incubated in the presence of an excess of insulin (approx. 3 μg of insulin/ml). Glucose-stimulated membranes can release approx. 1500 ng of insulin/ml before saturation of glucose-generated β-granule-binding sites takes place at 60 min incubation. When glucose 6-phosphate was added no further release of insulin took place despite the availability of 1.5 μg of insulin/ml in β-granules in the supernatant. It was possible that glucose 6-phosphate could not cause release under these circumstances because effector sites for glucose 6-phosphate had been diluted as a result of granule-membrane fusion with plasma membrane. It could also be that the membrane was not capable of responding to any other secretagogue. To test these propositions, 10 mM tolbutamide, a known insulin-releasing agent, was added to a membrane/granule incubation that had been similarly exposed to glucose. The system responded to tolbutamide. Furthermore the amount of insulin released was equal to that found when membranes were treated with tolbutamide in the absence of glucose, i.e. approx. 0.5 μg of insulin/ml.

Glucose and glucose 6-phosphate utilize different pathways in the generation of β-granule-binding sites on the plasma membrane. The fact that the release system in vitro becomes refractory to glucose 6-phosphate, but not to another secretagogue, tolbutamide, when the glucose-generated β-granule-binding sites are saturated, indicates that glucose and glucose 6-phosphate probably share a common final path. Tolbutamide must release insulin via a glucose-independent pathway.


HORMONAL CONTROL OF LIPID METABOLISM:
 a Colloquium organized on behalf of the Lipid Group
by R. M. Denton (Bristol)

Short-Term Hormonal Control of Fat Metabolism in the Liver
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Major processes of hepatic lipid metabolism

The predominant processes of fat metabolism in the liver of non-ruminant mammals are: (a) utilization of all species of circulating non-esterified fatty acids that circulate bound to plasma albumin, (b) synthesis de novo of long-chain fatty acids, (c) conversion of activated fatty acids into products, i.e. mainly glycerides, ketone bodies and CO₂, (d) synthesis of cholesterol and (e) export of micelles containing lipids and protein, VLD lipoprotein (very-low-density lipoprotein).

Hepatic lipid metabolism is under very fine control, over a time-scale from seconds to weeks. Some of the extraneous factors that can influence the rates of the above processes include diet and timing of meals, temperature, physical trauma and psychological factors. There are also changes in lipid metabolism in a variety of pathological states: diabetes and other endocrine disorders, vascular disease, nephrosis, obesity, catabolic disease, inborn errors, and liver disease.

In general, processes of lipid metabolism are slower in man than rodents (when calculated in units which could, in principle, be independent of weight, i.e. per mass of tissue, or as half-times in plasma). Nevertheless, there is no doubt about the crucial pathogenetic significance of events of lipid metabolism in the conditions enumerated above. Despite this fact, very little is known about the regulation of fat metabolism at the molecular level.
Precursors and pathways

Certain features of hepatic lipid metabolism are worth recapitulating, as they will form the likely sites of hormone action. In the order in which they were listed in the previous section, the major processes possess the following characteristics. (a) Non-esterified fatty acids enter the liver by a mechanism which is not understood (but involves permeases and binding proteins), and are converted into acyl-CoA derivatives in the extramitochondrial phase. (b) The major precursors of hepatic long-chain fatty acids synthesized de novo are glycogen and C3–C5 sources such as lactate; blood-borne glucose is not a significant precursor (Hems et al., 1975). (c) The pools of long-chain acyl-CoA in liver can encounter two major fates, namely entry to mitochondria via the carnitine system for β-oxidation, or conversion into glycerides, of which the major classes are phospholipids and triacylglycerols. This is clearly a vital control point in lipid metabolism, subject not only to long-term, but also to short-term control (McGarry et al., 1974). Much remains to be clarified about these reactions, and especially about the acyltransferase and phosphatidate phosphatase reactions, which are critical in glyceride transformations. It should also be noted that ketone bodies can be derived largely from the acetoacetyl-CoA produced at the C4 stage of β-oxidation, except in severe insulin deficiency. Thus there is no great requirement for net conversion of acetyl-CoA into acetoacetyl-CoA in mitochondria, and it is also not certain how much acetoacetyl-CoA is converted into acetyl-CoA.

(d) Synthesis of cholesterol, the precursors of which (not well clarified) could include fatty acid-derived acetyl-CoA and C4 and C6 intermediates, as well as acetyl-CoA from glycogen and lactate. (e) VLD lipoproteins exported by the liver contain apoproteins which are synthesized within the liver, and also recycled during glyceride conveyancing in plasma. The major lipids are triacylglycerol, and to a lesser extent phospholipid and cholesterol. The most rapid reaction of the VLD lipoprotein components is hydrolysis of the triacylglycerol, by the lipoprotein process in muscle and adipose tissue.

Hormonal control of fatty acid synthesis

One of the processes most susceptible to hormonal control is fatty acid synthesis. In particular, it is apparently insulin-dependent, being severely diminished in insulin-deficient diabetes. There seems no reason to doubt that such promotion of lipogenesis by insulin reflects a direct hepatic action, over hours or days. It is noteworthy though, that short-term stimulation of fatty acid synthesis in the liver by insulin has not been consistently demonstrable in any type of isolated preparation. Adrenal glucocorticoids promote hepatic fatty acid synthesis by increasing plasma insulin concentrations (Kirk et al., 1976).

Turning to inhibitory actions of hormones, some of these are listed in Table 1. Glucagon inhibits fatty acid synthesis in experiments lasting longer than 30 min, by a secondary mechanism, i.e. depletion in liver of favoured substrates of lipogenesis, such as glycogen and lactate. There is no evidence for a primary direct action of glucagon on lipogenesis, and therefore it is not surprising that cyclic AMP does not affect acetyl-CoA carboxylase.

There is a group of hormones which exert a catabolic action on the liver, which is not mediated by cyclic AMP. So far, this group has been shown to include α-adrenergic agonists, vasopressin, oxytocin and angiotensin II. These hormones inhibit fatty acid synthesis, at least in the mouse liver (Table 1), whereas in the rat liver, only an action on glycogen degradation has so far been documented (Hems et al., 1976).

Hormone effects on glyceride metabolism

Processes of glyceride metabolism are complex, and it is certain that hormones will affect these processes in liver (Hems, 1975). Insulin in particular can promote export of VLD lipoprotein by liver (Topping & Mayes, 1972). In a different approach to the question of glyceride dynamics, we have followed net changes in triacylglycerol and phospholipid contents during perfusion of the mouse liver. Insulin suppressed the breakdown of triacylglycerol in the liver, even in the absence of an effect on fatty acid
Table 1. *Short-term hormonal control of fatty acid synthesis*

Rates of fatty acid synthesis were measured in the mouse liver perfused with glucose (15 mM), lactate (10 mM) and $^3$H$_2$O. Data (calculated per h) are from Ma & Hems (1975), Ma et al. (1977), Salmon & Hems (1975) or are unpublished experiments of Dr. G. Y. Ma.

<table>
<thead>
<tr>
<th>Hormone (nM)</th>
<th>Fatty acid synthesis (µmol of C2 units/h per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>27</td>
</tr>
<tr>
<td>Insulin (30)</td>
<td>30</td>
</tr>
<tr>
<td>Vasopressin (3)</td>
<td>10</td>
</tr>
<tr>
<td>Angiotensin II (4)</td>
<td>8</td>
</tr>
<tr>
<td>Adrenaline (200)</td>
<td>12</td>
</tr>
<tr>
<td>Glucagon (0.1)</td>
<td>28</td>
</tr>
<tr>
<td>Glucagon (1)</td>
<td>23</td>
</tr>
<tr>
<td>Glucagon (10)</td>
<td>16</td>
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</tbody>
</table>

synthesis (Salmon & Hems, 1976), suggesting that this may be a major site of action of insulin. No other hormonal actions on glyceride metabolism have been adequately clarified. (This is a regrettable state of affairs, given the enormous importance of these pathways.) Glucagon can inhibit synthesis and export of glycerides (Heimberg et al., 1974), but the significance of this action is not clear.

**Mechanisms of hormone action**

It is established that glucagon and $\beta$-adrenergic agonists act via cyclic AMP in the liver, and it is still an acceptable notion that all their actions stem from this nucleotide. A larger group of hormones do not act via cyclic AMP, including $\alpha$-adrenergic agonists, vasopressin, oxytocin and angiotensin II (K. Siddle & D. A. Hems, unpublished work.) So far, there is no credible evidence that cyclic GMP has a significant role in any hormone action on liver. The mechanism of action of the second group of hormones (above) is obscure. It is relevant that the action of vasopressin on hepatic glycogen metabolism involves extracellular Ca$^{2+}$ (Stubbs et al., 1976). However, the action of small peptides on lipid metabolism may not resemble that on glycogen, as shown by the fact that the former action is completely deficient in the liver of genetically obese mice, whereas glycogen breakdown is stimulated normally (Hems & Ma, 1976).

**Functional significance of hormone actions: why is there fatty acid synthesis in the liver?**

The functional importance of the hormonal control of hepatic fatty acid synthesis requires to be appraised. Indeed, the basic question is: why is there fatty acid synthesis in the liver, when there is a constant supply of palmitic acid (the main product of synthesis) reaching liver via plasma?

A fundamental point is that during most of man’s evolution, there was not a superabundance of food, so that there would have been far less adipose tissue available for lipogenesis. Hence the role of the liver would have become crucial. The existence of adaptations in hepatic lipogenesis in response to diet is well recognized, and involves hormonal control of long-term type. Short-term responses to carbohydrate feeding are not well understood, largely because the role of insulin in hepatic lipogenesis is so problematical.

There are other environmental variables which can affect hepatic fatty acid synthesis. We have investigated one which is of major relevance in mammals, namely temperature. When mice are placed in a cold environment, there is acceleration of fatty acid synthesis, within 1 h (Fig. 1). This result suggests that hepatic lipogenesis has a major role in heat production. In general, mechanism of heat production in homoeotherms are not well understood.
Fatty acid synthesis was measured in the liver of intact fed female mice with $^3\text{H}_2\text{O}$ (over 1 h; Hems et al., 1975) between 11:00 and 13:00h. Measurements were made at various times after the start of exposure at 4°C, in a large cold-room. Control animals did not exhibit an increase in hepatic lipogenesis. Results are means±S.E.M. of three to five determinations.

**Role of adrenaline and angiotensin II in diabetes**

Another aspect of the control of hepatic fatty acid synthesis in the liver concerns the events of pathological states. In diabetes, there is a gross decline in hepatic fatty acid synthesis, which may be largely due to the decline in blood insulin concentration. In longer-term diabetes, the processes of glyceride assembly and export become impaired, through complex changes.

Hormones with catabolic actions, which increase in plasma in diabetes, may also be implicated in the decline in hepatic fatty acid synthesis in diabetes. These hormones include not only glucagon, but also adrenaline and angiotensin II, which can inhibit fatty acid synthesis (Ma et al., 1977; Table 1) and which do rise in blood in diabetes (see Hems et al., 1976, for references).

**Hormonal responses in genetic obesity**

Obesity is in a general sense the converse of diabetes, and is like diabetes in reflecting inherent endocrine alterations in the animal. Thus an individual can be designated as obese, regardless of his weight. Obesity is important because it can lead to diabetes, as a result of the strain on the endocrine pancreas that is imposed by insulin-resistance.

The pathogenesis of obesity offers one of the major conundra of biochemical endocrinology. The best hope of solving the riddle, and for investigating obesity in general, lies with the use of genetically obese rodents. Obese mice have an impairment in the hepatic response to hormones that inhibit fatty acid synthesis, such as vasopressin (Hems & Ma, 1976). This defect is intractable to severe food privation, and could clearly be relevant to the causation of obesity. If it may be taken to be representative of their inborn error (as seems warranted), then it appears that their inborn error residues in the cellular responses to extracellular effectors (e.g. in the hypothalamus and peripheral tissues).
This defect is not generalized, as glycogen metabolism responds normally to vaso-
pressin (Hems & Ma, 1976) and does not involve the adenylate cyclase system, both
because vasopressin does not act via this mechanism (Kirk & Hems, 1974) and because
glucagon acts normally on glycogen metabolism in genetically obese mice (G. Y. Ma &
D. A. Hems, unpublished work).

Conclusion

It is still axiomatic that the liver constitutes the main cross-roads for the processes of
interorgan lipid metabolism, i.e. those processes designed to serve the whole animal as
well as individual organ needs. Despite the prime importance of this role, knowledge of
mechanisms of regulation of hepatic fat metabolism is derisory. The preceding account
has been designed to highlight this state of affairs.

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Regulation of Liver Lipid Metabolism in Experimental Obesity

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The physiopathology of experimental obesity, studied in several animal models, has been
reviewed by Assimacopoulos-Jeannet & Jeanrenaud (1976). We summarize here data
obtained with livers from three types of obese animals, the obese–hyperglycaemic
(ob/ob) mouse, the albino rat made obese by electrolyte lesions of the hypothalamus
and the albino mouse made obese by chemical lesions of the hypothalamus.

Materials and methods

Animals used in these experiments were as follows: (a) 8–12-week-old male obese–
hyperglycaemic (C57 BL/6J ob/ob) mice and lean controls (C57 BL/6J ++/++); (b)
female Wistar rats weighing between 180 and 200 g; (c) Swiss albino male mice that were
either 9 or 20 weeks old. All animals were fed ad libitum with laboratory chow. In rats,
lesions of the hypothalamus were produced by stereotaxically guided electrolysis,
successful lesions of the ventromedial hypothalamus area being subsequently controlled
histologically. Hypothalamic-lesioned and unoperated control rats were used for liver
perfusion 10 days after the operative day (Karakash et al., 1977). In mice, lesions of the
hypothalamus were obtained by a single injection of gold thioglucose (150–350 mg/kg
1977