Glycogen Synthesis in the Perfused Liver of Adrenalectomized Rats

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1. A total loss of capacity for net glycogen synthesis was observed in experiments with the perfused liver of starved adrenalectomized rats. 2. This lesion was corrected by insulin or cortisol *in vivo* (over 2–5 h), but not by any agent tested in perfusion. 3. The activity of glycogen synthetase *a*, and its increase during perfusion, in the presence of glucose plus glucogenic substrates, were proportional to the rate of net glycogen accumulation. 4. This complete inherent loss of capacity for glycogen synthesis after adrenalectomy is greater than any defect in hepatic metabolism yet reported in this situation, and is not explicable by a decrease in the rate of gluconeogenesis (which supports glycogen synthesis in the liver of starved rats). The short-term (2–5 h) stimulatory effect of glucocorticoids in the intact animal, on hepatic glycogen deposition, may be mediated partly through insulin action, although neither insulin or cortisol appear to act directly on the liver to stimulate glycogen synthesis.

A well-established action of adrenal-cortical steroids in the intact animal is to stimulate hepatic glycogen deposition (Long *et al.*, 1940; for a review, see Landau, 1965), as may be inferred from the decline in liver glycogen after adrenalectomy (Britton & Silvette, 1937; Cori & Cori, 1927; see Landau, 1965). This effect of glucocorticoids is due to activation of glycogen synthetase to produce the *a* form (an active form of the enzyme; for reviews, see Hers *et al.*, 1970; Ryman & Whelan, 1971), as shown by measurements of pathway intermediates (Young, 1966; Hornbrook *et al.*, 1966; De Wulf & Hers, 1967; Kreutner & Goldberg, 1967) and of enzyme activities (Sie & Fishman, 1964; Hornbrook *et al.*, 1966; Kreutner & Goldberg, 1967; De Wulf & Hers, 1967, 1968; De Wulf *et al.*, 1970). Conversely, in starved adrenalectomized animals, activation of synthetase to the *a* form is impaired (Mersmann & Segal, 1969; Glinsmann *et al.*, 1970; Gruhner & Segal, 1970; Miller *et al.*, 1973), and the capacity for glycogen deposition is diminished (Friedmann *et al.*, 1965, 1967; Hornbrook *et al.*, 1966; Kreutner & Goldberg, 1967). This lesion in the synthetase system may be corrected by glucocorticoids, and also by administration of glucose or insulin to intact animals, and is impaired in insulin-deficient animals, suggesting that the impairment in glycogen synthesis may reflect insulin insufficiency (Kreutner & Goldberg, 1967; Nichols & Goldberg, 1972; Hornbrook, 1970).

Most of these investigations have involved intact animals, and partly for this reason, aspects of the role of the adrenal-cortical hormones in glycogen metabolism have not been fully clarified. For example, it is not certain whether the alterations on adrenalectomy

are inherent to the liver, or whether the decline in gluconeogenic capacity is relevant. Also, it is not clear whether glucocorticoids exert direct hepatic actions, and, in view of the possible role of insulin in glucocorticoid action (see above), it is puzzling that insulin itself (at least in the absence of other hormones) appears to exert no direct hepatic action on glycogen synthesis (Whitton & Hems, 1975).

This problem has been tackled in the perfused liver of starved adrenalectomized rats by using conditions that support rates of net glycogen synthesis in normal (starved) rats equal to those obtainable *in vivo* (Hems *et al.*, 1972; Whitton & Hems, 1975). The present study therefore constitutes an expansion of the work of, in particular, Mersmann & Segal (1969). The present results reveal a marked inherent impairment in net glycogen synthesis, which is more severe than any other alteration in hepatic metabolic processes after adrenalectomy so far reported.

It is suggested that this lesion is a consequence of insulin deficiency in the intact animal, reflecting loss of the indirect influence that insulin exerts (ultimately) on the liver (Whitton & Hems, 1975).

Methods and Materials

Animals

Albino male Sprague–Dawley rats, weighing about 170 g, were fed on a standard (Thompson's) supplemented cereal diet (Heygate and Sons Ltd., Northampton, U.K.). Bilateral adrenalectomy was performed under ether anaesthesia by means of two lateral incisions. If both adrenal glands were not clearly removed in their entirety, the animal was

discarded. The rats were then given 0.9% (w/v) NaCl to drink, and starved in separate cages for 48 h before use. Some fatalities occurred on starvation. All animals were used within 7–11 days of the operation.

Chemicals

Chemicals were the highest grade commercially available (for sources, see Hems & Whitton, 1973; Whitton & Hems, 1975). Acetylcholine was from BDH Chemicals Ltd. (Poole, Dorset, U.K.).

Perfusions of liver and preparation of samples

Liver perfusions and the preparation of samples were carried out as described by Whitton & Hems (1975). In some perfusions, a supplemented medium was used, containing in addition to carbohydrate substrates: insulin (initially 10 munits/ml), cortisol 21-succinate (sodium salt) (initially 20 µg/ml) and a mixture of normal circulating amino acids (initially at about four times normal concentrations); these additions were maintained by infusion (Whitton & Hems, 1975).

Analytical methods

Glycogen synthetase, in the presence and absence of glucose 6-phosphate (7 mM, i.e. total and synthetase *a* activities respectively), and phosphorylase *a* were assayed at 30°C, by following (respectively) the incorporation of ¹⁴C from ¹⁴C-labelled uridine diphosphoglucose (4.5 mM) or glucose 1-phosphate (16 mM) into glycogen (1%, w/v), essentially as described by Das & Hems (1974). Two methods for synthetase assay were described previously (Whitton & Hems, 1975); subsequent experiments have shown that most (or all) of the differences between Methods 1 and 2 were due to the centrifugation. Therefore in the present work, synthetase was assayed by Method 1 (Whitton & Hems, 1975), except that homogenates were not centrifuged. Glycogen phosphorylase was also assayed in non-centrifuged homogenates, diluted about 20-fold; to systematize the small contribution of phosphorylase *b* to the activity recorded (Stalmans & Hems, 1975), AMP (1 mM) was present in the assay mixture. However, at the low glucose 1-phosphate concentration used, activity of phosphorylase *b* would be negligible (Tan & Nuttall, 1975); therefore the activity measured may be presumed to be due to phosphorylase *a*.

Glycogen and glucose were determined as described elsewhere (Hems *et al.*, 1972).

Results

Rates of net glycogen deposition in the perfused liver of starved adrenalectomized rats

In perfused livers from adrenalectomized rats, there was no discernible net glycogen accumulation, in conditions that are optimal for synthesis in starved rats (Hems *et al.*, 1972), or in perfusions including

cortisol and insulin (Table 1). Sham-operated rat livers exhibited normal rates of synthesis (Table 1). After 50–60 min pretreatment of adrenalectomized rats with hexoses, and with insulin in some groups, there was a partial restoration of the capacity for net glycogen synthesis to about half the normal rate, regardless of the nature of any further additions to the perfusate (Table 1). In all livers, initial glycogen contents were conspicuously low (Table 1), in contrast with streptozotocin-diabetic rats (where pretreatment with hexoses or insulin fully restored synthesis rates, and initial glycogen contents in perfused livers were 10–50 µmol of glucose/g; Whitton & Hems, 1975).

In the perfusions described in Table 1 (or Fig. 1), the glucose concentration was initially 27–31 mM (which is optimal for glycogen synthesis in starved rats; Hems *et al.*, 1972). There was slight glucose output during perfusion of sham-operated or pretreated adrenalectomized rats (the increase between 20 and 50 min being 0.5–4 mM; Table 1). Thus the net carbon sources of glycogen in these perfusions were C₃ substrates (lactate, glycerol and pyruvate) or fructose (rather than glucose), as in similar perfusions of livers of starved (Hems *et al.*, 1972) or starved diabetic (Whitton & Hems, 1975) rats. The lowest glucose output occurred in perfusions with supplemented medium (Table 1; details of this supplementation with insulin, cortisol and amino acids are given in the Materials and Methods section and in Whitton & Hems, 1975). Other perfusions (Table 1) suggested that this may not reflect action of insulin or cortisol, but the influence of the complete amino acid mixture. In livers from untreated adrenalectomized rats (where there was no glycogen synthesis), there was no increase in glucose concentration during perfusion with C₃ substrates (Table 1). If glucose was not added to such perfusions (with the mixture of lactate, glycerol and pyruvate) the rate of net gluconeogenesis was about 1.5 µmol of fresh liver/min per g (C. J. Kirk, unpublished work). Thus the capacity for gluconeogenesis in these livers was sufficient to have supported normal net rates of glycogen synthesis.

Restoration of the capacity for net glycogen synthesis

Short periods of treatment of adrenalectomized rats with hexoses or insulin before perfusion produced a partial restoration of rates of net glycogen synthesis in the eventual perfusion (Table 1). Full restoration of rates of synthesis (in the subsequent perfusion) was attained after longer treatment (2 h) with hexoses plus either insulin or cortisol, or 4.5 h after cortisol alone (Fig. 1).

A general outcome of the experiments described in Table 1 and Fig. 1 is that there was no glycogen synthesis in perfusions, unless adrenalectomized rats were pretreated *in vivo* (not necessarily with cortisol). Further attempts to achieve full restoration of glycogen-deposition rates, particularly by manipu-

Table 1. *Rates of net glycogen accumulation in perfused livers of adrenalectomized rats*

Livers of 48 h-starved adrenalectomized (or sham-operated) rats were perfused with bicarbonate-buffered saline containing albumin and erythrocytes (Whitton & Hems, 1975) and glucose plus C₃ substrates (lactate, glycerol and pyruvate: Hems & Whitton, 1973). Further additions in some perfusions included insulin (initially 10 munits/ml) or cortisol (initially 20 µg/ml). Pretreatment of rats was with 2 ml of water (subcutaneously) containing fructose (0.25M) and glucose (1.25M); insulin (1 unit in 1 ml) was also given to two groups. The indicated pretreatment times are from the moment of injection to the start of anaesthesia of the rat (before liver perfusion). Glycogen and glucose synthesis were calculated (for each perfusion) from the contents in samples removed at 20 and 50 min. For other details, see the text. Results are means ± S.E.M. for the numbers of observations indicated. The experimental groups have been numbered 1–10 for identification in Tables 2 and 3 and Fig. 2: all groups were adrenalectomized, except group 1 (sham-operated). $P < 0.01$ for group 1 compared with group 2, 3 or 4.

Experimental group	Pretreatment, and time of pretreatment (min)	Extra additions to perfusate (plus C ₃ substrates)	No. of perfusions	Glycogen content at 20 min (µmol of glucose/g fresh wt. of liver)	Average increase in perfusate glucose (mM)	Rate of net glycogen accumulation (µmol of glucose/min per g fresh wt. of liver)
1 (Sham-operated)	None	None	11	19.3 ± 4.9	1.99 ± 0.72	0.65 ± 0.06
2	None	None	6	2.8 ± 1.5	-0.42 ± 1.05(5)	-0.02 ± 0.02
3	None	Fructose (instead of C ₃ substrates)	3	1.7 ± 0.2	3.57 ± 1.63	0.00 ± 0.02
4	None	Full supplement*	5	1.5 ± 1.0	0.50 ± 0.33(4)	0.01 ± 0.04
5	Hexoses, 50	None	6	2.8 ± 1.2	3.18 ± 0.75	0.16 ± 0.05
6	Hexoses, 50	Full supplement*	9	5.8 ± 2.0	0.64 ± 0.29	0.31 ± 0.09
7	Hexoses, 50	Insulin	3	6.0 ± 1.4	1.50 ± 0.38	0.15 ± 0.13
8	Hexoses, 50	Cortisol	4	3.2 ± 1.0	2.53 ± 0.45	0.21 ± 0.05
9	Hexoses plus insulin, 60	Cortisol	8	6.0 ± 4.8	1.71 ± 0.48	0.28 ± 0.09
10	Hexoses plus insulin, 60	Cortisol plus insulin	6	1.6 ± 0.5	1.82 ± 0.33	0.25 ± 0.10

* See the text for details (Methods and Materials section; also Whitton & Hems, 1975).

lations *in vitro*, were made (i) in four perfusions lasting 2–4 h, resembling those described in Table 1 (group 6) except that pretreatment included insulin, and fructose was also added to the perfusate (5 mM initially) with acetylcholine (200 µg/ml in two of the perfusions; see Mondon & Burton, 1971) and (ii) in three perfusions resembling those in Table 1 (group 2), and including fructose and insulin in the perfusate. In the latter group, the average rate of net glycogen deposition was 0.03 µmol of glycogen glucose/min per g. In group (i) no further restoration was achieved over the net rates (about 0.3 µmol of glycogen glucose/min per g) obtained in rats which received 1 h of similar pretreatment (Table 1). Cortisol added to the perfusate did not stimulate glycogen synthesis (Table 1; compare groups 4 and 2, 8 and 5).

Properties of hepatic glycogen synthetase and phosphorylase

To gain an insight into the impairment in glycogen accumulation in adrenalectomized rats, the activities

of glycogen synthetase and phosphorylase were assayed in perfused livers. As a prerequisite to interpreting activities in sequentially removed liver samples, enzymes were assayed simultaneously in various lobes in intact rats. There was a decline in the proportion of synthetase *a* in starved adrenalectomized rats, (23%, cf. 55% in control livers) confirming previous results (Mersmann & Segal, 1969). No differences between lobes were detected (results not shown).

In perfused livers of adrenalectomized rats, the synthetase *a* activity (assayed in non-centrifuged homogenates) was consistently lower than in sham-operated controls (Table 2). In animals that received no pretreatment, and net glycogen synthesis was zero, synthetase activities were the lowest recorded, and there was no increase in synthetase *a* during perfusion (between 20 and 50 min: Table 2). In the perfusions where some glycogen synthesis was manifest (i.e. after pretreatment *in vivo*), there were higher synthetase *a* activities, especially in the 50 min

Fig. 1. Restoration of glycogen synthesis (in perfusion) by administration of hormones to intact adrenalectomized rats

Adrenalectomized rats received various pretreatments, in 2ml of water (subcutaneously). Livers were eventually perfused with glucose plus C_3 substrates, and net glycogen accumulation was measured between 20 and 50min. The points on the Figure were calculated from the time of pretreatment to the mid-point of this period (i.e. to about 45 min after the anaesthetization of rats). Pretreatments were with; cortisol (10mg, ●); fructose and glucose plus either insulin (1 unit; ▲) or cortisol (10mg; ▼), or cortisol plus insulin (■). The latter two values (▼, ■, in which both groups received cortisol) are joined by a single line. ---- indicates the rate of synthesis in sham-operated rats (Δ; S.E.M. ± 0.06), and the rate in untreated adrenalectomized rats (○) is taken from group 2 in Table 1. For other details, see the text. Results are means of results from three or four perfusions (vertical bars indicate S.E.M.).

samples (Table 2). Taking all groups, there was a strong correlation ($P < 0.001$) between the rate of net synthesis and the activity of synthetase after 50min (plotted in Fig. 2a as percentage of synthetase *a*; but the absolute activity of synthetase *a* shows the same relationship). In contrast with the results obtained in diabetic-rat livers (Whitton & Hems, 1975), hexose pretreatment of rats, or fructose in perfusions, did not induce a high activity of synthetase *a* in the perfused liver samples (Table 2). Thus in adrenalectomized-rat livers, a relationship between synthetase *a* activity and glycogen synthesis (Hers *et al.*, 1970) was observed, without exception.

The sequential removal of two liver samples permitted calculation of changes in synthetase activity during perfusion. The capacity for glycogen synthesis (or its absence) was associated with a sensitivity (or lack thereof) of glycogen synthetase to activation by substrates during perfusions (Table 2). This correlation, observed previously in livers of starved or starved diabetic rats, except for livers of diabetic rats, where the perfusate contained fructose (Whitton & Hems, 1975), is illustrated for the present groups of perfusions (except those with fructose) in Fig. 2(b), which shows that the proportionality between net glycogen synthesis and synthetase activation ($P < 0.001$) in different groups of perfusions, resembles that in diabetic-rat livers (Fig. 2b).

Glycogen phosphorylase activities, assayed in sequential biopsies in adrenalectomized rat livers, were about $2 \mu\text{mol/min per g}$ in all samples (Table 3). There was no sign of the decrease in activity during perfusion which occurred in sham-operated rats

Table 2. Glycogen synthetase activities in perfused livers

Glycogen synthetase was assayed (in non-centrifuged homogenates) in liver samples removed from some of the perfusions described in Table 1. Rates of glycogen synthesis are from the relevant perfusions. Other details are in the text, or Table 1, where the experimental groups are identified. Results are means $\pm$ S.E.M. for three assays, except in group 6, for which four measurements were made.

Experimental group	Glycogen synthetase						Net glycogen synthesis ($\mu\text{mol of glucose/min per g}$)
	$10^2 \times \text{Activities } (\mu\text{mol/min per g})$						
	20 min		50 min		Form <i>a</i> (%)		
	Form <i>a</i>	Total	Form <i>a</i>	Total	20 min	50 min	
1	40 $\pm$ 13	65 $\pm$ 14	62 $\pm$ 10	78 $\pm$ 6	56 $\pm$ 11	79 $\pm$ 8	0.61 $\pm$ 0.08
2	14 $\pm$ 1	56 $\pm$ 3	16 $\pm$ 1	64 $\pm$ 5	25 $\pm$ 3	25 $\pm$ 2	-0.04 $\pm$ 0.03
3	12 $\pm$ 3	56 $\pm$ 9	16 $\pm$ 1	57 $\pm$ 4	21 $\pm$ 4	28 $\pm$ 4	0.00 $\pm$ 0.02
4	15 $\pm$ 1	50 $\pm$ 6	14 $\pm$ 1	51 $\pm$ 6	31 $\pm$ 3	27 $\pm$ 3	-0.01 $\pm$ 0.04
5	16 $\pm$ 6	61 $\pm$ 12	30 $\pm$ 6	77 $\pm$ 7	26 $\pm$ 5	38 $\pm$ 4	0.19 $\pm$ 0.10
6	24 $\pm$ 4	62 $\pm$ 3	41 $\pm$ 9	73 $\pm$ 2	38 $\pm$ 6	47 $\pm$ 11	0.31 $\pm$ 0.17
7	21 $\pm$ 6	72 $\pm$ 1	28 $\pm$ 8	78 $\pm$ 9	29 $\pm$ 8	34 $\pm$ 7	0.15 $\pm$ 0.13
8	22 $\pm$ 6	57 $\pm$ 4	29 $\pm$ 6	64 $\pm$ 3	37 $\pm$ 7	46 $\pm$ 9	0.34 $\pm$ 0.07

Fig. 2. Relationship between glycogen synthetase and net glycogen synthesis, in perfused livers

From the results in Table 2, for % of synthetase *a*, values have been plotted against net glycogen synthesis rates, for (a) activity in the final liver sample, (b) the change in % of form *a* between 20 min and 50 min. The symbols $\square$, $\triangle$, $\bullet$, $\blacksquare$, $\blacktriangle$, $\blacktriangledown$ apply respectively to groups 1, 2 and 4–8 in Table 2: solid symbols indicate groups that received pretreatment *in vivo* (see Table 1). Results are means $\pm$ S.E.M. from the number of assays given in Table 2 (where S.E.M. for glycogen-synthesis rates are given), and the regression lines take each value ($n = 22$) and variation in both planes into consideration: (a) $r = 0.89$, $P < 0.001$; (b) $r = 0.72$, $P < 0.001$. In (b), ---- applies to diabetic rats (Whitton & Hems, 1974, 1975).

(Table 3) and in similar liver perfusions of starved diabetic rats in which impaired glycogen synthesis was corrected by pretreatment (Whitton & Hems, 1974, 1975). This lack of a decline was manifested even in perfusions where some glycogen deposition was observed (Table 3).

Glycogen deposition in adrenalectomized rats *in vivo*

For comparison with results in Table 1, rates of net glycogen deposition were measured in intact starved adrenalectomized rats, by a procedure involving sequential liver biopsies, under anaesthesia (Hems *et al.*, 1972). In all rats, blood glucose was 30–50 mM at the end of 1 h of infusion of glucose (1.0–1.5 M) into a

Table 3. Glycogen phosphorylase activity in perfused livers

Glycogen phosphorylase was assayed in the samples for which synthetase activities are reported in Table 2. Other details are in Tables 1 and 2, or in the text.

Group	Glycogen phosphorylase ($\mu\text{mol/min per g}$)	
	Activity at 20 min	Change in 30 min
1	3.1 ± 0.1	-0.36 ± 0.06
2	1.7 ± 0.2	$+0.61 \pm 0.05$
3	2.3 ± 0.5	$+0.90 \pm 1.06$
3	1.9 ± 0.3	$+0.82 \pm 0.33$
5	1.8 ± 0.1	$+0.18 \pm 0.22$
6	2.3 ± 0.1	$+0.27 \pm 0.16$
7	2.0 ± 0.3	$+0.52 \pm 0.19$
8	2.4 ± 0.3	-0.12 ± 0.26

tail vein. Results were as follows (range of rates, in μmol of glucose/min per g, with the number of rats in parentheses): adrenalectomized rats, 0–0.08(4); sham-operated rats, 0.31–0.93(4); normal (starved) rats, 0.46–0.65(4); adrenalectomized rats after an extra glucose dose (2.5 ml of 1.5 M, 90 min before infusion), 0.01–0.32(5). These values confirm those obtained in perfusions (and in other experiments *in vivo*: Winternitz *et al.*, 1957; Friedmann *et al.*, 1965, 1967) in that there was negligible net glycogen synthesis during the first hour of glucose administration (compared with a rate of about 0.7 in normal starved rats; Hems *et al.*, 1972), whereas the capacity for synthesis was restored by more prolonged hexose treatment. Thus glycogen synthesis can occur in starved adrenalectomized rats in the absence of restored glucocorticoids, as would be expected from the results in Fig. 1, which showed that the intrinsic capacity for synthesis can be restored by insulin.

Discussion

Impairment of glycogen synthesis in adrenalectomized rats

The present experiments demonstrate that in starved adrenalectomized rats there is a total loss of the capacity for net glycogen deposition in the liver. In general this result confirms those of many experiments with intact animals, or with liver slices. However, the extent of the inherent impairment in glycogen synthesis has not been apparent, since the lesion is reversed within a few hours by substrate (or hormone) administration *in vivo*, and also because previous studies have not used a control preparation in which maximal net glycogen synthesis occurs.

The total loss of glycogen-synthetic capacity, revealed in the present experiments, represents a more severe inherent alteration in hepatic metabolism after adrenalectomy (plus starvation) than has been

reported so far for any other metabolic process (e.g. gluconeogenesis, ketogenesis, fatty acid metabolism). The impairment in gluconeogenesis (which process supports glycogen deposition in the present conditions) is not severe (Glenn *et al.*, 1961; Eisenstein *et al.*, 1966; Haynes & Lu, 1969; Exton *et al.*, 1972; Haft *et al.*, 1972) and is insufficient to explain the total loss of glycogen-synthetic capacity after adrenalectomy.

The hepatic capacity for net glycogen accumulation in starved adrenalectomized rats was restored by administration of cortisol to the intact rat, which is not surprising, since stimulation of hepatic glycogen synthesis is one of the best established actions of glucocorticoid hormones (see the introduction). This result shows that cortisol (used in hemisuccinate form, as a highly soluble derivative of a naturally occurring glucocorticoid) can substitute for corticosterone (the major circulating glucocorticoid in rodents). Hence the failure of cortisol to act in perfusions cannot be attributed to this factor.

Is insulin deficiency the explanation of the impairment on adrenalectomy?

Several series of experiments in which glycogen synthetase in particular was investigated have strongly suggested that the impairment in hepatic glycogen synthesis after adrenalectomy is a consequence of insulin deficiency (see the references cited in the introduction). In line with this notion, insulin concentration in blood (Van Lan *et al.*, 1974) and secretion (Malaisse *et al.*, 1967) are diminished after adrenalectomy. The present experiments confirm that glucocorticoids may stimulate glycogen synthesis via insulin, since insulin (plus hexoses, to prevent fatal hypoglycaemia) administered to the intact rat restored the capacity for net glycogen synthesis more rapidly than did cortisol alone. This restoration occurred in about 2–3 h, compared with 1–2 h in streptozotocin-diabetic rats (Whitton & Hems, 1975). Also, hexose treatment (in the absence of glucocorticoid) improved the capacity for glycogen deposition in intact starved adrenalectomized rats. This probably involved insulin action, as hexoses alone exerted no effect in perfused livers from adrenalectomized rats or diabetic rats (Whitton & Hems, 1975); also, the restorative effect of hexoses in diabetic rats is diminished by anti-insulin serum (Whitton & Hems, 1975).

At first sight it would be paradoxical for insulin deficiency to provide the sole explanation for the total loss of glycogen-synthetic capacity on adrenalectomy (plus starvation) in that the defect in starved diabetic animals is less marked, e.g. in its extent, in the sensitivity of synthetase to fructose, and in the rapidity of restoration by hexoses (alone) *in vivo* (Whitton & Hems, 1975). However, it is possible that lack of insulin in 48 h-starved adrenalectomized rats is indeed more severe than in the diabetic animals used in our

previous study (Whitton & Hems, 1975), which were only moderately diabetic, and did not require insulin maintenance. On starvation, glycogen mobilization in diabetic rats is slow (Friedmann *et al.*, 1963) and hyperglycaemia correspondingly slow to disappear. Thus insulin may still be detected in blood of starved streptozotocin-diabetic rats at about half the concentration observed in control starved rats (Schein *et al.*, 1971; Junod *et al.*, 1969), whereas the concentration in starved adrenalectomized rats is very low (Van Lan *et al.*, 1974), in contrast with fed adrenalectomized rats (Pericas & Jolin, 1974).

The possibility that insulin lack accounts for the impairment in glycogen synthesis after adrenalectomy would explain the fact that the defect in synthetase activation is much more severe in starved (than fed) animals (Mersmann & Segal, 1969; Miller *et al.*, 1973). In the starved adrenalectomized state, a near-total lack of circulating insulin would not be fatal; since glucocorticoids are required for maximal adipose-tissue-triglyceride mobilization in diabetes (Chernick *et al.*, 1972), serious ketonaemia (and acidosis) would not supervene in their absence. In the light of these considerations, it is not surprising that glucocorticoids (*in vivo*) did not restore the capacity for synthesis of glycogen in less than 3 h; such a period could be required for a full insulin effect, although insulin itself can act in 2 h.

In a parallel study of fatty acid synthesis in perfused livers from adrenalectomized (fed) rats, the data also suggested that the observed impairment in lipogenesis could reflect a moderate insulin lack (Kirk *et al.*, 1975, 1976).

If, however, insulin is not the sole explanation for the decrease in hepatic glycogen-synthetic capacity in adrenalectomized animals, then any separate short-term role of glucocorticoids in hepatic-glycogen synthesis (e.g. Gruhner & Segal, 1970) is clearly exerted in the same general sense as that of insulin, i.e. both hormones appear to promote glycogen accumulation as they do fatty acid synthesis [see the following paper (Kirk *et al.*, 1976)]. Thus there is no evidence for the production by glucocorticoids of 'insulin resistance' (i.e. antagonism to insulin action) in the liver, in connexion with the intrinsic hepatic capacity for glycogen synthesis or fatty acid synthesis.

With regard to the short-term action of insulin or glucocorticoids *in vivo*, the evidence so far suggests that in neither case do these involve hepatic action. The failure to detect any such direct hepatic action on glycogen accumulation or relevant enzymes, in the present study, is in keeping with a similar failure to detect a direct action of insulin or cortisol on glycogen synthesis in the perfused liver of starved or diabetic rats (Whitton & Hems, 1975). However, this issue remains open while effects *in vivo* are unexplained, and in view of the existence of longer-term direct actions of cortisol in cultured foetal liver (Plas & Nunez,

1975; Plas *et al.*, 1973; Monder & Coufalik, 1972).

As to the substrates that support hepatic glycogen synthesis, the present experiments show that glucogenic precursors contribute significantly to synthesis (after pretreatment that need not include glucocorticoids), as in livers from starved (Hems *et al.*, 1972) or diabetic (Whitton & Hems, 1975) rats. In the intact fed animal, a variety of precursors (including glucose) may provide carbon for glycogen accumulation in a manner which is not altered by adrenalectomy (Morikawi & Landau, 1963). The rapid action of glucocorticoids (*in vivo*) to bring about hepatic glycogen synthesis (Long *et al.*, 1940) is likely to involve increases in the provision of such substrates to the liver, and indirect insulin action, whereas on the evidence yet available, direct effects of either glucocorticoids or insulin on the liver do not appear to be implicated.

It is also noteworthy that deficiencies of insulin (Whitton & Hems, 1975) or glucocorticoids (the present work) do not lead to any long-term changes in glycogen metabolism, which can be restored to normal by both hormones (*in vivo*) in 2–5 h. This area of hepatic metabolism is apparently not susceptible to any long-term changes in quantity of enzyme protein (i.e. enzyme induction).

Properties of glycogen-metabolizing enzymes

In conditions such as those of the present perfusions (and in the absence of hormones), glycogen metabolism is controlled by the responses of the enzymic apparatus to substrates (Stalmans *et al.*, 1974a,b). Glucose can activate synthetase (i.e. increase the proportion and amount of the *a* form) and inactivate phosphorylase, in intact animals (Hers *et al.*, 1970) and in the perfused liver (Buschiazzo *et al.*, 1970; Glinsmann *et al.*, 1970). These effects could stem from an initial action of glucose to activate phosphorylase phosphatase (Stalmans *et al.*, 1970, 1971, 1974a,b; Hue *et al.*, 1975). An activating effect on synthetase can be also obtained with glucogenic substrates (Walli *et al.*, 1974; Whitton & Hems, 1975). Maximum rates of net glycogen synthesis are only observed when the response of synthetase to glucose plus C₃ substrates is operative (Whitton & Hems, 1974, 1975; the present work).

The experiments with livers from adrenalectomized rats described here revealed a defective capacity for net glycogen deposition, associated with impaired sensitivity of the synthetase system to blood-borne substrates, as in livers from diabetic rats (Whitton & Hems, 1975). The relationship between these two parameters resembled that observed in diabetic-rat livers (Whitton & Hems, 1974, 1975), which is compatible with the notion that insulin lack could explain the impairment in glycogen metabolism on adrenalectomy. However, in the perfusions of livers of starved adrenalectomized rats, activities of glycogen

synthetase *a* never attained those observed in livers of control (or diabetic) rats, although activities increased slightly if animals were pretreated with hexose (which partially restored glycogen-synthetic capacity). Thus the impairment in synthetase activation (i.e. lack of production of *a* form by phosphatase) was more severe in starved adrenalectomized rats (see Mersmann & Segal, 1969) than in diabetic rats studied previously (where fructose was able fully to activate synthetase but not net glycogen accumulation; Whitton & Hems, 1975). This could reflect the occurrence of a more severe insulin deficiency in starved adrenalectomized rats than in starved streptozotocin-diabetic rats (see above).

Phosphorylase activity (the *a* form in the present assay conditions) in the perfused liver of starved adrenalectomized rats also behaved differently from that of normal rats (Whitton & Hems, 1974, 1975), in that the normal decline during perfusion with a full complement of carbohydrate substrates was not observed. Impairments in phosphorylase inactivation in perfused livers from adrenalectomized animals have been reported previously (Miller *et al.*, 1973); further, glucocorticoids can stimulate phosphorylase phosphatase (Stalmans *et al.*, 1970). Conversely, there are impairments in hormonal activation of phosphorylase in perfused livers from adrenalectomized rats (Exton *et al.*, 1972; Saitoh & Ui, 1975). Thus glucocorticoids appear to promote a shift in the basal properties of the enzyme systems towards a greater capacity for glycogen synthesis, whereas they induce a change in the opposite direction with regard to hormone-dependent responses. The full significance of these complex actions of glucocorticoids and the mechanisms which underlie them, are not yet clear.

In livers from fed or starved rats, initiation of glycogen synthesis by glucose may depend largely on its (indirect) action on glycogen synthetase phosphatase (see above). At first the results obtained in adrenalectomized rats seem compatible with this view, in that neither glycogen synthesis nor inactivation of phosphorylase and activation of synthetase were observed. However, a more complex defect in the initiation of glycogen synthesis may underlie the severe impairment which follows adrenalectomy (plus starvation), if this does reflect extreme insulin lack. This is shown by the characteristics of glycogen metabolism in livers from starved streptozotocin-diabetic rats (Whitton & Hems, 1975), where there appears to be a 'receptor' defect in the glucose-dependent initiation of glycogen synthesis, since synthetase *a* can be formed in response to fructose (in contrast with adrenalectomized starved rats) but without an associated increase in net glycogen synthesis (Whitton & Hems, 1975). On the arguments advanced here, such a glucose 'receptor' lesion must also underlie the defect after adrenalectomy (plus starvation). These considerations of the events in

pathological states (due to insulin lack) show that the effect of glucose in stimulating glycogen synthesis (Hers *et al.*, 1970; Hems *et al.*, 1972; Hue *et al.*, 1975) may not consist solely of a simple effect on phosphorylase or synthetase phosphatase (Stalmans *et al.*, 1974a,b). Thus even in normal animals there may be more complex hepatic responses to glucose (and other substrates) than that of phosphorylase phosphatase described above. These responses could involve 'receptors' and the participation of protein in the initiation of glycogen synthesis (Krisman & Barengo, 1975).

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