Fat-Mobilizing Activity of Human Urine Extract

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IN 1947 Weil and Stetten[1] reported that an alkaline extract of urine from fasting rabbits was effective in increasing liver fat in mice. More recently[2,3] we have shown that fat-mobilizing activity is present in human urine under certain conditions including fasting.

CONDITIONS IN WHICH ACTIVITY IS FOUND

The conditions in which we have found activity in the urine are shown in Table I. Carbohydrate deprivation appears to be at least as important a stimulus as calorie deficiency. With diets of 1,000 calories, activity appears when the carbohydrate content is reduced below 100 gm. and increases progressively with further carbohydrate restriction.

In two patients with diffuse lipoatrophy, activity has been found in the urine although a normal mixed diet was being consumed. One of them was an adolescent girl with well controlled diabetes and accelerated growth in addition to lipoatrophy. The other patient was not diabetic.

As Table I also shows, activity has been found in diabetic ketosis, in widespread carcinoma with wasting, and during the first few days after major surgical operations. In all these patients the intake of calories was relatively low and there is some doubt about the interpretation of the finding.

A normally functioning anterior pituitary appears to be necessary for the production of the active material. Six patients with deficient function of the anterior pituitary, fasting for thirty-six hours or on a diet of 1,000 calories and 90 per cent fat, failed to produce any activity in the urine (Table III).

BIOLOGICAL EFFECTS

The effects of subcutaneous injection of active material into mice are summarized in Table II. Liver fat, blood lipids and blood ketones all increase, the effects being maximal about six hours after injection (Fig. 1, Table III). Values in this and subsequent tables are means ± standard error of the mean. The minimal dose of our most active extracts for obtaining the liver fat and ketone effects is about 10 μg. for each mouse. Larger doses (> 200μg.) are needed to produce unequivocal rises in blood lipids (total lipids, phospholipid, cholesterol and non-esterified fatty acids).

There is an early and transient fall in blood sugar (Fig. 1). Utilization as well as mobilization of body fat is increased. This has been demonstrated using labeled tripalmitate.[2] However, it is demonstrated more simply by the loss of body weight and carcass fat after single large doses or repeated small ones (Figs. 2 to 4, Table IV). No change in appetite and food intake accompanies this increased catabolism of body fat. On the simplest assumption, therefore, total expenditure of energy must increase. Alternatively, the efficiency of the utilization of energy may diminish. Measurements of the uptake of oxygen are in progress.

PREPARATION OF EXTRACTS

Our present method of extraction is illustrated in Figure 5. The preparation of the alkaline extract may be conveniently carried

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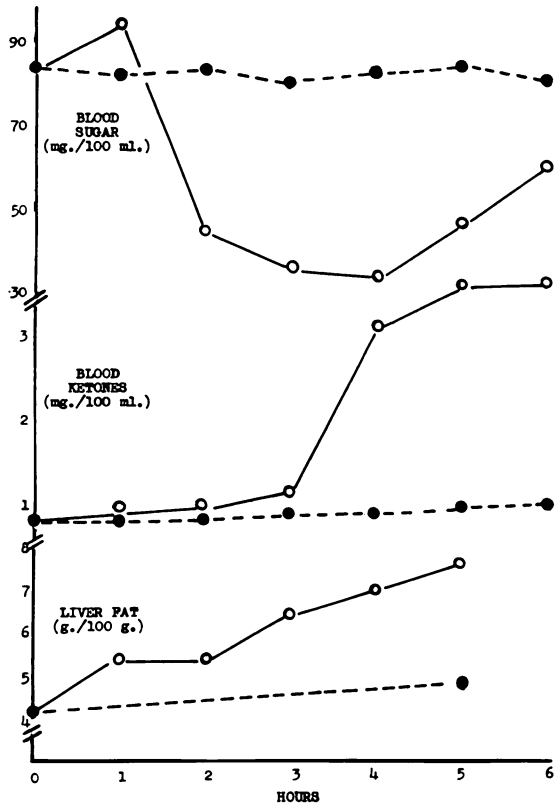

FIG. 1. Time-course of effects of fat-mobilizing substance (FMS) on blood sugar, ketone bodies (as acetone), and liver fat in mice. Groups of animals killed at hourly intervals after subcutaneous injection. Broken lines indicate results in control animals injected with saline.

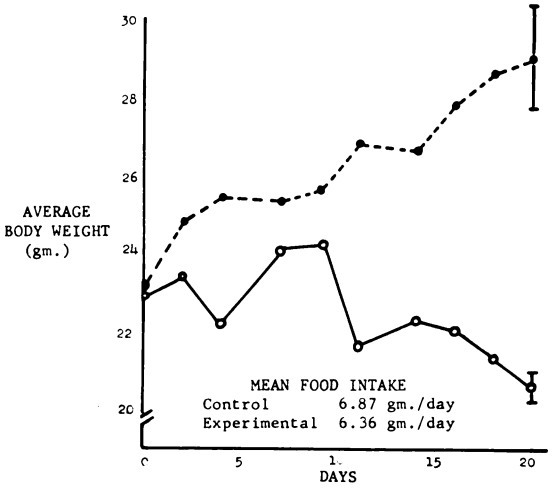

FIG. 2. Effect of injection of saline (broken line) or active extract (solid line) three times weekly for twenty days on body weight of mice (ten mice in each group). The difference in food intake is the largest observed in six experiments. For carcass analysis see TABLE IV.

TABLE I
Fat-Mobilizing and Ketogenic Activity in Urine

Present (Fasting)	Absent (Normal Diet)
1,000 Calories, 90 per cent Fat	1,000 Calories, 90 per cent CHO
1,000 Calories, 90 per cent Protein	Lipodystrophy } non-fasting
2,000 Calories, 83 per cent Fat	Late Pregnancy } non-fasting
Diffuse lipoatrophy (non-fasting)	Brief Exercise } non-fasting
Diabetic ketosis*	
Carcinomatosis*	
Postoperative*	

* Calorie intake low.

TABLE II
Effects of Active Extracts in Mice

Increased	Diminished
Liver fat	Blood sugar
Blood lipids	Body weight
Blood ketones	Carcass fat
Fat utilization	

TABLE III
Effects of Urine Extracts from Normal and Pituitary-Deficient Subjects on Liver Fat and Blood Ketones in Mice

Material Injected Six Hours Previously	Liver Fat (gm./100 gm.)	Blood Ketones (mg./100 ml. as acetone)
Saline	4.3 ± 0.14	1.68 ± 0.37
NFU	4.8 ± 0.24	2.10 ± 0.46
FU	7.2 ± 0.33	4.08 ± 0.45
FU (Hypopituitarism)	3.9 ± 0.17	1.35 ± 0.33

NOTE: NFU = Non-fasting urine extract. FU = extract of urine collected during fasting or 90 per cent fat, 1,000 calorie diet. Hypopituitarism = eight observations on six subjects, one hypophysectomized three weeks previously, two with postpartum pituitary necrosis and three with chromophobe adenomas. All patients were receiving maintenance doses of cortisone and thyroid.

out in an M.S.E. basket centrifuge. The use of oxycellulose (Eastman) increases the potency twenty to thirty times (compared with the ultrafiltrate). Our extract is shaken with 25 to 50 per cent of oxycellulose overnight

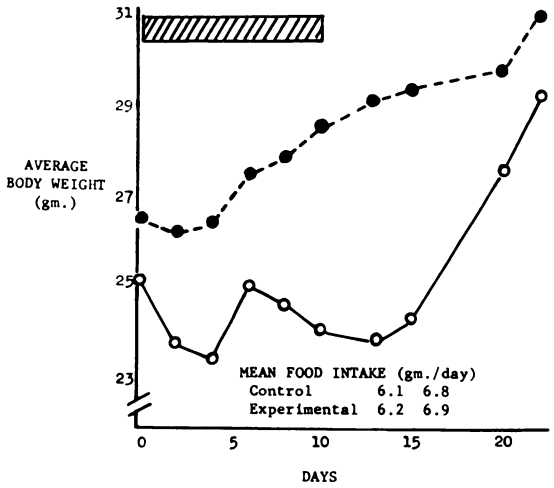

FIG. 3. The same experiment as in Figure 2 but the injections were stopped after ten days. Note recovery of body weight in treated group. No difference in food intake between control and experimental groups in either part of the study.

(sixteen hours). About 10 per cent of the original activity is not extracted during this single exposure: two or more extractions therefore will be needed for quantitative work. The yield for the excretion of urine over a twenty-four hour period has ranged from 1 to 7 mg.

TABLE IV

Carcass Analysis After Treatment with Active Extract for Three Weeks (gm./100 gm.)

Substances Analyzed	Control	Experimental	Average Net Change (gm./mouse)	Average Net Change (gm./100 gm.)
Fat	14.3 ± 0.5	9.9 ± 0.4	−1.25	−38
Water	64.1 ± 0.7	67.3 ± 0.4	−0.80	−5.5
Protein	11.8 ± 0.4	13.5 ± 0.3	+0.07	+2.5

NOTE: The same experiment as illustrated in Figure 2. The amount of water lost is somewhat greater than can be accounted for by depletion of fat depots. We have not observed any significant gain or loss of protein after prolonged treatment with active extracts.

CHEMICAL PROPERTIES

The oxycel extract has a nitrogen content of about 8 per cent. After acid hydrolysis, it yields the following amino acids: histidine, phenylalanine, leucine, serine, cystine, aspartic acid and a trace of alanine. It gives only a weakly positive Molisch test for carbohydrate material.

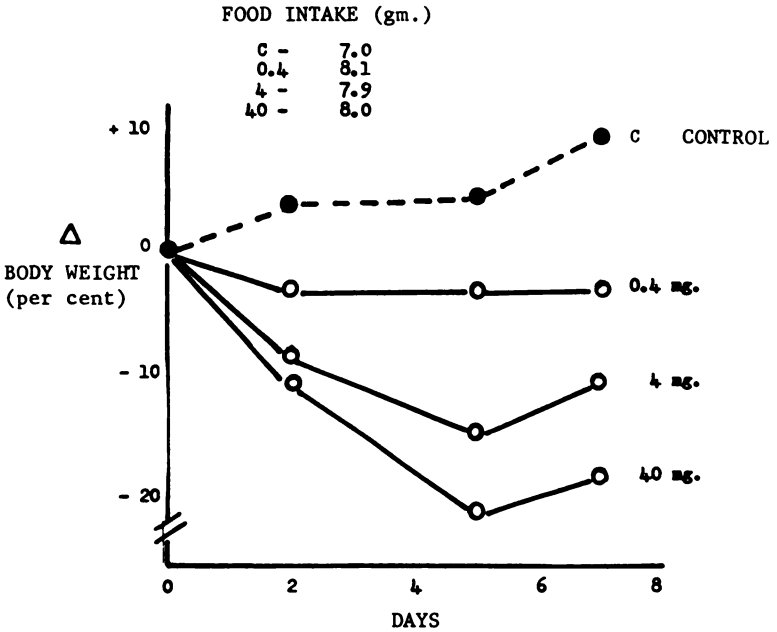

FIG. 4. Body weight as per cent of initial value plotted against time in days. Single mice receiving graded single doses of oxycel material. Fat content of carcasses on day 7: Control, 14.9 per cent; 0.4 mg., 12.4 per cent; 4 mg., 11.7 per cent; 40 mg., 10.3 per cent.

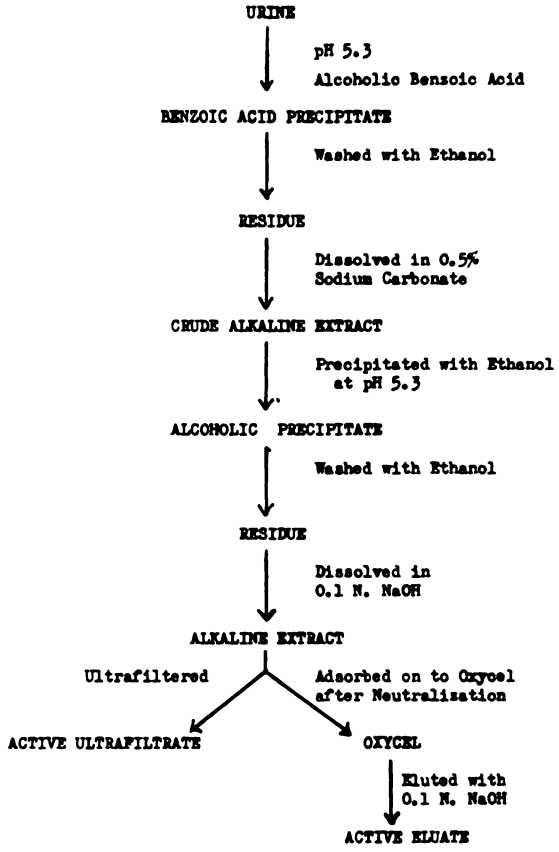

FIG. 5. Extraction procedure. The final eluate is neutralized and freeze-dried.

The biologically active material is thermostable up to 80°c. in 0.1 N alkali. It is destroyed by boiling for two minutes. It is ultrafiltrable through Visking membrane (i.e. molecular weight less than 18,000). After peptic digestion some activity remains, but activity is completely destroyed by trypsin and by chymotrypsin.

EFFECT ON ADIPOSE TISSUE *IN VITRO*

We have used a modification of the method of White and Engel.[4] Pieces of epididymal fat weighing about 50 mg. from rats weighing 90 to 110 gm. have been incubated in a bicarbonate medium containing 4 per cent bovine albumin. Non-esterified fatty acid (NEFA) release into the medium during a three hour incubation has been determined by Dole's[5] method. Addition of oxycel material increases NEFA release (Table v), the threshold concentration being 1 μg./ml. The response is linearly related to the logarithm concentration. Similar data have been obtained using the cruder ultrafiltrate preparation at concentrations about twenty times greater.

TABLE V

Release of Non-esterified Fatty Acid (NEFA) from Adipose Tissue Incubated with Oxycel Extract

Concentration of Extract (μg./ml.)	NEFA Released (μM./100 mg.)*
0 (18)†	0.18 ± 0.08
0.06 (4)	0.23 ± 0.16
0.6 (4)	0.44 ± 0.10
1.6 (5)	1.03 ± 0.39
6 (4)	2.20 ± 0.31
120 (3)	5.03 ± 0.45

* Values for NEFA are mean ± standard error of the mean.

† Figures in parentheses show the number of observations.

TABLE VI

Comparison of Effects of Urinary Material and Corticotropin on Blood Ketones, Liver Fat and Eosinophil Count in Mice*

Material Injected Six Hours Previously	Blood Ketones (mg./100 ml. as acetone)	Liver Fat (gm./100 gm.)	Eosinophils (cu./ mm.)
Saline	2.45 2.60	4.00 3.75	165 149
Oxycel Extract (50 μg.)	4.60 5.15	7.40 7.85	145 141
ACTH (0.5 U.)	3.75 4.15	5.95 6.50	75 60

NOTE: Absence of effect on eosinophils of urinary extract in a dose sufficient to produce relatively large effects on blood ketones and liver fat.

* Two mice per group.

RELATION TO CORTICOTROPIN AND GROWTH HORMONE

The active urinary substance has certain properties in common with corticotropin, namely, affinity for oxycellulose, lipolytic effect *in vitro* and capacity to lower blood

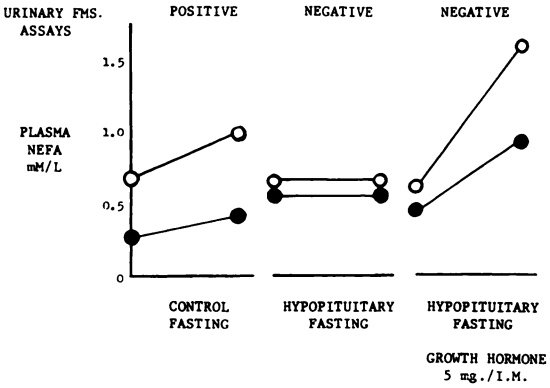

FIG. 6. Plasma NEFA concentrations at 8 A.M. and at noon in subjects fasted overnight: First column, two normal subjects; second column, two subjects with pituitary deficiency, one was recently hypophysectomized and the other had a chromophobe adenoma; third column, effect of human growth hormone (5 mg.) administered intramuscularly (I.M.) in the pituitary-deficient subjects. Urine collected between 8 A.M. and noon was assayed for fat-mobilizing substance (FMS). Activity was present in the normal fasting urines but absent in the urine from the pituitary-deficient subjects even after the growth hormone was administered. Urine collected for several days after administration of growth hormone was also negative.

sugar and to increase liver fat and blood ketones. The most obvious point of difference is in the effect on body weight and carcass composition. The urinary material also appears to be more stable in alkali. It does not depress the eosinophil count in the mouse (Table VI). See also Chalmers et al.[3]

This substance shares with the growth hormone the properties of lowering blood sugar and of increasing fat mobilization and catabolism. With respect to NEFA release it is more active *in vitro* and possibly less active *in vivo*. It does not increase the rate of growth nor the deposition of protein. It differs from the growth hormone also in its chemical properties, especially in its ultrafiltrability and its affinity for oxycellulose. We have examined the possibility that it is a fragment derived from the growth hormone. In two pituitary-deficient persons, urine collected after an intramuscular injection of the human growth hormone* has been assayed for fat-mobilizing activity. In neither case could any activity be detected by *in vitro* or *in vivo* methods (Fig. 6).

SUMMARY

In people who are actively mobilizing and utilizing fat, a substance can be extracted from the urine which will cause increased fat mobilization and catabolism in mice, with depletion of the body fat stores. The material is active *in vitro* in releasing NEFA from adipose tissue at a concentration of less than 1 μg./ml. The pituitary is necessary for its production. It is not corticotropin or the growth hormone. Its relation to these hormones is briefly discussed.

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