in the early hours (*e.g.*, 0-8), PL animals appear normally active while BLN animals are somewhat depressed which may be attributed to the effects of major surgery. The PL procedure offers several advantages besides sparing the mice from surgery. In this laboratory a skilled operator can prepare about 8 BLN animals in 1 hour. On the other hand, a relatively inexperienced operator can prepare 8 PL animals in 10 minutes. The economy of PL preparation in terms of time and required skill of the operator, therefore, argues strongly for use of this method when an anuric mouse is needed as a research animal.

Summary. Penile ligation (PL) of mice effected a state of anuria which is indistinguishable from anuria effected by bilaterial nephrectomy (BLN) with respect to survival of anuric mice and enhanced lethality when treated with digitoxin. PL-induced anuria probably results from production of a urine stop-flow situation as shown by PSP excretion studies. The PL operation is simpler and faster than the BLN operation and is recommended for production of anuric mice as a research tool.

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Metabolic Effects of Isoproterenol and Propranolol in Normal Subjects Before, During and After Triiodothyronine-Induced Hypermetabolism.* (31727)

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The precise mechanism of the metabolic and circulatory abnormalities of patients with spontaneous or drug-induced hyperthyroidism are unknown. Previous clinical and experimental observations(2-4) have supported the hypothesis that a hyperactive sympathetic nervous system may be responsible for the changes associated with hyperthyroidism. Among the metabolic abnormalities reported in individuals with hyperthyroidism are elevated levels of plasma free fatty acids (FFA) and blood glucose obtained in the fasting state(5). The FFA mobilizing effects of thyroid hormone could be mediated through stimulation of beta adrenergic receptors. It is also known that epinephrine, norepinephrine and isoproterenol increase plasma FFA concentrations by enhancing mobilization from adipose tissue(6,7). Adrenergic blocking agents inhibit the augmented FFA release from adipose tissue caused by catecholamines (8).

This study was designed to evaluate the effects of beta adrenergic receptor stimulation by isoproterenol and beta receptor blockade by propranolol on levels of plasma FFA and blood glucose of normal subjects before, during and after a period of triiodothyronine-induced hypermetabolism. We also wished to test the hypothesis that triiodothyronine may augment the metabolic responses to graded doses of isoproterenol.

Materials and methods. Eight normal men

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TABLE I. Effects of Triiodothyronine in 8 Normal Subjects.

Avg values ± S.E.	Periods of study: Initial control period (A)†	Hypermetabolic period (B)†	Final control period (C)†
Sleeping pulse rate, beats/min	66 ± 2	96 ± 4*	69 ± 2
Oxygen consumption, ml/min M^2	131 ± 4	189 ± 7*	131 ± 2
Body wt, lb	183 ± 7	178 ± 7*	179 ± 8
Serum cholesterol, mg/100 ml	202 ± 10	124 ± 10*	235 ± 16

* Significantly different from control values at 5% level or better.

† The metabolic studies were done during an initial control period (A), at the end of a hypermetabolic period (B), in which each subject received triiodothyronine for 14 days, and during a final control period (C) 3 wk after the triiodothyronine was stopped.

were the subjects in this study. They ranged in age from 30 to 40 years (mean, 35 years) and in body surface area from 1.76 to 2.14 M^2 (mean, 1.94 M^2). Each subject had a normal serum protein-bound iodine and normal 4 and 24 hour uptake of radioactive iodine by the thyroid gland. Each subject received a high caloric diet ranging from 3500 to 5000 calories throughout the study.

Metabolic responses to beta adrenergic receptor stimulation by isoproterenol hydrochloride were measured in the 8 men before and after propranolol (0.15 mg/kg iv). Non-heparinized venous blood was obtained from a small cardiac catheter, the tip of which was located in the right atrium, before and during the third minute of infusion of isoproterenol at 3 dose levels, before and after propranolol. Isoproterenol was infused at rates of 0.025, 0.05, and 0.1 μg/kg/min. Significant attenuation of the normal chronotropic, inotropic and vasodepressor effects of isoproterenol in these subjects was used as an index of beta adrenergic receptor blockade (9). Heart rate, cardiac output, and systemic arterial and mean right atrial pressures were recorded before and during the fourth minute of infusions of isoproterenol before and after propranolol.

These studies were done in each subject on 3 separate occasions. The first period was a pretreatment control (A). The second period (B) followed 14 days of treatment with triiodothyronine, 250 to 500 μg/day; the third period (C) was a post-treatment control and took place 21 days after triiodothyronine had been stopped.

Each patient had fasted and had not smoked for 12 hours before the study. No sedative was given. Measurements were made with the patient in the supine position. Pressures were recorded from the right atrium and brachial artery with Statham strain gauges. Cardiac output was measured by the indicator-dilution method using indocyanine green dye as the indicator. Duplicate measurements of output and pressures were made during the initial control period before propranolol. The details of the hemodynamic methods have been published previously(9). Plasma FFA were measured by the method of Dole and Meinertz(10) and blood sugar by the 'Glucostat'§ micromethod. Serum cholesterol was measured by the method of Pearson, Stern and McGavack(11).

The results obtained in the second period (during triiodothyronine administration) were compared to those of the two control periods using t-tests, and an analysis of variance(12). F values or t values derived from the analyses were considered statistically significant if $p<0.05$.

Results. Triiodothyronine produced a significant increase in the average sleeping pulse rate and oxygen consumption and a fall in body weight and serum cholesterol (Table I). Clinical signs of hypermetabolism such as tremor, irritability, heat intolerance and increased perspiration were present in each subject. Three weeks after triiodothyronine had been discontinued the laboratory signs and the clinical manifestations of hypermetabolism had disappeared.

The effect of propranolol on the hemody-

§ Worthington Biochemical Corp., Freehold, N. J.

We obtained generous supplies of propranolol from Dr. Alex Sahagian- Edwards, Ayerst Laboratories, New York.

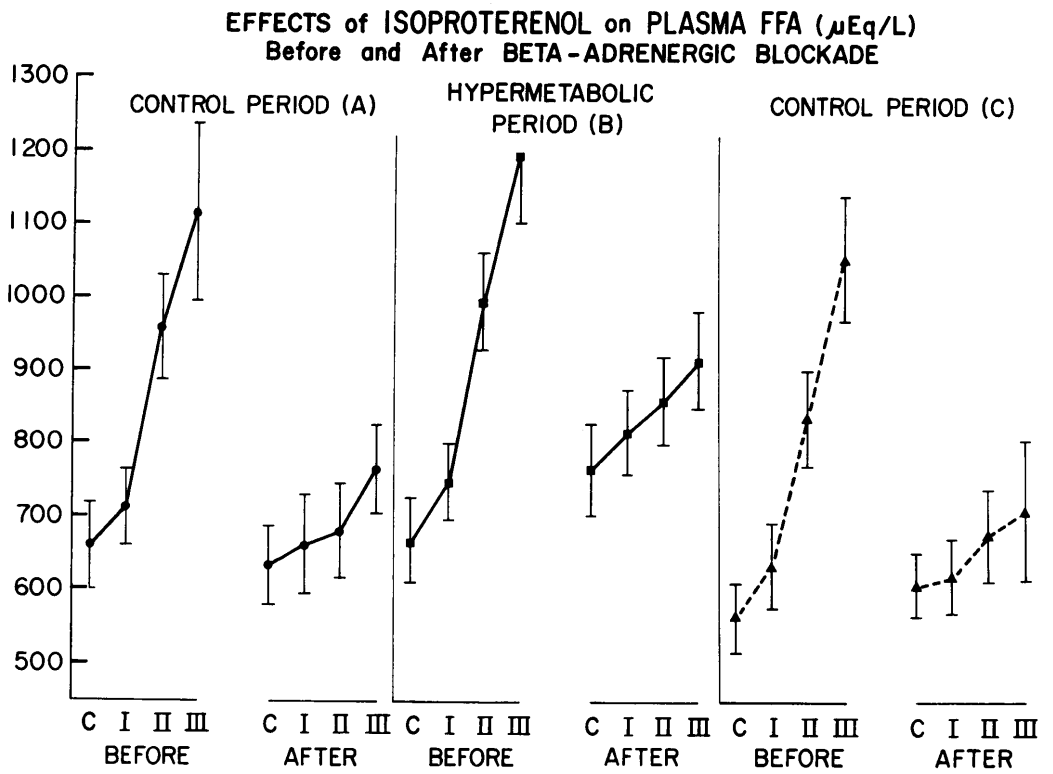

FIG.. 1. Effects of isoproterenol on plasma FFA μEq/L before and after beta adrenergic blockade. Mean values (±S.E.) are shown. On the abscissa, C refers to control measurements. I, II, and III refer to the responses to infusions of graded doses of isoproterenol. Effects of isoproterenol on plasma FFA before and after propranolol were measured during initial control period (A), at the end of a hypermetabolic period (B), in which each of the 8 subjects received triiodothyronine (250 to 500 μg/day) for 14 days, and during a final control period (C) 3 weeks after triiodothyronine was stopped.

namic responses to isoproterenol in the 3 periods has been published elsewhere(9). Briefly, the results indicate that effective beta adrenergic receptor blockade was achieved in these subjects as evidenced by significant attenuation of the chronotropic, inotropic and vasodepressor effects of isoproterenol in each period.

The effects of isoproterenol on the average levels of plasma FFA before and after propranolol are shown in Fig. 1. An analysis of variance of the changes in plasma FFA during isoproterenol infusions in the three periods before propranolol is shown in Table II. The initial control plasma levels of FFA before propranolol were not significantly different in the three periods (A, B, and C). Isoproterenol produced a significant dose-related increase in plasma FFA in each period before propranolol. These curves are linear and parallel. Thus the increments in FFA during isoproterenol infusions in the hypermetabolic period were not significantly different from those during the two control sessions.

Propranolol alone had no appreciable effect on the control levels of plasma FFA in each period but the slight change in FFA during isoproterenol infusions after the beta blocking drug in each period contrasted markedly with the significant rises in FFA during isoproterenol infusions before propranolol. Fig. 2 clearly shows that, in the doses used in this study, propranolol alone did not appreciably alter the plasma FFA levels at 15, 45 or 70 minutes after intravenous administration in either the hypermetabolic period or during the 2 euthyroid periods.

The effects of graded doses of isoproterenol on blood sugar before and after propranolol

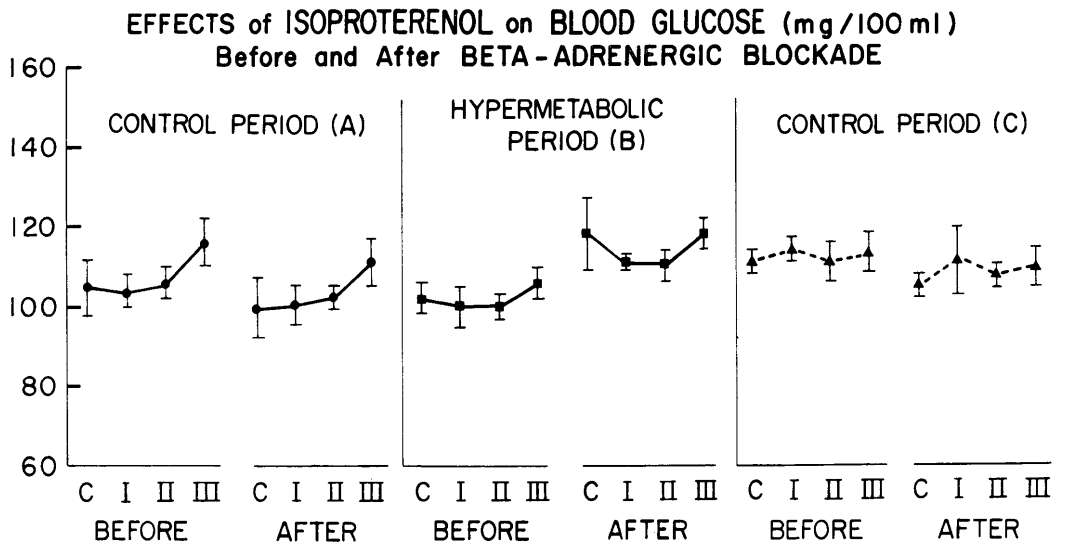

FIG. 3. Effects of isoproterenol on blood glucose before and after beta adrenergic receptor blockade. See legend for Fig. 1.

in each of the 3 periods are shown in Fig. 3. The control blood sugar levels before and after propranolol were similar in each period. Isoproterenol produced no appreciable changes in blood sugar levels in any period. The changes in blood sugar levels during isoproterenol infusions after propranolol were insignificant in each session and did not differ from those observed during isoproterenol infusion before beta adrenergic receptor blockade. The lack of effect of propranolol on control levels of blood sugar at 15, 45 and 70 minutes after its intravenous administration in each period is shown in Fig. 4.

TABLE II. Analysis of Variance of Changes in Plasma Free Fatty Acids (FFA) During Isoproterenol Infusions in the Three Periods Before Propranolol.†

Sources of variation‡	Plasma FFA dF	Mean square	F§
Periods	2	12,678	.73
A *vs* C	1	1,716	.10
A + C *vs* B	1	5,903	.34
Regression	1	2,109,249	122.20*
Nonparallelism	2	1,941	.11
Nonlinearity	1	6,162	.36
Error	56	17,261	
Total	71		

* $p<.01$; absence of an asterisk indicates $p>.05$.

† The 3 periods were the initial control study (A), the study after 2 wk of oral administration of triiodothyronine (B), and the final control study, which was done 3 wk after the triiodothyronine was stopped (C). Increases in plasma levels of FFA during isoproterenol infusions in the 3 periods were linear and parallel but not significantly different from each other.

‡ Only the pertinent sources of variation are shown.

§ F equals the variance ratio obtained by dividing the appropriate mean square by the "error" mean square.

Discussion. The subjects in this study developed definite clinical and laboratory signs of hypermetabolism. Tremor, irritability, heat intolerance and increased perspiration were noted in all the men. Definite and marked increases in the sleeping pulse rate and oxygen consumption were evident in each subject. The average weight loss for

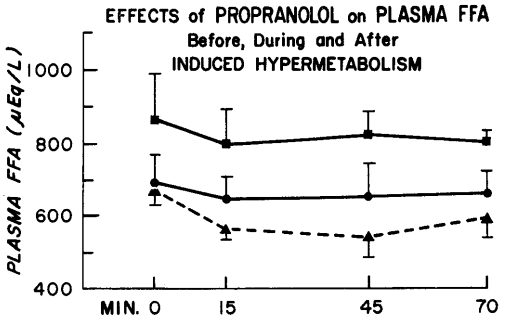

FIG. 2. Effects of propranolol on plasma FFA (μEq/L) before, during and after induced hypermetabolism. Solid dots and triangles represent average values during first and final control periods (A and C); squares indicate values during hypermetabolic period (B).

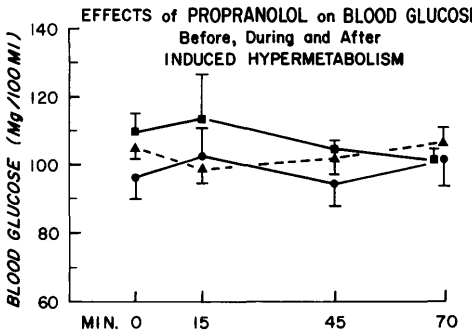

FIG. 4. Effects of propranolol on blood glucose before, during and after induced hypermetabolism. See legend for Fig. 2.

the 8 subjects during the hypermetabolic period was 5 lb. Nevertheless, the average fasting levels of plasma FFA and blood glucose were not significantly higher in the hypermetabolic period than during the control periods. One possible reason for the discrepancy between these observations and other reports(5,14) is that weight loss was minimal in our subjects as contrasted to that frequently seen in some patients with spontaneous thyrotoxicosis. More marked elevations of baseline plasma levels of FFA reported in patients with severe thyrotoxicosis may merely reflect the increased lipid mobilization from adipose tissue which occurs with weight loss. In this study an attempt was made to minimize weight loss by an increase in caloric intake during the hypermetabolic period.

The relatively small sample size of our study group ($N = 8$) might be another explanation. Another reason might be that the blood samples were not obtained under basal conditions, although a similar amount of time was allowed in each period after placement of the arterial needle and cardiac catheter. Additional studies in our laboratory of basal levels of plasma FFA in a larger number of subjects ($N = 14$) showed that the average resting level of plasma FFA of 613 ± 53 (S.E.) μEq/L during a hypermetabolic period was significantly higher ($p<0.05$) than the corresponding resting level of 450 ± 34 μEq/L in the same subjects when they were euthyroid (unpublished observations). Blood samples in this larger group of subjects were obtained at the bedside rather than in the test room. The fasting levels of blood glucose in these 14 subjects, however, were not significantly higher in the hypermetabolic period than during the time they were euthyroid.

We cannot exclude the possibility in the present study that the same unknown factors which prevented a significant rise in baseline plasma levels of FFA and an increase in blood glucose during hypermetabolism also prevented a fall in plasma FFA with propranolol or a rise in blood sugar during infusions of isoproterenol. Other investigators(15,16), however, have also reported that isoproterenol produces little, if any increase in plasma glucose in man, even when infused continuously for as long as 15 minutes. Neither propranolol (15) nor butoxamine(16) blocks the increments in plasma glucose induced by infusions of epinephrine or norepinephrine although butoxamine lowers the baseline concentration of plasma glucose significantly(16). The lack of appreciable changes in blood glucose following beta adrenergic receptor stimulation during isoproterenol or beta adrenergic receptor blockade with propranolol suggests that other receptors than the beta adrenergic receptor must be involved in mediating the hyperglycemic responses to catecholamines in man.

The striking increases in levels of plasma FFA during isoproterenol infusions in each period and the effective blockade of these increments by propranolol confirm the findings of other investigators(17,18). The results suggest that in man the effect of isoproterenol upon free fatty acid mobilization is mediated by beta adrenergic receptors. The findings do not support the hypothesis that the metabolic changes in triiodothyronine-induced hypermetabolism are mediated through stimulation of beta adrenergic receptors. The results are consistent with the recent studies in animals(19,20) and in man (21-23) indicating that thyroid hormone does not augment the cardiovascular or the metabolic responses to catecholamines.

Summary. Effective beta adrenergic receptor blockade was produced in 8 normal men before, during and after a 14-day period of triiodothyronine-induced hypermetabolism. Triiodothyronine did not augment the increases in plasma levels of free fatty acids

during infusions of graded doses of isoproterenol. The increments in plasma FFA during isoproterenol administration were significantly attenuated by propranolol in the hypermetabolic period as well as during the initial and final control periods. Neither isoproterenol nor propranolol produced any appreciable changes in blood glucose in any period. The results also suggest that beta adrenergic receptors may mediate the effects of isoproterenol upon free fatty acid mobilization but do not suggest a similar receptor role for regulation of blood sugar levels.

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Selective Suppression of Penicillin Hypersensitivity by Certain Phenoxy Propanediols. (31728)

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Little work has been reported on the broad concept of interference with immunological reactions by simple chemical compounds. It has been known for some time that certain simple dyes may specifically enhance or suppress the reaction between the syphilis reagin and the corresponding antibody(1). More recently it was shown that peritoneal mononuclear phagocytes obtained from guinea pigs that have been treated with meprobamate do not support the intracellular growth of *Brucella abortus* and thus behave like cells obtained from immunized animals(7). It was of interest to see whether meprobamate would also be able to affect certain other immune responses. The passive cutaneous anaphylaxis reaction (PCA) elicited in the guinea pig with penicillin conjugates and bovine serum albumin (BSA) was selected as the experimental model. Meprobamate did not interfere with this reaction. However, certain phenoxy propanediols selectively inhibited the penicillin induced anaphylaxis without affecting anaphylaxis induced by other proteins. This interesting observation was followed up not only because of theo-