system used in MA was specific for human IgM. A high incidence of positive results was obtained for IM sera in contrast to a low incidence in sera of patients suffering from other diseases and sera of healthy individuals. Good correlation was obtained between results in MA and tests with agglutination of NDV-modified RBC's. Positive results were obtained with IM sera using the VIC strain of NDV in MA, but not with the B1 strain.

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Failure of Methimazole to Affect Peripheral Thyroid Indices in Man* (32697)

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Thiouracil compounds have been extensively utilized both experimentally and therapeutically. Their antithyroid action at the level of the thyroid gland is well known (1). Extrathyroidal effects of these substances have been reported (2–4). Propylthiouracil (PTU) has been shown to produce a slowing of the plasma loss of radioactivity following the injection of ^{131}I-labeled L-thyroxine in man (2,3,5), with no effect on the thyroxine-binding serum protein carriers (3,5). In these studies a decrease in urinary and an increase in fecal radioactivity was noted (2,3). The excretory pattern of radioactivity in rats following radiothyroxine injection has been shown to be altered similarly by some but not all antithyroid compounds (4). The data in man and in the rat have been interpreted to indicate that PTU inhibits peripheral deiodination of thyroxine. Inhibition of thyroxine monodeiodinase obtained from rat kiddeiodination of thyroxine. Inhibition of thyroxine deiodination in rat kidney slices following thiouracil and PTU administration (7) have been reported. Methimazole did not appear to affect the excretory pattern of radioactivity in rats (4) nor the thyroxine deiodination by rat kidney slices (7). Slingerland and Burrows (2) found no alteration in radiothyroxine turnover studies in one myxedematous patient given methimazole.

Since methimazole has been used as an experimental tool in the study of peripheral thyroxine metabolism, this study was undertaken to elucidate what effects this compound has peripherally in man.

Material and Methods. The subjects studied included 16 adult volunteers ranging in age from 23 to 62 years. Each was considered to be free of endocrine disease by history and

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physical examination, and euthyroid by thyroid function studies. In addition two myxedematous patients (C.L. and A.T.) were studied while maintained in a euthyroid state with 0.2 mg of Na-L-thyroxine daily.

Methimazole was administered orally at a dosage of 20 mg every 8 hours. The subjects received no other medication unless indicated. Blood specimens were drawn in the morning following an 8-hour fast. No attempt was made to control the diet or the usual activities of the subjects.

Radiothyroxine turnover studies. Thyroxine turnover and degradation rates were studied in two normal volunteers and the two myxedematous patients.

(1) The two normal volunteers received a single intravenous injection of 20–25 μc ^{131}I-labeled thyroxine.[2] Potassium perchlorate, 300 mg every 8 hours, was begun immediately and continued throughout the study to prevent thyroidal reaccumulation of peripherally degraded radioiodine. Following a 7-day control period with potassium perchlorate alone, methimazole was administered for the following 10-day period. Plasma samples were obtained daily from days 2 to 17 after the radiothyroxine injection and were prepared in 3-ml duplicate samples and stored until the final day. The samples were then counted together with an aliquot of the dose injected in an automatic scintillation well counter.

(2) The two myxedematous patients received L-thyroxine replacement during the entire study. They each received two intravenous radiothyroxine injections in doses as above; the first followed by no medication other than their replacement therapy, the second, 4 weeks later, preceded by 4 days of methimazole which was continued for 10 days after the injection of radiothyroxine and were treated as described above. All measurements of radioactivity were done for a sufficient period of time to keep the counting error below 1%.

The slope of the log of plasma radioactivity against time in days was calculated by the method of least squares. The fractional loss of radioactivity is presented for convenience as the percent of radiothyroxine turnover per day (k). The thyroxine distribution space (TDS) was obtained by the isotope-dilution method. The extrathyroidal hormonal iodine pool (ETP) and quantity of thyroxine degraded daily (DD) were calculated by the methods previously reported (8).

Serum thyroxine-protein carrier binding

[2] Obtained from Abbott Laboratories, shipped as chromatographically pure L-thyroxine; specific activity ranged from 25 to 37 mc/mg. Human serum albumin was added on arrival to make 1% concentration and stored at 2–5°C. until used. Further dilutions were made with 1% albumin-normal saline solution just prior to administration. The identity of the administered ^{131}I-labeled L-thyroxine was checked in two chromatographic solvent systems (Tertiary amyl alcohol, 2N ammonium hydroxide and butanol, 2N acetic acid) each day of use. The dose injected was corrected for the liberated radioiodine present.

TABLE I. Effect of Methimazole on Thyroxine Turnover and Degradation.

		K (% per day)		TDS (liter)		ETP (μg)		DD (μg/day)	
Subject	Age, sex	Control	MTH	Control	MTH	Control	MTH	Control	MTH
P.J.	62, male	11.2	9.7	11.7	12.9	725	774	81.0	75.0
L.T.	43, male	9.0	9.0	13.2	13.1	832	904	74.9	81.4
C.L.[a]	70, male	8.6	8.4	13.1	14.4	838	806	72.1	67.7
A.T.[a]	66, male	10.4	10.1	9.3	10.2	642	775	66.8	78.3
Mean		9.8	9.3	11.8	12.7	759	815	73.7	75.6
p		NS		NS		NS		NS	

Note—Abbreviations: K = turnover rate; TDS = thyroxine distribution space; ETP = extrathyroidal thyroxine pool; DD = daily degradation; MTH = methimazole; NS = not significant ($p > .05$).

[a] Myxedematous patient on 0.2 mg Sodium L-thyroxine per day, studies performed 4 weeks apart.

studies. The method is essentially that described by Ingbar (9) with the modifications listed below. To an aliquot of serum, radiothyroxine was added together with various concentrations of sodium L-thyroxine so that the enrichment ranged from near physiological level of about 0.9–450 μg%. Electrophoresis of these dilutions on Whatman 3 mm paper strips in a Tris-maleate buffer system (pH 8.6 at room temperature, ionic strength 0.1) for 20–22 hours at 2–5°C was performed in a commercially available hanging strip unit (Spinco Durrum Cell) at 4–4.5 mA per unit with voltage readings at 65–70 V. The percentage distribution of thyroxine between the binding sites at near physiological levels of thyroxine and the binding capacity of thyroxine-binding globulin (TBG), and thyroxine-binding prealbumin (TBPA) were calculated.

Serum free-thyroxine studies and PBI determinations. The serum free-thyroxine levels (fraction and concentration) were determined in duplicate by the magnesium precipitation method of Sterling and Brenner (10) in six subjects. The serum protein-bound iodine (PBI) was determined in duplicate by a modified chloric acid digestion method with brucine end point stabilization (11) in 14 of the normal volunteers.

Samples for the thyroxine-binding carrier studies, serum free thyroxine, and PBI were obtained before and after 10 days of methimazole administration. All samples were stored at —20°C until used. Determinations of thyroxine-binding studies and free-thyroxine levels were always performed in the same daily run for any given individual. In the free-thyroxine determinations an aliquot of a large normal serum pool was also utilized to correct daily variations in the data.

Statistical methods. Statistical evaluation of data was performed according to methods described by Steel and Torrie (12).

Results. Methimazole was found to have no significant effect on the turnover and quantitative degradation of L-thyroxine in the four subjects studied (Table I). The extrathyroidal thyroxine pool, thyroxine distribution space, and quantity of thyroid hormone degraded daily were unchanged from control

TABLE II. Effect of Methimazole on Thyroxine-Binding Serum Protein Carriers and the Free-Thyroxine Level in Euthyroid Subjects.

	PBI (μg/100 ml)	Endogenous thyroxine distribution (%)[b]		Thyroxine-binding capacity (μg/100 ml)		Serum-free thyroxine	
		TBG	TBPA	TBG	TBPA	Percent	mμgI/100 ml
Before methimazole	5.6[a] (14)	57.6 ± 7.0 (11)	27.3 ± 7.3 (11)	26.6 ± 6.4 (11)	154.1 ± 42.8 (11)	0.046 ± .009 (6)	2.68 ± 0.53 (6)
After methimazole	5.9 (14)	57.8 ± 7.5 (11)	25.0 ± 10.3 (11)	25.0 ± 5.8 (11)	154.1 ± 38.3 (11)	0.047 ± .007 (6)	2.73 ± 0.21 (6)
p	NS	NS	NS	NS	NS	NS	NS

Note—Abbreviations: PBI = serum protein-bound iodine; TBG = thyroxine-binding globulin; TBPA = thyroxine-binding prealbumin; NS = not significant ($p > .05$).

[a] Values represent mean ± standard deviation. Numbers in parentheses indicate the number of subjects (ages 23–36) studied.

[b] Average enrichment 0.9 μg% thyroxine.

values when methimazole was administered.

The distribution of radiothyroxine at physiological levels of thyroxine between TBG and TBPA and the binding capacities of these carrier proteins were found to be unchanged by methimazole administration in 11 euthyroid subjects (Table II), nor was any change noted in the free-thyroxine values (percent or concentration). Methimazole was found to produce no significant change in the PBI.

Summary and conclusions. The effects of methimazole (60 mg/day) on peripheral thyroxine turnover and transport were investigated in 16 adult volunteers. In four subjects (two euthyroid individuals and two myxedematous patients in the euthyroid state on thyroxine replacement) using exogenously administered ^{131}I-labeled L-thyroxine, the radiothyroxine turnover was investigated. Simultaneously, the effects of methimazole administration (10 days) on thyroxine-binding serum protein carriers (11 subjects) and serum free-thyroxine levels (six subjects) were observed. Methimazole administration in man in these studies was shown to have no effect on peripheral thyroid indices: thyroxine binding to serum proteins, the serum free-thyroxine levels or the turnover and total quantitative degradation of thyroxine. This finding confirms a similar result on thyroxine turnover in one myxedematous patient reported earlier (2).

From the present data it would appear that methimazole does not alter the turnover, transport or quantitative degradation of thyroxine in man when compared to propylthiouracil.

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Effect of Phytohemagglutinin and Various Mycobacterial Antigens on Lymphocyte Cultures from Leprosy Patients (32698)

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Lymphocyte transformation in cultures from peripheral blood has been described as an *in vitro* reflection of immunological capacity. The phenomenon has been associated with the delayed type of hypersensitivity and with homograft histoincompatability. The morphological change is an *in vitro* transformation of small lymphocytes into large blast-like cells that are capable of mitosis; it can be brought about by a number of different stimulants (1, 2). Some stimulants, such as phytohemagglutinin (PHA) and streptolysin O, cause a profound response in

[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service, or by the U. S. Department of Health, Education, and Welfare.