Concentrations of Glycerol and Non-Esterified Fatty Acids in Plasma During Fat Mobilization

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The presence of fat-mobilizing activity in the pituitary gland was first demonstrated by Best & Campbell (1936). More recently a series of studies have been carried out on a lipid-mobilizing factor which has been extensively purified from the anterior pituitary gland (Rudman & Seidman, 1958; Rudman, Seidman & Reid, 1960; Di Girolamo, Rudman, Reid & Seidman, 1961; Rudman, Seidman, Brown & Hirsch, 1962) and its action appears to be adequately explained by the following sequence of events after its administration.

1. Increased hydrolysis of triglycerides in adipose tissue.

2. Release of the free fatty acids produced by this hydrolysis into the bloodstream, where they are bound to the albumin.

3. Transport of the albumin-bound free fatty acids to other tissues, including the liver.

4. Conversion of a proportion of free fatty acids carried to the liver into triglycerides which are returned to the plasma in the low-density lipoproteins, with a resultant rise in the concentration of blood triglyceride.

Little is yet known of the fate of the glycerol, which is also formed during the hydrolysis of the adipose-tissue triglycerides. In this paper the effect of a purified lipid-mobilizing factor from the anterior pituitary gland on the concentrations of glycerol and free fatty acids in the plasma is investigated.

MATERIALS AND METHODS

The starting material consisted of a crude fraction obtained from residues of the commercial production of adrenocorticotrophic hormone from pig pituitaries.

Starting material (210 g.) was extracted twice, with 4 and 1 l. of 0.1 N-ammonia respectively, for 1 hr. at room temperature. After centrifugation at 5600 rev./min. for 10 min., the pooled supernatants were adjusted to pH 5.0 by addition of 4 N-hydrochloric acid and an inactive precipitate was removed by centrifugation. The precipitate was washed twice with 300 ml. portions of water. The pH 5 purified extract and washings were pooled and concentrated by distillation *in vacuo* (temperature < 25°) followed by freeze-drying (yield: 81.3 g.).

Portions (2.4 g.) of this freeze-dried substance were subjected to gel-filtration on an annular column of 120 g. of Sephadex G-75 with height 27 cm., 9 cm. external and

4 cm. internal diameter, which was equilibrated and eluted with 0.01 M-pyridinium acetate, pH 5. The first peak absorbing at 280 m μ was collected and concentrated to a fifth of the original volume by distillation *in vacuo*. The concentrated eluates of two gel-filtration runs were pooled, cleared by centrifugation and placed on an annular column of 62 g. of DEAE-Sephadex A-25 with height 15 cm., 6 cm. external and 1.6 cm. internal diameter. The column was equilibrated and eluted with the same system as used for the gel-filtration. A first peak absorbing at 280 m μ and showing lipid-mobilizing as well as pressor activity was discarded. After elution of the first peak the activity remaining on the column was eluted with 1 l. of 0.1 N-acetic acid. This second eluate was concentrated *in vacuo* and freeze-dried, yielding 1.44 g. of a lipid-mobilizing substance (LM 610), 4.78 mg. of which was equivalent to 1 g. of dry anterior pituitary.

The elution of the DEAE-Sephadex column with 0.05 N-acetic acid shows the second eluate to consist of four peaks, two having lipid-mobilizing activity.

Other hormonal activities in LM 610 were of the following order: vasopressin, ≤ 7 milliunits/mg.; adrenocorticotrophic hormone, 35 milliunits/mg.; melanophore-stimulating hormone, 1 mg. of LM 610 was equivalent to 10 μ g. of synthetic α -melanophore-stimulating hormone in the *Anolis* assay (Burgers, 1961).

Glycerol was measured by a modification of the method of Lambert & Neish (1950) as described by Korn (1955). The method was adapted for use with serum in the following manner: 0.1 ml. of serum was diluted with 0.2 ml. of water, deproteinized by the addition of 0.3 ml. of 0.15 N-barium hydroxide and 0.3 ml. of 2.5% zinc sulphate and filtered. The estimation was then performed on a portion (0.5 ml.) of the clear filtrate.

In six experiments, the mean recovery of glycerol added to serum in amounts equivalent to 1000–3000 μ moles/l. of serum was 95%. This determination is liable to interference from any compound which on oxidation with periodate gives rise to formaldehyde. In normal serum and in serum after administration of lipid-mobilizing factor, glucose is the most commonly occurring substance of this type. The conditions of the assay, however, decrease glucose oxidation to a minimum, and 200 μ g. of glucose gives the same colorimeter reading as ± 3 μ g. of glycerol.

Non-esterified fatty acids were determined in serum by the method of Trout, Estes & Friedberg (1960).

RESULTS

Low doses of LM 610 were administered to rabbits, and the concentrations of glycerol and non-esterified fatty acids in the serum measured at

Table 1. Net increases in glycerol and non-esterified fatty acid concentrations after the subcutaneous injection of lipid-mobilizing factor

NEFA, Non-esterified fatty acids.									
Dose of LM 610 ... 150 µg.		300 µg.		500 µg.		750 µg.		3.0 mg.	
Time (hr.)	Rabbit no. 7490 (♂)	Rabbit no. 7759 (♂)	Rabbit no. 54 (♀)	Rabbit no. 163 (♀)	Rabbit no. 560 (♀)	Rabbit no. 9587 (♀)	Rabbit no. 7986 (♂)	Rabbit no. 9223 (♂)	Rabbit no. 560 (♀)
	Glycerol (µmoles/l. of serum)	NEFA (µequiv./l. of serum)	Glycerol (µmoles/l. of serum)	NEFA (µequiv./l. of serum)	Glycerol (µmoles/l. of serum)	NEFA (µequiv./l. of serum)	Glycerol (µmoles/l. of serum)	NEFA (µequiv./l. of serum)	Glycerol (µmoles/l. of serum)
0-50	245	1210	245	500	725	2500	440	1975	430
2-00	225	245	200	405	435	2355	820	2255	470
4-00	0	135	100	165	40	120	835	650	485
6-00	-25	180	25	-45			325	75	255
									320
									8820
									3010

regular intervals up to 6 hr. thereafter (Table 1). The net increases in the serum concentrations of glycerol and of non-esterified fatty acids became greater as the dose of lipid-mobilizing factor was increased. This response was generally of short duration and concentrations were tending to return to pre-injection values after 4-6 hr. It should be noted that, at the two higher doses particularly, the elevation of serum glycerol was appreciably less than that of serum non-esterified fatty acids.

With a dose of 3.0 mg. the magnitude and duration of the glycerol response were considerably increased. Maximum values of serum glycerol of 9350 µmoles/l. and of serum non-esterified fatty acids of 4200 µequiv./l. were obtained. Whereas the maximum serum non-esterified fatty acid concentrations were reached within approx. 1 hr., values for serum glycerol were still increasing up to 5-6 hr. after injection.

When 15.0 mg. of lipid-mobilizing factor was injected these results recurred, glycerol concentrations rising more slowly than those of non-esterified fatty acids, reaching a maximum 4-9 hr. after injection, and declining only slowly to control values after 12-14 hr. (Fig. 1).

To estimate the amount of glycerol that must be liberated into the circulation to cause serum patterns like those found, a model system was set up in which 3.5 g. of glycerol in 0.9 % sodium chloride

Fig. 1. Concentrations of glycerol (O) and non-esterified fatty acids (NEFA, Δ) in the serum after the subcutaneous injection of 15 mg. of LM 610 in saline. (a) Rabbit no. 222 (♀); (b) rabbit no. 192 (♀; death occurred at 15 hr.).

solution was infused intravenously into a rabbit over 3–4 hr. and the magnitude and duration of the increase in concentration in the serum followed (Fig. 2). Though the decrease in concentration after the cessation of infusion was more rapid than after the injection of 15.0 mg. of LM 610, the maximum concentrations of glycerol reached in the plasma were comparable.

A further two rabbits were injected with 7.5 mg. of LM 610 and samples of blood removed before and 2 hr. after the injection. High concentrations of non-esterified fatty acids indicated that con-

siderable fat mobilization had taken place. A sample of the serum was deproteinized and freeze-dried, and subsequently portions were submitted to thin-layer chromatography on silicic acid in two solvent systems [light petroleum (b.p. 60–80°)–diethyl ether (3:2, v/v) and propan-1-ol–ethyl acetate–water (7:1:2, by vol.)] with glycerol and glycerol monostearate as reference substances. While the magnitude and intensity of colour of the glycerol spot was always increased in the serum samples after administration of lipid-mobilizing factor, no traces of spots corresponding to a monoglyceride were seen. Monoglycerides are the only products of partial hydrolysis which could interfere with the glycerol determination.

DISCUSSION

The action of this lipid-mobilizing preparation from the anterior pituitary is considered to be hydrolysis of triglycerides in the fat depots. Assuming complete hydrolysis, since products of partial hydrolysis could not be shown by thin-layer chromatography to be present in appreciable amount, the products would be 1 molecule of glycerol and 3 molecules of fatty acid for each molecule of triglyceride mobilized. For several reasons, however, it would be little more than fortuitous if the theoretical ratio, glycerol:fatty acid 1:3, was ever found in the plasma.

First, after hydrolysis, part at least of the fatty acids will be re-esterified and returned to the 'triglyceride pool' without ever leaving the adipose-tissue cell. This is in contrast with the liberated glycerol, which is not utilized again for triglyceride synthesis, and can diffuse rapidly into the blood. Secondly, when these substances have passed into the circulation their dilution spaces differ widely, perhaps by a factor of 10–15 (Shafrir & Gorin, 1963). Glycerol will diffuse widely and rapidly throughout the total body water (Zilversmit & McCandless, 1957), whereas the distribution of the non-esterified fatty acids is limited to the total albumin space. Lastly, the rates of turnover in and disappearance from the plasma are also very different. The metabolic disappearance of non-esterified fatty acids is known to be extremely rapid, their half-life in the plasma being about $1\frac{1}{2}$ – $2\frac{1}{2}$ min. (Laurell, 1957; Fredrickson, Gordon, Ono & Cherkas, 1958), in contrast with that of glycerol which has been estimated at about 30 min. (Shafrir & Gorin, 1963).

With low doses of the lipid-mobilizing factor the resulting hydrolysis is of short duration and the amount of glycerol liberated when distributed throughout the total body water is not sufficient to be detected. The greater amount liberated and smaller dilution allow a short-lived accumulation of

Fig. 2. Concentrations of serum glycerol during and after the intravenous infusion of 3.5 g. of glycerol in saline. (a) Rabbit no. 9368 (○); rabbit no. 9456 (△). (b) Rabbit no. 9456 (○); rabbit no. 217 (△).

non-esterified fatty acids in the blood, despite their more rapid removal.

Different circumstances obviously operate after the administration of higher doses of lipid-mobilizing factor. Whereas concentrations of glycerol can rise to high values, a plateau is seen for the non-esterified fatty acids, the maximum of which never exceeds $\pm 4000 \mu\text{equiv./l.}$ of serum. We imagine that, as soon as all available binding sites on the plasma albumin molecule are occupied, no further transportation of non-esterified fatty acids occurs. Since, so far as is known, no similar mechanism exists for glycerol, no brake is put on its release into the bloodstream. Since glycerol is rapidly excreted in the urine the prolonged considerable elevation in serum concentrations of glycerol merits comment. While it is possible that this may be a biphasic phenomenon, with the later part of the increase sustained by the action of clearing factor lipase on a late triglyceridaemia (Robinson & French, 1960), the greatly elevated concentrations may simply arise from the hydrolysis of considerable amounts of fat. The results of the infusion experiments support the inference that in a 3 kg. rabbit about 3 g. of glycerol may have been liberated as a result of action of lipid-mobilizing factor. Although based on very rough calculations this would suggest the hydrolysis of quite large amounts of fat, perhaps up to 20–30 g., after a single injection.

At all doses of lipid-mobilizing factor the serum non-esterified fatty acids increase more rapidly than does the serum glycerol. In contrast with this Hagen & Hagen (1962), using an enzymic method of assay, found that during an intravenous infusion of noradrenaline the increase in serum glycerol, originating also from hydrolysis of triglyceride in fat depots, was more rapid than that of non-esterified fatty acids. This may be due to the greater sensitivity of their method or to some difference in the mechanism of release, but it is rather surprising in view of the known rapidity with which glycerol is distributed and also in view of the greater quantity of non-esterified fatty acids concurrently liberated.

SUMMARY

1. The concentrations of non-esterified fatty acids and glycerol were determined in the blood of rabbits after the administration of a fat-mobilizing substance of pig-pituitary origin.

2. When low doses ($150\text{--}300 \mu\text{g.}$) were injected, mainly an increase in the concentration of non-esterified fatty acids was observed. Higher doses ($500\text{--}750 \mu\text{g.}$) produced an increase in both these acids and glycerol, the glycerol remaining at a lower concentration.

3. At high doses ($3\text{--}15 \text{ mg.}$), high concentrations of glycerol are observed, whereas the non-esterified fatty acids do not increase further.

4. A rough calculation of the minimal amount of fat released is made.

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