however, the electrode tip was within $\frac{1}{2}$ mm. of the neurohypophysis.

The uterine responses to the electrical stimuli (1 min. duration) varied greatly with the strength of the stimulus (figures 10, 12), but even with very strong stimulation—sufficient to cause elevation of the upper eyelids, enophthalmos, closure of the rima palpebrarum and twitching of the vibrissae—the greatest uterine response obtained was equivalent to that seen following the injection of 100 mU of pituitrin and pitocin (figures 12, 13), a submaximal response as compared with those obtained from the rabbits in group C.

Group C rabbits

The four animals in this group had a total of 138 stimuli applied. The stimulating tip of the electrode in these animals was situated in two rabbits (17, 20) in the neurohypophysis at the junction of the median eminence with the stalk, and in the other two (21, 35) in contact with the right side of the infundibular stem. As stated above, two of these animals (rabbits 17, 21) were allowed to retain their ovaries, whereas the other two (20, 35) were ovariectomized and a tablet of stilboestrol di-*n*-butyrate implanted in their right flanks.

Under a variety of conditions, anoestrus, oestrus, pseudo-pregnancy, and following administration of progesterone, the reactions of the uterus to stimulation of the neurohypophysis were found to be similar to those following injections of posterior lobe extracts.

(i) *The response of the anoestrous uterus.* This is well exemplified by the results obtained from rabbit 21. At the time of the operation for vaginal transplantation, normal ripe follicles and a small, old, haemorrhagic follicle were observed in each ovary. Eight days later the first record of uterine motility was taken, and it was noted that the uterus showed no rhythmic waves and did not react to stimulation of the neurohypophysis. Similarly, on the ninth and eleventh post-operative days, the uterus was quiescent and failed to react to stimulation or injection of posterior lobe extracts. By the sixteenth day, slight rhythmic activity had developed, and a mild increase in tone was observed following stimulation of the infundibular stem and injection of pitocin (500 mU). On the seventeenth day, rhythmic activity had increased and a well-marked contraction, tending towards a tetanus, followed stimulation and the injection of pitocin (500 mU). It would seem that this animal, in oestrus at the time of the vaginal transplantation, was rendered anoestrous by the trauma of the operation and then slowly came on heat during the subsequent 2 or 3 weeks.

A similar sequence of events has been observed in rabbit 35 and in two of the animals in group B.

(ii) *The response of the oestrous uterus.* The response of the oestrous uterus to stimulations of the neurohypophysis has been observed many times (107) in the animals in group C. Before describing the response in detail, it is worth mentioning the facts that (*a*) the response varied slightly from animal to animal, but in any one animal was similar to that following injection of posterior lobe extracts, (*b*) the response could be graded by varying the intensity of the stimulus (i.e. by varying the I.C.D.), (*c*) a critical I.C.D. could be found, representing

a threshold stimulus (it is to be noted that the critical I.C.D. to elicit the oxytocic effect was slightly less than that for the anti-diuretic effect) and (*d*) the maximum uterine responses to stimulation of the neurohypophysis were approximately equal to those following intravenous injection of 500 mU pitocin.

The response of the oestrous uterus to stimulation of the neurohypophysis and to injections of pituitrin and pitocin is well typified by a series of records obtained from rabbit 35, between 13 June 1944 and 23 June 1944 (see figures 15–20). At the time these results were obtained, the animal had carried the buried coil *in situ* for 3 months and the operation for vaginal transplantation, ovariectomy and stilboestrol tablet implantation was performed 5 weeks previously. One uterine tracing was taken daily, during which either the neurohypophysis was stimulated or an intravenous injection of pituitrin or pitocin was given.

The response of the uterus to a maximal stimulus is shown by figure 15. The stimulation was applied continuously for 1 min., with an I.C.D. of 1·7 cm. The visible signs of this stimulus were marked elevation of the upper eyelids and enophthalmos. The effects on the uterus were clean cut. After a latent period of about 30 sec., there was a sudden marked contraction of greater magnitude than the previous spontaneous rhythmic waves. The uterine contraction was maintained at this level for nearly a minute, followed by a gradual fall in tone over a period of 4–5 min., during which time the rhythmic contractions reappeared with an increased frequency and a diminished amplitude. This response of the uterus compares very closely with that obtained by an intravenous injection of 500 mU of pituitrin and pitocin.

As regards the above reaction, there are several points that require further mention.

(*a*) The latent period of the uterine response to stimulation of the neurohypophysis was 20–30 sec. from the beginning of stimulation and to injection of posterior lobe extracts only 15–20 sec. Two factors may have played a part in producing this difference. The secretions of the pituitary gland had to traverse a sinusoidal capillary plexus before reaching the jugular vein, whereas the injected solution passed straight from the relatively large marginal vein of the ear into the jugular vein and thus probably took less time to reach the uterine musculature. And whereas the injection of extracts was usually completed within a few seconds from the insertion of the needle into the vein, the neurohypophysis may have taken considerably longer than this to secrete an equivalent quantity of hormone into the blood stream. This long latent period of 30 sec. seen in the stimulation experiments would afford confirmatory evidence, if any were needed, that the activation of the uterus was hormonal in nature.

(*b*) The response of the uterus to stimulation of the neurohypophysis resembled the response to pitocin more closely than that to pituitrin, in so far as both stimulation and pitocin caused less inhibition of the rhythmic contractions following the initial large contraction. This may be seen more easily on comparing the effects of stimulation with the responses to large doses (500 mU) of pituitrin and pitocin (figures 15–17), when the inhibitory effects are more marked than after small doses of these extracts (figures 18–20). This similarity of the responses following pituitary stalk stimulation to those evoked by pitocin rather than pituitrin has been noted by Ferguson (1941) in one experiment performed under chloralose-urethane anaesthesia. Ferguson suggested that the hormone liberated by stimulation of the pituitary stalk had little pressor activity. The question of

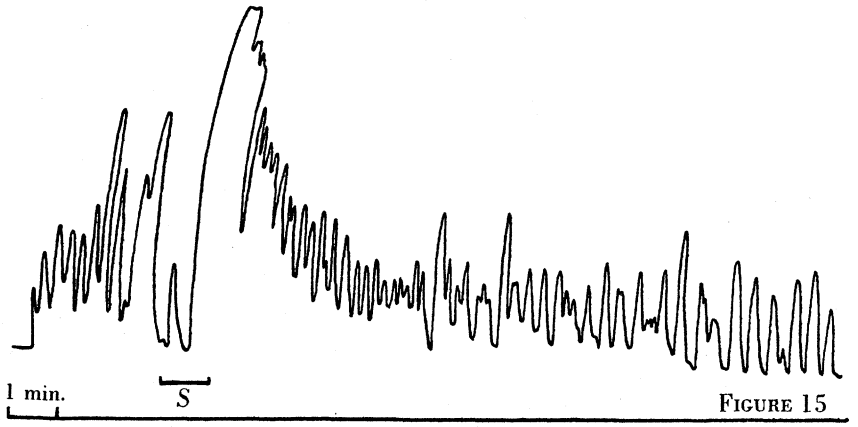

FIGURE 15

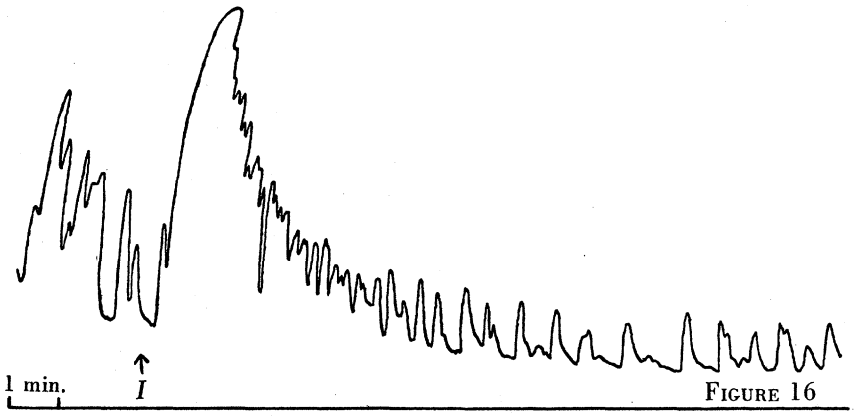

FIGURE 16

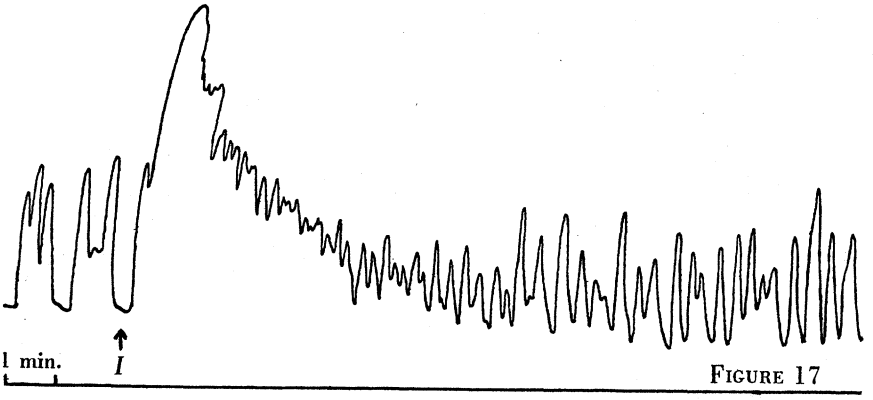

FIGURE 17

FIGURES 15–17. To illustrate response of oestrous uterus to stimulation of neurohypophysis and intravenous injection of 'pituitrin' and 'pitocin', rabbit 35 (group C; electrode tip in contact with neurohypophysis). Note closer similarity of response to stimulation to injection of 'pitocin' rather than 'pituitrin'.

FIGURE 15. 13 June 1944. Moderate stimulation, *S* (I.C.D. 1·7 cm.).

FIGURE 16. 14 June 1944. Intravenous injection 500 mU 'pituitrin', *I*.

FIGURE 17. 15 June 1944. Intravenous injection 500 mU 'pitocin', *I*.

(Tracings of kymograph recordings $\times \frac{1}{2}$.)

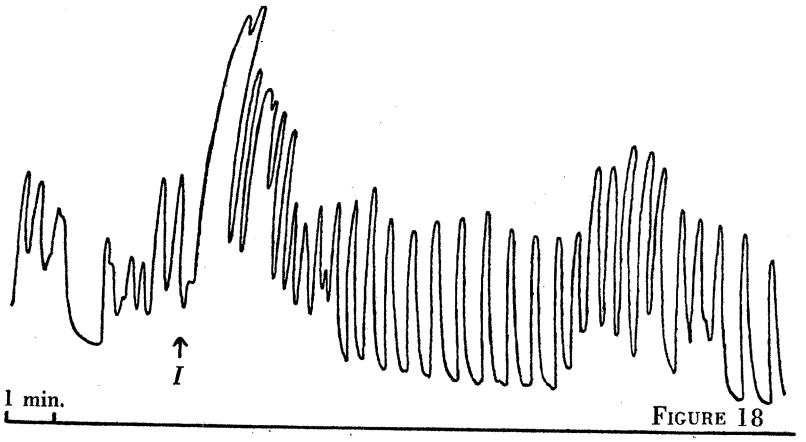

FIGURE 18

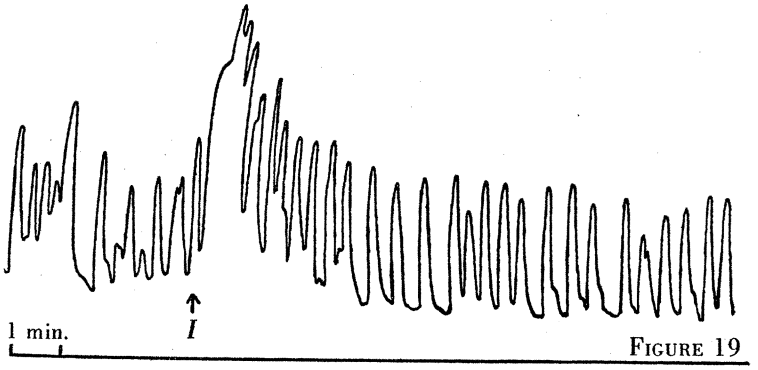

FIGURE 19

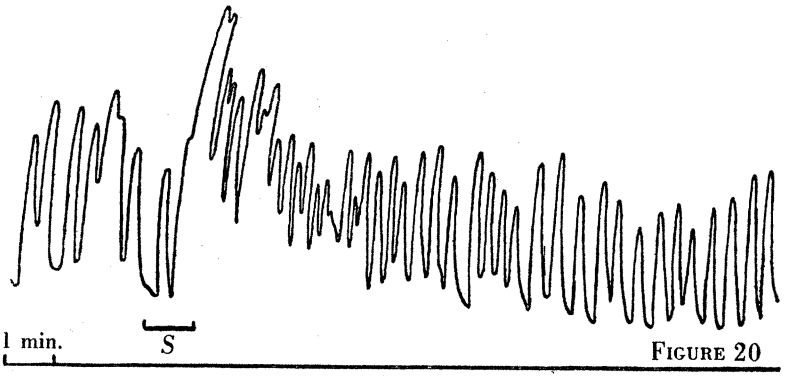

FIGURE 20

FIGURES 18–20. To illustrate response of oestrous uterus to stimulation of neurohypophysis and intravenous injection of 'pituitrin' and 'pitocin', rabbit 35 (group C; electrode tip in contact with neurohypophysis).

FIGURE 18. 18 June 1944. Intravenous injection 20 mU 'pitocin', *I*.

FIGURE 19. 19 June 1944. Intravenous injection 20 mU 'pituitrin', *I*.

FIGURE 20. 20 June 1944. Weak stimulation of neurohypophysis *S* (I.C.D. 2·2 cm.).

(Tracings of kymograph recordings $\times \frac{1}{2}$.)

the relative amounts of oxytocic to anti-diuretic (or pressor) activity of the natural secretion will be discussed in greater detail below.

(*c*) The amount of hormone liberated by the neurohypophysis on maximal stimulation was equivalent in oxytocic action to 200–500 mU of pitocin. Ferguson (1941) also found in anaesthetized post-partum rabbits and cats, that the uterine response evoked by stimulation of the pituitary stalk was equal to intravenous injection of 500 mU pitocin. Although at first sight this amount seems extraordinarily large, if compared with the hormonal content of the infundibular lobe of the rabbit it is less surprising. Rough assays have been performed on the infundibular lobes of four normal rabbits. These were removed within a few minutes of death, dissected and dehydrated in acetone, dried in a vacuum desiccator and extracted with $\frac{1}{4}$% acetic acid, so that each posterior lobe was equivalent to 5 c.c. of the final extract. These extracts were assayed on the conscious rabbit preparation described above, using small doses (20 mU) of 'pituitrin' as a standard. The results obtained by this method indicated that the oxytocic content of the infundibular lobe of the rabbit's pituitary was more than 500 mU and less than 1000 mU. Simon & Kardos (1934) estimated the amounts of the oxytocic principle in the pituitary of the rabbit to be 0·6–1·5 U. Jores & Von Wittern (1934), however, published lower figures; they found only 0·05–0·25 U of oxytocic principle as being present in the pituitary of the non-pregnant rabbit, figures which are almost certainly too low. It would seem, then, that the hormonal content of the neurohypophysis is sufficient to account for the amount of oxytocic principle liberated by a maximal stimulation.

(*d*) The constancy of the uterine response. The effects of repeated stimulations of the neurohypophysis were similar to the effects of repeated injections of posterior lobe extracts in so far as decreasing uterine responses were observed if the stimulus or injection was repeated within too short a time interval. For the purposes of comparison, intervals of at least 6 hr. and usually 24 hr. were allowed between stimulations and/or injections, in the ovariectomized, oestrogenized rabbit, the uterine responses then being comparable and reproducible with a fair degree of accuracy.

(*e*) Gradation of the uterine responses to stimulation of the neurohypophysis is easily obtained by varying the I.C.D. (see figures 15–20). This conforms well with the similar gradation observed for the anti-diuretic and chloruretic reactions described previously.

It should be mentioned that although for descriptive purposes one series of experiments on rabbit 35 has been referred to and figured, these results are typical of many obtained on this and other animals.

(iii) *The response of the pseudo-pregnant uterus.* The reactivity of the uterus to stimulation of the neurohypophysis and to injection of pituitary (posterior lobe) extract (Boots) was tested throughout one period of pseudo-pregnancy in rabbit 17. This animal had the secondary coil inserted on 20 May 1942, and the vaginal transplant (without ovariectomy) performed on 3 April 1943. On 3 June 1943 the uterus showed a marked response to intravenous injection of 50 mU, and on 4 June 1943 a marked response to stimulation of the neurohypophysis. On 7 June 1943 a buck was accepted twice. (Mating occurs normally in these animals with a vaginal transplant, since the caudal three-quarters of the vagina is intact and normally situated.) By 8 June 1943 (18 hr. post-coitus) the normal rhythmic contractions and the response of the uterus to both stimulation and posterior lobe extract

(500 mU) were very markedly decreased; on 11 June 1943 ($3\frac{3}{4}$ days post-coitus), the response to stimulation had disappeared and to posterior lobe extract (500 mU) was very slight. This condition persisted till 21 June 1943 (14 days post-coitus), when the uterus showed an increasing response to posterior lobe extract, but still no response to stimulation; the rhythmic waves were then increasing. By 29 June 1943 (22 days post-coitus) the spontaneous waves were moderate in size and the response to stimulation and injection of posterior lobe extract (500 mU) had both returned.

(iv) *The response after administration of progesterone.* A series of observations was made on the uterine reactivity of rabbit 20 before and after subcutaneous injection of progesterone. This animal had the secondary coil inserted on 27 November 1942 and the operation of vaginal transplantation, ovariectomy and stilboestrol implantation on 24 July 1943. Two courses of progesterone treatment were given with similar results. The observations made during one course are given below.

On 6 October 1943 the uterus was in a well-marked 'oestrous' state as shown by the rhythmic contractions and the responses to stimulation of the neurohypophysis and intravenous injection of 500 mU 'pitocin'. Three subcutaneous injections, each 0·2 mg. progesterone in an oily base, were given at 5.30 p.m. on 6 October, 7 October and 8 October 1943, after the observations on the uterine reactions had been made on the respective days. On 7 October 1943 (18 hr. after the first injection of progesterone) the spontaneous activity of the uterus was markedly diminished, but the responses to stimulation and injection 500 mU 'pitocin' had been only slightly inhibited. On 8 October 1943 (19 hr. after the second injection of progesterone) the response to stimulation of the neurohypophysis was very slight, although to injection of 500 mU 'pitocin', was fairly marked. On 9 October 1943 (19 hr. after the third injection of progesterone) the responses to both stimulation and 'pitocin' were very slight. By 11 October 1943 the spontaneous activity and the response to stimulation and 'pitocin' were again all well marked.

Thus it may be said that the responses of the uterus to stimulation of the neurohypophysis and to intravenous injections of posterior lobe extracts are similar qualitatively under a variety of conditions (anoestrus, oestrus, pseudo-pregnancy, and after progesterone treatment), and that by varying the strength of stimulus, the response of the oestrous uterus can be made quantitatively similar to that following injections of 'pitocin' in doses up to 500 mU.

(v) *The response under anaesthesia.* A series of observations have been made in two rabbits (20, 35) as to the effect of anaesthesia on the uterine responses to stimulation of the neurohypophysis and injection of posterior lobe extracts.

Deep ether anaesthesia (absent corneal reflexes; exophthalmos and the progression reflex present; respiration rapid with short, forced expiratory movements) exerts a very marked inhibitory effect on the uterine response to stimulation of the neurohypophysis, but much less inhibitory effect on the response to intravenous injection of 500 mU 'pitocin' or 'pituitrin'. It would appear, then, that the inhibitory effect of ether is exerted mainly (though not entirely) on central structures preventing the liberation of the oxytocic hormones, rather than peripherally on the uterine musculature.

Light anaesthesia induced by the intravenous injection of 2% chloralose in 10% urethane solution (3 c.c./kg. body weight) has little effect on either the uterine response to stimulation or that to intravenous injection of pitocin.

(vi) *The exclusion of the adrenal medulla as participating in the reaction.* It is well known that adrenaline has a pronounced motor effect on the uterus of the rabbit, and for this reason it was of interest to compare the responses of the uterus to adrenaline, stimulation of the neurohypophysis and posterior lobe extracts; and to exclude again any possibility of an emotional accompaniment of the stimulus acting through the adrenal medulla and so participating in the uterine response.

Intravenous injection of 1 c.c. of 1/150,000 solution of 'adrenalin chloride' (Parke, Davis and Co.) into the conscious rabbit was found to be quite consistent in its action in causing a sudden powerful uterine contraction followed by a fairly prolonged period of inhibition before the rhythmic waves reappeared. Large doses of 'pituitrin' commonly inhibit the activity of the uterus following the initial contraction, but never to the extent seen after the administration of adrenaline. It is a simple matter then to differentiate between the effects of adrenaline on the uteri of these rabbits and the effects of stimulation of the neurohypophysis or injection of posterior lobe extract.

The converse experiment was performed. This consisted in removing the left adrenal gland and denervating the right adrenal as described previously for rabbit 20. This rabbit showed as marked a uterine response to stimulation after the operation as before.

From both lines of evidence, then, it appears very unlikely that the adrenal medulla plays any important part in these uterine reactions.

(7) Some data on the relative pressor and oxytocic activity of the secretion of the neurohypophysis

The nature of the secretory product of the neurohypophysis has been a controversial subject since Oliver & Schafer (1895) first discovered the pressor activity of extracts of the pituitary gland. Dudley (1923) produced evidence indicating the existence of more than one active principle, one with oxytocic and the other with pressor activity. This idea of more than one principle has been termed the 'multiple hormone theory' and has been strongly contested since 1920 by Abel and his co-workers (see Abel & Rouiller 1922), who support the 'unitary theory' that the secretion is a single specific active substance with multiple actions. In 1928, Kamm, Aldrich, Grote, Rowe & Bugbee demonstrated a fairly complete separation of two potent preparations, one with oxytocic and the other with pressor and anti-diuretic activities. These two fractions are now marketed as commercial preparations, under the names 'pitocin' and 'pitressin', whilst the whole extract with full activity is known as 'pituitrin' (Parke, Davis and Co.). A further separation of activity has been claimed by Heller (1939), who reported that heating commercial posterior pituitary extract for 90 min. at pH 10·0 resulted in an extract in which the ratio of anti-diuretic to pressor activity was approximately 100 to 8. The current view that the pressor and anti-diuretic activities reside in the same complex molecule is thus not supported by Heller's work.

The unitary theory of Abel has recently received strong support in the work of Van Dyke, Chow, Greep & Rothen (1942), who have isolated a protein from the dried posterior lobe of frozen gland material, which behaves as a homogeneous substance, as shown by solubility, electrophoresis and ultracentrifugation tests. It has oxytocic, pressor and anti-diuretic activities present in the same proportion as in the standard posterior lobe powder

of the American Pharmacopoeia. These workers state, however, that even if the pars neuralis elaborates a single protein with multiple activities, it appears teleologically improbable that it is secreted unchanged, and they suggest the possibility that specific enzymes liberate one or the other active fragments of the parent molecule depending upon the demands of the organism.

Firm support for the claim that a substance extracted from an endocrine gland is the secretory product of the gland would be obtained if it were possible to show that this substance on appropriate administration to an animal has the full qualitative and quantitative activities produced by stimulation of the animal's own gland. Thus a claim that a single substance represents the secretion of the neurohypophysis would receive support if the substance were shown to produce multiple activities present in the same ratio as those produced in the animal by stimulation of its own neurohypophysis, under a variety of similar conditions.

Technique

Experiments were performed to investigate the quantitative similarity of multiple actions obtained by injection of posterior lobe extracts and stimulation of the neurohypophysis. The actions measured were the oxytocic and anti-diuretic. A rabbit was given water (50 c.c./kg. body weight) by stomach tube, and 1½ hr. later a second dose (40 c.c./kg. body weight). The bladder was then emptied by manual expression and urine samples collected at quarter-hourly intervals. When the urine flow had increased to about 10 c.c./¼ hr., an appropriate interval between collecting the urine samples was chosen, the animal tied in the supine position and a uterine tracing taken. During this period, either a submaximal stimulus of 1 min. duration was administered to the hypophysis, or an intravenous injection of posterior lobe extract given. The uterine tracing was continued till it was necessary to collect the next urine sample, when the animal was untied and the bladder emptied. The diuresis curve was then completed in the usual way. This procedure was repeated daily for several days, so that records of both the oxytocic and anti-diuretic activities were obtained for varied doses of posterior lobe extract and varied intensities of stimulation in the same animal. On these days the animals were fed after the experiments on green food only.

The extracts used were pituitary (posterior lobe) extract (Boots Pure Drug Co. Ltd.) in one series of experiments; 'pituitrin' (Parke, Davis and Co.), several different batches, in eight series of experiments; and in case there should be a species difference, extracts of the posterior lobe of the rabbit pituitary, in five series of experiments. The usual method of dehydrating and defatting in acetone followed by extraction with ¼% acetic acid was followed in preparing the extracts from the rabbit's gland. According to Kamm *et al.* (1928) there is very little loss in pressor or oxytocic activity if the glands are desiccated in acetone within a few minutes of death, and although glands extracted with water or saline show a significant loss of oxytocic and especially pressor activity, those extracted with ¼% acetic acid are comparatively stable.

Results

Experiments were performed on three animals (20, 26, 35) each with a coil implanted and the operation of vaginal transplantation and stilboestrol tablet insertion performed several months previously. Rabbit 20 had the tip of the electrode in the junction of the

median eminence and infundibular stem, rabbit 35 had the tip in contact with the right side of the infundibular stem, and in rabbit 26 the tip was situated deeply in the pars distalis, $\frac{1}{2}$ mm. distant from the infundibular stem. The first two animals (both in group C) had previously reacted to stimulation with maximal anti-diuretic and oxytocic responses, and the last rabbit (group B) had reacted with submaximal responses. For purposes of quantitative assay it is essential that the responses to be compared should be submaximal, and it was found that rabbit 26 gave responses on stimulation that were rather more easily balanced than the other two animals. The details of one typical series of experiments are given in table 6.

TABLE 6. RABBIT 35. EXPERIMENTS PERFORMED BETWEEN 9 AND 13 JULY 1944. COMPARISON OF THE EFFECTS OF STIMULATION OF THE INFUNDIBULAR STEM AND INJECTIONS OF SMALL DOSES OF 'PITUITRIN'

Date ...	9 July 1944	10 July 1944	11 July 1944	12 July 1944	13 July 1944
urine samples in c.c.	2·7	15·5	8·0	15·6	10·8
	18·8	13·6	15·8	21·5	13·6
	Inj.	Stim. I.C.D.	Inj.	Stim. I.C.D.	Stim. I.C.D.
	10 mU	2·0 cm.	30 mU	2·15 cm.	2·05 cm.
	14·6	18·2	14·1	22·2	15·7
	0·4	0·8	1·8	1·3	0·9
	0·3	1·0	0·6	0·4	0·5
	0·7	0·7	0·4	1·3	1·1
	1·0	0·5	0·5	3·0	1·1
	3·2	0·7	0·5	5·2	1·1
	7·2	0·6	1·0	8·7	1·1
	—	0·5	1·2	13·2*	1·8
	—	1·8	3·3	13·6	3·8
	—	5·0	6·0	18·8	6·0
	—	—	6·9*	—	—
	—	—	5·5	—	—
	—	—	7·0	—	—
relative magnitudes of oxytocic responses (see figures 21–25)	+	+++++	++	+++	++++

The table (reading from above downwards) shows the anti-diuretic effects produced by the various procedures. After giving water by stomach tube, two samples of urine were collected previous to stimulation or injection; subsequently, urine samples were collected till the flow had increased to at least 5·0 c.c./$\frac{1}{4}$ hr. The urine was collected at 15 min. intervals, except the two samples following injection or stimulation, which were collected over 20 and 10 min. intervals respectively, thus allowing more time for recording the uterine changes. At * the rabbit was strapped on its back as during the experimental period to control the effect of such handling on the water excretion. In the lower row the magnitude of the oxytocic (figures 21–25) responses are represented. It may be seen that the responses obtained by stimulation on 10 July 1944 may be discarded, as they do not balance with any responses to the injection of 'pituitrin'.

Fourteen series of experiments have been performed in which a balance was obtained between the effects of stimulation and injection of posterior lobe extracts. In all cases where the oxytocic responses were compared on a basis of anti-diuretic response, the injection of posterior lobe extract caused smaller oxytocic effects than did stimulation of the neurohypophysis.

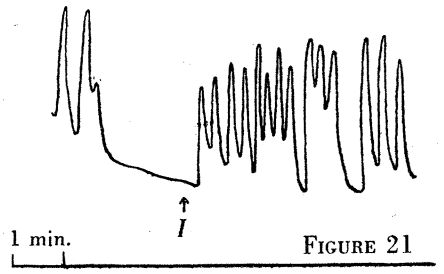

FIGURE 21

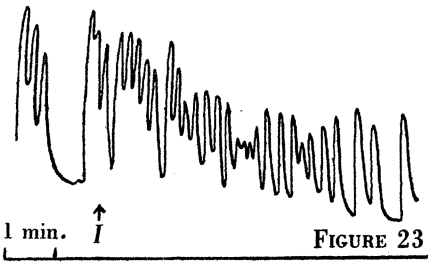

FIGURE 23

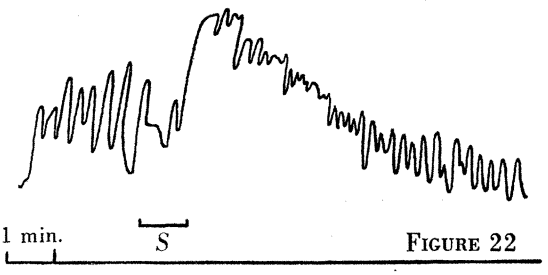

FIGURE 22

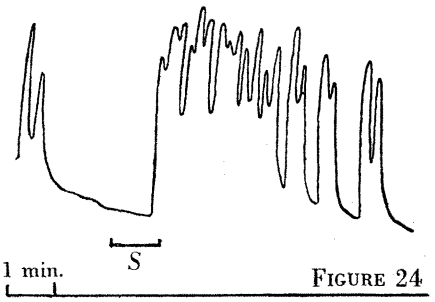

FIGURE 24

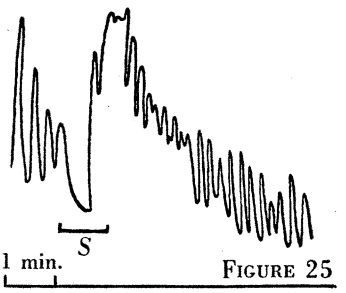

FIGURE 25

FIGURES 21–25. The uterine curves obtained in one series of experiments (oxytocic and anti-diuretic responses measured simultaneously), rabbit 35 (group C). See text for full description of these experiments and the anti-diuretic responses.

FIGURE 21. 9 July 1944. Intravenous injection 10 mU 'pituitrin', *I*.

FIGURE 22. 10 July 1944. Stimulation of neurohypophysis, *S* (I.C.D. 2·0 cm.).

FIGURE 23. 11 July 1944. Intravenous injection 30 mU 'pituitrin', *I*.

FIGURE 24. 12 July 1944. Stimulation of neurohypophysis, *S* (I.C.D. 2·15 cm.).

FIGURE 25. 13 July 1944. Stimulation of neurohypophysis, *S* (I.C.D. 2·05 cm.).

(Tracings of kymograph recordings $\times \frac{1}{2}$.)

Discussion

The above procedure is open to many criticisms. First, the difference in the portal of entry into the circulatory stream of the extracts and the secretions of the neurohypophysis may cause some variation in their actions. The rate of entry and, therefore, the time relations of the concentration of the two substances in arterial blood are probably not strictly comparable, as shown by the differences in the latent periods of the oxytocic response to stimulation and to injection of extracts. Secondly, the sensitivity of the animals may vary from day to day. Walker (1939) stated that the sensitivity of hydrated rabbits to the diuresis-inhibiting factor varied over daily periods. This variation in sensitivity also occurs in the case of the oxytocic reaction, although over daily periods with the standardized animals,

it is slight. In view of the above observations, slightly irregular results were expected and indeed obtained, but on comparing the results produced by stimulation of the neurohypophysis with those obtained by injection of posterior lobe extracts, the difference noticed was constant over many series of experiments and would, therefore, seem to be significant. Thirdly, the handling of the animal involved in recording the uterine reactions may by itself provoke sufficient emotional stress in the rabbits to cause an anti-diuresis. For this reason, the first three series of experiments were performed under constant sedative doses of chloralose and urethane. Later, however, it was found in a series of control observations in which conscious animals were tied supine and uterine recordings taken for a quarter of an hour without stimulation or injection, that the anti-diuretic effect of manipulations was negligible. All the later experiments were then performed on the fully conscious animal. Fourthly, the acetic acid in the posterior lobe extracts may have modified the hormonal action. Control experiments in which comparable doses of acetic acid were used showed that this substance exerted no diuresis-inhibiting action, did not augment the diuresis-inhibiting action of saline posterior lobe extracts, and did not inhibit the oxytocic action of saline posterior lobe extracts. And fifthly, a last point for consideration concerns the state of hydration of the animals; those from which the posterior lobe extracts were made in comparison with the rabbits that were submitted to pituitary stimulation. The posterior lobe extracts were obtained, at least, in the case of the rabbit extract, from non-hydrated animals, whereas the hypophysial secretion with which these extracts were compared was necessarily produced in a hydrated animal. A few control experiments in which the ratios of anti-diuretic to oxytocic factors present in the glands of hydrated and non-hydrated rabbits have been determined, show, however, no apparent difference.

If the above criticisms render the conclusions of these experiments unconvincing, there is another line of approach to the problem which also indicates that stimulation of the neurohypophysis in the non-hydrated rabbit produces a secretion more akin to 'pitocin' in its effect than 'pituitrin'. Ferguson (1941) described an experiment in which he stimulated the pituitary stalk of an anaesthetized animal whilst recording the uterine contractions and the blood pressure. The results of stimulation as regards both the uterine and vascular effects were more clearly simulated by an injection of 1 U 'pitocin' than 1 U 'pituitrin', and the conclusion was drawn that the hormone liberated by stimulation of the stalk has little pressor action. The observation made by Ferguson that the uterine response to stalk stimulation is more similar to the response obtained by intravenous injection of 'pitocin' than 'pituitrin' has been confirmed frequently in this present work (see figures 15–17). The difference in uterine effects lies in the greater inhibition of the 'tetanus' and of the rhythmic waves subsequent to the initial contraction which occurs after injection of 'pituitrin'. This inhibition is usually claimed to be due to the pressor fraction of the whole extract, and since the pressor activity parallels the anti-diuretic, it is probably justifiable to conclude from the uterine curves alone that the secretion of the neurohypophysis is relatively less rich in anti-diuretic activity than whole extracts of the posterior lobe.

The above results throw light on an apparent discrepancy in the dosage of posterior lobe extracts needed to produce its various actions. In the unanaesthetized rabbit, $\frac{1}{10}$ mU of extract will often excite an anti-diuresis, whereas 10 mU of extract exerts little oxytocic effect.

A word of caution must be added. These results should not be read as evidence for or against the unitary theory so strongly upheld by Abel. If it could be shown that the proportions of pressor (and/or anti-diuretic) and oxytocic activities as produced by stimulation of the neurohypophysis ran parallel under a variety of conditions (hydration and dehydration, anoestrus, oestrus, pregnancy, pseudo-pregnancy and post-partum, etc.), then strong evidence would be obtained for the unitary theory. If, however, the proportionate activities varied as the state of the animal, then again the data would be insufficient to warrant any conclusions regarding the number of hormones found, for as pointed out by van Dyke *et al.* (1942), even if the neurohypophysis elaborates a single compound, it may well be liberated into the blood stream as a series of active fragments of the parent molecule.

GENERAL DISCUSSION

These results may be most suitably considered under three headings: (i) the method of remote control stimulation, (ii) the nerve supply of the neurohypophysis, (iii) the neurohypophysis as an endocrine gland under neural control.

(i) *The method of remote control stimulation*

The method of remote control stimulation has proved to be a simple and effective technique for stimulating the hypothalamo-hypophysial region. All the apparatus used in this investigation was made in the Anatomy School, and could be similarly constructed in any department containing a good workshop, though one factor which might limit the general application of the method is the need for radiographic examination at regular intervals.

Many anticipated difficulties, such as the development of intracranial irritation and infection, interference with the venous drainage of the brain through damage to the superior sagittal sinus, and so on, were never met. Other difficulties and limitations became apparent in the course of this work, some of which have been mentioned and discussed previously. First, loosening of the screw in the skull with movement of the coil and electrode was seen in several of the preliminary animals, and overcome by using the minimum of wax to cover the coil. A second complication, one that is likely to cause more interference when stimulating other regions of the nervous system, is the development of sensory side-effects. In the anterior hypothalamus there are only two regions in which stimulation appears to cause the animal discomfort: dorsally, near the anterior thalamic nuclei, and anteriorly, near the optic chiasma. If strong stimulation was applied in either of these two regions, one of the first visible effects was a restless jerking movement of the head which developed, if stimulation was continued, into violent struggling movements. It is thought likely that this was not a direct motor effect, but was due to spread of current to some sensory field giving rise to unpleasant or painful sensations. It was fortunate for the present work that in the region of the hypophysis, even excessive stimuli could be applied without producing restlessness or signs of discomfort. However, it may be found that remote control stimulation of other structures has a limited field of use in the conscious animal, owing to this side-effect. A third difficulty encountered was the decreasing efficiency of the buried unit which occurred some 8 months after the implantation. This question has been discussed

already, and it is hoped that the trouble has been eradicated, wholly or in a large measure, by the use of platinum wire instead of silver for the stimulating electrode. The last difficulty to be mentioned here, one that has caused little inconvenience in the stimulation of the neurohypophysis but which seems likely to arise in experiments concerned with more lateral regions of the brain or the peripheral nerves, is the problem of fixation of the buried coil. In the experiments to date, the stimulating electrode has been placed in the median plane, and only through technical inexactitude was occasionally placed slightly to one side of the mid-line. In all these cases the coil was situated in the mid-line on the dorsal surface of the skull, a region where little movement of the tissue occurs, and where the lateral forces exerted on the coil are bilateral and, therefore, compensated. If the coil and electrode are placed laterally in an asymmetrical position, it is thought that some movement of the coil would occur with consequent loosening of the screw and shifting of the electrode, and that this shifting would form an even more acute problem in attempts to stimulate the peripheral nerves.

The advantages of the technique are manifold and obvious. Most of the experimental results recorded were only made possible by the freedom from anaesthesia which the remote control method confers, examples of such results being the effects of stimulation on a water diuresis, and the negative effects of stimulation on the blood sugar. Prolonged and repeated stimulation of one organ and repeatable stimulation of the same group of cells of that organ are possible by no other known method, and it is for this reason that the technique seems so admirably suited for investigating the effects of stimulating the adenohypophysis or its secretomotor innervations. The highly localized nature of the stimulus effective for the non-myelinated fibres of the hypothalamo-hypophysial region suggests the possibility that these fibres would respond more easily to an alternating current of a different wave form, but in some ways this would be a doubtful advantage. In the present experiments, for example, since the localized nature of the stimulus for the fibres of the neurohypophysis was quite certain, the spread of the stimulus to the third nerve, composed largely of somatic efferent fibres with a lower threshold value, gave a useful visual check on the stimulation. A final point of technical value is the ability to fix the tissues with the electrode *in situ*. If the diameter of the bared tip differs from the external diameter of the insulated part of the electrode, the circular hole seen in the final sections will vary in a similar measure, and it becomes a simple matter to locate the exact site of the stimulating tip of the electrode. It also renders superfluous any calculations of shrinkage of the tissues in histological preparation.

(ii) *The nerve supply of the neurohypophysis*

The anatomical studies of Ramon y Cajal (1894) laid the foundations of our present knowledge on this subject. He found fibres in the brain of 2-day-old mice running from the neural stalk to the neural lobe. These fibres he described as being in relation with a nucleus above the optic chiasma on either side, the nucleus perichiasmatique, or as it is now called, the nucleus supraopticus. Since Cajal, many workers have studied these fibres, amongst whom may be mentioned Gentes (1903), Gemelli (1906), Tello (1910), Pines (1925), Greving (1925, 1926), Croll (1928), Bucy (1930), Roussy & Mosinger (1933 *a*, *b*), Hair (1938), Rasmussen (1938) and Truscott (1944). A full list of references to the anatomy

of this tract in all types of vertebrates from cyclostomes to mammals can be found in the monograph by Fisher *et al.* (1938).

The sites of origin of this unmyelinated tract are not fully known. There is much evidence that a large part of it arises from the nucleus supraopticus. Evidence of a morphological nature has been produced by Pines (1925) and Greving (1925) amongst others, who studied silver preparations of human tissue and described a wealth of fibres running from this nucleus into the neural stalk. Further evidence accrues from the well-known work of Fisher *et al.* (1938) on experimental diabetes insipidus, and of Broers (1932) (dog), Hare (1937) (dog), Magoun & Ranson (1939) (monkey), and Rasmussen (1940) (rat, dog and man), all of whom described a loss of cells in the nucleus supraopticus following hypophysectomy or other lesions of the neurohypophysis. The number of cells in each supraoptic nucleus has been put at 50,000–70,000 for man (Rasmussen 1938), 30,000–40,000 for the monkey (Magoun & Ranson 1939), 35,000–41,000 for the dog and 7000 for the rat (Rasmussen 1940). The number of fibres in the neural stalk appears to be considerably less than would be expected from these figures. Rasmussen (1938) found in man that the supraoptic nuclei together contain twice as many cells as are necessary to account for the fibres in the neural stalk. Also in the Macaque monkey, Rasmussen (1938) found in an actual fibre count 40,000 unmyelinated fibres in the lower part of the stalk, whilst the total number of cells in the two supraoptic nuclei of this animal according to Magoun & Ranson (1939) would be about 70,000. These last workers offered the following suggestions to account for the discrepancy, first, that some fibres from the cells of the supraoptic nuclei may end in the median eminence proximal to the level at which the fibre count was made, and secondly, that the fibre counts are too low, owing to the technical difficulty of counting fine unmyelinated fibres. The fact that other hypothalamic nuclei probably contribute fibres to this system would appear to make this discrepancy still greater. In view of their common phylogenetic origin (Meyer 1935), and their similar morphological characters as regards cytoarchitecture and blood supply, it might be expected that the fibre connexions of the paraventricular nucleus with the hypophysis would be similar to those of the supraoptic nucleus. The evidence on this point is equivocal, though many workers have described paraventriculo-hypophysial fibres. Laruelle (1934) described two groups of these fibres, an external and internal set. Many fibres may be seen running from the paraventricular nucleus towards the tuber cinereum, but it is probable that most of these do not continue to the hypophysis but end in the supraoptic nucleus, or more caudally on cells scattered in the region of the tuber. Evidence that the paraventricular nucleus does not contribute a major component to the fibres of the neural stalk is seen from the lack of well-marked degenerative changes in this nucleus following lesions of the infundibular stem. Some retrograde degeneration has been described by Rasmussen (1940) in the rat and man, but it is clear that the cell-loss is much less in the paraventricular nucleus than in the supraoptic nucleus. Greater degenerative changes are seen in the paraventricular nucleus, however, if the median eminence of the tuber cinereum is damaged (Heinbecker & White 1941).

Scattered cells and nuclei of the tuberal region of the so-called substantia grisea centralis of Greving, are also often suggested as giving origin to the fibres in the more lateral and posterior parts of the stalk (Roussy & Mosinger (1933*c*) and others). No cellular loss has

been described in this region following the stalk sections, but the possibility remains that scattered cells of the tuber cinereum may give rise to axones passing to the neurohypophysis.

Fisher *et al.* (1938) have summarized the nerve supply of the neurohypophysis and state that the hypothalamo-hypophysial tract is divisible into two main parts:

(*a*) The supraoptico-hypophysial tract in the ventral wall of the neural stalk, arising mainly from the nucleus supraopticus and possibly from other anterior hypothalamic nuclei (viz. nucleus ventromedialis, anterior hypothalamic area and the nuclei periventricularis, dorsalis and ventralis).

(*b*) The tubero-hypophysial tract in the dorsal wall of the neural stalk appears to arise from cells just caudal and lateral to the infundibulum and possibly from the nucleus periventricularis ventralis.

A possible paraventriculo-hypophysial tract arising in the nucleus paraventricularis, must be added to this list.

Now the only one of these tracts on which there is any sound information is the supraoptico-hypophysial tract, this term being now used in the more restricted sense to include only those fibres arising from the nucleus supraopticus. Its nuclear origin, its course in the anterior region of the hypothalamus, tuber cinereum and neural stalk, are fairly well known. Most workers also accept the view of Ranson's school that the essential aetiological factor in diabetes insipidus is a lesion in some part of this tract, whereby it fails to exert a controlling influence on the secretion of the anti-diuretic hormone. The work reported here adds confirmatory evidence in support of these views. Stimulation of the antero-ventral part of the median eminence at its junction with the neural stalk, the site of the supraoptico-hypophysial tract, was effective in inhibiting a water diuresis and in causing other reactions attributable to the activation of the neurohypophysis, whereas stimulation of the lateral and posterior regions of the tuber cinereum was ineffective in producing any such effects. It appears likely that the chloruretic and oxytocic actions of the neurohypophysis are also under the control of the same nerve tract for stimuli capable of producing an anti-diuresis were equally effective in producing these other effects. The results obtained by stimulation of the neurohypophysis are, in themselves, insufficient to conclude that the secreto-motor innervation of this gland is the supraoptico-hypophysial tract. It is true that stimulation of the ventral part of the median eminence and the infundibular stem cause the liberation of hormones from the pars neuralis, but it is possible that tracts from nuclei other than the nucleus supraopticus are located here also. To investigate the possible secreto-motor activity of fibres from the other hypothalamic nuclei it is necessary to consider the results of stimulations made dorsal and rostral to the tuber cinereum where the individual tracts radiate and diverge from each other. From the negative results obtained by stimulation applied in the region of the paraventricular nucleus and ventromedian nucleus, it seems unlikely that these nuclei are concerned with the secretion of the anti-diuretic hormone.

From all the evidence available at the moment, the position may be summarized as follows. The supraoptico-hypophysial tract appears to be one important nervous pathway involved in regulating the secretions of the neurohypophysis; lesions in this tract produce a state of hypo-function of the gland (diabetes insipidus), whereas stimulation of this tract causes secretory activity of the gland. The nucleus of origin of the supraoptico-hypophysial

tract is well known from histological studies and from the results of degeneration experiments. The exact method of termination of the fibres is doubtful. As regards fibres arising from the other hypothalamic nuclei and entering the neural stalk, little is known. The anatomical evidence in most cases is open to the criticism that the fibres have not been traced definitely into the stalk and may possibly end and relay in the tuber cinereum. Degeneration experiments give no evidence that any other nuclei supply fibres to the pituitary with the possible exception of the paraventricular nuclei. Lesions in the posterior hypothalamus are not aetiologically important in the production of diabetes insipidus, and stimulation of the paraventricular and ventro-median hypothalamic nuclei does not cause secretory activity of the neurohypophysis.

If hypothalamic nuclei, other than the nucleus supraopticus, do send fibres into the neural stalk, what might be their function? The most likely hypothesis is believed to be the control of the adenohypophysis, either by a direct passage from the pars nervosa through the pars intermedia, or by means of a chemo-transmission through the hypophysial portal vessels. It must be admitted, however, that this is conjecture.

(iii) *The neurohypophysis as an endocrine gland*

The lack of obvious secretory cells in the neurohypophysis led many of the earlier workers to discredit the view that this organ was an endocrine gland; Cajal, for example, suggested the possibility that the 'superior' lobe of the pituitary was a sensory end-organ. At the beginning of the present century, when extracts of the pars neuralis were found to possess a high degree of pharmacological activity, the probability that this structure functioned as an endocrine gland was reviewed. But again, its apparently non-glandular nature threw doubt on it being the site of formation of any hormones, and the views of Herring (1908) were accepted by many authorities. Herring suggests that the cells of the pars intermedia invaded the posterior lobe and became transformed into hyaline bodies (later known as Herring-bodies) which represented the secretory principle. This material was further supposed to travel up the pituitary stalk towards the third ventricle, when it was assumed to act either on the nerve centres of the tuber or invade, through the ependyma, the ventricular cavity.

In the last ten years the site of formation of the active principles has been shown to be the neurohypophysis. The evidence on this point is quite conclusive in several forms and lies in the fact that in the armadillo, manatee, porpoise, whale and Indian elephant, the buccal hypophysis is separated from the neural by a capsular extension of the meninges, the only point of contact being a tongue-shaped pars tuberalis which rises above the capsule and comes into juxtaposition with the median eminence (see Oldham (1938), *Dasypus novemcinctus*; Oldham, McCleery & Geiling (1938), *Trichechus inunguis*; Geiling, Voss & Oldham (1940), *Tursiops truncatus*, *Prodelphinus pragiodon*; Wislocki & Geiling (1936), *Physeter megalocephalus*, *Balaenoptera physalis*, *Balaenoptera sibbaldi*; and Wislocki (1939), Indian elephant). In these animals, the hormonal content of the neurohypophysis is qualitatively the same as in other mammals as regards the vasopressor and oxytocic principles. These must therefore have been formed *in situ*, and do not owe their presence to the pars neuralis forming part of a transit route between the pars intermedia and hypothalamus. The views

of Herring must then be discarded in these animals and very probably in other forms as well, in which the pars intermedia lies in contact with the infundibular lobe. In the rabbit, the present stimulation experiments have shown that an anti-diuretic effect is obtained more readily when the electrode lies in the neurohypophysis than when it lies in the pars intermedia; and although the anti-diuretic response is only a criterion of release of the hormone into the blood stream and not of its intracellular formation, it is felt that these experiments substantiate the view that the active substances are forms in the neurohypophysis. Further evidence for this view may be found in the tissue-culture experiments of Geiling & Lewis (1935, rat and mouse) and in the results given by Fisher *et al.* (1938) on the hormonal content of the atrophic neural lobes of their cats with experimental diabetes insipidus.

The old problem of the apparent lack of secretory cells in the infundibular lobe remains to be solved. Gersh (1939) produced evidence that his parenchymatous glandular cells (pituicytes of Bucy) are the secretory elements. His evidence, however, has not been confirmed by Hickey, Hare & Hare (1941).

Although it now seems fairly certain that the active principles extractable from the neurohypophysis are formed in this organ, it is only lately that they have been allowed a physiological, as opposed to pharmacological, status. Van Dyke (1936) stated that the experimental evidence was insufficient to conclude that the pars neuralis was a gland of internal secretion. Since that time, however, sound data have accumulated in support of the view that this organ should be included amongst the endocrine glands, so far as its anti-diuretic and probably oxytocic activities are concerned. It is convenient at this point to consider these two activities shown by extracts of the neurohypophysis and the evidence that they have a physiological role in the body. In assessing the possible endocrine activity of the glands of the body three main lines of approach are available. First, the production of a hypo-functional state, either by damage, removal or denervation of the organ, or by changing the external or internal environment of the animal in such a way that the activity of the organ is inhibited. In many cases the effects of these deficiency states on distant organs are easily observed, though in some cases (e.g. the adrenal medulla) more delicate tests are necessary. If the administration of glandular extracts can be shown to prevent the onset of these effects or to abolish them when present, strong evidence is obtained as to the natural function of the gland. The second line of approach lies in the converse procedure, whereby a hyper-functional state of the gland is produced, either by stimulation of the gland or its nerve supply or by changing the external or internal environment of the animal in such a way that the secretory activity of the organ is excited. If these hyper-functional states can be duplicated by injection of extracts of the gland, it would seem that it has at least the potentiality of reproducing these states in the intact animal. Clinical material may be grouped in the same two categories of hypo- and hyper-functional states, there being little evidence for the old theory of dysfunctional activity. Thirdly, there is the method of subjecting the arterial and venous blood of the organ under consideration to chemical or biological assay. This last method of inquiry has borne less fruit in the case of the pituitary than the other two methods, and the results obtained by its use will be omitted in the following discussion.

Hypo-functional states and the anti-diuretic hormone

The first important experimental work performed on this condition was that of Starling & Verney (1925), who showed that the heart-lung-kidney preparation of the dog, in which the animal's own pituitary gland was excluded from the circulatory system, developed a diuresis and that the urine secreted had a low chloride content. Injections of posterior lobe extract reduced the rate of urine flow and raised its chloride content. Verney (1926, 1929) followed up these experiments by demonstrating that if the head of another dog was included in the heart-lung-kidney preparation, the rate of urine flow decreased and the chloride content of the urine increased, but not if the included head had been previously hypophysectomized.

The effect of hypophysectomy in producing the polyuric state of diabetes insipidus has long been a controversial subject. At the present time there appear to be two reasons for the discordant views drawn from the results of hypophysectomy. First, it seems likely that the neurohypophysis (that is, the median eminence, infundibular stem and lobe) acts as a functional unit and, therefore, removal of the posterior or infundibular lobe in many cases leaves sufficient secretory tissue intact to preserve a normal urine flow. And secondly, too little attention was paid previously to the presence or absence of anterior lobe tissue left in the operated animals. Fairly complete removal of the neurohypophysis and the presence of some normally secreting anterior lobe tissue are both essential for the production of the diabetic state (see, however, Heinbecker & White 1941). The results obtained by Fisher *et al.* (1938) by denervating the organ are more convincing than any results in the literature obtained by hypophysectomy. These workers revived the use of the Horsley-Clarke stereo-taxic instrument for producing discrete lesions in the floor of the third ventricle. They were able in many cats (sixty-four) and a few monkeys to cause a state of diabetes insipidus in which polydipsia, polyuria and a urine of low specific gravity were prominent features. They also found that the administration of posterior lobe extracts gave in many cases an efficient replacement therapy and reduced the polyuria to normal levels of urine output. Histologically, it was found that the lesions in the animals which had shown the diabetic condition were always situated so as to interrupt the supraoptico-hypophysial tract on both sides, and that lesions in other regions of the hypothalamus were ineffective in altering the fluid exchange. From these experiments, Fisher *et al.* concluded that the neurohypophysis elaborated a true internal secretion, their reason being that extirpation or secondary atrophy of this structure causes diabetes insipidus, that a potent extract can be prepared from the infundibular lobe, that the neural division itself appears to elaborate this active substance, and that administration of the extract formed a successful substitution therapy for the diabetic state.

The most accurate observations on substitution therapy up to date are those of Shannon (1942). This worker showed that dogs with diabetes insipidus required only small doses of posterior lobe extract to control the polyuria. The extract was administered by a method of continuous intravenous infusion into the marginal vein of the ear, this method being thought to afford the closest approach to the natural liberation of the principle into the blood stream. The dosage necessary to maintain an anti-diuresis in a moderate-sized dog (15 kg.) was found to lie in the range of 1·0–5·0 mU/hr. Shannon points out that this

surprisingly low rate of administration, which presumably is close to the normal rate of liberation, is in keeping with the effectiveness of pituitary preparations whose rate of absorption from the subcutaneous tissues has been considerably diminished (administration of pitressin tannate in pea-nut oil, see Greene & January 1940). It was also found that graded anti-diuretic effects could only be obtained over this range of dosage and that 5·0 mU/hr. was sufficient to produce a maximal effect, larger doses tending to increase the urine flow.

Hyper-functional states and the anti-diuretic hormone

Several experimental procedures are on record to produce secretory activity in the neurohypophysis. Exercise, emotional stress and afferent nerve stimulation have been shown to inhibit a water diuresis in conscious animals, usually dogs, and there is much evidence that these reactions are mediated through the central nervous system and the posterior pituitary. Conclusive evidence that this is so would prove that the neurohypophysis is an endocrine organ capable of secreting an anti-diuretic hormone into the blood stream when the organism was acted upon by a 'natural' stimulus.

Muscular exercise has been known for many years to cause an inhibition in the rate of urine flow. Klisiecki, Pickford, Rothschild & Verney (1933 *a*, *b*) showed in dogs that exercise inhibited the urine flow from a denervated kidney during a water diuresis in a manner comparable to that of the normally innervated fellow kidney of the same animal. It was also shown that administration of posterior lobe extract gave similar results. In 1938, Rydin & Verney showed that mild exercise of 4–5 min. duration caused a definite inhibition of water diuresis in dogs, but that repetition of the experimental procedure in any one animal resulted in progressive diminution of the response. For this and other reasons, they concluded that the inhibition of water excretion by short-lived exercise was due to the emotional accompaniment of that exercise. Emotional stress alone produced a similar type of inhibition, an inhibition which was independent of the endogenous release of adrenaline and yet humorally determined; and its course was accurately matched by a similar dose of post-pituitary extract. The evidence, therefore, was strongly in favour of a pituitary mechanism underlying the reaction. Rydin & Verney also pointed out that if such was the case, there could be little doubt that the supraoptico-hypophysial tract was the nervous pathway by which the pituitary was activated. The correctness of this inference was shown by O'Connor & Verney (1942) by the demonstration that the inhibition of a water diuresis by emotional stress in the dog was largely reduced by the removal of the infundibular lobe of the hypophysis. The operative approach to the pituitary was by Aschner's diasphenoid route, and the fact that the infundibular stem and median eminence were left intact probably accounted for the fact that the post-operative urinary output was within normal limits, and also for the slight inhibitory response that could still be evoked by an emotional stimulus. The evidence, then, for the pituitary hypothesis so far as the emotional inhibition of a water diuresis is concerned is very strong, and it seems likely that the same mechanism underlies, at least in part, the response to muscular exercise.

Another type of stimulus, an afferent nerve stimulus, has also been shown to inhibit a water diuresis, and there is much evidence that the response is mediated through the pituitary gland also. It was first shown by Theobald (1934), whilst repeating some experi-

ments described by Molitor & Pick, that the procedure of lumbar puncture was sufficient to inhibit a water diuresis for about 40 min. and, further, that the mere shaving of the skin over the animal's lumbar vertebrae sometimes gave similar results. Theobald & Verney (1935) showed that the response was unaltered by complete denervation of the kidneys and suggested a humoral agent as responsible for the reaction. Their observations made it unlikely that this agent was adrenaline, and Verney (1936) suggested the response was due to reflex release of the anti-diuretic principle from the posterior pituitary. Evidence in support of this hypothesis was obtained by Haterius (1940), who showed that similar lumbar irritation in rabbits anaesthetized with chloralose-urethane provoked a marked inhibition of a water diuresis, but not if the pituitary stalk had been interrupted 2–4 days previously. Complete agreement on the underlying mechanism has not been reached by all authorities however, for Hare (1939) states that similar inhibitions of urine flow follow restraint or painful stimuli in cats and dogs suffering from diabetes insipidus caused by hypophysectomy or pituitary stalk section. Whatever the explanation of Hare's observations may be, the balance of evidence is, at the moment, strongly in favour of the pituitary hypothesis.

Electrical stimulation of the neurohypophysis or its nerve supply promised more direct and convincing evidence on this problem than the rather circuitous methods of stimulation described above. The work of Haterius (1940) already described was unconvincing owing to the limitations imposed by the method of stimulation used. The anaesthetic and the necessity for clamping the animal's head in the stereotaxic instrument interfered with the water diuresis in many cases so that few reliable results were obtained. Remote control stimulation afforded a method of overcoming the difficulties inherent in other techniques, anaesthesia and disturbance to the animals in the form of head clamps and immediate operative procedures being rendered unnecessary. In the present work, the highly localized nature of the stimulus, making it certain that the neurohypophysis was the active region involved, and the possibility of repeating the experiment in any particular animal so that individual irregularities could be controlled, were both found to be factors of the greatest value. Other results which confirmed the belief that stimulation evoked a physiological endocrine response were the constancy of the anti-diuretic response as observed from day to day; the parallelism seen between the intensity of stimulation and the degree of anti-diuresis, that is, the gradation effect; and the close correspondence between the results of stimulation and the intravenous injection of posterior lobe extracts, both on the rate of urine flow and the concentration of urinary chlorides.

Only one other method of stimulating the posterior pituitary to secretory activity will be described, and that is the 'method of dehydration'. If the fluid intake of an animal is reduced, the fluid output likewise falls, and it was thought probable that the lowered output was due to a higher rate of secretion of the anti-diuretic hormone. Now since it has been shown that the intravenous injection of posterior lobe extracts into decapitated cats (Jones & Schlapp 1936), rabbits and rats (Heller 1937), is followed by the appearance of part of their pressor and anti-diuretic activities in the urine, several workers have examined the urine of severely dehydrated animals to see whether the supposed stimulus to pituitary secretion in the form of a lowered fluid intake is manifested by an excretion of substances with an anti-diuretic activity. Gilman & Goodman (1937) found that if fluid was withheld from normal rats for periods up to 96 hr. an anti-diuretic substance appeared in the urine

in marked quantity in 24 hr. and maximum in 48 hr. Their assays were made according to Burn's rat method. Hypophysectomized rats consistently failed to show a diuresis-inhibiting substance in their urine on similar treatment. Ingram, Ladd & Benbow (1939) found that dehydrated normal cats excreted a substance in the urine which depressed water diuresis in rats. In cats suffering from experimental diabetes insipidus, dehydration was ineffective in causing the appearance of anti-diuretic material in the urine. Walker (1939), however, failed to substantiate these results. He stated that normal cats with free access to drinking water excreted small amounts of anti-diuretic material in their urine, and that the amount of this substance was not always increased by dehydration and was unaffected by hypophysectomy. In experiments on rats, the results of Gilman & Goodman were confirmed in the main, but their conclusions were strongly criticized on the grounds that the evidence for the pituitary origin of the material was insufficient.

Further work is needed before any definite conclusions can be drawn regarding the urinary anti-diuretic material. It would be interesting to measure the excretion of this substance before and after stimulation of the neurohypophysis, for it seems likely that this procedure would furnish evidence for the origin of the material.

As regards the anti-diuretic activity of the neurohypophysis, there appears ample evidence for regarding this organ as an endocrine gland secreting an anti-diuretic hormone.

Hypo-functional states and the oxytocic 'hormone'

Over 30 years have elapsed since Dale (1909) first described the oxytocic action of post-pituitary extracts, and the physiological significance of this is still undecided, though the suggestion almost implicit in such an action is that the neurohypophysis forms part of the mechanism responsible for the uterine contractions at parturition.

Hypophysectomy of the pregnant animal has given varying results in the hands of different workers. The majority find that the operation performed early in pregnancy is liable to cause foetal death and absorption, and performed later may result in premature delivery, though in many cases the duration of pregnancy is unaffected, a normal litter being delivered at full term. After total hypophysectomy the young die within a short time of birth owing to the failure of the hypophysectomized mother to lactate. The noteworthy point for the present discussion is the claim that hypophysectomy does not necessarily prevent normal parturition from occurring. As pointed out by Fisher *et al.* (1938), results of this nature should not be used as evidence that the neurohypophysis is not concerned in the mechanism of normal labour without consideration of the following points:

(1) The completeness with which the neurohypophysis has been removed. Many workers have drawn conclusions from the results of simple hypophysectomy in which the infundibular stem and median eminence have been left intact. As mentioned previously, these two structures are similar to the infundibular lobe in function and may possibly account for the normal labour in the same way as they may prevent the onset of diabetes insipidus after removal of the infundibular lobe. Thus serial sections through the hypothalamus and sella turcica are essential to the proper interpretation of such work.

(2) The extent to which the pars distalis has been left intact. It is technically difficult or impossible to remove the neurohypophysis and leave the adenohypophysis intact, but

it is desirable that at least some secretory tissue of the glandular lobe should be left *in situ*. There is little work on record in which this condition has been observed.

(3) The type and duration of labour. The statement that delivery occurred at term is insufficient: a full account of the duration of the various stages and of any deviation from the normal is required.

With the above criteria in mind, the significance of the results obtained by many workers is rendered doubtful.

The experimental technique of Fisher *et al.* (1938), and Dey *et al.* (1941) afford more satisfactory results in estimating the part played by the neurohypophysis in labour. In these experiments, small localized lesions were placed in the hypothalamus, interrupting the supraoptico-hypophysial tracts, in pregnant cats and guinea-pigs. In the majority of animals disturbances of parturition were seen, many of the animals dying in labour. However, since about one-third of the guinea-pigs with these lesions delivered themselves normally, it was felt that the results could not be offered as convincing evidence that the secretory activity of the neurohypophysis played an essential part in labour. In view of the fact that the pars distalis was not subjected to direct interference and that the median eminence and infundibular stem were denervated, it would seem that these experiments are of greater value in the analysis of parturition than the work quoted above in which operative hypophysectomy was employed.

Hyper-functional states and the oxytocic 'hormone'

The effect of increased activity of the neurohypophysis on the uterus had received little attention until recently. The effect of stimulation of the pituitary stalk on uterine movements was described by Haterius & Ferguson (1938) and Ferguson (1941). These observers found a marked increase in uterine motility in anaesthetized post-partum rabbits and cats on stimulation of the stalk, which was abolished if an electrolytic lesion had been previously made in the stalk but was not abolished by crushing the neck so that only vascular connexions remained between the head and trunk. The response was also shown not to be due to concurrent blood-pressure changes. The stimulus used in these experiments was sufficient to produce a bilateral flutter of the eyelids and twitching of the whiskers. These signs have been noted during stimulation of the same region with the remote-control method, though a stimulus strong enough to produce twitching of the whiskers was rarely used, a much weaker stimulus being sufficient to produce a maximal uterine response providing the stimulating tip of the electrode was situated in, or in contact with, the infundibular lobe or stem. There seems little doubt, however, that the results obtained by Haterius & Ferguson were due to secretion of the oxytocic factor by the neurohypophysis.

If the experiments of Fisher *et al.* (1938) and Dey *et al.* (1941) failed to produce convincing evidence that the neurohypophysis plays an essential part in labour, the work of Haterius & Ferguson certainly demonstrates the potentiality of the gland to fulfil such a role and lends support to theories such as the oestrin-oxytocin synergism theory propounded by Marrian & Newton (1935).

The results of remote-control stimulation of the neurohypophysis on the motility of the uterus of the oestrous rabbit reinforce the results of the American workers. The reactions of the post-partum uterus to stimulation of the neurohypophysis were not studied in this

present work, but the responses from the oestrous and oestrogenized uteri were very similar to those described and depicted by Haterius & Ferguson for the post-partum organ. The endocrine state in some post-partum rodents appears to be similar in many ways to that of oestrus, as shown by the regularity with which mice and rats and non-suckling post-partum rabbits come into oestrus within a few days of delivery. Therefore, the similarity of the responses of the oestrous and post-partum uteri to stimulation of the pituitary is hardly surprising.

It is difficult at first sight to see any physiological significance underlying the fact that the rabbit possesses a means for increasing the activity of the empty oestrous uterus, a means that involves a nervous and glandular transmission of stimuli. One possible function served by this mechanism may be the transportation of spermatozoa from the vagina to the uterine cavity during and after coitus. It is generally admitted that coitus in the rabbit stimulates the activity of the adenohypophysis by a reflex pathway through the hypothalamus and infundibular stem. It seems likely that the neurohypophysis may be stimulated by a similar nervous reflex and that the resulting uterine activity may, by the creation of negative pressure waves, assist in the transmission of seminal fluid. That stimulation of the neurohypophysis occurs during coitus is rendered probable by the work of Verney and his colleagues, who showed that emotional stress causes liberation of at least the anti-diuretic hormone from this gland, and it cannot be doubted that an emotional disturbance is the normal accompaniment of coitus and the orgasm. Reynolds (1930*a*) showed that the activity of the rabbit's uterus is increased by coitus, and it seems likely that this is at least partly dependent on the secretion of the oxytocic factor by the pituitary gland. The part played by uterine movements in the carriage of seminal fluid up the female genital tract is undecided. In some forms, such as the rat, the uterine activity is probably an important factor, for Hartman & Ball (1930) found that in this animal large numbers of spermatozoa were present in the uterus within 30 sec. of coitus, and had reached the ovarian end of the uterus in less than 2 min. In the case of the rabbit the position is not so clear. The early observation of Heape (1905) that sperms reach the ovarian end of the rabbit's uterus about 2 hr. after copulation has been confirmed by many workers, amongst whom are Florey & Walton (1932), who also assert that uterine movements in the rabbit play no part in the transport of seminal fluid. The most detailed account of the passage of sperms and ova in this animal is, however, that given by Parker (1931). This worker studied the rate of entry of sperms into the uteri by killing female rabbits at measured intervals after coitus, and immediately severing the uterus near the upper boundary of the cervix. He found that the entry was too rapid to be accounted for by the rate of swimming of the sperms, estimated at 0·05 mm./sec., and from these and other observations concluded that the ascent of spermatozoa into the uterus is dependent upon a vigorous muscular reaction of the uterus excited by the act of coitus; and that infertile coitus may be caused by failure in this uterine reaction. Parker quotes an excerpt from an early paper by Heape (1898): 'It is not clear how the spermatozoa naturally find their way from the vagina to the uterus; but, from certain experiments which I have made on rabbits, I am inclined to think the greater part of it is drawn in by a sucking action of the uterus. The os, which is placed above the ventral wall of the vagina appears to dip down into the midst of the spermatozoa as they lie on the floor of the vagina and in conjunction with peristaltic contraction of the

uterus, to be withdrawn again, and this action appears to be repeated more than once at intervals....This sucking action of the uterus in the rabbit above mentioned, was induced by stimulating the erectile tissue of the vulva.' This last sentence of Heape's almost implies a neural reflex as underlying the uterine movements concerned, and it is possible that the neuro-hormonal transmission, as suggested above, may be the means used to stimulate the uterus to this sperm-acquiring activity.

It may be concluded that the evidence for the hormonal nature of the oxytocic activity of the posterior pituitary is weaker than that for the anti-diuretic action, but is still sufficient to render it probable that the neurohypophysis is an endocrine gland concerned with parturition (and possibly sperm transport). As regards the former function, further evidence is awaited as to the intimate nature and co-ordination of uterine contraction and relaxation involved in the delivery of a litter of young (see Newton 1937), and also confirmation, on different species, of the results obtained by Ranson and his co-workers on the dystocia produced by lesions in the supraoptico-hypophysial tract.

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Description of Plates

Plate 15

Figure 26. X-ray photograph of head of rabbit 2; 2 January 1941. ($\times \frac{2}{3}$.)

Figure 27. X-ray photograph of head of rabbit 2; 14 January 1944. ($\times \frac{2}{3}$.)

Figure 28. Photograph of rabbit 2. Coil unit implanted 1 January 1941, photograph taken 14 January 1944.

Figure 29. Photograph of rabbit 9 with litter. Coil unit implanted 12 December 1941. Photograph taken 20 February 1943.

Figure 30. Photograph of skull vault of rabbit 15 and skull of normal rabbit, to show the secondary formation of bone which occurs beneath the coil. Coil inserted 29 April 1942; rabbit killed 28 October 1942. ($\times \frac{2}{3}$.)

Figure 31. Photograph of skull vault and soft tissues of rabbit 29 to show the fibrous tissue capsule which forms over the implanted coil. The capsule has been opened by a cruciate incision, one segment turned back and the coil removed. Coil inserted 9 September 1943, rabbit killed 21 March 1944. ($\times \frac{2}{3}$.)

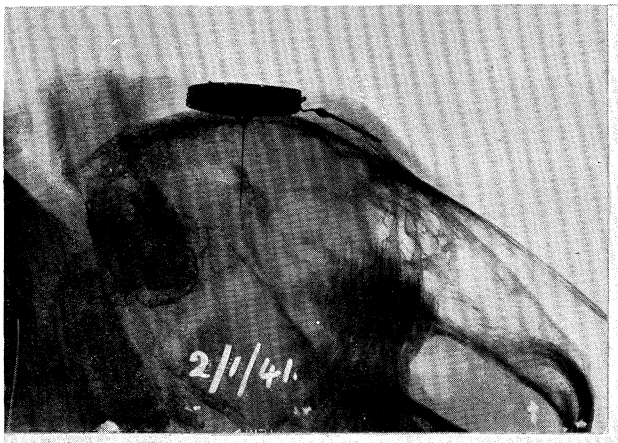
Plate 16

Figure 32. Photomicrograph of a horizontal section through the dorsal hypothalamus of rabbit 22 (group A). Note the site of the insulated electrode tip just posterior to the anterior part of the third ventricle. Toluidine blue stain. ($\times 7\frac{1}{3}$.)

Figure 33. Photomicrograph of the same brain as shown in figure 32; slightly more dorsal level. Note the larger hole left by the insulated region of the electrode. Toluidine blue stain. ($\times 7\frac{1}{3}$.)

Figure 34. Photomicrograph of the electrode site, shown in figure 32. To show the normal nerve cells and mild tissue reaction in the vicinity of the stimulating tip of the electrode. Toluidine blue stain. ($\times 163$.)

Figure 35. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 27 (group A), to show the site of the stimulating tip of the electrode just anterior ($\frac{1}{2}$–1 mm.) to the infundibular lobe. (An artificial tear is present in this section between the pars distalis and the pars intermedia.) ($\times 7\frac{1}{3}$.)



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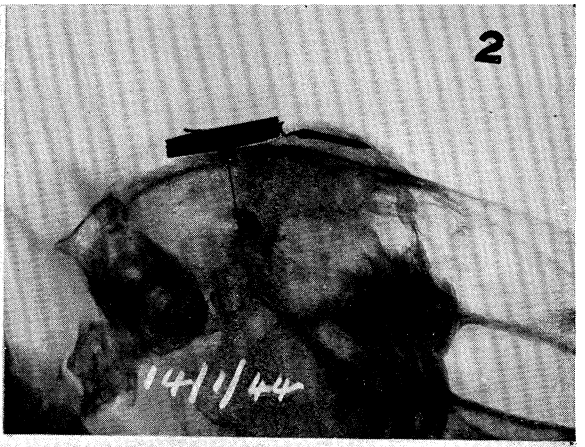

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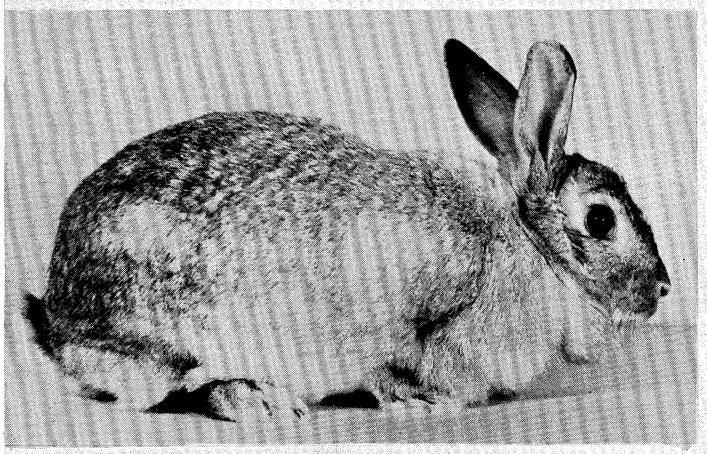

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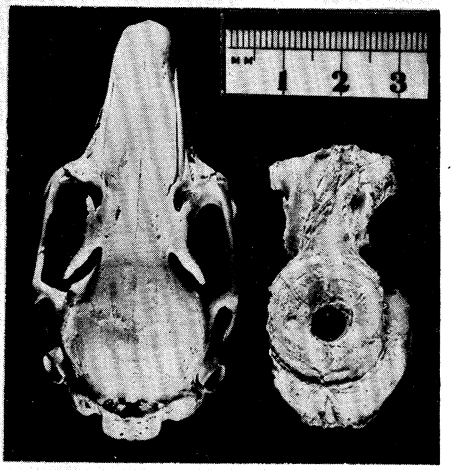

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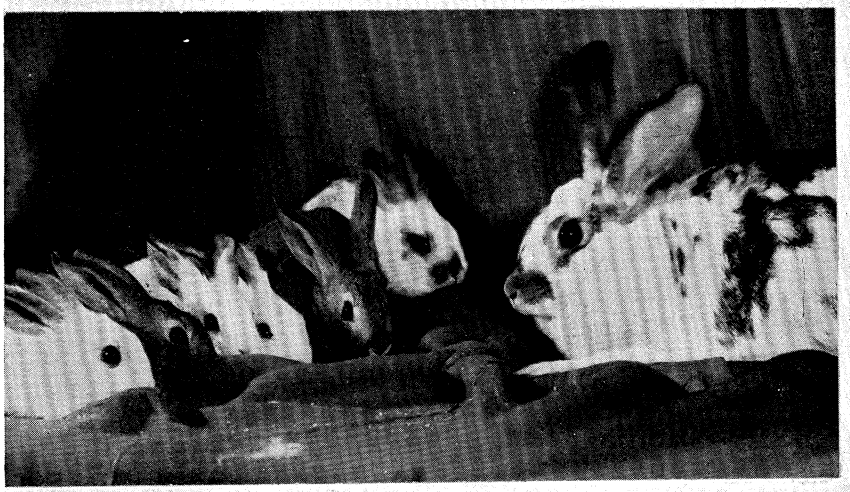

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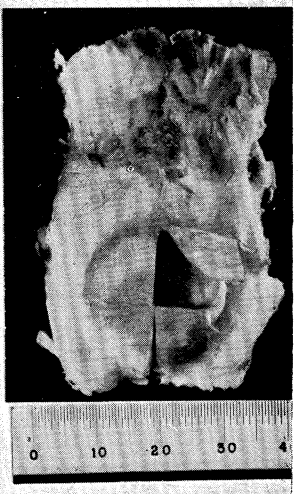

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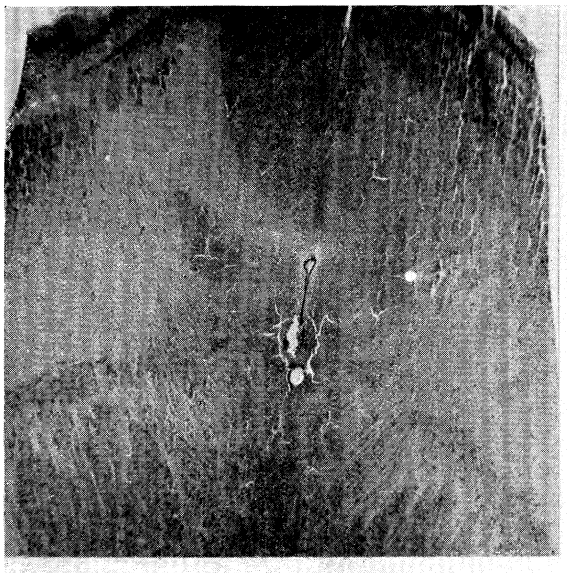

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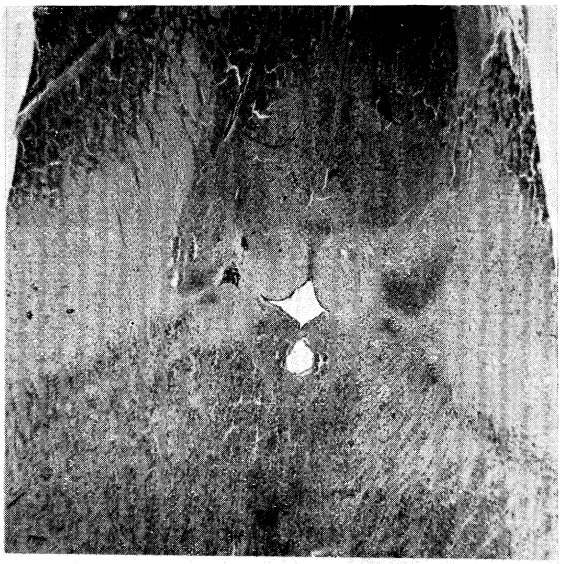

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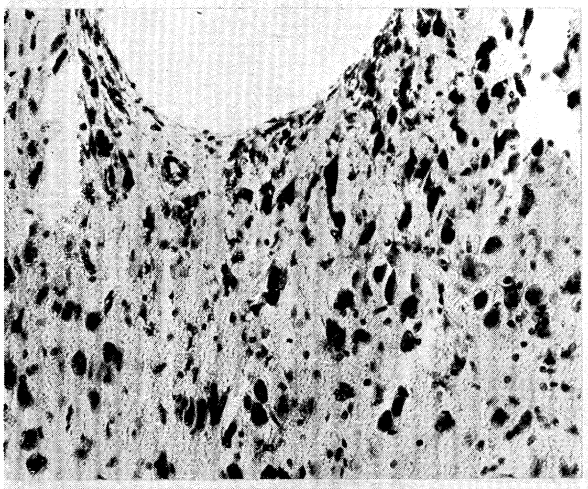

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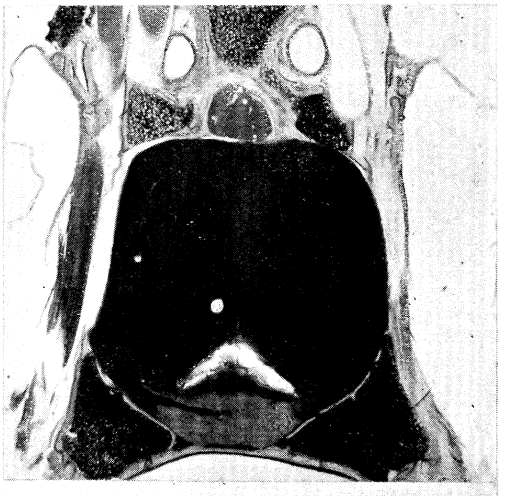

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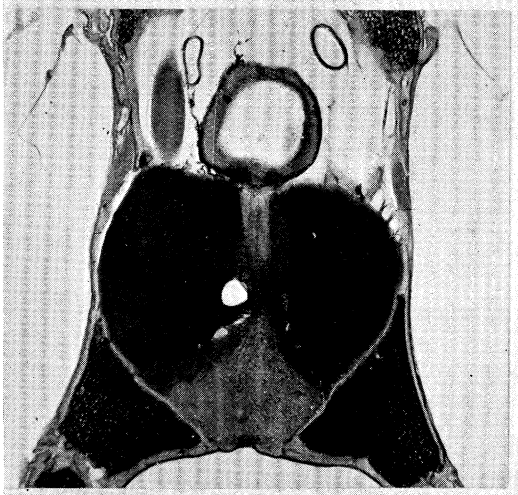

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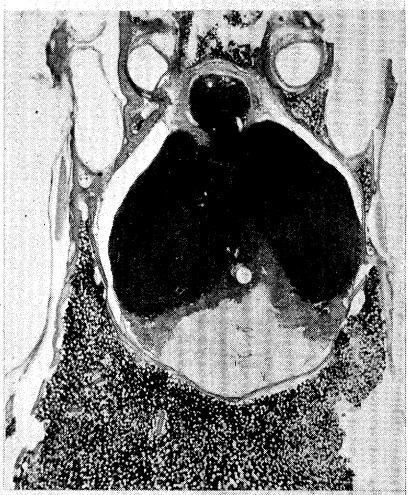

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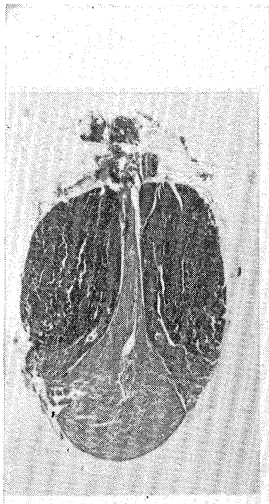

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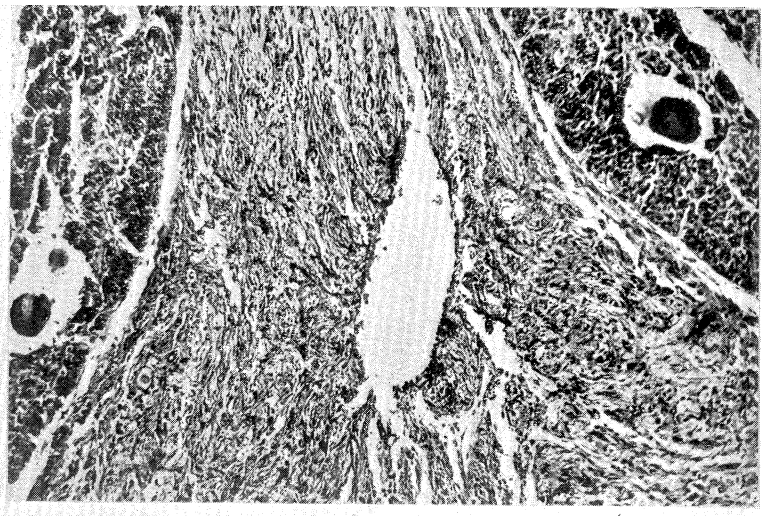

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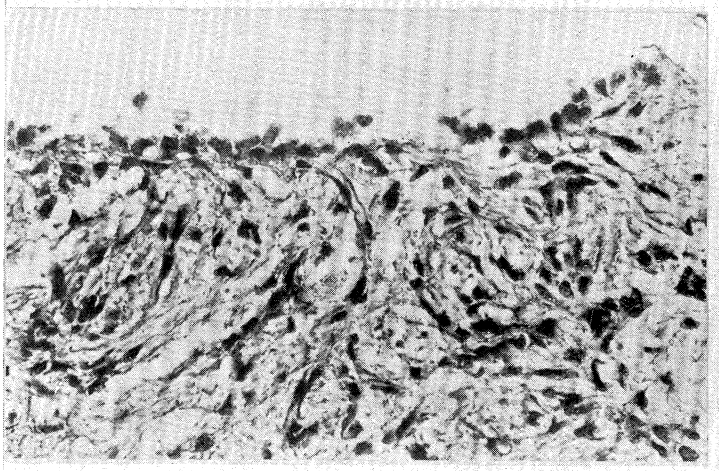

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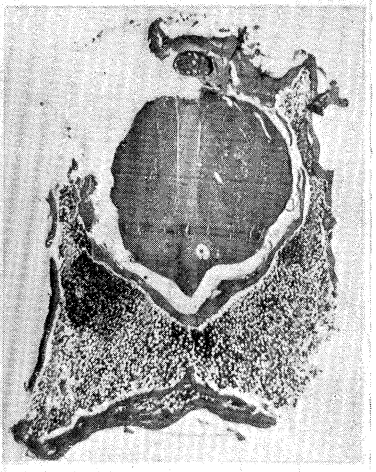

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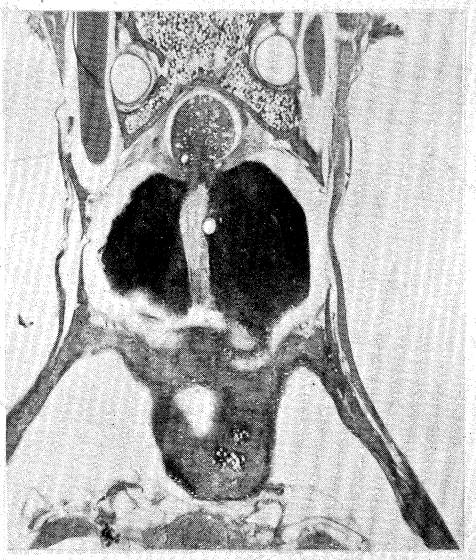

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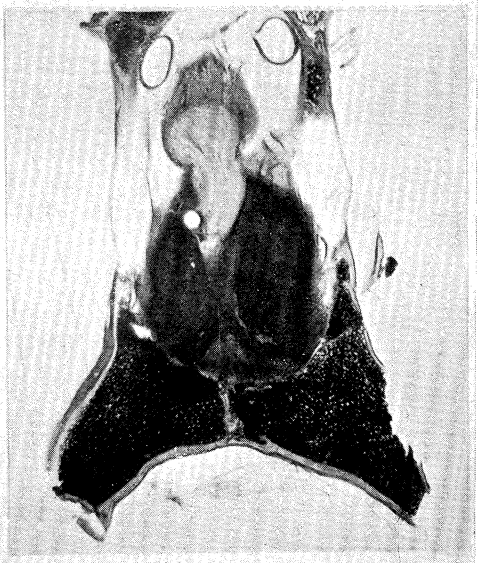

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FIGURE 36. Photomicrograph of the same pituitary as in figure 35, showing the increase in the size of the hole to accommodate the insulated part of the electrode. Note that in this rabbit the infundibular stem was in contact only with the insulated region of the electrode. (× 7⅓.)

FIGURE 37. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 25 (group B). Note the site of the stimulating tip of the electrode in the pars intermedia. (× 7⅓.)

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FIGURE 38. Photomicrograph of a horizontal section through the pituitary gland of rabbit 13 (group C). Note the site of the electrode tip in the junction of the infundibular stem with the infundibular lobe. (× 7⅓.)

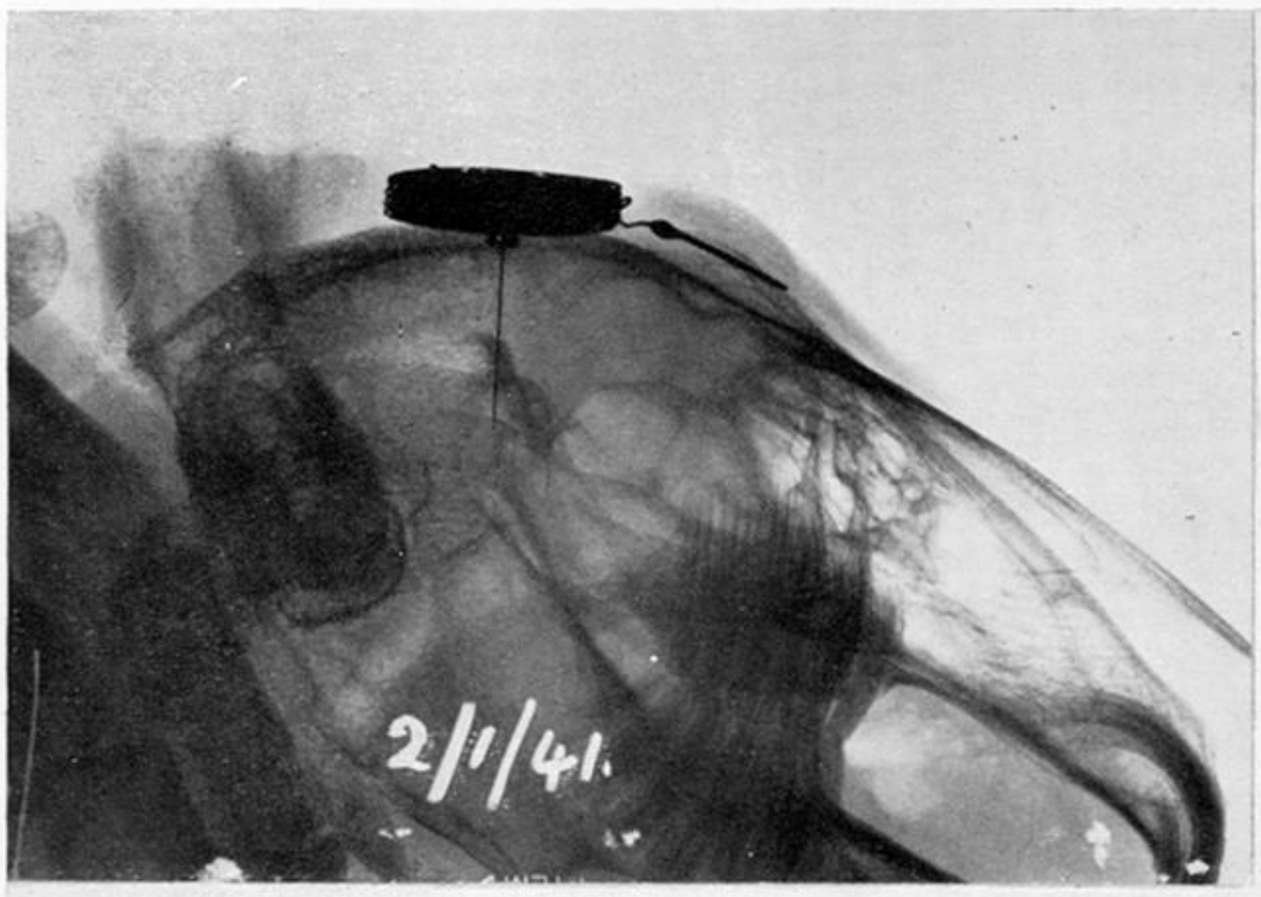
FIGURE 39. Photomicrograph of the electrode site shown in figure 38. (× 75.)

FIGURE 40. Photomicrograph of the electrode site shown in figures 38, 39. High-power view to show the very slight tissue reaction around the electrode. (× 291.)

FIGURE 41. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 15 (group C). The electrode tip was situated in the infundibular lobe, slightly to the left of the mid-line (reversed in photomicrograph). (× 7⅓.)

FIGURE 42. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 21 (group C). The electrode tip was situated in contact with the right side of the infundibular stem. (× 7⅓.)

FIGURE 43. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 35 (group C). The electrode tip was situated in contact with the right side of the infundibular stem (reversed in photomicrograph). (× 7⅓.)



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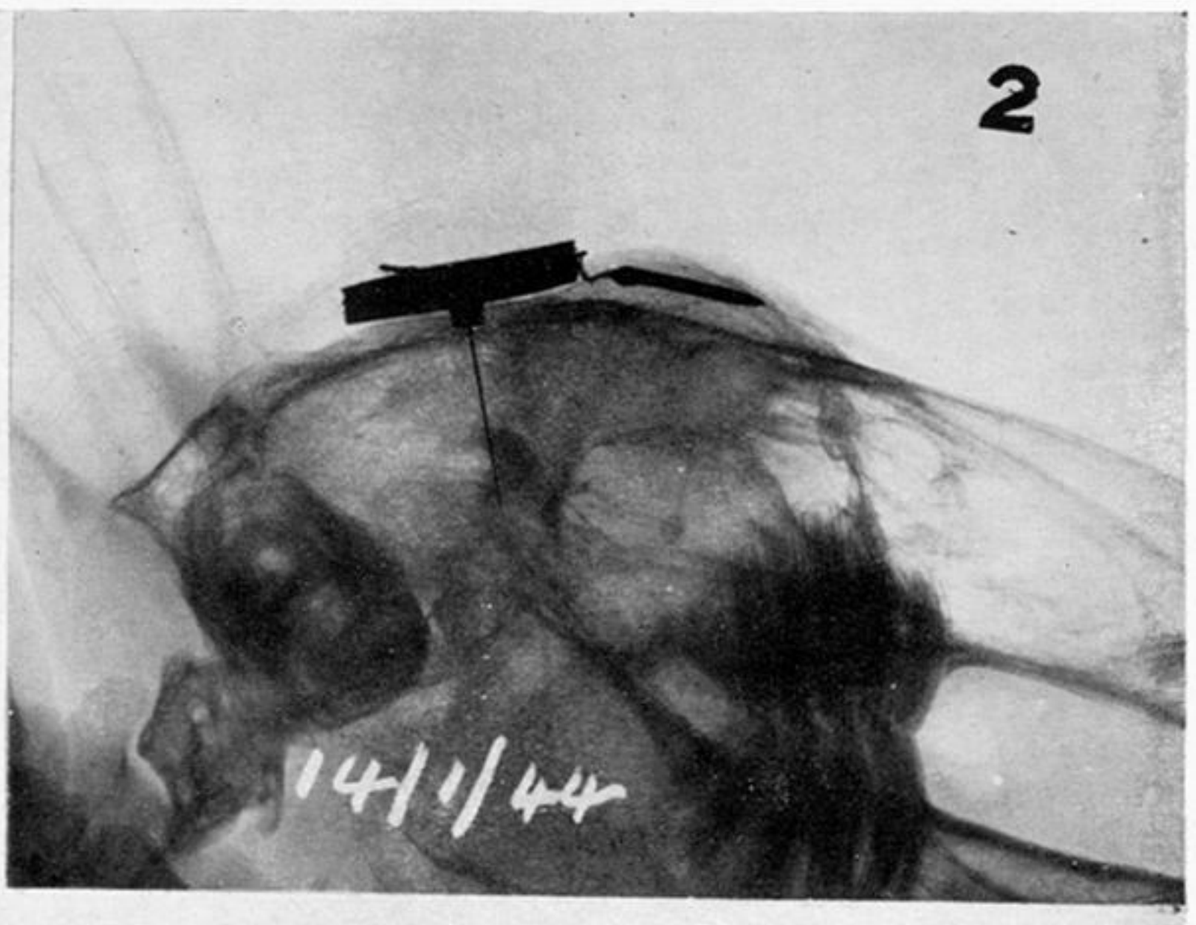

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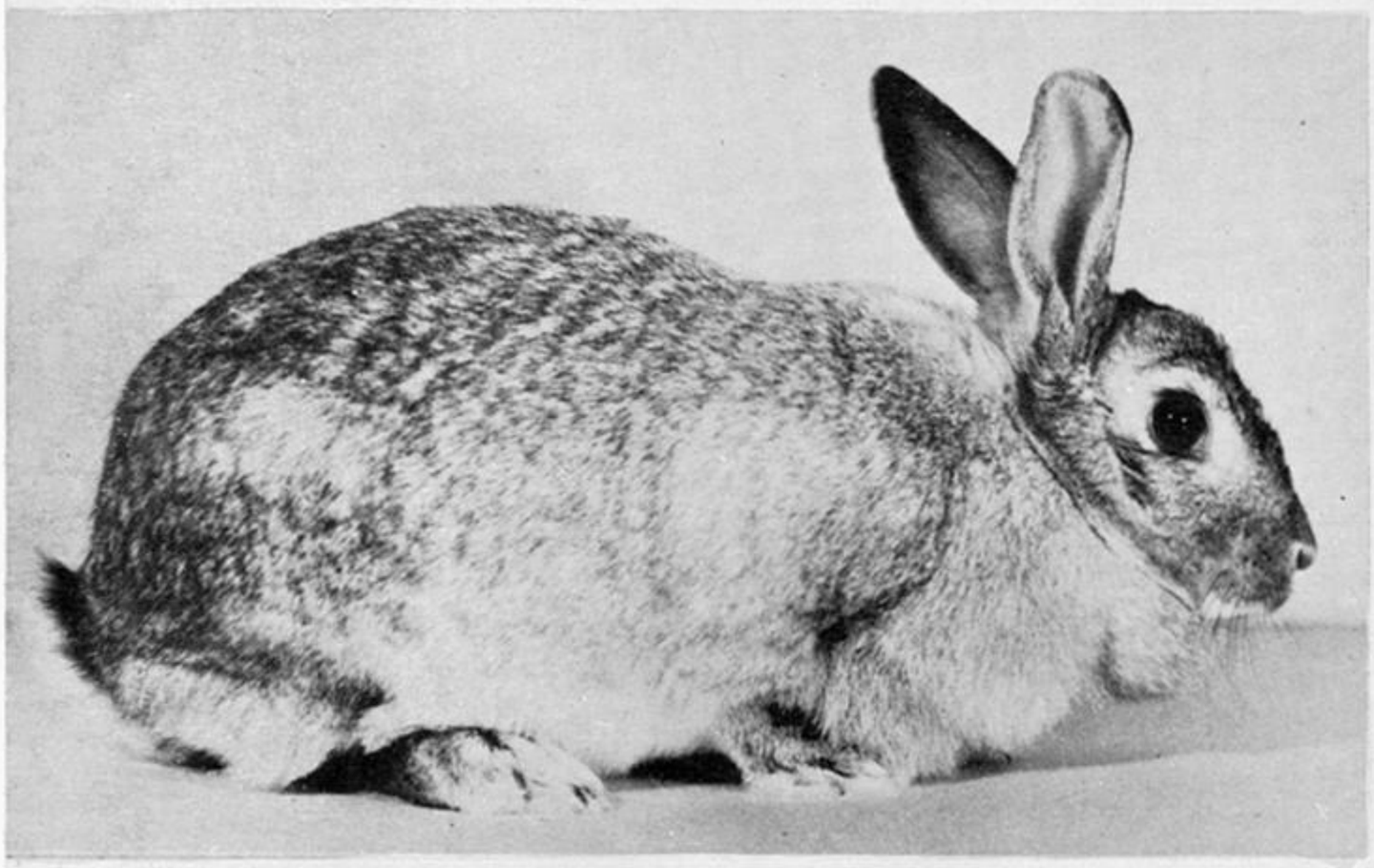

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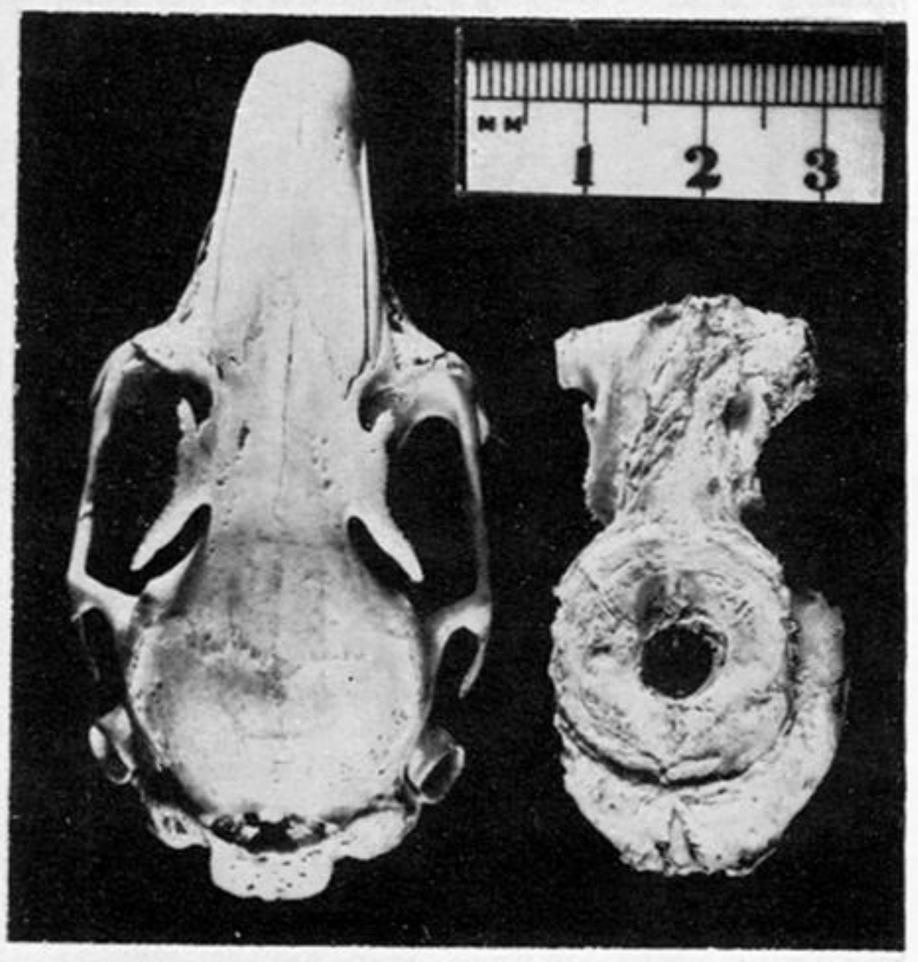

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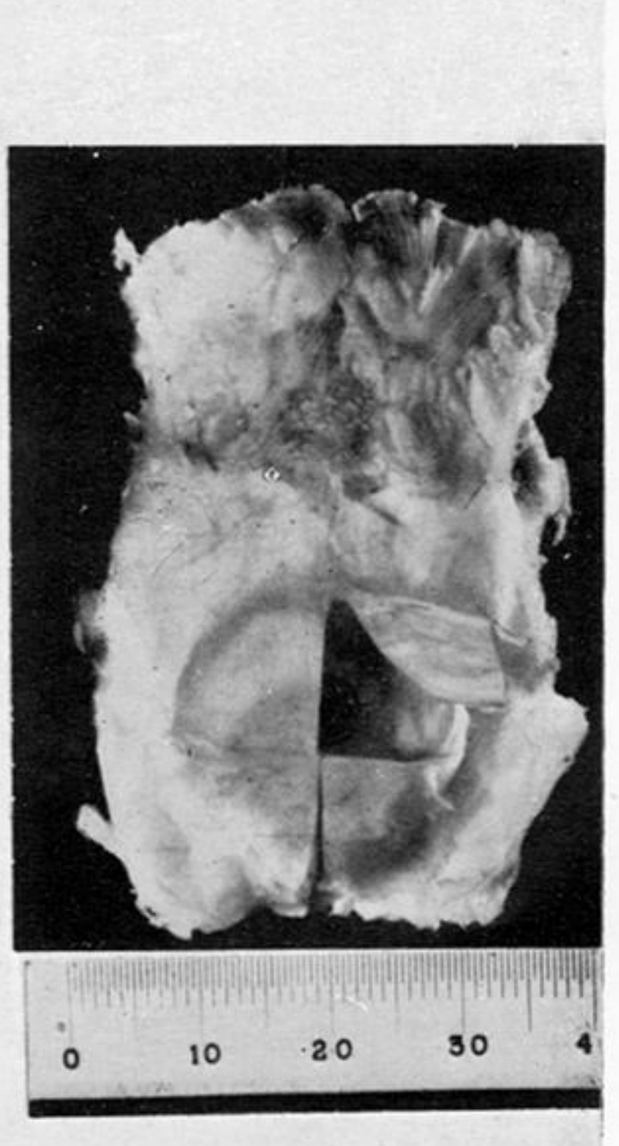

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Plate 15

Figure 26. X-ray photograph of head of rabbit 2; 2 January 1941. ($\times \frac{2}{3}$.)

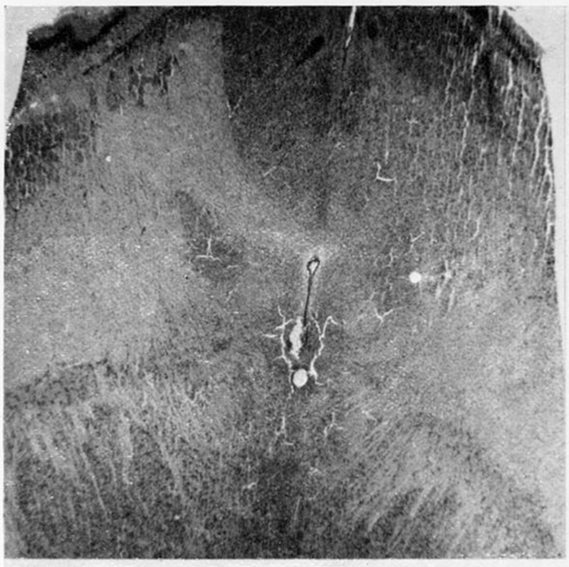
Figure 27. X-ray photograph of head of rabbit 2; 14 January 1944. ($\times \frac{2}{3}$.)

Figure 28. Photograph of rabbit 2. Coil unit implanted 1 January 1941, photograph taken 14 January 1944.

Figure 29. Photograph of rabbit 9 with litter. Coil unit implanted 12 December 1941. Photograph taken 20 February 1943.

Figure 30. Photograph of skull vault of rabbit 15 and skull of normal rabbit, to show the secondary formation of bone which occurs beneath the coil. Coil inserted 29 April 1942; rabbit killed 28 October 1942. ($\times \frac{2}{3}$.)

Figure 31. Photograph of skull vault and soft tissues of rabbit 29 to show the fibrous tissue capsule which forms over the implanted coil. The capsule has been opened by a cruciate incision, one segment turned back and the coil removed. Coil inserted 9 September 1943, rabbit killed 21 March 1944. ($\times \frac{2}{3}$.)



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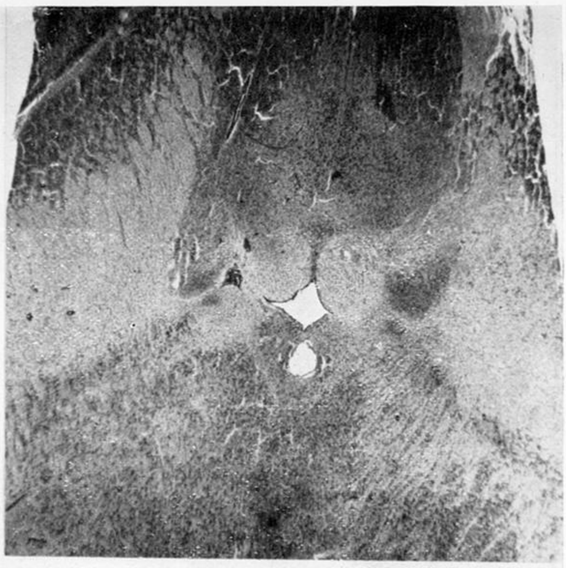

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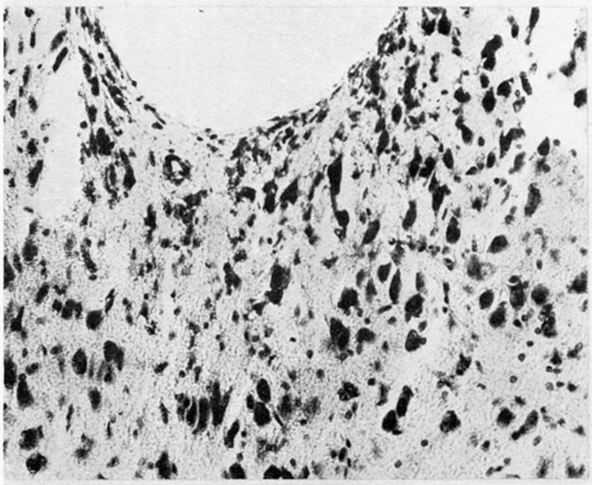

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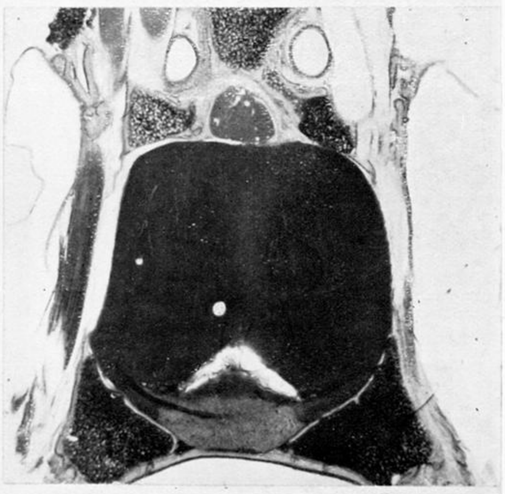

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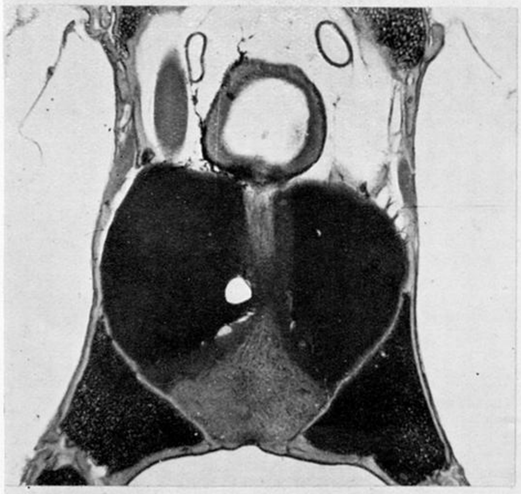

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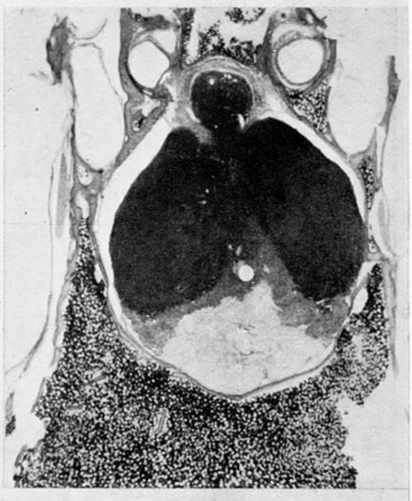

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Plate 16

Figure 32. Photomicrograph of a horizontal section through the dorsal hypothalamus of rabbit 22 (group A). Note the site of the insulated electrode tip just posterior to the anterior part of the third ventricle. Toluidine blue stain. ($\times 7\frac{1}{3}$.)

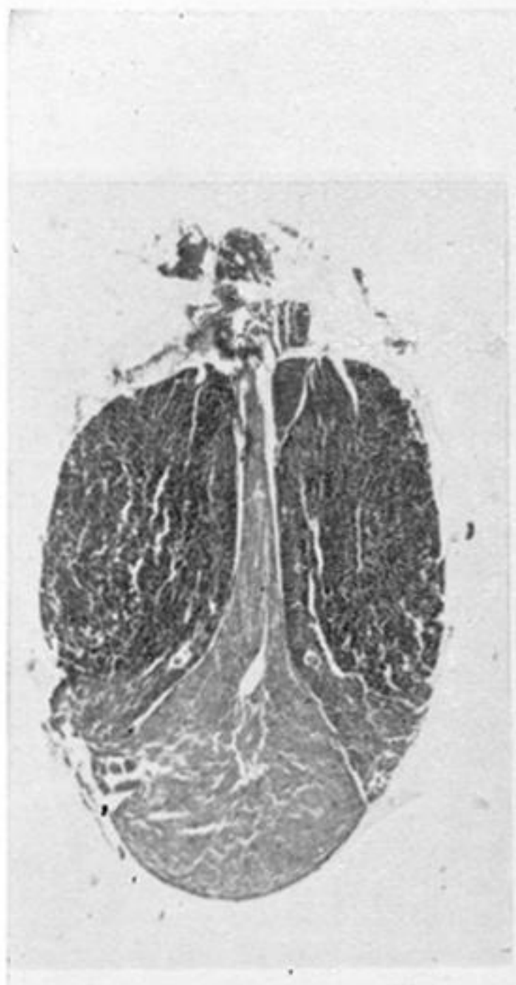
Figure 33. Photomicrograph of the same brain as shown in figure 32; slightly more dorsal level. Note the larger hole left by the insulated region of the electrode. Toluidine blue stain. ($\times 7\frac{1}{3}$.)

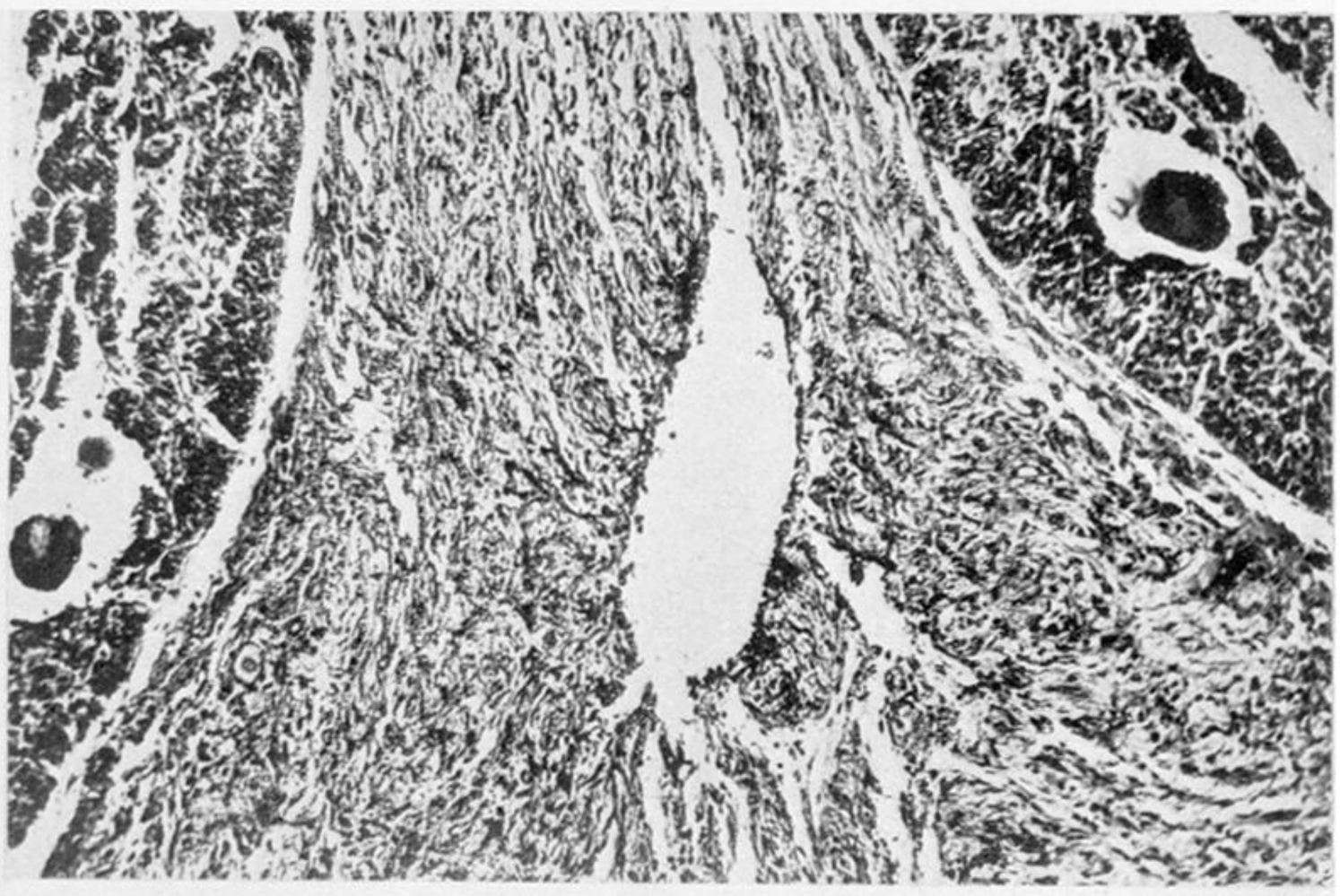
Figure 34. Photomicrograph of the electrode site, shown in figure 32. To show the normal nerve cells and mild tissue reaction in the vicinity of the stimulating tip of the electrode. Toluidine blue stain. ($\times 163$.)

Figure 35. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 27 (group A), to show the site of the stimulating tip of the electrode just anterior ($\frac{1}{2}$–1 mm.) to the infundibular lobe. (An artificial tear is present in this section between the pars distalis and the pars intermedia.) ($\times 7\frac{1}{3}$.)

Figure 36. Photomicrograph of the same pituitary as in figure 35, showing the increase in the size of the hole to accommodate the insulated part of the electrode. Note that in this rabbit the infundibular stem was in contact only with the insulated region of the electrode. ($\times 7\frac{1}{3}$.)

Figure 37. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 25 (group B). Note the site of the stimulating tip of the electrode in the pars intermedia. ($\times 7\frac{1}{3}$.)





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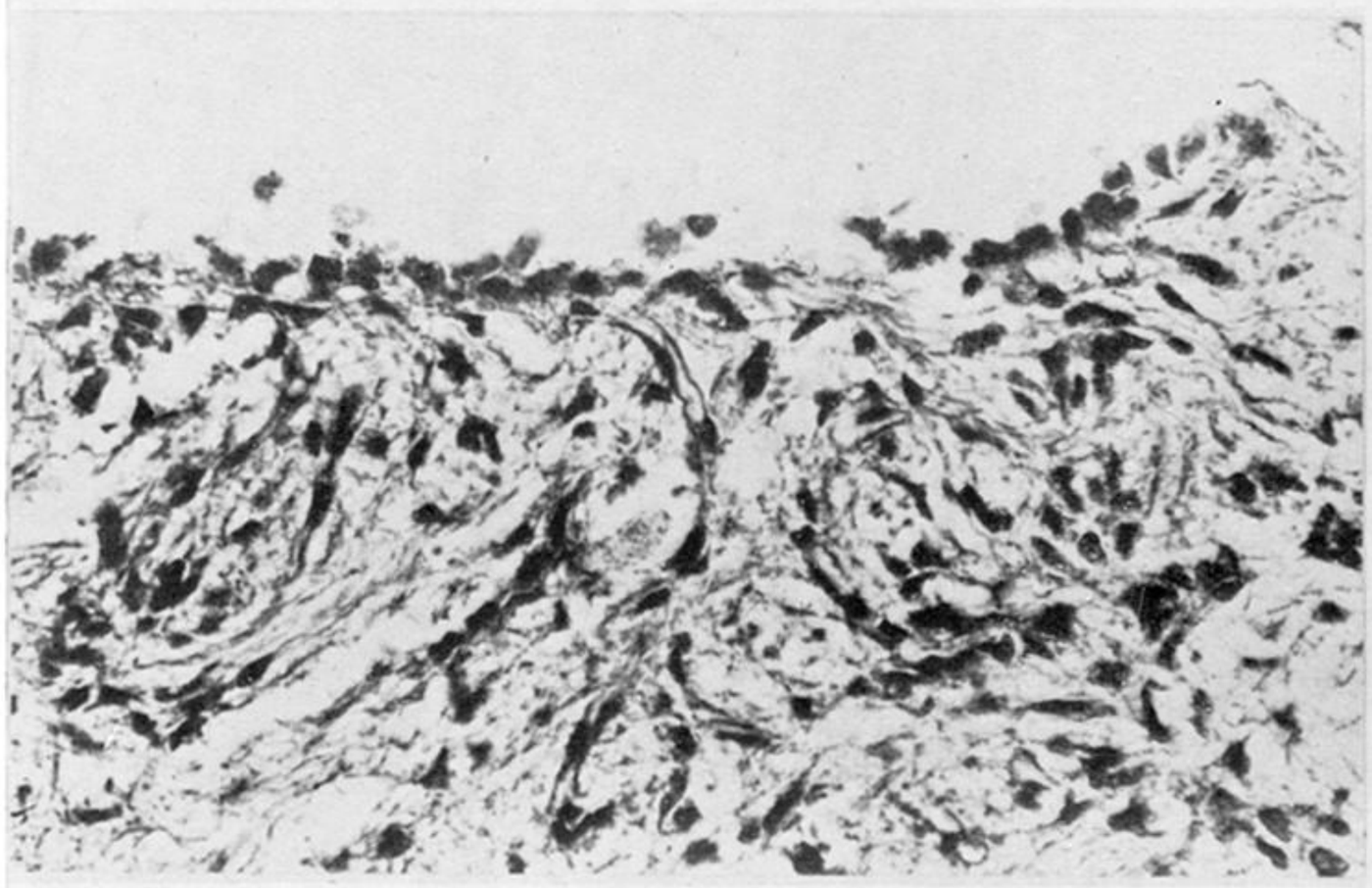

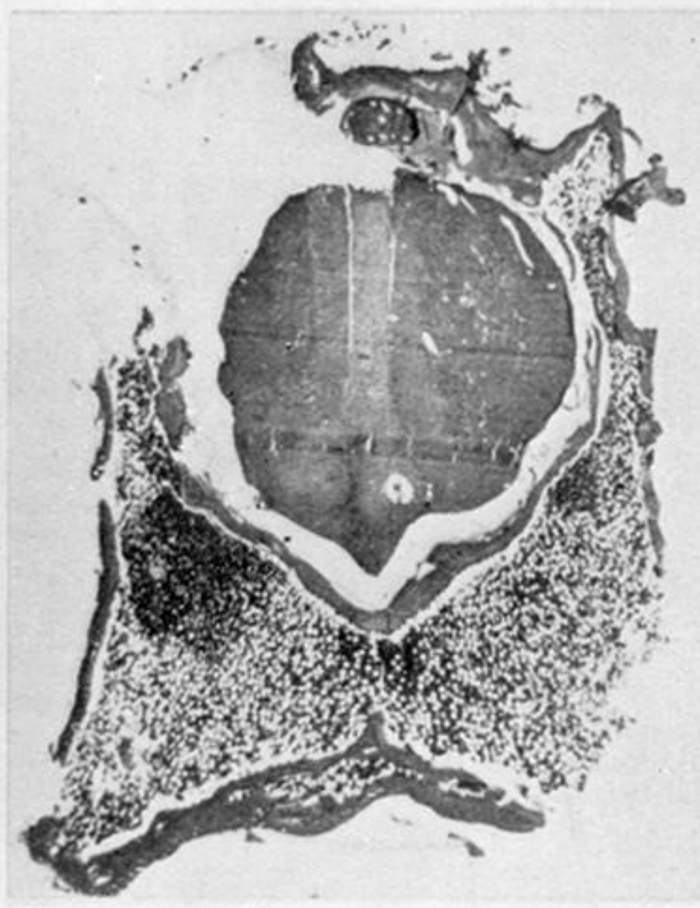

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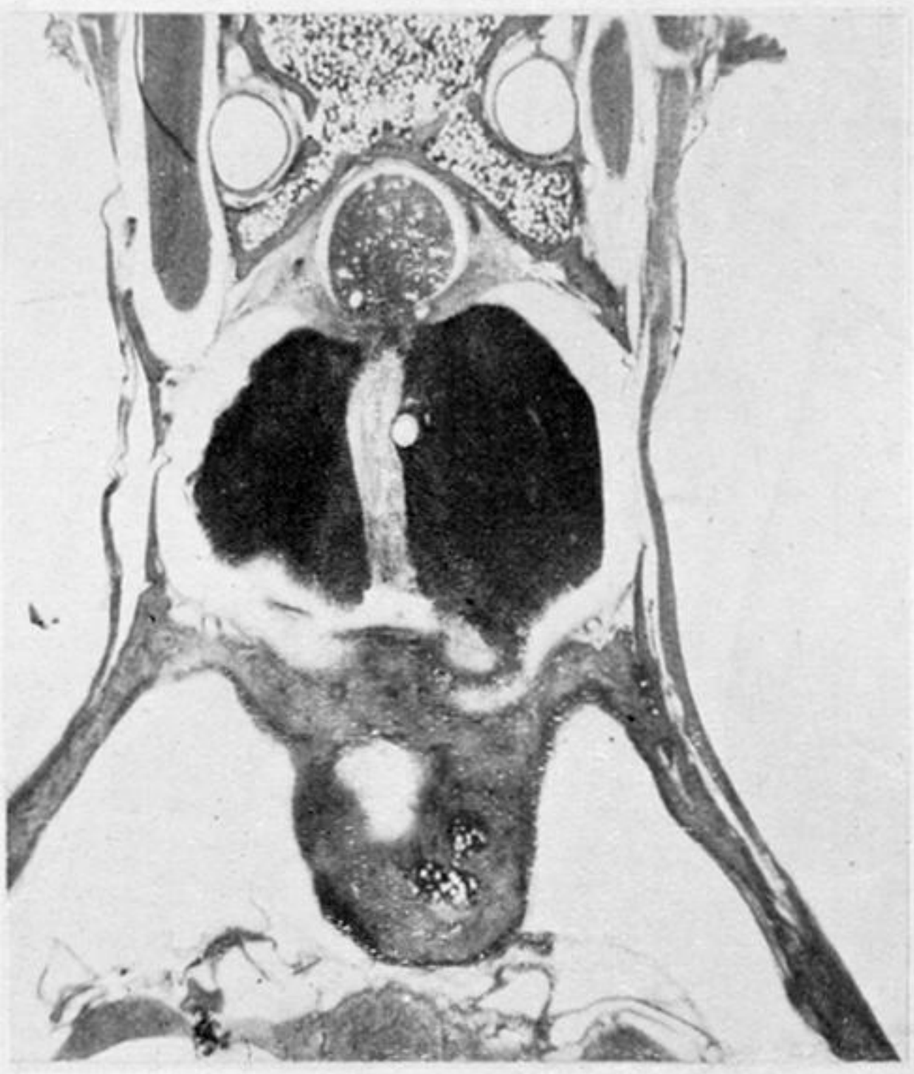

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PLATE 17

FIGURE 38. Photomicrograph of a horizontal section through the pituitary gland of rabbit 13 (group C). Note the site of the electrode tip in the junction of the infundibular stem with the infundibular lobe. ($\times 7\frac{1}{3}$.)

FIGURE 39. Photomicrograph of the electrode site shown in figure 38. ($\times 75$.)

FIGURE 40. Photomicrograph of the electrode site shown in figures 38, 39. High-power view to show the very slight tissue reaction around the electrode. ($\times 291$.)

FIGURE 41. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 15 (group C). The electrode tip was situated in the infundibular lobe, slightly to the left of the mid-line (reversed in photomicrograph). ($\times 7\frac{1}{3}$.)

FIGURE 42. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 21 (group C). The electrode tip was situated in contact with the right side of the infundibular stem. ($\times 7\frac{1}{3}$.)

FIGURE 43. Photomicrograph of a horizontal section through the pituitary gland and sella turcica of rabbit 35 (group C). The electrode tip was situated in contact with the right side of the infundibular stem (reversed in photomicrograph). ($\times 7\frac{1}{3}$.)