TABLE I—Distribution of ABO Blood Groups in Chronic Carriers and Non-carriers of the SH Antigen

Blood Group							Donors				Mentally Retarded				SH Antigenaemia Incidence*
							Carriers		Non-carriers		Carriers		Non-carriers		
							No.	%	No.	%	No.	%	No.	%	
O	..	..	..	..	..	78	44.1	3,571	45.1	77	45.3	449	47.5	11.8	
A	..	..	..	..	..	61	34.5	2,893	36.5	64	37.6	343	36.3	10.0	
B	..	..	..	..	..	25	14.1	1,104	13.9	19	11.2	128	13.6	10.9	
AB	..	..	..	..	..	13	7.3	351	4.4	10	5.9	25	2.6	10.3	
Total	..	..	..	..	..	177	100.0	7,919	100.0	170	100.0	945	100.0	10.8	

*Wassaic State School only.

TABLE II—Distribution of ABO Blood Groups in Mentally Retarded Patients in Relation to Race and Mental Diagnosis

Blood Group	Caucasians				Negroes				Mongols				Non-mongols			
	Carriers		Non-carriers		Carriers		Non-carriers		Carriers		Non-carriers		Carriers		Non-carriers	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O	68	47.9	369	46.1	9	32.1	80	55.2	45	46.4	67	40.1	32	43.8	382	49.1
A	51	35.9	306	38.3	13	46.5	37	25.5	39	40.2	71	42.5	25	34.3	272	35.0
B	15	10.6	101	12.6	4	14.3	27	18.6	7	7.2	25	15.0	12	16.4	103	13.2
AB	8	5.6	24	3.0	2	7.1	1	0.7	6	6.2	4	2.4	4	5.5	21	2.7
Total	142	100.0	800	100.0	28	100.0	145	100.0	97	100.0	167	100.0	73	100.0	778	100.0

however, be emphasized that an association with blood groups was not found in posttransfusion hepatitis studies carried out with large patient samples and adequate controls in the U.S.A. (189 patients) (Allen and Sayman, 1962), Japan (587 patients) (Shimada *et al.*, 1965), and Poland (70 patients) (Szmunn *et al.*, 1966). Thus it seems unlikely that serum hepatitis could have been a selective factor determining the distribution of ABO or Rh antigens in populations, or that these blood groups are associated with susceptibility to either acute or chronic forms of serum hepatitis virus infections.

This study was supported by NIH Grants AI 09516 and HE 09011 and a grant-in-aid from the Strasburger Foundation. A.M.P. is the recipient of a Career Scientist Award of the Health Research Council of the City of New York under Contract I-533. Requests for reprints should be addressed to W.S., The New York Blood Center, 310 East 67th Street, New York, N.Y. 10021.

References

- Allen, J. G., and Sayman, W. A. (1962). *Journal of the American Medical Association*, **189**, 1079.
 Lewkonja, R. M., and Finn, R. (1969). *British Medical Journal*, **3**, 268.
 Prince, A. M. (1968). *Proceedings of the National Academy of Sciences of the United States of America*, **60**, 814.
 Prince, A. M., and Burke, K. (1970). *Science*, **169**, 593.
 Shimada, N., *et al.* (1965). In *Proceedings of the 10th Congress of the International Society of Blood Transfusion*, p. 1066.
 Szmunn, W., Gawronowa, M., and Horoch Cz. (1966). *Epidemiological Reviews*, **20**, 201.
 Szmunn, W., Pick, R., and Prince, A. M. (1970). *American Journal of Epidemiology*, **92**, 51.
 Szmunn, W., and Prince, A. M. (1970). Intra-Institutional Spread of Serum Hepatitis Specific Antigen. Abstract of the American Public Health Association Annual Meeting, Houston, Texas, October.
 Vesely, K. T. (1969). *Vnitřní Lékařství*, **15**, 1089.
 Zuckerman, A. J., and McDonald, J. C. (1963). *British Medical Journal*, **2**, 537.

Further Observations on the Effect of Synthetic Thyrotrophin-releasing Hormone in Man

B. J. ORMSTON, J. R. KILBORN, R. GARRY, JACQUELINE AMOS, REGINALD HALL

British Medical Journal, 1971, **2**, 199-202

Summary

Synthetic thyrotrophin-releasing hormone (TRH) given intravenously in doses of 50 µg or more causes a significant rise in serum thyroid-stimulating hormone (TSH) levels but has no effect on serum growth hormone, plasma luteinizing hor-

mone, or plasma 11-hydroxycorticosteroids under carefully controlled basal conditions.

The peak TSH response to intravenous TRH occurs at 20 minutes. The mild and transient side effects, which occur only after intravenous TRH, include nausea, a flushing sensation, a desire to micturate, a peculiar taste, and tightness in the chest. There is considerable variability in response to a given dose of TRH in the same subject on different occasions and in different subjects. Oral administration of TRH in doses of 1 mg and above causes a rise in serum TSH, maximal at two hours, a consistent response being obtained at doses of 20 mg and above. A rise in serum protein-bound iodine (P.B.I.) follows that of TSH, a consistent response being observed at 40-mg doses of TRH orally. Measurements of serum TSH after intravenous administration of TRH or of serum TSH or serum P.B.I. after oral TRH should prove useful tests of pituitary TSH reserve.

University of Newcastle upon Tyne and Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP

B. J. ORMSTON, M.B., B.S., Research Fellow in Endocrinology
 J. R. KILBORN, M.B., PH.D., Research Officer in Medicine
 R. GARRY, M.B., B.S., Senior House Officer in Medicine
 JACQUELINE AMOS, Chief Technician
 REGINALD HALL, M.D., F.R.C.P., Professor of Medicine

Introduction

The structure of thyrotrophin-releasing hormone (TRH) has been defined and the molecule synthesized in several laboratories (Boler *et al.*, 1969; Burgus *et al.*, 1969; Gillesen *et al.*, 1970). It is a tripeptide, containing the amino-acids glutamic acid (glu), histidine (his), and proline (pro), which is active in the form L-pyrroglutamyl-L-histidyl-L-proline-amide. Intravenous injection of this hormone has been shown to produce a significant rise in serum thyroid-stimulating hormone (TSH) in normal subjects (Bowers *et al.*, 1970a; Fleischer *et al.*, 1970; Hall *et al.*, 1970). This paper extends these observations and describes the effects of oral administration of the hormone.

Subjects and Methods

Eight men and six women aged 18-38 years volunteered for these experiments. The purpose of the study was explained and informed consent obtained.

Thyrotrophin-releasing Hormone.—The TRH used in this study was supplied by Roche Products Ltd. in the form of the acetate 0.2798 g of TRH acetate is equivalent to 0.24 g of the free amide). All doses described subsequently refer to the acetate. The material used for intravenous administration was made up in 0.9% sodium chloride solution and stored at concentrations of 100 or 400 µg/ml at -20°C until use. All intravenous injections of TRH were given in a constant volume (2 ml) of normal saline. The material used for oral administration was prepared immediately before use. The required dose of pure TRH acetate powder was mixed with 10 ml of tap-water and then consumed immediately, the phial being rinsed with a further 10 ml of water.

Thyroid-stimulating Hormone Immunoassay.—Serum TSH levels were measured by a modification of the double antibody technique of Odell (Raud and Odell, 1969; Hall *et al.*, 1971) using guinea-pig antihuman TSH and rabbit anti-guinea-pig gammaglobulin as second antibody. Medical Research Council HTSH Standard A was used as the reference preparation.

Other Hormone Assays.—Serum growth hormone levels were measured in this laboratory as previously described (Wilkinson *et al.*, 1970). Results were expressed in terms of Medical Research Council Standard A HGH. Plasma luteinizing hormone (LH) was measured by immunoassay by Dr. W. M. Hunter, of the M.R.C. Clinical Endocrinology Unit, Edinburgh. Plasma LH is expressed in µU/ml of Medical Research Council ICSH Standard 68/40, assuming 40 U/ampoule. Plasma 11-hydroxycorticosteroids were measured by a modification of the method described by Murphy (1967) by Mr. D. Cook in the department of clinical biochemistry, University of Newcastle upon Tyne. Serum protein-bound

iodine (P.B.I.) levels were measured by the Technicon AutoAnalyzer technique of Riley and Gochman (1964).

Thyrotrophin-releasing Hormone Administration.—(1) *Intravenous administration:* (a) Extended test: After an overnight fast an indwelling cannula was inserted into a forearm vein. Thirty minutes later the first blood specimen was obtained (-15 minutes) and a further specimen was taken immediately

TABLE I—TSH Response to Intravenous TRH Extended Tests

Subject	Dose µg	TSH Response (µU/ml)										
		Time (minutes):	-15	0	+5	+15	+20	+25	+30	+60	+120	+180
1	50		1.1	0.7	1.2	3.6	4.1	4.4	4.6	2.0	0.5	1.7
1	100		<0.5	0.5	0.7	4.8	4.6	5.1	3.8	2.5	1.1	1.1
1	200		0.8	0.5	2.6	5.0	5.4	6.4	6.4	2.7	1.1	1.2
1	400		0.8	0.6	2.0	5.6	7.2	5.9	5.9	4.8	1.7	1.1
2	200		1.6	1.0	5.4	5.4	6.6	5.1	5.1	2.4	1.4	1.8
2	200		0.5	0.8	1.6	3.1	3.2	3.2	2.4	1.6	0.6	0.9
2	200		0.8	1.4	2.8	5.6	5.6	4.2	4.0	2.8	1.3	1.8

before the rapid intravenous injection of TRH. The subjects rested in the supine position throughout the test, serial blood samples being obtained over a subsequent three-hour period (Table I). (b) Short test: The subjects were not fasting and proceeded with their routine activities during the test. Specimens of blood were obtained before and 20 and 60 minutes after the injection of 100 µg of TRH. *Oral administration:* After an overnight fast a basal blood sample was obtained and the dose of TRH was ingested. Further blood samples were obtained at the times indicated (Table II). The subjects were allowed to proceed with their normal activities during the test and to have breakfast one hour after the dose of TRH.

Results

The normal range of absolute values for serum TSH obtained in this laboratory is 0.6-4.2 µU/ml, mean 1.6 µU/ml, standard error ± 0.15 µU/ml (Hall *et al.*, 1971). These results were derived from 29 subjects (18 men and 11 women) whose ages ranged from 20 to 79 years.

INTRAVENOUS INJECTION

Extended Test.—Doses of 50 µg and above caused a significant rise of serum TSH levels in all normal subjects. In a series of seven extended tests the mode time of the peak response was 20 minutes—20 minutes in 4, 25 minutes in 2, 30 minutes in 1 (Table I). The specificity of the response of the anterior pituitary to TRH injection is shown in Table III. In a series of five experiments on two fasting subjects who remained supine throughout the test TSH levels

TABLE II—Effect of Oral TRH on Serum TSH and Serum P.B.I. Levels

Subject	Sex	Dose of TRH (mg)	TSH (μU/ml)							P.B.I. (μg/100 ml)						
			Time (hours):							0	½	1	2	4	6	9
1	M.	3	1.8	1.4	1.4	0.9	1.3	—	0.7	6.1	6.0	6.2	6.0	6.0	—	6.0
		10	<0.5	<0.5	0.7	0.6	0.7	—	0.8	6.0	6.1	6.0	5.7	6.7	—	6.3
		40	1.1	2.0	2.6	5.0	6.2	—	0.8	5.7	6.1	6.2	6.6	6.7	—	6.4
		1	0.5	0.8	0.8	0.7	0.6	—	0.6	7.7	7.7	—	7.4	7.1	—	—
2	M.	5	1.0	2.5	3.5	5.1	1.3	—	<0.5	7.0	7.1	7.2	8.3	8.7	—	7.7
		20	0.8	8.6	7.2	7.1	1.8	—	0.5	7.2	7.2	7.6	7.7	5.7	—	6.9
		40	2.7	—	—	10.6	6.6	4.7	—	7.5	—	—	8.7	9.7	9.4	—
		1	0.8	1.5	0.9	<0.5	0.5	—	<0.5	6.2	6.0	—	6.0	6.2	—	6.0
3	M.	5	0.6	0.6	<0.5	0.5	0.8	—	<0.5	6.2	6.0	6.0	6.1	6.2	—	6.0
		20	0.6	1.8	5.4	1.4	1.7	—	0.7	—	7.4	6.5	7.0	7.2	—	6.7
		3	<0.5	0.8	1.5	7.1	4.0	—	1.6	7.0	7.6	7.4	8.3	8.6	—	7.6
		10	0.5	0.8	1.3	2.1	1.3	—	1.0	6.5	6.4	6.5	7.5	7.9	—	7.6
4	M.	40	0.9	3.6	6.5	11.4	10.6	—	2.8	6.7	6.9	7.1	8.1	8.6	—	8.7
		3	0.9	3.5	1.4	1.6	0.9	—	1.1	7.5	7.8	7.9	8.7	—	8.0	
		10	2.1	6.8	8.2	5.4	3.9	—	1.6	7.3	7.0	7.2	7.6	8.3	—	7.6
		40	0.5	5.1	7.3	15.0	6.4	—	0.7	7.4	7.5	7.5	8.0	9.2	—	—
5	M.	1	<0.5	1.0	1.9	1.6	1.0	—	0.8	6.6	6.6	7.4	8.0	6.8	—	6.1
		5	<0.5	6.1	7.1	3.9	1.6	—	<0.5	6.1	6.0	6.2	6.0	6.6	—	6.0
		20	<0.5	3.2	3.8	4.4	3.0	—	0.5	5.0	5.6	5.6	6.3	6.0	—	6.0
		40	0.8	—	—	14.5	12.1	6.1	—	5.9	—	—	6.6	7.9	8.4	—

rose in each case but there was no response in serum growth hormone, plasma cortisol, or plasma luteinizing hormone levels which could be attributed to TRH. The magnitude of the peak response to a given dose of TRH varied not only between subjects but also in the same subject given the same dose on different occasions (Fig. 1). Despite this variability in response to a single dose it was possible to show a dose-response relationship in one subject under carefully controlled basal conditions (Fig. 2).

FIG. 1—Variability in response to a single dose of TRH.

FIG. 2—Response to differing doses of intravenous TRH in Subject 1. (Dose of TRH in μg.)

Short Test.—As a result of these preliminary observations a simplified test of TSH reserve was devised, as described above. This procedure was followed in eight men and six women (Fig. 3). One man (Subject 7) who had finished a one-week course of triiodothyronine 120 μg daily five days

FIG. 3—Response of 100 μg of TRH (intravenously) in eight men and six women. (Subject 7 had finished a one-week course of triiodothyronine 120 μg daily five days before this TRH test. Subject 3 has shown consistently low responses to TRH in several experiments.)

before the TRH test showed no rise in TSH. He had previously responded well to TRH (Subject 3, Hall *et al.*, 1970). Another man (Subject 3, current investigation) showed a reduced response to TRH in this test and also in response to TRH 200 μg intravenously and 1, 5, and 20 mg of TRH orally. The two women who showed the greatest responses to TRH were in the premenstrual phase of the cycle.

ORAL ADMINISTRATION OF TRH

Doses of 1 mg and above produced a significant rise in serum TSH and P.B.I. levels (Table II). It can be seen that only doses of 20 mg and above caused a consistent rise in serum TSH and that only the 40-mg doses produced a rise in P.B.I. in every case. The mode time of the peak response of TSH in 13 experiments was two hours (30 minutes in two, one hour in four, two hours in six, and four hours in one). There was a considerable variation in TSH response between subjects at a given dose level, but in general there was a dose-response relationship in an individual subject (shown for Subject 2 in Fig. 4). The rise in P.B.I. closely followed that of TSH (Subject 2, 40-mg dose shown in Fig. 5).

SIDE EFFECTS

Side effects were mild and transient, occurring only after intravenous administration (Table IV). The onset of the side

TABLE IV—Side Effects of TRH in 14 Subjects

Nausea	..	..	9	Peculiar taste	..	..	3
Flushing sensation	..	..	8	Tightness in chest	..	..	3
Desire to micturate	..	..	5				

TABLE III—Response of Pituitary Hormones and Cortisol to Varying Doses of Synthetic TRH in Two Subjects

Time (min)	TSH (μU/ml)					GH (ng/ml)					LH (mU/ml)					Cortisol (μg/100 ml)				
	*	†	‡	§		*	†	‡	§		*	†	‡	§		*	†	‡	§	
-15	1.1	<0.5	0.8	1.4	0.5	0.5	0.8	0.7	0.8	2.1	2.1	3.4	4.0	2.3	5.6	9.0	8.5	7.5	6.0	10.5
0	0.7	0.5	0.5	1.4	0.8	0.5	1.0	0.7	0.5	1.6	1.9	4.1	3.9	2.4	6.5	6.0	5.5	8.5	6.5	10.5
+5	1.2	0.7	2.6	2.8	1.6	0.4	1.0	1.2	1.3	1.3	2.2	4.0	4.2	3.6	6.2	6.0	6.0	7.5	8.0	7.0
+15	3.6	4.8	5.0	5.6	3.1	0.6	1.6	1.1	1.2	1.1	2.3	4.1	5.9	3.2	5.7	6.0	6.0	9.5	7.0	7.5
+20	4.1	4.6	5.4	5.6	3.2	0.5	1.4	1.4	0.7	1.0	2.0	4.0	5.9	2.9	6.0	7.0	6.0	9.5	7.0	7.0
+25	4.4	5.1	6.4	4.2	3.2	0.3	1.5	1.6	0.3	0.9	1.8	4.0	6.0	3.2	6.0	8.0	5.9	9.0	7.0	7.0
+30	4.6	3.8	6.4	4.0	2.4	0.5	1.2	1.4	0.2	1.0	1.9	4.2	6.2	4.0	5.6	8	5.0	9.0	10.0	8.0
+60	2.0	2.5	2.7	2.8	1.6	0.5	1.5	1.3	0.8	1.1	1.8	3.8	6.2	3.6	4.9	8.5	5.0	9.0	—	8.5
+120	0.5	1.1	1.1	1.3	0.6	0.6	2.9	3.3	0.2	1.1	2.2	—	7.1	3.1	5.7	6.5	5.0	7.0	9.0	7.5
+180	1.7	1.1	1.2	1.8	0.9	0.7	2.3	1.2	0.9	1.0	3.1	4.1	6.1	3.5	6.5	7.0	6.0	6.9	7.5	16.5

*Subject 1, 50 μg. T.R.H. intravenously.
†Subject 1, 100 μg. T.R.H. intravenously.
‡Subject 1, 200 μg. T.R.H. intravenously.
§Subject 2, 200 μg. T.R.H. intravenously.
||Subject 2, 200 μg. T.R.H. intravenously.

effects was rapid, being experienced within 15 seconds of the injection. There was no relationship between the side effects and the TSH response at dose levels of 50 μg of TRH and above.

No change occurred in pulse rate, blood pressure, or respiration in any subject. Electrocardiographic and electroencephalographic recordings were unchanged in one subject. Estimations of haemoglobin, white cell counts, and liver function tests were unchanged when measured in 10 subjects from one to three weeks after TRH. No long-term side effects were noted in four subjects who had received the drug six months previously.

FIG. 4—Response to differing doses of oral TRH in Subject 2.

FIG. 5—Response of serum TSH and P.B.I. to 40 mg TRH (oral) for Subject 2.

Discussion

The results reported here confirm previous observations that synthetic TRH given intravenously to euthyroid subjects causes an increase in serum TSH levels (Bowers *et al.*, 1970a; Fleischer *et al.*, 1970; Hall *et al.*, 1970). The magnitude of the response appears to be dose-dependent, though there is a wide variation in response to a given dose even in the same subject. TRH appears to have a specific action on the anterior pituitary causing TSH release, since in our series of experiments, carried out under carefully controlled basal conditions, we were unable to find a rise in serum growth hormone, plasma luteinizing hormone, or plasma 11-hydroxycorticosteroids (used as an index of corticotrophin release). The rise in plasma cortisol reported by Bowers *et al.* (1970a) and of cortisol and growth hormone described by Fleischer *et al.* (1970) are likely to represent non-specific stress responses.

Following the observation that the biological action of TRH extracted from hypothalamic sources was not destroyed by a series of proteolytic enzymes (Burgus *et al.*, 1966), the same workers showed that the synthetic tripeptide was active on oral administration in mice (Vale *et al.*, 1970). Our obser-

vations have shown that TRH is active when administered orally to euthyroid human volunteers in doses of 1 mg and above. It produces a rise in serum TSH which is followed by an increase in serum P.B.I. Oral administration of TRH with estimations of P.B.I. may therefore be useful as an indirect test of pituitary TSH reserve where TSH assays are not available. Where TSH assays are available the short intravenous test provides a simple, effective, dynamic test of pituitary TSH release. We are unable to offer an explanation for the consistently poor response to TRH shown by Subject 3; he showed no clinical or biochemical evidence of pituitary or thyroid dysfunction. Further studies are required to define the frequency of such reduced responses to TRH in normal subjects.

The prolonged inhibition of the TRH response produced by triiodothyronine in one subject stresses the importance of defining the time course of this thyroid hormone effect. Bowers *et al.* (1970b) showed that 100 μg of triiodothyronine by mouth partially or completely suppressed the effect of 400 or 800 μg of injected TRH five to seven hours afterwards in three out of four normal men.

Bowers *et al.* (1970c) suggested that females are more sensitive to TRH than males, and two of the women we investigated, both in the premenstrual phase of the cycle, had higher peak TSH values than any other normal subjects. Further studies are under way to investigate the sensitivity of women to TRH at various stages of the menstrual cycle.

Fleischer *et al.* (1970) showed that TRH was without effect on serum TSH levels in three patients with well-documented hypopituitarism and had a subnormal action in another patient with a pituitary tumour who was clinically and biochemically euthyroid by other criteria. Our observations would support the view that administration of TRH either intravenously or orally (when TSH measurements may be supplemented or replaced by P.B.I. estimations) should prove a useful test of pituitary TSH reserve capacity.

The advice of Professor G. A. Smart and Professor A. L. Latner is gratefully acknowledged. The work was supported by grants from the Wellcome Trust, the North of England Council of the British Empire Cancer Campaign, and the Scientific and Research Subcommittee of the Royal Victoria Infirmary. We are grateful to Dr. Harold Holgate, of Roche Ltd., for supplying thyrotrophin-releasing hormone; to the Medical Research Council for human TSH and HGH; to Mr. J. Wilson for PBI estimations; and to Mrs. A. Hewison for serum growth hormone estimations. The assistance of our colleagues who volunteered to have TRH administered is gratefully acknowledged.

References

- Boler, J., Enzmann, F., Folkers, K., Bowers, C. Y., and Schally, A. V. (1969). *Biochemical and Biophysical Research Communications*, 37, 705.
- Bowers, C. Y., *et al.* (1970a). *Biochemical and Biophysical Research Communications*, 39, 352.
- Bowers, C. Y., *et al.* (1970b). Abstracts of the Endocrine Society, April 1970.
- Bowers, C. Y., Schally, A. V., Weil, A., Reynolds, G. A., and Folkers, K. (1970c). The Sixth International Thyroid Conference, Vienna.
- Burgus, R., Dunn, T. F., Desiderio, D., Vale, W., and Guillemin, R. (1969). *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, 269, 226.
- Burgus, R., Ward, D. N., Sakiz, E., and Guillemin, R. (1966). *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, 262, 2643.
- Fleischer, N., Burgus, R., Vale, W., Dunn, T. F., and Guillemin, R. (1970). *Journal of Clinical Endocrinology and Metabolism*, 31, 109.
- Gillessen, D., Felix, A. M., Lergier, W., and Studer, R. O. (1970). *Helvetica Chimica Acta*, 53, 63.
- Hall, R., Amos, J., Garry, R., and Buxton, R. L. (1970). *British Medical Journal*, 2, 274.
- Hall, R., Amos, J., and Ormston, B. J. (1971). *British Medical Journal*, 2, 582.
- Murphy, B. E. P. (1967). *Journal of Clinical Endocrinology and Metabolism*, 27, 973.
- Raud, H. R., and Odell, W. D. (1969). *British Journal of Hospital Medicine*, 2, 1366.
- Riley, M., and Gochman, V. (1964). In *Technicon Symposium. Automated Analytical Chemistry*, p. 62. Chertsey, Surrey, Technicon Instruments.
- Vale, W., Burgus, R., Dunn, T. F., and Guillemin, R. (1970). *Journal of Clinical Endocrinology and Metabolism*, 30, 148.
- Wilkinson, R., Hall, R., Cooper, A., and Newell, D. J. (1970). *Journal of Endocrinology*, 46, 167.