approximately coincide; (ii) successive transitions must not overlap; (iii) a transition must coincide approximately with the intersection of the two theoretical lines.

Exploration with a desk-top computer reveals that Cases 2 and 3 can yield single abrupt transitions. Case 1 cannot.

It has been possible to fit the data for glutamate dehydrogenase equally well with either Case 2 or Case 3 equations. Case 2 represents a simpler model and constitutes the minimal assumption needed to account for the data.

Case 2 for glutamate dehydrogenase requires an equation containing terms in S^7 . Physical interpretation of the mathematical deductions raises interesting questions with regard to the catalytic mechanism and the number of NAD^+ -binding sites.

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The Acylation of Endogenous Lysophospholipids in Isolated Rat Liver Microsomal Fraction: Influence of Glutathione and Ethylenediamine-tetra-acetate on this Process

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Although the acylation of various exogenous monoacylphosphoglycerides by rat particles has been extensively studied, less attention has been paid to the acylation of the endogenous lysophospholipids present in these membranes or generated during the incubation. Because lipids integrated in a membrane might not have the same micellar structure as that of similar substances dispersed in a watery phase and might therefore exhibit different susceptibility to enzymes, it was decided to reinvestigate this acylation process by incubating liver microsomal fraction with trace amounts of ^{14}C -labelled fatty acids and the necessary cofactors without the addition of any acyl acceptor.

The incubation conditions were essentially as described by Possmayer *et al.* (1969) except that the concentration of radioactive fatty acids was $4\mu\text{M}$ and that no glycerol 3-phosphate was added; the medium contained 7mg of protein/ml. Measurement of specific radioactivities at the zero time gave an estimate of the dilution factor, for each radioactive tracer, by the endogenous unlabelled fatty acids. The

dilution factor was 12 for palmitate acid and 4 for linoleate. Hence the amount of fatty acid incorporated into phospholipids was much greater than could be simply derived from the quantity of radioactivity recovered in the latter substances and the amount of radioactive fatty acids added to the medium. Over 95% of the labelled linoleate taken up into phosphatidylcholine was present at the 2-position, indicating the good positional specificity of this microsomal system.

Linoleate was incorporated preferentially into phosphatidylcholine, whereas radioactive palmitate taken up into phospholipids was distributed almost equally between phosphatidylcholine and phosphatidylethanolamine. The fairly high percentage of radioactive palmitate incorporated into the fully saturated phosphatidylcholines correlated with the fact that 20% of this labelled saturated fatty acid introduced into total phosphatidylcholines was recovered at the 2-position. This might perhaps reflect the predominance of the 1-acyl isomer of lysophosphatidylcholine over the other lysophosphoglycerides if one assumes that there was no absolute specificity in the acylation, as was suggested by Van Den Bosch *et al.* (1967).

EDTA and GSH were shown to inhibit the acyl transfer. However, when the concentration of these inhibitors was not greater than 5mM, a selective impairment of the transfer of palmitate into phosphatidylethanolamine was usually observed and this was accompanied by an increased incorporation of fatty acids into triglycerides. Cholesterol esterification was strongly inhibited in all cases. Experimental evidence showed that both inhibitors may act indirectly via the stimulation of a lipase, resulting in a noticeable accumulation of various acyl acceptors, which could then compete with the lysophospholipids for the acyltransferase. The triglyceride breakdown induced by EDTA was moderate but did increase the pool of diacylglycerols; on the other hand, GSH markedly stimulated the hydrolysis of triglycerides, causing some monoacylglycerol to accumulate and leading to a significant enlargement of the free fatty acid pool. However, a direct inhibition of the acyl transfer by higher concentration of inhibitors could not be ruled out. The selective decrease of the palmitate incorporation into phosphatidylethanolamine might indicate that the concentration of the 2-monoacyl isomer of lysophosphatidylethanolamine in the microsomal membranes is much smaller than that of the other lysophospholipids.

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