

The plasma free fatty acid rebound induced by nicotinic acid

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ABSTRACT The time course of the nicotinic acid-induced changes in levels of plasma free fatty acids (FFA) was examined. The plasma FFA response of fasted dogs to graded doses of nicotinic acid was shown to be biphasic: an initial depression of the level of plasma FFA was followed by a rebound elevation to supernormal levels. FFA rebound was not seen after the administration of the nicotinic acid homologue, pyridyl-acetic acid, or a variety of nicotinic acid metabolites.

A similar pattern of FFA response was observed in fasted, normal rats. Adrenalectomy did not abolish the secondary elevation of FFA but did cause a somewhat delayed response. Hypophysectomy modified the time course of the response—the initial FFA decrease was prolonged—and the intensity of the FFA rebound was diminished. No rebound was observed in hypophysectomized, adrenalectomized rats. In normal rats, nicotinic acid caused a significant rise in the level of plasma corticosterone.

A normal rebound pattern was observed in thyroidectomized rats. Reserpine, administered on a schedule designed to deplete catecholamine stores, altered the time course of plasma FFA changes only slightly.

The results indicate that both the pituitary and adrenal functions are required for the expression of the rebound phenomenon after nicotinic acid administration.

KEY WORDS nicotinic acid · plasma free fatty acid decrease and rebound · adrenalectomy · hypophysectomy · thyroidectomy · reserpine · reserpine · nicotinic acid metabolites · plasma corticosterone · dog · rat

THE HYPOCHOLESTEROLEMIC EFFECT of nicotinic acid in man was first demonstrated in 1955 by Altschul, Hoffer, and Stephen (1). Much speculation concerning the mechanism of action of nicotinic acid followed, and in 1962 Carlson and Öro (2) reported that the levels of

plasma free fatty acids (FFA) of fasting humans were markedly reduced by this compound. These authors suggested that the long-term hypolipemic effect of nicotinic acid was due to this action (2, 3). The reduction of plasma FFA could be attributed to the effect of nicotinic acid in adipose tissue since a profound inhibitory effect on norepinephrine-induced lipolysis could be demonstrated in adipose tissue *in vitro* (3). In addition it has been shown that nicotinic acid accumulates rapidly in adipose tissue; its rate of accumulation in the tissue is consistent with its FFA-reducing properties (4).

A study of the time course of the effects of nicotinic acid on FFA by Carlson and Öro revealed (2, 3) that FFA values tend to rise above control levels after an initial decrease. The mechanism of the FFA rebound phenomenon has not been elucidated. The present paper reports the results of studies designed to define the effects of nicotinic acid on plasma FFA in dogs and rats and to investigate the mechanism of FFA rebound.

MATERIALS AND METHODS

Unanesthetized adult mongrel dogs of both sexes, weighing 8–14 kg, and male Sprague-Dawley rats weighing about 150 g were used. All animals were fasted for 18–20 hr before the administration of nicotinic acid.

Reserpine, 1.5 mg/kg, when used, was administered intravenously 24 and 18 hr prior to nicotinic acid administration. Bilateral adrenalectomies were carried out on rats under ether anesthesia 7 days before they were used. Adrenalectomized rats were allowed free access to 0.9% saline drinking water.

Hypophysectomized rats were supplied by the Charles River Breeding Laboratories, Brookline, Mass., and used 2 wk after surgery. Bilateral adrenalectomies were carried out on hypophysectomized rats 2 wk after hypophysectomy. These rats were used 5–7 days after surgery.

Abbreviation: FFA, free fatty acid(s).

Thyroidectomized rats were supplied by the Charles River Laboratories and used 5–6 wk after surgery.

In the studies with dogs, blood samples for analysis were obtained from the jugular vein. Rats were anesthetized intraperitoneally with sodium pentobarbital, 30 mg/kg, and 5–7 ml of blood was withdrawn from the abdominal aorta into a heparinized syringe. Nicotinic acid was administered into the brachial vein of dogs and into the tail vein of rats.

Plasma FFA were determined by the method of Dole (5). Whole blood glucose was determined on the Technicon AutoAnalyzer by the ferricyanide reduction method of Hoffman (6). Plasma corticosterone levels were measured by the fluorometric procedure described by Guillemin (7).

RESULTS AND DISCUSSION

FFA Rebound in Dogs

The intravenous administration of nicotinic acid to adult mongrel dogs (five dogs per group) resulted in an initial decrease in plasma FFA over a dose range of 1–32 mg/kg (Fig. 1). An increase in dose from 1 mg/kg to 3.2 mg/kg resulted in a more pronounced FFA depression; increases in dose beyond 3.2 mg/kg did not intensify the FFA depression, but prolonged the effect. At doses of nicotinic acid above 3.2 mg/kg, plasma FFA values had a pronounced tendency to rebound from the depressed range (around 200 μ eq/liter) to values between 1500 and 2000 μ eq/liter. An increase in the dose of nicotinic acid delayed the onset of the secondary elevation of plasma FFA. 18 hr after the intravenous administration of nicotinic acid, 100 mg/kg, the plasma FFA values in fasting animals were elevated to 2216 ± 293 (mean \pm SD, $n = 5$). Control values remained within the normal range at 948 ± 209 .

Since a rebound response was obtained only after a pronounced depression of plasma FFA that lasted about 1 hr, the possibility existed that this response was evoked by the initial FFA decrease. If this were correct, one would expect closely related FFA-lowering compounds to induce a rebound response. To test this hypothesis we gave the nicotinic acid homologue, pyridyl-3-acetic acid, to dogs intravenously over the same 1–32 mg/kg dose range. The level of FFA was depressed to about the same extent as it was by nicotinic acid (Fig. 2), but there was no indication of rebound during the 6 hr observation period. Thus, FFA rebound does not seem to be simply a consequence of a decrease of plasma FFA to extremely low levels. However, the possibility remains that the rapid rate of metabolism of nicotinic acid is ideally suited to unmask the tendency of plasma FFA to reach elevated levels after an initial depression.

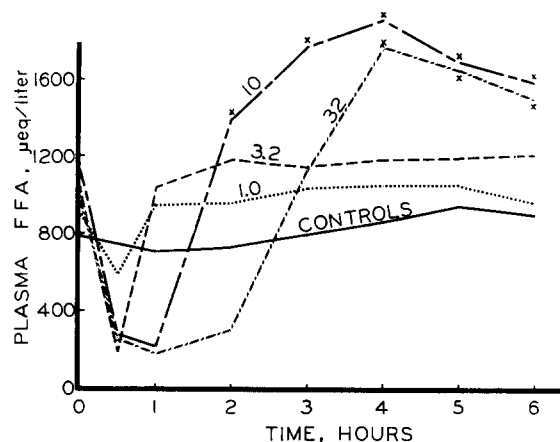


Fig. 1. Time course of the effects of various intravenous doses of nicotinic acid on the fasting plasma FFA of unanesthetized mongrel dogs. Each curve represents the mean response of four dogs; dose in mg/kg shown on each curve. X, significantly elevated from corresponding untreated controls ($P < 0.05$).

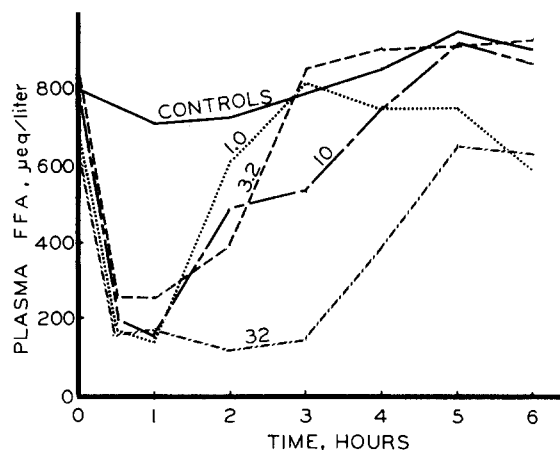


Fig. 2. Time course of the effects of various intravenous doses of pyridylacetic acid on the fasting plasma FFA of unanesthetized mongrel dogs. Each curve represents the mean response of four dogs; dose in mg/kg shown on each curve.

Another explanation for the rebound might be that nicotinic acid is converted to a metabolite capable of eliciting a pronounced FFA release. To examine this possibility, we administered intravenously to dogs a number of known metabolites of nicotinic acid, namely nicotinuric acid, trigonelline, *N*-methyl 2-pyridone-5-carboxamide, and *N*-methyl nicotinamide chloride (8, 9), at a dose of 10 mg/kg. None of these compounds produced significant changes in plasma FFA (Fig. 3).

A relationship between the cardiovascular effects of nicotinic acid (cutaneous flush) and FFA rebound appears unlikely in view of the findings of Carlson and Öro (10). These investigators reported a rise of plasma FFA values to above normal levels upon the injection of nicotinic acid even after long-term administration of the acid,

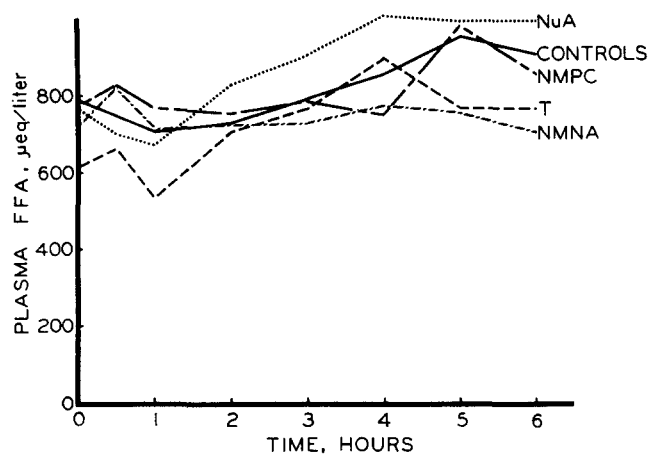


FIG. 3. Time course of the effects of several nicotinic acid metabolites on the fasting plasma FFA of unanesthetized mongrel dogs. Each curve represents the mean response of four dogs to 10 mg/kg, i.v., of drug. NuA, nicotinuric acid; T, trigonelline; NMPC, *N*-methyl 2-pyridone-5-carboxamide; NMNA *N*-methyl nicotinamide chloride.

when the cutaneous flush had been completely abolished. Furthermore, in the course of our studies on dogs, a cutaneous flush was obvious within 10 min after all doses from 1 to 100 mg/kg, whereas the plasma FFA rebound was delayed when the dose of nicotinic acid was increased (Fig. 1). An intravenous dose of 100 mg/kg delayed the FFA rebound until after the normal 6 hr observation period. This dissociation of the cardiovascular effects and rebound effects of nicotinic acid strongly suggests that the two phenomena are not causally related.

Rebound in Rats: Role of Adrenal and Pituitary

Similar studies were now undertaken in rats. An intravenous dose of nicotinic acid, 10 mg/kg, in normal, fasting rats produced a rapid and distinct fall in plasma FFA (Fig. 4). A maximum decrease was observed after 1 hr and was followed by a remarkable rise to elevated levels after 1.5 hr. These elevated levels were maintained for the remainder of the 4 hr observation period. After 4 hr the elevated FFA levels tended to return toward normal.

The well-established role of the endocrine system in the mobilization of fatty acids during fasting in rats (11) prompted a study of the effects of adrenal and hypophyseal ablation.

Adrenalectomized rats were given nicotinic acid, 10 mg/kg, intravenously (Fig. 5). The decrease in plasma FFA resembled that observed in normal rats but the peak increase in plasma FFA was observed at 2 hr instead of 1.5 hr. As in the normal rat, plasma FFA tended to return to normal values after 4 hr.

The most pronounced changes in the responses of plasma FFA to nicotinic acid were observed in the hypophysectomized rat. The initial FFA decrease was so prolonged that the values did not return to normal until

after 2–3 hr (Fig. 6). In addition, the FFA elevation observed 3 hr after nicotinic acid administration was modest in comparison with that in normal rats. The interpretation of this small elevation is complicated by the fact that the level of plasma FFA in the fasting, hypophysectomized animal is somewhat lower than in controls; nevertheless it is clear that hypophysectomy very much reduces the magnitude of the nicotinic acid-induced FFA rebound.

A number of pituitary factors—ACTH (12), TSH (13), α -MSH, adipokinetic hormone (14), and growth hormone (15)—have been reported to cause increases in

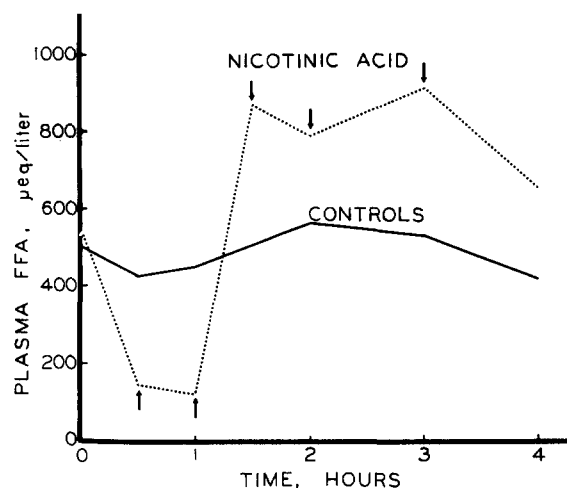


FIG. 4. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA of normal Sprague-Dawley rats. Each point represents the mean of six samples. Arrows indicate points significantly different ($P < 0.05$) from corresponding control points.

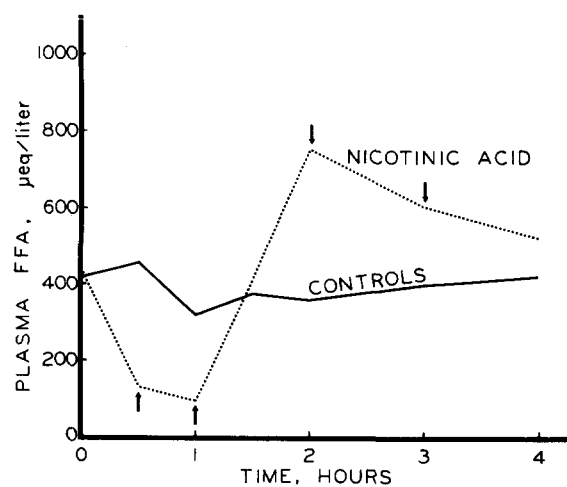


FIG. 5. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA of adrenalectomized Sprague-Dawley rats. Each point represents the mean of six samples. Arrows indicate points significantly different ($P < 0.05$) from corresponding control points.

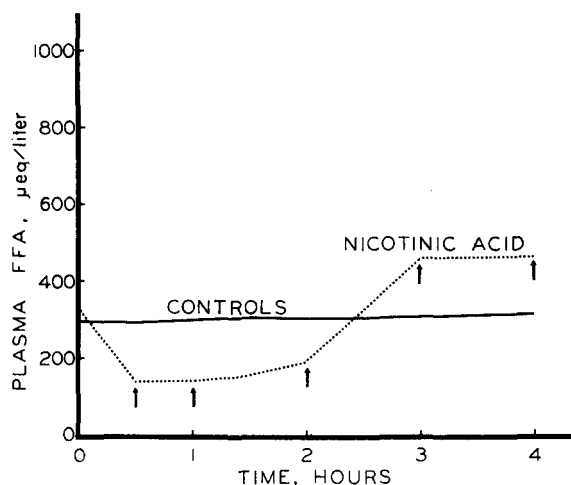


FIG. 6. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA of hypophysectomized Sprague-Dawley rats. Each point represents the mean of six samples. Arrows indicate points significantly different ($P < 0.05$) from corresponding control points.

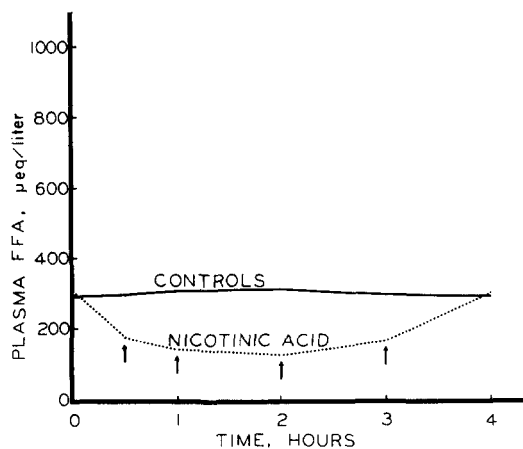


FIG. 7. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA of adrenalectomized, hypophysectomized Sprague-Dawley rats. Each point represents the mean of eight samples. Arrows indicate points significantly different ($P < 0.05$) from corresponding control points.

FFA release from mammalian adipose tissue *in vitro*. Lerner and McGuire (16) have described the lipolytic activity of ACTH and α -MSH in humans. The actions of both hormones were characterized by a delayed hyperglycemic effect. In addition, Knobil (15) has shown that the rate of FFA release from adipose tissue was stimulated by growth hormone. No short-term effects on blood glucose were observed. In this regard, it is interesting to note that no significant changes in blood glucose were observed in the nicotinic acid-treated rats and dogs during the peak increases in plasma FFA (Tables 1, 2).

Since adrenalectomy and hypophysectomy, separately, caused modifications in the FFA responses to nicotinic acid, studies were carried out in adrenalectomized-

hypophysectomized rats. Fig. 7 demonstrates that nicotinic acid, 10 mg/kg administered intravenously, reduced the plasma FFA of these fasting animals over a 4 hr period. The return to control levels was gradual and no evidence of FFA rebound was observed. Additional studies, in which the observation period was extended to 6 hr, also failed to reveal FFA values different from those of the control group.

In a further examination of the necessity for the pituitary and adrenal glands in FFA rebound, nicotinic acid (10 mg/kg) was administered intravenously to normal rats and plasma corticosterone values were monitored over the 4 hr observation period. Under these conditions, nicotinic acid caused a rapid and significant elevation of corticosterone levels at precisely the times when the rate of increase of plasma FFA was maximal (Fig. 8). The well-known permissive role of adrenocortical hormones in FFA mobilization (17) suggests that adipose tissue is sensitized to the action of pituitary lipolytic factors (probably ACTH) and therefore gives rise to a full expression of FFA rebound.

Role of Thyroid

Since hypophysectomized rats are likely to become hypothyroid after several weeks, nicotinic acid (10 mg/kg) was administered intravenously to rats that had been thy-

TABLE 1 EFFECT OF NICOTINIC ACID ON BLOOD GLUCOSE OF NORMAL FASTED RATS

Time	Nicotinic Acid*	Controls
hr	mg/100 ml	
0	67 \pm 6	69 \pm 5
0.5	74 \pm 4	74 \pm 6
1	68 \pm 10	67 \pm 10
2	61 \pm 11	62 \pm 8
3	59 \pm 5†	68 \pm 7
4	57 \pm 6†	70 \pm 6

Values are means \pm SD ($n = 6$).

* Administered intravenously, 10 mg/kg.

† Significantly lower than corresponding control group ($P < 0.05$).

TABLE 2 EFFECTS OF NICOTINIC ACID ON BLOOD GLUCOSE OF NORMAL FASTED DOGS

Time	Nicotinic Acid*	Controls
hr	mg/100 ml	
0	69 \pm 4	66 \pm 4
0.5	70 \pm 6	70 \pm 5
1	69 \pm 6	68 \pm 4
2	71 \pm 6	68 \pm 5
3	66 \pm 2	67 \pm 3
4	63 \pm 4	66 \pm 4
5	66 \pm 2	63 \pm 5
6	65 \pm 2	68 \pm 4

Values are means \pm SD ($n = 6$).

* Administered intravenously, 10 mg/kg.

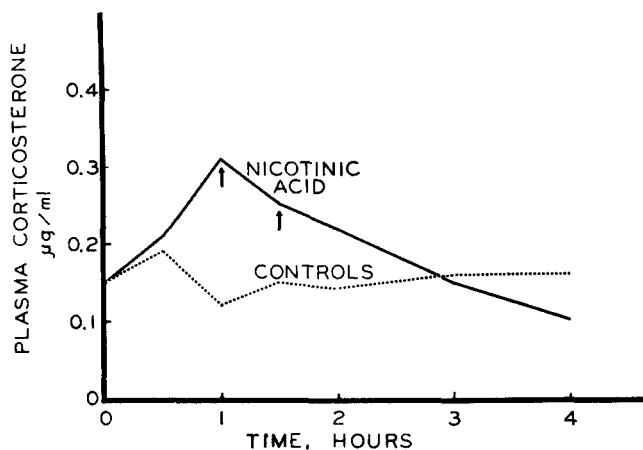


FIG. 8. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the plasma corticosterone levels of normal, fasted Sprague-Dawley rats. Each point represents the mean of eight rats. Arrows indicate points significantly different ($P < 0.05$) from corresponding control values.

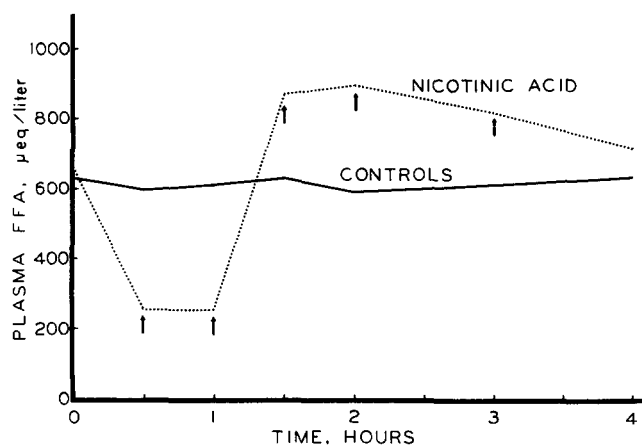


FIG. 9. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA values of thyroidectomized Sprague-Dawley rats. Each point represents the mean of six rats. Arrows indicate points significantly different from corresponding control points ($P < 0.05$).

roidectomized at least 5 wk previously. It is clear from Fig. 9 that the time course of plasma FFA changes induced by nicotinic acid in thyroidectomized rats is similar to that seen in normal intact rats. This finding indicates that the thyroid gland is not involved, in any significant way, in plasma FFA rebound.

Role of Catecholamines

In an attempt to determine the role of catecholamines in FFA rebound, rats were given reserpine intravenously, 1.5 mg/kg, 24 and 18 hr before nicotinic acid administration. This pretreatment has been reported (18) to deplete the norepinephrine stores of adipose tissue, brain, and heart in the rat. Under these conditions, nicotinic acid caused the expected fall in plasma FFA, which was followed by a rebound to levels comparable to those ob-

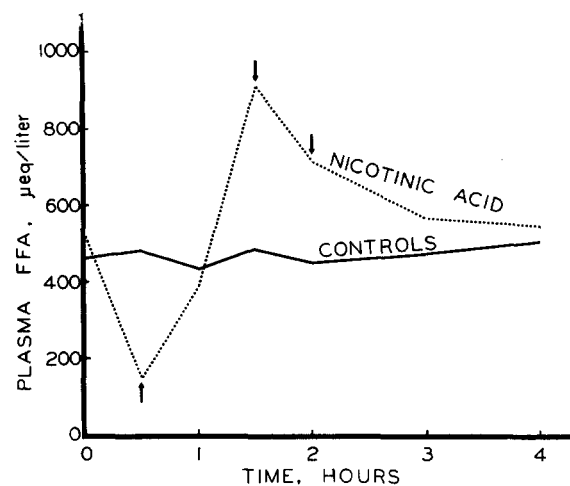


FIG. 10. Time course of the effects of nicotinic acid, 10 mg/kg i.v., on the fasting plasma FFA of reserpinized Sprague-Dawley rats. Each point represents the mean of six samples. Arrows indicate points significantly different ($P < 0.05$) from corresponding control points.

served in normal, intact rats (Fig. 10). The rebound response in reserpinized rats was evident after 1 hr instead of 1.5 hr. The apparently increased readiness to rebound may be related to the depletion of catecholamines from tissue stores as well as from adrenergic receptors. Burn and Rand (19) have demonstrated that reserpine, which depletes peripheral stores of norepinephrine, renders animals hypersensitive to several effects of norepinephrine: the pressor, vasoconstricting, and nictitating membrane-contracting effects. These authors reported (20) similar hypersensitivity after sympathetic nerve degeneration, which also depleted tissue stores of norepinephrine.

These results strongly suggest that the peripheral stores of tissue norepinephrine have no significant role in the secondary increase in plasma FFA after nicotinic acid administration. That reserpine depletes the catecholamine stores in the adrenal gland (21) further suggests that the adrenal medulla has a limited role in the induction of FFA rebound.

Conclusion

A prominent role for the pituitary-adrenal system is clear from the lack of an FFA rebound in adrenalectomized-hypophysectomized rats. Further evidence for such a role is found in the fact that an increase in plasma corticosterone values parallels the rebound elevation of plasma FFA. The manner in which nicotinic acid stimulates the pituitary-adrenal system is under investigation.

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