Glycogen Synthesis in the Perfused Liver of the Starved Rat

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1. In the isolated perfused liver from 48 h-starved rats, glycogen synthesis was followed by sequential sampling of the two major lobes. 2. The fastest observed rates of glycogen deposition ($0.68\text{--}0.82\mu\text{mol}$ of glucose/min per g of fresh liver) were obtained in the left lateral lobe, when glucose in the medium was $25\text{--}30\text{mM}$ and when gluconeogenic substrates were present (pyruvate, glycerol and serine: each initially 5mM). In this situation there was no net disappearance of glucose from the perfusion medium, although ^{14}C from $[\text{U-}^{14}\text{C}]\text{glucose}$ was incorporated into glycogen. There was no requirement for added hormones. 3. In the absence of gluconeogenic precursors, glycogen synthesis from glucose (30mM) was $0\text{--}0.4\mu\text{mol}/\text{min}$ per g. 4. When livers were perfused with gluconeogenic precursors alone, no glycogen was deposited. The total amount of glucose formed was similar to the amount converted into glycogen when 30mM -glucose was also present. 5. The time-course, maximal rates and glucose dependence of hepatic glycogen deposition in the perfused liver resembled those found *in vivo* in 48 h-starved rats, during infusion of glucose. 6. In the perfused liver, added insulin or sodium oleate did not significantly affect glycogen synthesis in optimum conditions. In suboptimum conditions (i.e. glucose less than 25mM , or with gluconeogenic precursors absent) insulin caused a moderate acceleration of glycogen deposition. 7. These results suggest that on re-feeding after starvation in the rat, hepatic glycogen deposition could be initially the result of continued gluconeogenesis, even after the ingestion of glucose. This conclusion is discussed, particularly in connexion with the role of hepatic glucokinase, and the involvement of the liver in the glucose intolerance of starvation.

The factors that control the synthesis of hepatic glycogen after starvation are poorly understood. For example, it is not clear whether insulin or other hormones exert a rapid direct effect on the liver in this situation [see Steele (1966) and Hers *et al.* (1970) for reviews]. Also, the circulating precursors which provide the carbon atoms of glycogen have not been identified. When material other than glucose is administered after starvation, hepatic glycogen deposition (see e.g., Winternitz *et al.*, 1957; Hornbrook *et al.*, 1965, 1966) is likely to be due to gluconeogenesis. If, however, glucose is ingested after starvation, the accumulation of hepatic glycogen could be due to either continued gluconeogenesis (defined as new synthesis of glucose in mono- or poly-saccharide form) or hepatic uptake of circulating glucose. From experiments *in vivo* with ^{14}C -labelled precursors, Olavarria *et al.* (1968) suggested that hepatic glycogen synthesis in starved-re-fed rats, even when they receive glucose, is mainly a result of gluconeogenesis, at least initially.

In the present paper, the results are reported of an investigation with the isolated perfused rat liver which support the suggestion of a major role for gluconeogenesis in the initial accumulation of hepatic glycogen in starved-re-fed rats. The data show that

during glycogen deposition in the perfused liver, there is no obligatory requirement for circulating hormones. A preliminary report of part of this work has appeared (Whitton & Hems, 1971).

Materials and Methods

Materials

Animals. Albino, male, Sprague-Dawley rats, weighing about 200 g before starvation, were fed on a standard supplemented cereal diet, and were starved for 48 h, from 10:00h, before use.

Chemicals. Enzymes for analytical procedures and sodium pyruvate were from C. F. Boehringer Corp. Ltd. (London W.5, U.K.). D-Glucose was from Fisons Ltd. (Loughborough, Leics., U.K.) and L-serine and glycerol were from BDH Chemicals Ltd. (Poole, Dorset, U.K.). Oleic acid was from Sigma Chemical Co. Ltd. (London S.W.6, U.K.). These and other chemicals were AnalaR or comparable grade. D-[U- ^{14}C]glucose was from The Radiochemical Centre (Amersham, Bucks., U.K.). Insulin was the highest-grade commercial ox preparation from Burroughs Wellcome and Co. Ltd. (Dartford, Kent, U.K.).

Perfusion of isolated liver

The perfusion technique was that of Hems *et al.* (1966), which was based on the method of Miller *et al.* (1951). Rats were anaesthetized with a fresh aq. Nembutal solution. The standard medium consisted of a NaHCO_3 -based saline (Krebs & Henseleit, 1932) containing 2.5% (w/v) bovine serum albumin (fraction V; Pentex Ltd., Miles-Seravac, Holyport, Maidenhead, Berks., U.K.) dialysed against four changes of bicarbonate-saline for 2 days. Fresh rat erythrocytes provided haemoglobin (3.5% w/v, during perfusion) either as whole fresh defibrinated blood (Baron & Roberts, 1963) or as a suspension of cells washed twice in 8 vol. of bicarbonate-saline containing 5 mM-glucose. Sufficient blood for a perfusion was obtained by aortic puncture from one fed rat (under ether anaesthesia), weighing 500–700 g. The gas phase was $\text{O}_2 + \text{CO}_2$ (95:5), i.e. pH was about 7.4. The perfusion cabinet was maintained at 38°C. Non-fat substrates were added to the medium as aq. 0.5–1.0 M solutions. Oleate was added as a 0.1 M-solution in warm NaOH solution (about 0.2 M), to medium containing albumin.

Care was taken to prevent drying of the liver, by the use of saline-wetted tissue supported over the organ. After perfusion, the liver could be cleared of blood immediately by perfusion with saline. Haemolysis after 50 min was less than 1%, of the initial haemoglobin.

The operation was carried out essentially as described by Hems *et al.* (1966). In addition the hepatic artery was tied immediately after the start of perfusion and the duodenum was cannulated to guarantee the free flow of bile. Initially, livers were perfused with 60 ml of warm oxygenated bicarbonate-saline containing albumin and 30 mM-glucose. The first 25 ml of medium was discarded. Perfusion was continued with the remaining 35 ml of saline, and erythrocytes in 10 ml of glucose-saline were added over the next 4–10 min. Blood was warmed to 38°C before addition. When whole blood was added gradually over 10 min there was no significant vasoconstriction.

The input pressure was 10–16 cm medium, which produced a flow rate of 2.1–2.5 ml/min per g after the addition of blood. A satisfactory perfusion showed the following characteristics: (i) rapid backflow of partially oxygenated blood from the portal vein cannula, during the 40–60 s before the start of perfusion (while the inferior vena cava was cannulated); (ii) the liver immediately cleared of endogenous blood at the start of perfusion; (iii) the liver was evenly brown-coloured, unswollen and moist, throughout perfusion; (iv) sufficient blood flow was obtained without excessive input pressure; (v) the flow of yellowish bile appeared to be unimpeded (0.3–0.6 ml after 50 min of perfusion).

Liver samples were obtained after snaring the base

of the lobes with a firm thread ligature. The median (notched, second largest) lobe was tied between the notch and the base on the side of the smaller half-lobe; the larger half-lobe then provided the sample.

Measurement of glycogen synthesis by sequential liver sampling in vivo

Rats were anaesthetized with fresh aq. Nembutal solution. Glucose (1.5–2.5 M) was infused intravenously into a tail vein from a burette, and was sometimes administered intra-gastrically (1.5 ml) under light ether anaesthesia 20 min before Nembutal anaesthesia. Each infusion comprised 1–5 ml in 30–90 min. Liver samples were removed through the smallest possible cut in the abdominal wall, after snaring the base of the lobes as described above. After the first sample a saline-soaked pad was inserted between the incision and the bowel. The rat was kept warm by a nearby light-bulb during infusion. In some experiments, the blood vessels to one kidney were tied; this appeared to make no difference to glycogen synthesis.

Analytical methods

Liver samples were immediately dropped into liquid N_2 , ground to a powder, and about 0.5 g of the powder was boiled for 30 min in 10 vol. of aq. 30% (w/v) KOH. Glycogen was precipitated with 3 vol. of ethanol (Good *et al.*, 1933), sedimented by centrifugation, suspended in 20 ml of water (with a motor-driven pestle) and hydrolysed with amyloglucosidase from *Aspergillus niger* (kindly prepared by Professor W. J. Whelan) as described by Lee & Whelan (1966). If ^{14}C -radioactivity in glycogen was counted, glycogen (15–50 μmol of glucose) was washed twice in aq. 70% (v/v) ethanol, hydrolysed in 1 ml of dilute amyloglucosidase overnight, and dissolved for liquid-scintillation counting in a toluene-5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole (butyl-PBD, Ciba) scintillator fluid (Elliott *et al.*, 1971).

Glucose in perchloric acid extracts of perfusion medium or blood, or after hydrolysis of glycogen, was determined with glucose oxidase (Krebs *et al.*, 1964).

Results

Variation in glycogen content in different areas of the liver

To follow hepatic glycogen synthesis by a sequential sampling procedure it was necessary to establish that glycogen contents in different areas of the liver resembled each other, at least initially. This aspect of hepatic glycogen metabolism was therefore investigated.

In the 48 h-starved rat, when the major areas of the liver were sampled simultaneously to include the bulky middle of the lobes, the glycogen contents (5–60 μmol of glucose/g of fresh liver) were usually within 10–20% of each other. This was true before or after infusion of glucose *in vivo* (results not shown) or after a short period of perfusion (Table 1). However, after a longer period of perfusion, differences between lobes in the glycogen content were observed (Table 1). The apparent rate of glycogen synthesis was greater in the left lateral (un-notched, largest) lobe (Table 1). Since the initial glycogen concentration in this lobe could reasonably be estimated by sampling any other part of the liver, the standard procedure adopted in subsequent perfusions was to sample the median (notched) lobe initially, followed by the left lateral lobe. Thus the preparation studied was the 'perfused left lateral lobe'. We cannot explain this apparent difference in rates of synthesis between lobes, but it need not reflect a phenomenon that occurs *in vivo*.

Glycogen deposition in the perfused liver

When the liver from 48 h-starved rats was perfused with glucose (30 mM) alone, the rate of glycogen deposition (0–0.4 $\mu\text{mol}/\text{min}$ per g of fresh liver) was variable. Glucose uptake was 0.4–0.7 $\mu\text{mol}/\text{min}$ per g, measured after the initial phase of equilibration of glucose between the liver and perfusion medium.

More rapid rates of glycogen accumulation were observed if gluconeogenic substrates were added to the perfusion medium. As a standard mixture, pyruvate, serine and glycerol (each initially 5 mM) were employed. This combination of substrates, which provided a substantial total quantity of available carbon atoms for glycogen synthesis with-

out the risk of inhibitory concentrations of any one substrate, was chosen in view of the rapid rates of gluconeogenesis obtainable from such substrate combinations (Ross *et al.*, 1967). If this group of substrates was added after 15 min, the rate of glycogen deposition when glucose was 30 mM was about 0.7 $\mu\text{mol}/\text{min}$ per g (Tables 2 and 5). In this situation, much less glucose was released into the medium than in the absence of glucose, but there was no decline in the glucose concentration (Tables 2 and 5).

The concentration of glucose in the medium was critical for glycogen synthesis. In perfusions with gluconeogenic precursors, where the glucose concentration was varied between 10 mM and 40 mM, maximal rates of glycogen deposition were observed when glucose was 25–30 mM (Fig. 1; Table 2). Increasing the glucose concentration in the medium did not cause a major change in the total amount of glucose formed, but rather a direction of the products of gluconeogenesis into glycogen (Table 3). In the perfusions with 35 mM- or 40 mM-glucose, the glucose concentration in the medium sometimes decreased markedly during the first 15 min of perfusion, by as much as 10 mM; these perfusions have not been included in the reported results. In the other perfusions with 40 mM-glucose plus gluconeogenic precursors (i.e. those reported in Table 2 and Fig. 1), the total rate of gluconeogenesis was faster than at lower glucose concentrations, and yet glycogen synthesis was submaximal; we have no explanation for this observation.

The time-course of glycogen deposition was investigated with the standard sampling technique, i.e. median followed by left lateral lobe, in two groups of perfusions. In these experiments the average rate of glycogen synthesis in the first 35 min of perfusion was not as rapid as that between 20 and 50 min (Fig. 2).

Table 1. *Glycogen content and synthesis in the major lobes of perfused liver from starved rats*

Livers were perfused with bicarbonate–albumin–saline plus fresh defibrinated whole blood, containing glucose (30 mM). After 15 min, pyruvate, serine and glycerol were added (each initially 5 mM). Other details are given in the text. Results are mean values $\pm$ S.E.M. with the number of observations in parentheses.

Glycogen content (μmol of glucose/g of fresh liver)			
Lobes sampled simultaneously after 20 min	Lobes sampled sequentially during perfusion		Rate of glycogen synthesis calc. from sequential liver samples (μmol of glucose/min per g)
	20 min	50 min	
Median lobe: 13.2 $\pm$ 2.0 (3)	Median lobe: 22.4 $\pm$ 4.8 (11)	Left lateral lobe: 47.0 $\pm$ 5.0 (11)	0.82
Left lateral lobe: 15.2 $\pm$ 1.5 (3)	Median lobe: 22.4 $\pm$ 4.8 (11)	Caudate lobes: 39.0 $\pm$ 5.4 (11)	0.55
Combined caudate lobes: 12.3 $\pm$ 3.7 (3)	Left lateral lobe: 14.6 $\pm$ 3.5 (5)	Median lobe: 28.3 $\pm$ 4.0 (5)	0.46

Table 2. *Glycogen synthesis, and changes in glucose in the medium, during perfusion of livers from starved rats*

Livers were perfused with bicarbonate-albumin-saline containing washed erythrocytes, and glucose at various concentrations (initially 10–40 mM). When present, gluconeogenic substrates (see Table 1) were added after 15 min. Glucose in the medium was determined in duplicate or triplicate. Other details are given in the text. Results are mean values $\pm$ S.E.M.

Gluconeogenic substrates	No. of perfusions	Approx. initial concn. of glucose (mM)	Glucose in medium (mM)			Glycogen content (μ mol of glucose/g of fresh liver)		Calc. average rate of glycogen synthesis (μ mol of glucose/min per g of fresh liver)
			20 min	35 min	50 min	After 20 min (median lobe)	After 50 min (left lateral lobe)	
+	3	10	11.0 $\pm$ 1.1	13.3 $\pm$ 1.1	13.7 $\pm$ 0.6	7.1	8.3	0.04 $\pm$ 0.02
+	7	20	18.1 $\pm$ 1.0	19.3 $\pm$ 1.0	20.5 $\pm$ 0.9	12.1	16.3	0.20 $\pm$ 0.06
+	11	30	27.9 $\pm$ 0.6	28.9 $\pm$ 1.9	28.5 $\pm$ 0.6	21.5	42.1	0.68 $\pm$ 0.05
+	6	40	38.2 $\pm$ 0.8	41.6 $\pm$ 1.0	42.8 $\pm$ 1.1	13.6	21.5	0.27 $\pm$ 0.05
—	5	30	31.6 $\pm$ 1.8	30.2 $\pm$ 0.1	28.7 $\pm$ 1.7	29.8	35.1	0.17 $\pm$ 0.09

This was possibly due to the general adjustment of liver function after the operative procedure.

Since there was no net uptake of glucose by the liver on the addition of gluconeogenic substrates, the net source of carbon atoms for the accumulated glycogen must have been the gluconeogenic precursors. For comparison with glycogen synthesis in the presence of glucose, gluconeogenesis from the standard substrate combination was followed in the absence of added initial glucose (Fig. 3 and Table 3). At maximum, the rate of gluconeogenesis was about 1.4 μ mol/min per g (Fig. 3). This experiment confirmed that gluconeogenesis was sufficient to support glycogen deposition, although the rate tended to decrease during perfusion, probably owing to a decline in concentrations of substrates.

During glycogen synthesis from gluconeogenic precursors, in the presence of glucose, there could be formation of free glucose by gluconeogenesis and also uptake of glucose to form glycogen, in which case there need be no significant net alteration of glucose concentration in the medium. The extent of this process was investigated with [U- 14 C]glucose (30 mM). The incorporation of 14 C into liver glycogen (Table 4) showed that, assuming that the specific radioactivity of the medium glucose did not change appreciably, about one-third of the total glycogen synthesized was derived from glucose in the medium.

The influence of fatty acid and insulin on glycogen synthesis was investigated in view of the interest in these as modifiers of carbohydrate metabolism. In the standard optimum conditions for glycogen synthesis, sodium oleate (1 mM initially) did not significantly affect glycogen deposition (Table 5). Insulin also did not affect glycogen synthesis in this situation, although in suboptimum conditions (absence of gluconeogenic precursors, or if glucose was less than 30 mM) there was moderate stimulation of glycogen synthesis (compare Tables 2 and 5). This could have been due to inhibition of simultaneous glycogen breakdown (Haft, 1967; Glinnsman & Mortimore, 1968; Mackrell & Sokal, 1969; Mondon & Burton, 1971; Williams *et al.*, 1971).

Hepatic glycogen deposition in the intact rat

To assess the physiological significance of the properties of hepatic glycogen synthesis observed in perfused liver, rates of glycogen synthesis were measured in intact 48 h-starved rats anaesthetized with Nembutal, by a serial liver sampling procedure. The left lateral lobe was removed first, followed by the median lobe, as this was technically the easier order of sampling. The rate of glycogen deposition was dependent upon the blood glucose concentration (Fig. 4), in a manner similar to that observed in perfusion experiments. The time-course of glycogen deposition during intravenous infusion of

Table 3. *Calculated total synthesis of glucose during perfusion of liver from starved rats*

The amounts formed of glucose in the medium and liver glycogen have been calculated from the results in Table 2 and Fig. 3.

Approx. initial concn. of glucose in medium (mM)	Gluconeogenic substrates	Observed metabolic changes between 20 and 50min perfusion			Total glucose synthesized ($\mu\text{mol}/30\text{min per g}$)
		Change in medium glucose (mM)	Glucose release ($\mu\text{mol}/\text{g}$)	Glycogen synthesis ($\mu\text{mol of glucose}/\text{g}$)	
0	+	+3.5	22.4	0	22.4
10	+	+2.7	20.6	1.2	21.8
20	+	+2.4	18.0	6.0	24.0
30	+	+0.6	5.6	20.4	26.0
40	+	+4.6	35.3	8.1	43.4
30	-	-2.9	-23.1	5.1	-18.0

Fig. 1. *Rates of glycogen synthesis in the perfused liver, at various concentrations of glucose in the medium*

Livers of starved rats were perfused with the standard medium, containing 0–40mM-glucose. After 15 min gluconeogenic substrates were added (see Table 2). Glucose concentration was measured after 20min of perfusion, and glycogen synthesis between 20 and 50min. Many of the experiments are also reported in Table 2. Each point represents a single perfusion.

Fig. 2. *Time-course of glycogen synthesis in the perfused liver*

Livers of starved rats were perfused as described in Table 2; the initial glucose concentration was 30mM. Liver samples were taken at 20 and 50min (■; 11 perfusions; gluconeogenic precursors added after 15min) or at 5 and 35min (●; three perfusions; gluconeogenic precursors added after 1min). The bars represent the S.E.M.

glucose was investigated in groups of infusions, and was similar to that in the perfusion experiments (Fig. 5). There was no alteration in glycogen synthesis if glucose was administered intra-gastrically 30min before the intravenous infusion, or if insulin was

present in the infusion fluid (100mU/ml), or if the adrenergic inhibitors pronethalol (I.C.I. Ltd., Cheshire, U.K.) or dihydroergotamine (Sigma) were administered (5 mg/kg; subcutaneously) 30 min before infusion of glucose. (The results of these experiments are not shown.)

Fig. 3. Time-course of glucose formation in the perfused liver

Livers of starved rats were perfused as described in Table 2, except that no glucose was added. Gluconeogenic substrates were added after 15 min. The larger part of the median lobe was removed after 20 min, as in the standard procedure for measuring glycogen synthesis; its glycogen content was less than 5 μmol of glucose/g. Results are the mean values of three perfusions of livers whose average weight (after the biopsy at 20 min) was 5.5 g.

Discussion

Circulating precursors of hepatic glycogen

The use of an isolated perfused liver preparation that deposits glycogen at physiological rates has permitted the characterization of this process in starved rats. The present experiments suggest that much of the hepatic glycogen that accumulates in the rat on re-feeding after a period of starvation could be initially derived from gluconeogenesis (defined as the new synthesis of glucose in either mono- or polysaccharide form). This follows from the facts that the fastest rates of glycogen deposition in the perfused liver from starved rats were obtained with gluconeogenic precursors in the perfusion medium as well as glucose, and that there was no net uptake of glucose by the liver in this situation, despite its high concentration (25–30 mm). These characteristics of hepatic glycogen synthesis may reasonably be presumed to exist *in vivo*, because the rate of glycogen deposition, its dependence on glucose concentration, and time-course, were similar in the perfused liver and in the intact rat.

It is reasonable to suppose that gluconeogenesis should have a major role in the re-deposition of hepatic glycogen after re-feeding with material other than glucose. It is also possible that a continuation of gluconeogenesis (from 'endogenous' precursors) could contribute to hepatic glycogen synthesis after the post-starvation ingestion of glucose. In this event, the increase in circulating glucose concentration would relieve the liver of its role in the release of free glucose into blood, and newly formed hepatic glucose phosphates would be directed to form glycogen. The present experiments suggest that this could be the initial sequence of events *in vivo* on re-feeding with glucose. This possibility was suggested from experiments on intact rats by Olavarria *et al.* (1968), and was also raised by Jeffcoate & Moody (1969) and Zaragoza-Hermans & Felber (1972).

Table 4. Incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]$ glucose (30 mm) into glycogen of perfused liver from starved rats

Livers were perfused as described in Table 2, except that a trace quantity of $[\text{U-}^{14}\text{C}]$ glucose was added to the medium at 15 min. Results are the mean values of three perfusions, in which the average initial specific radioactivity of the glucose in the medium was 6.36×10^3 d.p.m./ μmol .

	Analysis of liver glycogen			Calc. rate of glycogen synthesis (μmol of glucose/min per g of fresh liver)	
	Glycogen content (μmol of glucose/g of fresh liver)	^{14}C incorporated (d.p.m./ μmol of glucose)	Incorporation of $[\text{U-}^{14}\text{C}]$ glucose ($\mu\text{mol}/\text{g}$)	Net rate	From $[\text{U-}^{14}\text{C}]$ glucose
At 20 min (median lobe)	37.0 ± 12.0	69 ± 16	0.4	—	—
At 50 min (left lateral lobe)	59.1 ± 15.5	714 ± 274	6.6	0.74	0.21

Table 5. Influence of added sodium oleate and insulin on glycogen synthesis in perfused liver from starved rats

Livers were perfused as described in Table 2. When present, sodium oleate was initially 1 mM (after 15 min of perfusion). Insulin was added at 10 min, and subsequently at 10 min intervals, as 0.1 ml of a 1 U/ml solution. Other details are given in the text. Results are mean values $\pm$ S.E.M., with the number of measurements given in parentheses when these are different from the number of perfusions.

Gluconeogenic substrates	Other additions	No. of perfusions	Approx. initial concn. of glucose (mM)	Glucose in medium (mM)			Glycogen content (μ mol of glucose/g of fresh liver)		Rate of glycogen synthesis (μ mol of glucose/min per g of fresh liver)
				20 min	35 min	50 min	After 20 min (median lobe)	After 50 min (left lateral lobe)	
+	—	3	20	22.2 $\pm$ 0.6	21.4 (2)	21.8 $\pm$ 0.7	11.0	21.3	0.35 $\pm$ 0.11
+	Insulin	5	20	19.1 $\pm$ 0.9	19.0 $\pm$ 0.9	18.7 $\pm$ 1.0	22.7	31.3	0.28 $\pm$ 0.11
+	Insulin	5	20	19.4 $\pm$ 0.4	21.3 $\pm$ 0.4 (3)	21.6 $\pm$ 0.7	22.3	39.7	0.58 $\pm$ 0.14
+	Insulin	6	30	27.9 $\pm$ 0.9	27.5 $\pm$ 1.0	27.5 $\pm$ 0.8	39.6	51.6	0.40 $\pm$ 0.08
+	—	9	30	28.4 $\pm$ 0.3 (6)	30.1 $\pm$ 0.5 (4)	30.4 $\pm$ 0.5 (6)	23.6	43.3	0.66 $\pm$ 0.05
+	Oleate	5	30	29.0 $\pm$ 0.4	30.6 $\pm$ 0.3	30.2 $\pm$ 0.5	26.6	43.0	0.54 $\pm$ 0.05
+	Insulin	5	30	26.1 $\pm$ 0.7	27.4 $\pm$ 0.4 (4)	27.5 $\pm$ 0.6	37.6	58.5	0.69 $\pm$ 0.09
+	Insulin, oleate	3	30	30.2 $\pm$ 1.6	29.6 (2)	30.8 (2)	14.7	29.7	0.50 $\pm$ 0.10

Although the concentrations of circulating precursors in the present experiments were high compared with those in the intact rat, the findings may nevertheless be related to the starvation-re-feeding situation *in vivo*. The question of particular interest is whether glucose in the blood, after ingestion by a starved rat, is taken up by the liver to form glycogen. In the perfusion experiments, glucose and gluconeogenic precursors were present at near-saturating concentrations, and the liver exhibited a total preference for the gluconeogenic precursors. In the intact starved-re-fed rat, although the blood concentrations of gluconeogenic precursors are lower, so is that of glucose (i.e. less than 10 mM, unless a very large dose of glucose is ingested). Hence it is likely that in the initial phase of re-feeding with glucose *in vivo* a hepatic preference for gluconeogenic precursors would prevail and that net glucose uptake by the liver would be negligible.

On ingestion after starvation, glucose is degraded by extrahepatic tissues into potentially glucogenic precursors such as lactate and alanine. These precursors, diluted in the general pools of 'endogenous'

Fig. 4. Rates of glycogen synthesis *in vivo*, at various blood glucose concentrations

Glucose was infused intravenously, from 5 to 90 min after anaesthesia. Liver samples were taken after 30 and 90 min, and blood after 90 min. Other details are given in the text. Each point represents a single experiment.

Fig. 5. Time-course of glycogen synthesis *in vivo*

Glucose was infused intravenously into anaesthetized rats. In three groups of experiments, each with six rats, and each denoted by a different symbol, two sequential liver samples were taken: ●, at 5 and 35 min after the start of the infusion, or ▲, after 5 and 65 min, or ■, after 30 and 90 min. The blood glucose concentration at the time of the second sample was 23–36 mM.

precursors, would eventually become incorporated into liver glycogen. The extent of this process has been measured by Friedmann *et al.* (1965), and was estimated at 20–40% of the glycogen synthesis. This result is not incompatible with the present conclusion that hepatic glycogen synthesis, after ingestion of glucose, may initially derive largely from the continuing flux of 'endogenous' gluconeogenic precursors.

Role of hepatic glucokinase

Hepatic glucokinase activity is not always sufficient to account for the uptake of glucose to form glycogen after the post-starvation ingestion of glucose in the rat. This has given rise to the suggestion that alternative pathways of glucose uptake may exist [see Friedmann *et al.* (1967) and Ryman & Whelan (1971) for discussion]. The present results may resolve this difficulty, in suggesting that gluconeogenesis could make a major contribution to hepatic glycogen synthesis, immediately after the ingestion of glucose after starvation. Thus there is no requirement for glucokinase activity to be as rapid as the rate of glycogen deposition.

Activation of hepatic glycogen synthesis after starvation

When glucose is ingested after a period of starvation, the initiation of glycogen synthesis in liver is due

to activation of glycogen synthetase (see Hers *et al.*, 1970, for review; Gruhner & Segal, 1970; Hornbrook, 1970; Blatt & Kim, 1971). The present results suggest that this initiation of glycogen synthesis could largely be a direct consequence of an increase in glucose concentration in the hepatic portal vein, since maximal glycogen accumulation required the presence of glucose in the medium, but no added hormones. The rate of total gluconeogenesis (to form glucose plus glycogen) was not much altered by the presence of glucose in the medium, which is in accord with the possibility that glucose (or its metabolic products) may primarily activate the glycogen synthetase system. Such activation of glycogen synthetase by glucose has been demonstrated in the isolated perfused liver (Buschiazio *et al.*, 1970; Glinzmann *et al.*, 1970). It was also noteworthy that high concentrations of circulating glucose (40–50 mM) were inhibitory to glycogen deposition; this could reflect an osmotic action of such a high solute concentration, or more complex effects of glucose.

The question arises of how glucose initiates glycogen synthesis. The glucose-phosphorylating enzymes in liver after starvation (Walker & Rao, 1964; Salas *et al.*, 1963; Sharma *et al.*, 1963) are sufficient to support some phosphorylation of glucose, even if not at the full rate of glycogen deposition. The experiment with [^{14}C]glucose in the perfusion medium showed that glucose uptake did occur during optimum glycogen synthesis from gluconeogenic precursors. Since the rate of glycogen synthesis in the perfused liver was dependent on glucose concentration over the range 10–30 mM, which corresponds to the ' K_m ' (for glucose) of hepatic glucokinase (DiPietro *et al.*, 1962; Walker & Rao, 1964), phosphorylation of glucose may be implicated in the initiation of glycogen synthesis by glucose.

During glycogen deposition from gluconeogenic precursors it is also possible that glucose could act to direct tissue glucose 6-phosphate to glycogen, by inhibition of glucose 6-phosphatase (see Cahill *et al.*, 1959; Ryman & Whelan, 1971).

Action of insulin on hepatic glycogen metabolism

There is controversy about the possible existence of a rapid, direct action of insulin on the liver in post-starvation glycogen deposition. In support of this possibility, hepatic glycogen synthetase can be activated by insulin in certain conditions (Bishop *et al.*, 1971), glycogen synthesis can be prevented by anti-insulin serum (Steiner, 1964) and insulin can stimulate glycogen synthesis from glucose in the isolated perfused liver of starved rats (Sokal *et al.*, 1958). It is established that insulin can inhibit hepatic glycogen breakdown in the perfused liver in some conditions (see Mondon & Burton, 1971, for references), as it can in the intact animal [see Steele

(1966) and Madison (1969) for reviews]. However, there is also evidence against a direct hepatic action of insulin in the initial phase of post-starvation glucose assimilation. For example, insulin does not activate glycogen synthetase in the perfused rat liver (Glinsmann *et al.*, 1970), and anti-insulin serum does not alter the immediate hepatic fate of administered glucose in rats (Moody *et al.*, 1970). In rats with portal-caval anastomosis, a combination of high circulating insulin and normal glucose concentrations is associated with decreased hepatic glycogen synthesis (Assal *et al.*, 1971).

Since the immediate post-starvation synthesis of glycogen appears to be largely a result of gluconeogenesis (even after glucose ingestion), insulin, which can inhibit gluconeogenesis in the isolated liver (Exton *et al.*, 1970), would not be expected to stimulate this process. In the present experiments with the perfused liver, glycogen synthesis was rapid in the absence of added hormones, and insulin did not influence glycogen synthesis under optimum conditions. Hence in the immediate post-starvation situation it appears unlikely that circulating insulin exerts a major direct stimulatory effect on liver glycogen synthesis. This is in general agreement with the conclusions of Moody *et al.* (1970), Glinsmann *et al.* (1970) and Hers *et al.* (1970). However, it is possible that insulin may exert a moderate hepatic effect during post-starvation glycogen deposition, in view of its known action in preventing glycogen breakdown. In the present experiments insulin (albeit at a high concentration) produced a moderate stimulation of glycogen deposition in suboptimum conditions. Also, the present observations do not exclude a possible hepatic action of insulin *in vivo* in combination with other hormones (e.g. in counteracting the glycogenolytic action of glucagon: Glinsmann & Mortimore, 1968; Mackrell & Sokal, 1969). Regarding the effect of anti-insulin serum, obtained by Steiner (1964), this could reflect extrahepatic events that indirectly resulted in a glycogenolytic action on the liver, or the relatively long duration of the experiment (3.5 h), since the present results are not incompatible with a longer-term action of insulin on liver glycogen metabolism.

Role of the liver in glucose tolerance after starvation

During starvation glucose tolerance is impaired in comparison with the fed state. The present results suggest that, in the initial phase of assimilation of a glucose load by tissues of starved animals, there is no significant net uptake of glucose by the liver, although there may be a marked diminution in the hepatic release of glucose into blood, caused largely by the increased concentration of blood glucose and perhaps by insulin. This conclusion is in accord with the main

explanation for the glucose intolerance of starvation proposed by Mahler & Szabo (1970).

Adaptation of hepatic gluconeogenesis between the starved and fed states

After a period of deprivation of dietary glucose, hepatic metabolism adapts on eventual re-feeding with glucose, so as to decrease the rate of gluconeogenesis. The present experiments suggest that the initial phase of such a re-adjustment between 'starved' and 'fed' states (defined with regard to dietary glucose) need not involve a cessation of net hepatic gluconeogenesis, but rather its continuation for perhaps several hours, contributing to the re-accumulation of glycogen. During this time the activity of glucokinase in liver may slowly increase (Salas *et al.*, 1963; Sharma *et al.*, 1963). In the event of persistent repletion with dietary glucose, net uptake of glucose by the liver could eventually supervene. Such a slow adaptation to re-feeding with glucose is likely to be of greater value to an animal than a more rapid response to a possibly transient surge of glucose in the portal vein.

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