Effects of Glucagon and Insulin on Fatty Acid Synthesis and Glycogen Degradation in the Perfused Liver of Normal and Genetically Obese (*ob/ob*) Mice

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1. Rapid effects of hormones on glycogen metabolism and fatty acid synthesis in the perfused liver of the mouse were studied. 2. In perfusions lasting 2 h, of livers from normal mice, glucagon in successive doses, each producing concentrations of 10^{-10} or 10^{-9} M, inhibited fatty acid and cholesterol synthesis. In perfusions lasting 40–50 min, in which medium was not recycled, inhibition of fatty acid synthesis was only observed with glucagon at concentrations greater than 10^{-9} M. This concentration was about two orders of magnitude higher than that required for the stimulation of glycogen breakdown. Glucagon did not inhibit the activity of acetyl-CoA carboxylase, assayed 10 or 20 min after addition of glucagon (10^{-9} or 10^{-10} M). It is proposed that the action of glucagon on hepatic fatty acid biosynthesis could be secondary in time to depletion of glycogen. Insulin prevented the effect of glucagon (10^{-10} M) on glycogenolysis, but not that of vasopressin. 3. Livers of genetically obese (*ob/ob*) mice did not show significant inhibition of lipid biosynthesis in response to glucagon, although there was normal acceleration of glycogen breakdown. This resistance to glucagon action was not reversed by food deprivation. Livers of obese mice exhibited resistance to the counteraction by insulin of glucagon-stimulated glycogenolysis, which was reversible by partial food deprivation.

Among rapid catabolic effects of hormones on hepatic metabolism, the stimulation of glycogen degradation by glucagon, mediated via cyclic AMP, is the best understood (Exton *et al.*, 1972a). Insulin can counteract the effect of glucagon, this being one of the few potent effects which insulin exerts rapidly and directly on the liver (Exton & Park, 1972).

Some questions remain concerning the effects of glucagon and insulin on liver. Thus there is controversy about the action of glucagon on hepatic fatty acid synthesis. A series of reports have suggested that glucagon or cyclic AMP can inhibit fatty acid synthesis in the rodent liver, on the basis of experiments with intact rats (Rous, 1970; Klain & Weiser, 1973), liver slices (Haugaard & Stadie, 1953; Bloxham & Akhtar, 1972; Akhtar & Bloxham, 1970; Allred & Roehrig, 1973; Bricker & Levey, 1972a,b; Bricker & Marraccini, 1975; Meikle *et al.*, 1974; Raskin *et al.*, 1974) or cell suspensions (Capuzzi *et al.*, 1971, 1974; Harris, 1975; Edwards, 1975; Muller *et al.*, 1976). But studies with the perfused rat liver have been less conclusive. Glucagon

has been reported to inhibit fatty acid synthesis from acetate (Regen & Terrell, 1968) or lactate (Exton *et al.*, 1972b). However, glucagon does not exert significant inhibition of lipogenesis in short perfusion experiments (Raskin *et al.*, 1974), despite the fact that in similar conditions in slices, inhibition by glucagon is observed (Raskin *et al.*, 1974). This is a disconcerting result, as any true short-term effect of glucagon would be expected to appear in such perfusions.

Another question of interest concerns insulin-resistance in liver. Genetically obese mice provide a model of insulin-resistance in muscle and adipose tissue (Abraham & Beloff-Chain, 1971; see Czech *et al.*, 1977, for review). An investigation of counteraction by insulin of the effects of glucagon should reveal whether the livers of obese mice exhibit insulin-resistance.

In the present study, effects of glucagon and insulin on metabolic processes have been investigated in the perfused liver of normal and genetically obese (*ob/ob*) mice. The question of control of fatty acid synthesis has been tackled with the technique of measurement of fatty acid synthesis with $^3\text{H}_2\text{O}$ (Windmueller & Spaeth, 1966; Lowenstein, 1971; Salmon *et al.*, 1974; Hems *et al.*, 1975). The results show that glucagon at concentrations that obtain in

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blood *in vivo* exerts no significant short-term effect on the conversion of acetyl units into fatty acids, and that the liver of genetically obese mice exhibits reversible resistance to the effect of insulin (added with glucagon) on glycogenolysis. Finally we report that in contrast with its effect in the presence of glucagon, insulin does not counteract the actions of vasopressin on fatty acid synthesis and glycogenolysis in mouse liver.

Materials and Methods

Animals and chemicals

The origin and diet of lean and obese (*ob/ob*) fed female mice (aged 10–12 weeks) and sources of chemicals and materials for perfusion have been described (Ma & Hems, 1975; Hems & Ma, 1976). Lean mice homozygous for the normal allele were identified in the colony as described by Beloff-Chain *et al.* (1975). Diet-restricted obese mice received 4g of food/day and weighed 28–35g, compared with 25–30g for lean mice. Glucagon (insulin-free) was kindly donated by Eli Lilly Ltd. (Indianapolis, IN, U.S.A.). Insulin (crystalline, glucagon-free) was from Burroughs Wellcome Ltd. (Beckenham, Kent, U.K.) and vasopressin (grade VI) from Sigma Chemical Co. (Kingston upon Thames, Surrey, U.K.).

Perfusion of the liver

The liver was perfused as previously described (Ma & Hems, 1975) with 60ml of perfusate con-

taining albumin, aged human erythrocytes and, in most perfusions, glucose (15mM) and lactate (10mM). Two types of experiment were carried out. In the first type perfusate was not recirculated and hormones, when present, were added to the medium in a single dose. In a second group perfusate was recycled and glucagon, when present, was added in nine sequential doses (to correct for depletion of hormone by liver) at intervals of 15min. These two types of perfusion have been described previously (Ma & Hems, 1975; Hems & Ma, 1976). The average rate of flow of perfusate was 1.4ml/min per g in lean mice and 1.2ml/min per g in obese mice.

Analytical methods

The total rate of lipid synthesis was measured by the incorporation of ^3H from $^3\text{H}_2\text{O}$ into either fatty acids or cholesterol (Salmon *et al.*, 1974; Ma & Hems, 1975), and calculated as C_2 units (i.e. acetyl residues) incorporated into lipids, after taking into account the isotope discrimination effect between ^1H and ^3H (Brunengraber *et al.*, 1973; Ma & Hems, 1975). Conversion of lactate into glucose (Elliott *et al.*, 1971) or lipid (Salmon *et al.*, 1974) was followed with $[\text{U-}^{14}\text{C}]\text{lactate}$. Glycogen, glucose and lactate were measured by enzymic techniques (Elliott *et al.*, 1971). Acetyl-CoA carboxylase activity was assayed by the method of Halestrap & Denton (1973) with the following minor modifications. Liver was rapidly frozen, and a supernatant

Table 1. Response to glucagon of fatty acid and cholesterol synthesis in the perfused liver of normal and genetically obese (*ob/ob*) mice

Livers were perfused (during the summer) for 3h with recirculating perfusate containing glucose (15mM) and lactate (10mM) unless indicated. Glucagon, when present, was added after 45min and then at 15min intervals, each time to the concentration indicated. Radioactive precursors were added after 60min. Lean and obese mice were homozygous for their respective alleles. Other details are in the text. Diet-restricted mice were fed 4g/day (Hems & Ma, 1976). Results are means $\pm$ S.E.M.

Mouse	Glucagon (M)	No. of perfusions	Lipid synthesized (μmol of C_2 units/2h per g fresh wt.)				Lactate C converted into glucose (μg -atoms/ 2h per g)
			Total		From lactate		
			Fatty acid	Cholesterol	Fatty acid	Cholesterol	
Lean	Control	5	44.4 $\pm$ 3.7	2.3 $\pm$ 0.4	21.1 $\pm$ 2.0	1.1 $\pm$ 0.2	216 $\pm$ 8
Lean	10 ⁻¹⁰	3	16.7 $\pm$ 1.1*	1.8 $\pm$ 0.4	6.8 $\pm$ 0.8*	0.7 $\pm$ 0.2	—
Lean	10 ⁻⁹	5	9.2 $\pm$ 2.0*	0.6 $\pm$ 0.1*	3.8 $\pm$ 0.8*	0.3 $\pm$ 0.1*	295 $\pm$ 20*
Obese	Control	3	61.2 $\pm$ 7.3	2.2 $\pm$ 0.1	18.4 $\pm$ 2.2	0.5 $\pm$ 0.1	—
Obese	10 ⁻⁹	3	44.5 $\pm$ 2.2	2.0 $\pm$ 0.1	15.0 $\pm$ 1.1	0.5 $\pm$ 0.1	—
Obese (diet-restricted)	Control	4	49.7 $\pm$ 4.1	2.2 $\pm$ 0.4	18.7 $\pm$ 1.8	0.8 $\pm$ 0.1	—
Obese (diet-restricted)	10 ⁻⁹	4	36.8 $\pm$ 5.6	2.2 $\pm$ 0.3	15.3 $\pm$ 2.3	0.8 $\pm$ 0.2	—
Obese†	None	3	68.5 $\pm$ 18.0	—	—	—	—

* $P < 0.01$, compared with control value.

† Glucose (15mM) in perfusate, but no lactate added.

Fig. 1. Concentration-dependence of effects of glucagon in perfused liver of normal mice

Livers from fed normal mice (identified as homozygous for the normal allele, rather than heterozygous for the *ob* gene) were perfused (in the winter) for 30 min with recirculating perfusate containing glucose (15 mM) and lactate (10 mM). Then glucagon was added in one dose, to the concentration indicated, with [U-¹⁴C]lactate and ³H₂O, at the beginning of a non-recirculating period (a further 40–50 min). Glucose output (○) was measured by simultaneous analysis of input and effluent perfusate, 10 min after glucagon, and liver fatty acids were analysed for radioactivity at the end of the perfusion. The amount of fatty acid synthesis was calculated as μmol of C₂ units/h (▲, total C₂ units; ▼, C₂ units from lactate). Other details are in the text. Results are from three to five perfusions (bars indicate S.E.M.).

fraction was assayed before ('initial') and after ('total') treatment with Mg²⁺ plus citrate by following incorporation of ¹⁴C from H¹⁴CO₃⁻ (Gove & Hems, 1978).

Results

Effect of glucagon on lipid synthesis in recirculating perfusions

In perfusions of normal mouse liver, when perfusate was recirculated for 2 h, there was inhibition by glucagon of fatty acid synthesis (measured with ³H₂O or [¹⁴C]lactate: Table 1). This type of experiment, in conditions optimal for hepatic fatty acid synthesis (Salmon *et al.*, 1974), thus confirmed the inhibition reported previously with liver preparations. Cholesterol synthesis was also inhibited by glucagon and acceleration of the conversion of lactate into perfusate glucose was observed (Table 1). Fatty acid synthesis was slightly diminished by glucagon in obese mice; food deprivation made no difference to the extent of inhibition (Table 1). There was no significant inhibition by glucagon of the synthesis of cholesterol in livers from obese mice, even in food-deprived animals (Table 1).

Perfusions with glucose alone were also carried out with livers from obese mice. The rate of fatty acid synthesis was not significantly lower than when lactate was also present (Table 1), whereas livers from lean mice exhibit a several-fold decline in the rate on omission of lactate (Salmon *et al.*, 1974).

Effect of glucagon in flow-through perfusions of livers of normal mice

The above results show that glucagon can inhibit fatty acid synthesis in the perfused liver of normal mice. A possible explanation for this effect (and for reported inhibitory effects in slices or cell suspen-

Table 2. Effect of glucagon on acetyl-CoA carboxylase activity in the perfused liver

Livers of lean mice were perfused with non-recirculating perfusate. A single dose of glucagon, to the concentration indicated, was added after 40 min. Control perfusions received no addition. After a further period, the liver was freeze-clamped, and the enzyme was assayed. Only one (final) liver sample was taken from each perfusion. Other details are in the text. Results are means ± S.E.M.

Glucagon (M)	Time after hormone (min)	No. of observations	Acetyl-CoA carboxylase (μmol/min per g of fresh liver)	
			Initial	Total
None*	—	3	0.12 ± 0.02	0.52 ± 0.06
None†	—	3	0.12 ± 0.01	0.48 ± 0.04
10 ⁻⁹	10	4	0.12 ± 0.02	0.51 ± 0.03
10 ⁻⁹	20	4	0.15 ± 0.08	0.59 ± 0.05
10 ⁻⁸	10	4	0.11 ± 0.02	0.45 ± 0.03
10 ⁻⁸	20	4	0.12 ± 0.04	0.46 ± 0.13

* Sample taken after 50 min.
† Sample taken after 60 min.

Table 3. Response to glucagon of glycogen content and glucose output in perfused livers of normal and genetically obese (*ob/ob*) mice

In the perfusions described in Table 1, the glycogen content of the liver was measured at the end of perfusion, and glucose output was measured by simultaneous analysis of input and effluent perfusate, 10 min after hormone was added. Other details are in Table 1 or the text. Results are means $\pm$ S.E.M. All glucagon effects are significant ($P < 0.01$), compared with control values. The initial glycogen content was about $160 \mu\text{mol}$ of glucose/g, in lean or obese mice.

Mouse	Glucagon (M)	No. of perfusions	Final glycogen content (μmol of glucose/g)	Glucose output ($\mu\text{mol}/\text{min}$ per g)
Lean	None	5	170 ± 31	0.44 ± 0.10
Lean	10^{-10}	3	16 ± 2	3.70 ± 0.31
Lean	10^{-9}	5	21 ± 4	5.01 ± 0.65
Obese	None	3	117 ± 20	0.54 ± 0.18
Obese	10^{-9}	3	15 ± 3	5.52 ± 0.43
Obese (diet-restricted)	None	4	124 ± 25	0.59 ± 0.13
Obese (diet-restricted)	10^{-9}	4	12 ± 5	4.78 ± 0.57

Fig. 2. Effect of insulin on glucose release due to glucagon in perfused liver of normal mice

Livers were perfused as described in Fig. 1, except that perfusate contained only glucose (initially 10 mM). The glucose concentration of effluent perfusate was determined during the non-recirculating period. The glucagon concentration was either 10^{-9} M (a) or 10^{-10} M (b), and insulin was 10^{-8} M . Perfusions contained glucagon alone ($\circ$) or glucagon plus insulin ($\bullet$) or no additions (----). Other details are in the text. Results are means of three to six perfusions; bars indicate S.E.M.

sions) is that during perfusion with glucagon, in view of its effects on carbohydrate metabolism, there is depletion of favoured substrates for lipogenesis, namely glycogen and lactate (Salmon *et al.*, 1974). In this case, glucagon should exert less inhibition in shorter perfusions; conversely, a true short-term effect of glucagon on fatty acid synthesis should be manifest in these conditions. Hence non-recirculating perfusions were carried out, in which glucagon at selected (unchanging) concentrations reached the liver for only 40–50 min. No significant inhibition of lipogenesis (measured with either $^3\text{H}_2\text{O}$ or ^{14}C -lactate) was seen unless the glucagon concentration was greater than 10^{-9} M (Fig. 1). Glucose output was stimulated at lower glucagon concentrations (10^{-10} M ; Fig. 1). In these experiments, lactate provided about two-thirds of the carbon atoms for fatty acid synthesis.

Effect of glucagon on acetyl-CoA carboxylase activity on flow-through perfusions

Several reports have suggested that glucagon may inhibit fatty acid synthesis at the acetyl-CoA carboxylase reaction (see the introduction). A direct inhibitory effect on this enzyme (e.g. analogous to activation of phosphorylase) should be manifest within a short period after hormone addition. Therefore this enzyme was assayed 10–20 min after glucagon addition in flow-through perfusions. No inhibition of acetyl-CoA carboxylase was detected (Table 2), compared with the activity in control livers.

Effects of glucagon and insulin on glucose release and glycogen content of perfused livers

Glucagon produced the expected glucose release (Assimakopoulos-Jeannet *et al.*, 1973) in perfused

Table 4. Effect of insulin on glycogen degradation due to glucagon in the perfused liver of normal and genetically obese (*ob/ob*) mice

Livers of fed mice were perfused with recirculating perfusate containing 10mm-glucose for 30min. At this time, hormones were added. Perfusion was then continued without recirculation for 25min, and liver glycogen content was determined. Diet-restricted *ob/ob* mice received 4g of food/day, and weighed 28–35g. Results, from the same experiments as those described in Fig. 2, are means ± S.E.M. for the numbers of observations indicated.

Mouse	Hormone (M)	Number of perfusions	Final liver glycogen content (μmol of glucose/g of wet liver)
Lean	Control	7	197 ± 8
Lean	Glucagon (10 ⁻¹⁰)	5	57 ± 3
Lean	Glucagon (10 ⁻⁹)	3	49 ± 6
Lean	Glucagon (10 ⁻¹⁰); insulin (10 ⁻⁸)	5	141 ± 13
Lean	Glucagon (10 ⁻⁹); insulin (10 ⁻⁸)	3	46 ± 7
Obese	Control	3	231 ± 6
Obese	Glucagon (10 ⁻¹⁰)	4	92 ± 9
Obese	Glucagon (10 ⁻¹⁰); insulin (10 ⁻⁸)	4	102 ± 5
Obese*	Control	3	209 ± 23
Obese*	Glucagon (10 ⁻¹⁰)	3	22 ± 4
Obese*	Glucagon (10 ⁻¹⁰); insulin (10 ⁻⁸)	3	158 ± 14

* Diet-restricted.

Fig. 3. Effect of insulin on glucose release due to glucagon in perfused liver of genetically obese mice
Experiments resembling those described in Fig. 2 were carried out with livers from either freely fed genetically obese mice (a) or obese mice which received a restricted diet (b). The glucagon concentration was 10⁻¹⁰M. Symbols and other details are as in Fig. 2 and in the text.

livers of normal mice, and in genetically obese mice, whether they were fed *ad lib.* or severely deprived of food (Table 3). Insulin (10⁻⁸M) prevented glucose release in the presence of glucagon (10⁻¹⁰M), but not if the glucagon concentration was 10⁻⁹M (Fig. 2; Table 4).

From the data in Fig. 2, and the fact that perfusate flow rate was about 1 ml/min per g, the time course, extent and rate of glycogen degradation in the liver may be calculated. Glucose release at a glucagon concentration of 10⁻⁹M amounted to about 10 μmol/min per g of wet liver during the period of peak release, i.e. between 10 and 15 min after glucagon; a further 60 μmol of glucose/g was released during the next 15 min. These rates are of the same order as in the perfusions where lactate was also added (Table 3, Fig. 1). The glucose released in these experiments was largely a consequence of glycogenolysis (Table 4).

In perfused livers of genetically obese mice, insulin (10⁻⁸M) did not counteract the glycogenolytic effect of glucagon (10⁻¹⁰M); food deprivation reversed this hepatic insulin-resistance (Fig. 3, Table 4).

As insulin counteracts the glycogenolytic effect of glucagon in liver, it was of interest to assess whether this hormone would counteract the catabolic effects of vasopressin in liver. A concentration of vasopressin was chosen which was maximal for the effects on glycogenolysis and on fatty acid synthesis (Ma & Hems, 1975). Insulin did not counteract the catabolic

Table 5. *Effect of insulin on vasopressin action on glycogen content and fatty acid synthesis in the perfused liver*
Livers were perfused for 3 h with recirculating medium containing $^3\text{H}_2\text{O}$, $[\text{U-}^{14}\text{C}]\text{lactate}$ (initially about 12 mM) and glucose (initially about 15 mM). Radioisotope-labelled water and lactate were added after 60 min. Vasopressin (10^{-8} M) was added after 45 min. In one group, insulin (4.5 nmol) was added from the start of the perfusion, and subsequently every 60 min until the end of the perfusion. Livers were analysed after 3 h, and results are means $\pm$ S.E.M., for the numbers of perfusions indicated.

Treatment	No. of perfusions	Fatty acid synthesized (μmol of C_2 units/2h per g)		Final glycogen content (μmol of glucose/g of fresh liver)
		Total	From lactate	
Control	4	38 ± 4	14 ± 2	177 ± 25
Vasopressin	3	18 ± 4	7 ± 2	50 ± 12
Vasopressin plus insulin	3	23 ± 5	6 ± 1	45 ± 19

effects of vasopressin on liver (Table 5). Therefore this combination of hormones was not tested in genetically obese mice. This result suggests that the mechanisms of action of insulin and vasopressin on liver do not include any crucial common steps.

Discussion

Inhibition of hepatic lipid synthesis by glucagon

These experiments have documented the concentration-dependence of the short-term action of glucagon on murine hepatic fatty acid synthesis. Only at concentrations as high as 10^{-9} – 10^{-7} M was there inhibition by glucagon, whereas glucagon at concentrations that occur in plasma (10^{-11} – 10^{-10} M ; Unger, 1976) failed to exert a short-term effect on hepatic fatty acid synthesis, in conditions that provided a critical test of this possibility, i.e. relatively short non-recycling perfusions in optimal substrate conditions. Hence this study provides no support for the contention that glucagon exerts a short-term direct inhibitory effect on hepatic fatty acid synthesis (see the references in the introduction), but endorses the opposite conclusion of Raskin *et al.* (1974). Our investigation amplifies and complements this study (which featured the perfused rat liver and the use of ^{14}C -labelled acetate and octanoate) in the use of $^3\text{H}_2\text{O}$ and $[\text{U-}^{14}\text{C}]\text{lactate}$, which are two of the most satisfactory precursors for measuring fatty acid synthesis (Salmon *et al.*, 1974) and in the use of substrate conditions optimal for lipogenesis (Salmon *et al.*, 1974), in which at least one hormone can rapidly inhibit this process (i.e. vasopressin: Ma & Hems, 1975).

Glucagon at lower concentrations (10^{-10} M) can, however, produce inhibitory effects on hepatic fatty acid synthesis, in longer recycling perfusions in mice (present work) and rats (Regen & Terrell, 1968) or in incubations of rat liver slices or cell suspensions (see the introduction for references). One possible explanation is that, during these experiments, liver becomes deprived of glycogen, which is a preferred

carbon source for fatty acid synthesis (Salmon *et al.*, 1974; Clark *et al.*, 1974).

Glucagon can also inhibit glycolysis, by bringing about conversion of pyruvate kinase into a phosphorylated form (Blair *et al.*, 1976; Feliu *et al.*, 1976; Ljungstrom & Ekman, 1977; Van Berkel *et al.*, 1977; Riou *et al.*, 1978). This effect would also contribute to inhibition of fatty acid synthesis from glycogen. Such inhibition of glycolysis, presumably related to the established effect of glucagon to stimulate the incorporation of lactate carbon atoms into glucose (manifest in the present experiments), would tend to remove pyruvate in mitochondria from the dehydrogenase reaction on the pathway of lipogenesis.

These considerations imply that liver preparations treated with glucagon would gradually convert the favoured precursors for lipogenesis (glycogen, lactate) into a less-favoured precursor, glucose (Salmon *et al.*, 1974). In effect, a response to glucagon has often been sought in incubations which are not comparable with their control incubations, in terms of substrates offered to the tissue throughout the experiment. In hepatocyte suspensions, cyclic AMP exerts less effect on lipogenesis in the initial period of incubation (Harris, 1975), as the above explanation would predict.

From the lack of a short-term inhibitory effect of glucagon on acetyl-CoA carboxylase activity, described here, it follows that inhibitory effects of glucagon on the proportion of the active form of acetyl-CoA carboxylase, reported in experiments with liver slices (Allred & Roehrig, 1973), cell suspensions (Muller *et al.*, 1976) and the intact rat (Klain & Weiser, 1973), also may not reflect a primary direct short-term action of glucagon on cells. The effect in slices or cell suspensions could be due to secondary inactivation after alterations of carbohydrate metabolism (see above); thus acetyl-CoA carboxylase is susceptible to control by a variety of intracellular modifiers (Carlson & Kim, 1974b), but not cyclic AMP (Carlson & Kim, 1974a).

Inhibition of fatty acid synthesis by glucagon, secondary (in time) to alterations in carbohydrate metabolism, would still have significance in the intact animal, despite being slower than the most rapid effects of glucagon. An increase in the hepatic capacity for ketogenesis in response to glucagon has also been suggested to be secondary to glycogen depletion (McGarry *et al.*, 1975). Since ketogenesis is the result of β -oxidation of fatty acids (i.e. the opposing pathway to lipogenesis), it would be expected to exhibit parallel (but opposite) behaviour to lipogenesis.

With cholesterol synthesis, although inhibition by glucagon may involve specific effects of cyclic AMP (Bloxham & Akhtar, 1971; Bloxham *et al.*, 1971), it could also be secondary to glycogen depletion, and due to inhibition of glycolysis at pyruvate kinase. Until a rapid response to glucagon is demonstrated, such a view is simpler than any other, and could explain the lack of a close correlation between this inhibition and the hepatic content of cyclic AMP (Bricker *et al.*, 1976), and the relative insensitivity of cholesterol synthesis to glucagon, compared with that of glycogen metabolism.

Effects of glucagon and insulin on liver of obese mice

The stimulatory effect of glucagon on glycogenolysis may be counteracted by insulin, at appropriate concentrations of each hormone (Glinsmann & Mortimore, 1968; Exton & Park, 1972). This effect was confirmed in the liver of normal mice, whereas genetically obese mice showed resistance to this antagonistic effect of insulin. This is the first demonstration of insulin-resistance in the liver of obese mice, by experiments where insulin is added *in vitro*, although insulin-resistance has been inferred from experiments *in vivo* (Kreutner *et al.*, 1975). However, if obese mice were deprived of food, to an extent sufficient to keep their weight in the same range as that of the normal mice, the insulin effect returned to normal. Such reversibility is also observed for the decline in the number of insulin receptors in the liver of obese mice (Soll *et al.*, 1975; Le Marchand *et al.*, 1977), for the insulin-resistance observed in adipose tissue and muscle of freely fed genetically obese mice (Abraham & Beloff-Chain, 1971; for reviews see Loten *et al.*, 1974; Czech *et al.*, 1977) and for their increased rates of basal glycogenolysis (Elliott *et al.*, 1971).

In contrast, the process of hepatic fatty acid synthesis in genetically obese mice exhibits irreversible resistance to inhibition by vasopressin (Hems & Ma, 1976). The present data show that glucagon does not act normally on fatty acid synthesis in obese mice, in that the proportional inhibition of fatty acid synthesis by glucagon in the liver of genetically obese (*ob/ob*) mice was less than that in lean mice.

This could be because fatty acid synthesis in the liver of genetically obese mice is less susceptible to alterations in the pattern of precursors. Thus when glucose alone (15mM) was offered to the perfused liver from diet-restricted *ob/ob* mice, the rate of fatty acid synthesis was near maximal for these livers, and severalfold higher than the rate in livers from normal mice. Hence glucagon would be expected to exert a less-marked secondary inhibition of lipogenesis than that seen in normal mice, as the conversion of glycogen into glucose would not deprive the liver of a favoured precursor (glycogen) by converting it into a less-favoured precursor (glucose). This characteristic cannot yet be regarded as a straightforward impairment of hormone action in genetically obese rodents. The glycogenolytic response, which is established to be cyclic AMP-dependent, was not impaired in obese mice (see also Chan *et al.*, 1975).

Among hormones that can exert rapid catabolic effects on hepatic metabolism (Hems, 1977), only glucagon has so far been clearly shown to inhibit cholesterol synthesis (whether as a primary or secondary effect). In obese mice, such inhibition was not present. The impaired catabolic effect of glucagon in liver of genetically obese mice adds another 'hormone-resistance' to the expanding list of such defects in obese mice. This resistance to glucagon action, as for vasopressin, is confined to fatty acid and cholesterol synthesis (rather than glycogen breakdown). Resistance to catabolic hormone effects on lipogenesis could have a key role in obesity (Hems & Ma, 1976; Hems, 1979).

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