Resolution by Computer Simulation of Contradictory Experimental Findings as to the Effect on Gluconeogenesis of Oleate Addition in Perfused Rat Liver

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Different workers have obtained contradictory results regarding the effect of oleate addition on the rate of gluconeogenesis in perfused rat livers. Computer simulation of these experiments indicates that the different effects observed by the research groups of Williamson and of Exton and Park can be due to a small quantitative difference in fatty acid metabolism by the different strains of rat used.

There is disagreement in the biochemical literature over the effect on gluconeogenesis of adding oleate to the perfusate in perfused rat liver experiments. Williamson and associates (1-4) found that adding oleate stimulates gluconeogenesis from lactate, from about 2000 nanomoles of glucose/minute/gram dry weight of liver to about 4,160. On the other hand, Exton and Park and associates (5,6) found that perfusion with lactate resulted in glucose production rates of from 2300 to 4600 nanomoles glucose/minute/gram dry weight, and that oleate (added as an albumin complex) does not significantly increase this rate (7).

This difference was at first ascribed to differences in technique: Exton and Park use red blood cells in their perfusion medium, whereas Williamson et al do not. However, it has recently become apparent that the difference is due to differences in the strain of rats used, as a single worker using the same technique throughout has obtained the qualitatively different results using different rat strains (R. Scholz, personal communications). Computer simulation of liver gluconeogenesis suggests a resolution for this contradiction. A detailed report of

this simulation is being published elsewhere; however, the explanation for the experimental disagreement which this provides is of more general interest than the necessarily detailed description of the entire model.

A computer model of the Krebs cycle and related metabolism in perfused rat liver, including gluconeogenesis, has been constructed and fitted to the data of Williamson and associates (1-4), using the simulation language of Garfinkel (8). Details of this model, its fit to the experimental data, and its properties on manipulation have been described elsewhere (9). This model, which is described in Figure 1, indicates that the gluconeogenesis rate is primarily

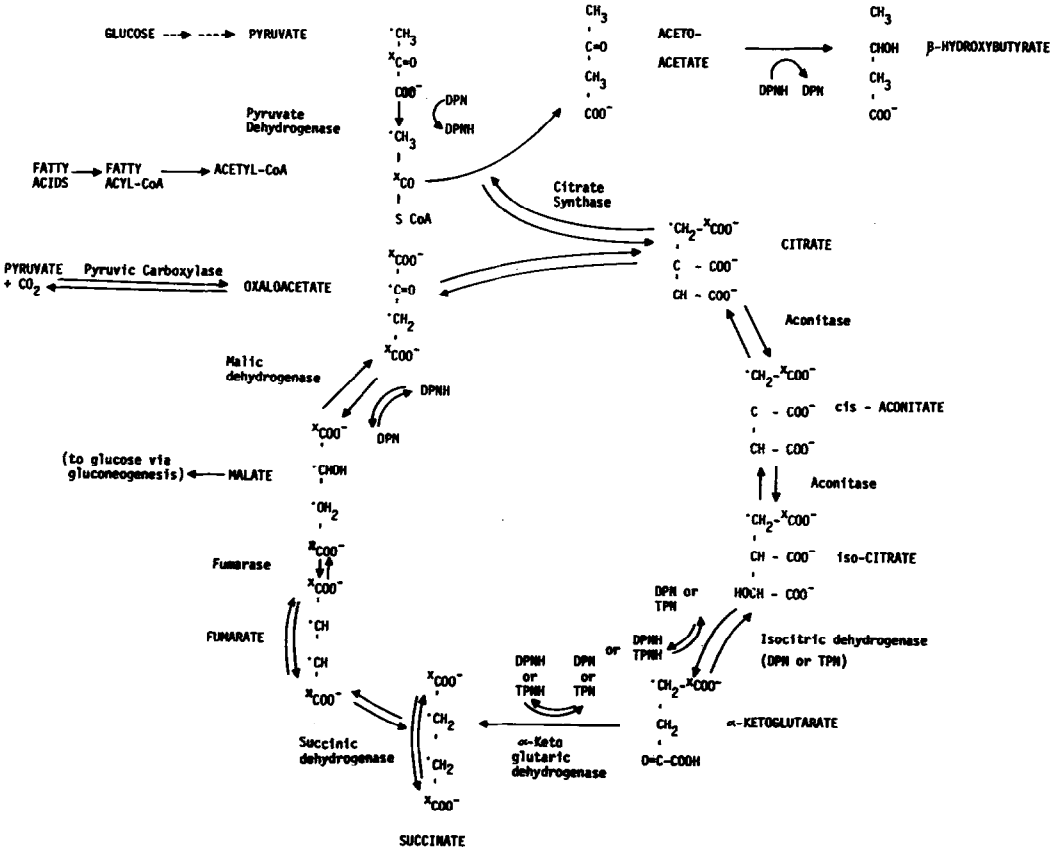

Figure 1. Overall structure of the model (including provision for tracing radioactive atoms).

determined by the rate at which material is fed into the pathway by pyruvate carboxylase. When the model was subjected to sensitivity analysis, variation of its parameters showed that in the physiological range pyruvate carboxylase activity is extremely sensitive to acetyl CoA concentration, which in turn is quite sensitive to the rate at which fatty acids (the principal precursor of acetyl CoA) enter the system. The shape of the curve in Figure 2, which shows the dependence of glucose production rate on fatty acid input rate, suggests that the discrepancy between the findings of Williamson and of Exton and Park might be accounted for if the livers from the different rat strains differ in their degree of saturation by endogenous fatty acids. The

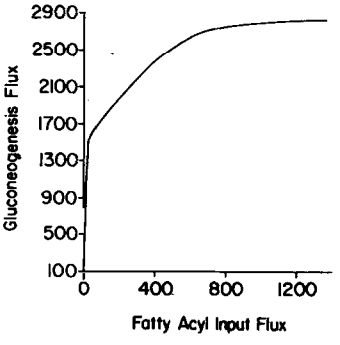

Figure 2. Dependence of gluconeogenesis on fatty acyl input (dimensions, nanomoles/g dry weight/min).

behavior of the model also suggests that the fatty acid feed-in pathway, perhaps extending back to the kinase step, is highly coordinated and controlled, perhaps even comparably to the fatty acid synthesis complex described by Lynen (10).

The critical computer experiment performed here is the simulation of the results of Exton and Park using a model developed for Williamson's data. This was done by starting with parameters which fit the data of Williamson (2) for lactate perfusion without oleate, and slightly

increasing fatty acid uptake rate. Compensating changes were made by supplying increased substrate to pyruvate carboxylase, since its rate of pyruvate fixation necessarily increases, and by moving its product in the direction of malate (the immediate glucose precursor in this model). Table I shows the parameters used to represent the

TABLE 1

Fit of the Model to the Data of Williamson and of Exton and Park

Numbers in nanomoles/g dry weight (min) unless otherwise indicated.

	Williamson				Exton & Park	
	Without oleate		With oleate		Without oleate	
Model Parameters Varied						
Oxaloacetate malate (rate constant)	1.0		2.0		2.3	
Lactate supply (rate constant)	5700		8500		8000	
Fatty acid feed-in (flux)	117		555		142	
Resulting parameters with References	Obs.	Calc'd	Obs.	Cal'd.	Obs.	Cal'd.
Glucose production (2)([a])	2040	1989	4160	3897	2320[a] to 4650	3225
Malate concentration (2)(12)	701	663	2142	2147	1400	1075

The only other changes of more than 10% on going from the left-hand column to the right-hand column are that acetyl CoA rises 17%, acetoacetate 33%, beta-hydroxybutyrate 40%.

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a Exton and Park give differing values from the experiments most nearly resembling those of Williamson (perfusion of livers from fasted rats using (defatted) Fraction V as the source of albumin, with substrate supplied immediately) in different publications: 4650 (5, Table I and Table VI); 2320 to 4120 (6, Table II). Much of this variation is due to exploration of perfusion conditions and albumin fractions.

two sets of Williamson data (with and without oleate) and the Exton and Park data. It is seen that a change of only 21% in the rate of endogenous fatty acid feed-in converts a model fitting Williamson's data to one fitting the data of Exton and Park. So small a quantitative change can very readily be ascribed to interstrain differences; much larger differences between rat strains are known, and it has recently been shown (11) that there are appreciably larger individual differences even within one (Wistar) strain of rat.

The model with these parameter changes also approximately fits the higher malate concentration (1400 nmoles/g dry wt) observed by Exton and Park (12). It also shows a qualitatively correct increase in ketogenesis; a quantitative fit could probably have been obtained at the price of manipulating additional parameters.

It therefore appears that the apparent qualitative contradiction between the findings of Williamson and associates and of Exton and Park and associates may be ascribed to a single small quantitative difference between the two strains of rat (Holtzman and Sprague-Dawley, respectively) used by the two groups of investigators.

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