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## Effect of Reserpine on Fatty Acid Mobilization.* (27644)

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The mobilization of free fatty acids (FFA) from adipose tissue is increased during fasting and in response to the exogenous administration of growth hormone, epinephrine and norepinephrine. In addition, corticotropin, thyrotropin and possibly growth hormone, as well as epinephrine and norepinephrine, are potent lipolytic agents when incubated with adipose tissue *in vitro* (see 1 for review). Because the mode of action of all of these agents is similar as judged by their effects on carbohydrate metabolism and oxygen consumption(1, 2), as well as their effects on FFA mobilization, the possibility arises that all of these agents may exert their action on adipose tissue through a common pathway or by stimulating the release of a common mediator within the adipose tissue. The recent finding that norepinephrine is present in significant amounts in adipose tissue(3,4) lends credence to the possibility that the lipolytic agents mentioned above might act simply by releasing the catecholamines bound presumably in nerve endings in adipose tissue. In a preliminary communication, Paoletti *et al.*(3) reported that not only could adipose tissue be depleted of norepinephrine by pretreating rats with reserpine, but that rats so treated failed to mobilize FFA in response to corticotropin.

The present experiments were performed in an attempt to assess the role of catecholamines in the FFA mobilization which results from fasting and growth hormone administration.

*Materials and methods.* Studies were performed using fed and fasted normal male rats of the Charles River strain weighing approximately 150 g. Blood was taken from the abdominal aorta for determination of plasma FFA concentration. Epididymal adipose tissue was removed and samples of the thin distal portions were weighed and placed in vials of Krebs-Ringer phosphate buffer containing 5% fat-free human serum albumin. The vials were incubated in a Dubnoff metabolic water bath at 37°C for 3 hours. Combined FFA content of the tissue plus incubation medium was determined in each case according to the procedure of Dole(5). Blood sugars were

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TABLE I. Effect of Reserpine on FFA Mobilization during Fasting.

| Animals | Adipose tissue FFA, μeq/g/3 hr | P* | Plasma FFA, μeq/ml | P* |
|---|---|---|---|---|
| Fed controls | 2.85 ± .40† | | .25 ± .03 | |
| Fasting | 11.29 ± .71 | <.001 | .96 ± .07 | <.001 |
| " + reserpine, 5 mg/kg | 6.35 ± .50‡ | <.001 | .45 ± .04‡ | <.01 |

* Compared to fed control.
† Mean ± S.E.—at least 7 rats/group.
‡ Significantly different (p <.001) from fasting untreated rats.

measured by the glucose oxidase method (Worthington Biochemicals). Reserpine was injected subcutaneously in doses of 5 to 20 mg kg 18 hours before death unless stated otherwise. Ovine growth hormone§ was given intraperitoneally (1 mg/rat) 4 hours before sacrifice. Corticotropin, when used, was added to the medium at a final concentration of 0.1 μg/ml.

*Results.* Treatment of normal rats with 5 mg/kg of reserpine significantly reduced but did not abolish the normal increase in FFA mobilization which accompanies fasting (Tables I and III). However, this effect was not always apparent even when other actions of reserpine were fully evident (*e.g.*, Table IV). This dose of reserpine produced severe changes in normal function characterized by somnolence, diarrhea, nasal discharge and an apparent decrease in body temperature. Mild but persistent hyperglycemia was also noted in these animals (Table II). Despite its relative efficacy in reducing FFA mobilization in response to fasting, reserpine treatment failed to block FFA mobilization in response to growth hormone (Table III).

Since Paoletti *et al.*(3) reported that adipose tissue which was depleted of catecholamines, as verified by direct chemical analysis, fails to produce FFA in response to corticotropin, it was felt that the response to corticotropin might be used as an indirect indicator of the catecholamine content of adipose tissue. However, corticotropin caused as great an increase in FFA production in adipose tissue from reserpinized rats as from normal animals even when the dose of reserpine exceeded the effective dose used by Paoletti by as much as 6-fold (Table IV).

*Discussion.* Even when administered at a dose sufficient to cause severe physiological disturbances, reserpine failed to block FFA mobilization in response to fasting. Since reserpine usually diminished the production of FFA during fasting, the possibility that endogenous catecholamines, and hence the sympathetic nervous system, influences the extent of FFA mobilization during fasting cannot be entirely ruled out. A more likely explanation for this effect, however, is offered by the observation by ourselves and others (6) that reserpine produces a mild but prolonged hyperglycemia. The inhibitory effect of glucose on FFA production is well known

TABLE II. Effect of Reserpine on Blood Glucose in Fasting Rats. Reserpine (5 mg/kg) given at zero time of fasting.

| Hr of fasting | Blood glucose, mg/100 ml Controls | Reserpine-treated | P |
|---|---|---|---|
| 0 | 85.1 ± 2.4* | 87.6 ± 2.1 | |
| 14 | 85.4 ± 3.0 | 115.4 ± 3.9 | <.001 |
| 24 | 77.3 ± 3.3 | 103.2 ± 1.4 | <.001 |

* Mean ± S.E.—8 rats/group.

§ Gift of Endocrinology Study Section, Nat. Inst. Health.

TABLE III. Effects of Growth Hormone on FFA Mobilization in Normal and Reserpine-Treated Rats.

| Animals | FFA production, μeq/g/3 hr | P |
|---|---|---|
| Normal | | |
| Control | 11.61 ± .82* | |
| Growth hormone | 15.38 ±1.36 | <.05 |
| Reserpine-treated | | |
| Control | 6.64 ± .83 | |
| Growth hormone | 12.29 ± .79 | <.001 |

* Mean ± S.E.—at least 10 animals/group.

TABLE IV. Response of Adipose Tissue from Fasting Reserpine-Treated Rats to Corticotropin Added *in Vitro*.

| Reserpine dose | No. of tissue pairs | FFA production, μeq/g/3 hr | | |
|---|---|---|---|---|
| | | Control | Corticotropin added (0.1 μg/ml medium) | P |
| None | 8 | 11.54 ± 1.47* | 28.91 ± 2.04 | <.001 |
| 5 mg/kg | 7 | 11.76 ± .97 | 27.90 ± 2.00 | <.001 |
| 20 " | 6 | 13.09 ± .58 | 29.72 ± 1.98 | <.001 |
| 10 " , daily × 2 | 7 | 11.99 ± 2.52 | 23.12 ± 2.73 | <.02 |

* Mean ± S.E.

(1), although the explanation for the hyperglycemia is not apparent. Furthermore, guanethidine, which is also known to deplete catecholamines(7), failed to modify this response to fasting or to influence blood glucose concentrations when given subcutaneously in doses of 20 mg/kg (Edmonson, unpublished). The finding that FFA mobilization can occur during fasting in rats even after catecholamine depletion is in accord with earlier observations of Levy and Ramey(9) and Goodman and Knobil(8) who found that autonomic blockade did not interfere with normal fat mobilization during fasting. These findings are, however, at variance with the observation of Shafrir and Wertheimer(10) who noted a decrease in FFA release from mesenteric adipose tissue removed from fasting rats pretreated with debenzyline.

Since the dosage of reserpine used in the present study should have been adequate to deplete the adipose tissue of catecholamines (3,4) it may be concluded that the FFA mobilizing action of growth hormone is not dependent on the mediation of epinephrine or norepinephrine. This conclusion is consistent with earlier findings (Goodman and Knobil, unpublished) that neither ganglionic blockade with hexamethonium nor adrenergic blockade with ergotamine interfered with FFA mobilization in the monkey following growth hormone administration.

Although it has been suggested(3) that FFA mobilization in response to corticotropin is mediated by norepinephrine, the present studies provide no evidence to support this thesis. Our data clearly indicate that pretreatment of donor rats with large doses of reserpine does not alter the ability of the adipose tissue to produce FFA when corticotropin is added *in vitro*. Since we were unable to increase plasma FFA concentration by giving corticotropin in gelatine according to the procedure of Paoletti *et al.*(3), we were also unable to confirm their conclusion that reserpine blocks the increase in plasma FFA induced by corticotropin. The reasons for these discrepancies are not readily apparent.

*Summary.* Reserpine treatment diminished but did not abolish the mobilization of free fatty acids (FFA) which occurs during fasting in rats. Reserpine also did not interfere with increased production of FFA resulting from administration of ovine growth hormone *in vivo*. Similarly reserpine failed to abolish this effect of corticotropin *in vitro* even when given in doses in excess of those known to deplete adipose tissue of norepinephrine. It may be concluded that the fat-mobilizing actions of these hormones are not dependent on the mediation of epinephrine or norepinephrine. Because the dose of reserpine used in these experiments also produced a mild but prolonged hyperglycemia, it cannot be definitely established whether the reduced response to fasting in reserpine-treated animals was due to the depletion of norepinephrine from adipose tissue or to the hyperglycemia.

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## Comparison of Cholesterol Uptake in Normal and Atherosclerotic Individuals. (27645)

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It is widely accepted that an elevated concentration of cholesterol in serum is related to an accentuation of the process of atherosclerosis(1). However, the mechanism by which cholesterol separates from serum lipoproteins and accumulates in plaques in vessel walls remains unclear(2).

Since atherosclerosis is frequently associated with an elevated concentration of serum cholesterol, the question arises as to what extent the capacity of serum lipoproteins to bind and transport cholesterol is normally utilized, *i.e.*, in normal persons, is the capacity of lipoproteins to bind cholesterol saturated, or is there a reserve cholesterol binding capacity? If a reserve cholesterol binding capacity exists, is it increased, decreased, or unchanged in persons with atherosclerosis?

To investigate these questions, we measured the concentration of serum cholesterol before and after incubation of the serum with solid cholesterol. Serum was obtained from putatively normal young men and from patients hospitalized for some form of arteriosclerotic cardiovascular disease such as angina pectoris, myocardial infarction, or hypertension. Preliminary results confirmed that human serum can dissolve additional cholesterol and revealed that serum from the group of patients dissolved as much cholesterol as did serum from healthy young men.

Some of the data reported herein were the basis of a previous preliminary report(3).

*Materials and methods.* Cholesterol was USP grade, recrystallized twice from 95% ethanol. To prepare batches of crystals of similar size, the recrystallized product was dissolved in the minimum volume of boiling 95% ethanol required to effect solution and precipitated by rapid addition of 3 volumes of distilled water at 20°C. After cooling to 15°C, the crystals were filtered off, washed with copious volumes of ethanol-water (1:3, v/v), and sucked dry on the filter. The product was then air dried for 24 hours and stored in a vacuum desiccator.

The procedure for incubation of serum with solid cholesterol was somewhat different from that used by the French workers(4,5,6) or by Avigan(7). After investigating the results of incubating serum for various periods with various amounts of cholesterol(8), the following procedure was adopted:

1. Pipette a suitable volume (*e.g.*, 4 ml) of serum into each of six 16 × 125 mm screw-cap test tubes.

2. Add sufficient cholesterol, recrystallized as described above, to provide 40, 80, 120, 160, and 200 mg cholesterol/ml serum in tubes 1 through 5, respectively. Tube 6 is a control; no cholesterol is added to it.

3. Incubate all tubes at 37°C for 4 hours with gentle inversion of all tubes at 15-minute intervals.

4. Filter the contents of all tubes through Whatman No. 41 filter paper. Use only gravity flow.

5. Determine cholesterol concentration in the filtrate. Tube 6 is an incubated control. As an additional control, determine concen-