J. Physiol. (1956) 131, 125–136

THE THYROXINE 'RECEPTOR' OF THE THYROID-PITUITARY SYSTEM

BY CURT VON EULER AND BJÖRN HOLMGREN*

From the Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden

(*Received* 9 *June* 1955)

The main principle of homoeostasis implies the existence of specific detectors or 'receptors' which govern the corresponding regulator mechanisms. The interaction of the anterior pituitary gland with the target glands constitutes a feed-back circuit preserving homoeostasis of the target hormones. This was first demonstrated for the pituitary-thyroid system (Aron, Caulaert & Stahl, 1931) and then for the glands (Hohlweg & Dohrn, 1931; Kuschinsky, 1931). Ingle, Higgins & Kendall (1938) first demonstrated that large amounts of cortin produced atrophy of the adrenal cortex. Although an extensive literature has confirmed these findings, very little is known about the underlying mechanisms. Concerning the thyroid-pituitary axis (Salter, 1940) facts have accumulated which indicate that thyroxine inhibits secretion of thyrotropic hormone by acting on hypothalamic structures, and in this respect it is interesting that the tuber cinereum and the median eminence of the posterior pituitary have been found to possess a specific affinity for thyroxine (Courrier, Horeau, Marois & Morel, 1949, 1951; Harper & Mattis, 1950, 1951; Jensen & Clark, 1951; see the discussion by Marois, 1951).

The present paper deals with the question of whether the thyroxine-sensitive 'receptors' of the thyroid-pituitary system constitute an adeno-hypophysial mechanism or whether they are located in the brain stem. Evidence in favour of the first alternative will be presented. This conclusion has been confirmed by a different experimental approach, reported in the succeeding paper (Euler & Holmgren, 1956). A preliminary account has already appeared (Euler & Holmgren, 1954).

METHODS

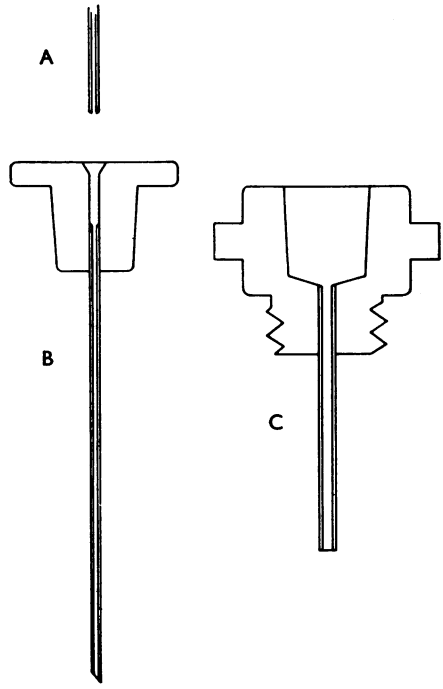
Healthy adult female rabbits have been used throughout this work. Thyroid activity, as indicated by the rate of thyroidal release of ^{131}I, has been followed for periods of 1–2 weeks or more. The method used has been described in detail by Brown-Grant, Euler, Harris & Reichlin (1954) and

* On leave of absence from the Institute of Physiology, University of Chile, Santiago de Chile.

now slightly modified. Two to three days after the rabbits had received a dose of 2–5 μc of carrier-free radio-iodine (^{131}I) intramuscularly the observations started. The unanaesthetized rabbit was placed in a hammock and immobilized by restriction clamps (Harris, 1948*a*). The neck rested against the lead collimator of a scintillation counter. The latter consisted of a thallium-activated NaI crystal 4 cm in diameter and 25 mm thick in front of an E.M.I. photomultiplier type 6260. Together with the preamplifier it constitutes a 'Gamma Counter' Type 1186 A (AERE, Harwell). The scaling device, which had an input stage with adjustable bias voltage for pulse discrimination, consisted of an electronic scaler of 64 (L.K.B. type no. 6413) and a rapid mechanical counter. The efficiency of the detecting equipment was controlled regularly by means of a ^{137}Sc standard of 0·5 μc activity (half life 33 years). Provided the bias voltage of the input stage of the scaler or the high tension to the photomultiplier had been adjusted to give constant counts of the ^{137}Sc standard, the counts from a given source of ^{131}I in the thyroid of a rabbit were also constant. The radio-activity of the thyroid region was usually measured twice a day. Geometrical errors due to small differences in the position of the rabbit's thyroid were avoided by taking the average of four to six counts preceded by replacement of the rabbit's head and neck. The accuracy of the counts varied from rabbit to rabbit. The number of counts made each time was determined after a rough estimation of the deviations between the individual counts. The level of the counting rate determined the counting time. The best way of estimating the accuracy of the measurements was to ascertain the deviations from the theoretical values under periods of complete thyroid inhibition which should plot as a horizontal line. In the present work the standard deviation of the differences from the mean under complete inhibition (the horizontal line) was calculated from twenty rabbits. The values stayed within the range $\pm 1{\cdot}2\,\%$ to $\pm 2{\cdot}4\,\%$. The counts were corrected for background, physical decay and efficiency.

The rabbits were kept under standardized conditions, i.e. in an environmental temperature of $29 \pm 1°$ C and a constant diet of oats, carrots and tap water *ad lib.*

In order to be able to repeat injections of minute volumes of a drug at a predetermined spot in the hypothalamus or in the pituitary gland of the conscious rabbit, cannulas were chronically implanted into the rabbit's head. The procedure was as follows. Under urethane-chloralose anaesthesia (5 ml./kg body weight of a solution of 10 % urethane and 1 % chloralose) the rabbit's head was firmly fixed in a stereotaxic apparatus and orientated so that the plane of the tangents through the bregma became horizontal (cf. Harris, 1947). A small hole was drilled vertically through the skull and a thread made to fit the 'guide' (see text-fig. 1). The 'guide' was screwed down vertically. It was found necessary to strengthen the attachment of the 'guide' to the skull. This was done by means of a self-apolymerizing methacrylate for dental use ('Swedon'). The 'guide' implanted in that way was as rigidly attached to the skull after 6 months as it was on the day of operation. The skin was sutured round the guide and sealed with 'Swedon'. There was no trouble with skin infections. The head of the rabbit with the implanted 'guide' was then X-rayed in order to verify that the direction of the guide was the intended one and, further, to permit an accurate calculation of the length of the cannula to be inserted. The cannula consisted of a stainless hypodermic needle, 0·45 mm in external diameter, and a Perspex head as illustrated on Text-fig. 1. When the cannula had been adjusted to the right length it was inserted by hand through the 'guide' into the brain and left there. The head of the rabbit was then X-rayed again (Pl. 1, fig. 1) to determine the final position of the bevel of the cannula. The injections were performed with an 'Agla' micrometer syringe to which was attached a short hypodermic needle of the same external diameter as that of the implanted cannula and ground to a point of the shape shown in Text-fig. 1. This, with an extremely small volume displacement due to the coupling, made a perfect fit with the Perspex head of the cannula. The volume of the cannula was less than 0·001 ml. After the cessation of the experiments in any one rabbit the animal was killed, the head and the brain fixed in 10 % formaline and the skull decalcified with equal parts of 40 % formic acid and 7·5 % Na-formate. A block containing the hypothalamus and pituitary region was embedded in paraffin wax and serially sectioned at 15 μ. The sections, stained with thionine, served to identify the site of the injections (Pl. 1, fig. 2).



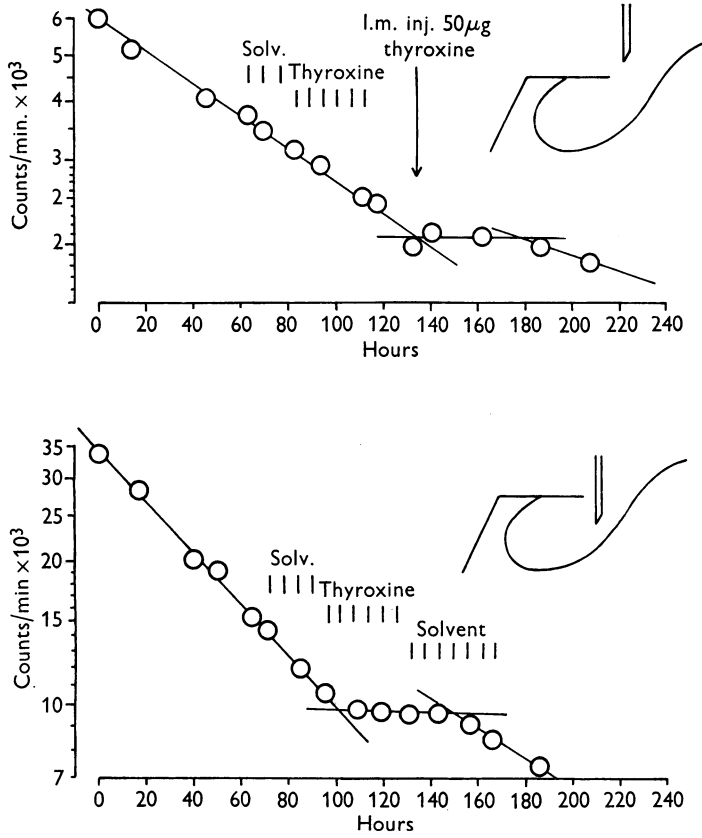
Text-fig. 1. Gear for microinjections in the conscious rabbit. The 'guide' C is screwed into the skull after stereotaxic positioning. The cannula B is inserted by hand through the 'guide'. A represents the specially ground end of the hypodermic needle attached to the microsyringe. A fits tightly in the Perspex head of the cannula.

RESULTS

Local injections of thyroxine. The experiments consisted in local administration of minute volumes of thyroxine solution into the anterior pituitary gland or adjacent parts of the brain. The influence of these injections on the release of radio-iodine from the thyroid gland was followed.

In most cases the guide for the cannula was implanted before the radioiodine was given, i.e. before the beginning of the actual experiment. In a few cases, however, the implantation was done during the course of a 'release curve'. In these cases the operation caused a flattening of the curve, i.e. inhibition of thyroid release of radio-iodine from the thyroid for a period of 6–24 hr. This was probably due to the physical stress of operation and anaesthesia (Brown-Grant, Harris & Reichlin, 1954*a*). The experiments were carried out in the manner illustrated in Text-figures 2 and 3. As soon as the successive estimation of thyroid content of ^{131}I, plotted on a logarithmic scale against time, had stabilized to give a straight line, the intracranial injections were begun. The

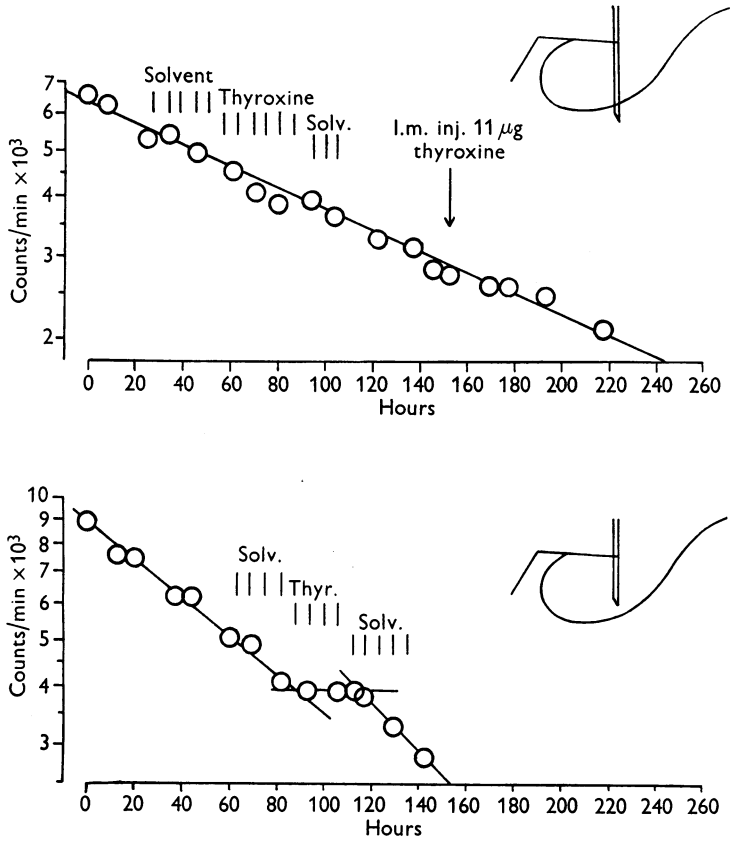
injections were done every 6th hour. To begin with, the solvent without thyroxine (0·015 N-NaOH) was injected as a control. Usually 0·002 ml. was injected each time. Since the volume of the cannula was of the order of, but always less than, 0·001 ml. the first injection was made bigger than the following ones by that amount. In a few experiments 0·005 ml. was injected each time. When it had been established that the solvent itself had no effect whatsoever on the



Text-fig. 2. The effects on the release of ^{131}I from the thyroid of local injections of L-thyroxine at the sites indicated by inset diagrams. In the upper curve an intramuscular injection of 50 μg L-thyroxine was given after the local injections.

release of thyroidal ^{131}I, it was exchanged for solvent plus thyroxine (0·002 ml. contained 2 μg Na L-thyroxinate). The injections were continued every 6th hour until it had been established whether or not a break in the curve illustrating liberation of radio-iodine from the thyroid was present. Then followed a new series of injections of solvent without thyroxine. Of the eighteen rabbits in which the effect of microinjections of thyroxine was studied only two had to be discarded because of a non-specific effect of the solvent. In some cases

the cannula was removed after the first experiment and exchanged for another cannula of different length. Thus two experiments were performed on the same animal at two different sites of injection. In Text-figs. 2 and 3 such double experiments are illustrated. Injections of thyroxine were effective in inhibiting the output of thyroid ^{131}I with one position of the cannula but not with the other (see, for example, Text-fig. 2). Every experiment was checked by demon-

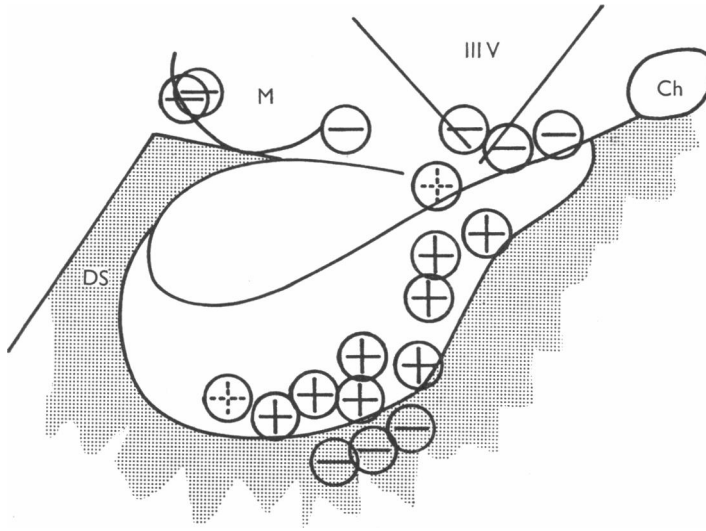


Text-fig. 3. The effect on the release of ^{131}I from the thyroid of local injections of L-thyroxine at the sites indicated in the inset diagrams. The upper curve shows absence of effect of 11 μg L-thyroxine intramuscularly.

strating that the total amount of thyroxine injected in this manner had no effect when given intramuscularly. In most cases the daily endogenous secretion of thyroxine was estimated according to the principles given by Perry (1951). Repeated small doses of thyroxine were administered intramuscularly and the smallest dose sufficient to inhibit the thyroidal release of ^{131}I for 24 hr determined (cf. Brown-Grant & Gibson, 1955). The daily secretion of thyroid hormone is considered to be equivalent to this amount of L-thyroxine.

The total of thyroxine injected through the implanted cannula was in any experiment always well below the value determined for the daily production of thyroid hormone of that animal.

The results of the experiments are summed up in Text-fig. 4. All the injections at sites outside the anterior lobe of the pituitary were 'negative', i.e. thyroxine injected locally into these spots had no inhibitory effect on the thyroidal release of radio-iodine. On the other hand, when thyroxine was injected locally into the anterior lobe the inhibitory effect was nearly always present.

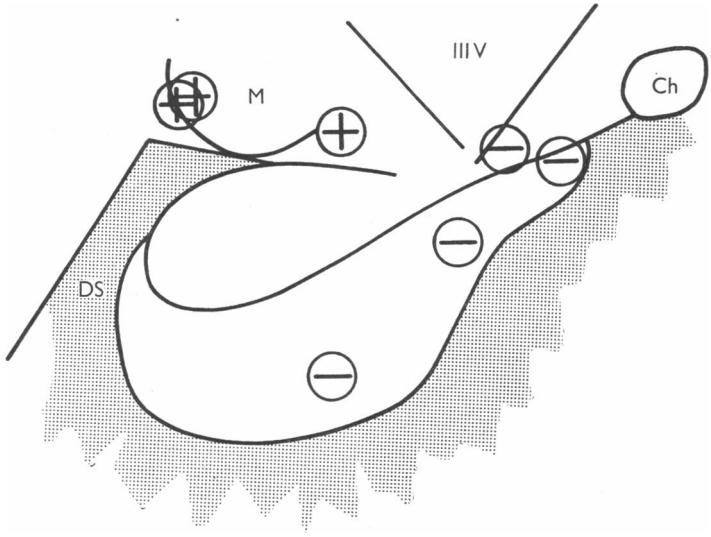


Text-fig. 4. A summary of the results obtained with local injections of L-thyroxine. ⊕ indicates definite inhibition of the release of ^{131}I from the thyroid; ⊖, no effect; ⊕, doubtful effect. Ch, optic chiasma; DS, dorsum sellae; M, corpus mamillare; III V, third ventricle.

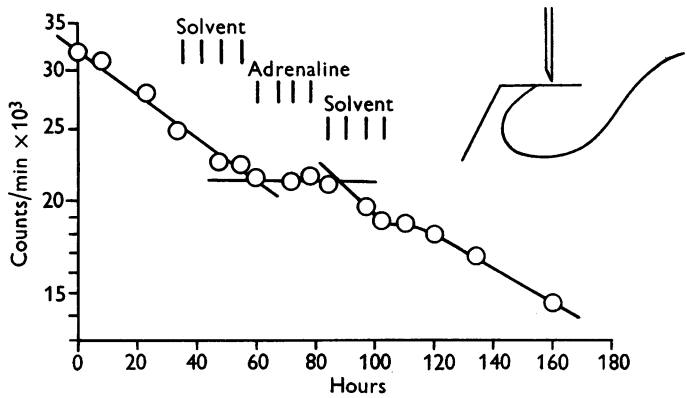
In order to obtain an estimate of the spread of thyroxine injected through the cannula, 0·002 ml. osmic acid was similarly injected and the distribution studied in the histological sections. Pl. 1, fig. 3, shows the spread of osmic acid injected 30 min before the rabbit was killed.

Local injections of adrenaline. In seven experiments on five rabbits adrenaline was injected locally into the anterior pituitary or adjacent parts of the hypothalamus. The procedure was the same as described for the local injection of thyroxine. The series of injections started with 0·003 ml. and continued with 0·002 ml. of the solvent only (0·001 N-HCl). When the first series of control injections proved to be without effect on the thyroidal release of iodine, adrenaline solution was injected (1 mg adrenaline as adrenaline-HCl per ml. of 0·001 N-HCl). Thus 2μg adrenaline in 0·002 ml. was injected every 6 hr until a clear result was obtained. Text-fig. 5 summarizes the results. Adrenaline of

that huge concentration injected locally into the anterior lobe of the pituitary gland, the tuber cinereum or the median eminence had no effect on the release of ^{131}I from the thyroid. However, injected into the vicinity of the



Text-fig. 5. A summary of the results obtained with local injections of adrenaline. Markings and abbreviations as in Fig. 4.

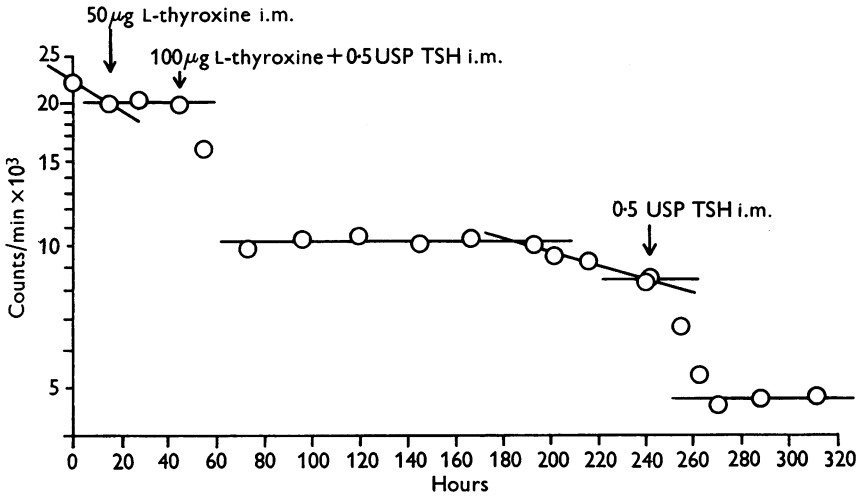


Text-fig. 6. The effect on the release of ^{131}I from the thyroid of local injections of adrenaline at the site indicated by inset diagram.

mammillary body adrenaline, even in the somewhat smaller dose of 0·2μg, had a definite inhibitory action on the thyroidal release of ^{131}I, as shown in Text-fig. 6.

Effect of thyroxine on the action of thyroid-stimulating hormone (TSH). It has frequently been held (e.g. Salter, 1950) that thyroxine inhibits the thyroid

itself or the thyrotropic stimulation of the thyroid. Although the evidence presented in favour of this hypothesis is extremely poor, it was considered worth while to find out whether this constituted a significant part of the feedback control of thyroxine secretion. In experiments on four rabbits, one of which was hypophysectomized, the effect of a certain dose of TSH on the



Text-fig. 7. Rabbit 236 (see Table 1). Changes in the release of ^{131}I from the thyroid in response to intramuscular injections of L-thyroxine plus thyroid-stimulating hormone (TSH), and to TSH alone.

TABLE 1. The effect of thyroxine on the thyroid response to 0·5 USP units of TSH

Rabbit no.	Dose of L-thyroxine (μg)	With thyroxine % fall	Without thyroxine % fall
167 Hypophysect.	250	39 (2)	42 (1)
230	200	46 (1)	38 (2)
236	100	41 (1)	35 (2)
249	200	43 (2)	48 (1)

The figures in parentheses indicate the order in which the tests were made.

release of ^{131}I from the thyroid was compared when given under heavy thyroxine treatment and in the untreated state. Since the effect of exogenous thyrotropin (TSH) diminishes, when repeatedly given to one and the same animal (cf. Brown-Grant, Harris & Reichlin, 1954*b*), thyroxine was sometimes combined with the first TSH injection and sometimes with the second one (Text-fig. 7). The percentage decrease of thyroidal ^{131}I was calculated and the results tabulated in Table 1. There was no significant change in the response of the thyroid to TSH when the thyroxine concentration of the blood was increased.

DISCUSSION

There is good evidence for the conclusion that the release of ^{131}I from the thyroid is equivalent to secretion of labelled thyroid hormone (Albert, 1951; Perry, 1951; Wolf, 1951; Brown-Grant, Euler, Harris & Reichlin, 1954). Likewise it seems justified to assume that the secretion of the thyroid is governed solely by the thyrotropic hormone (TSH) of the anterior pituitary gland (cf. Harris, 1948*b*), although under certain circumstances vasomotor reactions of the thyroid vessels may modify its activity (Brown-Grant & Gibson, 1954). Thus, changes in the rate of percentage loss of ^{131}I from the thyroid follow from a preceding change in the level of circulating thyroid-stimulating hormone. However, it must be kept in mind that there is no quantitative relationship between this rate and total thyroid hormone secretion. Since the rate of secretion is subject to feed-back control (thyroxine inhibiting pituitary) it may, however, be quantitatively assayed by determining the minimum exogenous dose of thyroxine that completely inhibits the endogenous thyroxine secretion per unit time. Sayers & Sayers (1947) used the principle of this method for determining the daily output of adreno-cortical hormone, Perry (1951) described the method for determining the rate of thyroxine secretion in the rat, and Brown-Grant & Gibson (1955) for the rabbit. The daily output of thyroid hormone in terms of L-thyroxine was estimated above and compared with the amount of thyroxine injected locally. These comparisons show that the effects elicited by the local injections of thyroxine were due to its specific action at the site of injection and not to a generalized effect. This conclusion is supported by the fact that injections into different regions gave different results as well as by the distribution of 'positive' and 'negative' sites.

The histological examination of the sites of injection revealed that the chronically implanted cannulas as well as the injections had caused very little damage to the tissues (cf. Pl. 1, fig. 2).

Local administration of thyroxine to the anterior pituitary gland provoked an inhibition of thyroxine secretion similar to the effect caused by an increased level of circulating thyroid hormone. Injections outside the anterior lobe failed to give such a response. These findings strongly indicate that the anterior pituitary gland itself is the strategic point of action of the thyroid hormone in suppressing secretion of thyrotropic hormone. This contradicts the conclusions drawn from the facts that the median eminence and tuber cinereum have a specific affinity for thyroxine (see Marois, 1951). Moreover, this high affinity for thyroxine is found only in some species and not in others (see Gross & Pitt-Rivers, 1952). Greer and co-workers (Greer, 1952; Greer, Scow & Grobstein, 1953; Scow & Greer, 1953) have reported that hypothalamic lesions reduce or block the growth of thyroid in response to goitrigens. This finding suggests a hypothalamic link in the mechanism controlling thyroid weight.

However, the present work does not deal with this question. The possibility that the *posterior* pituitary might be the site of the structures sensitive to thyroxine and inhibiting thyrotropic secretion has been excluded by experiments reported in the succeeding paper (Euler & Holmgren, 1956).

However, we do not consider the level of circulating thyroxine as the only regulator of thyroxine secretion. Euler & Holmgren (1956), on hypophysectomized rabbits with pituitary transplants in the anterior chamber of the eye, have shown that the changes in thyroid activity in response to cold (see Brown-Grant, Euler, Harris & Reichlin, 1954) and to stress (Brown-Grant, Harris & Reichlin, 1954*b*) are largely due to the central nervous control of the anterior pituitary. These effects are probably mediated by the hypothalamo-hypophysial portal vessels (see Harris, 1948*b*), although the details of the transmitter mechanisms are still unknown. However, the results of local injections of adrenaline indicate that this substance is not concerned with the secretion of thyrotropin. It might be objected that the concentrations used were unphysiological. However, there are good reasons for believing that both time and space gradients of the concentration after a local injection are steep enough to give large areas with much lower concentrations of adrenaline.

The inhibitory effect of adrenaline on thyrotropin secretion when injected locally in the vicinity of the mammillary bodies may be related to the stimulating action of adrenaline on ACTH secretion mediated by the posterior hypothalamus as reported by Porter (1952).

Our conclusion that the anterior pituitary itself is the site where thyroxine exerts its inhibiting effect on the secretion of the thyrotropic hormone appears to be confirmed by work reported in the following paper (Euler & Holmgren, 1956).

SUMMARY

1. A method is described for injecting minute volumes of drugs into various places in the hypothalamus and in the pituitary gland of the conscious rabbit.

2. Thyroxine injected locally in the anterior pituitary inhibited the release of ^{131}I from the thyroid gland. No effect, however, was obtained from thyroxine injected locally in the hypothalamus or median eminence.

3. The release of thyroidal iodine in response to exogenous thyrotropin was uninfluenced by simultaneous thyroxine administration.

4. It is concluded that thyroxine inhibits thyrotropin secretion by a direct action on the anterior pituitary gland and that this is the only route whereby thyroxine regulates thyroid secretion.

This work has been aided by grants from the Foundations 'Therese och Johan Anderssons Minne' and 'Magnus Bergwalls Stiftelse'.

REFERENCES

ALBERT, A. (1951). The in vivo determination of the biological decay of thyroidal radio-iodine. *Endocrinology*, **48**, 334–338.

ARON, M., VAN CAULAERT, C. & STAHL, J. (1931). L'équilibre entre l'hormone préhypophysaire et l'hormone thyroïdienne dans le milieu intérieur, à l'état normal et à l'état pathologique. *C.R. Soc. Biol., Paris*, **107**, 64–66.

BROWN-GRANT, K., VON EULER, C., HARRIS, G. W. & REICHLIN, S. (1954). The measurement and experimental modification of thyroid activity in the rabbit. *J. Physiol.* **126**, 1–28.

BROWN-GRANT, K. & GIBSON, J. G. (1954). Factors affecting the uptake of radio-iodine by the rabbit thyroid. *J. Endocrin.* **12**, ii.

BROWN-GRANT, K. & GIBSON, J. G. (1955). A comparison of thyroxine and triiodothyronine as inhibitors of pituitary thyrotrophic hormone secretion in the rabbit. *J. Physiol.* **127**, 352–357.

BROWN-GRANT, K., HARRIS, G. W. & REICHLIN, S. (1954*a*). The effect of emotional and physical stress on thyroid activity in the rabbit. *J. Physiol.* **126**, 29–40.

BROWN-GRANT, K., HARRIS, G. W. & REICHLIN, S. (1954*b*). The influence of the adrenal cortex on thyroid activity in the rabbit. *J. Physiol.* **126**, 41–51.

COURRIER, R., HOREAU, A., MAROIS, M. & MOREL, F. (1949). Étude quantitative de la pénétration de la radio-thyroxine dans les cellules hypophysaires. *C.R. Soc. Biol., Paris*, **143**, 935–937.

COURRIER, R., HOREAU, A., MAROIS, M. & MOREL, F. (1951). Sur le pénétration de la thyroxine dans le lobe postérieur de l'hypophyse. *C.R. Acad. Sci., Paris*, **232**, 776–778.

VON EULER, C. & HOLMGREN, B. (1954). The feed-back control of thyroid secretion. *Acta physiol. scand.* **31**, suppl. 114, 13–14.

VON EULER, C. & HOLMGREN, B. (1956). The role of hypothalamo-hypophysial connexions in thyroid secretion. *J. Physiol.* **131**, 137–146.

GREER, M. A. (1952). The role of the hypothalamus in the control of thyroid function. *J. clin. Endocrin.* **12**, 1259–1268.

GREER, M. A., SCOW, R. O. & GROBSTEIN, C. (1953). Thyroid function in hypophysectomized mice bearing pituitary transplants. *Proc. Soc. exp. Biol., N.Y.*, **82**, 28–30.

GROSS, J. & PITT-RIVERS, R. (1952). Experimental study of thyroid metabolism with radioactive iodine. *Brit. med. Bull.* **8**, 136–140.

HARPER, E. O. & MATTIS, P. A. (1950). Comparison of rate of uptake of radioactive thyroxine by tuber cinereum, hypophysis and thyroid. *Fed. Proc.* **9**, 282–283.

HARPER, E. O. & MATTIS, P. A. (1951). Uptake of radioactive thyroxine by the tuber cinereum and hypophysis in thyroidectomized rabbits. *Fed. Proc.* **10**, 306.

HARRIS, G. W. (1947). The innervation and actions of the neurohypophysis; an investigation using the method of remote-control stimulation. *Phil. Trans.* B, **232**, 385–441.

HARRIS, G. W. (1948*a*). Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. *J. Physiol.* **107**, 418–429.

HARRIS, G. W. (1948*b*). Neural control of the pituitary gland. *Physiol. Rev.* **28**, 139–179.

HOHLWEG, W. & DOHRN, M. (1931). Beziehungen zwischen hypophysenvorderlappen und Keimdrüsen. *Wien. Arch. inn. Med.* **21**, 337–350.

INGLE, D. J., HIGGINS, G. M. & KENDALL, E. C. (1938). Atrophy of the adrenal cortex in the rat produced by administration of large amounts of cortin. *Anat. Rec.* **71**, 363–372.

JENSEN, J. M. & CLARK, D. E. (1951). Localization of radioactive L-thyroxine in the neurohypophysis. *J. Lab. clin. Med.* **38**, 663–676.

KUSCHINSKY, G. (1931). Über die Bedingungen der Hypophysenvorderlappensekretion und ihre Folgen für den Ablauf des Zyklus. *Arch. exp. Path. Pharmak.* **162**, 183–196.

MAROIS, M. (1951). Quelques aspects de la régulation de la fonction thyroïdienne étudiés à l'aide de radioéléments. *Ann. Endocrin., Paris*, **12**, 333–359.

PERRY, W. F. (1951). A method for measuring thyroid hormone secretion in the rat with its application to the bioassay of thyroid extracts. *Endocrinology*, **48**, 643–650.

PORTER, R. W. (1952). Alteration in electrical activity of the hypothalamus induced by stress stimuli. *Amer. J. Physiol.* **169**, 629–637.

SALTER, W. T. (1940). Fluctuations in body iodine. *Physiol. Rev.* **20**, 345–364.

SALTER, W. T. (1950). Chemistry and physiology of thyroid hormone. In *The Hormones*, Vol. II, ch. 4. New York: Academic Press.

SAYERS, G. & SAYERS, M. A. (1947). Regulation of pituitary adrenocorticotrophic activity during response of rat to acute stress. *Endocrinology*, **40**, 265–273.

SCOW, R. O. & GREER, M. A. (1953). Observations on the control of thyrotropin secretion of intraocular pituitary implants in hypophysectomized mice. *J. clin. Endocrin.* **13**, 855.

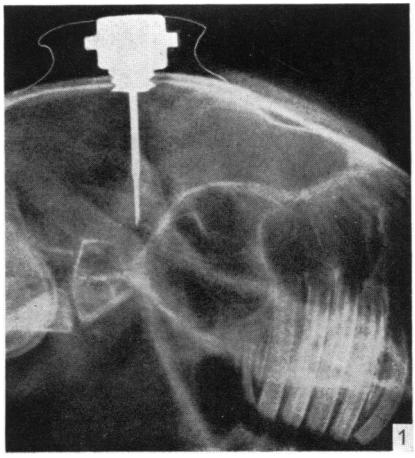
WOLF, J. (1951). Some factors that influence the release of iodine from the thyroid gland. *Endocrinology*, **48**, 284–297.

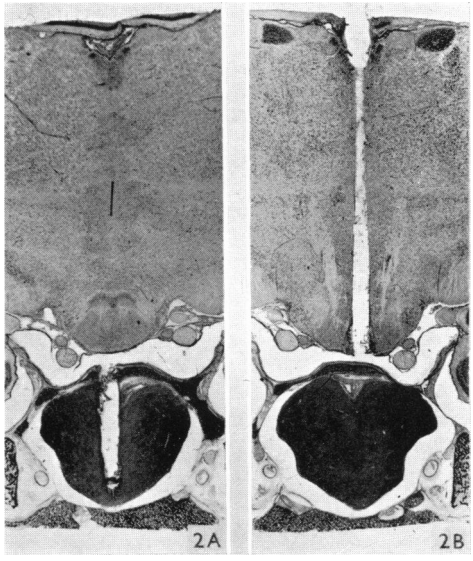
EXPLANATION OF PLATE

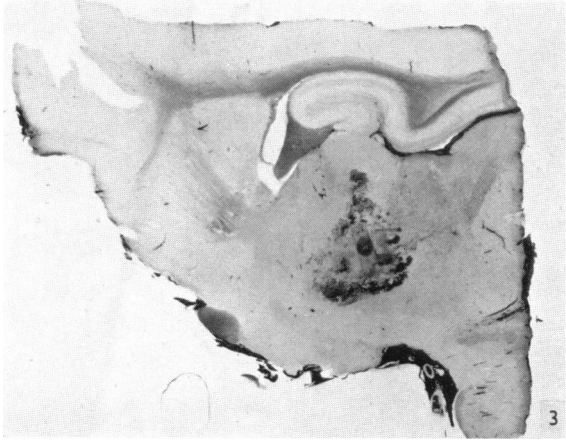
Fig. 1. X-ray of rabbit's head with 'guide' and cannula in position. The top of the head of the cannula is not visible. Outlines of the methacrylate superconstruction drawn in white. Retouched.

Fig. 2. Sections through the skull and brain of rabbit which had a cannula implanted. 200 microns in between A and B. Slight dislocation of the pituitary relative to the brain during the histological procedures has caused a step in track. Formol. Thionine.

Fig. 3. Paramedian section through the brain of a rabbit. 0·002 ml. of osmic acid injected through implanted cannula 30 min prior to sacrifice of the rabbit. Frozen section. Unstained. 100 microns.







(*Facing p.* 136)