hibition (HI), and gel-diffusion. The HA reactions in general were quite specific, although cross-reactions were observed. The extent of cross-reaction was greater with B antitoxins and A botulinal toxoid-sensitized red blood cells. The different antitoxins behaved similarly in HI and gel-diffusions, though the reactions of the NCDC and International antitoxins were weaker than those of rabbit antitoxins in gel-diffusions. Hemagglutination inhibition and gel-diffusion reactions with toxic cultures suggested that the *in vitro* specificity of the A toxin-antitoxin did not involve neutralizing antibodies. Hemagglutination inhibitions and gel-diffusions with B toxins did not resolve the question of the involvement of neutralizing antibodies in *in vitro* specificity, since fairly toxic cultures inhibited HA and formed bands believed to be associated with type specificity, whereas a virtually non-toxic B culture neither inhibited HA nor formed a line of identity with the type specific systems in gel-diffusion.

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Thyroid State and Vascular Reactivity in Rats.* (32589)

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The assumption that changing the thyroid state alters the responsiveness of the organism to catecholamines has been prevalent for many years(1). Arterial blood pressure studies have suggested that the vascular responses to catecholamines, and perhaps to other agents as well, are enhanced by hyperthyroidism and diminished in hypothyroidism(1,2). However, recent reports indicate that the responsiveness of myocardial and vascular smooth muscle to catecholamines may not always be augmented by hyperthyroidism in dogs(3,4).

The following studies describe the effects of altered thyroid state on the sensitivity of rats to the vasoconstrictor actions of norepinephrine and angiotensin. Changes in vascular resistance, independent of changes in systemic arterial blood pressure, were estimated using an auto-perfused hindquarter preparation.

Materials and methods. Male Sprague-Dawley rats (initially weighing 200 to 250 g) were used in this study. Hypothyroidism was produced in 9 rats by radiothyroidectomy (850 μc NaI^{131} I.P.) at least 2 weeks prior to the experiments. These rats were fed a Remington iodine deficient diet for 1 week prior to radiothyroidectomy and Purina Lab Chow thereafter. Rats of other groups were similarly fed the iodine deficient and then the standard diet. At autopsy, no functional thyroid tissue could be detected histologically in these animals. Hyperthyroidism was in-

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duced by 12 to 16 daily injections of L-thyroxine (100 μg/day, S.C.). The average increase in basal metabolic rate in 6 representative hyperthyroid animals (3 different determinations) was 27%. The average daily weight loss of each of these rats was 4 g. Euthyroid rats were given the solvent in the same manner as those receiving thyroxine and gained, on the average, 3 g per day.

Each rat was anesthetized with pentobarbital sodium (60 mg/kg, I.P.) and the trachea was cannulated. A segment of abdominal aorta was isolated, cannulated, and the hindquarters perfused with blood using a finger pump (Harvard, Model 500-1200) by the method described by Brody *et al*(5).

In this procedure, blood was passed from the centrally cannulated aorta through the pump and returned to the aorta at constant flow. Perfusion pressure, measured distal to the pump, was adjusted to be approximately equal to systemic aortic pressure. Drugs (0.05 ml) were injected into the perfusion stream. Pressures were measured with Statham P23Db transducers coupled to a Grass Model 7 polygraph recorder. All drugs were prepared in saline. Angiotensin was obtained in commercially available form (Hypertensin®, Ciba).

Statistical analyses were performed by standard methods(6) using the program EXBIOL(7) at the Common Research Computer Facility.§

Results. Relationship of perfusion pressure to vascular response. The mean aortic blood pressures and perfusion pressures determined in rats from each thyroid-state group are illustrated in Fig. 1. The perfusion pressures were slightly lower than the corresponding aortic pressures in all cases. The aortic blood pressures, and hence the perfusion pressures, were significantly higher in hyperthyroid and lower in hypothyroid rats than in euthyroid animals. Linear correlation analysis indicated that angiotensin and norepinephrine-induced vasoconstrictor responses (increases in perfusion pressure) were directly related in magnitude to the level of perfusion pressure. The vasoconstrictor responses elicited by angiotensin and norepinephrine were therefore

§ USPHS FR 00254.

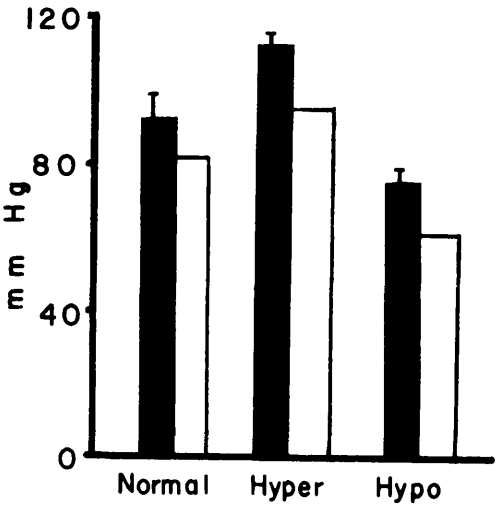

FIG. 1. Initial systemic aortic blood pressure measured proximal to the perfusion pump (solid bars) and initial hindquarter perfusion pressures measured distal to the pump (light bars). Bars represent group means of: normal = 9, hyperthyroid = 11, hypothyroid = 9 rats. The vertical T-bars represent standard errors.

adjusted by analysis of covariance to remove the influence of initial perfusion pressure. These adjusted responses were used for subsequent comparisons.

Responses to vasoactive agents. The linear relationships between increasing doses of norepinephrine or angiotensin and the vascular resistance of hindquarter preparations of euthyroid, hypothyroid, or hyperthyroid rats are illustrated in Fig. 2. A regression equation for each log dose-effect curve was calculated and the dose needed to induce a designated mm Hg increase in perfusion pressure (ED) was determined from the equations. Parameters related to these calculations are summarized in Table I. The slopes of the curves obtained with angiotensin or with norepinephrine from euthyroid, hypothyroid, or hyperthyroid rats were not significantly different from each other. However, norepinephrine dose-effect curves were steeper in all circumstances than those obtained with angiotensin. Angiotensin and norepinephrine were more active in hypothyroid than in euthyroid rats as shown by the dose-effect curves in Fig. 2 and the ED values in Table I.

The responses from euthyroid and hyperthyroid animals treated with angiotensin or

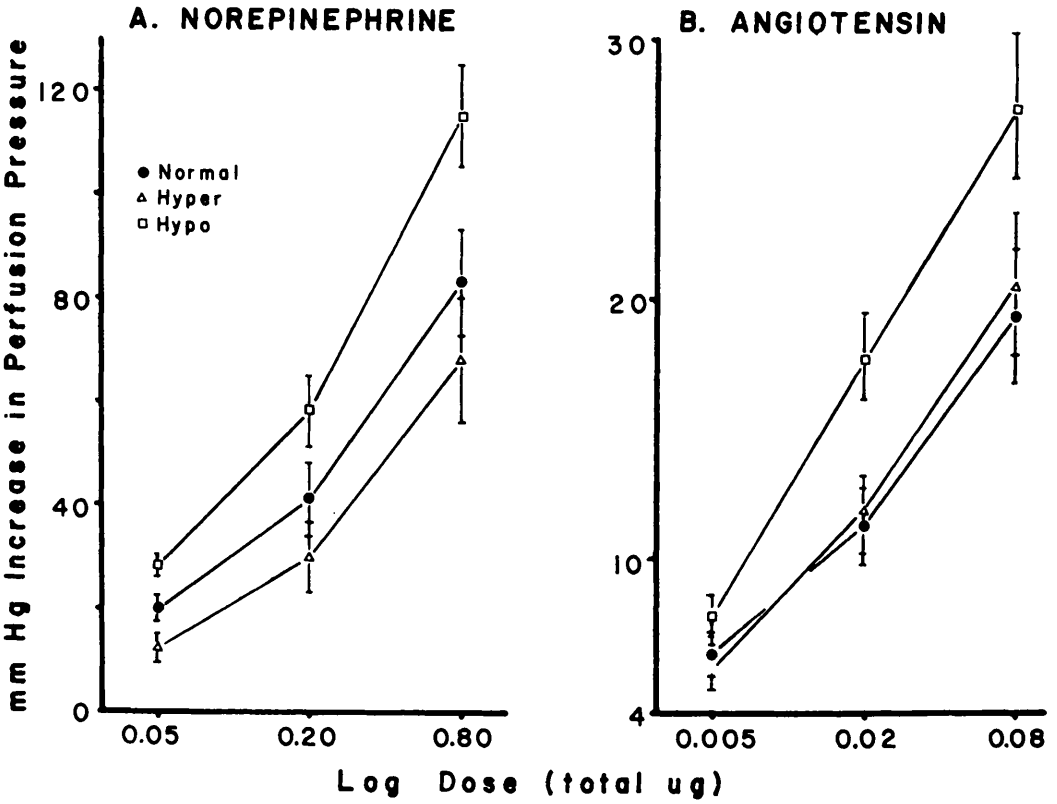

FIG. 2. Dose-effect curves relating perfusion pressure increases to log doses of norepinephrine or angiotensin. The vertical lines at each point represent standard errors. The perfusion pressure responses were adjusted to a common initial perfusion pressure by analysis of covariance. Normal = 9 rats, hyperthyroid = 11 rats, hypothyroid = 9 rats.

norepinephrine were not significantly different ($P>0.05$), although the data suggests that the hyperthyroid rats were less responsive to norepinephrine than controls. The vascular responses to norepinephrine or angiotensin before covariance adjustment for perfusion pressure were also compared, but not tabulated in this report. The unadjusted responses of euthyroid, hypothyroid, or hyperthyroid rats to angiotensin were not significantly different ($P>0.05$). Norepinephrine was most active in hypothyroid preparations although the dif-

TABLE I. Summary of Data Used to Estimate Vasoconstrictor Potency.

Vaso-constrictor	Parameter	Thyroid state		
		Euthyroid	Hypothyroid	Hyperthyroid
Angiotensin	Dosage range (μg)	0.005–0.08	0.005–0.08	0.005–0.08
	No. observations	24	27	33
	Regression coefficient ± SE	10.8 ± 2.2	16.4 ± 1.4	12.2 ± 2.1
	ED_{15} (μg) with SE range	0.035 (0.028–0.045)	0.013**(0.012–0.015)	0.032 (0.026–0.038)
Norepi-nephrine	Dosage range (μg)	0.05–0.8	0.05–0.8	0.05–0.8
	No. observations	24	27	33
	Regression coefficient ± SE	51.7 ± 8.3	72.2 ± 8.1	46.1 ± 7.3
	ED_{50} (μg) with SE range	0.22 (0.18–0.26)	0.11* (0.10–0.13)	0.39 (0.32–0.48)

No. observations refers to number of responses used to calculate the regression equation. ED_{15} and ED_{50} refer to the dose calculated from the regression equation needed to induce a 15 or 50 mm Hg increase in perfusion pressure. SE refers to standard error. * or ** significantly differs from euthyroid controls, $P<0.05$ and 0.01 respectively.

ferences were not statistically significant ($P>0.05$).

An additional radiothyroidectomized group of rats received replacement injections of L-thyroxine (1 μg/100 g bw/day, S.C.) until use. The adjusted vascular responses to norepinephrine were not significantly different from euthyroid controls. However, thyroxine maintenance did not alter the response of the athyroid rats to angiotensin and the replacement animals were significantly more responsive ($P<0.05$) than the euthyroid and hyperthyroid preparations.

The vasodilator responses to isoproterenol and papaverine were also studied with the rat hindquarter preparation. Hypothyroid animals seemed more sensitive than euthyroid controls although the dilator responses were too small and too variable for definitive conclusions. Similar studies seem indicated, using preparations more suitable for evaluating vasodilation.

Discussion. The results of this study, when considered with those obtained by others, suggest that hyperthyroidism does not enhance the reactivity of resistance vessels to vasoconstrictors in certain species. Using perfused dog hindlimbs, Zsöter *et al*(4) reported that the vasoconstrictor activity of norepinephrine was diminished in hyperthyroid animals, although the responses to angiotensin and vasopressin were not altered. These findings are similar to our results obtained with rats. Van Der Schoot and Moran(3) reported that the vascular response to norepinephrine was similar in the perfused hindlimbs of euthyroid and hyperthyroid dogs.

Page and McCubbin(2) noted that systemic arterial blood pressure responses to norepinephrine and angiotensin were diminished in hypothyroid dogs. In our studies with hypothyroid rats, the responses of the hindquarter resistance vessels to norepinephrine and angiotensin were enhanced. It is interesting that Buccino *et al*(8) reported that papillary muscles taken from hypothyroid cats were more responsive to the positive inotropic actions of norepinephrine and strophanthidin than euthyroid controls. The responses to these drugs seemed to be reduced in muscles taken from hyperthyroid cats.

Although the auto-perfused rat hindquarter preparation provided an indication of the vascular reactivity to vasoconstrictors, it was quite unsatisfactory for evaluating vasodilators. Other studies on the effect of thyroid state on vascular reactivity seem indicated, particularly if larger animals were used and specific regional vascular beds were studied. Less surgical trauma would be involved if non-cannulating electromagnetic flow sensing devices were employed. Vasodilators such as isoproterenol should be further studied and the effect of thyroid state on the sensitivity of specific adrenergic *alpha* and *beta* vascular receptors better defined.

Summary. The sensitivity of resistance vessels to the action of norepinephrine and angiotensin in hypothyroid, hyperthyroid, and euthyroid states was determined using auto-perfused rat hindquarter preparations. The vasoconstrictor responses were adjusted for perfusion pressure using analysis of covariance. Hypothyroid animals were more responsive to the vasoconstrictors than euthyroid controls. The responsiveness to angiotensin was unaltered in hyperthyroid rats but the sensitivity to norepinephrine was slightly reduced.

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