A SWITCH MECHANISM IN THE REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS IN RAT LIVER

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It is generally accepted that in a rat which is fed upon a carbohydrate-containing diet the liver will remove glucose from the portal blood and part of this glucose will be degraded by the process of glycolysis. However, when such an animal is starved the direction of carbohydrate metabolism in the liver must be reversed in order that glucose may be synthesised from non-carbohydrate precursors. It is now well established that glycolysis and gluconeogenesis can be regulated at least in part at the enzymatic level of phosphofructokinase (PFK) (EC 2.7.1.11) and fructose diphosphatase (FDPase) (EC 3.1.3.11). By analogy with the situation in kidney cortex, it has been suggested that in starvation this switch in carbohydrate metabolism could be effected by an increase in the intracellular concentration of citrate [1, 2]. The latter would inhibit PFK [3] and, providing that PFK and FDPase are simultaneously active (and thus catalyse a cycle between fructose-6-phosphate and fructose disphosphate) the rate of fructose diphosphate hydrolysis would be automatically increased. However it has been shown that the hepatic content of citrate decreases during starvation [4], and therefore the above mechanism cannot operate in liver. An alternative mechanism for the inhibition of hepatic glycolysis during starvation is proposed in this paper.

The catalytic activity of PFK and FDPase could be modified either by the effect of changes in the concentrations of regulatory metabolites or by changes in the concentrations of the enzymes *per se*. The latter possibility has been investigated by measuring the maximum catalytic activities of the enzymes, which it is assumed will provide some indication of their concentrations. Previously it has been shown that the

Table 1
Effect of starvation upon the maximum activities of PFK and FDPase in rat liver.

Treatment of rat	PFK activity		FDPase activity	
	μmoles/min/g fresh liver	μmoles/hr/100 g body weight	μmoles/min/g fresh liver	μmoles/hr/100 g body weight
Fed control	2.46 ± 0.09	680 ± 23 (6)	3.56 ± 0.16	1019 ± 57 (4)
Starved 24 hr	2.38 ± 0.05	513 ± 11 (4)	3.71 ± 0.29	800 ± 64 (4)
Starved 48 hr	2.00	358 (2)	2.78	507 (2)

Rats were stunned and killed by cervical fracture. The liver was rapidly excised, rinsed in ice-cold extraction medium, blotted dry and weighed. Portions of liver were quickly extracted in 5–10 vols ice-cold extraction medium. The extraction media and assay methods used were those of Underwood and Newsholme [7], and Opie and Newholme[8]. Results are expressed as mean ± S.E.M. Number of animals are given in parentheses.

maximum activity of PFK (calculated on the basis of body weight) is decreased by 80% after 48 hr starvation [5], whilst that of FDPase remains relatively constant [6]. However in the present investigation it was found that the maximum activities of both PFK and FDPase were decreased by a similar amount on starvation (table 1). It would therefore seem unlikely that changes in enzyme concentrations could be responsible for the switch from glycolysis to gluconeogenesis under these conditions.

The metabolic factors that might regulate the activity of either of these enzymes should become evident in a study of their properties. Thus hepatic FDPase is inhibited by AMP, whereas PFK is inhibited by citrate and ATP and these effects are abolished by AMP [3]. In an earlier study [4] it was found that the directions of the changes in these metabolites during starvation were not consistent with the role for increasing gluconeogenesis and decreasing glycolysis. However there is one other factor which can reduce the ATP inhibition of PFK, namely the other substrate, fructose-6-phosphate [3], and it has been shown that the content of fructose-6-phosphate is decreased approximately 50% on starvation [4]. Therefore, this change may play a role in reducing the activity of PFK and, because of the proposed cycling between fructose-6-phosphate and fructose diphosphate, may act to inhibit glycolysis and stimulate gluconeogenesis. It may be questioned whether such a decrease in the content of fructose-6-phosphate would be sufficient to induce a reversal of the direction of carbohydrate metabolism. However one advantage of cycling between such intermediates is the great sensitivity it confers upon the regulatory system [2]. Furthermore the marked fall in the contents of glucose-6-phosphate and fructose-6-phosphate occurs between 27 and 35 hr of starvation when the hepatic glycogen stores are depleted [4]: this is precisely the period in which stimulation of gluconeogenesis would be required by the animal because glucose can no longer be provided from liver glycogen. Moreover such a temporal mechanism of control may be exceedingly important for ensuring that the rate of glucose release is in accord with the demands for glucose. If excess glucose was released due to simultaneous glycogenolysis and gluconeogenesis the blood glucose level must increase which would cause changes in metabolism similar to those seen on refeeding (e.g. increased insulin release, decreased fatty acid mobilisation); such changes would be highly undesirable. The available evidence does indeed suggest that the rate of glycogen breakdown is sufficient to supply most, if not all, of the glucose required by the animal in the early stages of starvation; thus the maximum rate of glycogenolysis in rat liver *in vivo* is approximately 0.5 μmoles/min/g liver [4, 9] and the rate of glucose release which has been measured in dog liver *in vivo*, appear to be of the same order [10, 11]. The depletion of the glycogen stores and perhaps the decrease in activity of glucokinase [12, 13] would appear to be the main factors contributing to the decrease in hexose monophosphate in the liver and thus indirectly may be responsible for the switch from glycolysis to gluconeogenesis.

Furthermore, this hypothesis provides an explanation for the difference between liver and kidney cortex in relation to the control of these processes during starvation. Thus there is an increase in the contents of both citrate and glucose-6-phosphate in kidney cortex slices from starved animals, so that citrate inhibition of PFK could explain the enhanced gluconeogenesis and depressed glycolysis in this tissue [1]. Furthermore in the kidney cortex there is very little glycogen and glycogenolysis will be of no importance during starvation so that there is no problem about temporal control of the two glucose-forming processes, glycogen breakdown and gluconeogenesis. Moreover glucokinase is absent from the kidney cortex so that the major signal for increased hydrolysis of glucose-6-phosphate during starvation may be the elevation in its concentration. Thus the large glycogen store and the presence of glucokinase in liver may have necessitated the development of a different mechanism for the control of carbohydate metabolism in starvation at the level of PFK and FDPase. It is interesting that the behaviour of kidney cortex in recept to the changes in glucose-6-phosphate and citrate during starvation is similar to heart muscle [14, 15] whereas the behaviour of liver resembles that of adipose tissue [16].

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