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THE EFFECT OF PITUITARY STALK SECTION ON THYROID FUNCTION IN THE RABBIT

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There is much evidence that the central nervous system may influence the activity of the endocrine glands; in most cases this influence appears to be mediated via the hypothalamus and anterior pituitary gland. A considerable body of experimental evidence has accumulated which indicates that this control, in the case of the adrenal cortex and gonads, is exercised via the hypophysial portal vessels of the pituitary stalk (Harris & Fortier, 1954; Harris, 1955*a*). There is also much evidence of a hypothalamic control of thyroid activity. Recent work in this laboratory has been concerned with the development of methods for the detailed study of thyroid function in the rabbit (Brown-Grant, von Euler, Harris & Reichlin, 1954; Brown-Grant, Harris & Reichlin, 1954*a*, *b*). The present paper is concerned with the application of these methods to the investigation of the role of the pituitary stalk and portal vessels in the control of thyroid activity. The pituitary stalk has been divided in rabbits and the animals subjected to tests of adrenocortical (Fortier, Harris & McDonald, 1957) and thyroid function. A study of the changes in the volume of the pituitary gland and its individual lobes after stalk section was also made (Campbell & Harris, 1957). A preliminary report of these findings has been given (Harris, 1955*b*).

MATERIAL AND METHODS

Adult female rabbits of mixed breeds (2·0–3·5 kg body weight) were used; the animals were kept under the same standard conditions as in the previous studies and were fed on a pellet diet (M.R.C. diet no. 18) and tap water *ad lib*. Thyroid activity was determined by measurements *in vivo* of the uptake of ^{131}I 48 hr after the subcutaneous injection of a carrier-free tracer dose, and measurements of the rate of release of thyroidal radio-iodine in the conscious animal. The dose of radio-iodine was usually 3 or 6 μc in normal animals. In hypophysectomized and stalk-sectioned animals the dose was increased to as much as 40 μc, in some cases, to compensate for the

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reduced uptake and to obtain the same absolute thyroid counting rate at 48 hr after injection (2000–3000 counts/min) as in normal animals. For further details of the radio-iodine technique an earlier publication (Brown-Grant, von Euler *et al.* 1954) may be consulted.

The animals subjected to pituitary stalk section are divisible into two groups. (*a*) 'Simple stalk-sectioned' rabbits: those in which the pituitary stalk was cut and in which no attempt was made to prevent vascular regeneration across the site of section. Post-mortem examination of these animals revealed the presence of direct and obvious vascular connexions between the median eminence of the tuber cinereum and the anterior lobe of the pituitary. (*b*) 'Stalk-sectioned rabbits with plates': those animals in which a plate of waxed-paper was inserted between the cut ends of the stalk, and which were found post-mortem to possess no, or only slight, regeneration of the hypophysial portal vessels. Details of the operative and histological techniques, and of the method of estimating pituitary volumes, are described in the preceding papers (Campbell & Harris, 1957; Fortier *et al.* 1957). Studies were also made on a group of hypophysectomized animals in which the completeness of operation was checked by examination of serial sections through the sella turcica.

The procedure used to produce 'emotional stress' was forced immobilization; 'physical stress' consisted of laparotomy under ether anaesthesia or, in a few cases, of bleeding (20% of the estimated blood volume). Details of these procedures and of their effect in normal rabbits are given by Brown-Grant, Harris *et al.* (1954*a*).

The following drugs and hormones were used: thyrotrophic hormone (TSH) (Armour Laboratories Ltd., Lot no. R 377157), dissolved in 0·9% NaCl solution immediately before use; synthetic sodium L-thyroxine (THY) ('Eltroxin', Glaxo Laboratories Ltd), administered as a fine suspension in 0·9% NaCl solution; cortisone acetate (25 mg/ml.) (Merck and Co., Roussel Laboratories Ltd.); stilboestrol (5 mg/ml. in oil) (British Drug Houses Ltd). Dosage and route of administration are given in the relevant sections of the text.

RESULTS

Histology

The stalk-sectioned animals used in the present study fall into two groups: 14 'simple stalk-sectioned' animals and 34 'stalk-sectioned with plates.' The majority of these animals were also used for adrenal function studies and the histological findings in all animals of the present groups are similar to those previously described and depicted (see Fortier *et al.* 1957; Pls. 1 and 2). The main points may be summarized as follows: (*a*) the pituitary stalk had been completely divided in all cases, (*b*) the neural lobe of the pituitary showed marked atrophy (to about 26% of the normal volume), (*c*) the anterior lobe of the pituitary showed some atrophy (to about 75% of the normal volume; there was no significant difference in this respect between the two groups of stalk-sectioned rabbits), (*d*) the pars intermedia showed no significant change in volume, though in many cases it appeared thick and wrinkled, (*e*) the vascularity of the gland was well maintained and, except in one animal (referred to by Fortier *et al.* 1957), there was no sign of fibrosis or other ischaemic changes. The source of the blood supply to the pars distalis in the stalk-sectioned animals with plates appeared to be mainly from systemic vessels from the internal carotid artery and arterioles from the capsule of the gland. It was found difficult to evaluate the significance of fine capillaries in the fibrous

capsule surrounding the plate, which possibly formed a meagre vascular connexion between the median eminence and pars distalis. The simple stalk-sectioned rabbits, as also the normal rabbits, showed highly developed vascular connexions between the median eminence and the pars distalis, which appeared to form a major part of the blood supply to the gland. The changes in volume of the median eminence and pituitary gland after stalk section are described in detail in a previous paper (Campbell & Harris, 1957).

The level of thyroid activity in the stalk-sectioned rabbit

Two indices of the level of thyroid activity were used: (*a*) the uptake of radio-iodine by the thyroid gland 48 hr after the subcutaneous injection of ^{131}I, expressed, after correction for isotope decay, as a percentage of the administered dose, and (*b*) the rate of release of thyroidal radio-iodine expressed as the percentage of the gland ^{131}I content lost per day, which was determined directly from the graph of the release curve. Both these measurements were made on several occasions on each animal. The averages of the individual values for each animal were calculated to provide an uptake and a release index; in this way each animal contributed equally to the group mean.

TABLE 1. Thyroid activity as measured by the 48 hr uptake of ^{131}I and the percentage loss of thyroidal radioactivity per 24 hr in normal, 'simply stalk-sectioned', 'stalk-sectioned with plates' and hypophysectomized rabbits. In brackets, number of animals in groups

	Thyroid activity, 48 hr uptake	Loss %/day
Normal (24)	$22{\cdot}7 \pm 1{\cdot}4$	$18{\cdot}7 \pm 1{\cdot}5$
Stalk section, no plate (14)	$25{\cdot}0 \pm 2{\cdot}8$	$8{\cdot}7 \pm 1{\cdot}1$
Stalk section + plate (34)	$14{\cdot}4 \pm 1{\cdot}3$	$8{\cdot}7 \pm 0{\cdot}6$
Hypophysectomized (22)	$9{\cdot}1 \pm 0{\cdot}8$	$3{\cdot}8 \pm 0{\cdot}5$

Table 1 shows the uptake and release indices for the two groups of stalk-sectioned animals and the normal and hypophysectomized controls. It may be seen that the stalk-sectioned animals with plates show a reduced level of thyroid activity relative to the normal controls. The rate of release of the simple stalk-sectioned rabbits is reduced to the same extent, though the uptake of this group appeared normal. Both groups of stalk-sectioned rabbits show values significantly above the hypophysectomized group.

Since the volume of anterior pituitary tissue was found to be reduced to about 75% of normal in the stalk-sectioned animals, a possible relationship between the estimated volume of either the anterior or neural lobe of the pituitary gland and the uptake or release index in any one stalk-sectioned animal was examined graphically. No such correlation was observed. Similarly, there was no obvious relationship between these indices for thyroid activity and adrenal weight in the stalk-sectioned rabbits.

The experimental modification of thyroid activity in the stalk-sectioned rabbit

The rate of release of ^{131}I-labelled hormone from the thyroid gland is a measure of the rate of secretion of thyroid hormone, if the total gland content of hormone remains constant. Over the period of a single 'release curve', variations in hormone content can be neglected, and measurement of the rate of release of ^{131}I gives a direct index of the rate of hormone secretion, i.e. of thyroid activity (Brown-Grant, von Euler *et al.* 1954). The effects were investigated of thyroxine, emotional and physical stress, stilboestrol and, in a few instances, of cortisone. It appeared probable, on the basis of previous work (Brown-Grant, von Euler *et al.* 1954; Brown-Grant, Harris *et al.* 1954*a*, *b*; Brown-Grant, 1955) that these stimuli are effective in the normal rabbit by virtue of changes brought about in the rate of pituitary TSH secretion. Where the response to such stimuli is abolished by section of the pituitary stalk, the conclusion is reinforced that these stimuli act via the hypothalamus and pituitary gland. If, however, such stimuli are found to affect thyroid activity after effective stalk section, it is necessary to exclude a direct effect on the thyroid gland and possible changes in the sensitivity of the thyroid gland to TSH before it can be concluded that such stimuli act directly on the pituitary gland. In addition to experiments on stalk-sectioned rabbits, certain other experiments relevant to the considerations discussed above will be described. The groups of stalk-sectioned animals tested comprised 14 without plates and 34 with plates.

The effect of thyroxine. The dose of thyroxine used was 100 μg injected subcutaneously or intraperitoneally. No difference was seen in the response of normal animals injected by these two routes; the effect was a prompt (within 12 hr), complete (reduction to a rate of 1%/day or less) and reversible inhibition of the release of thyroidal ^{131}I. The duration of the response in 7 normal rabbits was 54–120 hr, with an average value of 83 ± 10 S.E.M. Thirty-four stalk-sectioned rabbits with plates were tested in 49 experiments; 40 positive responses were obtained, 4 equivocal responses and 5 negative ones. Every rabbit tested gave a clear positive response on at least one occasion; the characteristics of these responses differed from those obtained in normal animals only in their duration (Fig. 1). An average value of 100 hr $\pm$ 6 (S.E.M. of 40 experiments) was obtained, which is rather longer than in normal animals. Positive responses were obtained in animals with doubtful or absent portal vessel connexions. Three rabbits subjected to simple stalk section were similarly tested with thyroxine and all three responded.

It appears, therefore, that thyroxine can reduce the level of thyroid activity in stalk-sectioned rabbits as judged by the effect on the rate of release of thyroidal ^{131}I. To determine whether this effect is brought about by a direct action on the pituitary to reduce the level of TSH secretion, experiments were

performed on completely hypophysectomized animals. In 15 experiments on 8 animals the injection of 100 μg of thyroxine had no effect on the rate of release of thyroidal ^{131}I in 14 experiments (Fig. 1) and an equivocal effect in 1 experiment. In this dosage thyroxine does not further reduce the already low rate of release in the hypophysectomized rabbit. There is thus no evidence for a direct effect on the thyroid of the rabbit at this dose level.

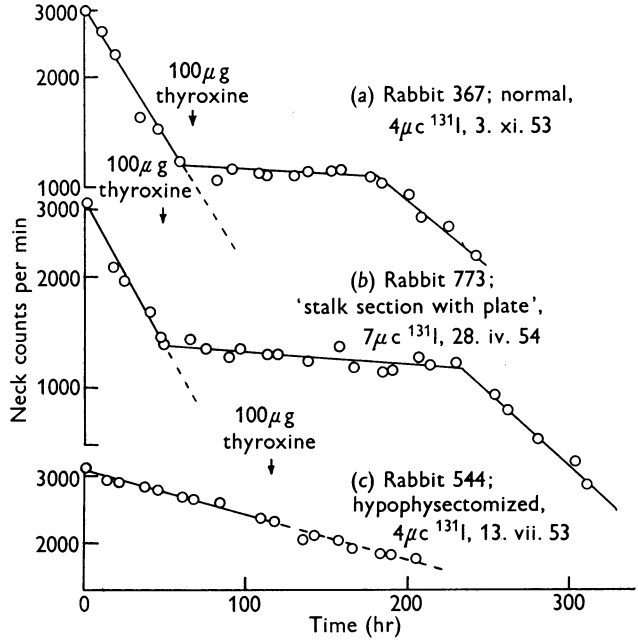

Fig. 1. The effect of thyroxine (100 μg subcutaneously) on the rate of release of thyroidal ^{131}I: (*a*) normal rabbit, (*b*) 'stalk-sectioned rabbit with plate', (*c*) hypophysectomized rabbit.

The effect of thyroxine on the response of the rabbit thyroid to exogenous TSH was investigated. Eight hypophysectomized animals, 1 stalk-sectioned and 1 normal animal were used in 11 experiments (2 experiments were performed on one of the hypophysectomized animals). The spontaneous rate of release of ^{131}I was very low in each case. The procedure was as follows. After an initial control period, a subcutaneous injection of TSH was given. There was a prompt discharge of ^{131}I from the thyroid, lasting 18–24 hr, followed by a return to the previous slow rate of release. The fall in ^{131}I content was determined from the graph and expressed as a percentage of the content at the time of the TSH injection. After a further control period a second injection of the same dose of TSH was given 6–24 hr after the administration of thyroxine, and the magnitude of the fall in ^{131}I content determined as before. Finally, a third control injection of TSH was given and the response determined in all experiments but one. One experiment is illustrated in Fig. 2 and details of all

experiments are given in Table 2. The response to TSH plus thyroxine should be compared with the average of the preceding and subsequent responses to TSH alone, as there may be a progressive decline in the response of the rabbit to repeated injections of TSH (Brown-Grant, Harris *et al.* 1954*b*). In order to compare the responses of the group as a whole, the initial response in each case

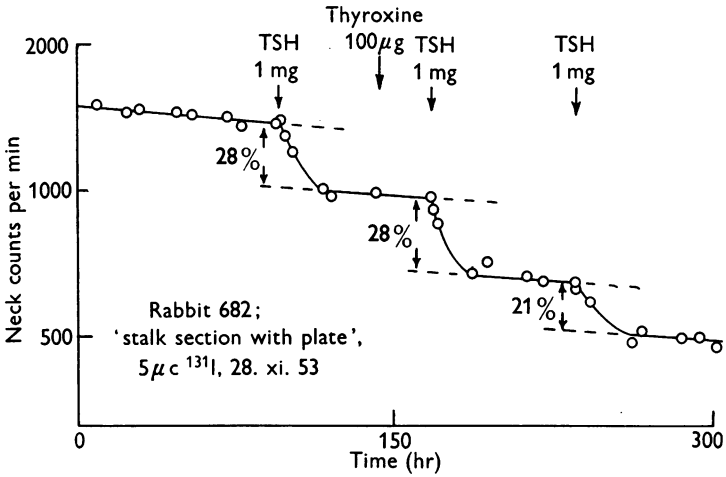

Fig. 2. The effect of TSH administration alone and of TSH administration after thyroxine on a rabbit after 'stalk section with plate'.

TABLE 2. The response of the thyroid to exogenous TSH with and without thyroxine

Rabbit no.	Dose of TSH (μg)	Dose of thyroxine (μg)	2nd injection TSH; interval after thyroxine (hr)	Response: gland's ^{131}I discharge (%) Control 1	After THY 2	Control 3
561N*	250	75, 25, 100	68, 54, 23	22·0	10·2	12·9
682SS	1000	100	24	28·0	28·0	21·0
701H	100	100,100	59, 11	11·7	0·0	10·7
738H	100	100	10	12·7	10·5	7·3
740H	100	100	6	19·0	14·5	13·0
803H	100	100	11	12·5	7·5	5·9
818H	100	100	6	9·5	3·3	6·3
818H	10,000	100	7	39·7	37·8	—
823H	5,000	100	8	45·3	33·2	13·8
834H	1,000	100	8	21·2	23·4	18·9
842H	1,000	100	8	26·1	31·0	20·3

* N, normal; H, hypophysectomized; SS, stalk-sectioned.

was taken as 100% and the second and third responses expressed as a percentage of this. The average response to TSH plus thyroxine was 72·5% ± 10·7 (S.E.M.); the average of the responses to the first and third doses of TSH was 83·1% ± 3·0 (S.E.M.). Thyroxine in this dosage has no significant effect on the response of the rabbit thyroid to exogenous TSH.

It appears, therefore, that thyroxine produces a decrease in the rate of release of ^{131}I in the rabbit after effective stalk section by a direct effect on the anterior pituitary to decrease the rate of TSH secretion.

The effect of laparotomy or haemorrhage. Laparotomy, haemorrhage, or the intraperitoneal injection of turpentine have been shown to produce a reversible inhibition of short duration of the release of ^{131}I in the rabbit. In a previous study 4 normal rabbits showed a complete inhibition of release for 29, 30, 44 and 46 hr following laparotomy, and 6 other animals showed inhibitions of 24–48 hr after haemorrhage (Brown-Grant, Harris *et al.* 1954*a*).

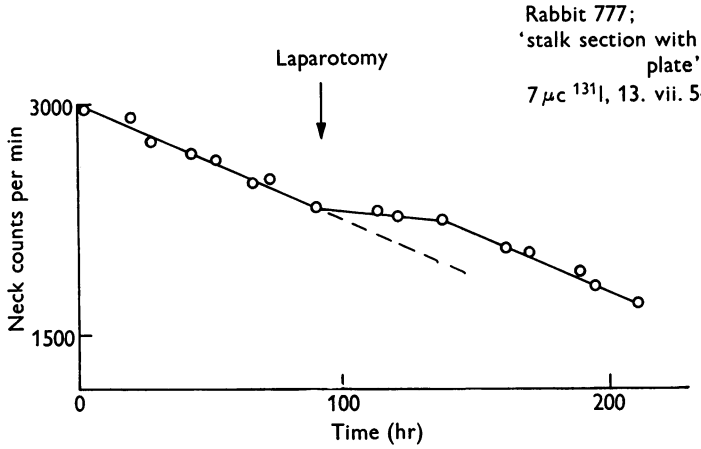

Fig. 3. The effect of laparotomy on the rate of release of thyroidal ^{131}I in a rabbit after 'stalk section with plate'.

In 15 rabbits (stalk-sectioned with plates) on which laparotomies were performed during a release curve, an inhibition similar in characteristics to that seen in normal rabbits was produced in 12 instances (Fig. 3); 2 responses were equivocal and 1 negative. Two rabbits were bled; 1 showed a normal response and 1 no response. The average duration of the 12 positive responses to laparotomy was 39 hr $\pm$ 4 S.E.M., and to haemorrhage 36 hr. Three laparotomies during release curves were performed on 3 completely hypophysectomized rabbits. Two showed no response and the other a response of doubtful significance. A direct effect of laparotomy on the thyroid does not seem likely.

The effect of laparotomy on the response to exogenous TSH was investigated in 5 normal rabbits; the animals were injected with 50 μg of thyroxine subcutaneously at 0 hr of the release curve and every 48 hr thereafter for the duration of the experiment. The suppression of endogenous TSH resulted in a flat release curve, and the effect of a standard dose of TSH (220 μg U.S.P. equivalent in 0·2 ml. saline subcutaneously) was determined as described in the previous section. The response to TSH alone and TSH administered 50–70 min after the end of a laparotomy was determined. The experimental

design and the responses obtained are shown in Table 3. The response to TSH plus laparotomy was 48·5% of the response to TSH alone.

The fact that laparotomy reduces the rate of release of thyroidal ^{131}I in stalk-sectioned rabbits, but has no effect on the rate of release of ^{131}I in the hypophysectomized rabbit, suggests a direct action on the anterior pituitary gland to reduce TSH secretion. However, laparotomy appears to interfere with the action of exogenous TSH administered 1 hr after the trauma, by reducing the response of the thyroid by half. While the present findings are inconclusive, it seems probable that in addition to this peripheral interference with the action of TSH, it is necessary to postulate a decrease in pituitary TSH secretion to explain the complete inhibition of the release of ^{131}I observed to follow laparotomy in normal and stalk-sectioned animals.

TABLE 3. The effect of laparotomy on the response to exogenous TSH (200 μg) in normal rabbits treated with 50 μg thyroxine subcutaneously every 48 hr. Responses are given in the order of performance. (L), TSH 1 hr after laparotomy; all TSH injections 24 hr after preceding injection of thyroxine.

Rabbit no.	Response: gland's ^{131}I discharged (%)			Response to TSH with laparotomy as % of response to TSH alone
	1	2	3	
1	13·1	4·2 (L)	18·5	32·0, 22·5
2	14·3	7·9 (L)	—	55·0
3	1·6 (L)	11·7	0·0 (L)	13·5, 0·0
4	11·0	8·4 (L)	12·4	76·0, 67·5
5	12·2 (L)	10·0	—	122·0

The effect of restraint. Forced immobilization was found to produce a temporary decrease in the rate of release of thyroidal ^{131}I in 21 out of 23 experiments on 19 normal rabbits (Brown-Grant, Harris *et al.* 1954*a*). The thyroid response to restraint was determined in 52 experiments on 33 rabbits (stalk-sectioned with plates). The results of 7 experiments were equivocal and have been discarded; the other results are given in Table 4. Twenty-two of the 33 rabbits failed to respond to the restraint stimulus. Similar tests were applied on 13 occasions to 13 rabbits subjected to simple stalk section. One result was equivocal and has been discarded; the other results, given in Table 4, stand in contrast to those obtained in stalk-sectioned animals with plates (see Fig. 4).

The effect of stilboestrol. Relatively large doses of natural or synthetic oestrogenic compounds have been shown to produce a reversible inhibition of the release of thyroidal ^{131}I in normal or gonadectomized male or female rabbits. Oestrogens do not interfere with the response of the thyroid gland to exogenous TSH (Brown-Grant, 1955).

Eight normal female rabbits were injected with 5 mg stilboestrol in 1 ml. oil subcutaneously. In 6 cases the release of ^{131}I was reduced to less than 1%/day and strikingly reduced in the other 2 for periods of 30–140 hr (average 76 ± 14 S.E.M.). The effect of similar injection of stilboestrol was tested in 28 experiments on 21 rabbits (stalk-sectioned with plates). Three responses

were equivocal and have been discarded, the other responses are shown in Table 4. Only 4 rabbits responded. Eleven rabbits that had been submitted to simple stalk section were injected with stilboestrol: 6 showed thyroid inhibition (Table 4). Out of the 10 stalk-sectioned animals that responded to stilboestrol, only 2 failed to respond to the restraint stimulus.

TABLE 4. Thyroid inhibitory responses of 'stalk-sectioned rabbits with plates' to laparotomy, restraint and injection of stilboestrol, and of 'simply stalk-sectioned' rabbits to restraint and stilboestrol.

	Stimulus	No. of rabbits	Total expts.	Thyroid response: Inhibition	Thyroid response: No effect
Stalk section + plate	Laparotomy	13	13	12	1
	Restraint	33	45	11	34
	Stilboestrol	20	25	4	21
Stalk section—no plate	Restraint	12	12	11	1
	Stilboestrol	11	12	6	6

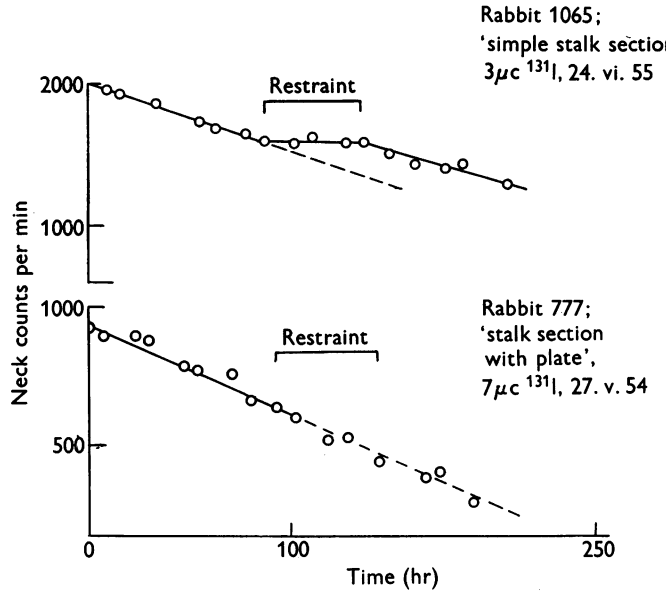

Fig. 4. The effect of restraint after 'simple stalk section', and after 'stalk section with plate'.

The effect of cortisone. Cortisone (5 or 10 mg twice daily subcutaneously) reduces the rate of release of thyroidal ^{131}I to less than 1%/day in normal or adrenalectomized animals. At this dose level there is no significant decrease in the thyroid response to exogenous TSH (Brown-Grant, Harris *et al.* 1954*b*). Cortisone (10 mg twice daily) was administered to 5 stalk-sectioned rabbits while the rate of release of ^{131}I was determined. In each case there was a prompt and complete temporary inhibition of the rate of release of ^{131}I, identical to that seen in normal animals.

DISCUSSION

Measurements of the uptake and the rate of release of ^{131}I by the thyroid provide indices of the level of thyroid activity in the rabbit (Brown-Grant, von Euler *et al.* 1954). The hypophysectomized rabbit shows a striking reduction in the 48 hr uptake, and in the rate of release, of ^{131}I. Rabbits subjected to stalk section with plates showed evidence of reduced thyroid activity as judged by both methods, but not to the level of the hypophysectomized animals. The smaller group of stalk-sectioned animals without plates showed a rate of release of thyroidal ^{131}I that was identical with the stalk-sectioned animals with plates, but no significant change from the normal in the 48 hr uptake. Since other factors than thyroid activity may influence these indices, it is not possible to use either as representing the absolute level of thyroid function. However, it would appear that the thyroid activity of effectively stalk-sectioned rabbits is intermediate between that of normal and hypophysectomized animals. These findings are in agreement with the majority of studies in which some criterion of thyroid activity has been measured after pituitary stalk section or pituitary transplantation. Uotila (1939) found the thyroid gland of pituitary stalk-sectioned rats to be histologically normal. However, lack of good control procedures for assessing hypophysial portal vessel regeneration leaves this finding open to doubt (see Harris, 1955*a*). Other workers (including Westman & Jacobsohn, 1938; Westman, Jacobsohn & Okkels, 1942; Schweizer & Long, 1950; Barrnett & Greep, 1951; Harris & Jacobsohn, 1952) have found that separation of the pituitary from the hypothalamus, by stalk section or transplantation, results in histological signs of thyroid atrophy. Greer, Scow & Grobstein (1953) observed that pituitary transplants in hypophysectomized mice did not maintain the thyroid weight above the level of hypophysectomized controls but found an uptake of ^{131}I per unit thyroid weight and a thyroid/serum iodide ratio of two-thirds the level of the intact controls. Donovan & Harris (1956) working on stalk-sectioned ferrets, found that effective stalk section resulted in a thyroidal uptake of ^{131}I of about one-third normal, though stalk section with portal vessel regeneration did not decrease the uptake below normal. von Euler & Holmgren (1956*b*) estimated the secretion rate of the thyroid gland in hypophysectomized rabbits bearing pituitary transplants and found a reduced rate as compared with normal, although they also point out that the rate of release of thyroidal ^{131}I is higher in the grafted than in the hypophysectomized control animals.

The effects of various procedures known to influence the rate of release of thyroidal ^{131}I were investigated in stalk-sectioned animals.

Injection of thyroxine. All the stalk-sectioned animals tested responded to injection of thyroxine with thyroid inhibition. This response was obtained in animals in which no apparent vascular connexions existed between the median

eminence and pars distalis, and in these and other stalk-sectioned rabbits the inhibition following a given dose of thyroxine seemed to be more prolonged than in normal animals. At this dose level thyroxine had no effect on the rate of release of thyroidal ^{131}I in hypophysectomized animals and did not significantly alter the thyroid response to TSH (see also von Euler & Holmgren, 1956*a*). This finding strongly suggests that a rise in the blood concentration of thyroid hormone affects TSH secretion by a direct action on the pars distalis of the pituitary. It is therefore confirmatory of similar conclusions drawn by von Euler & Holmgren (1956*a*, *b*), who made micro-injections of thyroxine directly into the pituitary gland and tuber cinereum of conscious rabbits, and who studied the ability of thyroxine to inhibit the thyroid gland in hypophysectomized rabbits bearing ocular pituitary transplants. The effect of reduced levels of thyroxine has been studied by other workers and there exists a number of observations indicating that the increased TSH secretion which occurs under these conditions is dependent upon the integrity of various areas of the hypothalamus and upon certain hypothalamo-hypophysial connexions. Brolin (1947) observed that the appearance of thyroidectomy cells in the pituitary was prevented by stalk section in the rat. Also in the rat Uotila (1940) and Barrnett & Greep (1951) found stalk section did not abolish thyroid hypertrophy after partial thyroidectomy or propylthiouracil feeding, respectively. However, it should be pointed out that some regeneration of the hypophysial portal vessels may have occurred in these experiments. Large anterior hypothalamic lesions in the rat (Greer, 1951, 1952; Bogdanove & Halmi, 1953) prevent the usual goitrogenic response to propylthiouracil feeding, as does transplantation of the pituitary to the anterior chamber of the eye in the mouse (Scow & Greer, 1953). Anterior hypothalamic lesions in the rat also prevent compensatory hypertrophy in partially thyroidectomized rats (S. Reichlin, unpublished). This evidence suggests that the hypothalamus is involved in the growth response of the thyroid gland to a lowered concentration of thyroid hormone in the blood. On the other hand, the increase in the thyroid:serum (T/S) ratio for ^{131}I which follows propylthiouracil feeding in normal animals is not dependent upon the hypothalamus or its connexions (Greer, 1951, 1952; Bogdanove & Halmi, 1953; Scow & Greer, 1953). The dissociation of the growth response, and the increase in T/S ratio seen in animals with hypothalamic lesions treated with propylthiouracil, have been attributed at least in part to a potentiation of the action of TSH by the goitrogen (Halmi & Spirtos, 1954; Vanderlaan & Caplan, 1954). That this is not the full explanation, however, is indicated by the fact that a similar dissociation has also been observed in rats with anterior hypothalamic lesions which have been subjected to partial thyroidectomy (S. Reichlin, unpublished). On the basis of the dissociation between the growth and TSH responses of the thyroid, Greer (1951) has postulated the existence of two thyroid-stimulating

pituitary hormones, one controlling the concentration of iodine by the gland and the other the growth of the gland. Greer's suggestion that the secretion of the hormone concerned with thyroid growth is controlled by the nervous system is substantiated by the recent studies mentioned above. His claim for a separate hormone concerned with the iodine-concentrating mechanism is less easy to evaluate. The early assumption that the sole action of goitrogenic drugs is via a change in the thyroid hormone concentration in the blood is almost certainly erroneous (see discussion by Halmi & Spirtos, 1954). Further, the T/S ratio, which is undoubtedly influenced by the level of TSH stimulation of the thyroid (Vanderlaan & Greer, 1950), may also be affected by many other factors (Halmi, Spirtos, Bogdanove & Lipner, 1953; Wollman & Scow, 1953, 1954*a*, *b*; Vanderlaan & Caplan, 1954). Until these aspects of thyroid function are better understood, the results obtained by goitrogen treatment and the use of T/S ratio in studies designed to elucidate the hypothalamic-pituitary control of thyroid function are difficult to interpret.

The question has been raised of an action of thyroxine on thyroid activity, independent of the pituitary gland. Cortell & Rawson (1944) claimed that in the rat thyroxine reduced the response of the hypophysectomized animal to exogenous TSH as judged by the acinar cell height and 24 hr ^{131}I pick-up. Rawson & Money (1949) showed that large doses of iodine had a similar effect. Overbeek, Fokkens, Querido, de Visser & Canninga (1953) also found a depression of ^{131}I uptake and a decrease in the response to exogenous TSH in the hypophysectomized rat fed on iodo-casein. Conversely, Chapman (1941), and more recently, Goldberg, Greep & Nay (1953) have described increased thyroid activity in the hypophysectomized rat maintained on a diet low in iodine. Eartly & Leblond (1954) found no change in the thyroid weight resulting from thyroxine injections in hypophysectomized rats. In the present work no direct effect of thyroxine on thyroid activity in the hypophysectomized rabbit was seen, and no significant alteration in the response to exogenous TSH was seen. It seems likely that under physiological conditions the major action of thyroxine on the thyroid is mediated via the anterior pituitary.

Laparotomy. This procedure resulted in an inhibition of the release of thyroidal ^{131}I in 12 out of 13 rabbits subjected to stalk section with plates. Since some evidence was obtained that laparotomy results in a reduced response to exogenous TSH in normal rabbits it cannot be stated that the whole inhibitory effect of such trauma is due to an action on the pituitary. However, the fact that the thyroid inhibition following laparotomy is complete and may persist for 40 hr suggests that a reduction in pituitary TSH secretion is the main factor underlying this response.

Restraint and stilboestrol. Since stalk-sectioned rabbits with plates were clearly capable of responding to injection of thyroxine with an inhibition of thyroid activity, and to laparotomy both with a decrease in thyroid activity

and an increase in adrenocortical activity (Fortier *et al.* 1957) the simple stalk-sectioned rabbits were not tested with these stimuli. However, the operation of stalk section with plate insertion largely abolished the inhibitory effect of emotional stress or injection of stilboestrol. Histological examination of the pituitary region in these animals revealed the presence of capillaries in the fibrous capsule around the plates, and since the significance of these vessels was unknown a group of rabbits was tested in which the pituitary stalk had been cut but no precautions taken to prevent portal vessel regeneration. Eleven out of 12 of these animals responded with thyroid inhibition following the restraint stimulus, and 6 out of 11 responded to injection of stilboestrol. Histological examination of the pituitary region in the rabbits in this group revealed the presence of marked vascular regeneration between the median eminence and pars distalis. There would seem to be a good correlation between the regeneration of portal vessels across the site of stalk section and the restoration of the responses to emotional stress and stilboestrol.

Cortisone. Administration of cortisone was found to depress thyroid function in the stalk-sectioned rabbits with plates as in the normal rabbit. It has previously been shown that at the dose of cortisone used there is no significant decrease in the thyroid response to exogenous TSH in the rabbit (Brown-Grant, Harris *et al.* 1954*b*). However, a direct effect of cortisone on the thyroid gland cannot at the present time be excluded, although the major action of this steroid on thyroid function would appear to be mediated by an effect on TSH secretion by the anterior pituitary gland. A similar conclusion has been reached by von Euler & Holmgren (1956*b*) after a study of the effect of cortisone on thyroid activity in hypophysectomized rabbits with intraocular pituitary transplants.

A comparison may be drawn between the type of stress stimuli which evoke adrenal cortical excitation (Fortier *et al.* 1957) and thyroid inhibition in the rabbits subjected to stalk section with plate. In both cases the effect of an emotional stress is greatly reduced, though the effect of stress involving physical trauma appears little changed. These findings are consistent with the hypothesis of Fortier (1951).

It is of interest to speculate why the anterior pituitary gland, divorced from hypothalamic control by effective stalk section, maintains a higher degree of thyroid activity than is seen in a normal animal subjected to an emotional stimulus. One possible explanation is that the hypothalamus can exert both an inhibitory and an excitatory effect on the anterior pituitary release of TSH. An excitatory effect has recently been demonstrated to follow electrical stimulation of the hypothalamus in unanaesthetized rabbits (Harris & Woods, 1956*a*, *b*).

Portal vessel regeneration across the site of pituitary stalk section (when no precautions are taken to prevent regeneration) occurs more slowly and is less

effective in restoring normal anterior lobe function in the rabbit than in the rat (Harris, 1950) or ferret (Donovan & Harris, 1956). It seems likely that the reason for this lies in the operative technique used in these three forms. In the rat and ferret the stalk was divided at about its mid point where it lies in the subarachnoid space. In the present study the pituitary stalk of the rabbit was divided at its point of emergence through the diaphragma sellae. Stalk section at this site may be followed by fibrosis across the small foramen in the diaphragm (see Pl. 1, Fortier *et al.* 1957) which may possibly form a partial blockade of the route regenerating vessels would traverse. There can be little doubt that in the rabbit anterior pituitary activity may be very largely restored after stalk section as shown by the few animals in which apparently full gonadotrophic function recurred, and by the return of adrenal cortical and thyroid responses to emotional stress, which were so markedly affected in the stalk-sectioned animals with plates.

From the present study it may be concluded that the pituitary divorced from anatomical connexion with the hypothalamus retains a residuum of autonomous activity with regard to secretion of thyrotrophic hormone, and that this activity can still be inhibited in an apparently normal way by a raised blood level of thyroid hormone and by the stress of physical trauma. The function of the hypothalamus in relation to TSH secretion seems to be to *maintain* the normal rate of secretion and to *modify* this rate of release in response to stimuli acting through the central nervous system.

SUMMARY

1. The thyroid activity of rabbits in which the pituitary stalk has been surgically interrupted has been studied by means of radio-iodine. In 14 animals the stalk was simply divided and in 34 it was cut and a plate of waxed-paper placed between the cut ends.

2. The 48-hr uptake of ^{131}I by the thyroid was not reduced in the simply stalk-sectioned rabbits, but was reduced to about two-thirds the normal value in the animals stalk-sectioned with plates. In both groups the rate of release of thyroidal radio-iodine was reduced to about one half the normal figure. All these values for stalk-sectioned animals were significantly above those found in hypophysectomized controls.

3. Section of the pituitary stalk leads to atrophy of the pars distalis to about 75% its normal volume. No correlation was found between the reduction in thyroid activity and pars distalis atrophy.

4. The release of thyroidal radio-iodine is inhibited in the normal rabbit by restraint, injection of stilboestrol, surgical trauma and injection of thyroxine. The operation of stalk section with plate largely abolished the thyroid inhibition to restraint or injection of stilboestrol, but had little effect on the

inhibition following laparotomy or injection of thyroxine. In the simply stalk-sectioned rabbits thyroid inhibition occurred in 11 out of 12 animals after restraint and in 6 out of 11 after stilboestrol.

5. Regeneration of the hypophysial portal vessels had occurred to a marked extent in the simply stalk-sectioned group. In the 'stalk-sectioned animals with plates' a fine capillary network in the fibrous capsule of the plate appeared to unite the vascular fields of the median eminence and pars distalis in the majority of animals.

6. It is concluded that the hypothalamus, through the mediation of the hypophysial portal vessels, maintains the activity of the thyroid gland and modifies this activity in response to stimuli acting through the nervous system. An increased concentration of thyroid hormone in the blood, and physical trauma, appear to affect the release of TSH by a direct action on the pituitary gland.

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