The Effects of Dietary Conditions and Glycerol Concentration on Glycerol Uptake by Rat Liver and Kidney-Cortex Slices

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1. Glycerol utilization by rat liver and kidney-cortex slices was studied in an attempt to define factors that might be important in the regulation of glycerol utilization by these tissues *in vivo*; the formation of glucose from glycerol by kidney-cortex slices was also studied. 2. The rate of glycerol uptake by liver slices was not changed (in comparison with the normal fed control) by starvation (48 hr.), feeding with a low-carbohydrate diet (4–8 days) or feeding with a diet containing 25% glycerol (up to 18 days). Similarly, starvation or a low-carbohydrate diet had no effect on uptake by kidney-cortex slices; however, feeding with the glycerol diet increased glycerol uptake by kidney-cortex slices. 3. The rates of glycerol uptake by slices from both tissues were increased on raising the glycerol concentration from 0.2 mM to 2.5 or 5.0 mM. 4. Starvation increased the conversion of glycerol into glucose by kidney-cortex slices, but there was no effect of the low-carbohydrate diet; the rate of glucose formation was increased by feeding with the 25% glycerol diet and was proportional to the increase in glycerol uptake. The rate of glucose production by these slices was increased by raising the glycerol concentration in the incubation medium from 0.2 mM to 1.0 mM, but, except for the slices from animals receiving the 25% glycerol diet, there was no effect above 1.0 mM glycerol. 5. The significance of plasma glycerol concentration in regulating glycerol uptake by these tissues is discussed.

During starvation and conditions of carbohydrate deprivation triglycerides are broken down to provide fatty acids, which form the primary fuel of the animal under these conditions. The other product of lipolysis is glycerol, which, although quantitatively much less important than fatty acids, plays a vital role as a precursor for gluconeogenesis (see Owen *et al.* 1967); this latter process provides glucose for the metabolism of the brain and other tissues under these conditions (see Krebs, 1964; Newsholme & Gevers, 1967).

Liver and kidney cortex are the two tissues in higher animals that are capable of carrying out gluconeogenesis; and, generally, the utilization of glycerol by these tissues has been studied in an attempt to understand the control of glycolysis and gluconeogenesis (for review see Newsholme & Gevers, 1967). In the present investigation glycerol metabolism was studied in an attempt to discover what factors may be important in the regulation of glycerol utilization by these tissues. The effects of

starvation and of a casein-margarine (low-carbohydrate) diet on the rates of glycerol uptake by liver and kidney-cortex slices were studied. Under these conditions the conversion of glycerol into glucose is of great physiological importance (as no dietary glucose is being supplied), and it seemed possible that a comparison of these conditions with the normal fed animal might provide information on the mechanism of control of glycerol utilization. The effect of a diet containing 25% of glycerol was also investigated because this provides a fairly continuous large increase of the plasma glycerol concentration; such an increase could produce changes in glycerol metabolism that might throw some light on the normal mechanism of regulation of glycerol uptake by liver and kidney cortex.

MATERIALS AND METHODS

Chemicals and enzymes. Unless otherwise indicated all chemicals were analytical grade from British Drug Houses Ltd., Poole, Dorset. ATP (sodium salt), phosphoenolpyruvate (tricyclohexylammonium salt) and NADH (sodium salt) were purchased from Boehringer Corp. (London) Ltd., London W.5. Glucose oxidase was obtained from Sigma (London) Chemical Co., London S.W.6, and all

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other enzymes were obtained from Boehringer Corp. (London) Ltd.

Animals. Female Wistar rats weighing about 200 g. were used. The normal diet for the rats was laboratory small-animal diet (Spillers Ltd., Gainsborough, Lincs.). The casein-margarine diet was 3 parts of casein and 1 part of margarine with vitamin and mineral supplements as described by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963). The 25%-glycerol diet contained 25% of glycerol mixed with 75% of powdered rat cake; rats were usually starved for 24 hr. before being given the glycerol diet.

Incubation procedures. For kidney-cortex slices the incubation procedure of Krebs *et al.* (1963), as modified by Underwood & Newsholme (1967), was used. About 50 mg. wet wt. of washed kidney-cortex slices was incubated in 4 ml. of bicarbonate-buffered saline (Krebs & Henseleit, 1932) in an atmosphere of $O_2 + CO_2$ (95:5). Incubations were carried out in 25 ml. conical flasks in a Gallenkamp metabolic shaker at 40° for periods of up to 1 hr. At the end of the period the slices were removed and dried to constant weight. A sample (3 ml.) of the medium was deproteinized with $HClO_4$ (final concn. 2%, w/v) and a portion of the acid supernatant used for glucose determinations. The remainder of the supernatant was neutralized with $KHCO_3$ (with an internal indicator) and then used for glycerol determinations.

For liver slices the method of Krebs, Notten & Hems (1966) was used. Liver slices were cut dry and weighed, and about 50 mg. of tissue was incubated by the same method as was used for slices of kidney cortex. Glycerol was determined in the neutralized deproteinized supernatant. As results of glycerol uptake are expressed on a dry-weight basis, a piece of liver was weighed wet and after drying to constant weight to obtain a wet wt./dry wt. ratio. In experiments in which glucose formation from glycerol was measured, endogenous glucose formation was measured with kidney-cortex slices incubated in the absence of glycerol, and this endogenous rate has been subtracted from glucose formation rates presented in this paper.

Analytical methods. Glucose was assayed by the method of Krebs *et al.* (1963) and glycerol by the method of Garland & Randle (1962).

RESULTS

Time-course of glycerol uptake by liver and kidney-cortex slices. At high initial concentrations of glycerol (2.5 or 5.0 mM) rates of glycerol uptake by both liver and kidney-cortex slices were linear for 1 hr. (Figs. 1 and 2); but at lower concentrations (0.1 or 0.2 mM) the rates of glycerol uptake decreased during the 1 hr. period of incubation (Figs. 1 and 2). In general, at the low initial glycerol concentrations the rate calculated from an incubation period of 1 hr. was found to be approx. 50% of the initial rate. All values quoted in this paper refer to an incubation period of 1 hr. without correction for this non-linearity; but in the determination of the K_m for uptake (see below) such a correction was applied.

Effects of concentration of glycerol and diet of the animal on glycerol uptake by kidney-cortex slices. The

Fig. 1. Progress curve of glycerol uptake by kidney-cortex slices. Conditions of incubation were as described in the text. Incubations were stopped at the times indicated and glycerol was determined in the medium. ○, Initial concn. of glycerol 0.1 mM (ordinate scale 0-25); ●, initial concn. of glycerol 5 mM (ordinate scale 0-500).

Fig. 2. Progress curve of glycerol uptake by liver slices. Conditions of incubation were as described in the text. Incubations were stopped at the times indicated and glycerol was determined in the medium. ○, Initial concn. of glycerol 0.2 mM (ordinate scale 0-60); ●, initial concn. of glycerol 2.5 mM (ordinate scale 0-150).

Table 1. *Effect of dietary conditions and glycerol concentration on glycerol uptake by kidney-cortex slices*

Kidney-cortex slices were incubated in Krebs–Henseleit saline containing 0.2 mM-, 1 mM-, 2.5 mM- or 5 mM-glycerol for 1 hr. at 40°. Glycerol was determined in the medium at the beginning and end of the incubation. The results are expressed as means $\pm$ S.E.M. with the number of observations in parentheses. Significance of differences from values with normal diet: * $P < 0.005$.

Concn. of glycerol (mM)	Diet of animals ...	Glycerol uptake (μ moles/hr./g. dry wt.)			
		Normal	25% glycerol for 11–18 days	Starved for 48 hr.	Casein–margarine for 6–8 days
0.2		50.7 $\pm$ 1.4 (16)	47.5 $\pm$ 3.6 (3)	52.8 $\pm$ 1.2 (6)	50.6 $\pm$ 3.2 (6)
1.0		160 $\pm$ 6.9 (15)	214 $\pm$ 14.0 (3)*	152 $\pm$ 7.7 (9)	170 $\pm$ 9.5 (6)
2.5		210 $\pm$ 7.9 (8)	390 $\pm$ 18.4 (9)*	238 $\pm$ 24 (3)	212 $\pm$ 7.6 (6)
5.0		297 $\pm$ 9.6 (9)	541 $\pm$ 47 (6)*	—	277 $\pm$ 22 (6)

Table 2. *Effect of the time-period on a 25%-glycerol diet on glycerol uptake and glucose production by kidney-cortex slices*

Kidney-cortex slices were incubated in Krebs–Henseleit saline containing 2.5 mM-glycerol for 1 hr. at 40°. Glycerol was determined in the medium before and after incubation, and glucose determined in the medium after incubation. Endogenous glucose formation was subtracted. The values are expressed as means $\pm$ S.E.M. with the numbers of observations in parentheses. Significance of differences from values at zero day of diet: * $P < 0.005$; ** $P < 0.05$.

Days on the diet	Glycerol uptake (μ moles/hr./g. dry wt.)	Glucose production (μ moles/hr./g. dry wt.)
0	210 $\pm$ 7.9 (8)	46.9 $\pm$ 2.9 (8)
1	238 $\pm$ 13 (3)	68.2 $\pm$ 4.6 (3)*
2	252 $\pm$ 13 (3)**	42.8 $\pm$ 7.1 (3)
3	280 $\pm$ 14 (3)*	58.4 $\pm$ 3.7 (3)
4	310 $\pm$ 22 (6)*	85.9 $\pm$ 7.5 (5)*
5	301 $\pm$ 12 (6)*	77.1 $\pm$ 8.5 (6)*
7	330 $\pm$ 9.5 (6)*	86.0 $\pm$ 5.8 (3)*
11	394 $\pm$ 23 (3)*	141.7 $\pm$ 12.4 (3)*
14	340 $\pm$ 10 (3)*	129.6 $\pm$ 14 (3)*
18	433 $\pm$ 33 (3)*	168 $\pm$ 4.7 (3)*

four dietary conditions investigated during this study were normal feeding, starvation for 48 hr., feeding with a casein–margarine diet for 4–8 days and feeding with a diet containing 25% of glycerol for periods up to 18 days. The effects of these dietary conditions on the uptake of glycerol (at four initial concentrations of glycerol) are shown in Table 1. The rate of glycerol uptake, in comparison with the fed control, was not affected by starvation or the casein–margarine diet; but the diet containing 25% of glycerol increased the glycerol uptake (by 34% at 1 mM-glycerol and by approx. 85% at 2.5 mM- and 5.0 mM-glycerol). The time-course of the effect of the 25%-glycerol diet is shown in Table 2: glycerol uptake was significantly increased after 3 days on the diet and a gradual increase was maintained throughout the experimental period (18 days).

Fig. 3. Double-reciprocal plot of rate of uptake of glycerol by kidney-cortex slices against the concentration of glycerol. Kidney-cortex slices were incubated as described in the Materials and Methods section. After 1 hr. the glycerol content of the medium was determined. The activity, v , is expressed as m-moles of glycerol removed/hr./g. dry wt.

The effect of increasing the glycerol concentration from 0.2 mM to 5.0 mM on glycerol uptake by these slices is also shown in Table 1; in all cases the uptake of glycerol increased as the concentration was raised. The data for kidney-cortex slices from a normal fed animal are shown as a double-reciprocal plot in Fig. 3; this provides a K_m value for glycerol uptake of approx. 1×10^{-3} M.

Effects of concentration of glycerol and diet of the animals on production of glucose by kidney-cortex slices. The casein–margarine diet had no effect on glucose production from glycerol by kidney-cortex slices (when compared with the normal fed animal); however, starvation increased glucose production

Table 3. *Effect of dietary conditions and concentration of glycerol on glucose formation from glycerol by kidney-cortex slices*

Kidney-cortex slices were incubated in Krebs-Henseleit saline containing 0.2mm-, 1mm-, 2.5mm- or 5mm-glycerol for 1 hr. at 40°. Glucose was measured in the medium at the end of the incubation. Endogenous glucose formation, in the absence of substrate, was subtracted to give the values below. The results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses. Significance of differences from values with normal diet: * $P < 0.005$; ** $P < 0.05$.

Concn. of glycerol (mm)	Diet of animals ...	Glucose formation (μ moles/hr./g. dry wt.)			
		Normal	25% glycerol for 11-18 days	Starved for 48 hr.	Casein-margarine for 6-8 days
0.2		13.5 $\pm$ 1.2 (16)	12.3 $\pm$ 1.0 (3)	18.9 $\pm$ 1.1 (6)**	13.3 $\pm$ 2.5 (6)
1.0		48.5 $\pm$ 2.5 (12)	61.1 $\pm$ 5.1 (3)**	60.6 $\pm$ 4.0 (9)**	55.1 $\pm$ 3.2 (6)
2.5		46.9 $\pm$ 2.9 (8)	146.5 $\pm$ 6.5 (9)*	61.1 $\pm$ 2.3 (5)**	53.1 $\pm$ 3.3 (6)
5.0		52.3 $\pm$ 5.5 (7)	209 $\pm$ 19 (6)*	62.6 $\pm$ 14.4 (3)	47.9 $\pm$ 7.2 (6)

Table 4. *Effect of glycerol concentration and of diet on glycerol uptake by liver slices*

Liver slices were incubated in Krebs-Henseleit saline containing 0.2mm- or 2.5mm-glycerol for 1 hr. at 40°. Glycerol was determined in the medium at the beginning and end of incubation. The results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses. Significance of difference from values with normal diet: ** $P < 0.05$.

Concn. of glycerol (mm)	Diet of animals ...	Glycerol uptake (μ moles/hr./g. dry wt.)				
		Normal	25% glycerol for 5-7 days	25% glycerol for 18 days	Starved for 48 hr.	Casein-margarine for 4-6 days
0.2		23.5 $\pm$ 0.96 (6)	25.8 $\pm$ 1.59 (5)	31.0 $\pm$ 1.19 (3)**	22.6 $\pm$ 1.12 (5)	25.8 $\pm$ 2.55 (5)
2.5		152 $\pm$ 9.3 (6)	162 $\pm$ 5.6 (6)	155 $\pm$ 8.3 (3)	151 $\pm$ 8.6 (6)	193 $\pm$ 25 (6)

(although the uptake of glycerol by the slices was unchanged); the 25%-glycerol diet increased glucose production proportionately with the increase in uptake of glycerol (see Table 3).

The effects of increasing the glycerol concentration on glucose production are shown in Table 3. For slices from normal rats, starved rats and rats on the casein-margarine diet increasing the glycerol concentration from 0.2mm to 1.0mm increased the production of glucose; however, increases in glycerol concentration above 1.0mm had no further effect on glucose production. On the other hand slices from animals given the 25%-glycerol diet showed increased glucose formation as the concentration of glycerol was increased above 1mm. Thus, except for the latter condition, raising the glycerol concentration from 1.0mm to 5.0mm had no effect on glucose production (Table 3), although glycerol uptake was increased (Table 1).

Effects of the concentration of glycerol and of diet of the animals on the uptake of glycerol by liver slices.

The effects of the four different dietary conditions at two concentrations of glycerol on the uptake of glycerol by liver slices are shown in Table 4. In comparison with the fed control there was no effect of starvation or the casein-margarine diet on glycerol uptake by the liver slices; these were

similar results to those obtained in the experiments with kidney-cortex slices (Table 1). However, with liver slices there was no effect of the 25%-glycerol diet on glycerol uptake; this contrasts with the kidney-cortex slices (see Table 2). The small increase in glycerol uptake on the 25%-glycerol diet observed at 18 days (but only at a low concentration of glycerol; see Table 4) can be explained by the fact that, after about 7 days on this diet, high concentrations of glycerol are found in the liver; this increased the initial concentration of glycerol in the incubation medium by about 0.1mm. This would be expected to increase glycerol uptake at an initial glycerol concentration of 0.2mm, because of the large effect on the initial glycerol concentration at this lower concentration.

As with kidney-cortex slices, increasing the initial concentration of glycerol from 0.2mm to 2.5mm increased glycerol uptake by liver slices from animals on all diets (Table 4).

DISCUSSION

The conditions of starvation and low-carbohydrate diet (casein-margarine) are such that the animal requires to synthesize glucose from non-carbohydrate sources (gluconeogenesis). One im-

portant precursor for glucose is glycerol, as lipolysis in various tissues (and therefore the production of glycerol) is increased under these conditions; but, as the present experiments demonstrate, there appears to be no increase in the ability of the liver or kidney cortex to increase the uptake of glycerol at a given concentration of this substrate.

The reason for the increased uptake of glycerol by these tissues under conditions of starvation etc. would appear to be the increased plasma concentration. The plasma concentrations of glycerol are 0.098 ± 0.015 and 0.136 ± 0.049 mM for normal fed and 48 hr.-starved rats respectively (C. Start & E. A. Newsholme, unpublished work). The uptake of glycerol by these tissues could be very sensitive to changes in plasma concentrations because the normal concentration is about tenfold lower than the K_m for glycerol uptake (Fig. 3): at such a concentration the substrate concentration-activity plot is almost linear so that changes in uptake would be directly proportional to changes in plasma glycerol concentration. Larsen (1963) has shown *in vivo* that the rate of glycerol removal from the blood of cats was constant only above a concentration of 1.1 mM; below this the rate was dependent on the concentration of glycerol. From the present experiments it seems likely that increased glycerol utilization during the first few days of starvation (or during a low-carbohydrate diet) is due to the effect of an increased plasma glycerol concentration on the uptake process of liver and kidney cortex.

The experiments with the 25%-glycerol diet demonstrate that kidney cortex, but not liver, can respond to an increased glycerol load with an increased glycerol uptake at a constant glycerol concentration. An adequate explanation for this increased uptake requires a knowledge of the properties and activity of the enzyme glycerol kinase, and this is discussed in the following paper (Robinson & Newsholme, 1969).

The uptake of glycerol by these tissues is not the

only factor that can influence the formation of glucose. Thus, once the glycerol has been converted into triose phosphate, both the pathways of glycolysis and gluconeogenesis are, theoretically, available. The present results demonstrate that at physiological concentrations of glycerol (i.e. 0.1 to 0.2 mM) a greater percentage (72% in comparison with 53%) of glycerol is converted into glucose in kidney-cortex slices from starved animals than from normal fed animals or those given the casein-margarine diet. This suggests that the pathway from triose phosphate to glucose is stimulated under conditions of starvation, perhaps by a mechanism described by Underwood & Newsholme (1967) and discussed in detail by Newsholme & Gevers (1967).

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