Production of Unesterified Fatty Acids from Isolated Rat Adipose Tissue Incubated *in vitro*. (23672)

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Recent studies of the concentration of unesterified fatty acid (UFA) in the circulating plasma of intact human subjects have given evidence that UFA is derived from adipose tissue, and that the amount in circulation and the rate of its production from adipose tissue vary in response to the metabolic requirements of the organism(1,2,3). The administration of carbohydrate, insulin, or both, to fasting subjects was shown to be a potent influence leading to a reduction of circulating UFA concentrations. Administration of epinephrine or fasting led to marked increases. The present study was undertaken to discover if corresponding effects could be demonstrated using isolated adipose tissue fragments incubated *in vitro*, since such a simplified system might lend itself to the demonstration of the role of the various factors concerned with the regulation of the liberation of UFA from depot fat.

Materials and methods. Adipose tissue for study was taken from the epididymal fat bodies of young male rats weighing 150 to 250 g; the animals were anesthetized with intravenous nembutal and the tissue removed prior to sacrifice. This tissue occurs in thin leaves and does not require slicing. The tissues were rinsed in a 1% solution of bovine serum albumin (Armour Fraction V) in saline, and stored briefly in this medium until all rats of a given group had been sacrificed. The tissues were then transferred into 25 ml Erlenmeyer flasks each containing 5 ml incubating medium, and agitated gently at 37°C. The flasks were covered with aluminum foil to prevent evaporation of the medium. The wet weight of each tissue fragment was determined at the end of the experiment, and suitable aliquots of the incubation medium were analyzed for UFA content at the beginning and end of each incubation, using the method previously published(2). The period of incubation was 4 hours except where otherwise noted. Incubations were carried out in a medium prepared by dissolving 5% by weight of bovine serum albumin in Krebs-Ringer phosphate buffer, and readjusting the pH to 7.3-7.4 with sodium hydroxide. Results of UFA analyses were calculated in terms of micromoles of UFA liberated per gram wet weight of tissue per hour. Whenever possible, one epididymal fat body from a rat served as a control for comparison with the behavior of the contralateral tissue under the influence of the agent being tested.

Results. The effect of nutritional state was tested by comparing UFA production rate from the tissues of rats fasted overnight with the behavior of tissues from rats maintained overnight without solid food, but with 10% glucose in their water. The tissues from the carbohydrate-fed rats produced significantly less UFA on incubation than did those from the fasted animals (p less than 0.01). Data from one representative experiment are given in Table I.

The effect of epinephrine added to the medium at a concentration of 0.01 μg per ml of solution was tested on the tissues of both fasted and carbohydrate-fed rats. In these experiments, incubation times of one hour were sufficient to demonstrate acceleration of UFA release in the epinephrine-treated tissues. The data from one typical experiment are given in Table II. This experiment alone is significant on statistical analysis (p equals 0.05); with 3 other experiments yielding similar results one cannot doubt the effectiveness of epinephrine at this concentration. At the concentration of 0.1 μg per ml of medium epinephrine showed a highly significant effect

TABLE I. UFA Production from Tissues of Fasted and Fed Rats.

State	UFA production (μM/g tissue/hr) Mean	S.D.
Fasted (4 tissues)	6.4	.75
Fed (4 ")	−.2	.38

TABLE II. Effect of Epinephrine (0.01 μg/ml) on UFA Production from Tissues of Fasted Rats.

Treatment	Mean UFA production (μM/g tissue/hr)
None (4 tissues)	1.31
Epinephrine (4 ")	2.31

Differences between outputs of treated tissues and contralateral controls:

Mean 1.00; S.D. .62

(p less than 0.01); whereas at a concentration of 0.001 μg per ml, the effect was questionable.

The effect of insulin was tested on the tissues of fasted rats incubated in media fortified by the addition of 1 mg glucose per ml. 0.01 unit of insulin per ml of medium caused a significant decrease in the UFA output of tissues so treated. In Table III are presented the data from one experiment demonstrating this effect; the results of this one experiment are highly significant (p less than 0.01). Repetitions of the same experiment yielded confirmatory results.

Discussion. The demonstration of liberation of UFA from adipose tissue fragments incubated *in vitro* serves to support the conclusion, derived from studies of intact fasting human subjects, that the source of the circulating UFA is adipose tissue. It must be noted, however, that if transport of fatty acids out of the depots as UFA is to account for the mobilization of stored fat to supply the caloric needs of the organism during periods of fasting, a rate of UFA release on the order of 100 micromoles per gram of tissue per hour would be necessary. (This calculation applies to rats of approximately 200 g weight, and employs unpublished data of Dr. Joseph Bragdon for the average total body fat and caloric requirement of Sprague-Dawley rats.) It is evident, therefore, that the observed metabolic activity of adipose tissue *in vitro* falls short of its postulated activity *in vivo*. Such impairment of activity is not surprising, since the incubated tissues are exposed to the medium only on their external surface, whereas *in vivo* the surface available for exchange of metabolites with plasma is much greater. In spite of this objection, it seems likely that this *in vitro* system constitutes a satisfactory model for the study of chemical and physiologic factors influencing UFA production.

TABLE III. Effect of Insulin (0.01 unit/ml) on UFA Production from Tissues of Fasted Rats.

Treatment	Mean UFA production (μM/g tissue/hr)
None (4 tissues)	1.58
Insulin (4 ")	−1.03

Differences between treated tissues and contralateral controls:

Mean 2.61; S.D. .43

The nutritional state of the donor animal, and 2 hormonal substances added *in vitro*, have been shown to influence UFA production in the model system in a manner compatible with their observed influences *in vivo*. The epinephrine effect has been demonstrated with hormone concentrations which are low enough to be similar to those effective *in vivo;* insulin has so far been tested only at a concentration 2 orders of magnitude higher than that effective *in vivo*. Nevertheless, the effectiveness of insulin in this simple system suggests that it operates in the intact organism by affecting the adipose tissue directly rather than through the mediation of some other physiologic system.

Summary. Adipose tissue from rats, incubated *in vitro* in a medium of known composition, was found to release unesterified fatty acids (UFA). The rate of evolution of UFA was increased by the addition of epinephrine to the medium or by fasting the donor animal. It was decreased by the addition of glucose and insulin to the medium, or by feeding the donor animal with carbohydrate.

1. Gordon, R. S., Jr., Cherkes, A., *J. Clin. Invest.*, 1956, v35, 206.

2. Gordon, R. S., Jr., *ibid.*, 1957, v36, 810.

3. Dole, V. P., *ibid.*, 1956, v35, 150.

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