addition of THC in a 20-μl volume of ethanol, to the incubation medium, in order to achieve THC concentrations of 0.25, 2.5, 12.5, or 25 μg/ml resulted in 25, 18, 29, or 86 percent inhibition, respectively, in the accumulation of T in the incubation medium. In another experiment, with testes obtained from immature mice, the dose of 25 μg THC per milliliter resulted in a 53 percent decrease ($P < .001$) in the accumulation of T (Table 1). In a subsequent experiment with adult animals, the addition of 25 or 250 μg CBN per milliliter of incubation medium significantly ($P < .001$) inhibited the production of T (Table 2). These results complement the findings of Jacubovic and McGeer (*11*), who demonstrated that THC, CBN, and other cannabinoids can inhibit the synthesis of nucleic acids, proteins, and lipids in testicular slices in vitro.

Recently, THC has been shown to possess estrogenic activity (*15*), and some investigators have suggested that estrogens may inhibit testosterone synthesis by a direct action on the testis (*16*). However, in this incubation system, the addition of estradiol, at doses as high as 5 μg/ml, did not affect T accumulation in the incubation medium (*17*).

It is always difficult to extrapolate from experiments conducted in vitro to conditions in vivo. Although THC can concentrate in testicular tissue (*18*), the actual in vivo concentrations in human marihuana users, or in experimental animals treated with cannabinoids, may vary considerably from those used in this study. However, the results do indicate that (i) THC, in a wide range of doses (including the relatively low level of 0.25 μg/ml), can significantly reduce T biosynthesis in the decapsulated mouse testis and (ii) CBN can have a similar effect.

The ability of either cannabis or THC to lower plasma T levels in vivo has been described (*1, 2*). We have observed that subcutaneously injecting male mice with 100 μg CBN per day for 4 days significantly reduced T levels in the plasma sampled approximately 5 hours after the last injection (1.73 ± 0.75 versus 8.50 ± 2.26 ng/ml; $P < .02$) (*19*). The similarity of the response of mouse testes in vitro to both THC and CBN suggests that nonpsychoactive constituents of cannabis can contribute to its effects on the endocrine system. Furthermore, suppression of testicular function by CBN, or other nonpsychoactive components of marihuana, could account for some of its effects on androgen-dependent behaviors.

The mechanism of action of marihuana on testicular function remains to be elucidated, but our results indicate that the reduction in peripheral T levels observed in vivo (*1, 2*) impaired spermatogenesis (*3, 4*), and decreases in androgen-dependent behaviors (*6, 7*) may be due, at least in part, to a direct inhibitory effect of cannabinoids on the production of T by the testis.

Table 2. The effect of treatment with cannabinol (CBN) in vitro on the production of testosterone (T) by the decapsulated testes from adult mice. The results represent mean (± S.E.) concentration of T in the incubation medium at the end of a 4-hour incubation.

CBN concentration (μg/ml)	T			
	ng/ml	*N*	Inhibition (%)	*P*
	368 ± 27	9		
25	99 ± 10	9	73	< .001
250	18 ± 5	8	95	< .001

S. Dalterio
A. Bartke, S. Burstein
Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

References and Notes

1. A. M. Symons, J. D. Teale, V. Marks, *J. Endocrinol.* **68**, 43P (1976); R. C. Kolodny, W. H. Masters, R. M. Kolodner, G. Toro, *New Engl. J. Med.* **290**, 872 (1974); R. C. Kolodny, P. Lessin, G. Toro, W. H. Masters, S. Cohen, in (*2*), p. 217.
2. M. C. Braude and S. Szara, Eds., *The Pharmacology of Marihuana* (Raven, New York, 1976).
3. V. P. Dixit, V. N. Sharma, N. K. Lohiya, *Eur. J. Pharmacol.* **26**, 111 (1974); W. C. Hembree, III, P. Zeidenberg, G. G. Nahas, in (*4*), p. 521; C. N. Stefanis and M. R. Issidorides, in (*4*), p. 533.
4. G. G. Nahas, Ed., *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Springer-Verlag, New York, 1976).
5. H. Rosenkrantz and M. C. Braude, in (*2*), p. 571; R. Collu, *Life Sci.* **18**, 223 (1976).
6. E. L. Abel, *J. Behav. Biol.* **15**, 255 (1975).
7. A. Merari, A. Barak, M. Plavis, *Psychopharmacologia* **28**, 243 (1973); M. G. Cutler, J. H. Mackintosh, M. R. A. Chance, *ibid.* **45**, 129 (1975); M. E. Corcoran, Z. Amit, C. W. Malsbury, S. Daykin, *Res. Commun. Chem. Pathol. Pharmacol.* **7**, 779 (1974).
8. H. Kolansky and W. T. Moore, *J. Am. Med. Assoc.* **222**, 35 (1972); J. Harmon and M. A. Aliapoulios, *New Engl. J. Med.* **287**, 936 (1972).
9. B. H. Marks, *Prog. Brain Res.* **39**, 331 (1973); I. Nir *et al.*, *Nature (London)* **343**, 470 (1973); J. Kramer and M. Ben-David, *Proc. Soc. Exp. Biol. Med.* **147**, 482 (1974).
10. G. R. Thompson, M. M. Mason, H. Rosenkrantz, M. C. Braude, *Toxicol. Appl. Pharmacol.* **25**, 373 (1973); M. K. Birmingham and A. Bartova, in (*4*), p. 425; R. K. Kubena, J. L. Perhach, Jr., H. Barry, III, *Eur. J. Pharmacol.* **14**, 89 (1971); B. Loscalzo, *Proc. Int. Congr. Pharmacol.* **5**, 142 (1972).
11. A. Jakubovic and P. L. McGeer, in (*4*), p. 223.
12. The gonadotropin was manufactured under the trade name Follutein (Squibb). The experimental conditions have been described by A. Bartke, D. Kupfer, and S. Dalterio [*Steroids* **28**, 81 (1976)]; M. P. Van Damme, D. M. Robertson, P. Romani, and E. Diczfalusy [*Acta Endocrinol. (Copenhagen)* **74**, 642 (1973)]; and M. L. Dufau, K. J. Catt, and T. Tsuruhara [*Biochim. Biophys. Acta* **252**, 574 (1971)].
13. A. Bartke, R. E. Steele, N. Musto, B. V. Caldwell, *Endocrinology* **92**, 1223 (1973); F. M. Badr and A. Bartke, *Steroids* **23**, 921 (1974).
14. To minimize the effects of between-animal variation on the results, the two testes incubated in each flask were obtained from two different animals. In some experiments, a control group and several treatment groups were included, and the significance of the differences between group means were compared by a one-way analysis of variance and Duncan's multiple-range test. In other experiments, cannabinoid-containing and control incubations were individually matched by using testes removed from the same mice, and the results were compared by a *t* test for paired comparisons.
15. J. Solomon, M. Cocchia, R. Gray, D. Shattuck, A. Vossmer, *Science* **192**, 559 (1976).
16. L. T. Samuels, J. G. Short, R. A. Huseby, *Acta Endocrinol. (Copenhagen)* **45**, 487 (1964); M. Chowdhury, R. Tcholakian, E. Steinberger, *J. Endocrinol.* **60**, 375 (1974).
17. A. Bartke and S. Dalterio, in preparation.
18. B. T. Ho *et al.*, *J. Pharm. Pharmacol.* **22**, 538 (1970).
19. Our preliminary observation of an antifertility effect of CBN in pregnant female mice suggests that gonadal steroidogenesis can be inhibited by treatment with CBN in both sexes.
20. Supported by NIH Research Career Development Award 5K04 HD70369 to A.B. and grant 1R01 HD09584, National Institute of Drug Abuse grant DA01170 to S.B., and by the Mabel Louise Riley Charitable Trust. We thank Dr. B. V. Caldwell for antiserum to testosterone.

30 November 1976; revised 17 January 1977

Suppression of Sympathetic Nervous System During Fasting

Abstract. *Two days of fasting in rats significantly reduces the turnover of norepinephrine in the heart. In contrast to the effects of ganglionic blockade in fed controls, similar blockade in fasted animals is without significant effect on [^{3}H]-norepinephrine retention or endogenous norepinephrine in the heart. These data are consistent with suppression of centrally mediated sympathetic activity in the fasted state. The decrease in norepinephrine turnover during fasting is completely reversed by 1 day of refeeding.*

Measurement of norepinephrine (NE) turnover provides a direct, in vivo means of quantifying the activity of the sympathetic nervous system in different sympathetically innervated organs. The sympathetic neurotransmitter, NE, is synthesized and stored within the peripheral sympathetic nerve endings. In response to nerve impulses NE is released from the nerve ending to stimulate the effector cells within its microenvironment. The action of released NE is terminated by an active transport mechanism within the membrane of the sympathetic nerve terminal. This uptake process conserves transmitter and, together with de novo biosynthesis of NE, serves to maintain the level of NE within a narrow range. The endogenous level of NE within a particular tissue does not, therefore,

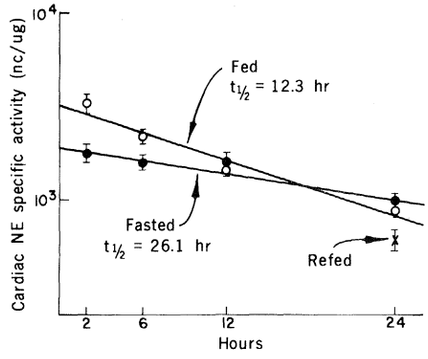

Fig. 1. [^{3}H]NE turnover in rat heart in fed, fasted, and refed animals. Female Sprague-Dawley rats (150 to 200 g) were separated into two groups 2 days before the start of the turnover experiment. Fed animals were continued on their diet of free access to rat chow and water; fasted animals were deprived of food but given unlimited access to both water and a hypotonic electrolyte solution containing 78 meq of Na and 15 meq of K per liter. (Estimated, voluntary Na^+ intake was 1 to 2 and 3 to 4 meq per rat per day for fed and fasted animals, respectively.) Refed animals, similarly starved for 2 days before the turnover study, were given rat chow and water after the study began. At the start of the turnover study, each animal received an intravenous injection of [^{3}H]NE (250 μc per kilogram of body weight; 32.5 c/mmole, New England Nuclear) by tail vein. At 2, 6, 12, and 24 hours after injection, five rats from each group were killed, and their hearts were removed and quickly frozen on Dry Ice. The organs were stored at −20°C until analyzed within 2 to 3 days. After the organs were homogenized in 0.4*N* perchloric acid at 4°C, NE was isolated chromatographically with alumina and the eluates were analyzed for [^{3}H]NE and NE as described (*4*). Values were corrected for recovery of 80 to 90 percent, as determined for each experiment. Specific activity of cardiac NE was determined for each time point and expressed as mean ± standard error of mean. The line representing the decline in specific activity with time was calculated by the method of least squares. For both fed and fasted groups, the significance of each slope is $P < .001$. For the fed group the slope or fractional turnover rate is 5.66 ± 0.14 percent with an NE turnover rate of 23.1 ± 1.5 ng per heart per hour [95 percent confidence interval (*4*)]. For the fasted group the fractional turnover rate is 2.66 ± 0.15 percent per hour, and the NE turnover rate is 13.1 ± 1.2 ng per heart per hour (95 percent confidence interval). The slopes are significantly different [$P < .005$, Student's t-test (*4*)]. In the single refed group the specific activity at 24 hours is significantly lower than that in either the fed or fasted groups ($P < .005$ and .025, respectively).

vary widely despite marked fluctuations in sympathetic activity because changes in the activity of membrane transport and in the rate of NE biosynthesis parallel changes in sympathetic activity (*1*). The kinetic measurement of NE turnover is therefore necessary for the assessment of sympathetic activity.

Two techniques of estimating NE turnover are employed in the present study. In the first, tracer doses of [^{3}H]NE are used to label intraneuronal NE stores. Following intravenous injection [^{3}H]NE is taken up by the axonal membrane uptake process; it rapidly equilibrates with intraneuronal NE stores and is released in response to sympathetic nerve impulses. The rate of disappearance of [^{3}H]NE is thus an indication of NE turnover in sympathetic nerves (*2*). This technique provides a reliable and reproducible measure of NE turnover (*3–5*). In the second method of assessing NE turnover, α-methylparatyrosine (αMPT) is used to inhibit tyrosine hydroxylase, the rate-limiting enzyme in NE biosynthesis (*6*). With NE biosynthesis inhibited, NE levels cannot be maintained and the rate of decrease of endogenous NE is an indication of NE turnover (*6*).

A variety of physiologic and pathophysiologic states known to increase sympathetic activity are associated with increased NE turnover as well. Hypothyroidism, hypopituitarism, cage restraint, exercise, and cold exposure, for example, stimulate NE turnover (*3, 4, 7*). Although pharmacologic agents such as ganglionic blocking drugs that decrease sympathetic outflow also decrease NE turnover, physiologic and pathophysiologic states that consistently reduce NE turnover have not been described. This report provides evidence that fasting for 2 days reduces cardiac NE turnover in rats.

The effect of 2 days of fasting on NE turnover as measured by the rate of disappearance of [^{3}H]NE is shown in Fig. 1. In this experiment fed rats and rats fasted for 48 hours received an intravenous injection of [^{3}H]NE at t_0. At 2, 6, 12, and 24 hours after injection, five animals from each group were killed and their hearts analyzed for [^{3}H]NE and endogenous NE. As shown in Fig. 1, fasting is associated with a highly significant ($P < .005$) increase in the calculated half-time of disappearance ($t_{1/2}$), from 12.3 ± 0.3 hours in the fed group to 26.1 ± 1.5 hours in the fasted group (*8*). The fractional NE turnover rate decreased significantly ($P < .005$) during fasting (from 5.66 ± 0.14 percent per hour in the fed rats to 2.66 ± 0.15 percent per hour in the fasted animals). Endogenous cardiac NE increased significantly ($P < .001$) in the fasted rats compared to the fed animals (492 ± 16.9 and 407 ± 16.3 ng per heart, respectively). The NE turnover rate, calculated as the product of the fractional turnover rate and endogenous NE content, was decreased ($P < .05$) in the hearts of fasting animals (23.1 ± 1.5 and 13.1 ± 1.2 ng per heart per hour in fed and fasted animals, respectively). In the refed group, which had been fasted for 48 hours before the start of the experiment but allowed access to food and water during the 24 hours after injection of [^{3}H]NE, there is a significant reduction ($P < .025$) in cardiac NE specific activity compared to that of animals fasted for 72 hours. This difference between fasting and refed animals is consistent with reversal of starvation-induced suppression of NE turnover by refeeding. Fasting for 2 days, therefore, is associated with decreased [^{3}H]NE turnover which is reversed by a single day of refeeding.

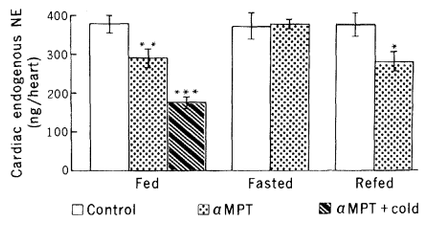

Fig. 2. Effect of inhibition of NE biosynthesis on endogenous NE in rat heart from fed, fasted, and refed animals. Animals were prepared as described in Fig. 1, except that refed animals after a 2-day fast were given access to rat chow and water 1 day before the experiment. Animals were injected intraperitoneally with the methyl ester of αMPT (Sigma) dissolved in saline, 250 mg/kg at t_0 and 125 mg/kg 3.5 hours later; controls received the same volume of saline in two injections. Animals were killed at 7 hours, and the endogenous NE levels in the heart determined as described for Fig. 1. A group of αMPT-treated animals was placed in cages in a cold room (4°C) during the experiment. Each group contained six to eight animals: $*P < .05$, $**P < .02$, $***P < .001$ for comparisons to the corresponding saline control.

The effect of inhibition of NE biosynthesis on endogenous NE in hearts of fed, fasting, and refed animals is shown in Fig. 2. In both fed and refed groups, significant ($P < .02$ and $< .05$, respectively) decreases in NE content in the heart were found after 7 hours of inhibition of NE biosynthesis; in the fasted animals, however, no decrease in cardiac NE occurred. The lack of change in cardiac NE after inhibition of NE biosynthesis in the fasted animals provides further evidence of decreased NE turnover in rats after a 2-day fast. Fed animals exposed to cold (4°C) exhibit a further decrease in cardiac NE after inhibition of NE biosynthesis ($P < .001$ and $< .005$ for comparison with control animals and αMPT-treated animals at room temperature, respectively) and provide an internal, positive control for the experiment. Thus, two independent methods for as-

sessing NE turnover demonstrate suppressed turnover during fasting which is reversed by refeeding.

Ganglionic blockade decreases sympathetic activity by interrupting central sympathetic outflow (*9*). The effect of such blockade on endogenous NE and on the residual [^{3}H]NE remaining in the hearts of fed and fasted animals 10 hours after administration of labeled NE is shown in Fig. 3. In this experiment chlorisondamine, a ganglionic blocking agent, was administered 5 minutes after injection of [^{3}H]NE into fed and fasted animals and again 5 hours after injection. The animals were killed 10 hours after the first injection. In the fed animals, the residual [^{3}H]NE is 61 percent greater ($P < .001$) and the endogenous NE is 25 percent greater ($P < .05$) in the group treated with ganglionic blockade than in the untreated control group. These differences are manifestations of reduced release of NE (both labeled and unlabeled) at the nerve terminal by pharmacologic reduction in impulse traffic. In the fasted animals, ganglionic blockade leads to only a 15 percent increase in residual [^{3}H]NE and a 5 percent increase in endogenous NE (neither difference statistically significant). The observation that ganglionic blockade produces smaller changes in cardiac NE in fasted animals than in fed controls is consistent with reduced impulse traffic in cardiac sympathetic nerves in fasting; the combination of fasting and ganglionic blockade yields little further decrease in impulse traffic beyond that produced by fasting alone.

Previous attempts to assess sympathetic activity during fasting have produced variable results. In studies of the effects of infusions of blocking drugs on tissues potentially responsive to catecholamines, such as pancreatic islets and adipose tissue, drug effects distant from the site of interest cannot be avoided (*10, 11*). Measurements of urinary or plasma catecholamines or their metabolites have likewise yielded no consistent results (*11, 12*). One factor apparently ignored in such studies is the natriuresis of fasting that occurs within the first week of a fast (*13*). Such a phenomenon in man, if not taken into account, might be expected to stimulate the sympathetic nervous system sufficiently on an orthostatic basis to obscure changes due solely to caloric deprivation. In the rat, an animal in which postural considerations are minimal, a significant decrease in urinary NE during the first of two 48-hour fasts in control animals has been reported (*14*). Thus, while no consensus exists concerning the functional state of the sympathetic nervous system during fasting, this question has apparently not been studied before in the direct manner described here.

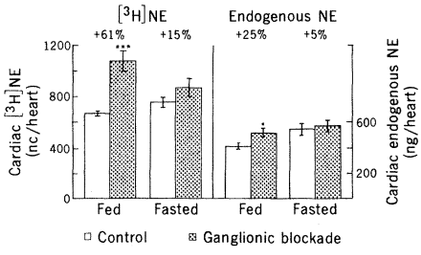

Fig. 3. Effect of ganglionic blockade on cardiac endogenous NE and [^{3}H]NE in fed and fasted rats. Animals were fasted for 2 days as described in Fig. 1. At 5 minutes and 5 hours after the intravenous injection of [^{3}H]NE (200 μc/kg), chlorisondamine (5 mg/kg; Ecolid, Ciba), a ganglionic blocking agent, was administered intraperitoneally. Control animals received intraperitoneal injections of saline. Animals were killed 10 hours after injection of [^{3}H]NE. Each group contained seven or eight rats. Percentages shown are increases in blockade group relative to control; $^*P < .05$, $^{***}P < .001$ for comparison to control.

Work in our laboratory suggests that the suppression of sympathetic activity reported here in heart occurs in other sympathetically innervated organs (*15*). In all organs examined, including liver, pancreas, spleen, small intestine, and salivary gland, fasting decreases sympathetic activity as measured by NE turnover. Thus, the fasting-related suppression of NE turnover in the heart, reported here, appears to reflect changes throughout the organism.

It is generally thought that the sympathoadrenal system is important in the adaptation to fasting. Glycogenolysis, gluconeogenesis, and lipolysis are stimulated by catecholamines, and the release of stored fuels and increase in hepatic glucose production during fasting have been thought to be controlled, in part, by circulating or locally released catecholamines (*16*). Although the suppression of NE turnover by fasting is evidence against that widely accepted belief, the possibility that circulating epinephrine may mediate some of the metabolic changes during fasting was not tested in the present study.

The association of fasting with decreased sympathetic activity has at least three significant implications. (i) This reduction in sympathetic activity may be a mechanism whereby the body attempts to conserve calories; therefore, manipulation to increase sympathetic activity may be useful in treatment of obesity. (ii) Since the sympathetic nervous system is important in the body's "fight or flight" response to stress, individuals who are suffering from caloric deprivation may have an impairment in this survival response, especially as it pertains to cardiovascular reflexes. (iii) Since hypertension probably involves the sympathetic nervous system, dietary factors may be important in the pathogenesis and treatment of some patients with high blood pressure.

JAMES B. YOUNG
LEWIS LANDSBERG
Department of Medicine and *Thorndike Laboratory, Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts 02115*

References and Notes

1. B. Bhagat and H. Zeidman, *Am. J. Physiol.* **219**, 691 (1970); G. C. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Ther.* **159**, 274 (1968); N. Weiner and M. Rabadjija, *ibid.* **160**, 61 (1968).
2. N. H. Neff, T. N. Tozer, W. Hammer, E. Costa, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **160**, 48 (1968).
3. L. Landsberg and J. Axelrod, *Circ. Res.* **22**, 559 (1968).
4. H. Taubin, B. Djahanguiri, L. Landsberg, *Gut* **13**, 790 (1972); B. Djahanguiri, H. Taubin, L. Landsberg, *J. Pharmacol. Exp. Ther.* **184**, 163 (1973).
5. The assumptions inherent in the [^{3}H]NE technique are that the NE content of the organ remains relatively stable throughout the turnover study and that the particular organ handles newly taken in [^{3}H]NE similarly to previously synthesized and stored NE. Although these assumptions may not be absolutely correct, the validity of this method has been demonstrated (*2–4*).
6. B. B. Brodie, E. Costa, A. Dlabac, N. H. Neff, H. H. Smookler, *J. Pharmacol. Exp. Ther.* **154**, 493 (1966).
7. R. Gordon, S. Spector, A. Sjoerdsma, S. Udenfriend, *ibid.* **153**, 440 (1966); A. Oliverio and L. Stjärne, *Life Sci.* **4**, 2339 (1965); A. Rubenson, *J. Pharm. Pharmacol.* **21**, 877 (1969); L. Landsberg, J. de Champlain, J. Axelrod, *J. Pharmacol. Exp. Ther.* **165**, 102 (1969).
8. Plotting cardiac [^{3}H]NE semilogarithmically against time yields similar results. Fasting is associated with a highly significant ($P < .001$) increase in $t_{1/2}$ (13.6 ± 0.3 and 29.1 ± 1.7 hours for fed and fasted animals, respectively). The data are plotted as specific activity versus time to remove minor fluctuations caused by slight changes in endogenous NE during the experiment.
9. G. Hertting, L. T. Potter, J. Axelrod. *J. Pharmacol. Exp. Ther.* **136**, 289 (1962).
10. R. P. Robertson and D. Porte, *Diabetes* **22**, 1 (1973); R. M. Walter, R. J. Dudl, J. P. Palmer, J. W. Ensinck, *J. Clin. Invest.* **54**, 1214 (1974); C. J. Goodner, D. J. Koerker, J. H. Werrbach, P. Toivola, C. C. Gale, *Am. J. Physiol,* **224**, 534 (1973); D. J. Koerker, C. J. Goodner, E. W. Chideckel, J. W. Ensinck, *ibid.* **229**, 350 (1975).
11. E. J. Pinter and C. J. Pattee, *J. Clin. Endocrinol. Metab.* **28**, 106 (1968); R. I. Misbin, P. J. Edgar, D. H. Lockwood, *Diabetes* **19**, 688 (1970).
12. W. Januszewicz, M. Sznajdelman-Ciswicka, B. Wocial, *J. Clin. Endocrinol. Metab.* **27**, 130 (1967); E. J. Drenick, L. C. Alvarez, G. C. Tamasi, A. S. Brickman, *J. Clin. Invest.* **51**, 2757 (1972); N. J. Christensen, *Diabetes* **23**, 1 (1974); R. G. Brodows, R. G. Campbell, A. J. Al-Aziz, F. X. Pi-Sunyer, *Metabolism* **25**, 803 (1976).
13. P. R. Boulter, R. F. Spark, R. A. Arky, *J. Clin. Endocrinol. Metab.* **38**, 248 (1974).
14. E. Herrera, R. H. Knopp, N. Freinkel, *Endocrinology* **84**, 447 (1969).
15. J. Young and L. Landsberg, *Diabetes* **25** (Suppl. 1), 391 (1976); in preparation.
16. J. Himms-Hagen, in *Catecholamines*, H. Blaschko and E. Muscholl, Eds. (Springer-Verlag, Berlin, 1972), p. 363–462.
17. We thank M. B. Berardino, D. Kuchmy, and P. Drain for excellent technical assistance. This work was supported in part by grants AM 05060-20, ML 11414, and RR 76 from the National Institutes of Health.

23 September 1976; revised 3 December 1976