position to both the cytoplasmic extensions of the myoepithelial cells and the secretory cells (Figs. 2 and 3). In addition, membrane evaginations of the myoneural junctions are also seen (Figs. 2 and 3). There appear to be fewer dense-cored vesicles in the nerve endings associated with the myoepithelial and secretory cells than in the nerve endings found in the connective tissue. The innervation of the Harderian gland we observed is similar in both male and female hamsters.

Fourman and Ballantyne (*8*) showed by histochemical studies that nerve fibers are associated with blood vessels of the Harderian gland of the Aylesbury duck. They also suggested that the secretory activity of the gland is influenced by blood flow to the gland. Our observations of the presence of nerve fibers associated with blood vessels of the interlobular connective tissue support Fourman and Ballantyne's suggestion. Cohn (*3*) showed that in mice numerous blood vessels are present in the lobar and interlobular connective tissue of the Harderian gland, but he did not report any innervation of the gland.

Tashiro *et al.* (*9*) reported that injection of rats with the neurotransmitter acetylcholine will produce, in a matter of minutes, a copious secretion of "bloody tears" from the Harderian gland. Chiquoine (*10*) suggested that myoepithelial cells may respond to acetylcholine by contraction, thus squeezing out the contents from the secretory cells. This is analogous to the contractile response of the myoepithelial cells of the mammary gland to oxytocin that is responsible for the phenomenon of "milk let down." The presence of nerve endings in apposition to the myoepithelial cells and the myoneural junctional folds (Fig. 3) indicates that the neurotransmitter passes a myoneural junction instead of diffusing through the connective tissue. This may also explain the fast response of the Harderian gland to the injection of acetylcholine.

It has been observed that the nature of the secretion of the Harderian gland is intermediate between the apocrine and holocrine types, with a greater tendency toward being apocrine (*5*). It is difficult at present to explain the significance of the relationship between the nerve fibers and the secretory cells which we observed. However, it appears that the process of secretion is affected directly by nervous stimulation of the myoepithelial cells.

Derrien and Turchini (*11*) and Strong (*12*) reported the appearance of high concentrations of porphyrin compounds in the Harderian glands of mice and rats soon after their birth. A recent study by Wetterberg, Geller, and Yuwiler (*2*) suggested that the Harderian gland might serve as an extraretinal photoreceptor in young rats. Our observations do not provide any direct evidence to support their suggestion.

CORAZON D. BUCANA
MATHEW J. NADAKAVUKAREN
Department of Biological Sciences, Illinois State University, Normal 61761

References and Notes

1. F. A. Davis, *Trans. Amer. Ophthalmol. Soc.* **27**, 461 (1929).
2. L. Wetterberg, E. Geller, A. Yuwiler, *Science* **167**, 884 (1970).
3. S. A. Cohn, *Histochem. Cytochem.* **3**, 342 (1955).
4. N. Björkman, L. Nicander, B. Schantz, *Z. Zellforsch.* **52**, 193 (1960).
5. K. C. Kanwar, *Cellule* **61**, 129 (1960).
6. M. A. Woodhouse and J. A. G. Rhodin, *J. Ultrastruct. Res.* **9**, 76 (1963).
7. G. Kelenyi and S. Orban, *Acta Morphol. Acad. Sci. Hung.* **13**, 155 (1964).
8. J. Fourman and B. Ballantyne, *Anat. Rec.* **159**, 17 (1967).
9. S. Tashiro, C. C. Smith, E. Badger, E. Kezur, *Proc. Soc. Exp. Biol. Med.* **44**, 658 (1940).
10. A. D. Chiquoine, *Anat. Rec.* **132**, 569 (1958).
11. E. Derrien and J. Turchini, *C. R. Soc. Biol.* **91**, 637 (1924).
12. L. C. Strong, *Proc. Soc. Exp. Biol. Med.* **57**, 78 (1944).
13. We thank Professor Arthur Merrick for reviewing the original manuscript for us.

18 October 1971

High-Resolution Proton Magnetic Resonance Spectra of a Rabbit Sciatic Nerve

Abstract. *Proton magnetic resonance spectra (220-megahertz field) of an isolated rabbit sciatic nerve in its native state have been observed and assigned to the extracellular water, intracellular water, and phospholipids of the nerve. This study indicates that the nerve fibers contain fluid-like hydrophobic regions, in agreement with the results of recent electron spin resonance spin-labeled studies of excitable membranes of nerve and muscle.*

Most mammalian nerve fibers are surrounded by a sheath of insulating material known as myelin, which consists of multilayers of membranes of the Schwann cells. Because of its lamellar structure, the myelin sheath has served as an important model for the lamellar membrane and has been the subject of numerous investigations, including extensive histological examinations and electron microscopy and x-ray diffraction studies (*1*). As a result of these efforts, the structure of the myelin sheath is perhaps better understood than that of any other membrane system.

In recent years, magnetic resonance spectroscopy has also been applied to the study of nerve tissues. Most of these studies, however, have been concerned with the state of intracellular water in the nerve. Fritz and Swift (*2*) have studied the state of intracellular water in frog sciatic nerves coated with mineral oil and have suggested that this intracellular water can exist in two states, depending on whether the nerve

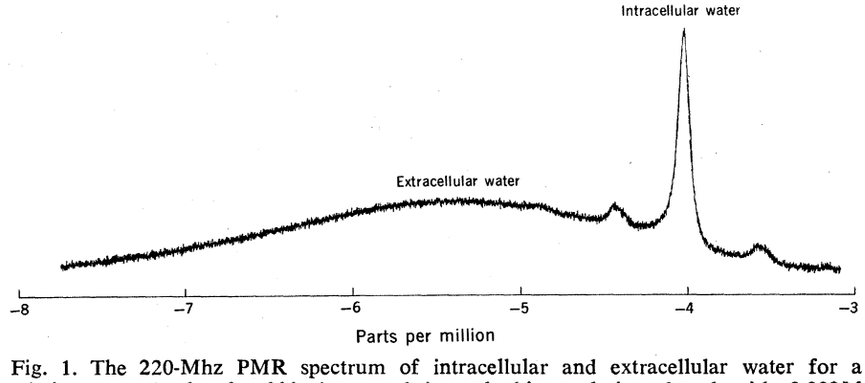

Fig. 1. The 220-Mhz PMR spectrum of intracellular and extracellular water for a sciatic nerve trunk of rabbit immersed in a bathing solution doped with 0.002*M* Mn^{2+}. The two smaller peaks adjacent to the intracellular water resonance are spinning side bands.

is polarized or depolarized. Chapman and McLauchlan (*3*) have examined sliced rabbit sciatic nerves on a spectrometer (Varian A-60) and have concluded that the bulk of the water inside the nerve is in a partially oriented state. More recently, however, Klein and Phelps (*4*) have performed similar measurements on rat phrenic nerves using deuteron magnetic resonance spectroscopy, but these experiments failed to produce evidence for any specific orientation of intracellular water.

Although Jenkinson *et al.* (*5*) have reported proton magnetic resonance (PMR) studies of both whole and sonicated beef brain myelin, a similar PMR study of a nerve in its native state has not appeared. We report here a 220-Mhz PMR study of an isolated rabbit sciatic nerve in its native state. Female rabbits (New Zealand White), weighing 1.4 to 1.8 kg each, were killed with Dry Ice. The sciatic nerves were excised and rinsed in normal saline, and the connective tissues were removed under a microscope. The nerve fibers, which were approximately 3 cm long, were then tied at both ends to a capillary with filaments from nylon stockings. This capillary contained tetramethylsilane (TMS), which was used as an external reference for measurements of chemical shift. The entire assembly was then inserted with Teflon spacers into a nuclear magnetic resonance (NMR) tube (outside diameter, 5 mm) containing modified Locke's physiological solution. This solution was prepared in deuterated water (99.5 percent) and was glucose-free. The samples were examined on an NMR spectrometer (Varian HR-220) at the normal probe temperature of 18°C. A 4096-channel computer (Fabri-Tek 1074) of average transients was used to enhance the signal-to-noise ratio where necessary.

The strongest signal in the PMR spectrum is a sharp singlet 2 hz in width and arises from the water in the nerve trunk and the bathing Locke's solution. The intensity of this resonance can be reduced significantly by rinsing the nerve trunk successively in deuterated Locke's solution. As shown in Fig. 1, this water signal can be resolved into two distinct peaks upon the addition of a paramagnetic ion such as Mn^{2+} into the bathing solution. The sharp resonance can be assigned to intracellular water and the broadened resonance at lower field to the bathing solution containing the paramagnetic ion. The use of paramagnetic ions to distinguish intracellular and extracellu-

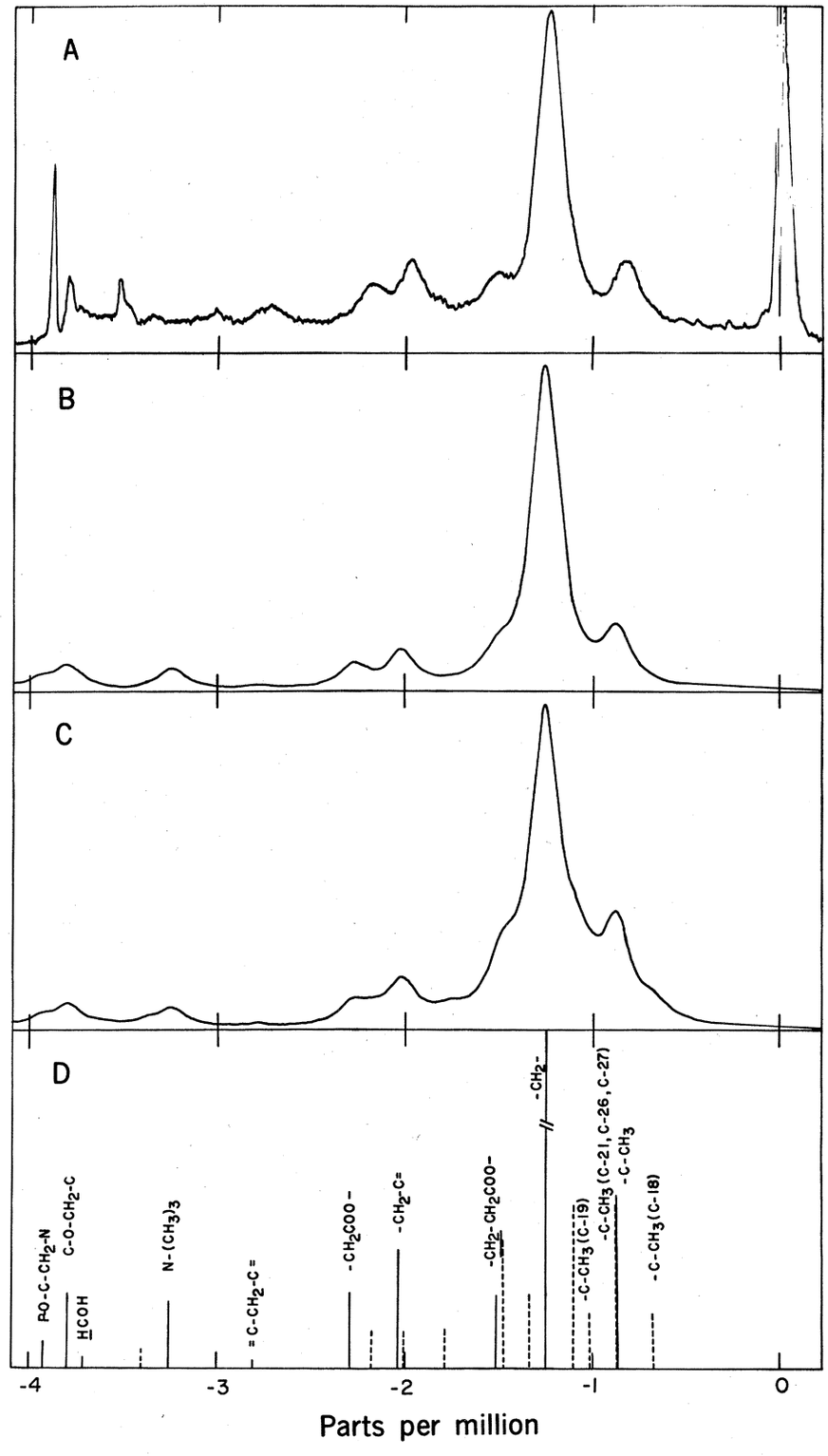

Fig. 2. (A) Proton magnetic resonance spectrum (220 Mhz) of an intact sciatic nerve in the region from 0 to 4 ppm downfield from TMS. (B) A computed spectrum of the phospholipids in the rabbit sciatic nerve. (C) A computed spectrum of the phospholipids and cholesterol in the rabbit sciatic nerve. (D) Proton spectral assignments and expected intensities. The phospholipid protons are indicated by solid lines; the cholesterol protons are depicted by dotted lines. Except for the methylene peak, all intensities are drawn to scale.

lar water was first reported by Fritz and Swift (*2*) in their studies of frog sciatic nerves. However, since their measurements were made on a Varian A-60 spectrometer, where the polarizing magnetic field is perpendicular to the axis of the sample, the paramagnetic susceptibility of the solution doped with Mn^{2+} shifts the extracellular water resonance to the high-field side of the intracellular water resonance. In the case of the HR-220 magnet-sample geometry, the polarizing magnetic field is along the long axis of the sample and the bulk susceptibility effect is twice in magnitude [in parts per million (ppm)] and opposite in sign to that observed in conventional spectrometers (*6*). The assignment of the water resonances can therefore be confirmed if one examines the spectrum of the nerve at the two different magnet-sample configurations. The larger bulk susceptibility shifts on the HR-220 spectrometer together with the additional dispersion of chemical shifts at the higher NMR frequency also lead to better resolution of the extracellular and intracellular water resonances. As anticipated, a nerve homogenate prepared in deuterated water gave a water peak that was not resolvable upon the addition of paramagnetic ions.

In addition to the strong water signals, we have observed several relatively sharp, although weaker, resonances in the region from 0 to 4 ppm downfield from TMS. A cumulative spectrum of this region after 36 scans is shown in Fig. 2A. No temperature dependence of this spectrum was observed, either with respect to spectral intensity or spectral position. The spectrum was also unaffected by the presence of 0.01*M* paramagnetic ions in the bathing solution. Also, the line width of the resonance at −1.26 ppm was found to vary linearly with the strength of the magnetic field, increasing from 18 hz at a field of 100 Mhz to 38 hz at a field of 220 Mhz.

There are several possible origins for this spectrum. One explanation is that it is due to phospholipids in the nerve trunk. Another possibility is that the spectrum is due to either triglycerides indigenous to the nerve fiber or to interstitial fatty substances that we have failed to remove from the nerve trunk. In order to ascertain whether this latter possibility was the case, we examined the 220-Mhz PMR spectrum of a nerve homogenate prepared in chloroform (Fig. 3). Here, the resonance widths are narrower; for example, the strong methylene resonance at − 1.26 ppm is only 15 hz wide. The choline methyl resonance as well as those due to the cholesterol are also readily discernible. The well-resolved peaks at − 0.68 ppm and − 1.02 ppm can be assigned to the C-18 and C-19 angular methyl groups of cholesterol.

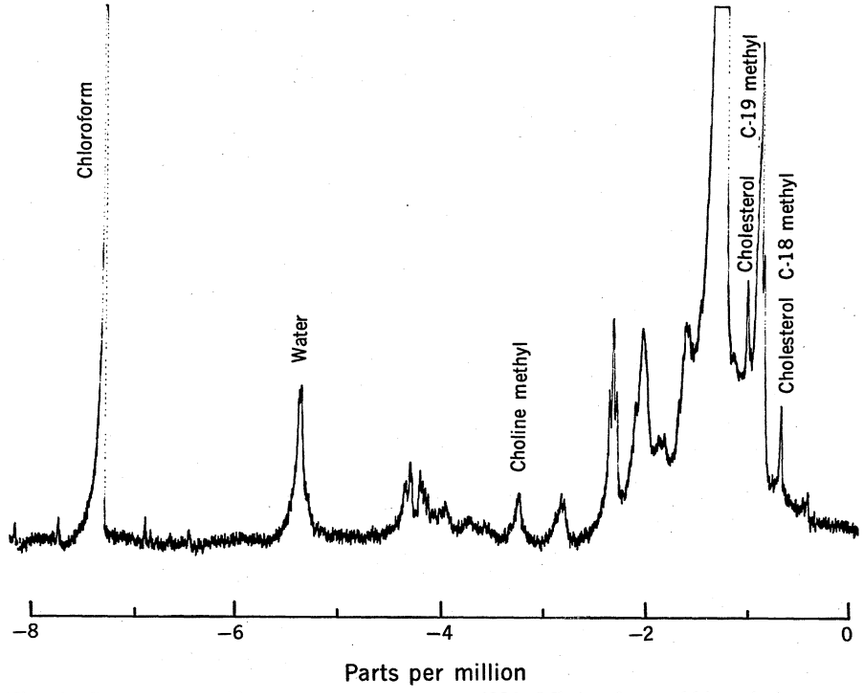

Fig. 3. Proton magnetic resonance spectrum (220 Mhz) of a rabbit sciatic nerve homogenate prepared in chloroform.

The methyl resonance at −0.88 ppm is due to the cholesterol C-21, C-26, and C-27 methyl groups as well as to the terminal methyl protons of the fatty acid side chains. We have used the relative intensities of these methyl resonances to ascertain the concentration of triglycerides and free fatty acids that might be present in the nerve fiber. Comparison of the observed relative intensities with those expected on the basis of the known lipid composition determined by Sheltawy and Dawson (*7*) by means of thin-layer chromatography indicates that triglycerides and free fatty acids can be present only in trace quantities. We confirmed this conclusion by carrying out a separate lipid analysis by thin-layer chromatography of a sciatic nerve homogenate prepared in a chloroform-methanol mixture (1:1). On a Brinkmann G plate, using a solvent system that consisted of heptane, diethyl ether, and acetic acid (70:10:0.5), we found the cholesterol band to be somewhat more intense than the phospholipid band whereas the band attributable to triglyceride and fatty acid was barely visible under hot sulfuric acid spray.

In view of the trace amount of triglycerides and fatty acids found, we have assigned the observed nerve spectrum in the methyl and methylene region to the phospholipids in the nerve. Since cholesterol might also contribute to the spectrum, an attempt was made to synthesize the observed spectrum expected for the phospholipids and the total lipids in the nerve on the basis of the known lipid composition reported by Sheltawy and Dawson and the known spectral positions for the various protons. These are shown in Fig. 2, B and C, respectively. Our assignments and the contributions of the various proton resonances are summarized in Fig. 2D. Comparison of the theoretical and the observed spectra suggests that there is greater resemblance of the nerve spectrum to that of the phospholipids without the cholesterol, but the differences between the two computed spectra are not really sufficiently pronounced to warrant an unequivocal conclusion on this point.

This NMR study reveals that the sciatic nerve contains hydrophobic regions that are sufficiently fluid to give a high-resolution PMR spectrum. These liquid-like hydrophobic regions have been attributed to the fatty acid side chains of the phospholipids of the nerve. These observations are consistent

with the results of recent electron spin resonance spin-labeled studies of excitable membranes of nerve and muscle (*8*). Additional experiments, however, are needed in order to determine whether the phospholipid spectrum observed here is due to excitable membranes in the nerve or to myelin membranes of the Schwann cells.

PHOEBE DEA
SUNNEY I. CHAN
Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena 91109

FRANK J. DEA
Department of Clinical Pharmacology and Pharmacokinetics, University of Southern California, Los Angeles 90033

References and Notes

1. J. D. Roberts, *Progr. Biophys. Biophys. Chem.* **10**, 343 (1960).
2. O. G. Fritz, Jr., and T. J. Swift, *Biophys. J.* **7**, 675 (1967).
3. G. Chapman and K. A. McLauchlan, *Nature* **215**, 391 (1967).
4. M. P. Klein and D. E. Phelps, *ibid.* **224**, 70 (1969).
5. T. J. Jenkinson, V. B. Kamat, D. Chapman, *Biochim. Biophys. Acta* **183**, 427 (1969).
6. D. H. Live and S. I. Chan, *Anal. Chem.* **42** 791 (1970).
7. A. Sheltawy and R. M. C. Dawson, *Biochem. J.* **100**, 12 (1966).
8. W. L. Hubbell and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.* **61**, 12 (1968); M. Calvin, H. H. Wang, G. Entine, D. Gill, P. Ferruti, M. A. Harpold, M. P. Klein, *ibid.* **63**, 1 (1969); W. L. Hubbell and H. M. McConnell, *ibid.*, p. 16.
9. Supported in part by grant GM-14523 from the U.S. Public Health Service and grant GP-8540 from the National Science Foundation. Contribution No. 4315 from the Noyes Laboratory of Chemical Physics.

23 August 1971

Thyrotropin-Releasing Hormone: Evidence for Thyroid Response to Intravenous Injection in Man

Abstract. *Administration of thyrotropin-releasing hormone to normal subjects causes a prompt rise in plasma thyrotropin concentration, followed by a significant increase in circulating plasma triiodothyronine. These observations may prove to be of value in simultaneously assessing the ability of the pituitary and thyroid glands to respond to their trophic hormones.*

Discovery of the hypothalamic releasing and inhibiting factors has broadened understanding of the control mechanisms of hormone release. Isolation and synthesis of thyrotropin-releasing hormone (TRH) (*1*) has permitted a closer scrutiny of this particular releasing factor in man. Studies from many laboratories have shown that intravenous administration of TRH causes prompt release of thyrotropin (TSH) from the pituitary gland (*2, 3*). This effect is probably mediated both by increased release of preformed TSH from the pituitary and by increased de novo synthesis (*4*). Since intramuscular administration of TSH in man leads to a rise in circulating thyroxine (T_4) concentrations after several hours, it would be anticipated that intravenous administration of TRH followed by a rise in endogenous TSH would also be followed by a rise in circulating thyroid hormone. Surprisingly, the effects of TRH on circulating thyroid hormone have not yet been clearly established. Some workers have found an increase in serum T_4 (*5*), but others have been unable to confirm the results (*6*). Failure to demonstrate a clear-cut rise in circulating thyroid hormone or any other definite effect of the TRH-induced elevations of TSH on thyroid gland function in man constitutes the most puzzling remaining question with regard to TRH action.

To clarify this important problem we administered TRH intravenously to normal male subjects and closely examined the changes in thyroid hormone during the first hour.

Eight males, aged 28 to 50 years, with no endocrinological problems, were studied. All subjects were clinically euthyroid, none had palpable enlargement of their thyroid glands, and all had normal concentrations of thyroxine and free thyroxine. After an overnight fast, an intravenous cannula was inserted in the antecubital fossa, and the subjects were allowed to rest quietly in bed for half an hour. Two baseline blood samples were collected at 15-minute intervals; then TRH (100 μg) was adminstered intravenously as a bolus. Blood samples were collected in heparinized syringes at 10, 20, 40, and 60 minutes, centrifuged immediately, and stored at −4°C until assayed.

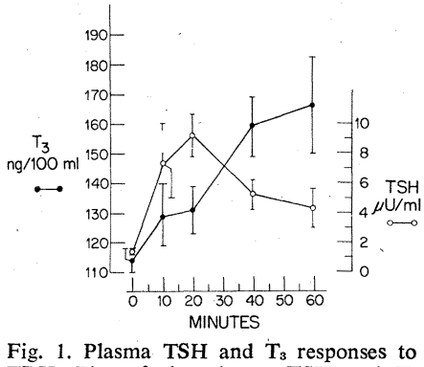

Fig. 1. Plasma TSH and T_3 responses to TRH. Plot of the plasma TSH and T_3 concentrations after an intravenous injection of 100 μg of TRH. Each point represents the mean value for the eight patients, and the height of the vertical line indicates the standard error about the mean.

Triiodothyronine (T_3) in plasma was measured by radioimmunoassay (*7*) as was plasma TSH (*8*). Plasma T_4 was assayed by competitive protein binding analysis (*9*) by the Boston Medical Laboratories. The percentages of free T_4 were determined by equilibrium dialysis in a dilute system (*10*) modified by adding T_3 labeled with ^{125}I as well as T_4 labeled with ^{131}I, prior to dialysis in order to simultaneously measure percentage of free T_3.

All eight subjects showed a rise in plasma TSH and T_3 (Fig. 1). Plasma TSH rose from 1.3 ± 0.8 μU/ml (mean ± standard error) to a maximum of 9.2 ± 1.4 μU/ml at 20 minutes and then fell toward control values during the remainder of the observation period. Plasma T_3 rose from a baseline of 118 ± 4 ng/100 ml to a plateau of 159 ± 10 ng/100 ml at 40 minutes. The change was highly significant ($P < .001$). Plasma T_4, not shown in Fig. 1, also appeared to rise at 60 minutes (from a mean of 4.9 ± 0.5 μg/100 ml to 5.7 ± 0.5 μg/100 ml). Although this increase was statistically significant ($P < .01$) by the paired *t*-test, it was not as great or as rapid as that of TSH or T_3. Indeed, individual changes were small: 6.5, 6.5, 3.5, 3.0, 4.0, 4.3, 6.8, and 4.5 μg/100 ml before TRH administration and 6.8, 8.3, 5.3, 4.3, 4.3, 5.0, 7.0, and 5.0 μg/100 ml afterward. No changes were found in percentage free T_3 and percentage free T_4.

Our results confirm previous reports of a prompt increase in TSH concentration in response to intravenous administration of TRH and extend these observations to include a rise in circulating T_3. The elevation in T_3 was seen in all subjects and occurred 20 minutes after the elevation in TSH. Plasma T_3 remained elevated throughout the observation pe-