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THE EFFECT OF ELECTRICAL STIMULATION OF THE HYPOTHALAMUS OR PITUITARY GLAND ON THYROID ACTIVITY

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It is clear that the central nervous system influences the activities of the ovary, adrenal cortex and thyroid gland. Brown-Grant, Harris & Reichlin (1954*a*) found that procedures calculated to give rise to emotional stress in rabbits (painful stimuli and restraint) result in a prompt and marked decrease of thyroid activity, and that sudden changes in the conditions of environmental lighting, whether from light to dark or vice versa, also result in temporary inhibition of the thyroid. The findings of von Euler & Holmgren (1956*b*), that hypophysectomized rabbits bearing pituitary transplants do not respond to environmental cold with an increase in thyroid activity and do not respond to the stress of 24 hr anaesthesia with thyroid inhibition, also indicate a neural mechanism underlying these reactions.

It is likely that the means whereby the central nervous system affects thyroid activity involves the hypothalamus and hypophysial portal vessels of the pituitary stalk. Brown-Grant, Harris & Reichlin (1957) found that simple stalk section in the rabbit was followed by regeneration of the portal vessels and a return of the thyroid inhibitory response to the stress of restraint or injection of stilboestrol, whereas stalk section with the placement of a plate between the hypothalamus and pituitary gland was associated with a permanent loss of the thyroidal responses to restraint and stilboestrol. These findings are compatible with those of von Euler & Holmgren (1956*b*) mentioned above. The inhibition of secretion of the thyrotrophic hormone (TSH) by the anterior pituitary which follows a rise in concentration of thyroxine in the blood may be independent of the hypothalamus, since direct injection of thyroxine into the pars distalis of the pituitary, but not into the hypothalamus

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(von Euler & Holmgren, 1956*a*), results in inhibition of thyroid activity, and administration of thyroxine to the effectively stalk-sectioned rabbit also results in thyroidal inhibition (Brown-Grant *et al.* 1957). On the other hand, the observations of Greer (1951, 1952) and Bogdanove & Halmi (1953), that certain hypothalamic lesions may prevent the hypertrophy of the thyroid that follows the administration of goitrogenic drugs, indicate that a decreased blood concentration of thyroid hormone requires the mediation of the hypothalamus in order to result in an increased discharge of TSH. The findings of Reichlin (1957), that compensatory hypertrophy of the thyroid does not occur in the presence of anterior hypothalamic damage, is also in accord with this view.

The hypothalamus appears necessary, not only to effect certain reflex changes in thyroid activity in response to environmental stimuli, but also to maintain thyroid activity under conditions of a constant environment. Several procedures have been used to isolate, completely or partially, the pituitary gland from the central nervous system. Pituitary transplantation (von Euler & Holmgren, 1956*b*), pituitary stalk section (Brown-Grant *et al.* 1957), and destruction of the anterior part of the median eminence (Ganong, Frederickson & Hume, 1955), or of the anterior hypothalamus (D'Angelo & Traum, 1956) have all been found to result in reduced thyroid function. The converse experiment of applying electrical stimulation to the hypothalamus and observing any change in thyroid activity has rarely been attempted. No increase in oxygen consumption of rabbits was seen by J. D. Green & G. W. Harris (unpublished), following prolonged stimulation of the hypothalamus, but definite conclusions were not drawn from this owing to the limitations of the method for measuring metabolic rate in rabbits. Histological signs of increased thyroid activity in rats and rabbits were reported by Colfer (1949) to follow stimulation of the hypothalamus for four 1 hr periods on each of 2 days. No optimum site in the hypothalamus was found but control stimulation of the thalamus or corpus callosum gave negative results. Somewhat similar results were obtained by Del Conte, Ravello & Stux (1955), who found that diffuse electric shocks applied through the cranium of the guinea-pig resulted in an increase in the TSH concentration in the blood within half an hour of the stimulus. In the experiments described below electrical stimulation was applied for prolonged periods, 24–72 hr, to various regions of the hypothalamus and pituitary gland in rabbits. The rate of release of ^{131}I from the gland was used to detect changes in thyroid activity. Since hypothalamic stimulation is known to evoke release of the adrenocorticotrophic hormone (ACTH) (de Groot & Harris, 1950; and others), and since a rise in concentration of blood adrenal corticoids is known to depress thyroid activity (Brown-Grant, Harris & Reichlin, 1954*b*), both normal and adrenalectomized animals were studied.

METHODS

Adult female rabbits (mainly chinchilla) 2·0–3·5 kg body weight, were used. The animals were maintained at a constant room temperature of 27–29° C, and under constant conditions of lighting. For a few days before and during the course of the experiments the rabbits were fed on a standard diet of pellets (M.R.C. diet 18, supplied by A. C. Taylor, Ltd, London) and tap water, *ad lib.*

Thyroid activity

In vivo. This was assessed by the method described by Brown-Grant, von Euler, Harris & Reichlin (1954) which makes use of the rate of biological decay of ^{131}I. Doses of 2–5 μc of ^{131}I were generally used. In some of the adrenalectomized animals with a subnormal uptake of iodine it was necessary to use up to 10 μc of radioactive iodine in order to produce a thyroid counting rate of about 3000 counts/min at the time of the initial measurement (48 hr after injection). Twice daily counts of the radioactivity of the thyroid region were made, the average of six to ten 1-min counts being used on each occasion. In all the findings presented below correction has been made for the physical decay of ^{131}I. A standard source of radioactivity (0·5 μc ^{137}Caesium, supplied by the Radiochemical Centre, Amersham) was used to test the conventional scaling equipment at intervals. The equipment used had a straight-line frequency response up to 30,000 counts/min. In all other respects the technique used was as described previously.

In vitro. In some experiments blood was drawn from the marginal vein of the ear at intervals and the radioactive protein-bound iodine ($PB^{131}I$) and, in some cases, the total plasma radioactivity, were measured. In these experiments, which were usually performed as a terminal procedure, 30 μc ^{131}I was administered initially in order to obtain sufficient $PB^{131}I$ in the blood stream to count accurately. It was found that the radioactivity in the protein-bound fraction constituted about 85% of the total plasma radioactivity (see also Brown-Grant, 1955). The procedures used have been described by Brown-Grant *et al.* (1954).

Stimulation of the hypothalamus

Electrical stimulation of the hypothalamus was performed in unanaesthetized and unrestrained rabbits by a remote control (induction) method similar to that described by de Groot & Harris (1950).

Implanted unit. (Pl. 1, figs. 1–3). The coil was made of 3000 turns of 'Formvar' insulated copper wire, S.W.G. 42 (Leumex wire, type X, London Electric Wire Co.), wound on to a spindle which was removed after winding, leaving an open core 1·0 cm in diameter. The outside diameter of the coil was 4·5 cm. It was 0·6 cm thick. The resistance of such a coil was about 500 Ω. After winding, the coil was impregnated with 'Formvar' varnish, baked at 100° C and gently pressed into a saddle shape. The outer turn of the coil was then carried across its upper surface until it ended in the central core. Three coats of 'Welvic' paste were applied and the coil baked after each coat at temperatures of 100, 100 and 150° C respectively. Two leads of many-stranded copper tinsel wire, insulated with polyvinylchloride sheathing (Multitone Electric Co. Ltd., London), were then soldered to the ends of the coil in the core. The core was then filled, and the entire coil covered, with ceresine wax. The coil was finally covered with three layers of polythene ribbon (0·003 in. = 76 μ thick), which was kept in place by cotton thread ties, later used as ligatures. The other ends of the leads were soldered to platinum wire (S.W.G. 34) electrodes, insulated with glass capillary tubing, and the solder joint covered with dental cement ('Simplex' acrylic resin, Dental Fillings Ltd, London). For bipolar stimulation, two electrodes with 0·5 or 1 mm of their tips bare, and 0·5 or 1 mm apart, were maintained in position by the dental cement at the solder joint. For unipolar stimulation one lead was soldered to an electrode as above, and the other lead soldered to a small oval plate of 'Staybrite' steel (18/8 FMB–Firth Vickers Stainless Steel Ltd, Sheffield) which served as the indifferent electrode.

To implant the units the following procedure was used. A mid-line incision was made in the skin over the lower thoracic region of the vertebral column, the mobile skin retracted caudally and

the ties around the coil loosely sutured to the lumbodorsal fascia so that the centre of the coil was situated over the space between the third and fourth lumbar spinous processes. A mid-line incision was made in the scalp and a hollow glass tube passed up under the skin from the incision in the back to emerge from the incision in the scalp. The electrodes and leads were then threaded up the tube until they emerged at the cranial incision and the glass tube removed in a rostral direction. Seclomycin (penicillin and streptomycin, Glaxo Laboratories Ltd.) was applied to the region of the coil. The wound in the back was closed with interrupted eversion sutures and the skin allowed to return to its normal position so that the coil itself was no longer in contact with the incision. The rabbit's head was now orientated in a stereotaxic machine (Pl. 2, figs. 5, 6), a small trephine hole bored with a dental drill at bregma and four fixing screws ($\frac{3}{16}$ in. 6 B.A. thread, 'Staybrite' 18/8 FMB steel) inserted a few millimetres from the trephine hole. The electrodes were then inserted into the desired region of the hypothalamus, using stereotaxic co-ordinates as an approximate guide and radiography to localize the site of the electrode tip with accuracy. Such X-ray control for accurate placement of electrodes in the hypothalamus of the dog has been recently described by Hume & Ganong (1956), who injected radio-opaque material into the third ventricle. This latter procedure is unnecessary in the rabbit in which the bony landmarks in the region of the sella turcica are sufficiently well seen in radiographs to act as guides (Pl. 1, fig. 3). Once the electrodes were in the desired position dental cement was applied as a mound to incorporate the upper ends of the electrodes, the first few millimetres of the insulated leads and the four fixing screws in the skull. After applying Seclomycin to the wound the scalp was sutured with interrupted eversion sutures and the rabbit removed from the stereotaxic machine. The wounds healed without difficulty, sepsis did not occur, the electrodes did not move, and the rabbits showed no sign of inconvenience from the implanted units for as long as they were kept alive (for periods up to 10 months).

Stimulating circuit and primary coil (Pl. 3, fig. 7). The circuit used was the same as that described by de Groot & Harris (1950) with the exception that a grid bias applied to valves *T*2 and *T*3 (Text-fig. 1, de Groot & Harris, 1950), by means of a mercury switch and rotating cam allowed stimulation to be applied for periods of $\frac{1}{4}$ min on and $\frac{1}{4}$ min off. Also a primary coil of 40 turns $\frac{1}{4}$ in. (6 mm) copper tubing was substituted for the coil of 5 turns of $\frac{1}{2}$ in. tubing in many of the later experiments. This latter change gave an increased duration of the stimulating pulse from 0·5 msec to 1·4 msec (Pl. 1, fig. 4). At the termination of some experiments, the animal was anaesthetized before killing, the leads brought to the surface through a small incision in the back of the neck and connected to an oscilloscope by means of needles inserted through the polyvinyl sheaths of the leads. The rabbit was then placed in a cage in different positions in the stimulating rack (Pl. 3) and the induced voltages between the electrodes measured.

Operative procedures

Adrenalectomy was performed as described by Brown-Grant *et al.* (1954*a*). During the first stage, i.e. the removal of the right adrenal, through a large mid-line abdominal incision, opportunity was taken (i) to investigate the retroperitoneal region for nodules of accessory cortical tissue—these were removed if found —(ii) to remove both ovaries (twelve rabbits), (iii) to remove the splanchnic nerves and upper lumbar sympathetic chain of both sides (three rabbits).

Thyroid transplantation. In one animal the thyroid was carefully and gently dissected off the trachea, the capsule lacerated with a razor blade, and the gland transplanted to the anterior surface of the pretracheal muscles to which it was lightly sutured. The completeness of the transplantation was checked after death by measuring the radioactivity of the thyroid region before and after removing the muscles lying in front of the trachea.

General plan of experiment

In the first ten animals used in this study, the coil and electrodes were implanted first, the thyroid response to hypothalamic stimulation ascertained, the two-stage adrenalectomy (plus ovariectomy) performed on seven, and the experiments repeated with the animals maintained on constant daily doses of cortisone. Since the first stage of the operation, the removal of the right

adrenal, necessitates removal of a section of the right wall of the inferior vena cava and involves some mortality, the experimental plan in the later animals, thirty-three in number, was modified as follows. Right adrenalectomy (in five cases together with ovariectomy and in three other cases with lumbar sympathectomy) was performed as an initial procedure. Several weeks after full recovery, which should allow time for any compensatory adrenal hypertrophy, the stimulating unit was implanted. The effect of hypothalamic stimulation on thyroid activity was then observed. In seven of these animals further experiments were undertaken to determine the effects of administration of high doses of cortisone upon the thyroid response to hypothalamic stimulation. The removal of the left adrenal gland was then performed and the stimulation experiments repeated with the animals maintained on small constant daily doses of cortisone.

Drugs. The following drugs were used: 11-dehydro-17-hydroxycorticosterone (Cortisone acetate, Upjohn, London); adrenocorticotrophic hormone (ACTHAR Gel, Armour Laboratories, Chicago, Lot No. N31605); thyroxine (L-thyroxine-sodium, Eltroxin, Glaxo Laboratories Ltd), and thyrotrophic hormone (Thyrotrophin, Armour Laboratories, Chicaco, Lot No. R377157).

Histology. The animals were killed with an overdose of Nembutal (pentobarbitone sodium, Abbott Laboratories Ltd) and the heads were perfused with 100 ml. of 10% formalin through each carotid artery. After removing the skin, lower jaw and orbital contents, the heads (with electrodes *in situ*) were placed in 10% formalin. When fixation was complete the dental cement on the cranial vault was dissolved in chloroform, the electrodes were removed and the skulls decalcified in equal parts 40% formic acid and 7% sodium formate solution. Blocks of tissue containing the hypothalamus, pituitary gland and base of skull were dehydrated and embedded in low-viscosity nitrocellulose. Serial sections 100μ thick were cut in the horizontal plane and stained with haematoxylin and eosin, or with Weigert's iron-haematoxylin.

The thyroid glands were removed immediately after death and fixed in a saturated solution of corrosive sublimate in 10% formalin. In some cases questionable fragments of accessory adrenal cortical tissue, or pieces of ovary containing ruptured follicles, were fixed in 10% formalin, embedded in paraffin wax, sectioned at 5–7 μ, and stained with haematoxylin and eosin.

RESULTS

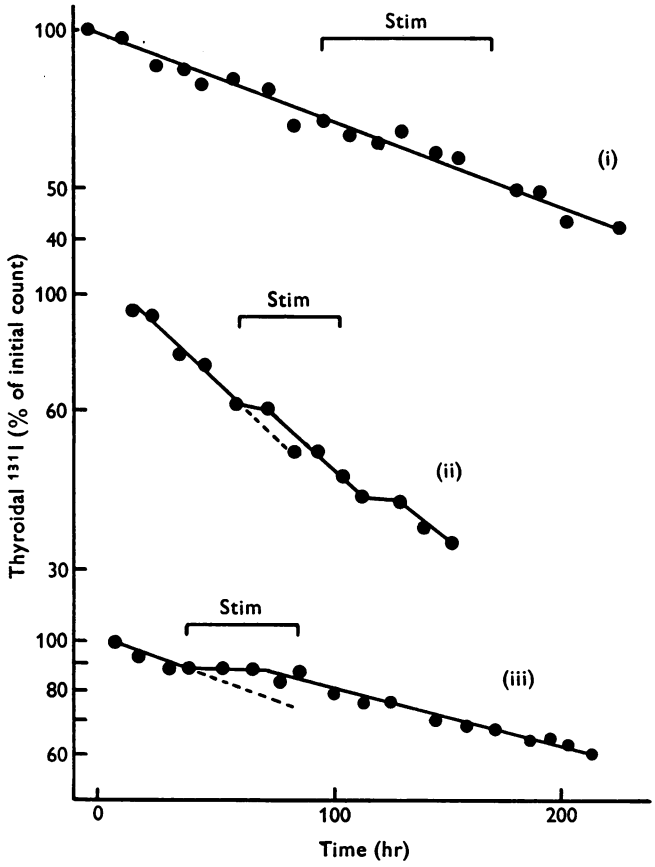
Rabbits with intact adrenal tissue

Results of stimulation on thyroid activity. In preliminary accounts of this work (Harris & Woods, 1956*a*, *b*) it was reported that in only two out of twenty-three animals with intact adrenal cortical tissue was an increase in thyroid activity observed to follow electrical stimulation of different regions of the hypothalamus or pituitary gland. Since the time of these publications a more detailed study of the effects of stimulation in the more anterior region of the hypothalamus has been made and further data obtained regarding the site in which stimulation results in increased thyroid function in animals with intact adrenal cortical tissue.

Table 1 shows the distribution of the animals in the various groups. It may be seen that electrical stimulation of various regions of the hypothalamus or pituitary gland for periods of 24–72 hr in forty-three normal or unilaterally adrenalectomized rabbits ($\pm$ ovariectomy) resulted in thyroid inhibition or no change in thyroid activity in thirty-four, group (*a*); and in thyroid acceleration in nine, group (*b*). There was no correlation between the results observed on the thyroid gland and the presence or absence of the right adrenal gland, or the presence or absence of the ovaries.

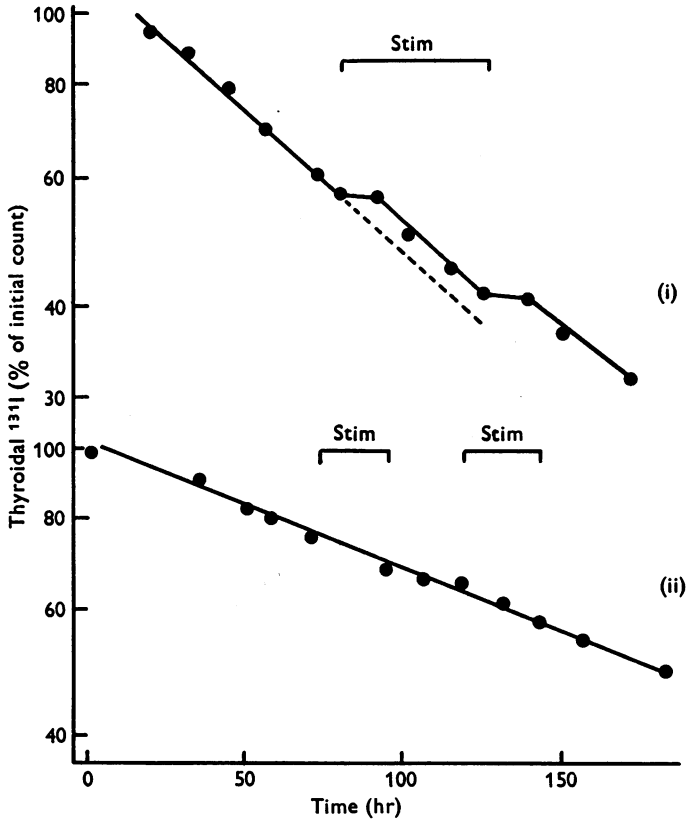
TABLE 1. Illustrates the number of animals in the different groups *a*, *b*, *c* and *d*, and the relationship of the groups

	No. of animals	The effect of hypothalamic stimulation on thyroid activity: Inhibition or no effect	Questionable effect	Excitation
With intact adrenal tissue	43	34 (group *a*)	0	9 (group *b*)
After complete adrenalectomy	32	12 (group *c*)	3	(13) (4) 17 (group *d*)



Text-fig. 1. Rabbit 1076. Three release curves. (i) Intact animal; no effect of stimulation on thyroid activity. (ii) After removal of right adrenal gland and ovaries; slight inhibition of thyroid activity at beginning and end of period of stimulation. (iii) After removal of the remaining left adrenal gland; thyroidal inhibition during the period of stimulation (see Pl. 5, fig. 18).

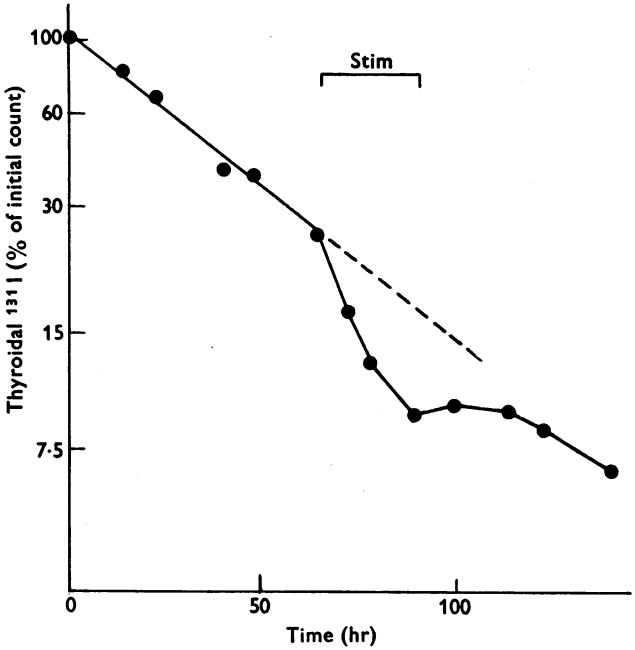
Sixty-two experiments on the animals in group (*a*) showed that thyroidal inhibition was obtained in forty-one, and no effect on thyroid activity in twenty-one experiments. The degree and duration of thyroid inhibition, when obtained, were variable. In some cases a temporary (about 12 hr) phase of inhibition was seen at the beginning of the period of stimulation and again following the end of the stimulation (Text-figs. 1(ii), 2(i)), whilst in others a more prolonged period of inhibition (Text-fig. 5(i)) persisted for the duration of the stimulus.



Text-fig. 2. Rabbit 1093. Two release curves. (i) After removal of right adrenal gland and ovaries; short periods of thyroidal inhibition at beginning and end of period of stimulation. (ii) After removal of the remaining left adrenal gland; no effect of stimulation on thyroid activity.

Thirty-three experiments on the animals in group (*b*) showed that excitation of thyroid activity occurred on twenty-eight occasions, no change in thyroid activity on three and some degree of thyroidal inhibition on two. The results of the five experiments on three rabbits, in which no increase in thyroid function was observed, are of doubtful significance, since on these

occasions the strength of the electrical stimulus was decreased in an attempt to define a threshold stimulus. The character of the thyroid response to electrical stimulation of the hypothalamus (Text-figs. 3, 7 (i) and 8 (i)) are typically those of the response seen following injection of exogenous thyrotrophic hormone (TSH) (Text-fig. 5 (i)), i.e. a short latent period (few hours), a rapid and marked increase in the rate of discharge of thyroidal ^{131}I (about 24–48 hr),

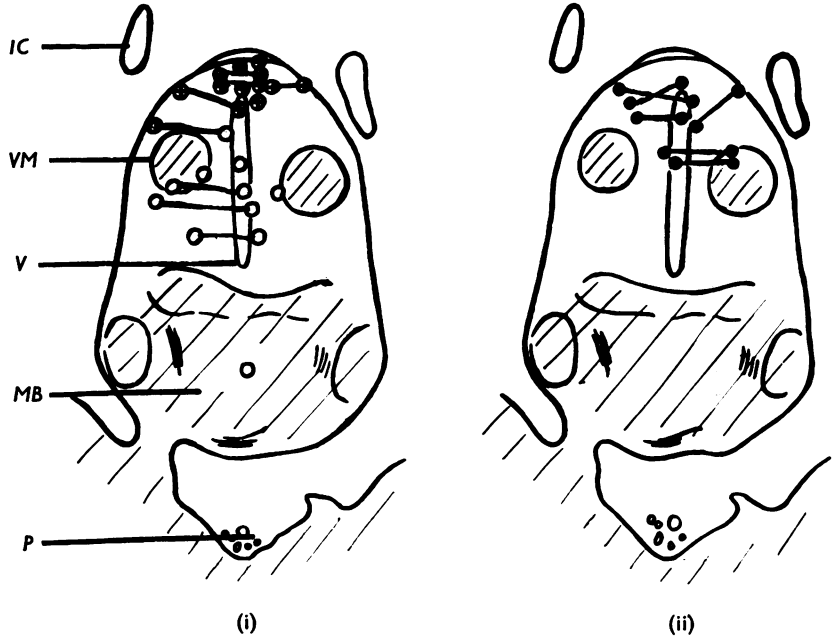


Text-fig. 3. Rabbit 1127. Right adrenal gland, only, removed; stimulation results in thyroid activation. Compare the release-curve pattern of the response with those shown in Text-figs. 5 and 8. (The position of the electrodes in this animal is shown in Pl. 4, fig. 9.)

followed by a period (24–48 hr) after stimulation in which thyroidal radioactivity remains constant or may even rise. It is likely that the cessation of secretion of thyroid hormone during this latter phase is due to the cessation of the pituitary release of TSH following the raised blood concentration of blood thyroxine. The fact that the thyroid gland may even accumulate ^{131}I over this period may be explained on the grounds that a fall in the blood concentration of TSH results in a reduction of the thyroid secretion of hormone before a reduction in the thyroidal uptake of iodine, and that about 10% of the iodine contained in secreted radioactive hormone is reaccumulated by the thyroid gland (Brown Grant *et al.* 1954; Brown-Grant & Gibson, 1955*a*).

Electrode position. In order to compare the electrode positions in different animals a standard series of horizontal sections through the hypothalamus and pituitary gland of a rabbit was prepared, projection tracings made of these

sections and the electrode positions plotted on these tracings. From the maps so obtained (for example, see Text-fig. 4) it is possible to say that in rabbits with intact adrenal cortical tissue stimulation in the region of the supra-opticohypophysial tract in the anterior part of the tuber cinereum (see Pl. 4, figs. 8–10) results in increased thyroid activity, whereas stimulation in more posterior regions of the median eminence (Pl. 4, figs. 11, 12), in the region of



Text-fig. 4. Diagram of a horizontal section extracted from a standard series of microphotographs upon which the electrode positions have been projected. Position of unipolar electrodes marked singly, and bipolar electrodes joined by a line. *IC*, internal carotid artery; *MB*, mammillary body; *P*, region of posterior perforated substance; *V*, third ventricle; *VM*, ventromedial nucleus of hypothalamus. (i) ⊕ and ⊕—⊕, electrode position in animals that responded to stimulation with thyroidal activation before adrenalectomy; ○ and ○—○, electrode position in animals that did not show thyroidal activation either before or after adrenalectomy. (ii) ● and ●—●, electrode position in animals that responded to stimulation with thyroidal activation but only after adrenalectomy.

the mammillary bodies (Pl. 5, fig. 14), in the tuberal region of the hypothalamus (Pl. 4, fig. 13; Pl. 5, figs. 16–19), more dorsally in the hypothalamus or in the pituitary gland directly (Pl. 5, fig. 15) does not.

Antidiuretic responses to stimulation. Whilst the present study was in progress it became apparent that the region of the supraopticohypophysial tract was that in which stimulation resulted in increased thyroid activity, even in the animal with intact adrenal cortical tissue. It was then decided to compare the antidiuretic and thyroidal responses of twelve animals to hypothalamic

stimulation. It had been shown previously (Harris, 1947) that electrical stimulation of the supraopticohypophysial tract results in release of antidiuretic hormone and an inhibition of a water diuresis. The assessment of an antidiuretic response was performed as described by Harris (1947). Out of the twelve rabbits investigated, seven gave an antidiuretic response to 1–2 min stimulation (five of these showed thyroidal acceleration to stimulation before adrenalectomy, and two showed thyroidal acceleration but only after adrenalectomy), three showed neither an antidiuretic nor a thyroidal response, one showed no antidiuretic but a probable thyroidal response after adrenalectomy, and one showed a marked antidiuretic response but no change in thyroidal function on stimulation. This latter animal was later found to have the electrodes in the posterior and superior region of the pars distalis, that is in a position to stimulate the nerve fibres of the infundibular stem but not those of the median eminence.

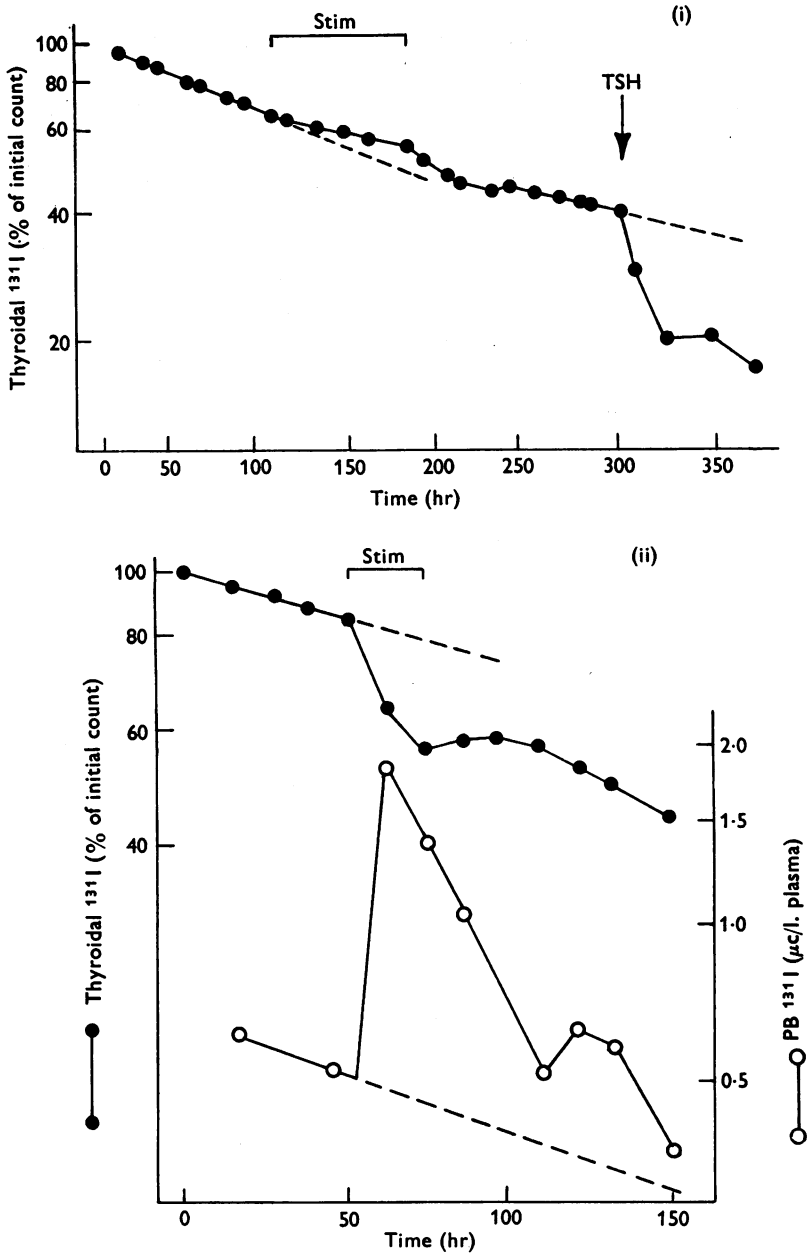
Completely adrenalectomized rabbits on constant cortisone therapy

Results of stimulation on thyroid activity. Since hypothalamic stimulation is known to evoke release of ACTH (de Groot & Harris, 1950), and since administration of ACTH or cortisone to the rabbit has been found to depress thyroid function (Brown-Grant *et al.* 1954*b*), thirty-two out of the above forty-three rabbits were re-studied after complete adrenalectomy and replacement therapy with *constant daily doses of cortisone.* Of these thirty-two animals, twenty-eight had previously shown no increased thyroid activity on hypothalamic stimulation and four had shown increased thyroid function.

Of the twenty-eight animals that before complete adrenalectomy failed to show thyroid acceleration in response to stimulation, twelve (group *c*) still gave the same negative response after adrenalectomy, three animals gave a doubtful response and thirteen (in group *d*) now showed a reversal of response and exhibited a clear-cut acceleration of thyroid activity on hypothalamic stimulation.

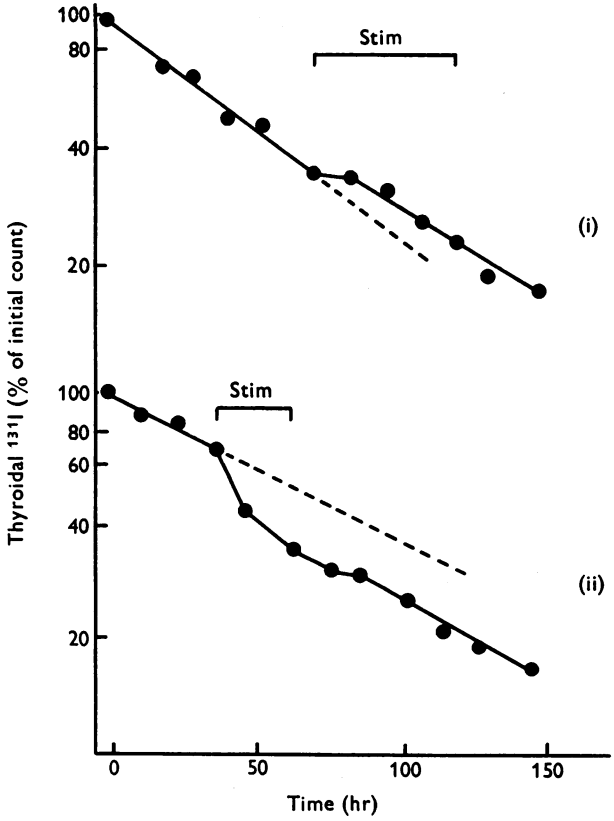
The twelve animals in group (*c*) showed, in thirty-two experiments, thyroidal inhibition on eight occasions, and no change in thyroid activity on twenty-four. These same twelve animals had, before adrenalectomy, shown thyroidal inhibition in seventeen experiments and no change of thyroid activity in five, figures which are significantly different from those obtained after adrenalectomy ($P < 0{\cdot}001$). The three animals that showed a dubious response after adrenalectomy did not survive the procedure of adrenalectomy for a sufficient length of time to enable the experiment to be repeated.

Thirteen animals showed a reversal of their previous response after adrenalectomy. Instead of thyroidal inhibition or no effect on thyroid activity, they showed a uniform and marked acceleration of thyroid function consequent on hypothalamic stimulation (compare Text-fig. 5, (i) and (ii); and Text-fig. 6 (i)



Text-fig. 5. Rabbit 1071. (i) Release curve: adrenal glands intact; stimulation results in slight inhibition of the thyroid gland. Intramuscular injection of TSH, 5 mg (equivalent U.S.P. standard) results in a clear increase in thyroid activity. (ii) Release curve ●—●, and curve representing PB^{131}I ○—○, after complete adrenalectomy. Note the increased thyroid activity, and the simultaneous increase in organically bound ^{131}I in the plasma, during the period of stimulation.

and (ii)). Out of thirty-five experiments these animals showed thyroid acceleration in twenty-nine; on the other six occasions the intensity of the stimulus had been decreased in an attempt to ascertain the threshold value of stimulus.

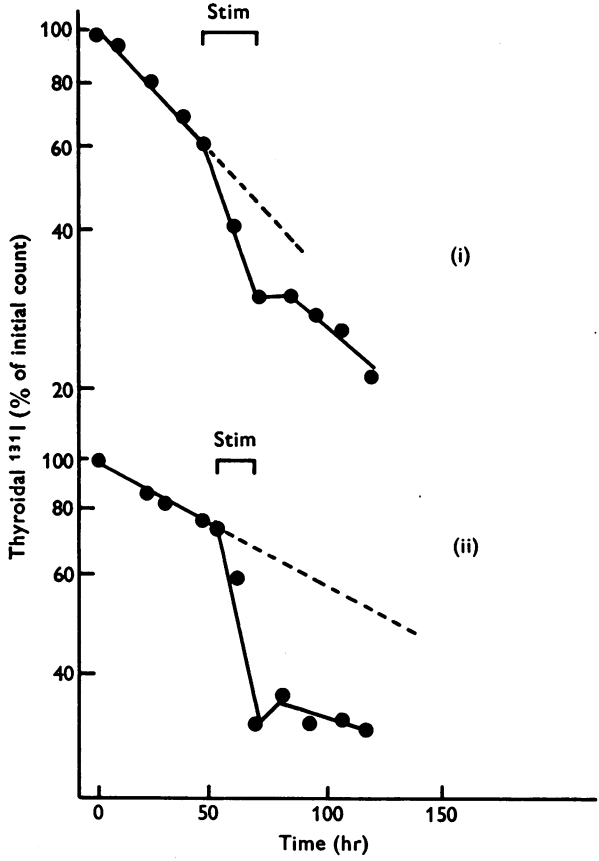


Text-fig. 6. Rabbit 1073. (i) Release curve after removal of right adrenal gland and the ovaries; slight inhibitory effect on thyroid gland at the beginning of the period of stimulation. (ii) Release curve after complete adrenalectomy; stimulation now evokes an increased rate of release of thyroidal ^{131}I, that is, an increase in thyroid activity.

Four animals in group (*b*), that is, rabbits in which stimulation had resulted in an increased release of thyroidal ^{131}I before complete adrenalectomy, had the remaining left adrenal gland removed and the experiments repeated. It was found that the thyroid response to stimulation was increased by complete adrenalectomy. Typical results are shown in Text-fig. 7(i) and (ii) and Text-fig. 8(i) and (ii).

In order to assess by a further method the acceleration of thyroidal activity resulting from stimulation, it was decided to measure the concentration of PB^{131}I in the blood during some experiments. Brown-Grant (1955) has shown

that the concentration of PB^{131}I in the blood of the rabbit declines exponentially during the course of a release curve, and that the curve expressing PB^{131}I roughly parallels the curve of thyroidal radioactivity. In the present work, such an experiment was usually made as a terminal experiment in any given animal, since the dose of ^{131}I necessary to obtain an accurate estimate of



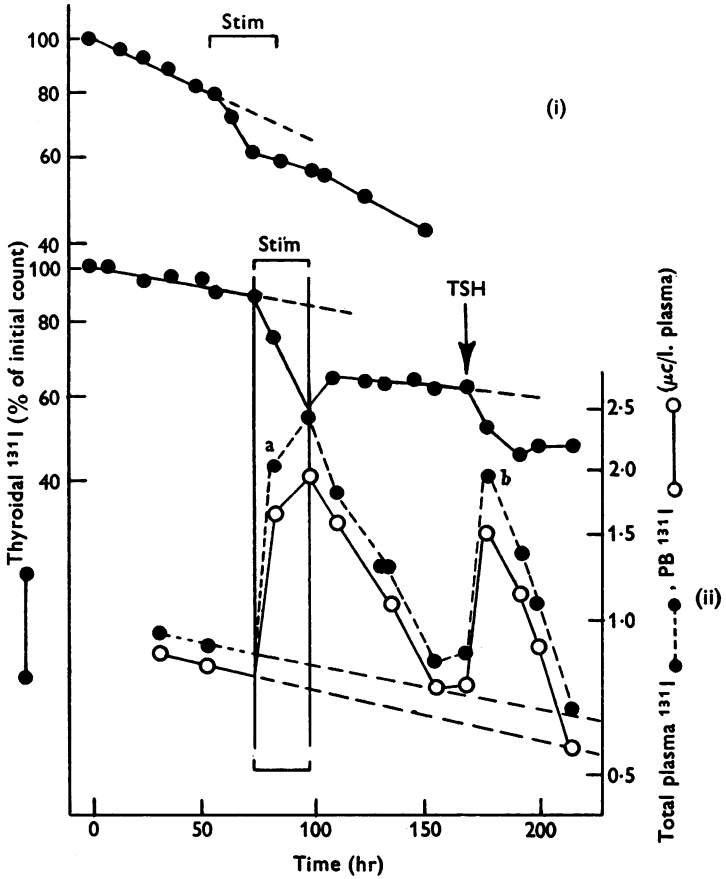
Text-fig. 7. Rabbit 1135. Two experiments, (i) before, and (ii) after complete adrenalectomy: note the increased effect of stimulation after adrenalectomy. Before complete adrenalectomy, stimulation resulted in $\times 2$ increase of thyroid function; after complete adrenalectomy, stimulation resulted in $\times 5$ increase in thyroid function.

PB^{131}I was high (about 30 μc ^{131}I). Out of twelve experiments on eleven rabbits, a clear increase in blood concentration of PB^{131}I occurred in eight (Text-figs. 5, 8), a slight increase in one, no change in one, and a decrease in two. These results were all in agreement with those observed from the thyroidal release curve, except in one instance in which stimulation resulted in a fall in PB^{131}I in association with a doubtful change in the rate of release of thyroidal ^{131}I.

A more detailed analysis of one such experiment, depicted in Text-fig. 8(ii), results in the following observations:

(*a*) the logarithmic curves representing the rate of fall of thyroidal radioactivity and of PB^{131}I are roughly parallel during the initial control period.

(*b*) The curves expressing the total radioactivity of the plasma and of PB^{131}I in the plasma, are also roughly parallel. Averaging all the figures obtained in this experiment the PB^{131}I constitutes 83% of the total plasma radioactivity.



Text-fig. 8. Rabbit 1138. Two experiments: (i) After right adrenalectomy only; the release curve shows that an increased thyroid activity follows stimulation. (ii) After complete adrenalectomy. The three curves show, from above down, the thyroid release curve and the curves of the total plasma radioactivity and the organically bound ^{131}I of the plasma. Note (*a*) the effect of stimulation is increased by complete adrenalectomy, and (*b*) the increased thyroid activity in response to stimulation is reflected both by the release curve and by the study of plasma radioactivity. The comparison of the effect of 24-hr stimulation and the subcutaneous injection of TSH (0·5 mg equivalent U.S.P. standard) is discussed in the text. The electrode position in this rabbit is shown in Pl. 4, fig. 8.

(*c*) The latent period of the thyroid responses and the responses of the $PB^{131}I$ are short (as measured in hours) both to hypothalamic stimulation and to injection of TSH. Recent unpublished results (R. George & G. W. Harris), in which the radioactivity in the venous blood from the thyroid gland has been measured, give a latent period of 0·25–0·5 hr for the action of TSH, injected intravenously, on the thyroid gland.

(*d*) At point '*a*' in Text-fig. 8(ii), 8 hr after commencing stimulation, the blood concentration of $PB^{131}I$ is raised. In spite of this fact the continued stimulation over the next 16 hr still maintains an increased thyroid function. The excitatory effect of hypothalamic stimulation thus appears to preponderate over the inhibitory effect of the 'feed-back' of a raised blood level of thyroid hormone.

(*e*) From previous calibration of the equipment used (see Brown-Grant *et al.* 1954), it may be calculated that the loss of neck counts during the first 8 hr of hypothalamic stimulation corresponds to a release of about 0·36 μc of radioactive iodine. Assuming a total plasma volume in the body of 100 ml., the rise in $PB^{131}I$ over this same period amounts to about 0·1 μc. After allowing for the rapid disappearance of radioactive thyroxine in the blood of the rabbit (Brown-Grant & Gibson, 1955*b*), these figures seem to show a rough correspondence.

(*f*) The increase in thyroidal radioactivity during the 12 hr following the cessation of stimulation represents an increase of 600 counts/min in neck counting rate. Since it has been found that only about 10% of the ^{131}I that leaves the gland as radioactive hormone eventually recirculates to the gland (Brown-Grant *et al.* 1954) this figure would seem high, since 10% of the radioactivity secreted in the previous 24 hr (i.e. the 24 hr period of stimulation) is represented by 320 neck counts/min.

(*g*) The effect of hypothalamic stimulation during the first 8 hr of stimulation is very similar to the effect of the injection of 0·5 mg (equivalent U.S.P. standard) TSH (s.c.) during a similar period, in that the latent period of the effect is short, the rate of fall of thyroidal radioactivity (36%/24 hr) is the same in both cases, and the increase of $PB^{131}I$ is $\times 2{\cdot}06$ and 2·04 respectively. The increase in thyroid activity, as measured from the release curve, results in both cases from a release rate of 5%/24 hr to 36%/24 hr, i.e. by a factor of $\times 7{\cdot}2$.

Electrode position. The electrode position in some of the animals that responded to hypothalamic stimulation with thyroid acceleration after, but not before, adrenalectomy is illustrated in Text-fig. 4(ii) and in Pl. 4, figs. 11–13. The electrodes in animals in this group were found to be in the tuberal region of the hypothalamus, and tended to be situated more posteriorly than those in

the rabbits in group *b* (that showed thyroidal excitation both before and after adrenalectomy) (Text-fig. 4(i) and Pl. 4, figs. 8–10); and more anteriorly than in those animals in group *c* (that showed no thyroidal excitation either before or after adrenalectomy) (Text-fig. 4(i) and Pl. 5, figs. 14 and 16–19). It should be pointed out, however, that some overlap was found in the electrode positions in the animals in the different groups.

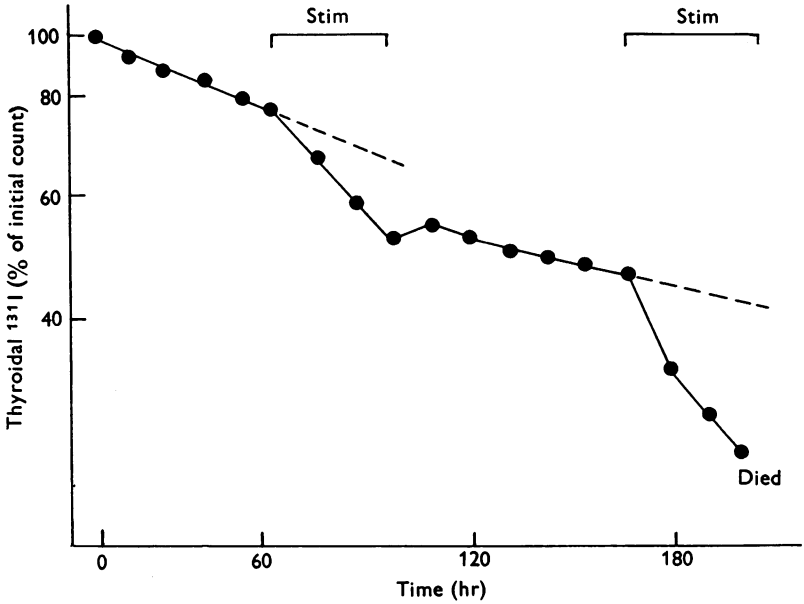
Completeness of adrenalectomy. As mentioned above, adrenalectomy was performed as a two-stage operation. The removal of the right gland through a mid-line abdominal incision was performed first. During the wide exposure of the abdominal contents necessary for this operation a careful search was made of the retroperitoneal area for accessory cortical tissue and in the few cases in which such was found, usually along the inferior vena cava on the right side or around the kidney on the left, it was removed. At a later date the left adrenal was removed through a small lumbar incision. This latter wound was found to heal well in spite of cortisone substitution therapy. The maintenance of adrenalectomized rabbits on cortisone for long periods is not an easy procedure. An average daily dose of cortisone is 1–2 mg/day. However, on this regime a proportion of rabbits showed wasting of muscles and lost weight owing to excess dosage of cortisone, and a number died from adrenocortical insufficiency. In the present study six animals (out of thirty-two) showed signs of adrenal insufficiency (became weak, ataxic, unable to stand, or comatose), and recovered with cortisone and glucose-saline therapy. Some of these, and other, animals died suddenly. In all, fourteen deaths in animals previously healthy were attributable to a hypoadrenocortical state. The occurrence of a particular thyroid response to hypothalamic stimulation was not related to the occurrence at any time of a state of adrenocortical insufficiency in the different animals.

The data regarding the completeness of adrenalectomy may be summarized as (*a*) the clear visualization of the removal of the two adrenal glands and surrounding tissue at the time of operation, (*b*) the careful examination for, and removal of, any accessory adrenal cortical tissue at the time of the right adrenalectomy, (*c*) the fact that fifteen out of thirty-two animals developed, at some time, a state of adrenocortical insufficiency, and (*d*) the careful naked-eye search, post-mortem, for any accessory adrenal tissue.

Deaths during hypothalamic stimulation

Six rabbits died during, and two shortly after, prolonged or repeated periods of hypothalamic stimulation (Text-fig. 9). Such an occurrence had not been observed in previous studies in which prolonged periods of stimulation (up to 1 week) had been used (G. W. Harris, unpublished). Six of the above animals were adrenalectomized; one had the left adrenal removed and one had both adrenals intact but was being given 60 μg thyroxine per day. Seven out of these

eight rabbits had shown thyroid acceleration following stimulation. One additional (adrenalectomized) rabbit was found dead 45 hr after a subcutaneous injection of TSH (2 mg equivalent of U.S.P. standard).



Text-fig. 9. Rabbit 1074. Both adrenals and ovaries removed. Two periods of stimulation, 37 hr and 36 hr, resulted in thyroid excitation and death of this animal.

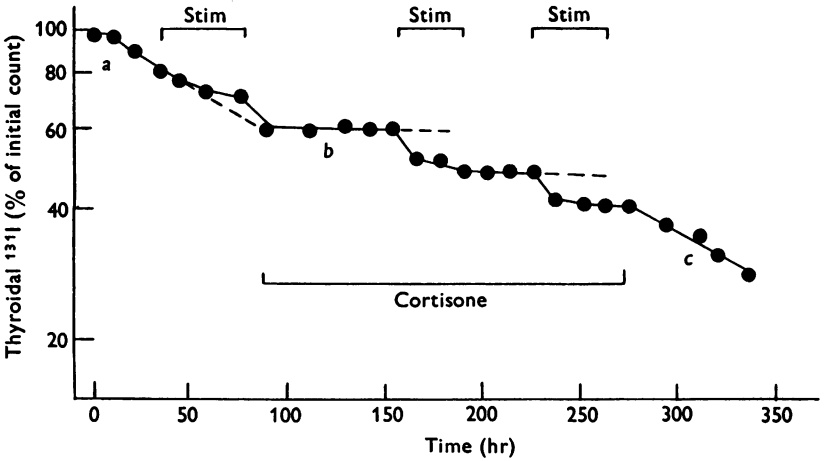
The mechanism of the change in response after adrenalectomy

Since thirteen rabbits showed a reversal of thyroid response to hypothalamic stimulation after adrenalectomy, it became of interest to see whether this effect of adrenal removal was due to loss of the adrenal medulla or cortex. Two procedures were used in trying to answer this question.

Adrenal medullary denervation. At the time of removal of the right adrenal gland the left adrenal was denervated by removal of the splanchnic nerves and upper lumbar sympathetic chain on both sides. Six experiments on these three animals showed thyroidal inhibition in four cases and no effect on thyroid activity in two. The left adrenal glands were then removed and the experiments repeated during maintenance with cortisone. Three experiments on two of these rabbits failed to show thyroid excitation. One experiment on the third animal showed that stimulation now evoked acceleration of thyroid function. This animal died in a state of adrenal insufficiency before the experiment could be repeated.

Adrenal cortical 'blockade'. Since administration of large doses of exogenous cortisone is known to inhibit the pituitary release of ACTH and to result in adrenal cortical atrophy, it was decided to stimulate the hypothalamus in

rabbits with an intact adrenal gland following the administration of large doses of cortisone. The doses of cortisone varied in the different animals from 3·75 to 30·0 mg/day. These were administered during the course of a release curve for several days until the slope of the curve showed marked flattening, that is, until the function of the thyroid gland had been markedly reduced. Stimulation was then performed as usual during the continued administration of cortisone. Seven rabbits, that had previously been observed to show either thyroidal inhibition or no change in thyroid function during stimulation, were submitted to the above procedure. In four animals a clear-cut acceleration of thyroid activity was seen during the period of stimulation (Text-fig. 10),



Text-fig. 10. Rabbit 1106. This release curve shows the effect of hypothalamic stimulation before and during the administration of large daily doses (10 mg) of cortisone. Note (i) the reduced rate of release of thyroidal ^{131}I whilst under cortisone administration (compare slopes at *a*, *b*, and *c*); and (ii) the reversal by cortisone of the effect produced by hypothalamic stimulation.

whereas in the other three no change in activity occurred. It is noteworthy that these rabbits showed similar results in later experiments when studied after complete adrenalectomy.

Effect of thyroid transplantation

One adrenalectomized rabbit, that had shown thyroidal acceleration during stimulation, had the thyroid gland transplanted to the superficial surface of the infrahyoid muscles. Twenty days later a dose of 30 μc ^{131}I was administered. The 48 hr uptake of ^{131}I was found to be only 4 % of the amount accumulated before transplantation of the thyroid. Stimulation of the hypothalamus (see Pl. 4, fig. 12) during the course of the release curve showed that the rate of release of radioactivity from the transplanted gland was increased in a similar measure to that observed before transplantation. At the completion

of the experiment the animal was killed and the radioactivity in the neck measured after removing the muscles bearing the transplant. No measurable activity was present.

Stimulation and release of gonadotrophic hormone

At post-mortem examination of the forty-three animals involved in the present investigation, a number of them were found to have corpora lutea of various ages in their ovaries. Although the animals were kept isolated during the several months of the experiment, some of them must be eliminated from the series in appraising the significance of the ovulation that had occurred. (i) Five had the ovaries removed before any hypothalamic stimulation was applied; (ii) one animal was injected with a preparation of thyrotrophic hormone (possibly contaminated with gonadotrophic hormone) shortly before killing; (iii) four animals were killed too long after the last period of stimulation for any corpora lutea to persist in their ovaries; and (iv) in one animal the ovaries were atrophic. Out of the remaining thirty-two animals, twenty showed the presence of corpora lutea in the ovaries (five of these had two sets of corpora, an old atrophic set and a fresh set) and twelve showed ovaries containing ripe follicles but no corpora lutea. In the animals that ovulated the electrodes were found to be situated in all cases in some part of the anterior or middle tuber cinereum. In the animals that did *not* show corpora lutea, the electrodes were found in different regions of the tuber cinereum and in various areas of the pituitary gland. The absence of ovulation in these latter rabbits cannot be held to be significant, since the animals were subjected to repeated experiments. It is possible that during the last experiment before death any particular animal may have been pseudopregnant from the stimulation associated with a previous experiment, and ovulation would therefore have been suppressed.

DISCUSSION

The technique of remote control stimulation has been modified from that described previously (Harris, 1947; de Groot & Harris, 1950). In earlier studies the coil and electrode system was made as a solid unit and implanted under the scalp, whereas in the work described above the coil was buried under the skin over the lumbar region of the vertebral column and flexible insulated leads ran to electrodes fixed to the cranium. This method had been attempted on previous occasions (G. W. Harris, unpublished) but had failed from fracture of the leads or insulation in the cervical region. The success of the present units depended on the use of many-stranded tinsel wire insulated with two sheaths of polyvinylchloride. The advantages of the method may be summarized as follows. (1) It is possible to implant a larger coil under the skin of the back than under the scalp; this makes it easier to induce the required voltage, and so to economize on the electrical components of the primary

circuit. (2) The present method makes it as easy to implant bipolar as unipolar electrodes; it also makes it possible to use X-ray control when implanting the electrodes, a procedure which greatly increases the accuracy of electrode placement. (3) The use of stainless steel screws and dental cement for fixing the electrodes to the bones of the skull has been found eminently satisfactory; no movement of the electrodes has been detected in any of the forty-three animals used in the present series. (4) After obtaining any given response by stimulation, it would be a simple matter to anaesthetize the animal, extract a portion of one lead through a small incision, cut the lead, pass a direct current of a few milliamperes through the unit and thus produce a lesion around the tips of the electrodes, reunite and reinsulate the lead, and repeat the experiment; such a procedure would seem to offer a good internal control for experiments on any one animal. (5) Whilst the animals are being killed with Nembutal the occasion is taken to extract the leads through a small incision in the skin of the neck and to record the voltages on an oscilloscope with the animal in similar positions in the field of the primary coil as were used in the previous experiment. In this way the voltages between the electrodes were measured for the individual rabbits. (6) After killing the animal, the leads are cut near the skull, and the coil and attached parts of the leads removed, cleaned, sterilized and used in further experiments. After formol fixation of the head, the dental cement is dissolved in chloroform and the electrodes and stainless steel screws are recovered for further use.

Rabbits with intact adrenal tissue respond to hypothalamic stimulation in the region of the supraopticohypophysial tract with an increase of thyroid activity. Many workers have reported that lesions in the hypothalamus may interfere with the normal secretion of TSH (Bogdanove & Halmi, 1953; Bogdanove, Spirtos & Halmi, 1955; Greer & Erwin, 1956; Ganong *et al.* 1955; D'Angelo & Traum, 1956; Reichlin, 1957). Ganong *et al.* (1955) found that lesions in the anterior part of the median eminence reduced thyroid activity in dogs to the level seen in hypophysectomized animals. Greer (1951), in commenting on the region of the hypothalamus in which lesions result in reduced thyroid function, says: 'The impression gained so far, however, is that the area is anterior to the ventro-median nucleus and lies along or near the ventral surface of the hypothalamus, possibly near the ventral extension of the supraopticohypophysial tract.' The evidence derived from studies of lesions and stimulation are in good agreement. Both indicate that it is the anterior part of the median eminence that is involved in the control of TSH secretion and thyroid activity. Although stimulation in the region of the supraopticohypophysial tracts resulted in increased thyroidal secretion, it is not certain that this result can be ascribed to the supraopticohypophysial tracts themselves. The microanatomy of the hypothalamus is highly complex and there is little knowledge of the detailed pathway of the multitude of unmyelinated

fibre tracts in this region. It is likely that other tracts run closely associated with the supraopticohypophysial tract, and this probability should be taken into account before associating the present results with any particular nervous pathway. In the twelve rabbits in which both thyroidal and antidiuretic responses were studied, the association between the two responses in the different animals was suggestive but not entirely uniform. In one case, in which a marked antidiuretic response but no increase in thyroid activity followed stimulation, the electrodes were found to be situated in the pars distalis in a position to stimulate the nerve fibres of the infundibular stem but not those of the median eminence. The data from this animal then cannot be related to the question of which nerve tracts of the hypothalamus are concerned with the regulation of TSH secretion. One other rabbit, however, showed a thyroidal response to stimulation but no antidiuretic response. In this case the electrodes were situated in the anterior tuber cinereum, slightly posterior to the supraopticohypophysial tract (Pl. 4, fig. 11). Since an antidiuretic response forms a very sensitive test of electrical stimulation of the supraopticohypophysial tract, this evidence suggests that stimulation of the tract is not essential in evoking a thyroid response. On the other hand, the findings of Dubreuil & Martini (1956), that the uptake of radioactive iodine by the thyroid gland of male rats is increased by previous administration of vasopressin, is compatible with the view that the supraopticohypophysial tract regulates pituitary secretion of TSH by releasing a vasopressin-like substance into the hypophysial portal vessels.

The evidence that hypothalamic stimulation causes increased thyroid activity through the agency of thyrotrophic hormone may be summarized. First, hypothalamic stimulation is effective in exciting the activity of the transplanted thyroid as well as of the normal gland: the effect is thus humorally mediated. Secondly, the effect of hypothalamic stimulation on thyroid activity may be closely simulated by injection of appropriate doses of TSH (see Text-fig. 8). And thirdly, it may be recalled that von Euler & Holmgren (1956*b*), and Brown-Grant *el al.* (1957) have shown, by different methods, that the central nervous system may influence thyroid activity through the mediation of hypothalamo-hypophysial connexions.

It has been suggested that the hypothalamus may be 'mapped', three-dimensionally, in terms of pituitary hormones (Harris, 1955). In such a map the control of TSH secretion would be localized in the anterior parts of the median eminence and tuber cinereum. It would seem inadvisable to term such an area a 'centre'; it probably represents some neural mechanism akin to a 'final common path' by which the nervous system acting through this area and the pituitary stalk controls the secretion of TSH. This region of the hypothalamus then would give the maximum effects on TSH release when damaged or stimulated. It is likely, however, that many reflex paths from the

brain stem, hippocampus, amygdaloid nuclei, thalamus, cerebral cortex and other parts of the central nervous system play upon and influence this final common path. If this is so, then it is to be expected that a more detailed investigation will reveal further hypothalamic areas, through which pass such reflex paths, that may exert an influence on TSH discharge. It is of interest that the anterior part of the median eminence, concerned with thyroid activity, is adjacent to the anterior hypothalamic and preoptic region known to be concerned with the regulation of body temperature (Magoun, Harrison, Brobeck & Ranson, 1938; von Euler, 1952).

Complete adrenalectomy, with maintenance on small and constant doses of cortisone, was performed in thirty-two out of the total of forty-three rabbits studied. The difference in the thyroid response seen after adrenalectomy, as compared with that in the same animal in experiments before adrenalectomy, may be summarized as follows: (*a*) Four animals that showed an increase in thyroid activity to stimulation before complete adrenalectomy, showed a greater increase after. (*b*) Thirteen animals that had shown inhibition, or no change of thyroid activity, previous to complete adrenalectomy, exhibited a clear-cut acceleration of thyroid function afterwards; the increased function of the thyroid was assessed by measurements of the release curve of thyroidal radio-iodine, and by measurements of the blood concentration of $PB^{131}I$; the changes recorded by the two methods were in good agreement. (*c*) Twelve animals that previous to complete adrenalectomy had shown inhibition, or no change of thyroid activity, gave responses of the same two types afterwards but showed a significant decrease in the number of responses of the inhibitory type.

The reason for the differences observed before and after adrenalectomy is not entirely clear. The most likely hypothesis is that some substance is liberated from the adrenal gland during hypothalamic stimulation which in turn inhibits either TSH secretion or the thyroid gland itself. It is known that electrical stimulation of certain areas of the hypothalamus evokes secretion of adrenaline (Magoun, Ranson & Hetherington, 1937) and that adrenaline may inhibit thyroid activity in the rabbit, probably by a vasoconstrictor action on the vessels of the gland (Haigh, Reiss & Reiss, 1954; Brown-Grant *et al.* 1954*a*; Brown-Grant & Gibson, 1956). However, it is unlikely that adrenal medullary secretion is the only factor involved in the present experiments since (*a*) the median eminence is not a particularly effective site for localized electrical stimulation to excite adrenaline secretion (Magoun *et al.* 1937), (*b*) the dose of adrenaline necessary to produce prolonged inhibition of the thyroid gland of the rabbit is high (Brown-Grant *et al.* 1954*a*), (*c*) in the case of one rabbit adrenal denervation did not have the same effect as adrenal removal, and (*d*) rabbits with an adrenal gland intact may show a reversal of the thyroid response to stimulation if under high cortisone administration.

It is likely that adrenal cortical secretion is the major factor involved, since: (i) hypothalamic stimulation is known to evoke release of ACTH (de Groot & Harris, 1950; Hume & Wittenstein, 1950; Hume, 1953; Porter, 1954); (ii) the dose of adrenal steroids necessary to produce prolonged inhibition of the thyroid gland in the rabbit seems to be within the physiological range (Brown-Grant *et al.* 1954*b*; Brown-Grant, 1956); and (iii) rabbits which possess an intact adrenal gland may show a reversal of thyroid response, so that hypothalamic stimulation results in thyroidal acceleration, if given large doses of cortisone in an attempt to 'blockade' ACTH secretion. It is possible that the adrenal steroids affect thyroid activity by suppressing the secretion of TSH from the anterior pituitary, since cortisone was found not to influence the response of the thyroid gland of the hypophysectomized rabbit to injection of exogenous TSH (Brown-Grant *et al.* 1954*b*).

Hypothalamic stimulation might evoke a rise of adrenal steroid concentration in the blood for a variety of reasons. First, stimulation of hypothalamic nerve fibres may excite release of anterior pituitary ACTH. Secondly, the slight noise and flickering of lights from the thyratron valves during stimulation may evoke some emotional disturbance and so some ACTH secretion; and thirdly, any rise in the blood level of thyroid hormone might in itself stimulate the pituitary-adrenal axis (Wallach & Reineke, 1949; Timiras & Woodbury, 1955). It is likely that the most important of these factors is the first, since the type of response both before and after adrenalectomy was related to the electrode position in the hypothalamus.

Stimulation of the most anterior part of the tuber cinereum resulted in thyroidal acceleration both before and after adrenalectomy, and similarly stimulation of the superior region of the hypothalamus or areas posterior to the median eminence failed to result in thyroidal acceleration either before or after complete adrenalectomy. In the intermediate zone between the above two areas, that is, in the region of the median eminence, stimulation after adrenalectomy in many rabbits evoked increased thyroid activity though stimulation before had given inhibition of, or no change in, thyroidal function. A possible explanation of this finding is that the median eminence is a zone where the ACTH and TSH 'fields' of the hypothalamus overlap. De Groot & Harris (1950) have shown in rabbits that stimulation of the more posterior parts of the tuber cinereum and mammillary region excites secretion of ACTH, whilst in the present study stimulation of the anterior area of the tuber cinereum excited TSH release. Since the evidence is strongly in favour of the view that both neural mechanisms act on the anterior pituitary through the pituitary stalk, it seems likely that in the region of the median eminence there is overlap of the areas in which stimulation might evoke both ACTH and TSH release. In that case it is possible that stimulation at this site would act rapidly to raise the blood level of adrenal steroids, and that this might inhibit any TSH

secretion that would otherwise have been elicited. Such a view would at the moment fit the experimental findings.

A point of interest arising from the above results is that eight rabbits died during or shortly after hypothalamic stimulation. Six of these animals were adrenalectomized, and seven had shown thyroid acceleration following the start of stimulation. It is well known that thyrotrophic hormone or thyroid hormone should be administered with caution to patients suffering from Addison's disease. It is possible that, in the present work, the thyroid acceleration resulting from hypothalamic stimulation was responsible for precipitating a state of acute adrenal insufficiency.

The presence or absence of the ovaries was not related to the type of thyroid response following hypothalamic stimulation. Further, the occurrence of ovulation following stimulation could not be correlated with the thyroid responses. The incidental observation, that ovulation had occurred in a proportion of the rabbits following stimulation in the region of the median eminence, confirms the older observations of Harris (1937, 1948), Haterius & Derbyshire (1937) and Markee, Sawyer & Hollinshead (1946).

Animals in which the stimulating tips of the electrodes were situated deeply in the pars distalis of the pituitary gland failed to show any sign of thyroidal activation on stimulation, either before or after complete adrenalectomy. This is again in conformity with previous results that electrical stimulation of the anterior pituitary gland, at a strength that does not produce spread of current to the hypothalamus, fails to excite release of gonadotrophic hormone (Markee *et al.* 1946; Harris, 1948), or adrenocorticotrophic hormone (de Groot & Harris, 1950). This suggests that the stimulus from the hypothalamus which evokes anterior pituitary secretion is not transmitted to the gland cells by nerve fibres. Taken in conjunction with the findings of Brown-Grant *et al.* (1957), who reported the loss of nervous reflex control of TSH release from the adenohypophysis after pituitary stalk section, but the return of such control if regeneration of the hypophysial portal vessels occurred, the evidence would suggest that hypothalamic regulation of TSH discharge occurs through the mediation of the hypophysial portal vessels.

In a preliminary report of this work (Harris & Woods, 1956*b*), the possible relationships between the present findings and the aetiological factors involved in Graves's disease have been discussed in some detail. The two factors that have been described many times as of importance in the development of exophthalmic goitre, emotional trauma and adrenal cortical deficiency, may be compared with the electrical stimulation of the central nervous system and the bilateral adrenalectomy utilized in the present experiments. Such a comparison might imply that the onset of Graves's disease is associated with an increase in the blood concentration of TSH. This has been reported by severa groups of workers (Querido & Lameyer, 1956; Gilliland & Strudwick, 1956).

The results of McCullagh, Clamen & Gardner (1957) are directly related to the hypothesis that central nervous factors and some degree of adrenal cortical insufficiency are connected with Graves's disease. These workers found that the exophthalmos and other features of hyperthyroidism improve or completely disappear on treatment by (*a*) administration of ACTH and hydrocortisone or (*b*) operative section of the pituitary stalk. Also pertinent is the observation of Purves (1957) that patients in whom a raised blood concentration of TSH has been demonstrated possess also a raised plasma protein-bound iodine, and that the simultaneous increase in TSH and thyroid hormone in the blood is strong evidence that some pathological condition exists in the 'feed-back mechanism', probably at a hypothalamic or pituitary level.

SUMMARY

1. Various areas in the hypothalamus and pituitary gland have been electrically stimulated for periods of 24–72 hr, using a remote control method, in forty-three conscious rabbits. The effect of such stimulation on thyroid activity has been assessed by measuring the rate of release of ^{131}I-labelled hormone from the gland and by studies of the blood concentration of PB^{131}I.

2. Stimulation in rabbits with intact adrenal tissue results in increased thyroid activity if the electrode tips are situated in the anterior part of the median eminence adjacent to the supraopticohypophysial tract. Stimulation of more posterior and superior areas of the hypothalamus, and different regions of the pituitary gland, did not elicit thyroid activation. That the supraopticohypophysial tract is the neural path involved in the thyroid response is not certain, since concurrent experiments in which both the thyroid and antidiuretic responses were measured in twelve rabbits revealed a discrepancy in the responses in the case of one animal.

3. Stimulation was repeated in thirty-two of the above forty-three animals after complete adrenalectomy and maintenance with small daily doses of cortisone. In four cases in which thyroid activation had occurred before adrenalectomy, a greater thyroidal response was observed after adrenalectomy. In thirteen other animals in which no increased thyroid function was observed before adrenalectomy, constant and marked thyroidal activation in response to stimulation was observed after adrenalectomy. In these latter thirteen rabbits the electrode positions were found to be in the median eminence, but posterior to the supraopticohypophysial tract.

4. The mechanism of the change in thyroidal response after complete adrenalectomy was investigated in animals subjected to adrenal denervation or to adrenal cortical 'blockade' with high doses of cortisone. The evidence indicates that it is removal of the adrenal cortex, rather than the adrenal medulla, which is effective in reversal of the thyroid response after adrenalectomy. Since stimulation of the hypothalamus is known to activate release of

pituitary ACTH, and since adrenal corticoids have been shown to inhibit TSH secretion, it is suggested that the effect of adrenalectomy is to prevent a sudden rise of adrenal steroids in the blood simultaneous with the period of stimulation.

5. In one animal a typical thyroid acceleration was observed in response to hypothalamic stimulation after the thyroid gland had been transplanted to the anterior surface of the infrahyoid muscles.

6. Eight rabbits died during, or shortly after, prolonged or repeated periods of hypothalamic stimulation. An increase in thyroid hormone in the blood tends to a state of adrenal cortical insufficiency.

7. It was observed incidentally that twenty rabbits had ovulated after periods of stimulation. In these cases the electrodes were situated in the anterior or middle tuber cinereum. These observations confirm previous reports.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. The implanted unit. The coil, with the cotton threads used for suturing to the lumbo-dorsal fascia, is on the right and the bipolar glass-insulated electrodes (together with the carrier for insertion in the stereotaxic machine), are in the centre.

Fig. 2. Lateral view of a rabbit, a few days after insertion of the unit.

Fig. 3. Lateral radiograph of a rabbit's skull showing the electrodes and the fixation screws. The mound of dental cement which anchors the electrodes to the screws is not apparent in an X-ray photograph.

Fig. 4. Photograph of an oscilloscope tracing of the pulse developed in the implanted coil. The duration of the pulse is 1·4 msec, and the voltage varies with the position of the animal's cage in the rack (depicted in Pl. 3).

PLATE 2

Figs. 5, 6. Anterior and lateral views of the stereotaxic machine used to implant the electrodes.

PLATE 3

Fig. 7. Photograph of the stimulating circuit (on the right), the primary coil (left and below) and the rotating rack carrying four rabbit cages. The cages can be individually adjusted so that they lie at various heights above the primary coil.

PLATES 4 AND 5

Photomicrographs of horizontal sections through the hypothalamus or pituitary gland, and surrounding structures in different animals. The sections are 100μ thick and are stained with haematoxylin and eosin or Weigert's iron-haematoxylin. *A*, anterior wall of third ventricle; *E*, site of uninsulated tip of electrode(s); *IC*, internal carotid artery; *MB*, mammillary body; *NL*, neural lobe of pituitary; *O*, oculomotor nerve; *OT*, optic tract; *PD*, pars distalis; *PI*, pars intermedia; *V*, third ventricle; *ZT*, zona tuberalis.

PLATE 4

Fig. 8. Rabbit 1138. Bipolar electrodes in anterior wall of median eminence; thyroid excitation before adrenalectomy; response increased by adrenalectomy (see Text-fig. 8).

Fig. 9. Rabbit 1127. Unipolar electrode in anterior wall of median eminence (section obliquely cut); thyroidal excitation before adrenalectomy (see Text-fig. 3).

Fig. 10. Rabbit 1125. Unipolar electrode in anterior wall of median eminence; thyroidal excitation before adrenalectomy. Response increased by adrenalectomy.

Fig. 11. Rabbit 1144. Bipolar electrodes in median eminence; thyroidal excitation only after adrenalectomy.

Fig. 12. Rabbit 1102. Unipolar electrode in lateral wall of median eminence; thyroidal excitation only after adrenalectomy.

Fig. 13. Rabbit 1104. Unipolar electrode adjacent to wall of third ventricle; thyroidal excitation only after adrenalectomy.

PLATE 5

Fig. 14. Rabbit 1101. Unipolar electrode in mammillary body; no thyroidal activation by stimulation

Fig. 15. Rabbit 1114. Unipolar electrode in pars distalis of pituitary gland; no thyroidal activation by stimulation.

Fig. 16. Rabbit 1077. Bipolar electrodes in the tuber cinereum, just posterior to the ventromedial hypothalamic nuclei; no thyroidal activation by stimulation.

Fig. 17. Rabbit 1165. Unipolar electrode in mid tuber cinereum; no thyroidal activation by stimulation.

Fig. 18. Rabbit 1076. Bipolar electrodes in mid tuber cinereum; no thyroidal activation by stimulation (see Text-fig. 1).

Fig. 19. Rabbit 1119. Bipolar electrodes in mid tuber cinereum. The position of one electrode is shown only by an indentation in the wall of the third ventricle; no thyroidal activation by stimulation.

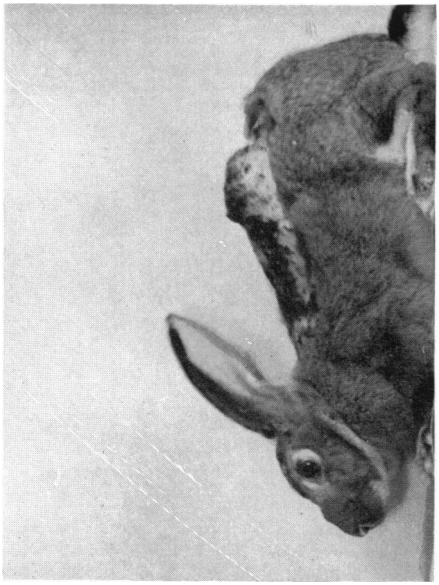
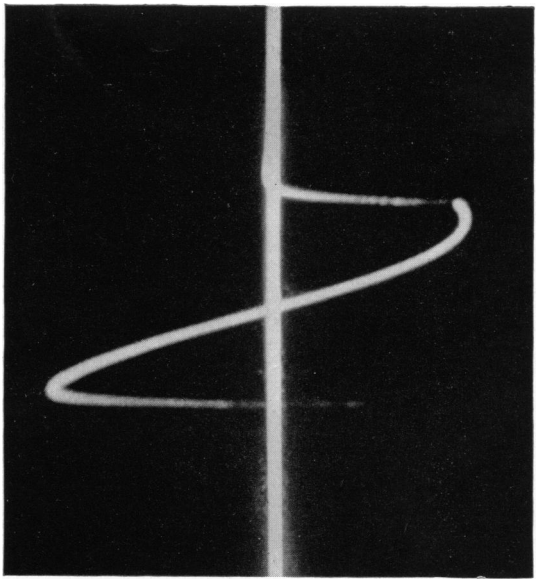


Fig. 2



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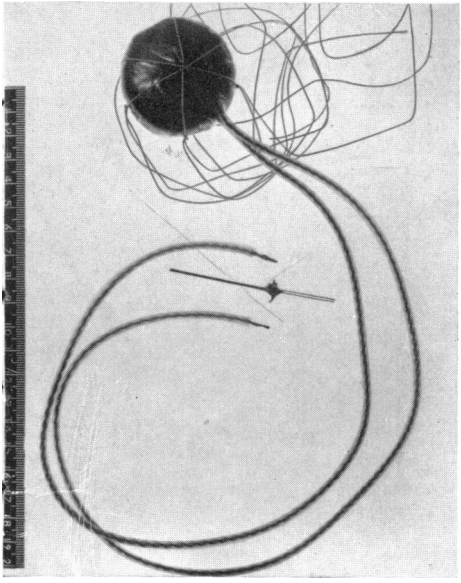

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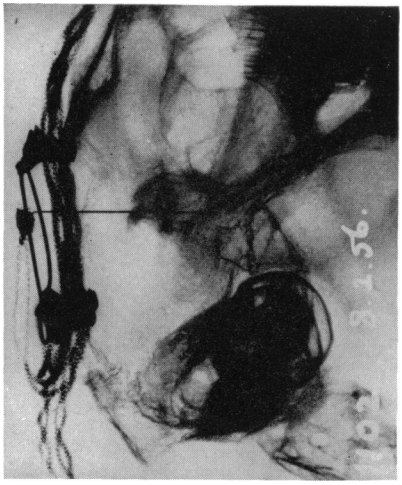

Fig. 3

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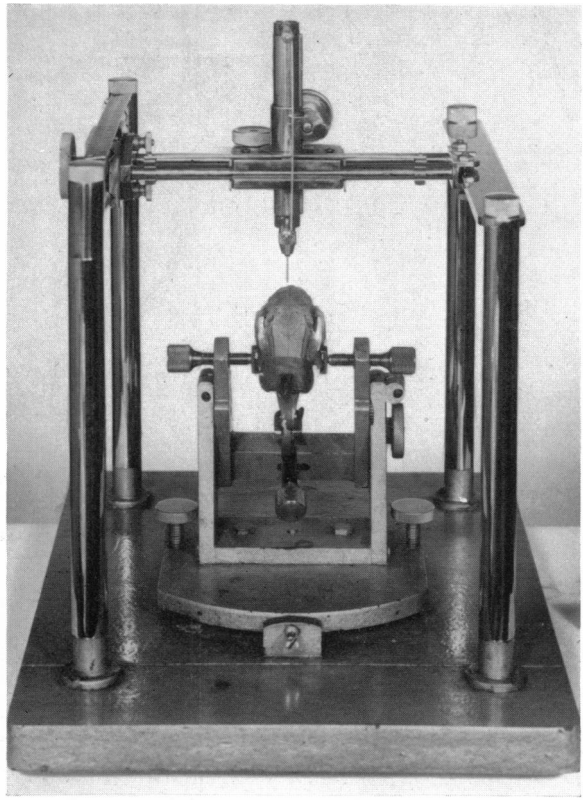

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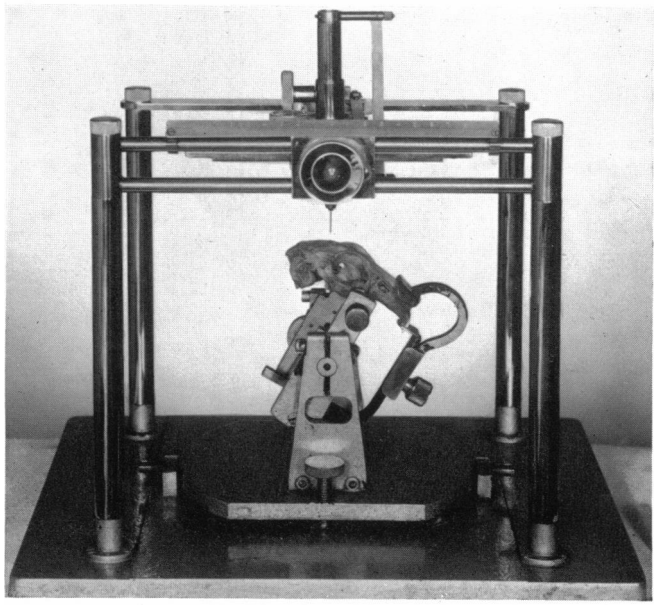

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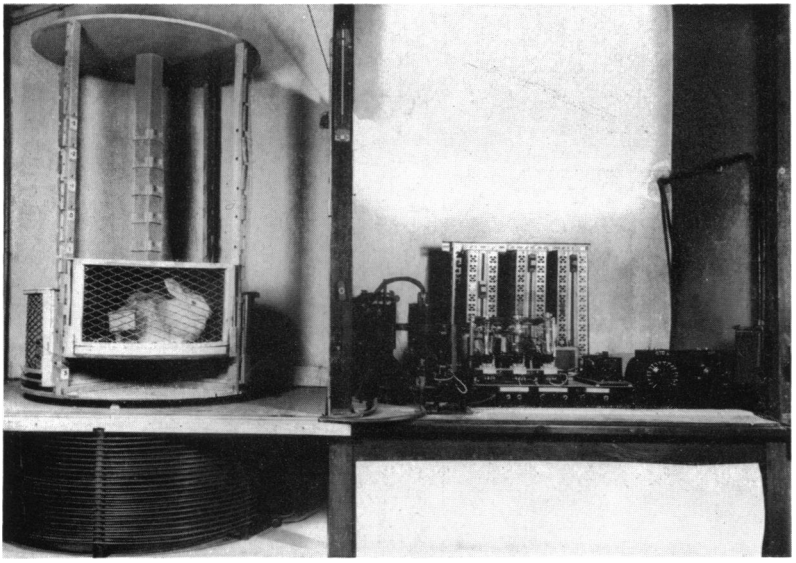

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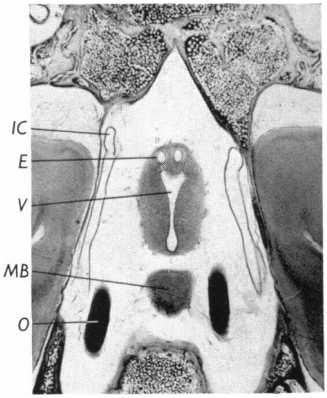

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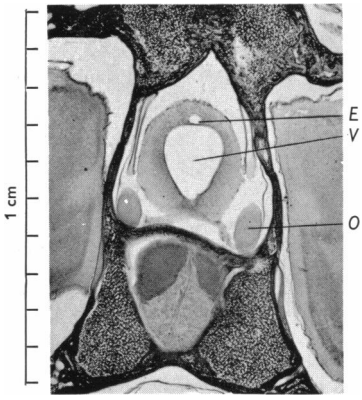

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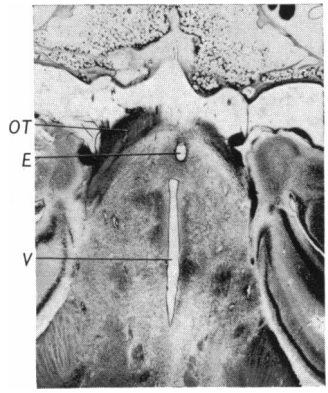

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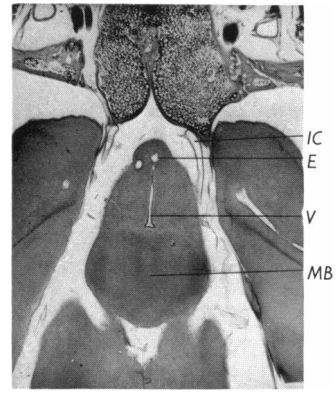

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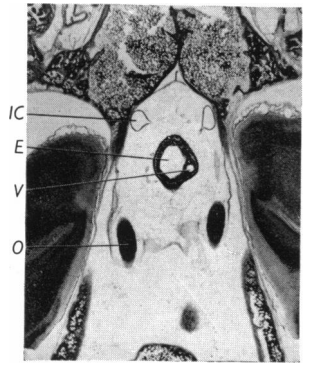

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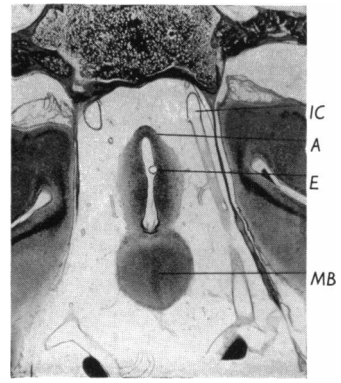

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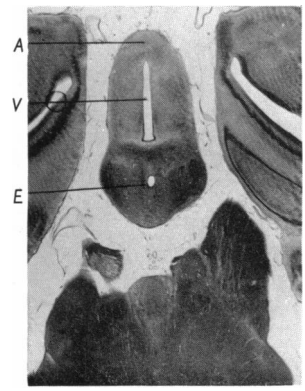

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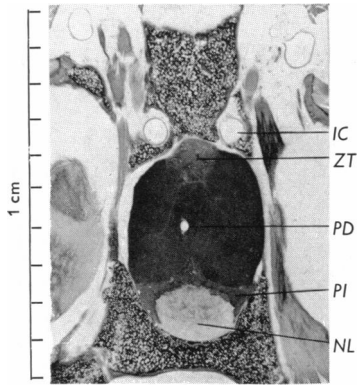

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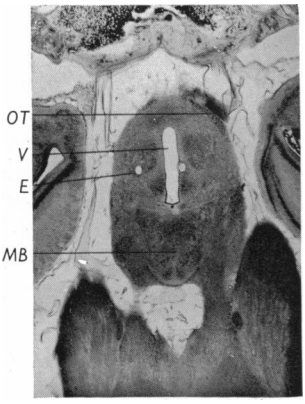

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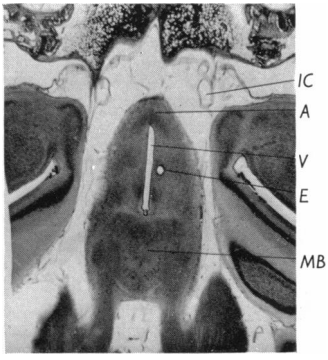

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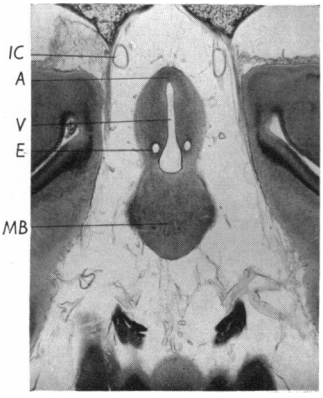

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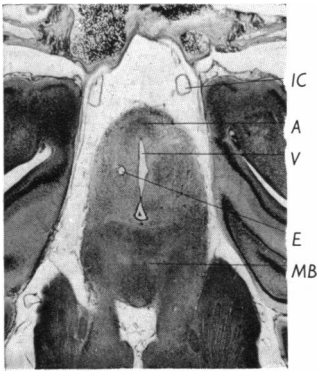

Fig. 19