

The role of the sympathetic nervous system in the metabolism of free fatty acids*†

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SUMMARY

Norepinephrine and epinephrine increased circulating levels of free fatty acids in humans and dogs. The increase was sustained in dogs during infusion of norepinephrine, but transient during epinephrine infusion, which also increased plasma sugar concentration. Anxiety or discomfort in humans and decrease in depth of pentobarbital anesthesia in dogs were accompanied by increased free fatty acid concentration, whereas ganglionic blockade with hexamethonium in dogs resulted in a prompt and sustained fall. In intact and adrenalectomized dogs receiving constant infusions of palmitic acid-1-C¹⁴, the decrease in free fatty acid concentration following hexamethonium was shown to result from a reduction in the rate at which they were added to plasma. Only a slight additional reduction was produced by insulin. The effects of norepinephrine and epinephrine on free fatty acid concentration were readily demonstrated during ganglionic blockade, whereas peripheral adrenergic blockade with dibenamine inhibited the response to these amines. The response of free fatty acids to sympathetic amines was also diminished in adrenalectomized dogs maintained with desoxycorticosterone alone. The concept is proposed that the sympathetic nervous system exerts a tonic action on the mobilization of fatty acids from adipose tissue which may be altered by central stimuli as well as by hormonal factors.

Recent studies (1) indicate that lipids are transported to peripheral tissues from adipose tissue depots mainly in the form of free fatty acids (FFA). These fatty acids are released from adipose tissue triglycerides and circulate in the blood plasma chiefly in a complex with albumin. The plasma level of FFA is increased by fasting and by administration of epinephrine (2, 3), growth hormone (4), and thyroid hormone (5). Epinephrine apparently acts by increasing the rate of mobilization of FFA. Partial glycerides accumulate in adipose tissue after administration of epinephrine (6), and *in vitro* studies have shown that epinephrine increases the rate of liberation of FFA from adipose tissue (7). Insulin, on the other hand, results in a fall in the plasma level of FFA (2, 3) and decreases the rate of FFA liberation from

adipose tissue *in vitro* (7). It appears likely that a number of hormones can alter the rate of mobilization of FFA from adipose tissue. Results of earlier studies, however, have suggested that mobilization of fat from adipose tissue is influenced by the nervous system. Peripheral nerve endings supply not only the blood vessels of adipose tissue but also the fat cells themselves (8). A number of investigators have shown that interference with the innervation of adipose tissue may impair the ability of the depot to mobilize fat. Wertheimer (9) found that section of the spinal cord above the sixth thoracic segment prevented fatty liver in phlorhizinized dogs. According to Beznák and Hasch (10), Hausberger (11), and later Clément (12), unilateral denervation of various bodies of fat in the mouse, rat, cat, and rabbit diminishes the rate of depletion of triglycerides from the depot on the denervated side in fasting animals. In view of these findings, further characterization of the interrelationship between fat mobilization and the sympathetic nervous system seemed pertinent.

To assess the role of the sympathetic nervous system in the metabolism of plasma FFA, the effects of norepinephrine (the physiologic neurotransmitter re-

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leased at postganglionic sympathetic nerve endings [13]), ganglionic blockade, and peripheral adrenergic blockade were studied. These experiments indicate that the metabolism of FFA may be altered strikingly by changes in the activity of the sympathetic nervous system, as well as by administration of norepinephrine, and therefore furnish considerable support for the concept that the sympathetic nervous system may play an important role in the regulation of the mobilization of fat from adipose tissue.

METHODS

Experimental Subjects. Studies were performed on men and male dogs in the postabsorptive state. Human subjects were kept at rest in bed during study with indwelling needles in place in an antecubital vein. Most of the animals were anesthetized with pentobarbital; a few experiments were performed on unanesthetized animals to determine the effects of anesthesia per se. Dogs were adrenalectomized in two-stage operations and maintained on desoxycorticosterone (2.5 mg. daily, intramuscularly). In some instances cortisone (5 to 40 mg. daily, intramuscularly) was also given. In anesthetized dogs, indwelling catheters in the femoral artery were utilized for recording blood pressure and withdrawing blood samples. In unanesthetized dogs, blood samples were taken from an antecubital vein.

Agents Administered. All agents were injected intravascularly. Standard pharmaceutical preparations of epinephrine hydrochloride and norepinephrine bitartrate were used. Hexamethonium was administered as the bromide or chloride. Dibenamine was dissolved in equal parts of ethanol and propylene glycol to make a 5 per cent solution which was acidified with 0.5 N HCl 1:100. This solution was diluted with 0.15 M saline for injection. Palmitic acid- $1-C^{14}$, specific ac-

tivity 5 mc. per mmole, was obtained from a commercial source. The acid was converted to the sodium salt and complexed with dog serum freed of lipoproteins less dense than 1.21, as described previously (14). The final molar ratio of fatty acid to albumin did not exceed 2.

Analytical. Blood samples were mixed with 1 mg. per ml. of sodium oxalate, chilled immediately in ice water, and centrifuged at 3°C. The plasma was kept at -19°C until analyses were performed. No change in analytical values was observed in tests on plasma frozen for as long as 1 month. Packed cell volume was determined by centrifuging the blood samples at $1800 \times g$ for 30 minutes in standard Wintrobe tubes, total protein by the method of Gornall *et al.* (15), and re-

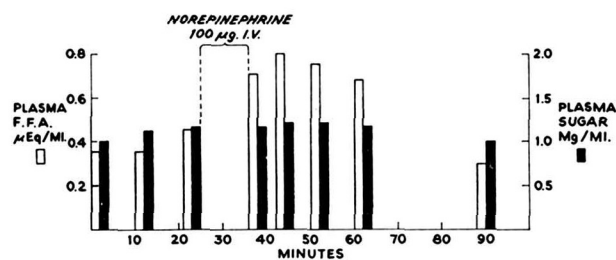


Fig. 2. Effect of norepinephrine on plasma levels of FFA and sugar in a healthy 35-year-old male subject.

ducing substances by the method of Folin and Wu (16). FFA were extracted from plasma by the method of Davis (17). The ether extract was evaporated under an air stream at room temperature, and the lipids were taken up in heptane. FFA were titrated according to the method of Dole (2); radioactivity was determined in a Packard liquid scintillation spectrometer with 0.3 per cent diphenyloxazole in toluene as phosphor.

RESULTS

Human Studies. The results of infusion of epinephrine and norepinephrine in a healthy subject are shown in Figure 1. The two agents had a similar effect on plasma FFA concentration, but epinephrine had a considerably more pronounced influence on plasma sugar than norepinephrine. In a second subject, as shown in Figure 2, infusion of 100 µg. of norepinephrine produced a rise in plasma FFA concentration which persisted for approximately 30 minutes. Absence of anxiety or discomfort in the subject was found to be essential for proper evaluation of the action of sympathetic amines on FFA. In three separate studies FFA concentrations rose 0.1 to 0.3 µeq. per ml. during a 30-minute "control" period following placement of indwelling needles.

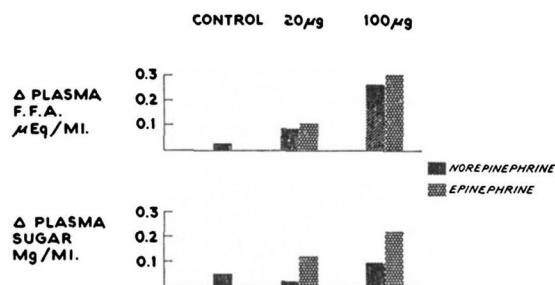


Fig. 1. Effect of graded doses of norepinephrine and epinephrine on plasma levels of FFA and sugar in a healthy 33-year-old male subject. The 20 µg. doses were administered in 2 minutes and the 100 µg. doses in 6 minutes. The increments plotted were those occurring 10 to 13 minutes after start of the infusions.

Dog Studies. The depth of anesthesia was found to affect markedly the concentration of FFA in the plasma. In general, decrease of anesthetic effect to the point where the dog shivered or developed tachypnea was associated with a significant rise in FFA concentration. In four experiments plasma FFA levels rose 0.28, 0.30, 0.55, and 0.68 $\mu\text{eq. per ml.}$, respectively, over a period of from 20 to 40 minutes as depth of anesthesia decreased. When anesthesia was properly maintained, single injections of small amounts of sympathetic amines produced detectable effects (Fig. 3).

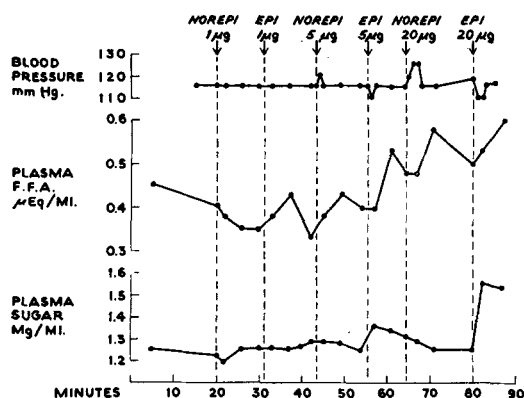


FIG. 3. Effect of graded doses of norepinephrine and epinephrine on plasma levels of FFA, sugar, and blood pressure in an anesthetized fasting male dog weighing 15 kg.

Prolonged infusion of norepinephrine (Fig. 4) resulted in persistent elevation of FFA concentration but no appreciable increase in sugar concentration, whereas similar infusion of epinephrine resulted in transient elevations of FFA and sustained elevation of sugar (Fig. 5). Packed cell volume rose significantly during these infusions, but plasma protein concentration was not significantly altered. The hemodynamic effects of

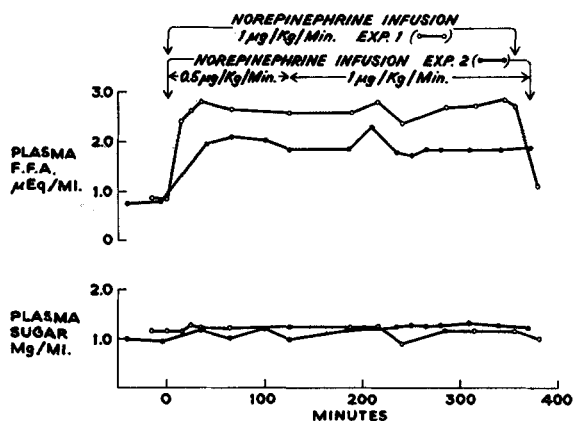


FIG. 4. Effect of norepinephrine infusion on plasma levels of FFA and sugar in 2 anesthetized fasting male dogs weighing approximately 20 kg.

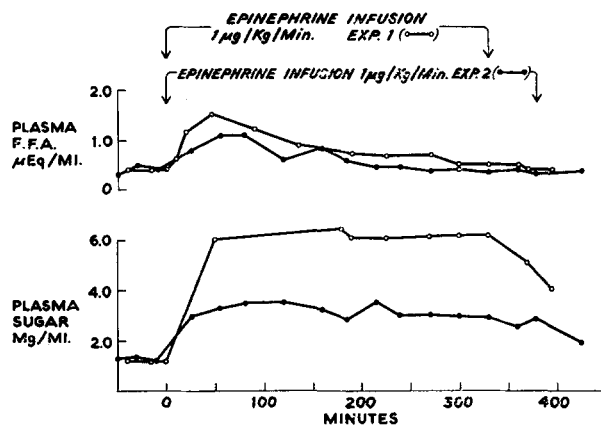


FIG. 5. Effect of epinephrine infusion on plasma levels of FFA and sugar in 2 anesthetized fasting male dogs weighing approximately 20 kg.

the infusions were transient; blood pressure remained elevated for only a few minutes.

Administration of hexamethonium uniformly resulted in a rapid and profound fall in plasma FFA concentration whenever the initial level was normal or high. The decrease persisted for long periods when ganglionic blockade was maintained (Fig. 6). Infusion

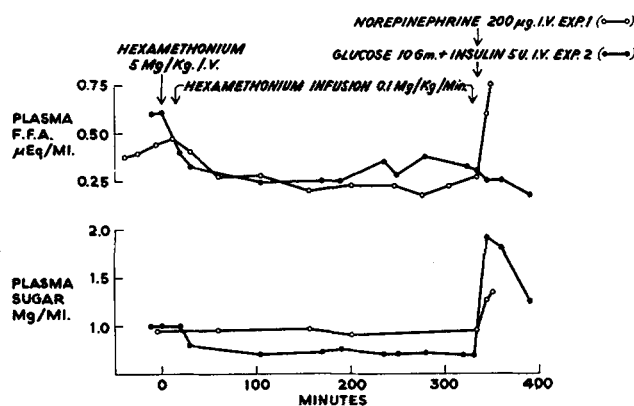


FIG. 6. Effect of hexamethonium administration on plasma levels of FFA and sugar in 2 fasting male dogs weighing approximately 20 kg. Pentobarbital anesthesia. Additional doses of 1 mg. per kg. of body weight were given at intervals during experiment 1 to maintain ganglionic blockade. The subsequent effects of glucose and insulin in one experiment and norepinephrine in another are also shown.

of glucose and insulin after administration of hexamethonium produced only a slight additional fall in FFA concentration, but the effects of norepinephrine and epinephrine were readily demonstrated. The action of hexamethonium was not dependent on anesthesia since similar results were obtained in unanesthetized dogs. In four anesthetized dogs given hexamethonium decreases in depth of anesthesia to the point where they shivered produced no significant change in plasma FFA levels ($<0.05 \mu\text{eq. per ml.}$).

To determine whether the effects of hexamethonium resulted from alterations in the rate at which FFA was added to plasma or removed from it, the following studies were performed. A solution of palmitic acid-1-C¹⁴, complexed with serum freed of lipoproteins less dense than 1.21, was infused into anesthetized fasting dogs at the constant rate of 1.4×10^5 cpm. per minute (0.15 μ eq. FFA per minute). After an appropriate control interval hexamethonium was given intravenously. After another interval a second agent was administered. Serial samples of blood plasma were extracted and assayed for radioactivity and FFA. Fractionation of the extract (14) showed that almost all the radioactivity was present in FFA. The results of one such experiment are shown in Figure 7. The level of radioactivity was stable during the

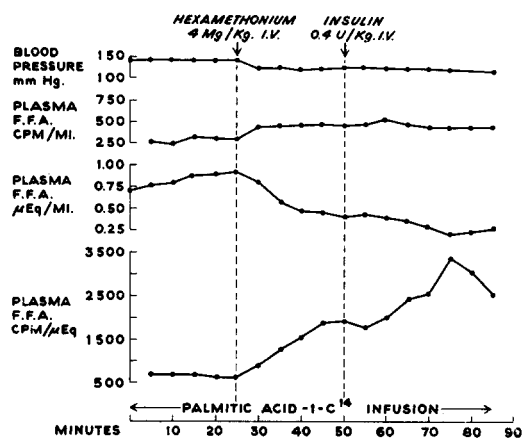


FIG. 7. Effect of hexamethonium and subsequent administration of insulin on blood pressure, plasma level of FFA, radioactivity in lipid extract of plasma, and calculated specific activity of FFA during constant infusion of palmitic acid-1-C¹⁴ in an anesthetized fasting male dog weighing 20 kg.

control period. After hexamethonium, total radioactivity rose slightly, indicating a decrease in the rate of removal of FFA from plasma. In spite of this, FFA concentration fell rapidly, showing that the rate of addition of FFA to plasma was greatly reduced. Administration of insulin was followed by a further slight fall in FFA concentration and a rise in FFA specific activity, suggesting an even greater decrease in the rate of addition of FFA to plasma. Additional studies showed that hexamethonium had little effect on plasma levels of FFA in dogs pretreated with glucose and insulin. Similar experiments were performed on two adrenalectomized dogs on a maintenance regimen of 2.5 mg. of desoxycorticosterone acetate and 40 mg. of cortisone daily. In the first dog (Fig. 8) FFA concentration was low initially, and only a slight fall was observed after hexamethonium. At the same time total radioactivity rose, so that FFA specific activity was

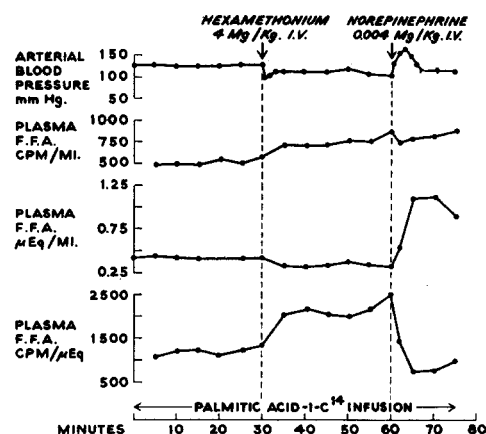


FIG. 8. Effect of hexamethonium and subsequent administration of norepinephrine on blood pressure, plasma level of FFA, radioactivity in lipid extract of plasma, and calculated specific activity of FFA during constant infusion of palmitic acid-1-C¹⁴ in an anesthetized fasting adrenalectomized male dog weighing 22 kg.

doubled, indicating a sharp reduction in the rate at which FFA was added to plasma. Administration of norepinephrine resulted in a striking increase in the rate at which FFA was added to plasma, as shown by a rise in FFA concentration with no change in total radioactivity. In the second adrenalectomized dog (Fig. 9) a decrease in anesthetic effect during the con-

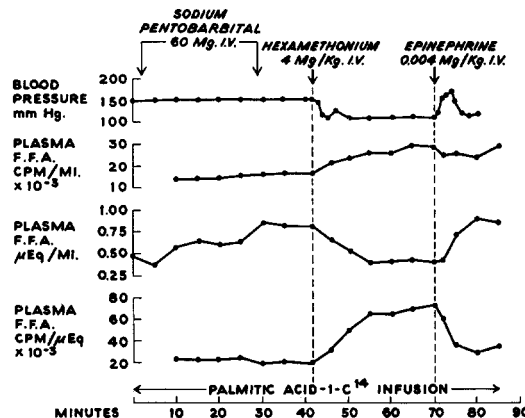


FIG. 9. Effect of hexamethonium and of subsequent administration of epinephrine on blood pressure, plasma level of FFA, radioactivity in lipid extract of plasma, and calculated specific activity of FFA during constant infusion of palmitic acid-1-C¹⁴ in an anesthetized fasting adrenalectomized male dog weighing 18 kg. Additional doses of pentobarbital were required to maintain anesthesia during the control period.

control period was associated with a significant rise in FFA concentration. The effects of hexamethonium and epinephrine were similar to those noted in the previous experiment.

In contrast to their striking action in adrenalectomized animals maintained with cortisone in addition to desoxycorticosterone, epinephrine and norepinephrine had only a relatively slight effect on adre-

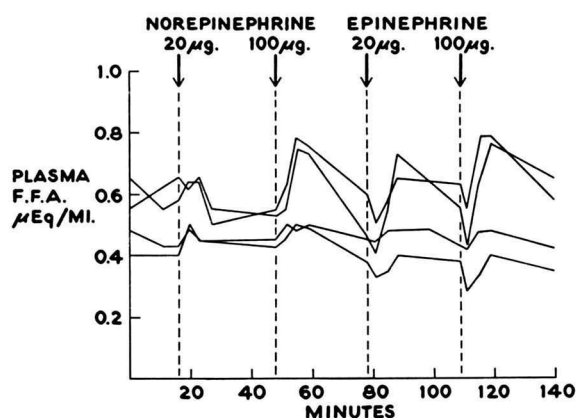


Fig. 10. Effect of graded doses of norepinephrine and epinephrine on plasma level of FFA in anesthetized adrenalectomized male dogs weighing 17 to 23 kg. Therapy: 2.5 mg. desoxycorticosterone acetate intramuscularly daily for 6 to 12 weeks prior to study.

nalectomized dogs maintained with desoxycorticosterone alone. Only two of the four dogs studied showed a significant rise in plasma FFA concentration after administration of either agent in doses up to 100 μ g. (Fig. 10). Administration of cortisone (10 mg. daily) restored the sensitivity to catechol amines in a dog that had shown no response when receiving desoxycorticosterone alone. The level of FFA increased 0.33 and 0.25 μ eq. per ml. of plasma 10 minutes after administration of 100 μ g. of norepinephrine and epinephrine respectively. The transient fall in FFA concentration after epinephrine (Fig. 10) was a consistent finding; it took place at the time the hemodynamic effects of epinephrine were subsiding. No such fall was observed after administration of norepinephrine.

The effects of norepinephrine and epinephrine on plasma FFA were blocked by prior intravenous ad-

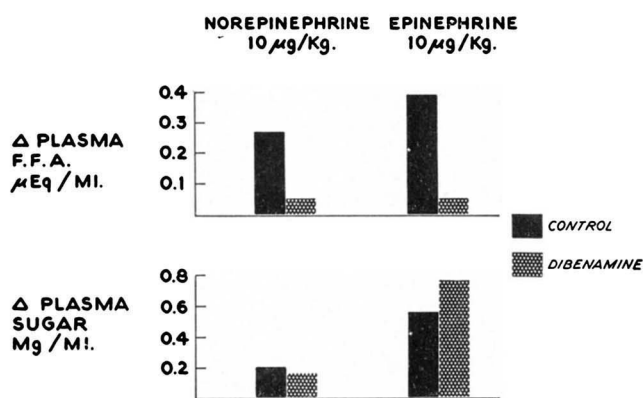


Fig. 11. Effect of administration of dibenamine on response of plasma levels of FFA and sugar to large doses of norepinephrine and epinephrine. Anesthetized fasting male dog weighing 16 kg. Control experiment performed 3 weeks prior to administration of dibenamine. The changes plotted were those occurring 10 minutes after administration of the amines.

ministration of dibenamine (20 mg. per kg. of body weight). Because dibenamine was found to produce a prompt rise in plasma FFA and sugar concentration, several hours were allowed to elapse before experiments were carried out on the treated animals. The results of one such experiment in an unanesthetized dog, given in Figure 11, showed that dibenamine had blocked the effect of sympathetic amines on FFA, whereas the effect of epinephrine on plasma sugar was unaltered. Two other experiments in anesthetized dogs gave similar results, and blood pressure measurements showed reversal by dibenamine of the usual effect of the sympathetic amines.

DISCUSSION

The observation that norepinephrine, like epinephrine, results in a rapid increase in the concentration of plasma FFA indicates that norepinephrine has important metabolic functions. Brewster *et al.* (18), in a study on anesthetized dogs under total epidural sympathetic blockade, found that infusion of epinephrine and norepinephrine led to an equal rise in oxygen consumption, but that only epinephrine produced a rise in serum lactate, pyruvate, and sugar concentration. We also observed an increase in oxygen consumption associated with an increase in plasma FFA concentration in anesthetized dogs after administration of norepinephrine.¹ The possible importance of the effect of norepinephrine on plasma FFA is suggested by the profound decrease in the rate at which FFA is added to plasma after administration of hexamethonium. This change, presumably reflecting decreased mobilization of adipose tissue triglycerides, is most likely the result of decreased liberation of norepinephrine from sympathetic nerve endings in the parenchyma of adipose tissue. Blockade of adrenal medullary secretion cannot be the decisive alteration, since the response to hexamethonium was similar in intact and adrenalectomized dogs. The perfusion of adipose tissue after ganglionic blockade has not been studied. Therefore the role of changes in blood flow in adipose tissue cannot be assessed in these experiments. Regardless of the mechanism, however, the action of hexamethonium on FFA mobilization must be assumed to result from prevention of the release of norepinephrine, since the action of injected norepinephrine is unaltered in the animal under ganglionic blockade.

Our interpretation of the experiments in which palmitic acid-1-C¹⁴ was infused requires the assumption that palmitic acid is a representative tracer for FFA

¹ Unpublished observations.

as a whole. In man the bulk of plasma FFA consists of palmitic, oleic, and stearic acids (19), the first two of which have a similar turnover rate (20). If these results can be extended to dogs, this assumption would be reasonable.

The rise in plasma FFA accompanying anxiety or discomfort in humans and decreasing depth of anesthesia in dogs provides further evidence of the role of the sympathetic nervous system in the mobilization of fatty acids from adipose tissue. Pentobarbital anesthesia in dogs is accompanied by diminished adrenal secretion of norepinephrine and epinephrine. Decreases in the depth of anesthesia are associated with increases in medullary secretion.² This effect of anesthesia may apply to the extra-adrenal sympathetic nervous system as well, since lessening of anesthesia in an adrenalectomized dog was accompanied by a rise in plasma FFA concentration (Fig. 9). Furthermore, alterations in depth of anesthesia did not result in changes in plasma FFA concentration in dogs given hexamethonium.

Administration of hexamethonium to dogs receiving a constant infusion of palmitic acid-1-C¹⁴ resulted in an increase in plasma radioactivity, indicating a decrease in the rate of removal of FFA from plasma. This decrease might occur as the result of reduced blood flow. In addition, the transient fall in FFA concentration after injection of epinephrine may well result from the increased cardiac output produced by this agent, particularly since norepinephrine, which presumably does not alter cardiac output appreciably, did not produce a similar reduction in FFA. Considering the extremely rapid turnover of FFA in the blood (14), it is not surprising that the rate of blood flow may limit the rate of their removal from the circulation.

In our experiments administration of dibenamine blocked the response of FFA to norepinephrine and epinephrine, but failed to lower FFA concentration to levels observed after administration of hexamethonium. This observation is in keeping with previous ones that this agent blocks the hemodynamic effects of injected sympathetic amines more effectively than the effects of spontaneous sympathetic activity (21). Since dibenamine must also block the effects of epinephrine and norepinephrine liberated from the adrenal medulla, it follows that adrenal medullary secretion was not an important factor in maintenance of plasma FFA levels in the dogs studied. Furthermore, we have observed that adrenalectomized dogs maintained with desoxycorticosterone acetate and cortisone

have normal plasma FFA levels.³ The failure of dibenamine to block the effects of epinephrine on plasma sugar has been reported previously (22). This dissociation, in conjunction with the fact that epinephrine and norepinephrine have similar effects on FFA but quite different effects on sugar concentration, suggests that these agents utilize different cellular mechanisms to effect release of FFA from adipose tissue and to promote glycogenolysis in the liver. The rise in plasma FFA and sugar concentration during the period of development of dibenamine blockade is consistent with other evidence which suggests that adrenal medullary discharge occurs during this time (23).

The response of the plasma FFA of adrenalectomized dogs to administration of sympathetic amines is consistent with the concept that the effects of sympathetic amines are decreased in the absence of adrenal cortical function (24). Shafir *et al.* (25) obtained somewhat similar results with epinephrine.

The importance of extra-adrenal sympathetic activity in fat mobilization is supported by the recent demonstration by White and Engel (26) that L-norepinephrine is approximately as effective as L-epinephrine in promoting release of FFA from rat epididymal adipose tissue incubated *in vitro*, and the report of Levy and Ramey (27) that administration of ergotamine tartrate prevented loss of lipid from epididymal adipose tissue in fasting adrenalectomized rats.

The physiologic significance of the effect of sympathetic nervous activity on the mobilization of fatty acids from adipose tissue is not defined precisely by this study. It is clear, however, that this mechanism can provide for rapid mobilization of fatty acids for energy needs of peripheral tissues. The reduction in plasma FFA levels in fasting dogs produced by hexamethonium suggests that the tonic activity of the sympathetic nervous system may provide for continuous release of FFA at a level which can be modified by insulin and other humoral agents. These observations indicate the need for further investigation of the interplay of nervous and humoral factors in the homeostatic control of fatty acid mobilization in various physiologic and pathologic states, and of the secondary metabolic effects in liver and peripheral tissues of altered mobilization of FFA.

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