Arterial blood pressure was unchanged by He-O_2 breathing.

The relationship between adrenergic activity and cardiac arrhythmias in experimental myocardial infarction is well known. Increases in circulating catecholamine concentrations, and an increase in sensitivity to their arrhythmic action, have both been demonstrated (*7*). The increased secretion of catecholamines after experimentally induced infarction is abolished by adrenalectomy, which is also followed by a return of normal cardiac rhythm. Cardiac sympathectomy protects against ventricular fibrillation after coronary artery ligation in anesthetized dogs (*7*). Thus, an agent which reduces sympathetic activity or circulating catecholamines might also reduce the likelihood of ventricular arrhythmias. We have some evidence of both effects and we suggest the possibility of a causal relationship.

These effects of helium are puzzling (*8*). In the traditional view, helium has no physiological effects except those attributable to its physical properties, such as its density, its thermal conductivity, or its acoustic velocity (*9*). Such properties are not likely to account for helium's antiarrhythmic action for several reasons: (i) any benefit in reduced airway resistance due to its low density would be partly offset by its higher viscosity (*10*); (ii) the effect of any change in the hydrodynamic properties of the breathing mixture was minimized, both in earlier work (*1*) and in ours, by the use of mechanical ventilation; (iii) the effect of thermal conductivity was minimized in our study by maintaining constant body temperature; (iv) maximal protection against ventricular fibrillation occurs with only 20 percent helium in the breathing mixture (*1*). This last finding also provides evidence that the putative helium effect is not merely due to the absence of nitrogen. It seems likely therefore that the antiarrhythmic property of helium in the anesthetized dog represents a pharmacologic action whose mechanism may involve altered adrenergic activity.

LAWRENCE RAYMOND
RICHARD B. WEISKOPF
MICHAEL J. HALSEY, ALAN GOLDFIEN
EDMOND I. EGER III
JOHN W. SEVERINGHAUS
Cardiovascular Research Institute and *Departments of Medicine, Anesthesia* and *Obstetrics and Gynecology, University of California, San Francisco 94122*

References and Notes

1. R. Pifarré, T. K. Raghunath, R. M. Vanecko, F. S. Chua, J. U. Balis, W. E. Neville, *J. Thorac. Cardiov. Surg.* **60**, 648 (1970).
2. Mechanical ventilation was provided with a respirator (Bird), using 5 cm of water as positive end-tidal pressure. The dogs breathing N_2-O_2 received, per kilogram of body weight, 3.0 ± 2.8 mEq of sodium bicarbonate, and 50 ± 11 mg of sodium pentobarbital during surgical preparations for arterial ligation. Dogs breathing He-O_2 received, per kilogram of body weight, 3.6 ± 1.9 meq of sodium bicarbonate and 58 ± 14 mg of sodium pentobarbital. The difference in dosage is not significant. Circumflex ligation was delayed until 40 minutes after the administration of any medication.
3. Acute ligation of the circumflex coronary artery in the anesthetized dog occludes blood flow to about one-third of the myocardial mass [F. W. Quattlebaum, S. Victorine, V. O'Malley, R. F. Edlich, *Circulation* **40**, 111 (1969)]. Schlesinger mass is a radiological contrast substance composed of barium sulfate in a gelatin and potassium iodide base [M. J. Schlesinger, *Lab. Invest.* **6**, 1 (1957)].
4. T. A. Joas and W. C. Stevens, *Anesthesiology* **35**, 48 (1971). In this technique, epinephrine, in successive doses of 0.5, 1.0, 1.5 μg/kg and higher, is given in 5 ml of saline through a catheter into the inferior vena cava, at a steady rate, during 60 seconds. The dose is increased until the end point—2 or more PVC's—is reached. After each dose, blood pressure and heart rate are observed until they return to baseline, and a period of 5 minutes then elapses before the next higher dose is given.
5. H. Weil-Malherbe, *Methods Med. Res.* **9**, 130 (1961).
6. R. F. Klein, W. G. Troyer, H. K. Thompson, M. D. Bogdonoff, A. G. Wallace, *Arch. Intern. Med.* **122**, 476 (1968); J. Staszewska-Barczak, L. Ceremuzynski, *Clin. Sci.* **34**, 531 (1968). Increased myocardial sensitivity to catecholamines has been reported by H. M. Maling and N. C. Moran [*Circ. Res.* **5**, 409 (1957)] and confirmed by others, including V. A. Kurien, P. A. Yates, M. F. Oliver [*Eur. J. Clin. Invest.* **1**, 225 (1971)]. The role of the nervous system in arrhythmias which follow coronary occlusion in the cat has been discussed by R. A. Gillis [*Amer. Heart J.* **81**, 677 (1971)].
7. A. S. Harris, A. Estandia, R. F. Tillotson, *Amer. J. Physiol.* **165**, 505 (1951).
8. Our experiments provide no information on the way in which helium might act to reduce the concentration of catecholamines in arterial blood. With recordings from the superior cervical sympathetic ganglion in the anesthetized dog and cat, we have found no loss of sympathetic activity with helium (R. A. Mitchell, M. J. Halsey, D. A. Herbert, L. W. Raymond, R. B. Weiskopf, unpublished observations).
9. R. D. Dripps, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, London, ed. 4, 1970), p. 926.
10. J. H. Comroe, Jr., R. E. Forster II, A. B. Dubois, W. A. Briscoe, E. Carlsen, *The Lung* (Yearbook Medical Publishers, Chicago, 1967), pp. 296–297.
11. R. A. Fisher, *Statistical Methods for Research Workers* (Oliver & Boyd, Edinburgh, 1928), p. 84. Alternatively, $P < .01$ if the incidence of VF in dogs breathing He-O_2 is compared with that of larger series of dogs breathing air at similar arterial P_{O_2}, that is, about 50 percent VF [G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, 1969), p. 214]. The PVC's in dogs breathing He-O_2 and those breathing N_2-O_2 were compared with Wilcoxon's sum of ranks test, with tables from R. Langley [*Practical Statistics* (Dover, New York, 1971), pp. 166–170]. Other mean values were compared by paired or unpaired *t*-tests, as appropriate.
12. We thank Dr. F. G. Standaert for helpful suggestions, and Merry Nishimura, Lou Aycock, and Virginia Vilnis for laboratory assistance. Supported in part by NHLI grant He 06285, PHS grants 5 TI GM0063 12, 1P01 GM 15571 0 1A1, 2F03 GM 29932 03, and the U.S. Navy Bureau of Medicine and Surgery (Code 3161).

26 November 1971; revised 21 January 1972

Specific Triiodothyronine Binding Sites in the Anterior Pituitary of the Rat

Abstract. *Studies with* L-[^{125}I]*triiodothyronine and* L-[^{125}I]*thyroxine, and with equilibrium dialysis of plasma proteins indicate that rat pituitary binds* L-*triiodothyronine 9.8 times as strongly as it does* L-*thyroxine. Injection of even small doses of nonradioactive* L-*triiodothyronine reduces the pituitary/plasma ratio of radioactive* L-*triiodothyronine, an indication of the existence of pituitary binding sites with a limited capacity for* L-*triiodothyronine. Limited capacity binding sites for* L-*thyroxine could not be demonstrated.*

The principal site of the hormonal feedback regulating secretion of the thyroid gland appears to be situated within the cells of the adenohypophysis, although ancillary sites within the hypothalamus have not been excluded (*1*). Selective localization of L-triiodothyronine (T_3) and L-thyroxine (T_4) in the adenohypophysis of the rat has previously been noted (*2*). In order to analyze the mechanism by which thyroid hormones interact within the pituitary to modulate the secretion of thyroid-stimulating hormone (TSH) we performed experiments to define and quantitate the kinetics of interchange of T_4 and T_3 between the plasma and the adenohypophysis of the rat. The results of these studies reveal the existence of a set of pituitary binding sites, apparently specific for T_3, which have a high affinity and a low capacity for this iodothyronine.

The kinetics of interchange of thyroid hormones between tissues and plasma were analyzed according to techniques previously described (*3*). Male Sprague-Dawley rats (150 to 250 g), on a diet of Wayne laboratory chow, were injected intravenously with a combined dose of either [^{125}I]T_3 (60 to 80 μc/μg) and [^{131}I]albumin (0.5 to 1 μc/mg), or [^{125}I]T_4 (50 to 70 μc/μg) and [^{131}I]albumin (0.5 to 1

μc/mg). Groups of animals were killed at designated intervals by exsanguination from the abdominal aorta. The anterior hypophysis and the whole brain were removed in all experiments and the liver and kidney in some experiments as well. The tissues were homogenized, and the homogenates of plasma and tissue were subjected to trichloroacetic acid (TCA) precipitation to remove radioiodide. Carrier protein (2.5 ml of human banked plasma) was added to each pituitary sample. The radioactivity within the pituitary, which was precipitable by TCA, consisted of the injected iodothyronine; this was confirmed by chromatographic studies indicating that over 85 percent of the ethanolic extracts of the pituitary, obtained 3 hours after the injection of T_3 or T_4, consisted of the injected iodothyronine. A correction was made for iodothyronine bound to plasma proteins trapped within tissues from the tissue [^{131}I]albumin counting rate and the simultaneous ratio of [^{125}I]-iodothyronine to [^{131}I]albumin in plasma. The net strength of hormone binding by plasma proteins was determined with equilibrium dialysis of plasma (*4*).

Secular equilibrium between T_4 in plasma and T_4 in the pituitary was established within 30 minutes after injection; the ratio of the concentration of T_4 in pituitary to the concentration of T_4 in plasma (pituitary/plasma isotopic activity ratio) was approximately 0.09. On the other hand, after the injection of tracer quantities of [^{125}I]T_3 the ratio of pituitary T_3 to plasma T_3 did not achieve its equilibrium value of 10.5 until 3 hours after injection. Of incidental interest was the finding, both in the case of T_4 and T_3, that the brain did not achieve secular equilibrium within the period of these experiments.

The partition of iodothyronine between plasma and tissue depends upon the relative strength of its binding by the tissues and by the plasma (*5*). It is possible to calculate, from previously developed equations, the relative strength of binding of T_3 to T_4 by any given tissue (*6*). In one group of animals (Fig. 1), the mean relative strength of binding by plasma $(b_p)_4/(b_p)_3$, determined by equilibrium dialysis, was 12.0. From the isotopic concentration ratios of the iodothyronines (Fig. 1), we calculated the ratio of the relative strengths of binding by the pituitary of T_3 and T_4, $(b_i)_3/(b_i)_4$, to be 9.8. This is considerably larger than corresponding ratios in other tissues studied; the relative cellular binding strengths, $(b_i)_3/(b_i)_4$, have been determined to be 2.0 in total carcass, 0.9 in liver, and 2.1 in kidney (*6*). The pituitary cells thus exhibit a great capability for the preferential accumulation of T_3.

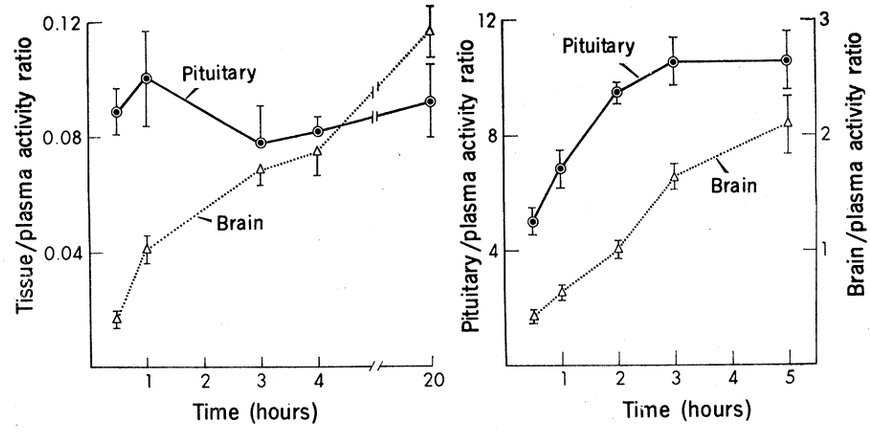

Fig. 1. (Left) Tissue/plasma activity ratios (percent of dose per gram/percent of dose per milliliter) after intravenous injection of [^{125}I]T_4 (0.5 ng) at time $t = 0$. The pituitary equilibrates with plasma rapidly after the injection; whole brain has not equilibrated with plasma at the end of the study. Each point represents the mean (± standard error of the mean) of five animals. (Right) Tissue/plasma activity ratios after intravenous injection of [^{125}I]T_3 (0.13 ng). Pituitary equilibrates with plasma by the third hour after injection. In the case of pituitary, the correction for trapped plasma protein accounts for 3 percent of the observed counting rate of T_3 and 50 percent of the observed counting rate of T_4.

In order to assess the saturation characteristics of the hormone binding sites, we determined the effect of injecting increasing quantities of nonradioactive T_4 and T_3 on the tissue/plasma isotopic activity ratio for pituitary, liver, kidney, and brain at 3 hours after the injection of the iodothyronines. With T_4, no significant changes were noted in any of the tissues studied when the

Table 1. Effect of loading doses of T_4 and T_3 on radioactive and nonradioactive iodothyronine partition between pituitary and plasma. All observations were made 3 hours after the injection of a combined dose of labeled and nonradioactive T_3 and T_4. Each result is the mean of a group of four to five animals. Concentrations of iodothyronines in plasma and in pituitary were estimated on the assumptions that (i) endogenous T_3 concentration is 1.76 pmole/ml (that is, 1.15 ng/ml), (ii) that the endogenous T_4 concentration is 43.1 pmole/ml (that is, 30.3 ng/ml) (*11*), and (iii) that the specific activity of iodothyronine in pituitary is equal to that in plasma. Significance of the difference in activity ratio between a given dose and the lowest dose injected in the series was evaluated by the Student's *t*-test.

Iodothyronine	Experiment dose (pmole)	Percent of dose per gram of pituitary	Activity ratio*	Estimated iodothyronine concentration in:	
				Plasma (pmole/ml)	Pituitary (pmole/g)
		Experiment A			
T_3	200	1.2	9.6	2.0	19
	415	1.1	8.8	2.2	19
	768	0.89	7.0†	2.8	19
	3,070	0.47	5.7†	4.4	25
	15,350	0.37	2.8†	22.1	62
		Experiment B			
T_3	384	1.9	9.2	2.6	23
	768	1.4	7.7	3.2	24
	3,070	1.0	3.8†	9.8	37
		Experiment C			
T_3	200	1.2	12.6	2.0	25
	15,350	0.5	4.2†	18.0	77
		Experiment D			
T_4	206	0.16	.09	47	4.2
	412	0.21	.11	51	5.8
	771	0.27	.10	64	6.2
	3,070	0.16	.09	99	8.8
	15,350	0.21	.09	410	37.0
		Experiment E			
T_4	206	0.17	.09	47	4.1
	15,350	0.16	.08	510	42.0

* Activity ratio is the percent of the injected dose of iodothyronine per gram of pituitary over the percent of the injected dose per milliliter of plasma. † Significantly different at $P < .01$.

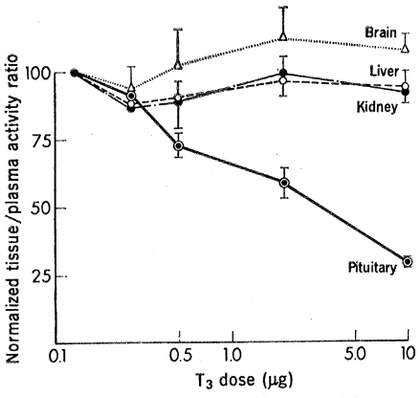

Fig. 2. Isotopic tissue/plasma activity ratios measured 3 hours after intravenous injection of [^{125}I]T_3 (0.13 ng) plus varying quantities of nonradioactive T_3. Determinations were made after trichloroacetic acid precipitation and correction for trapped plasma proteins within tissue. Ratios at the lowest total injected dose (0.13 ng) were normalized to 100, and those at higher doses are expressed as a percent of the low dose ratio. Of the tissues examined, only the pituitary tissue/plasma activity ratio shows a definite decrease with increasing doses of injected T_3.

injected dose was increased from 0.16 to 11.9 μg. Similarly, there were no changes in the tissue/plasma activity ratios for liver, kidney, and brain when increasing amounts of nonradioactive T_3 were injected (Fig. 2). In contrast, there was a progressive and marked reduction in the pituitary/plasma activity ratio as the dose of T_3 was increased from 0.1 to 10 μg.

The results of three separate experiments with increasing doses of T_3 are summarized in Table 1 and are compared with the results of two experiments in which T_4 was injected. In order to compare the amount of T_4 and T_3 bound to the pituitary, the doses and concentrations are expressed in molar units. Estimation of the nonradioactive T_3 and T_4 in plasma and pituitary is described in the legend to Table 1.

Changes in the pituitary/plasma activity ratio of T_3 could be attributed exclusively to changes in pituitary binding because no significant alterations in plasma binding were detected either for T_3 or T_4 when equilibrium dialysis was performed with serums from these animals. The results (Fig. 2, Table 1) indicate the existence of a set of binding sites in the pituitary, which are characterized by high affinity but low capacity for T_3. To the best of our knowledge, this is the first demonstration of apparently specific, and easily saturable, cellular binding sites for any of the iodothyronines. The absence of significant changes in the pituitary/plasma activity ratio for T_4 suggests that the T_4 binding sites are nonsaturable, and therefore probably nonspecific. The highest molar concentrations of T_4 in the pituitary were similar to the molar concentrations in the pituitary attained in the T_3 experiments.

When saturation experiments with loading doses of T_3 were repeated 5 hours after the injection of T_3, the same general dose-response relation was observed as in the 3-hour experiment. On the other hand, no changes in the pituitary/plasma concentration ratios were observed with increasing doses of T_3 when measurements were made 15 minutes after injection. It is possible that specific T_3 binding sites equilibrate relatively slowly with plasma, and that the early 15-minute uptake by the pituitary is mediated by rapidly equilibrating nonsaturable sites (*7*).

The results of experiment A (Table 1) indicate that as the concentration of T_3 in plasma was increased from 2.0 pmole/ml to 2.8 pmole/ml, a statistically significant decrease ($P < .01$) in the pituitary/plasma concentration ratio occurred without a change in the estimated pituitary content of T_3. These findings suggest that the pituitary binding sites are close to saturation at endogenous concentrations of T_3. As the T_3 concentration was further increased above 4.4 pmole/ml, there was a progressive increase in pituitary content of T_3, possibly due to the participation of secondary nonsaturable binding sites.

Injection of both T_4 and T_3 can inhibit pituitary secretion of TSH (*1*). Because it has been shown by Braverman, Ingbar, and Sterling (*8*) that T_4 can be converted to T_3 in man, the possibility must be considered that the inhibitory action of T_4 is mediated by T_3. Moreover, studies in our laboratory (*9*) have indicated that similar conversion occurs in the rat and is sufficiently large to account for most, if not all, of the hormonal potency of T_4. The existence of specific T_3 pituitary binding sites and the failure to find such binding sites for T_4 would be compatible with the concept that T_3 is the primary hormone. If the T_3 binding sites in the pituitary participate in the negative feedback system with TSH, the data in Table 1 would suggest that the binding sites are very close to being saturated at normal endogenous concentrations of T_3. One would therefore expect that under normal circumstances the pituitary would release TSH in an on-off fashion, the release being triggered by desaturation of the pituitary binding sites as a consequence of a small decrease in the concentration of circulating T_3. Additional studies are required to determine whether, in fact, the pituitary operates in this postulated fashion. It is of interest, however, that specific binding sites with limited capacity for estradiol have been demonstrated in rat pituitary by Kato and Villee (*10*).

ALAN R. SCHADLOW
MARTIN I. SURKS,
HAROLD L. SCHWARTZ
JACK H. OPPENHEIMER
Endocrine Research Laboratory, Department of Medicine, Montefiore Hospital and Medical Center, and *Albert Einstein College of Medicine, Bronx, New York 10467*

References and Notes

1. S. Reichlin in *The Thyroid*, S. C. Werner and S. H. Ingbar, Eds. (Harper & Row, New York, 1971), p. 95.
2. D. Ford, S. Kantounis, R. Lawrence, *Endocrinology* **64**, 977 (1958); D. Ford, H. Corey, J. Gross, *ibid.* **61**, 426 (1957).
3. J. Hasen, G. Bernstein, E. Volpert, J. H. Oppenheimer, *ibid.* **82**, 37 (1968).
4. J. H. Oppenheimer, R. Squef, M. I. Surks, H. Hauer, *J. Clin. Invest.* **42**, 1769 (1963).
5. J. H. Oppenheimer, M. I. Surks, H. L. Schwartz, in *Recent Progress in Hormone Research*, E. B. Astwood, Ed. (Academic Press, New York, 1969), p. 381.
6. J. H. Oppenheimer, H. L. Schwartz, H. C. Shapiro, G. Bernstein, M. I. Surks, *J. Clin. Invest.* **49**, 1016 (1970). Briefly, it was shown

$$\text{that } \frac{(b_i)_3}{(b_i)_4} = \frac{(b_p)_3}{(b_p)_4} \times \frac{(c_i{}^*)_3/(c_p{}^*)_3}{(c_i{}^*)_4/(c_p{}^*)_4}$$

where b_i is the cellular binding for an individual tissue or organ; b_p is the strength of plasma binding, which can be experimentally defined by equilibrium dialysis to be $b_p = (1 - DF)/DF$, where DF is the dialyzable fraction, that is, the fraction of labeled iodothyronine in the dialysis system in the free or nonprotein bound form; $c_i{}^*$ is the radioactive concentration of hormone in any tissue i; and $c_p{}^*$ is the radioactive concentration of hormone in plasma. The subscripts 3 and 4 refer to T_3 and T_4, respectively.
7. Strictly speaking, 3 to 5 hours after injection of loading doses of T_3, secular equilibrium between pituitary and plasma probably has not been established. Since T_3 is rapidly metabolized ($t_{1/2} = 6$ hours), the pituitary/plasma activity ratio for loading doses should gradually increase as the ambient concentration of T_3 in plasma decreases. Initial experiments confirm these theoretical considerations.
8. L. E. Braverman, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 855 (1970).
9. H. L. Schwartz, M. I. Surks, J. H. Oppenheimer, *ibid.* **50**, 1124 (1971).
10. J. Kato and C. Villee, *Endocrinology* **80**, 1133 (1967).
11. R. W. Heninger, F. C. Larson, E. C. Albright, *J. Clin. Invest.* **42**, 1761 (1963).
12. Supported by USPHS grant NSO-3000 and U.S. Army research contract DA-49-MD-2967. J.H.O. is a career scientist of the Health Research Council of New York City (contract I-222). A.R.S. is a Fellow in Endocrinology, Department of Medicine, Montefiore Hospital and Medical Center.

15 December 1971; revised 24 February 1972