Biochem. J. (1979) **182**, 727–734
Printed in Great Britain

Glycogen Synthesis by Hepatocytes from Diabetic Rats

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(*Received 5 March* 1979)

Hepatocytes prepared from streptozotocin- and alloxan-diabetic rats starved for 24h contain 0.5–2% wet wt. of glycogen. Glycogen synthesis in the hepatocytes from such rats, after prior depletion of the glycogen by glucagon injection, was studied. As distinct from cells from normal animals, there was no glycogen synthesis from glucose as sole substrate, even at concentrations of 60mM. When supplied with glucose, a gluconeogenic precursor (lactate, dihydroxyacetone or fructose), and with glutamine there was concurrent synthesis of glucose and of glycogen. Without glutamine there was little or no glycogen synthesis. The rate of glycogen formation was in the same range as for cells from control rats. Glutamine addition markedly activated glycogen synthase in cells of starved diabetic rats, but there was no effect on phosphorylase. We obtained very little synthesis of glycogen with hepatocytes from fed diabetic rats, whereas with normal animals, synthesis by such cells equals or exceeds that obtained from starved rats. The conversion of synthase *b* (inactive) into the active form was studied in rat liver homogenates. The activation of the synthase in cells from starved diabetic rats is somewhat less than that from normal animals, but that from fed diabetic rats is markedly decreased compared with that in livers of fed control animals or that of starved diabetic animals.

The hepatic glycogen content of diabetic fed rats is rather low and insulin treatment of diabetic as well as normal rats leads within a few hours to a dramatic increase in liver glycogen. Thus it is generally accepted that there is a defect in the mechanism of glycogen synthesis in diabetics. Several investigators have provided evidence for the impairment in the activation of glycogen synthase in diabetes (for reviews see Nuttal, 1972; Stalmans, 1976; Hers, 1976). However, Langley *et al.* (1957) showed that depancreatomized starved rats deposit liver glycogen after feeding as rapidly as normal animals. These studies were extended by Weinhouse and his co-workers (Friedman *et al.*, 1963, 1965, 1967), who convincingly demonstrated that starved alloxan-diabetic rats depleted of liver glycogen replenish it at normal rates for 4–5h when re-fed after starvation, until hepatic glycogen concentrations reach 3–4% (wet wt.) of liver, but do not attain normal concentrations, which are about 8%. These results were confirmed by Hornbrook (1970).

Hems *et al.* (1972) have shown that livers of normal starved rats perfused with a medium containing glucose and a mixture of amino acids and gluconeogenic precursors *in vitro* synthesize concurrently glucose and glycogen at rates similar to those observed *in vivo*. No synthesis of glycogen occurred under these conditions in perfused livers of starved streptozotocin-diabetic rats (Whitton & Hems, 1975). Glycogen synthesis returned to normal if these rats were injected with insulin or fed fructose (but not glucose) several hours before surgery.

We have shown (Katz *et al.*, 1976, 1979) that hepatocytes from starved or fed rats form glycogen at rapid rates from gluconeogenic precursors in the presence of glucose and several amino acids. In view of the discrepancy in the findings *in vitro* and *in vivo* we investigated glycogen synthesis by hepatocytes from streptozotocin- and alloxan-diabetic rats. We show in the present paper that such hepatocytes prepared from starved animals synthesize glycogen in the presence of glutamine at rates that are within the normal range.

Methods

Male Wistar rats (180–240g) were used. They were made diabetic by injecting streptozotocin either intraperitoneally (110mg/kg body wt.) or intravenously (70mg/kg body wt.), or with alloxan (140mg/kg body wt.) injected subcutaneously. The rats were used from 3 to 28 days after injection. The animals were starved overnight. In most experiments, to deplete liver glycogen, the animals were injected subcutaneously 1h before surgery with glucagon (0.05mg/kg body wt.) Blood was taken from the portal vein for the determination of plasma glucose. The methods for cell preparation, incubation and analysis were previously described (Katz *et al.*, 1976, 1979). Briefly, about 0.15ml of packed cells

(20–25mg of cell protein) were incubated for 1h at 37°C in a volume of 3ml of Krebs bicarbonate buffer in an atmosphere of O_2/CO_2 (19:1, v/v). The medium was always 10mM in glucose, and contained as gluconeogenic precursor either 13.5mM-lactate/1.5mM-pyruvate (i.e. a concentration ratio of 9:1), 20mM-dihydroxyacetone, or 10mM-fructose. Glutamine when present was 10mM. Also glucose was used as sole substrate at a concentration of 60mM. The preparation of cell extracts for enzyme assay was as previously described (Katz *et al.*, 1979). The assay of glycogen synthase and phosphorylase in hepatocyte extracts was by the method of Golden *et al.* (1977). The concentration of UDP-glucose was 1mM, sodium sulphate (10mM) was present in the assay of the *a* form and 2mM-glucose 6-phosphate, but no sulphate, was present in the assay of the total (*a*+*b*) form. Total phosphorylase was assayed after pretreatment with phosphorylase kinase, to convert the *b* form into the active *a* form, and 5.0mM-AMP was present during the assay, as described by Katz *et al.* (1979).

The activation of glycogen synthase was examined in liver extracts of normal and streptozotocin-diabetic rats. The rats were anaesthetized with Nembutal, the abdomens opened, the livers excised and at once frozen between steel blocks cooled by solid CO_2. The frozen liver was stored at −20°C until assayed. The enzyme activities were unchanged for over 2 weeks in storage. The extraction medium was 50mM-imidazole buffer, pH7.0, 1% in glycogen and contained 5mM-dithiothreitol. Frozen liver was homogenized for 10s in 4vol. of ice-cold medium in a Polytron homogenizer (Brinkman Co.). To a portion of the extract an equal volume of the above medium (but 100mM in NaF) was added, and the extract kept on ice. Another portion was incubated at 22°C for 30min, after which an equal volume of the NaF solution was added to block further dephosphorylation of synthase *b*. The two extracts were assayed for active and total synthase as described above, except that the concentration of UDP-glucose was 10mM, and that of glucose 6-phosphate (for total synthase) was 10mM.

Results are expressed either as μmol of glucose or glucose equivalents/100mg of protein, or as μmol/ml of packed cells. The protein content of liver is approx. 20%, and 1ml of packed cells is equivalent to approx. 0.7g of liver.

Results

Glycogen synthesis

Cells from about 60 diabetic rats were used in the present study. The great majority had plasma glucose concentrations of over 300mg/100ml. Rats with plasma glucose concentrations below 240mg/100ml were excluded. Within a day or two after

Table 1. *Effect of glutamine on glucose and glycogen synthesis by hepatocytes from starved diabetic rats*

Alloxan (140mg/kg body wt.) was injected subcutaneously and streptozotocin (110mg/kg body wt.) was injected intraperitoneally (no glucagon treatment). Substrates were added in Expts. 1 and 3 at the beginning, and in Expts. 2 and 4 after a 30min preincubation, at which time the initial glycogen content was determined. The concentration of glucose was 10mM, 13.5mM-lactate/1.5mM-pyruvate (9:1), dihydroxyacetone at 20mM, fructose at 10mM and glutamine at 10mM. − indicates loss of glycogen. Each experiment was carried out with a different rat.

Inducing agent for diabetes ...	Streptozotocin				Alloxan			
Expt. no. ...	1		2		3		4	
Body wt. (g) ...	250		220		180		250	
Plasma glucose after starvation (mg/ml) ...	2.9		3.8		4.4		4.1	
Initial cell glycogen (μmol/100mg of protein) ...	73		31		33		35	
	Change in glucose or glycogen content (μmol/100mg of protein per h)							
Added substrate / Glutamine	Glucose	Glycogen	Glucose	Glycogen	Glucose	Glycogen	Glucose	Glycogen
Glucose plus −	44	−24	41	−11	42	−15	59	−14
lactate/pyruvate +	30	−11	26	10	33	0.6	45	9.5
Glucose plus −	88	−18	73	−11	75	−13	100	−22
dihydroxyacetone +	79	6.5	60	25	77	6.0	76	18
Glucose plus −	88	−15	88	1.2	85	−4.7	92	−2.5
fructose +	72	1.0	62	14	72	8.1	80	15

injection there was cessation of growth and after a week most rats showed a progressive loss of body weight. Rats surviving beyond 20 days were often emaciated, losing a third or more of the initial body weight and appeared to be ill. Cells from these rats showed high rates of glucose synthesis, but formed only a little glycogen. Some rats survived up to 4 weeks after injection with a moderate weight loss, and cells from these animals formed glycogen. We summarize in the present paper results with rats up to 18 days after injection, whose loss of body weight did not exceed 20% of the initial (fed) weight.

Cells from normal starved rats contained little or no glycogen, below 3 μmol of glucose equiv./100 mg of protein. However, cells from 24 h-starved diabetic rats contained considerable glycogen. The amount was quite variable, and as high as 80 μmol/100 mg of protein (about 2.5% wet wt. of liver). The paradox that livers of starved diabetic rats have a high glycogen content [about 3% (Friedman *et al.*, 1963), compared with 0.3% in normal animals] has been repeatedly reported in the literature (for numerous references of the older literature see Friedman *et al.*, 1963), but we find no mention of this in recent reviews on glycogen, and many investigators seem unaware of this fact.

The presence of glycogen caused great variability in our results. Without glutamine there was always glycogen breakdown. Glutamine suppressed breakdown and frequently there was a net deposition of glycogen. Initial glycogen content and breakdown varied greatly between cell preparations. There was a 10–15 min lag period in the onset of glycogen synthesis, and there was breakdown during this period, so that the net increase represents a balance of initial breakdown and subsequent synthesis, and is variable. Examples of such results are shown in Table 1. To decrease variability in net glycogen deposition, it was necessary to deplete initial glycogen content. For this we resorted to glucagon injection. In more recent work only glucagon-depleted animals were used, with a glycogen content in the cells of less than 15 μmol/100 mg of protein.

Table 2 summarizes results of glucose and glycogen production by a series of animals rendered diabetic by streptozotocin and alloxan and partially depleted of glycogen by glucagon treatment. There is little difference in net synthesis between these two types of diabetes. In the absence of glutamine there was a small amount of net glycogen synthesis from fructose, but with dihydroxyacetone and lactate only breakdown of the endogenous glycogen occurred. Addition of glutamine reversed breakdown into net gain in glycogen, and stimulated the formation of glycogen from fructose by three to five times.

Glycogen synthesis in cells of glucagon-treated starved normal rats is decreased by 20–25% compared with the value obtained in cells from untreated

Table 2. *Glucose and glycogen synthesis by hepatocytes from diabetic rats*

Streptozotocin and alloxan were injected as described in the legend to Table 1. Control and diabetic rats were pretreated for 1 h with glucagon (see the text). Results are means ± S.D. with ranges in parentheses. – indicates loss of glycogen.

		Change in glucose or glycogen content (μmol/100 g of protein per h)					
		Controls		Streptozotocin		Alloxan	
Injection	...	—		(6–16)		(3–17)	
Time after injection (days)	...	6		8		9	
Number of rats	...	(185–230)		(120–220)		(150–250)	
Body wt. (g)	...	—		3.7 ± 0.6		4.7 ± 1.6	
Plasma glucose after starvation (mg/ml)	...	(0.05–0.2)		(0.8–13)		(0.4–15)	
Cell glycogen (μmol/100 mg of protein)	...						
Added substrate	Glutamine (10 mM)	Glucose	Glycogen	Glucose	Glycogen	Glucose	Glycogen
Glucose plus 13.5 mM-lactate/ 1.5 mM-pyruvate	–	33 ± 5	1.1 ± 2.8	42 ± 6	–4.5 ± 3.5	48 ± 11	–0.2 ± 2.0
	+	27 ± 4	15 ± 4.3	39 ± 9	8.9 ± 1.3	40 ± 10	10 ± 3.8
Glucose (10 mM) plus dihydroxyacetone (20 mM)	–	70 ± 5	6.6 ± 2.7	72 ± 8	–3.8 ± 3.6	76 ± 7	–0.7 ± 3.6
	+	62 ± 1	24 ± 4.2	72 ± 12	18 ± 8.0	81 ± 10	15 ± 4.8
Glucose (10 mM) plus fructose (10 mM)	–	85 ± 5	6.0 ± 1.7	87 ± 16	3.2 ± 2.4	90 ± 16	4.6 ± 2.1
	+	70 ± 4	16 ± 2.2	76 ± 17	15 ± 8.9	79 ± 8.0	12 ± 6.1
Glucose (60 mM)	–		9.2 ± 3.3		–1.2 ± 1.5		–5.6 ± 4.2

Table 3. *Glycogen synthesis by streptozotocin-diabetic rats at* 3 *to* 21 *days after injection*

One batch of rats was injected intravenously with streptozotocin (70mg/kg body wt.) and at the intervals shown rats were treated with glucagon and hepatocytes were prepared. Results are means or ranges (shown in parentheses) for each group, and means ± S.D. for the combined groups for 3–14 days. – indicates loss of glycogen.

		Change in glycogen content (μmol/100mg of protein per h)				
Days after injection ...		3–4	7–9	14	21*	3–14
Number of rats ...		5	4	3	2	12
Plasma glucose after starvation (mg/ml) ...		(2.4–4.9)	(2.5–4.1)	(3.3–3.7)	3.8;3.6	3.4±0.7
Initial cell glycogen (μmol/100mg of protein) ...		(0.4–16)	(3.1–9.8)	(0.6–11)	11;2.9	
Additions	Glutamine					
Glucose (10mM) plus 13.5mM-lactate/1.5mM-pyruvate	–	–0.2 (–2.3–2.3)	–1.2 (–3.5–1.3)	–2.7 (–5.0–3.0)	–6.0;–1.8	–1.2±2
	+	12 (5.1–18)	13 (6.7–19)	6.4 (1.1–11)	10;3.2	11±5.8
Glucose (10mM) plus dihydroxyacetone (20mM)	–	2.6 (0–7.8)	–0.5 (–3.8–3.3)	–3.6 (–7.0–0.2)	–4.4;–2.2	—
	+	18 (6.7–28)	20 (10–25)	9.0 (3.9–16)	11;1.0	17±8.8
Glucose (10mM) plus fructose (10mM)	–	6.8 (1.3–12)	6.0 (1.2–9.7)	4.3 (9.3–10)	1.5;0.4	5.9±4
	+	15 (5.2–22)	15 (10–19)	12 (6.4–21)	12;4.3	14±6.5
Glucose (60mM)	–	2.9 (0.3–7.0)	1.7 (–1.5–4.4)	0.2 (–2.3–3.0)	–1.9;–1.1	1.7±4

* The weight of the first animal was, at the time of injection, 200g, and 21 days later (after starvation for 24h) was 175g (12% loss). The second rat weighed 230g initially and 155g after 21 days (33% loss).

Table 4. *Glucose and glycogen synthesis by hepatocytes from fed diabetic rats*
Results are means ± S.D. – depicts loss of glycogen.

		Change in glucose or glycogen content (μmol/100mg of protein per h)	
Number of rats ...		7*	
Plasma glucose (mg/ml) ...		4.7±0.7	
Initial cell glycogen (μmol/100mg of protein) ...		5.9±3.5	
Substrates	Glutamine (10mM)	Glucose	Glycogen
Glucose (10mM) plus	–	56±6	−2.6±3.8
lactate (15mM)	+	59±10	2.5±4.1
Glucose (10mM) plus	–	75±14	−2.8±5.2
dihydroxyacetone (20mM)	+	86±15	0.6±4.1
Glucose (10mM) plus	–	91±10	1.0±2.7
fructose (10mM)	+	100±20	3.2±3.6
Glucose (60mM)	–		−2.1±3.6

* Three streptozotocin-treated and four alloxan-treated animals.

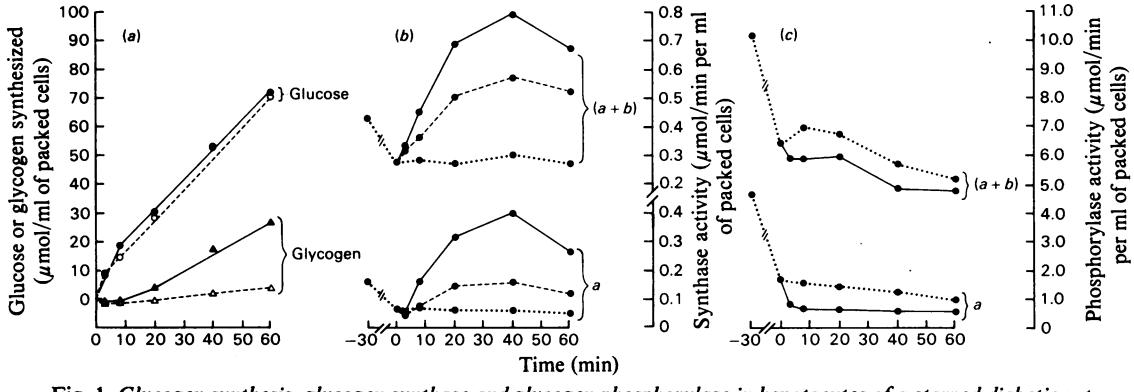

Fig. 1. *Glycogen synthesis, glycogen synthase and glycogen phosphorylase in hepatocytes of a starved diabetic rat*
The rat was injected with alloxan and starved for 24h at 15 days after injection. The rat was treated with glucagon for 1 h before anaesthesia. The cells were preincubated for 30 min before addition of substrates. Plasma glucose concentration was 4.3 mg/ml, cell glycogen was 15 μmol/100 mg of cell protein at the start, and 5.9 μmol/100 mg after 30 min. Substrates were glucose (10 mM) plus 13.5 mM-lactate/1.5 mM-pyruvate (9:1), with 10 mM-glutamine (solid line) or without glutamine (broken line). · · · ·, no substrate added (in *b*). (*a*) Synthesis of glucose and glycogen; (*b*) synthase *a* and synthase (*a*+*b*); (*c*) phosphorylase *a* and phosphorylase (*a*+*b*). In the absence of substrate, the phosphorylase curves (results not shown) were virtually the same as with glucose plus lactate/pyruvate.

animals (Katz *et al.*, 1979). Mean glycogen synthesis in the glucagon-treated diabetic rats from lactate and dihydroxyacetone was about 70% that of the glucagon-treated control rats, but there was little difference in synthesis from fructose. However, the values from the diabetic rats were within the range observed with cells from normal animals. It is noteworthy that the stimulation of glycogen synthesis by glutamine (above values without glutamine) was about the same in the streptozotocin-diabetic rats as the stimulation by glutamine in the control rats. Insulin addition had no effect on glucose nor on glycogen synthesis by hepatocytes from either normal or diabetic rats (results not shown).

Table 3 shows results with one batch of rats injected intravenously with streptozotocin, with the rats used at intervals from 3 to 21 days. The pattern of carbohydrate synthesis and response to glutamine was the

same at 3 days as at 9 days after injection. With protracted diabetes there tends to be a decline in the mean glycogen synthesis. After 2 weeks most of the animals lost a great deal of weight and there was considerable mortality after 20 days. There was much variability of synthesis in these animals, as shown by two animals who survived for 21 days (Table 3).

Hepatocytes from normal starved or fed rats take up glucose when present at concentrations above 15–20mM and form glycogen (Katz *et al.*, 1979). Glycogen synthesis from 60mM-glucose, present as sole substrate, is nearly equal to that from lactate in the presence of 10mM-glucose and 10mM-glutamine (Katz *et al.*, 1979). However, hepatocytes from starved diabetic rats take up very little glucose even at high concentrations and form little or no glycogen from 60mM-glucose (Tables 2 and 3). Owing to the low activity of glucokinase in the diabetic rats, the phosphorylation of glucose at a concentration of 20mM by cells from diabetic rats is negligible (Katz *et al.*, 1975).

Hepatocytes from normal fed rats form glycogen at a rate equal to or greater than that of cells from starved animals (Katz *et al.*, 1979). Surprisingly we observed very little glycogen formation with cells of fed diabetic rats (Table 4). These cells were virtually free of glycogen compared with normal cells, which contain 60–120μmol of glucose equiv./100mg of protein.

Enzymes of glycogen metabolism

Fig. 1 shows glucose and glycogen synthesis and the concurrent activities of the active and total forms of glycogen synthase and phosphorylase by cells from an alloxan-diabetic rat. A similar pattern was obtained also in streptozotocin-treated rats. In these experiments, cells were preincubated for 30min without substrate and the experiment was continued for 60min. The activities of synthase and phosphorylase decreased during preincubation. The decrease in both active and total phosphorylase activities is most pronounced, and occurs most likely, as previously described (Katz *et al.*, 1979), within a few minutes after the onset of incubation. There is, in the presence of glutamine, marked activation of synthase preceding the onset of glycogen synthesis, and also an increase in assayable total synthase, similar to patterns observed with cells from normal starved rats (Katz *et al.*, 1979).

Several investigators (Gold, 1970; Bishop, 1970; Tan & Nuttal, 1976; Miller, 1978) have reported that the conversion of the inactive synthase into the active form is impaired in livers of diabetic animals, suggesting a defect in glycogen synthase phosphatase. We have therefore compared the activation of endogenous synthase *b* in liver homogenates of fed and starved normal and streptozotocin-diabetic rats. Homogenates were incubated at 22–24°C for 30min, the dephosphorylation of the inactive phosphoenzyme was blocked before and after incubation by the addition of fluoride (see the Methods section) and extracts were assayed for active and total synthase. Entire homogenates were used, since we found that centrifugation or treatment with exchange resins (as employed by Tan & Nuttal, 1976) caused a marked decrease in total and active synthase.

Table 5. *Glycogen synthase activation in liver homogenates from normal and streptozotocin-diabetic rats*

Rats were made diabetic by streptozotocin injection and were used 4–5 days later. Liver homogenates were prepared as described in the text, and active and total synthase activities were assayed before and after 30min incubation at room temperature. Activities (means ± S.E.M.) are reported as μmol of UDP-glucose/min per ml of packed cells with the observed range for activation as a percentage increase in activity given in parentheses.

Group	Condition	Number of rats	Incubation (min)	Synthase *a* (μmol/min per ml)	Synthase *a* (% increase)	Synthase (*a*+*b*) (μmol/min per ml)	*P** values for synthase *a* activity compared with that in: Group 1	Group 2
1	Normal starved	5	0	0.52 ± 0.11	(74–156)	1.90 ± 0.20	—	<0.01
			30	1.04 ± 0.33		1.97 ± 0.33	—	N.S.
2	Normal fed	5	0	0.30 ± 0.04	(118–333)	1.98 ± 0.43	<0.01	—
			30	0.98 ± 0.30		2.10 ± 0.44	N.S.	—
3	Diabetic starved	11	0	0.43 ± 0.17	(34–260)	2.83 ± 0.84	<0.015	<0.05
			30	0.96 ± 0.36		2.94 ± 0.90	N.S.	N.S.
4	Diabetic fed	11	0	0.28 ± 0.07	(38–180)	2.52 ± 0.44	<0.05	N.S.
			30	0.60 ± 0.23		2.80 ± 0.52	<0.005	<0.01

* *P* values for synthase group 3 compared with group 4 were <0.01 and <0.005 before and after activation respectively.
Abbreviation: N.S., not significant.

Conversion of the *b* into the *a* form was only partial and only up to half of the total synthase became activated in extracts of normal rats, and there was no further increase when incubations were extended beyond 30min. Incubations at 37°C caused an extensive loss of enzyme activities. EDTA (2mM) was inhibitory, decreasing conversion by about half and confirming the observations of Gilboe & Nuttal (1978). Addition of 10mM-glucose or of 5mM-$MgCl_2$ had little effect on the conversion.

Our results are shown in Table 5. Total synthase was slightly higher in the diabetic rats compared with normal animals, but there was no difference between fed and starved animals. The fraction of the total synthase in the active form was on the average 15 and 25% respectively in livers of fed and starved control animals ($P>0.05$), and 11 and 15% in livers ($P>0.01$) of fed and starved diabetic animals respectively. After incubation the activity of synthase approximately doubled for liver extracts from the starved normal and starved diabetic rats and increased 3-fold for that from normal fed rats. About half of the synthase in liver extracts of normal rats was in the active form and about one-third was in that of the starved diabetics, but the activity of the *a* form was about the same for all three conditions, about 1.4μmol of UDP-glucose/min per g wet wt. of liver. On the other hand the activation of the synthase in the fed diabetic rats was significantly decreased compared with all other groups ($0.005<P<0.01$). The fraction in the active form was 21% and the mean activity was about 60% of that in other groups. However, the variability in the extent of activation was rather large, especially in the diabetic animals (see the ranges in Table 5) and it is noteworthy that in about 20% of either the fed or starved diabetic rats there was only a small increase in active synthase on incubation. We found no correlation between the severity of diabetes, as judged by blood glucose concentration and loss of body weight and the extent of activation.

It appears thus that conversion of synthase *b* into synthase *a* is somewhat impaired in livers of starved diabetic rats, but markedly decreased in livers of fed diabetic rats. Our findings are similar to those of Tan & Nuttal (1976) with alloxan-diabetic rats, but are at variance with those of Miller (1978). He found virtually no activation of endogenous synthase in liver extracts of starved streptozotocin-diabetic rats. It is noteworthy that his extracts contained 10mM-EDTA (which is inhibitory; see above) and he did not include sulphate in his synthase *a* assay.

Our results do not provide any evidence as to the mechanism for decreased activation of synthase in diabetic animals. Synthase phosphatase may be low, or synthase *b* altered in some way, or inhibiting (or activating) proteins or other ligands may be present (or absent).

Discussion

The present results establish that hepatocytes from starved diabetic rats respond to glutamine by activation of glycogen synthase and form glycogen from gluconeogenic precursors. The formation of glycogen is in the same range as with normal starved control rats (treated with glucagon), but it is more variable, and the mean net rate decreased compared with cells of control rats. The mean net synthesis from lactate was about 70% of the control values, but since the cells from the diabetic rats contained initially glycogen (which was broken down in the absence of glutamine), the stimulation of glycogen formation by glutamine was much the same as in control rats. Quantitative comparison of metabolic parameters between diabetic and normal controls is difficult. Diabetic rats differ greatly nutritionally from normal rats. In spite of their high food consumption, they lose weight and waste, and it is thus rather difficult to have a suitable control for the diabetic state. The present results are consistent with the findings *in vivo* that starved glycogen-depleted rats retain a near normal capacity for glycogen formation that persists for 4–5h after re-feeding (Friedman *et al.*, 1963, 1965, 1967). These workers concluded that the glycogen synthase system is intact in these animals, but there is a defect in the ability to accumulate and store glycogen.

Paradoxically livers of starved diabetic rats contain glycogen, whereas there is virtually none in diabetic rats fed *ad libitum*. Although cells of normal fed rats can synthesize glycogen at rates equal to or higher than those from starved normal animals (Katz *et al.*, 1979) glycogen synthesis by cells from fed diabetic rats was very low or absent. The activation of glycogen synthase was slightly decreased in livers of starved diabetics, but markedly impaired in those of fed animals (Table 5). It appears thus that the capacity for glycogen synthesis from gluconeogenic precursors is substantially retained in the starved diabetic rat, but it is almost abolished by feeding.

In cells of normal rats glucose present at concentrations above 20mM and as sole substrate is incorporated into glycogen, and at 60mM net glycogen synthesis is about equal to that from lactate in the presence of glutamine (Katz *et al.*, 1979). Cells from starved diabetic animals, however, cannot form glycogen from glucose even at concentrations of 60mM. The inability of diabetic liver to utilize glucose is expected in view of the low activity of glucokinase and the virtual absence of glucose phosphorylation (Katz *et al.*, 1975). If glucokinase were active there would be excessive futile cycling in the diabetic rat, and the phosphorylation in the presence of rapid glucose 6-phosphate dephosphorylation would be energetically rather wasteful.

The metabolic abnormalities of the diabetic

syndrome are promptly alleviated by the injection of insulin. However, it is unsettled whether hepatic glucose and glycogen synthesis are under direct primary control by insulin. It is established that in perfused liver and hepatocytes insulin antagonizes the effects of glucagon, counteracting the stimulation of gluconeogenesis by glucagon, and acts as an anticatabolic agent (see reviews by Exton & Park, 1972; Hers, 1976; Stalmans, 1976; Pilkis *et al.*, 1978). Insulin *in vitro* decreases glycogenolysis, protein breakdown and urea synthesis, and it enhances the retention of K^+ by liver tissue. On the other hand, in spite of numerous attempts, a reproducible quantitatively significant direct effect *in vitro* of insulin on hepatic gluconeogenesis and glycogen synthesis has not been demonstrated. This is in contrast with non-hepatic organs, such as adipose tissue and muscle, where a dramatic stimulation by insulin of carbohydrate metabolism and glycogen synthesis is readily shown *in vitro*.

We, as many other investigators, have not been able to demonstrate an effect of insulin on glycogen synthesis or glycogen synthase in hepatocytes. Recently Witters *et al.* (1976) have claimed that activation of glycogen synthase by insulin requires incubation of rat hepatocytes in Eagle's tissue-culture medium. This complex medium contains numerous amino acids, vitamins and cofactors. We found that with glucose, gluconeogenic precursors and glutamine present, glycogen synthesis and activation of glycogen synthase in this medium was no better than in bicarbonate buffers, and we could not detect a significant effect of insulin.

Kreutner & Goldberg (1967) and, more recently, Miller (1978) found that glucose infusion activates synthase in the perfused livers of normal starved rats, but there was no activation in perfused livers of starved diabetic rats. However, activation of glycogen synthase by glucose in diabetic animals, where blood glucose concentrations are very high, would serve no physiological purpose and would lead only to energy waste by futile cycling. The lack of synthase activation by glucose in the diabetic rat appears to be a metabolic adaptation rather than a lesion. Gold (1970), Bishop (1970) and Miller (1978) also reported an impairment in the activity of glycogen synthase phosphatase. We confirm in the present paper the marked decrease in the conversion of synthase *b* into synthase *a* in hepatocytes from fed diabetic rats, but find only a moderate decrease in activation in cells of starved diabetic rats. In the latter group there is in the presence of glutamine extensive conversion of synthase *b* into synthase *a* and substantial glycogen synthesis (Fig. 1).

To conclude, the fact that livers of overnight-starved diabetic rats contain glycogen and that such rats form liver glycogen at normal rates for several hours after re-feeding, as well as our present findings, establish the capacity for glycogen synthesis in the overnight-starved diabetic animals. Also, in our opinion, attempts by numerous investigators to demonstrate an effect of insulin *in vitro* on hepatic glycogen synthesis were unsuccessful. It appears therefore that the defects in glycogen synthesis in alloxan- or streptozotocin-diabetes are not due to the specific absence of insulin, but are secondary symptoms of the complex metabolic derangement in diabetes. Short-term starvation lowers blood glucose and may ameliorate the symptoms of the diabetic syndrome, and permit near normal glycogen synthesis. The mechanism of the impairment of glycogen synthase activation and glycogen synthesis in the fed diabetic still requires elucidation.

This work was supported by National Institutes of Health grants AM 12604 and AM 19576.

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