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VASOPRESSIN AND THYROID FUNCTION IN THE RABBIT

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Considerable evidence exists that the hypothalamus regulates the rate of secretion of thyrotrophic hormone (TSH) by a humoral mechanism involving the hypophysial portal vessels of the pituitary stalk. It seems likely that hypothalamic nerve fibres liberate some transmitter agent into the primary plexus of the portal vessels, situated in the median-eminence, and that this substance is carried to excite or inhibit the secretion of TSH by the cells of the anterior pituitary gland. In some preliminary studies it was found that dilute acetic acid extracts of rabbit and cattle median-eminence tissue, infused directly into the pituitary gland or intravenously, in rabbits resulted in increased thyroid activity (Garcia, Harris & Schindler, unpublished observations). In view of the fact that the median-eminence forms part of the neurohypophysis, it is very likely that the crude acidic extracts used in this study contained vasopressin.

Several investigators have reported that administration of vasopressin results in an increased secretion of TSH in the dog (Fraja & Martini, 1953) and the rabbit (Bottari, 1957), or an increased thyroid function in the rat (Ogawa, Arai & Shibata, 1956; Dubreuil & Martini, 1956), dog (Lipscomb, Hathaway & Gard, 1961) and man (Peterson, Cech & Johnson, 1960). On the other hand, various workers have administered vasopressin and failed to find any increased discharge of TSH (Rose, Nelson & Bradley, 1960) or any increase in thyroid activity (Arimura, Takagi & Ueno, 1956; Reichlin, 1957; Crosson, Falch & Reichlin, 1960; Moses, Lobovsky, Chodos & Lloyd, 1961) in the rat or man.

Further evidence relating vasopressin secretion to TSH discharge lies in the fact that hypothalamic lesions in the path of the supraoptico-hypophysial tract result in cessation of vasopressin secretion (Fisher, Ingram & Ranson, 1938) and also decreased thyroid activity (Greer &

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Erwin, 1954; D'Angelo & Traum, 1956). Conversely, electrical stimulation of the supraoptico-hypophysial tract region evokes increased secretion of vasopressin (Harris, 1947) and also increased thyroid function (Harris & Woods, 1958; D'Angelo, 1962).

In view of the above evidence the effect of administration of vasopressin on thyroid function in the rabbit was investigated by studying the acute release of ^{131}I-labelled compounds from the thyroid gland.

METHODS

Adult female Chinchilla rabbits (2·5–3·5 kg body weight) were used unless otherwise stated; 440 rabbits were used in all. The animals were isolated for at least a month before use, under standardized conditions of lighting and environmental temperature (28–29° C). They were fed on a pellet diet (M.R.C. diet 18, supplied by E. Dixon and Sons, Ware) and tap water *ad lib.*

Experimental procedure. The rabbits were injected subcutaneously with 50 μc carrier-free ^{131}I (iodide in dilute sodium thiosulphate solution) and a suitable period (6 days) was allowed for nearly all the inorganic ^{131}I to be excreted. At the end of this time the great majority of the radioactive iodine is present in organic combination in the thyroid gland and blood. On the 2 days preceding, and on the day of, the experiment each animal was injected intravenously with 25 μg L-thyroxine, in order to suppress the release of endogenous TSH. Throughout the experiments the animals were conscious and unrestrained. On the morning of the experiment the left marginal ear vein was cannulated with polyethylene tubing (0·5 mm internal diameter, 0·9 mm external diameter) to facilitate the withdrawal of blood samples, and an injection of heparin (500 u. 'Liquemin', Roche Products Ltd) was given intravenously. Any material to be tested was then infused over a 2 hr period into the right marginal ear vein, at a rate of 1·15 ml/hr, by means of a slow injection apparatus (Palmer and Co., Brixton). Blood samples (about 1·2 ml. each) were taken before the infusion began (zero time sample) and at hourly intervals after the start of the infusion. The radioactivity per millilitre of blood was determined in a well-type scintillation counter (Ekco, type N 550 A) with an automatic scaler and pulse analyser (Ekco, type 610 A). Observations were usually continued for 6 hr after the start of the infusion. The animals were killed with an overdose of pentobarbitone sodium (Nembutal, Abbott Laboratories), the thyroid glands immediately removed and placed in 10 ml. 2N-NaOH, and the state of the ovaries and genital tracts carefully observed. The following day the hydrolysate of the thyroid gland was diluted to an appropriate volume and the radioactivity of a 1 ml. portion determined as above. All measurements of radioactive samples were corrected for background and physical decay.

Estimation of plasma protein-bound ^{131}I ($PB^{131}I$) was performed by precipitating the proteins in 1 ml. of plasma with 3 ml. 10% trichloroacetic acid, centrifuging, washing the precipitate twice with 10% trichloroacetic acid and recentrifuging, dissolving the precipitate in 2N-NaOH to a volume of 1 ml. and counting the radioactivity. The PB ^{131}I was then expressed as a percentage of the total plasma radioactivity.

Thyroxine space. For the purpose of expressing the thyroid response in terms of the percentage of thyroid hormone released from the gland by any given stimulus, it is necessary to know the thyroxine space of the animal. This was determined in a series of animals, as follows. Rabbit plasma, containing radioactive thyroid hormone, was obtained by injecting normal rabbits with 100 μc of ^{131}I. Six days later the animals were heparinized, anaesthetized and bled. The blood was centrifuged, the plasma separated and the radioactivity of the protein-bound fraction (PB ^{131}I) of one portion of the plasma was measured. The

activity, measured in the scaling equipment described above, was approximately 6000–12,000 c/100 sec/ml. plasma. 10-ml. portions of this plasma were then injected intravenously into the experimental animals, and the plasma PB ^{131}I of these rabbits determined at 5 min, 30 min, 1 hr, 2 hr, 4½ hr, 12 hr, 22 hr, 28 hr after injection. After the rapid initial decline in blood concentration of PB ^{131}I, lasting about 3–5 hr, the rate of disappearance of PB ^{131}I from the plasma follows an exponential slope with a half-life of about 15 hr. Extrapolation of this slope to the ordinate gives a figure from which the thyroxine space of the animal may be calculated. In another series of rabbits the determination was repeated with radioactive synthetic L-thyroxine.

Assay of plasma TSH. Plasma TSH concentration was measured by Dr D. El Kabir, using the *in vitro* method based on the TSH-induced release of ^{131}I from guinea-pig thyroid tissue (El Kabir, 1960; Bottari, Donovan & El Kabir, 1963).

Chromatography. Thyroid glands were extracted for 14 hr at 2° C in Krebs–Ringer–bicarbonate solution and a portion of the extract hydrolysed with pancreatin for 8 hr at 37° C (Taurog, Potter & Chaikoff, 1955). Descending paper chromatography was carried out with a butanol:acetic acid:water (100:15:37·5) system. The proportions of the radio-iodinated components of the glands were determined by cutting the chromatographic strip in consecutive 1 cm sections, placing each section in a vial and counting the radioactivity in a well-type scintillation counter.

Plasma was hydrolysed overnight with papain and the radio-iodinated components determined by a method of column chromatography, using an anion-exchange resin, and eluting with increasing concentrations of acetic acid, as described by Pitt-Rivers & Sacks (1962).

Operative procedures. These were performed 4 days after giving the ^{131}I, and 2 days before the experiment. The animals were anaesthetized with pentobarbitone sodium, 30 mg/kg body wt. i.v., followed by ether, given by tracheal intubation as required.

Hypophysectomy was performed by the parapharyngeal method of Jacobsohn & Westman (1940). 'Sham hypophysectomy' consisted of similar operative procedures up to the stage of the exposure of the base of the cranium and the prevertebral region. In the sham operation care was taken not to injure the veins draining the sphenoidal venous sinus, since they form part of the venous paths draining the pituitary gland.

Thyroidectomy was performed through a mid-line skin incision. During the dissection of the gland care was taken to ligate the thyroid arteries before the thyroid vein, and to preserve the recurrent laryngeal nerves.

Cannulation of the common carotid artery was carried out in order to infuse directly into the thyroidal circulation. A polythene cannula (outside diameter 0·75 mm) was inserted into the common carotid artery so that the tip was pointing toward the heart and situated a few millimetres distal to the origin of the thyroid artery. Any small muscular branches between the ligature around the cannula and the point of penetration of the thyroid vessel into the gland were tied and cut. After completion of the operation the cannulae were flushed twice daily with saline (NaCl 0·9 g/100 ml. H_2O) containing heparin 100 i.u./ml.

Histology. After killing the hypophysectomized rabbit, and removing the thyroid gland, the head was perfused through each common carotid artery with 100 ml. 10 % formol-saline. After removing the skin, lower jaws and orbital contents, the heads were placed in 10 % formol. When fixation was complete, the skulls were decalcified in equal parts of 40 % formic acid and 7 % sodium formate solution. Blocks of tissue containing the hypothalamus, pituitary region and base of skull were dehydrated and embedded in low-viscosity nitrocellulose. Serial sections 100 μ thick were cut in the sagittal plane and stained with Weigert's iron-haematoxylin.

Drugs. The following hormone preparations and drugs have been used. Thyrotrophic hormone; International Standard Thyrotrophin (13·5 mg/i.u.) (National Institute for Medical Research, London); and, in some cases, Armour Bovine Thyrotrophin (lot No.

216-172-6), dissolved in saline immediately before use. Vasopressor hormone; highly purified lysine vasopressin (from Drs A. V. Schally and R. Guillemin, AVS-7-21, 242 u./mg, and AVS-13-76, No. 9, 274 u./mg) was used for most studies. It was made up in a concentration of 20 u./ml. 0·5 % (v/v) acetic acid and kept frozen in sealed vials in a volume sufficient for each experiment. This concentrated solution was diluted to a suitable volume with saline immediately before use. Other vasopressin preparations used included highly purified arginine vasopressin (from Professor H. Heller); synthetic lysine vasopressin (Sandoz Ltd, Basle; lot no. 006-02); and synthetic arginine vasopressin (from Dr V. du Vigneaud, batch No. AVS 4, 419089). In some experiments vasopressin was used which had been reduced with sodium thioglycollate (0·02 M, pH 7·5) for 2·5 hr at room temperature according to the procedure of Ames, Moore & van Dyke (1950). Synthetic oxytocic hormone (Syntocinon, Sandoz Ltd, Basle; OTS 68-69005); adrenocorticotrophic hormone (Corticotrophin, B.P., Crookes Laboratory Ltd, London, P 75–U 0503); adrenaline bitartrate (Burroughs Wellcome and Co, C 462 52712); L-noradrenaline bitartrate (Bayer Products Ltd, Nk 478); crystalline glucagon hydrochloride (Eli Lilly and Co., U.S.A., No. 666); insulin B.P. (Burroughs Wellcome and Co., No. 5402/A); 3′5′ cyclic adenosine monophosphate (L. Light and Co. Ltd., Colnbrook) were also used, and diluted with saline immediately before use. Radioactive L-thyroxine (Radiochemical Centre, Amersham, IB 14) was diluted in a methanol:ammonium-hydroxide:sodium-chloride solution before use.

RESULTS

The first parameter of thyroid function detectably increased after administration of, or secretion of, TSH is the release of thyroid hormone from the gland. Previous work (Söderberg, 1958; Campbell, George & Harris, 1960) demonstrated an increased radioactivity in the venous blood from thyroid glands 15–20 min after intravenous injection of TSH. The hormone in these glands had been previously labelled with ^{131}I. Such observations require the use of anaesthesia. In the present study the radioactivity of systemic venous blood has been followed in conscious rabbits after infusions of TSH, vasopressin and other substances. Pretreatment of the animals with thyroxine for 48 hr before the beginning of an experiment produced inactive glands that were more sensitive to the action of a given dose of TSH. To calculate the total radioactivity released from the thyroid gland by any infused substance it is necessary to know the increase in radioactivity per millilitre of systemic blood, and the thyroxine space of the animal. Following an experiment the animals were killed and the radioactivity of the thyroid gland measured. From these data it is possible to express the results in terms of the proportion of stored radioactivity released from the gland by any given stimulus.

Measurement of the thyroxine space

In a series of eight experiments on seven rabbits, with rabbit plasma endogenously labelled with radioactive thyroid hormone, the thyroxine space was found to be equivalent to $144 \pm 7{\cdot}6$ (S.E. of mean) ml. blood/kg body weight. This value has been used in calculating the results presented

in all the sections below. In a further series of five animals given synthetic radioactive L-thyroxine the thyroxine space was found to be equivalent to 161 ± 14·4 ml. blood/kg body wt. There is no significant difference between these results ($P > 0{\cdot}2$). The thyroxine space has been here expressed as equivalent to blood volume instead of plasma volume, since the responses described in the next two sections were routinely followed by measuring the increase in radioactivity per millilitre of blood.

TSH infusions

The period of infusion of thyrotrophic hormone, and other substances described below, has been in all cases for 2 hr. The material infused over this time was dissolved in saline to a total volume of 2·29 ml. (0·019 ml./min). This infusion time was chosen in order to allow comparison with intrapituitary infusions of various substances (the results of which will be reported elsewhere), and because it was thought that a 2 hr infusion period approximated more closely to the normal rate of release of hormone from the pituitary gland than does a single injection.

As described above, thyroxine was administered to all animals, starting 48 hr before the beginning of any experiment. It is known (Brown-Grant, 1955) that a dose (I.V.) of 15–20 μg or more of thyroxine to an adult female rabbit maintained in an environment of 28° C results in a rapid and complete inhibition of thyroid activity. On these grounds 25 μg of L-thyroxine was administered by a daily intravenous injection to each experimental animal, so that the secretory activity of the thyroid at the time of the experiment would be suppressed to a similar extent in all cases. Further, a preliminary series of experiments showed that TSH administered at four different dose levels (1, 10, 100, 1000 m-u.) gave a three- to five-fold greater response in animals pre-treated with thyroxine.

The thyroidal response to the 2 hr infusion of TSH, or any other substance, has been expressed in the following way. An increased blood radioactivity was detectable 30 min after the start of TSH infusion, and generally reached a peak value at 3–5 hr after the beginning of the infusion (with large doses of TSH the peak value occurred later) (Fig. 1). Hourly samples were taken for at least 2 hr after the peak value had been reached. In calculating the results the difference in radioactivity between the zero time sample (before infusion) and the peak sample was used (*B–A*, Fig. 2) and considered to be the increased radioactivity per 1 ml. blood released from the thyroid gland by the infused material. Reference to Fig. 2 will indicate minor errors in taking these values. First, the decay rate of thyroxine (half-life about 15 hr in the rabbit, as determined from the experiments involving injection of radioactive thyroxine described above) in the circulation would indicate that a decrease in the radioactivity of the

zero time sample would have occurred by the time the peak value of the response had been reached (*A–C*, in Fig. 2) if the thyroid gland had been unstimulated. Secondly, it is likely that at the time of the peak sample the released radioactivity has not fully equilibrated throughout the entire

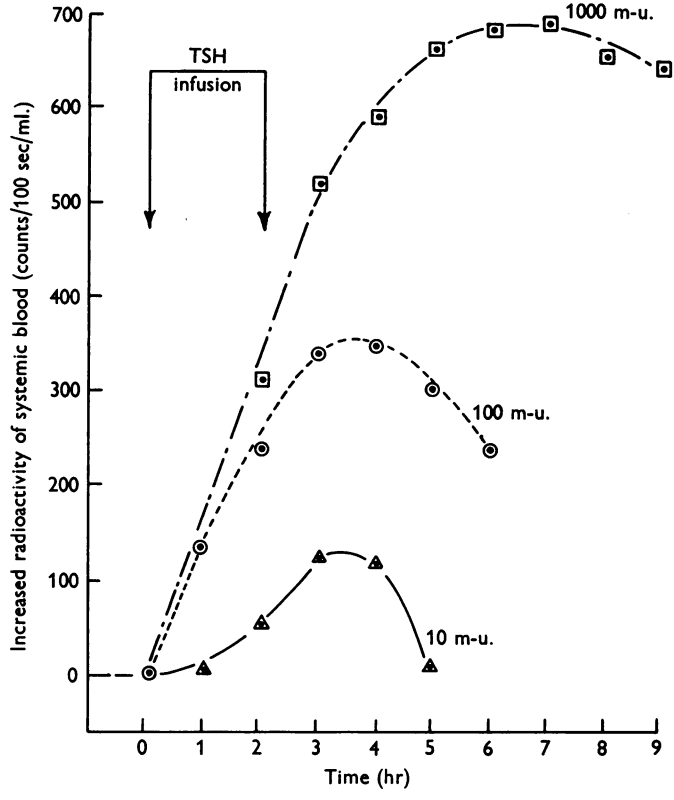

Fig. 1. Increased radioactivity per ml. systemic blood of rabbits following 2 hr intravenous infusions of International Standard TSH at rates of 0·0833, 0·833 and 8·33 m-u./min (total doses of 10, 100 and 1000 m-u., respectively). The blood radioactivity of the sample taken before the start of the infusion (zero time sample) is shown as zero. The actual count in this sample is subtracted from all subsequent counts; thus the graph is plotted as increase over the zero time sample in blood radioactivity. Note the rise in blood radioactivity reaching a peak value generally at 3–5 hr after the start of the infusion (but later in the highest doses) and then declining.

thyroxine space, as indicated by the transient and relatively rapid fall in blood radioactivity which follows the peak value (*B–D*, in Fig. 2). The best estimate of the released thyroidal radioactivity would probably be obtained by measurement of the value *F–E* in Fig. 2. However, the time

taken to establish the slope of the curve over the period *D–F* renders this measurement impractical, and it is thought that good estimates of the value are obtained by taking the difference between the zero-time and peak samples. Further, to use these latter values allows a linear log. dose-response curve to be plotted following infusion of TSH in doses of 10–1000 m-u. (see below, p. 494). The method of calculation of the response of the

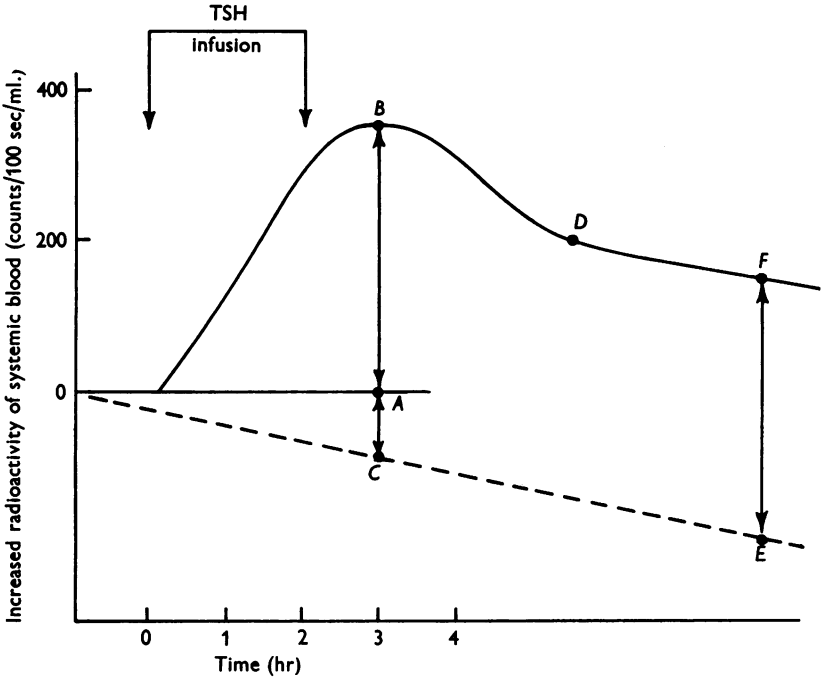

Fig. 2. To show the theory upon which calculations of thyroid responses are based; ordinate and abscissa as in Fig. 1. Secretory activity of the thyroid gland had been blocked by thyroxine injections, therefore blood radioactivity would be falling at an exponential rate depending on the half-life of circulating ^{131}I-labelled compounds (interrupted line). Following stimulation of the thyroid gland (as by an infusion of TSH), blood radioactivity will increase to a peak value (*B*) and then decline. Calculations are based on the increase in radioactivity (per ml. blood) of the peak sample over the zero time sample (*B–A*). An error in this calculation would be due to a decrease in blood radioactivity if the thyroid had not been stimulated (*A–C*). During thyroid stimulation radioactive thyroid compounds are released from the gland and diffuse through the whole thyroxine space of the body. After some time equilibrium will be reached and blood radioactivity will decrease exponentially (*D–F*) at the same rate and parallel to the slope at which it was decreasing before the infusion, but at a higher level. The ideal measurement would be the difference in blood radioactivity before and after the infusion when equilibrium had been reached (*F–E*); however, the time required for establishing these slopes accurately makes it impractical.

thyroid gland, in terms of the percentage of stored radioactivity released from the gland by any infusion, is as follows:

$$R = \frac{W \times X \times Y}{Z} \times 100,$$

where

R = percentage of stored thyroidal radioactivity released by the 2 hr infusion,
W = increased radioactivity per ml. blood (B–A, Fig. 2),
X = thyroxine space, expressed as ml. blood/kg body wt (viz. 144 ml./kg body wt),
Y = body wt (kg),
Z = total radioactivity in thyroid gland.

The total thyroidal responses to 2 hr intravenous infusions of TSH have been studied at three different dose levels (10, 100 and 1000 m-u. total

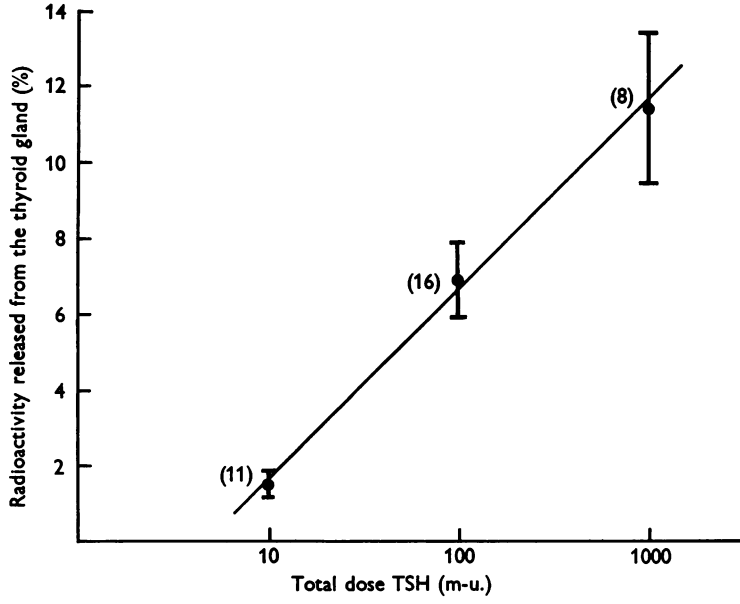

Fig. 3. Shows the log. dose-response curve to International Standard TSH. The thyroid response is expressed as the percentage of radioactivity released from the rabbit thyroid gland. All animals were pre-treated with thytoxine for 48 hr before the experiment to suppress endogenous thyroid activity. The total doses of TSH, 10, 100 and 1000 m-u. (0·0833, 0·833 and 8·33 m-u./min respectively) on a log. scale. The points shown are mean responses ± S.E. mean, and the number of animals in each group is shown in parenthesis. The responses are 1·5 ± 0·34, 6·9 ± 1·04 and 11·4 ± 2·06 % release for 10, 100 and 1000 m-u. TSH respectively. It may be noted that the thyroid response to various doses of TSH was linear over the range 10–1000 m-u. when plotted against log. TSH dose.

doses; 0·0833, 0·833 and 8·33 m-u./min). The log. dose-response curve is given in Fig. 3.

It may be said that the thyroid response to the various doses of TSH was linear over the range 10–1000 m-u. TSH when plotted against the logarithm of the dose of TSH, and that differences in the responses at each dose are significant statistically ($P < 0{\cdot}001$). Control experiments were performed in an identical manner in six normal rabbits, a 2 hr intravenous infusion of saline being given. Figure 4 illustrates one of these experiments. No increase in radioactivty in the blood occurred during or after these infusions.

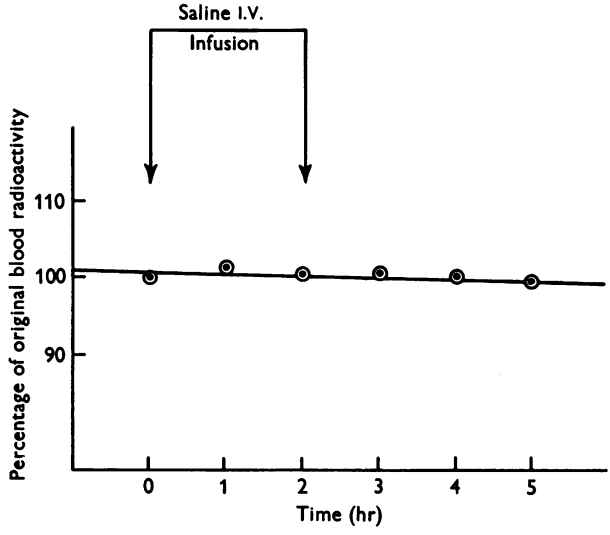

Fig. 4. Shows blood radioactivity in a normal rabbit blocked with thyroxine following a 2 hr control infusion of saline (the diluent for all substances used in these experiments). The radioactivity of the zero time sample is shown as 100 % and all subsequent values are shown as a percentage of this value. Note that there is no increase in blood radioactivity due to the infusion.

Vasopressin infusions

The initial finding that injection of Pitressin results in an increased discharge of radioactivity from the thyroid gland came from observations on anaesthetized rabbits, in which measurements of the radioactivity in thyroid vein and arterial blood were being made by the technique described by Campbell *et al.* (1960). In these preliminary experiments the responses varied from animal to animal and might have been due to TSH contamination of the posterior lobe extract used. For these reasons the experiments to be described below were conducted on conscious rabbits

and only highly purified or synthetic preparations of vasopressor hormone were used.

The majority of the experiments were conducted with a highly purified preparation of lysine vasopressin (242 or 274 u./mg). In the early stages of the investigation this material was assayed for TSH by Fortier & Schindler (1963) using an *in vitro* method and found to possess less than 0·02 m-u. TSH/i.u. of vasopressor activity. Further tests for TSH have

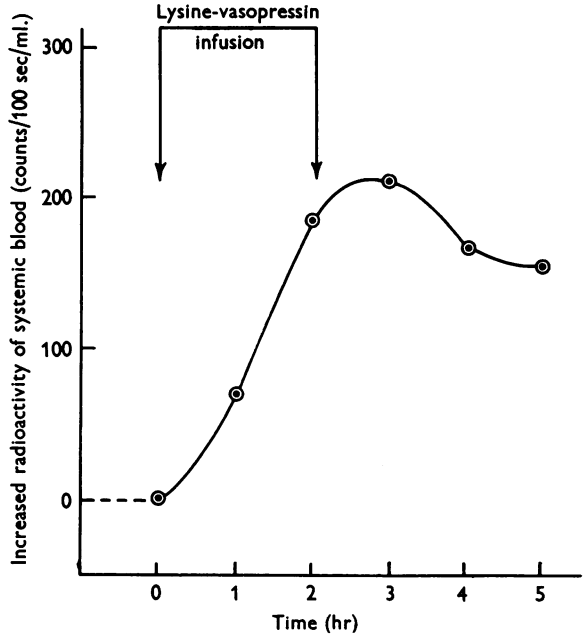

Fig. 5. Shows the increased radioactivity per ml. systemic blood of rabbits following 2 hr intravenous infusions of highly purified lysine vasopressin. Note the similarity in thyroid response to that seen following TSH infusions (Fig. 1).

since been made (Schindler & Levine, unpublished) using a modification of the McKenzie method (McKenzie, 1958) on immature rats. This modified assay can detect 0·25 m-u. TSH. No TSH-like activity was found in 125 vasopressor m-u. of this purified vasopressor preparation. It should be stated, however, that although most experiments have involved the use of this highly purified lysine vasopressin preparation, confirmatory experiments have been conducted with synthetic lysine vasopressin, and highly purified and synthetic arginine vasopressin.

The response of the rabbit thyroid gland to infusions of the different highly purified and synthetic vasopressor preparations have been studied in sixty-one normal female rabbits. The responses were similar in all

respects to those following infusion of TSH (see Figs. 1 and 5). The latent period, the time of the peak and the duration of the responses were indistinguishable from those seen following TSH administration. A 2 hr infusion of vasopressin, at rates of 2·8–22·4 m-u. pressor activity/min, elicited a discharge of thyroidal radioactivity which is comparable in

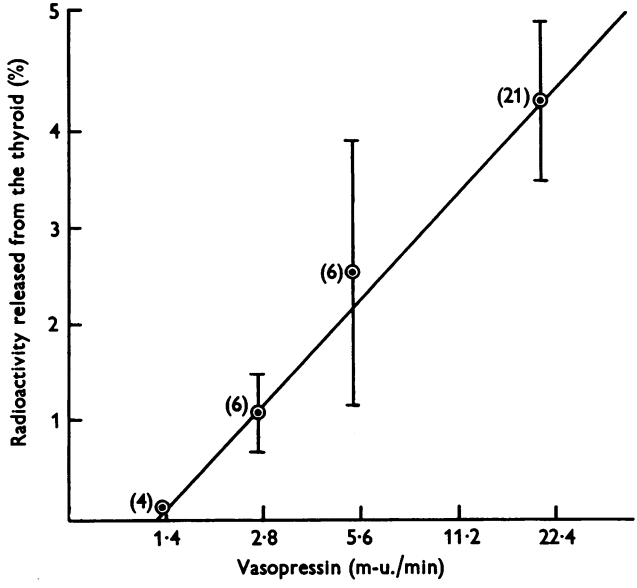

Fig. 6. The dose response-curve to various doses (log. scale) of highly purified lysine-vasopressin infused over a 2 hr period. The thyroid response is expressed as the percentage of radioactivity released from the rabbit thyroid gland. All animals were pre-treated with thyroxine for 48 hr before the experiment to suppress endogenous thyroid activity. The points represent the mean response ± S.E. mean and the number of animals in each group is shown in parenthesis. The slope of the line is linear and significant ($P = < 0{\cdot}05$). The responses to 1·4, 2·8, 5·6 and 22·4 m-u./min are respectively 0·14; 1·11 ± 0·40; 2·60 ± 1·44; and 4·38 ± 0·86 % discharge.

magnitude with that seen after similar infusions of 0·0833–0·833 m-u. TSH/min. At this rate of administration of vasopressin no pressor response was seen in two rabbits anaesthetized with pentobarbitone sodium and ether, or in one rabbit allowed to recover from ether anaesthesia, with a cannula in a femoral artery connected to a mercury manometer.

The thyroid responses observed to follow vasopressin at different dose levels were variable. The variability within each group is great (Fig. 6). However, an analysis of variance to test linear regression of the log. dose-response curve showed that the slope was linear and significant ($P < 0{\cdot}05$). The threshold dose of vasopressin under the present conditions appears

to be about 1·4 m-u./min, since only one out of four rabbits responded when infused at this rate.

To see whether a sex difference is apparent in the response, four male Chinchilla rabbits were infused for 2 hr at a dose rate of 22·4 m-u. vasopressin/min. These animals showed a percentage discharge of thyroid hormone of $4{\cdot}16 \pm 1{\cdot}27$. These responses are indistinguishable from those seen in female rabbits.

The nature of the radioactive substance released into the blood stream by vasopressin was investigated by studies of the protein-binding of the material and by chromatography. In seven experiments it was found that the percentage of blood radioactivity that was protein-bound in the zero time sample was $86{\cdot}13 \pm 1{\cdot}83\,\%$, and following vasopressin infusion the percentage in the peak sample was $83{\cdot}59 \pm 1{\cdot}37\,\%$. These values are not significantly different ($P > 0{\cdot}1$). Similar results were also observed in five cases after infusions of TSH [$PB^{131}I$ in zero time samples $91{\cdot}02 \pm 1{\cdot}93\,\%$, and in peak samples $85{\cdot}78 \pm 1{\cdot}40\,\%$; again no significant difference, $P > 0{\cdot}1$]. These findings indicate that both vasopressin and TSH excite the release of inorganic iodide, as well as organic compounds, from the thyroid gland (see below, p. 500). To investigate the nature of the material released and the composition of the organic fraction in more detail, the radioactive compounds present in the blood plasma of five animals before and after infusion of vasopressin were studied by chromatographic methods. After hydrolysis the plasma was submitted to column chromatography on an anion-exchange resin and eluted with 3 ml. portions of increasing concentrations of acetic acid (Pitt-Rivers & Sacks, 1962). This method as used separated the iodotyrosines, iodothyronines and inorganic iodide. Four to six days after administration of 200 μc ^{131}I the radioactive components in the blood plasma of the rabbit were found to be the iodothyronines $67{\cdot}8 \pm 6{\cdot}45\,\%$ (thyroxine and triiodothyronine, which are not separated by this method), inorganic iodide $21{\cdot}0 \pm 5{\cdot}61\,\%$, and the iodotyrosines $11{\cdot}2 \pm 2{\cdot}53\,\%$ (mono- and di-iodotyrosines were eluted together) (see Fig. 7). Following infusion of vasopressin, analysis of the peak blood samples showed that the plasma levels of the iodotyrosines were unchanged, while that of the inorganic iodide and the iodothyronines increased. $24{\cdot}8 \pm 3{\cdot}1\,\%$ of the total increase was due to a rise in plasma concentration of inorganic iodide, and $75{\cdot}2 \pm 3{\cdot}1\,\%$ of the total increase due to a rise in the iodothyronines (Fig. 8*b*). Similar results were seen, in the one animal studied, after infusion of 10 m-u. TSH.

In order to compare the iodinated compounds which increased in the plasma after vasopressin or TSH infusions with the proportion of such compounds present in the thyroid gland, each of the thyroid glands of 12 rabbits, 6 days after a tracer dose of ^{131}I, were extracted, hydrolysed and

submitted to paper chromatography. The radio-iodinated components were found to be distributed as follows: 16·71 ± 2·07 % inorganic iodide; 22·88 ± 1·29 % monoiodotyrosine; 29·05 ± 2·26 % diiodotyrosine; 19·66 ±

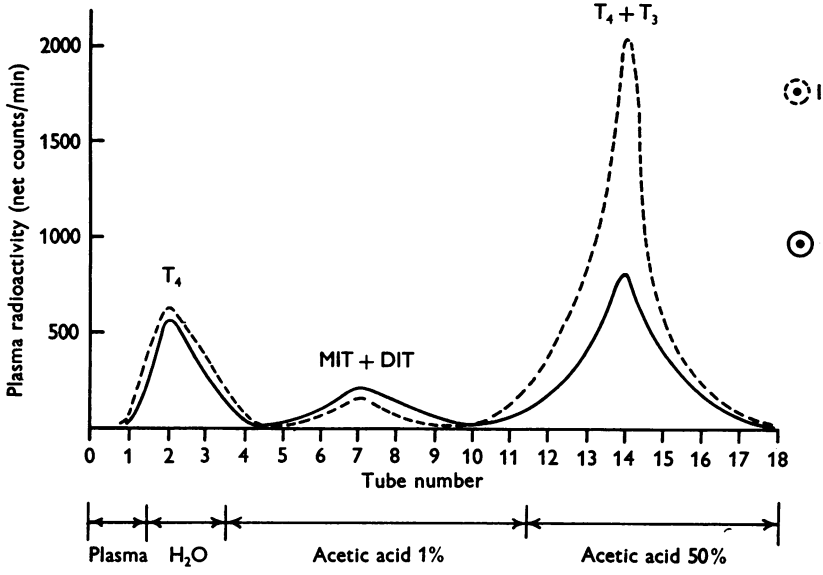

Fig. 7. The radioactive compounds present in the plasma of a rabbit taken before (——) and after (– – –) a 2 hr infusion of lysine-vasopressin. The plasma was hydrolysed with papain and separations were made by column chromatography on an anion-exchange resin and eluting with increasing concentrations of acetic acid. The plasma radioactivity is plotted against the serial number of the elution tube. Each tube contained 3 ml. of the eluate shown below the graph. The first peak to come off is non-hydrolysed protein (shown as T_4), which probably represents thyroxine still bound to plasma proteins. The method as originally described by Pitt-Rivers & Sacks (1962) can separate the iodotyrosines; however, in this case they were eluted together with 1 % acetic acid shown as MIT+DIT. The iodothyronines are then eluted with 50 % acetic acid (T_4+T_3). Inorganic iodide remains on the resin, which can then be placed quantitatively in a tube for determining the radioactivity (shown as I^-). It can be seen that the plasma levels of the iodotyrosines remained the same, while those of iodothyronines and inorganic iodide increased. The amounts of inorganic iodide and iodotyrosines present in the plasma of this animal were considerably higher than one would normally expect. This is probably due to a high dose of ^{131}I (200 μc) and a short time (4 days) between injection of the ^{131}I and the experiment. Endogenous thyroid activity was suppressed 48 hr before the experiment with exogenous thyroxine injections.

1·38 % thyroxine (including triiodothyronine which does not separate from thyroxine in the system used); and 11·74 ± 3·07 % of an incompletely hydrolysed fraction. In samples of unhydrolysed thyroid tissue from these animals the inorganic iodide was found to be 0·61 ± 0·06 %. Thus about

16 % of the inorganic iodide in the hydrolysed fractions was due to de-iodination. Figure 8*a* depicts graphically the proportion of the iodinated compounds present in the thyroid gland. Comparison of Fig. 8*a* and 8*b* shows that the proportions with which the different iodinated compounds contribute to the increase in blood radioactivity is different from the proportions in which they are found in the thyroid gland.

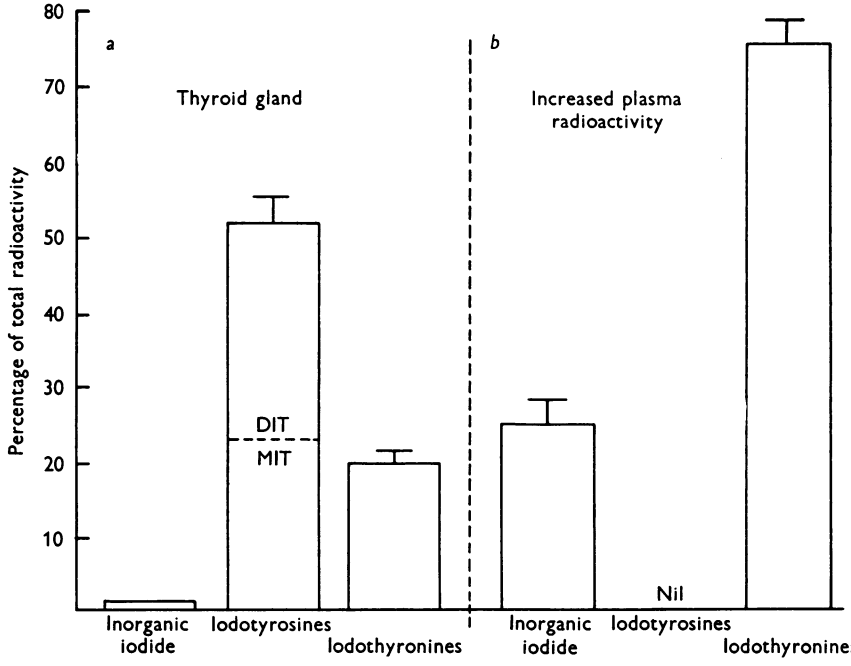

Fig. 8. The radio-iodinated compounds of the rabbit thyroid gland 6 days after a tracer dose of ^{131}I (*a*) and the iodinated compounds which increased in the plasma after vasopressin infusions (*b*). The bars in *a* represent the mean ±S.E. mean of 12 rabbits. The glands were extracted with Krebs–Ringer–bicarbonate and portions of the extract hydrolysed with pancreatin before being chromatographed on paper in a butanol:acetic–acid:water system. The proportions of the various compounds were as follows: 22·88 ± 1·29 % mono-iodotyrosine (MIT); 29·05 ± 2·26 % di-iodotyrosine (DIT); 19·66 ± 1·38 % thyroxine (including triiodothyronine and shown in the graph as iodothyronines). MIT and DIT are grouped together in the graph as iodotyrosines. The inorganic iodide shown represents 0·61 ± 0·06 % of the total radioactivity. This value was taken from chromatograms of non-hydrolysed aliquots of each of the glands. The radioactivity represented by an incompletely hydrolysed fraction and inorganic iodide which resulted from de-iodination of organic compounds during the hydrolysis procedure are not shown. As can be seen in *b*, the increase in plasma radioactivity following vasopressin infusions is accounted for by the iodothyronines and inorganic iodide, representing respectively 75·2 ± 3·1 % and 24·8 ± 3·1 % of the total increase. Although the iodotyrosines make up the largest proportion of the gland compounds, they are not released into the plasma following the vasopressin infusions.

Control infusions

Experiments have been conducted in which control substances—saline, thioglycollate inactivated vasopressin, oxytocin, insulin and noradrenaline —have been infused intravenously over a 2 hr period.

Saline. As described above, infusion of saline (the diluent for all infusions) in six rabbits evoked no thyroidal response.

Thioglycollate-inactivated vasopressin. Three rabbits have been infused with thioglycollate-inactivated lysine vasopressin at a rate equivalent to '22·4 m-u./min'. In two cases no response, and in one case a slight response (0·19 % discharge) were observed.

Oxytocin. Two rabbits were infused with synthetic oxytocin at a rate of 5·6 m-u./min. No response was seen in one case and a questionable response in the other. Two further rabbits were infused at 22·4 m-u./min and neither responded.

Insulin. Two rabbits were infused with insulin at rates of 2·1 and 4·2 m-u./min respectively. Neither showed a thyroid response.

Noradrenaline. Two animals received noradrenaline bitartrate at a rate of 2μg/min. Neither showed a thyroid response.

Analysis of the mechanism of action of vasopressin

The increased blood level of thyroid hormone which follows infusions of vasopressin could be brought about in several ways: (1) by an action of vasopressin on the central nervous system or pituitary gland to excite an increased discharge of TSH and so an increased thyroid secretion; (2) by an action on peripheral tissues, to increase the reabsorption from the intestine or tissue fluids and to reduce the rate of destruction and excretion of thyroid hormone; and (3) by a direct action on the thyroid gland to increase thyroid secretion.

Possible action via TSH secretion

Hypophysectomy. To see whether vasopressin infusions exert their action by increasing the pituitary secretion of TSH, the thyroid responses of eleven hypophysectomized rabbits have been compared with eleven sham-operated animals. The operation was performed 48 hr before the experiment. The hypophysectomized animals did not receive the routine administration of thyroxine during this 48 hr period, since it was found that thyroxine increased the post-operative mortality greatly, and since the purpose of thyroxine administration was to inhibit the secretion of TSH.

Both the hypophysectomized and sham-operated groups showed an

increased thyroidal activity following intravenous infusion of lysine-vasopressin at a rate of 22·4 m-u./min (Fig. 9). The hypophysectomized rabbits gave a response of $1{\cdot}92 \pm 0{\cdot}51$ % discharge of thyroid hormone, and the sham-operated $2{\cdot}36 \pm 0{\cdot}38$ % (Fig. 10*a*). These responses are not significantly different ($P = 0{\cdot}5$). Two further hypophysectomized rabbits

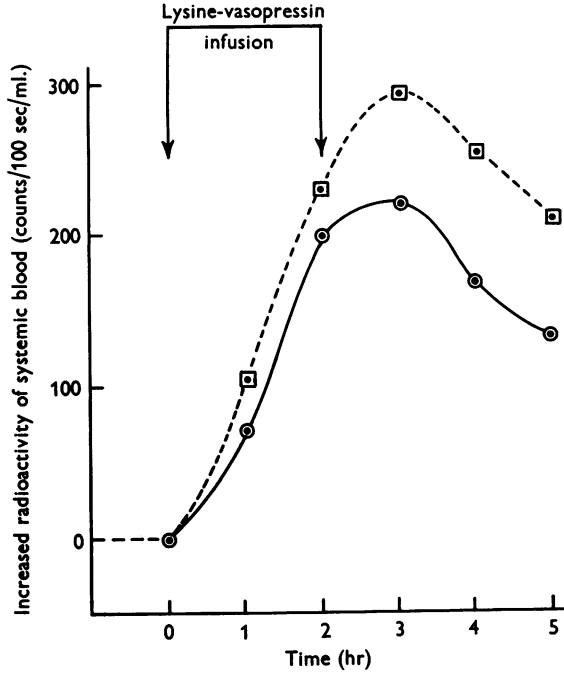

Fig. 9. Increased radioactivity per ml. systemic blood of a hypophysectomized (– – –) and sham-operated (——) rabbit following a 2 hr intravenous infusion of lysine-vasopressin at a rate of 22·4 m-u./min; ordinate and abscissa as in Fig. 1. The thyroid response is to be compared with that to the same dose of vasopressin given to a normal rabbit (Fig. 5). All operations were performed 48 hr before the experiment and the animals were alert and unrestrained throughout. The sham-operated animal's thyroid activity was suppressed for 48 hr before the experiment by injections of exogenous thyroxine. The hypophysectomized animal received no thyroxine, but its thyroid activity was suppressed by removal of the pituitary. It may be noted that vasopressin stimulates the thyroid gland to secrete even in the absence of the pituitary gland.

received infusions of synthetic arginine vasopressin at a rate of 4·17 m-u./min and showed comparable thyroid responses.

Examination of serial sections through the sellae turcicae of the hypophysectomized animals revealed a complete absence of pituitary cells in two rabbits, minute fragments of a few cells only in the case of five, and small to appreciable amounts in the others. It is likely that the remaining

fragments were non-functional, since they were thrombosed and embedded in blood clot. Retrograde thrombosis was observed in the blood vessels of the median eminence. The thyroid response of these animals to vasopressin showed no relationship with the completeness of hypophysectomy.

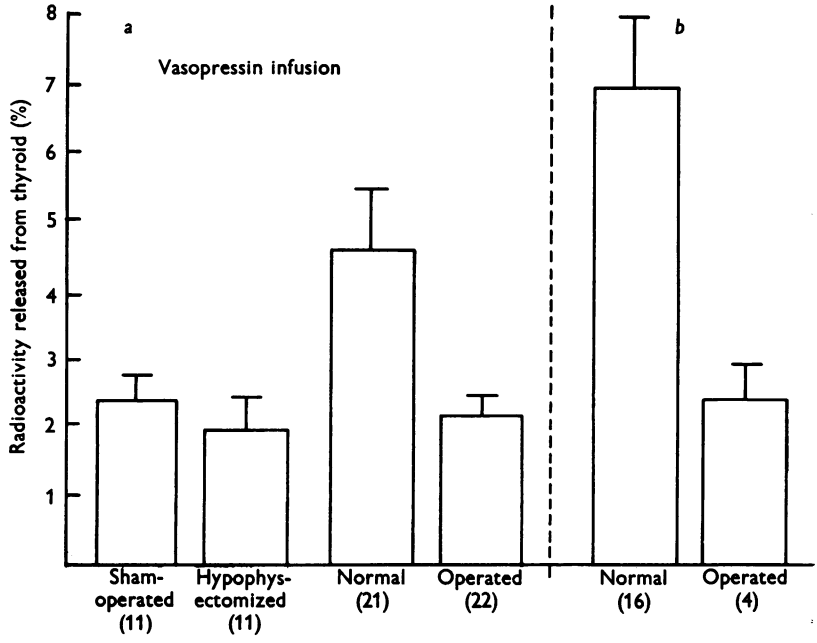

Fig. 10. Thyroid responses (mean+S.E.) to a standard 2 hr infusion of lysine-vasopressin at a rate of 22·4 m-u./min (*a*) or of TSH at a rate of 0·833 m-u./min (*b*) in groups of normal or operated rabbits. The thyroid responses are shown as the percentage of thyroidal radioactivity released by the infusion; the number of animals in each group is shown in parenthesis. It may be noted that there is no significant difference in the thyroid responses in sham-operated as compared to hypophysectomized rabbits ($2{\cdot}36 \pm 0{\cdot}38$ % and $1{\cdot}92 \pm 0{\cdot}51$ % respectively, $P = 0{\cdot}5$). All operations were performed 48 hr before the experiment. The thyroid responses in the operated animals (11 hypox+11 sham) when grouped ($2{\cdot}14 \pm 0{\cdot}31$ %) are significantly lower than those of normal rabbits ($4{\cdot}38 \pm 0{\cdot}86$ %, $P < 0{\cdot}02$) infused with the same dose of vasopressin. Similarly thyroid responses to TSH are lower in sham-operated rabbits ($2{\cdot}40 \pm 0{\cdot}51$ %) than in normal rabbits ($6{\cdot}92 \pm 1{\cdot}04$ %).

TSH assays. The blood concentration of TSH has been assayed in eight rabbits during infusion of vasopressin. Blood samples were taken before the start of the infusion and at 15 min, 1 hr and 2 hr after the start. These blood samples were immediately centrifuged, the plasma drawn off and frozen for subsequent assay. In seven of these animals no change was observed in the plasma TSH concentration. In the eighth animal an increase was seen in the 15 min blood sample.

Possible action on peripheral tissues

To see whether the increase in blood concentration of thyroid hormone following vasopressin infusion was due to some action on peripheral tissues, such as an increased rate of reabsorption of thyroxine from the intestine or tissue fluids, combined perhaps with a decreased rate of degradation or excretion of thyroxine, a study was made on three rabbits

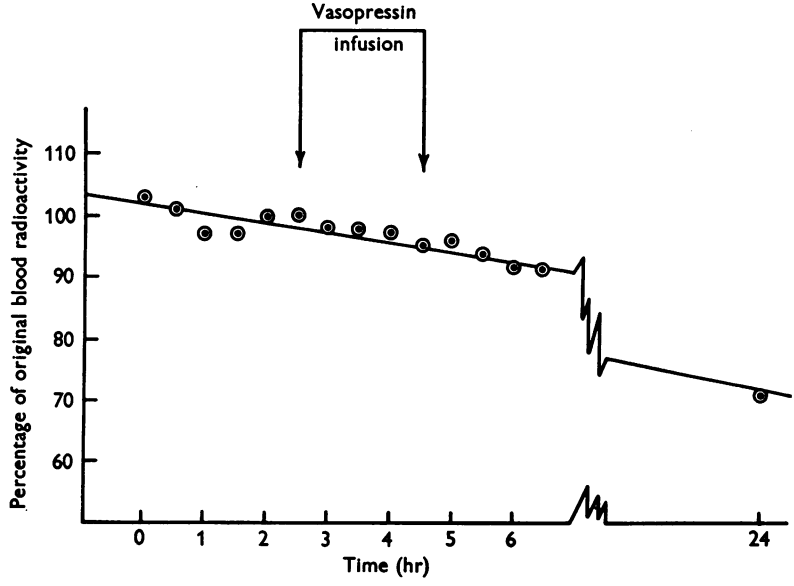

Fig. 11. Blood radioactivity in a rabbit which was injected with ^{131}I 6 days previously and thyroidectomized 48 hr before a 2 hr vasopressin infusion at a rate of 22·4 m-u./min. The radioactivity of the zero time sample is shown as 100% and all subsequent values are shown as a percentage of this value. Note that there is no increase in blood radioactivity due to the vasopressin infusion and the blood radioactivity fell at an exponential rate representing the half-life of circulating thyroxine.

injected with 100 μc ^{131}I 6 days before the experiment, and thyroidectomized 24 or 48 hr before the experiment. Sufficient PB ^{131}I remained in the blood to give high counting rates. Infusion of vasopressin at a rate of 22·4 m-u./min failed to evoke any increase in the blood radioactivity in these animals (Fig. 11) which continued to fall slowly according to the exponential rate of decay of thyroid hormone in the circulation.

Further evidence that vasopressin is not producing its effects by an action on peripheral tissues comes from measurement of the thyroxine space before and after 2 hr infusions of vasopressin. As described above, the thyroxine space of seven untreated rabbits was found to be equivalent

to 144 ± 7·6 ml. of blood/kg body wt. The thyroxine space was determined in two rabbits following a 2 hr infusion of vasopressin at a rate of 20 m-u./min. In both these animals the space was found to be equivalent to 145 ml. of blood/kg body wt. From this it would seem that vasopressin, at this dose rate, does not reduce the thyroxine space.

Possible direct action on the thyroid gland

The fact that hypophysectomy does not abolish the thyroidal response to vasopressin, together with the observation made in the initial experiments that injection of vasopressin evokes an increased concentration of radioactivity in thyroid-vein blood before a rise in systemic blood, indicates strongly that vasopressin is exerting an action directly on the thyroid gland.

Infusions into the thyroidal circulation. In an attempt to obtain more direct evidence as to whether vasopressin acts directly on the thyroid gland, polythene cannulae were implanted in the common carotid arteries (so as to be able to infuse solutions into the thyroidal circulation) 48 hr before the experiments. Animals with carotid cannulae were then infused either via the carotid artery, or intravenously (for control purposes), with different doses of vasopressin. Infusion at a dose rate of 1·4 m-u./min of lysine vasopressin into the carotid produced a questionable response in one animal and no response in two. The same dose, given intravenously to three rabbits bearing similar carotid cannulae, failed to elicit any response. As already described, this dose rate has been found to be about threshold in intact animals. Infusions of vasopressin at a rate of 22·4 m-u./min into the carotid arteries of four animals evoked a similar thyroidal response to that seen in two animals (bearing carotid cannulae) infused with this dose intravenously. However, before any conclusion can be drawn from these results, it must be noted that three rabbits infused with a total dose of 10 m-u. TSH in the carotid gave responses of 1·00, 1·50 and 1·39 % thyroid discharge as compared with two rabbits (bearing carotid cannulae) infused intravenously with this dose, with responses of 1·32, and 0·00 % discharge. Intact animals infused at this dose level gave a mean response of 1·47 ± 0·34 % thyroidal discharge.

Direct observation of the thyroidal circulation during vasopressin infusion

Two rabbits were anaesthetized (one with pentobarbitone sodium, I.V., and ether; the other with a solution of 2 % chloralose and 10 % urethane, I.V., and ether); the thyroid glands were exposed and observed under a binocular Zeiss operating microscope. After studying the visible thyroid vessels and observing the colour of the gland, an intravenous infusion of vasopressin was given for 5 min at a rate of 22·4 m-u./min. No obvious

change was observed to occur in either the diameter of the vessels or colour of the gland. Intravenous infusion at higher rates (up to 179·2 m-u./min) likewise produced no obvious change. Following this a cannula was tied in the left common carotid artery, with its tip pointing toward the heart and situated just distal to the origin of the thyroid artery. Infusion of vasopressin in this manner, at rates of 22·4 and 89·6 m-u./min, resulted in no signs of thyroidal vasoconstriction, although a final infusion of Indian ink resulted in a blackening of the left lobe of the thyroid as compared with the right, thus indicating that the previously infused solution had been entering the thyroidal circulation directly.

Possible action of vasopressin on a phosphorylase system

It is known that certain hormones, such as adrenaline and glucagon, activate hepatic phosphorylase systems (Rall, Sutherland & Wosilait, 1956; Rall, Sutherland & Berthet, 1957) and that ACTH activates the adrenal cortical phosphorylase system (Haynes & Berthet, 1957; Haynes, 1958; Haynes, Koritz & Péron, 1959). In all these cases stimulation of the production of 3′5′-adenosine monophosphate, which in turn stimulates the production of the phosphorylase, may be concerned. Hilton (1960) has found an increased adrenal cortical secretion following vasopressin infusions in the dog, and suggests that vasopressin may, in some way, activate the adrenal phosphorylase system.

To see whether substances (other than vasopressin) which are known to stimulate phosphorylase production in various tissues also stimulate the secretion of the thyroid gland, rabbits have been infused over the usual 2 hr period with 3′5′-adenosine monophosphate (3′5′-AMP), glucagon, adrenaline, and ACTH.

3′5′-AMP. Three rabbits received total doses of 1 mg 3′5′-AMP and showed no thyroid response. Two other animals received doses of 9 mg each and also failed to show a thyroidal response.

Glucagon. A total dose of 2·29 mg of glucagon was infused into each of two rabbits. One animal failed to respond, whereas the other showed a 2·1 % discharge of thyroid hormone.

Adrenaline. Six rabbits have each been infused with a total dose of 240 μg of adrenaline bitartrate. In two cases there was no thyroidal response, while in the remaining four, an elevation of blood radioactivity was observed (equivalent to 0·36–1·38 % discharge of thyroidal hormone). However, the nature of the response was different from that following infusions of vasopressin or TSH. The time of the peak of the response occurred within 1–2 hr after the start of infusion (instead of at 3–5 hr). Further the typical pattern of response, with blood radioactivity rising smoothly to a peak and then falling slowly, was not observed.

ACTH. Two rabbits were each infused with a total dose of 1·35 u. ACTH. In neither case was a thyroid response seen.

Observations on a reduced sensitivity of the thyroid gland to vasopressin and TSH following anaesthesia and operative trauma

Reference to Fig. 10 shows that the response of the thyroid gland of recently operated rabbits (both hypophysectomized and sham-operated) to infusions of vasopressin or TSH is less than normal. If the responses of the eleven animals in each of the hypophysectomized and sham-operated series are grouped, then the response of this 'operated group' to infusions of lysine-vasopressin at a rate of 22·4 m-u./min is significantly lower ($P < 0·02$) than that of the normal animals to similar infusions. Six further sham-hypophysectomized rabbits were infused with TSH and gave responses less than normal. Four of these were infused with TSH at a rate of 0·833 m-u./min (total dose 100 m-u.) and gave a response of $2·40 \pm 0·51$ % as compared with a response in 16 normal rabbits of $6·92 \pm 1·04$ %. This difference is significant ($P < 0·05$) (see Fig. 10*b*). The two other operated animals were infused with a total dose of 1000 m-u. and gave responses of 5·2 and 6·7 % thyroid discharge as compared with a response in eight normal rabbits to this dose of $11·39 \pm 2·06$ %.

DISCUSSION

The technique used in this study for detecting an increase in thyroidal function is similar, in many ways, to that evolved by Adams & Purves (1955) in the guinea-pig, and by McKenzie (1958) in the mouse, for the bioassay of thyrotrophic hormone. However, use of the rabbit enables serial blood samples to be obtained from any one animal, which allows detection of the peak of the response with greater precision. Conversion of the increased blood radioactivity into terms of percentage discharge of stored thyroidal radioactivity, by means of calculations involving the thyroxine space and thyroid-gland content of ^{131}I, would seem to give a more physiological measure of thyroid-gland response than figures based solely on percentage increase in blood radioactivity. It was found that the thyroid response to various doses of TSH was linear over the range 10–1000 m-u. when plotted against the logarithm of the dose of TSH.

The thyroidal responses obtained after vasopressin infusions were qualitatively similar to those following TSH infusions. The latent period of approximately 30 min, the time of the peak response after the beginning of the infusion, and the duration of the response were similar in all respects. Further, the *increased* blood radioactivity following either type of infusion was also similar, in that it was due to an increase in both inorganic iodide

and the iodothyronines, 25 % of the increase being due to iodide and 75 % due to iodothyronines. In neither case did the blood content of the iodotyrosines increase. In view of these similarities it is difficult to avoid the conclusion that vasopressin produces its effect either by increasing the effective blood concentration of TSH or by acting on the same thyroidal mechanism as that stimulated by TSH. A puzzling feature is the greater variability of the thyroidal response to a given dose of vasopressin, as compared with TSH. This variability is not solely due to strain or seasonal variation. It is possible that it may be due to variations in the blood concentration of oestrogens. The ovaries and uteri of all the animals were examined carefully at post-mortem, and in the great majority of cases were found to be typical of a state of oestrus. However, the blood concentration of oestrogen may well have varied in different animals, and from the findings reported by Harris, Levine & Schindler (1963), from experiments conducted on rats, the blood oestrogen level may be a factor of some importance in determining the thyroidal response to vasopressin.

The specificity of the thyroidal response to vasopressin is indicated by the results of the 17 control infusions of saline, thioglycollate-inactivated vasopressin, oxytocin, insulin and noradrenaline. No response was seen in 15 cases, a slight response in one and a questionable response in one.

It is interesting to speculate why the action of vasopressin on the thyroid gland is not detected by the release curve method in rabbits. Brown-Grant (1957) states that the only effect observed following injection of Pitocin, Pitressin or Pituitrin was a slight inhibition of thyroidal activity at high doses (5 or 10 u. three times a day). Similar results have been obtained in this laboratory (unpublished). It is possible that the time period of the measurements is of importance in this connexion, since responses persisting for only a few hours, especially if followed by periods of inhibition, may not be detected by a method depending on twice daily measurements of thyroidal radioactivity. Harris & Woods (1958) found that stimulation applied to the hypothalamus of the rabbit for a 48 hr period resulted in a substantial (48 hr) increase in thyroid activity as seen with the release curve method. The fact that repeated injections of vasopressin do not result in an increased slope of a thyroid release curve, as measured in this way, indicates that the thyroidal activation which followed electrical stimulation was not due to stimulation of vasopressin secretion. Further, in the same work an incomplete correlation was found between animals showing thyroidal and antidiuretic responses.

The mechanism by which vasopressin infusion excites thyroid activity has been investigated in several ways. The similarity of the vasopressin response to that evoked by TSH made it seem likely, during the early phase of this study, that vasopressin was in some way stimulating the

release of anterior pituitary TSH, which in turn was acting on the thyroid. The fact that hypophysectomized rabbits infused with vasopressin gave thyroid responses which in magnitude were not significantly different from those seen in the sham-operated rabbits rules out this possibility. Although only two of the eleven hypophysectomized rabbits had the pituitary completely removed, a further five had fragments so small in size that they would have been undetected except by microscopic examination of serial sections through the sella turcica. In all cases the fragments were thrombosed, imbedded in blood clot and probably non-functional. The magnitude of the responses seen in the hypophysectomized animals could not be correlated with the presence of any residual pituitary tissue; the largest thyroidal response in this series (6·13 % discharge of thyroid gland hormone) was observed in one of the completely hypophysectomized animals. It thus seems clear that vasopressin can exert a thyroid-stimulating action in the absence of pituitary tissue.

The conclusion that vasopressin does not stimulate thyroid activity by exciting secretion of pituitary TSH is reinforced by the measurements of the blood concentration of TSH before and during the vasopressin infusions. The assays were performed 'blindly' by Dr D. J. El Kabir, who used the *in vitro* guinea-pig thyroid method (El Kabir, 1960; Bottari *et al.* 1963). This method is sufficiently sensitive to measure the concentration of TSH normally present in the blood of the rabbit, and requires only a total of 0·3 ml. plasma for determinations in duplicate at each of two dose levels. The assays were made on blood samples obtained from eight rabbits infused with vasopressin. In seven cases no increase of TSH plasma concentration was observed; in one animal a slight increase was detected in one of three blood samples withdrawn during the infusion. The significance of the increase detected in this one sample is in doubt. These findings indicate that vasopressin is not acting by causing release of pituitary TSH, and further that it is not acting by releasing TSH from peripheral binding sites in the tissues. It may be mentioned, however, that, in contrast to the above findings, Bottari (1957) reported that an increase in plasma concentration of TSH followed injection of Pitressin in rabbits. One possible explanation that may account for this divergence of results is that Pitressin may be contaminated, to some extent, with thyrotrophic hormone. In the present study only highly purified or synthetic preparations of vasopressin were used. Further the present technique for TSH assay is felt to be more reliable than that used earlier by Bottari (see Bottari *et al.* 1963). Having reached the conclusion that vasopressin is not producing an increased blood concentration of TSH, there still remained the possibility that it was potentiating the action of circulating TSH on the thyroid gland. However, simultaneous infusions

of vasopressin (22·4 m-u./min) and TSH (0·083 m-u./min) did not result in a greater thyroidal response than did the infusion of either substance alone. Thus there appears to be no potentiation or synergism between these two hormones.

A further possible mode of action of vasopressin is that it was acting in some way to decrease the metabolism or excretion of thyroxine. Thus in the presence of a uniformly acting thyroid gland, a shrinkage in the thyroxine space, or a decreased rate of degradation or excretion of thyroxine, would act to raise the blood concentration of this hormone. Further, it could be supposed that in the presence of an inactive thyroid gland (produced by previous administration of thyroxine) vasopressin might stimulate increased absorption of thyroxine into the circulation from the intestine or some tissue depot, thus again causing an increased blood concentration. These theoretical considerations were shown not to be true, since infusion of vasopressin into animals recently thyroidectomized (96 hr after ^{131}I administration and 48 hr before the experiment) did not result in any increase in blood radioactivity. Further, measurements of the thyroxine space in rabbits following vasopressin infusion did not show any difference from the results obtained in untreated rabbits. In the case of one animal the thyroxine space was measured with and without vasopressin infusion on two separate occasions. No differences in the result were found. It would thus seem that vasopressin is not producing a rise in the blood concentration of thyroid hormone by an action on the peripheral tissues concerned with the metabolism or excretion of the hormone.

It seems probable that vasopressin exerts a direct action on the thyroid gland. In one attempt to demonstrate this rabbits were infused with vasopressin through cannulae previously implanted in the common carotid arteries in such a way that the infused solution passed directly into the thyroidal circulation. The thyroidal responses observed to follow vasopressin infusions in the carotid artery were not greater than those after similar infusions in the marginal vein of the ear. However, it was also found that the thyroid response to infusions of TSH made in a similar way in the carotid artery were likewise no greater than the responses to intravenous infusions of TSH. These results following infusions of TSH into the thyroidal circulation therefore stand in contrast to the results of similar experiments on the adrenal gland, in which markedly greater adrenal cortical responses follow injection of ACTH into the adrenal circulation as compared with the response to the same dose given systemically (Lipscomb & Nelson, 1962). Possible factors that may account for this difference are (1) that the thyroid gland seems less sensitive to both vasopressin and TSH following operative trauma (the arterial cannulae

were implanted 48 hr before the infusion, see below); (2) that infusion into one carotid artery will reach only one lobe of the thyroid gland, and since the thyroid artery of the rabbit gives a large branch to the larynx only a proportion of the infused dose will reach this lobe directly; and (3) the longer half-life of TSH or vasopressin, as compared with ACTH, may tend to reduce the difference in the effects of close arterial versus systemic intravenous infusions. In spite of the failure to demonstrate an increased thyroidal response on infusion of a given dose of vasopressin (or TSH) into the thyroidal circulation, there would seem to be little reason to doubt that it exerts a direct action on the thyroid gland.

In considering the action vasopressin exerts on the thyroid, consideration was first given to a possible vasoconstrictor effect on thyroid vessels. It might be suggested that vasopressin was exerting such a marked action of this type that the iodinated compounds of the gland were 'leaking out' into the general circulation under the influence of, perhaps, ischaemic changes in permeability. That a mechanism of this sort is not involved is indicated by the results of chromatographic analysis of the compounds in the plasma and thyroid gland. The major iodinated compounds of the gland were found to be the iodotyrosines. These compounds were found not to contribute in any way to the increase in blood radioactivity following vasopressin infusion, thus indicating that the infusion did not cause any abnormal or non-physiological release of stored iodotyrosines. The iodotyrosines are not normally found in appreciable amounts in the peripheral circulation. The detectable amounts found in the plasma in the present study, both before and after infusions of vasopressin or TSH, are probably to be accounted for by radiation damage to the thyroid due to the high dose of ^{131}I (200 μc) necessary for the chromatographic analysis. The increase in blood radioactivity that followed infusions of both vasopressin and TSH was found to be due partly (25 %) to an increase in inorganic iodide but mainly (75 %) to a rise in blood concentration of the iodothyronines. The fact that vasopressin exerts a similar action in this respect to TSH suggests that it may be acting on a similar mechanism in the gland. No conclusions can be drawn from the present work as to whether the inorganic iodide released from the gland originates from the iodide store or is due to de-iodination of organic compounds. A similar release of inorganic iodide, stimulated by an action of TSH, has been described by Rosenberg, Athans & Behar (1960), and Rosenberg, Ahn & Chalfen (1961) in the dog, man and rat, and by other workers. Further evidence indicating that vasopressin does not exert a marked thyroidal vasoconstrictor action is, first, that infusion of vasopressin in the doses used is not followed by a general pressor response and, secondly, that direct observation of the thyroid in anaesthetized rabbits did not reveal any

obvious change in colour of the gland or constriction of its vessels during vasopressin infusion.

Another possible mode of action of vasopressin on the thyroid is by an action on a thyroid phosphorylase enzyme system. Such a suggestion was considered by Hilton (1960) to explain the action of vasopressin on the adrenal cortex. No convincing evidence was found in the present study, from the results of infusions of substances known to stimulate phosphorylase systems, such as 3′5′-AMP, glucagon, adrenaline or ACTH, that such a system is involved in the effect of vasopressin exerts on the thyroid gland.

The question arises as to whether the action of administered vasopressin on the thyroid gland of the rabbit represents a physiological phenomenon. The threshold dose of vasopressin given by infusion, necessary to elicit thyroidal activation, is about 1–2 m-u./min. This is a large dose when compared with that necessary to elicit an antidiuretic effect. On the other hand, this dose is insufficient to evoke a pressor response in the anaesthetized or conscious rabbit. The solution of this problem depends upon experiments in which thyroid function is studied following the application of stimuli known to evoke reflex release of endogenous vasopressor hormones. A physiological role for vasopressin in the regulation of thyroid activity would be implied if such stimuli result in thyroidal activation in normal rabbits, but not in animals with the neurohypophysis chronically denervated.

A point of further interest arising from the present study concerns the reduced sensitivity of the thyroid gland to both vasopressin and TSH following operative trauma (Fig. 10*a*, *b*). This had seemed indicated in preliminary experiments (not included in this paper) and was the reason why the responses of all operated animals in the present study were compared with those of sham-operated animals, and why all surgical procedures were performed 48 hr before the experiment. These findings may also reflect on the conclusions drawn in a previous study by Brown-Grant, Harris & Reichlin (1954), in which inhibition of thyroid function was observed to follow surgical trauma. This thyroidal inhibition was thought to be secondary to inhibition of TSH secretion, but from the present findings may have been due in part to a reduced sensitivity of the thyroid gland.

SUMMARY

1. A method is described for measuring changes in thyroid activity in conscious unrestrained rabbits, based on the acute release of ^{131}I-labelled compounds from the thyroid gland, as determined by measurements of blood radioactivity. With this method straight-line log. dose-response

curves have been obtained to TSH given by intravenous infusion in doses between 10 and 1000 m-u.

2. The action of synthetic and highly purified preparations of vasopressin on thyroid function in the rabbit have been studied. An increased thyroid activity is stimulated by 2 hr infusions of vasopressin at rates of 1·4–22·4 m-u./min.

3. It has been shown that vasopressin does not act as a hypothalamic neurohumour increasing the discharge of pituitary TSH; nor does it act to release TSH from peripheral binding sites or to potentiate the action of circulating TSH. The evidence also indicates that vasopressin infusions do not result in a decreased thyroxine space and do not affect the rate of degradation or excretion of circulating hormone.

4. It is concluded that vasopressin exerts a direct action on the thyroid gland. This action is identical with that exerted by appropriate doses of TSH in its time relations, and in the nature of the compounds released from the gland (inorganic iodide and the iodothyronines, but not the iodotyrosines).

5. Anaesthesia and operative trauma (48 hr previously) have been found to decrease the sensitivity of the thyroid gland to vasopressin or TSH.

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