RESEARCH PAPERS

THE EFFECTS IN RABBITS OF THYROIDECTOMY AND TREATMENT WITH TRIIODOTHYRONINE ON THE SENSITIVITY TO NORADRENALINE AND THE CONTENT OF NORADRENALINE IN AORTA AND SPLEEN

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The sensitivity of spiral strips of aorta and strips of isolated spleen to sympathomimetic amines and nicotine has been studied in the thyroidectomised rabbit and in rabbits treated with triiodothyronine. Aortic strips from hyperthyroid rabbits were less sensitive to noradrenaline and less able to contract than those from normal rabbits; they also usually failed to respond initially to nicotine or tyramine although the nicotine response could be restored by exposure of the tissues to noradrenaline. Strips from hypothyroid rabbits were slightly less sensitive to low doses of noradrenaline than the normal although their maximal responses to high doses was greater. The dose-response curves for noradrenaline of spleen capsules from hyperthyroid and hypothryoid rabbits were more shallow than the normal. While spleen capsules from both experimental groups responded to nicotine with tachyphylaxis, they failed to respond to tyramine. Exposure of the capsules to noradrenaline temporarily restored the nicotine response. The noradrenaline content of aorta and spleen from hyperthyroid and hypothyroid rabbits was determined and in each case was found to be reduced to about 50 per cent of normal. These observations indicate that not all sympathetically innervated tissues of the hyperthyroid rabbit develop an increased sensitivity to sympathomimetic amines. Changes in tissue noradrenaline levels during periods of altered thyroid activity do not explain the altered sensitivity of the aorta and spleen to noradrenaline.

SOME of the effects of increased amounts of thyroid hormones resemble the effects produced by injection of sympathomimetic amines. It is generally held that in hyperthyroid states there is an increased sensitivity to adrenaline and noradrenaline, while in hypothyroid states there is a decreased sensitivity. Some of the evidence for this hypothesis is reviewed by Brewster, Isaacs, Osgood and King (1956). However, the evidence from the literature does not unanimously support the hypothesis (see Discussion), nor do our own experiments.

Recent investigations have led to the suggestion that the depletion of tissue stores of noradrenaline by sympathetic denervation or by reserpine treatment leads to an increase in sensitivity to noradrenaline (Burn and Rand, 1959a). Moreover, some sympathomimetic drugs, such as tyramine and nicotine, depend on the presence or availability of tissue stores of noradrenaline for their action (Burn and Rand, 1958a, b;

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Macmillan, 1959). Therefore we have now investigated the effect of thyroidectomy and of treatment with triiodothyronine, in rabbits, on the sensitivity of isolated preparations of spleen and aorta to noradrenaline, tyramine and nicotine. We have also determined the content of noradrenaline in these tissues to see if the altered sensitivity of tissues from the hypothyroid and hyperthyroid animals to the sympathomimetic amines and nicotine was related to the tissue noradrenaline levels.

METHODS

Male rabbits of 2·5 to 3·5 kg. were selected at random from a colony fed on Purina Laboratory Chow. Thyroidectomies were performed with sterile precautions and the animals allowed to recover from the operation for 8–20 days; the drinking water of these rabbits contained 2 per cent of calcium gluconate to counteract possible parathyroid damage. Rabbits were made hyperthyroid by daily subcutaneous injections of 100 μg. of triiodothyronine, using a solution of 1 mg./ml. of triiodothyronine in slightly alkaline saline. Injections were continued for 8 to 20 days until the day before the rabbit was killed. Reserpine treatment was given as described by Macmillan (1959).

Rabbits were killed by a blow on the head and then bled out. The entire thoracic aorta and the spleen were dissected out rapidly and washed in Locke's solution.

A spiral strip was prepared from the upper thoracic aorta (Burn and Rand, 1958a) and the remainder of the aorta was cleaned and stored at −8° for assay of its noradrenaline content. As far as possible the pitch of the spiral was the same in all experiments. The width of the final band of tissue was approximately 2 mm. It was arranged in a 9 ml. organ bath at 35° in Locke's solution gassed with O_2 so that its length was 7–7·5 cm. under a tension of 12 g. The spiral strip was allowed to remain untouched for 1 hr. Before each application of a drug the tension was reduced to 8 g., and the strip allowed to come to a new resting length. After the effect of the drug had been observed the bath was washed four times and the tension was again increased to 12 g. Noradrenaline was allowed to act for 2 min. and a further 6 min. elapsed before the next addition of noradrenaline. Tyramine and nicotine were allowed to act for 5 min. and a further 10 min. elapsed before the next addition of drug. Records were made on the smoked drum with a gimbal side writing lever with a magnification of 17:1.

The contractions of the isolated spleen capsule were observed by attaching threads to each pole of the spleen and cutting away the two lateral borders. The pieces removed from the spleen were stored at −8° for subsequent assay of their noradrenaline content. The central portion of the spleen was suspended in Locke's solution at 35° in a 40 ml. organ bath. Records were made with a frontal writing lever with a magnification of 17:1. The tension on the spleen capsule was 4·0 g.

The aorta and spleen fractions reserved for assay of noradrenaline were extracted and estimated using the methods of Burn and Rand (1959a).

RESULTS

Treatment of Rabbits

The mean increase in weight of the thyroidectomised rabbits was 310 g. and the mean decrease in weight of the rabbits injected with triiodothyronine was 500 g. No other special observations were made on the efficacy of the treatments, but the general appearance of the animals indicated that the hypo- and hyperthyroid states had been achieved; for

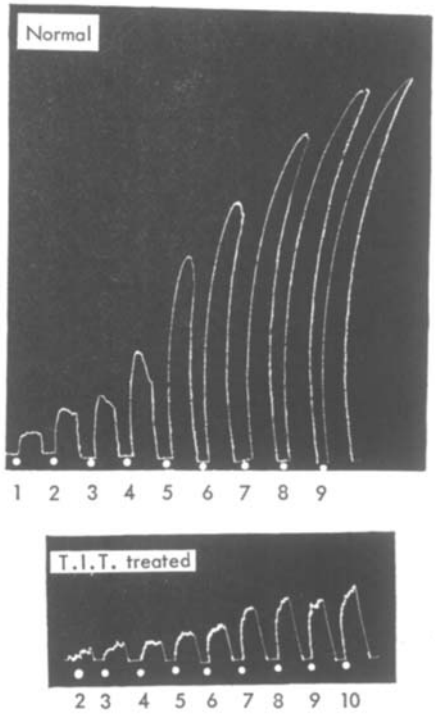

FIG. 1. Contractions of spiral strips of aorta to noradrenaline. Upper record, aorta strip from control rabbit; lower record, aorta strip from rabbit pretreated with triiodothyronine. The numerals refer to the dose of noradrenaline (base) in micrograms given into a 9 ml. bath. Contact time, 2 min.

example the triiodothyronine-treated rabbits were obviously hyperactive and their body temperature was higher than normal, while the opposite held for the thyroidectomised rabbits.

Noradrenaline on Aorta Strips

Typical records of the contractions to noradrenaline of isolated aorta strips from a hyperthyroid and from a normal rabbit are shown in Fig. 1, which illustrates the finding that the aorta strips from the hyperthyroid rabbits were less sensitive to noradrenaline and less well able to contract than those from normal rabbits. Strips from hypothyroid rabbits were slightly less sensitive to noradrenaline than normal as judged by the

higher mean threshold dose required, but they were able to give responses greater than the maximal responses obtained with normal strips. The results for all the experiments with noradrenaline on isolated aorta strips

TABLE I

MEAN RESPONSES OF RABBIT AORTA STRIPS TO NORADRENALINE

	Number in group	Threshold dose (μg./ml.). Geometric means	Dose for maximal response (μg./ml.). Geometric means	Maximal response (mm. contraction on kymograph). Means	Regression of response on log dose. Means
Normal	4	0·015	0·76	108	16·1
Treated with triiodothyronine	5	0·032	2·56*	40**	6·0*
Thyroidectomised ..	5	0·023	5·0**	149**	19·5

* Significantly different from normal ($P < 0·05$).
** Significnatly different from normal ($P < 0·01$).

are brought together in Table I. In each experiment the threshold dose for noradrenaline was determined by extrapolation to zero response from the calculated regression line relating response to the logarithm of the noradrenaline dose.

Nicotine and Tyramine on Aorta

Strips of aorta from normal rabbits responded with regular contractions to successive doses of nicotine bitartrate (60–250 μg./ml.) and of tyramine (25–200 μg./ml.) added to the bath, but strips from the hyperthyroid rabbits usually failed to respond to 10 times these doses of

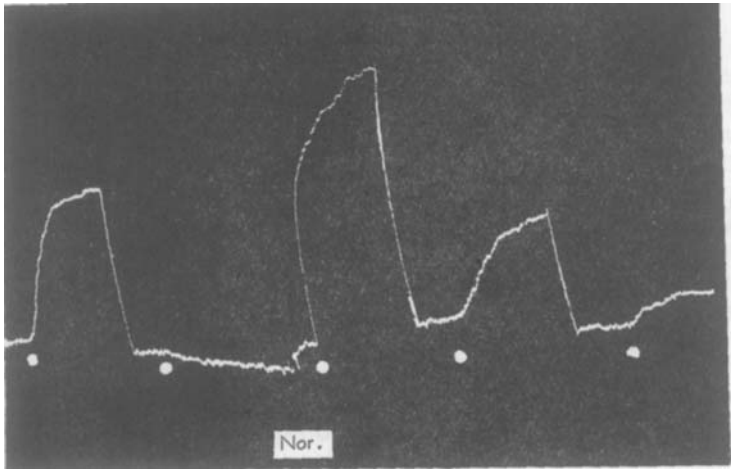

FIG. 2. Effect of nicotine on spiral strip of aorta from rabbit pretreated with triiodothyronine. At dots, 1 mg. of nicotine bitartrate was added to 9 ml. bath and allowed to act for 5 min. The second addition of nicotine did not cause a contraction, but after soaking the strip in noradrenaline (100 μg.) for 3 hr. (at Nor) and then washing out the noradrenaline the response to nicotine was restored.

nicotine or tyramine. However, in some experiments, a small contraction was produced by the first injection of nicotine, but no response was seen with subsequent doses (Fig. 2). When the response to nicotine was absent in an aorta strip from a hyperthyroid rabbit, it was restored after the addition of 10 to 100 μg. of noradrenaline to the bath for periods of time ranging from 5 min. to 3 hr. Fig. 2 shows an experiment in which there was a temporary restoration of responses to nicotine after soaking the aorta strip with noradrenaline. We were unable to restore the response of aorta strips to tyramine when it was absent as a result of

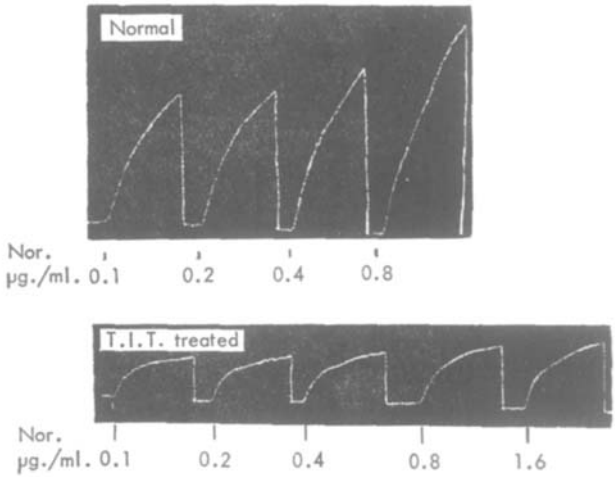

FIG. 3. Contractions of isolated spleen capsule produced by noradrenaline. Upper record from a normal rabbit, lower record from a rabbit pretreated with triiodothyronine. Contact time, 3 min.

pre-treatment of the rabbit with triiodothyronine. Aorta strips from thyroidectomised rabbits responded to nicotine and tyramine like strips from normal rabbits.

Noradrenaline on Spleen Capsule

The central part of the spleen capsule suspended in an isolated organ bath contracted in response to noradrenaline. Fig. 3 shows the contractions of spleen from a normal and a triiodothyronine rabbit to a range of concentrations of noradrenaline. The mean responses from all experiments are given in Fig. 4, which shows that the dose-response curves for noradrenaline obtained from the spleen capsules of both triiodothyronine-treated and thyroidectomised rabbits were more shallow than that obtained from the spleen capsules of the normal animals. The threshold doses of noradrenaline on the spleen capsule are given in Table II. Although the spleen capsules from the hyperthryoid rabbits were less contracted than the normal by large doses of noradrenaline, their sensitivity to low doses of noradrenaline was the most, and the capsules from the hypothyroid rabbits were the least sensitive. The

differences in threshold dose of noradrenaline between the three groups were shown to be significant by an analysis of variance at P less than 0·05.

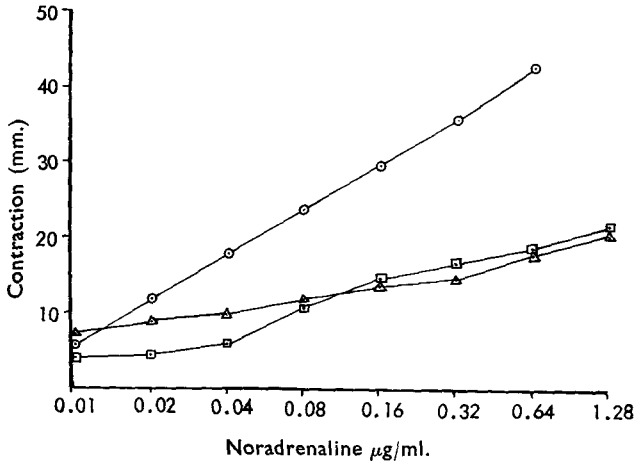

FIG. 4. Mean contractions of isolated spleen to noradrenaline. Ordinate, contractions of spleen in mm. on kymograph record (magnification of writing lever 17: 1). Abscissa, concentration of noradrenaline in μg./ml. (log. scale). Mean results: from 3 normal rabbits, ⊙; from 6 rabbits treated with triiodothyronine, △; from 5 thyroidectomized rabbits, ⊡.

Nicotine and Tyramine on Spleen Capsule

The contractions of spleen capsules from normal rabbits in response to 10–80 μg./ml. of nicotine bitartrate were graded with dose and were reproducible. In spleens taken from both triiodothyronine-treated and thyroidectomised rabbits the responses to nicotine were less well shown and exhibited a pronounced tachyphylaxis, so that eventually even high

TABLE II

THRESHOLD CONCENTRATION (ng./ml.) NORADRENALINE REQUIRED TO PRODUCE CONTRACTION OF SPLEEN

	Normal	Triiodothyronine-treated	Thyroidectomised
	5	1	10
	2	0·3	4
	2	0·3	15
		0·05	6
		2	4
		1·5	
Means	3	0·8	8

doses were without effect. Fig. 5, shows an experiment in which increasing doses of nicotine were given to a strip of spleen capsule from a triiodothyronine treated animal; the responses became smaller. After noradrenaline was added to the organ bath (0·1 μg./ml. for 10 min.) and then washed out a further addition of nicotine produced a well marked

contraction, but this restoration was only temporary. Strips of spleen capsule taken from reserpine-treated rabbits behaved in a similar way to those from triiodothyronine-treated rabbits. They contracted in response

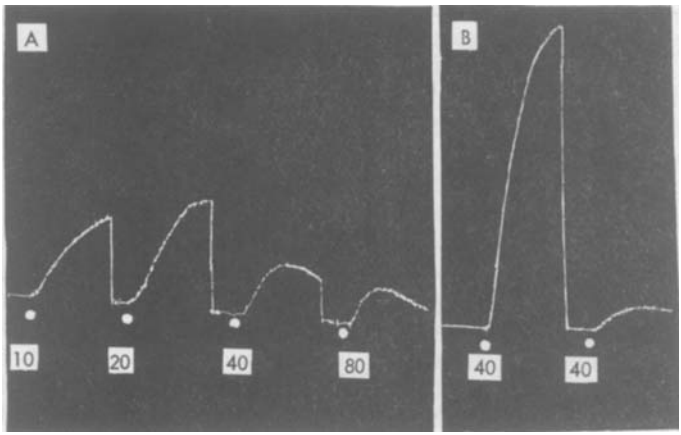

FIG. 5. Effect of nictotine on isolated spleen capsule from a triiodothyronine treated rabbit. At dots, nicotine bitartrate was added to the bath in the concentrations shown (μg./ml.). Panel A shows that there is marked tachyphylaxis to nicotine. Between A and B the spleen was soaked in noradrenaline (0·1 μg./ml.) for 10 min. and then noradrenaline was washed out. The next response to nicotine was potentiated.

to noradrenaline, but failed to contract with nicotine. After treating the reserpinised spleen in the organ bath with noradrenaline the response to nicotine was restored (Fig. 6). Tyramine did not contract the reserpinised spleen, nor the spleens from triiodothyronine-treated or thyroidectomised rabbits. We were unable to restore responses to tyramine by soaking the spleen with noradrenaline.

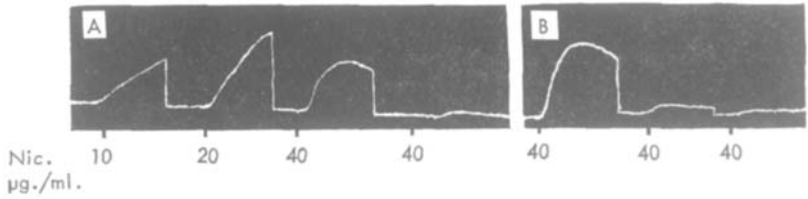

FIG. 6. Responses of isolated spleen strip from reserpine-treated rabbit to nicotine. Between A and B, noradrenaline (0·01 μg./ml.) was present in the bath fluid for 10 min. and then washed out.

Noradrenaline Content of Aorta and Spleen

The noradrenaline content of the aorta and spleen from normal, hyperthyroid and thyroidectomised rabbits was assayed by matching the pressor activity of tissue extracts with that of noradrenaline standards on the blood pressure of the pithed rat. In addition, we examined extracts

from the spleens of reserpine-treated rabbits. The results are shown in Table III.

TABLE III

SUBSTANCES PRESENT IN EXTRACTS OF RABBIT AORTA AND SPLEEN ESTIMATED AS NORADRENALINE (ng./g.)

	Aorta			Spleen			
	Normal	Triiodo-thyronine-treated	Thyroidect-omised	Normal	Triiodo-thryonine-treated	Thyroidect-omised	Reserpine-treated
	280	125		500	575		38
		129	138		206	425	75
	200	175	150	1500	550	700	180
	225	262	194	900	825	400	
	250	190	150	1140	500	310	
		200	125		335	500	
Means	239	180**	151***	1010	499*	467*	98*

* Significantly different from normal ($P < 0.05$).
** Significantly different from normal ($P < 0.01$).
*** Significantly different from normal ($P < 0.001$).

DISCUSSION

The effect of treatment with triiodothyronine on the aorta and spleen resembles, in some respects, the effect of treatment with reserpine. Thus both treatments lead to a decrease in the noradrenaline content of these tissues and to a loss of the action of nicotine and tyramine. The threshold dose of noradrenaline to contract the cat spleen *in situ* is decreased after reserpine treatment (Burn and Rand, 1959a), and after treatment with triiodothyronine a decrease in threshold was seen in the spleen, but not in the aorta. Suprathreshold doses of noradrenaline were less effective in strips of aorta and of spleen taken from hyperthyroid rabbits than in strips from the normal controls. This decreased responsiveness may in part explain the diminished effects of nicotine and tyramine. However the observation that soaking the strip in noradrenaline temporarily restored the response to nicotine suggests that the decreased store of noradrenaline was also partly responsible for the impaired response to nicotine.

There was less noradrenaline stored in aorta and spleen taken from thyroidectomised rabbits than in those taken from normal rabbits. Isolated spleen strips from these rabbits did not respond to tyramine and to nicotine, but after soaking with noradrenaline the response to nicotine was increased. Therefore it can be concluded that the reduction in noradrenaline stores in the spleen was concerned in the loss of response of spleen strips to nicotine. However, aorta strips from thyroidectomised rabbits responded to nicotine and tyramine as did strips from normal rabbits. The threshold dose of noradrenaline on both the aorta and spleen strips was raised, and the responsiveness of the spleen to larger doses of noradrenaline was decreased. However, the responsiveness of aorta strips from thyroidectomised rabbits to larger doses of noradrenaline was greater than that of control strips.

These results show that an inverse relationship between tissue stores of noradrenaline and the sensitivity to exogenous noradrenaline does not

always hold. Such a relationship was demonstrated by Burn and Rand (1959a) when they investigated the hypersensitivity to noradrenaline of the cat's dilator iris muscle and spleen, produced by both sympathetic denervation and by reserpine. Fleming and Trendelenburg (1961) have pointed out that the time factor must also be considered, although this factor seemed to be more important for the appearance of supersensitivity of the nictitating membrane than of the cardiovascular system. Burn and Rand proposed that the supersensitivity was a result of the unmasking of more receptor sites when the normal transmitter output fell as a result of the depletion. Axelsson and Thesleff (1959) found that in denervated, hypersensitive, skeletal muscle, which has been deprived of its neurohormone by denervation, a greater acetylcholine sensitive area could be detected than in innervated muscle which is continually exposed to its neurohormone. On the other hand, if larger amounts of acetylcholine are applied to the end plate region, the receptors become refractory and the sensitivity of the muscle is decreased (Katz and Thesleff, 1957). Conceivably, a similar situation may exist in sympathetically innervated tissues with respect to the sympathetic transmitter, noradrenaline, and the receptors to noradrenaline.

A lack of correlation between depleted tissue stores of noradrenaline and increased sensitivity might occur for a variety of reasons. Macmillan (1959) suggested that if the exogenous noradrenaline was prevented from entering stores, for example, by cocaine, more would be available to act on receptors, and Vane (1961) suggested that if acidic non-specific binding sites in tissues were occupied by pharmacologically inert basic substances, then a greater portion of a subsequently injected dose of a pharmacologically active base would be available for receptors. In either case there would be an increased sensitivity without a decrease in the store. The converse possibility, decreased store without increased sensitivity might occur if the decreased store were a reflection of increased output of transmitter. The observation that infusion of noradrenaline leads to a loss of sensitivity to noradrenaline (Burn and Rand, 1959b) supports the possibility that bombardment of receptors by endogenous noradrenaline may lead to a decreased sensitivity to endogenous noradrenaline, as well as to a reduction in the size of the store of endogenous noradrenaline.

Our experiments do not allow us to say by what mechanism the noradrenaline stores are depleted by triiodothyronine and by thyroidectomy, but it is probable that the mechanisms are different in each case. The effect of reserpine treatment (Thier and Gravenstein, 1960) and of guanethidine (Gaffney, Braunwald and Kahler, 1961) in abolishing those effects of hyperthyroidism which resemble the responses to sympathetic stimulation suggests that in the hyperthyroid state there is an increased output of noradrenaline. The decrease in noradrenaline stores in thyroidectomised rabbits may be due to impaired synthesis since Goodall (1951) found that the amino-acid precursor dihydroxyphenylalanine was present in the adrenals of thyroidectomised but not of normal sheep.

Our findings that artery strips from hyperthyroid rabbits were less sensitive to noradrenaline and that their ability to contract was considerably less than that of strips from normal rabbits confirms the earlier observations of Miculicich (1931), who investigated the effect of previous thyroid feeding on the vasoconstriction produced by injecting 20 μg. of adrenaline into the blood perfused leg of the dog. In legs from normal dogs the mean increase in pressure was 39 mm.; while legs from thyroid fed dogs were less sensitive with a mean increase in pressure of 35 mm. Smith (1954) found that isolated pig arteries stored for 18–24 hr. in a solution containing thyroxine were less sensitive to adrenaline than were control arteries stored for the same period. Smith (1953) also found that arteries from thyroidectomised pigs were insensitive to adrenaline, although thyroxine added to the perfusing fluid produced an immediate increase in sensitivity.

The effect of experimentally produced hyperthyroid states on the pressor response to adrenaline and noradrenaline has been investigated many times with somewhat contradictory results. In the dog it has been reported that the pressor response is less in the hyperthyroid than in the euthyroid state (Chamberlain, 1928; Hepler and Simonds, 1936; Riggs, Stanbury and Carr, 1951); on the other hand Brewster, Issacs, Osgood and King (1956) reported an increased pressor response after the hyperthyroid state had been produced. In the cat the pressor effect of adrenaline is reported as being greater in the hyperthyroid state (Blau and McNamara, 1930). In the hyperthyroid rabbit the pressor response to adrenaline has been reported as unchanged (Konig, 1928) or increased (Santesson, 1919; Spinks, 1952). To the extent that the responses of the isolated tissues we have chosen, the aorta and the spleen, may be taken as representative of the tissues which are responsible for pressor responses, our results indicate a decreased sensitivity to noradrenaline. However, in the whole animal the factors involved are certainly more complex. The conclusion we wish to draw is that not all effector organs show an increased sensitivity to noradrenaline in the hyperthyroid state.

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REFERENCES

Axelsson, J. and Thesleff, S. (1959). *J. Physiol.*, **149**, 178–193.
Blau, N. F. and McNamara, H. (1930). *Proc. Soc. exp. Biol. N.Y.*, **27**, 997–998.
Brewster, W. R., Isaacs, J. P., Osgood, P. F. and King, T. C. (1956). *Circulation*, **13**, 1–20.
Burn, J. H. and Rand, M. J. (1958a). *Brit. med. J.*, **1**, 903–908.
Burn, J. H. & Rand, M. J. (1958b). *J. Physiol.*, **144**, 314–336.
Burn, J. H. and Rand, M. J. (1959a). *Ibid.*, **147**, 135–143.
Burn, J. H. and Rand, M. J. (1959b). *Brit. med. J.*, **1**, 394–497.
Chamberlain, B. (1928). *Proc. Soc. exp. Biol. N.Y.*, **26**, 459–460.
Fleming, W. W. and Trendelenburg, U. (1961). *J. Pharmacol.*, **133**, 41–51.

Gaffney, T. E., Braunwald, E. and Kahler, R. L. (1961). *New Eng. J. med.*, **265**, 16–20.
Goodall, M. (1951). *Acta physiol. scand.*, **24**, Supp. 84.
Hepler, O. E. and Simonds, J. P. (1936). *Proc. Soc. exp. Biol. N.Y.*, **34**, 534–535.
Katz, B. and Thesleff, S. (1957). *J. Physiol.*, **138**, 63–80.
König, W. (1928). *Arch. exp. Path. Pharmak.*, **134**, 36–43.
Macmillan, W. H. (1959). *Brit. J. Pharmacol.*, **14**, 385–391.
Miculicich, F. G. (1931). *Arch. exp. Path. Pharmak.*, **162**, 484–487.
Riggs, D. S., Stanbury, J. B. and Carr, E. A. (1951). *J. Pharmacol.*, **101**, 31 (P).
Santesson, C. G. (1919). *Skand. Arch. Physiol.*, **37**, 185–215.
Smith, D. J. (1953). *Amer. J. Physiol.*, **172**, 118–128.
Smith, D. J. (1954). *Ibid.*, **177**, 7–12.
Spinks, A. (1952). *Ibid.*, **117**, 35–36P.
Thier, D. M. and Gravenstein, J. S. (1960). *The New Physician*, **9**, 39–41.
Vane, J. R. (1961). Communication to summer meeting of the Brit. Pharmacol. Soc., Edinburgh.