tubular mechanism for creatinine secretion. An alteration in the serum creatinine resulting in enhanced tubular transport, or reducing apparent serum creatinine concentration without affecting filtration, is less probable. The high pre-infusion ratio in patients with renal insufficiency, and the failure to respond to exogenous creatinine, suggests that the mechanism for increased tubular excretion may continue to function as long as the stimulus of an elevated serum creatinine persists.

1. Shannon, J. A., J. Clin. Invest., 1935, v14, 403.
2. Shannon, J. A., Ranges, H. A., ibid., 1941, v20, 169.
3. Pitts, R. F., Physiology of the Kidney and Body Fluids, Year Book Medical Publishers, Inc., 1963, 116.
4. Doolan, P. D., Alpen, E. L., Theil, G. B., Am. J. Med., 1962, v32, 65.
5. Miller, B. F., Winkler, A. W., J. Clin. Invest., 1938, v17, 31.
6. Schreiner, G. E., Proc. Soc. Exp. Biol. & Med., 1950, v74, 117.
7. Hare, R. S., ibid., 1950, v74, 148.
8. Brod, J., Sirota, J. H., J. Clin. Invest., 1948, v27, 645.
9. Miller, Z., Miller, B. F., Proc. Soc. Exp. Biol. & Med., 1951, v78, 471.
10. Abdon, N. O., K. Fysiogr. Sällsk, i Lund Forh., 1935, v5, 140 (Cited by Shannon and Ranges).
11. Rehberg, P. B., Internationale physiologenkongresses, 16 ste., Zurich, Kongressbericht I, 1938, p4 (Cited by Shannon and Ranges).
12. Goldman, R., Studies on the Clearance of Exogenous Creatinine by Rats. From Renal Metabolism and Epidemiology of Some Renal Diseases, National Kidney Foundation, 1964.
13. Pitts, R. F., Physiology of the Kidney and Body Fluids, Year Book Medical Publishers, Inc., 1963, 124.

Received December 6, 1966. P.S.E.B.M., 1967, v125.

Competition Between Thyroxine and TRF at the Pituitary Level in the Release of TSH.* (32051)

WYLIE VALE, ROGER BURGUS, AND ROGER GUILLEMIN
Department of Physiology, Baylor University College of Medicine, Texas Medical Center, Houston

In the first note in which we reported on the existence and early purification of a TSH releasing factor (TRF) of hypothalamic origin(1), we had shown that the TSH-releasing activity of a given dose of TRF could be decreased and eventually abolished by previous administration of increasing doses of thyroxine. Subsequently we also demonstrated that minute doses of thyroxine (T_4) added to the medium in which pituitary fragments were pre-incubated for 15 minutes would inhibit the effects of TRF added to the incubation system(2); also the administration of T_4 *in vivo* to the animal prior to removal of the pituitary for *in vitro* incubation was shown to decrease or inhibit the activity of TRF subsequently added to the incubating tissue. These results have been confirmed by others(3,4).

The results from *in vivo* experiments reported here show that some sort of a competition between thyroxine and purified TRF seems to exist at the level of the pituitary for the release of TSH, as increasing doses of TRF can overcome the blockade of TSH-release produced by increasing doses of thyroxine. In the course of these studies, we became intrigued by the possibility that the molecule of TRF might contain iodine; this was studied by neutron activation analysis of a sample of highly purified TRF as reported below.

Materials and methods. A. All studies were conducted on rats, male, 40-50 g B.W., obtained from Cheek-Jones Farms, Houston. After 3 days of adaptation to the environment of the animal quarters, the animals received a single i.p. injection of 5 μc of ^{131}I. Three days later, they were submitted to the

* Supported by USPHS Research Grants AM 08290-03, HD 02577-01, Endocrinology Training Grant 5T1-GM-956-03 and FR 00254 to Common Research Computer Facility, Texas Medical Center, Houston.

TABLE I. Effects of Increasing Doses of Thyroxine (T_4) on TSH-Releasing Activity of a Constant Dose of TRF.

Treatment	N	Adjusted mean ± S.E.†	P
No T_4 + No TRF	5	2.354 ± .017	
No T_4 + TRF 8 U	5	2.474 ± .018	**
.25 μg T_4 + TRF 8 U	5	2.440 ± .016	**
2.5 μg T_4 + TRF 8 U	5	2.359 ± .005	—
5.0 μg T_4 + TRF 8 U	5	2.367 ± .013	—
10.0 μg T_4 + TRF 8 U	5	2.321 ± .017	—
15.0 μg T_4 + TRF 8 U	5	2.306 ± .016	—

† Abridged $\log_{10}$ of original data (cpm of second blood samples adjusted by covariance to cpm of first blood samples—see ref. 9); ± S.E., standard error.

N, number of animals/group; P, probability of statistical significance for the difference between the adjusted mean of an experimental group and the adjusted mean of the control group, calculated by the multiple comparison test of Dunnett(9); (—), P not significant; (*), P = .05; (**), P = 0.01.

various experimental protocols described below (B,C). Stimulation of endogenous release of TSH was assessed by measurements of blood radioactivity levels before and after injection of TRF or saline as previously described(9).

B. *Increasing doses of thyroxine, one dose of TRF.* Thirty-five animals prepared as above (A), were divided into 7 groups. At 2100 hr, doses of T_4 L-thyroxine Na) ranging from 0.25 μg to 15.0 μg were administered s.c. to the various animals of 6 groups, the remaining rats receiving saline as controls (Table I). The next day at 0900 hr all animals received an s.c. injection of 250 μg dexamethasone-21-PO_4.† At 1300 hr, (*i.e.*, 16 hours after T_4 injection and 4 hours after dexamethasone-21-PO_4) a blood sample was taken for measuring radioactivity and a dose of 8 units(6) of purified TRF was injected i.v. to each animal, except for those of the absolute control group (see Table I). Two hours later, a second blood sample was obtained for radioactivity counting. All injections and blood samplings were done in and from the jugular vein.

C. *Increasing doses of TRF, one dose of thyroxine.* Fifty-one rats prepared as above (A), were divided into 9 groups; the animals of 5 groups (7 rats/group) received s.c. 10 μg of T_4 at 2100 hr; all others (4 groups of 4 rats each) received a control injection of saline; the next day at 0900 hr all animals received an s.c. injection of 250 μg dexamethasone-21-PO_4.† At 1300 hr (*i.e.*, 16 hours after T_4 injection and 4 hours after dexamethasone administration) a blood sample was obtained from each animal for measuring radioactivity and doses of TRF ranging from 3.6 to 57.4 units(6) were injected along with saline controls to the various groups as described in Table II. Two hours later a second blood sample was obtained for radioactivity count.

D. *TRF.* One single batch of TRF was used in these experiments. It was prepared from acid extracts of sheep hypothalamus by gel filtration on Sephadex G25 in 0.1 M pyridine acetate, followed by ion exchange chromatography on substituted CM-Sephadex C50 and refiltration on Sephadex G25 in 1 N acetic acid (see 1,7,8); the material had a specific activity of 80 TRF units/mg(6).

E. *Neutron activation of TRF.* A 1.0 μg sample of a highly purified TRF preparation (15,000 U/mg) (see 8) was exposed to neu-

† The single injection of dexamethasone-21-PO_4 is part of our current bioassay for TRF in the rat. The rationale for this treatment in a test studying release of TSH has been explained in an earlier report(5). The administration of dexamethasone is not a requisite for obtaining the results reported here, as has been observed in other experiments not included in this note; administration of dexamethasone increases the sensitivity to TRF and decreases the variance of the response of the assay animals to TRF (see5)—hence its inclusion in the protocols of this series of experiments.

TABLE II. Effects of Increasing Doses of TRF on TSH Release in Animals With or Without Pretreatment With Thyroxine (T_4).

Treatment	N	Adjusted mean ± S.E.†	P
No T_4 + no TRF	4	2.357 ± .026	
No T_4 + TRF 3.6 U	4	2.439 ± .024	—
No T_4 + TRF 9.18 U	4	2.480 ± .024	*
No T_4 + TRF 22.95 U	4	2.578 ± .023	**
10 μg T_4 + no TRF	7	2.182 ± .021	
10 μg T_4 + TRF 3.6 U	7	2.204 ± .022	—
10 μg T_4 + TRF 8.18 U	7	2.227 ± .021	—
10 μg T_4 + TRF 22.95 U	7	2.226 ± .021	—
10 μg T_4 + TRF 57.37 U	7	2.343 ± .022	**

Legend as in Table I.

tron activation,‡ using the 100 KW pool-type nuclear reactor of Texas A&M University, College Station, Texas. Following a pilot experiment to determine optimal conditions of sensitivity, the sample of TRF dissolved in 1.0 ml deionized glass distilled water was subjected to a flux of *ca* 1×10^{12} neutrons/sec/cm^2 for 1 hour. The irradiated material was digested and the extracted iodine was counted at 0.46 and 0.54 Mev in a multichannel γ-spectrometer assuming the reaction I^{127} (n,γ) I^{128}.

Results. Increasing amounts of T_4 injected in animals given an invariable dose of TRF (8 U) lead to a dose of T_4 (2.5 μg) which completely inhibits the TSH releasing activity of that dose of TRF (Table I).

In the second experiment in which increasing doses of TRF were administered to animals with or without pretreatment with T_4, the analyses of variance on the dependent and independent variables (which precede our analysis of covariance—see 9) revealed a highly significant heterogeneity of the independent variables, which of course precluded further adjustment by covariance. Observation of the original data immediately confirmed that this effect was due to the discrepancy in the radioactivity levels of the first blood samples in the two populations created by the administration of the large dose of T_4 to some of the animals. The design of the analysis of these data was then changed to represent the two populations, with and without T_4 treatment (see Table II). As could be expected, independent analyses of variance for each population on dependent and independent variables showed that the incompatibilities to covariance adjustment had now completely disappeared; covariance analyses were then performed on the transformed data(9) and the final results with their statistical significance are shown in Table II. In the animals not pretreated with T_4, increasing doses of TRF from 3.6 to 9.18 and 22.95 units produced a linear response in terms of TSH release. Pretreatment with 10 μg T_4 completely inhibited or prevented the effects of 3.6, 9.18 and 22.95 units of TRF; the dose of 57.37 U of TRF was able however to overcome the inhibition due to T_4 and to produce a highly significant response (release of TSH).

Calculations based on reference standard I_2 samples of 0.1, 1.0 and 10.0 ppb (parts per billion) treated under the same conditions as those described above for the neutron irradiation of TRF showed that the sample of TRF contained less than 1.0 ppb iodine. This means that for this sample of TRF to contain one atom of I^{128}/molecule or one gram-atom I^{128}/Mole TRF, the *minimal* molecular weight of TRF would have to be 1.28×10^{11}, a figure grossly incompatible with the known properties of TRF(1,7,8,12).

Discussion. The results obtained here would appear to be the most direct evidence reported so far in favor of the theory that there exists a negative interaction between TRF and T_4 at the pituitary level with a "variable set point" for T_4 level at which TSH secretion takes place. According to this theory and in agreement with the results reported here, persistent increased secretion of TRF due to one mechanism or another will rapidly cease to stimulate release of TSH as plasma T_4 concentrations rise. The level at which this occurs will be determined by the secretion rate of TRF—the greater the secretion of TRF, the higher the plasma concentration of T_4 necessary to inhibit its TSH releasing activity. The results obtained in the experimental situations produced in the present report had been forecasted(10,11) on theoretical grounds and were considered as necessary characteristics of a (then hypothetical) TRF.

Some time ago we demonstrated and reported(7,8) that TRF is not a simple polypeptide as had been thought for some years, a conclusion that was later confirmed by Schally *et al*(12). These new observations and those reported here on the competition between T_4 and TRF for stimulation of the release of TSH led us to wonder whether some similarity might exist between the molecules of T_4 and TRF; hence the search for iodine in TRF. The results reported above on the neutron activation of the highly purified sample of TRF did not support the hypothesis that TRF might be an iodinated compound.

‡ Under contract with Hastings Radiochemical Works, Houston, Texas.

Summary. When increasing amounts of thyroxine are administered to rats otherwise given an invariable dose of TRF, a dose of thyroxine is found to completely inhibit the TSH-releasing activity of that dose of TRF. Conversely, when increasing amounts of TRF are administered to animals pretreated with an invariable quantity of thyroxine known to inhibit the TSH releasing activity of several doses of TRF, a dose of TRF is eventually reached that will overcome the inhibitory effect of that amount of thyroxine. Neutron activation of a highly purified preparation of hypothalamic TRF failed to reveal presence of iodine in the TRF molecule.

1. Guillemin, R., Yamazaki, E., Jutisz, M., Sakiz, E., C. R. Acad. Sci. (Par.), 1962, v255, 1018.

2. Guillemin, R., Yamazaki, E., Gard, D. A., Jutisz, M., Sakiz, E., Endocrinology, 1963, v73, 564.

3. Sinha, D., Meites, J., Neuroendocrinology, 1965, v1, 4.

4. Bowers, C. Y., Redding, T. W., Hawley, W. D., Program 40th Meeting Endocrine Society, 1966, p48.

5. Sakiz, E., Guillemin, R., Endocrinology, 1965, v77, 797.

6. Guillemin, R., Sakiz, E., Nature, 1965, v207, 297.

7. Guillemin, R., Burgus, R., Sakiz, E., Ward, D. N., C. R. Acad. Sci. (Par.), 1966, v262, 2278.

8. Burgus, R., Stillwell, R. N., McCloskey, J. A., Ward, D. N., Sakiz, E., Guillemin, R., The Physiologist, 1966, v9, 149.

9. Sakiz, E., Guilemin, R., Proc. Soc. Exp. Biol. & Med., 1964, v115, 856.

10. Purves, H. D., The Thyroid Gland, Pitt-Rivers, R., Trotter, W. R., Ed., Butterworth, 1964, v2, 1.

11. Reichlin, S., Endocrinology, 1960, v66, 327.

12. Schally, A. V., Bowers, C. Y., Redding, T. W., Barrett, J. F., Biochem. Biophys. Res. Com., 1966, v25, 165.

Received December 8, 1966. P.S.E.B.M., 1967, v125.

Myocardial Lesions: Spontaneous Development in Captive Ground Squirrels.* (32052)

JEROME P. SCHMIDT AND JACK A. REHKEMPER†
(Introduced by J. D. Fulton)
USAF School of Aerospace Medicine, Brooks Air Force Base, Texas

This investigation stems from the earlier incidental discovery of myocardial lesions in untreated, apparently healthy captive ground squirrels(1,2) and monkeys(3). It was suggested that the lesions observed may have resulted from naturally acquired microbial infections or from the stress of captivity. The duration of the captive period was not reported but is presumed to have been several months. This is the first report of studies in which tissues from noncaptive animals were available for comparison with those from animals held captive for definitive periods.

Methods. Hearts of 29 arctic ground squirrels *(Citellus undulatus)* shot or captured in Central Alaska were examined. Captured animals were individually caged and isolated from other animals and from known sources of infection. They were provided with water and a nutritionally adequate diet which included fresh, leafy vegetables and apples daily. The caged animals were sacrificed with pentobarbital sodium solution after 2, 3 or 4 weeks in captivity. Hearts of all animals were fixed in neutral 10% formalin. Representative portions of cardiac muscle were imbedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin, periodic acid-Schiff, Brown-Brenn, or Masson's trichrome. These preparations were then studied by light microscopy.

* The research reported in this paper was conducted by personnel of the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas, and the 2794 USAF Dispensary, Kelly Air Force Base, Texas. Further reproduction is authorized to satisfy needs of the U. S. Government.

† Present address: 2794 USAF Dispensary, Kelly Air Force Base, Texas.