EFFECT OF OCTANOATE *IN VITRO* ON RAT LIVER SUPERNATANT PHOSPHOFRUCTOKINASE ACTIVITY*

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Both phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) activity and lipid concentration were found significantly increased in the livers of rat fed thrombogenic diets (Simard-Duquesne, 1968). These results were difficult to reconcile with the findings of Weber *et al* (1966) and Lea and Weber (1968) that octanoate inhibits *in vitro* liver supernatant phosphofructokinase activity. Attempts to repeat these experiments with liver supernatants prepared in buffered sucrose media were unsuccessful. The experiments reported here demonstrate that the inhibition of phosphofructokinase activity in rat liver supernatants by octanoate is dependent on the experimental conditions used.

Male albino rats (Holtzman Co., Madison Wisconsin) were used. They were fed laboratory chow and given water *ad libitum*. All were fastened overnight before the experiment.

Rat liver homogenates were prepared in isotonic KCl (0.15M) and centrifuged 45 minutes at 96,600 x g. Aliquots of the supernatants (with or without additions) were incubated 20 minutes in a constant temperature water-bath at 37° with shaking. After the incubation, the samples were cooled at 4° and the determination of phosphofructokinase was done immediately. The rate of phosphofructokinase activity was measured at 30° and at 340 mμ in a medium containing: Tris buffer 83.3 mM pH 8.6, $MgCl_2$ 1.3 mM, ATP 2.7 mM, DPNH 0.2 mM, glucose-6-phosphate 5 mM, excess aldolase, α-glycerophosphate

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dehydrogenase, triosephosphate isomerase, phosphohexoseisomerase, and the supernatant fraction. A Unicam spectrophotometer SP. 800 with scale expander, automatic four cell changer, and program controller accessories was used. The pH changes during the incubation were followed with a Beckman Zeromatic pH meter (with temperature compensator).

Octanoate 1.2M was prepared by suspending octanoic acid (Nutritional Biochemicals Co.) in water and adding NaOH until the acid was completely dissolved, and the pH was adjusted to 7.4.

Using rat liver supernatants prepared in isotonic KCl, the activity of phosphofructokinase was found to be inhibited by octanoate, and the results of Weber *et al* (1966) were easily reproduced: after 20 minutes incubation at 37° in the presence of 6mM octanoate, almost half the activity was inhibited (Table I). These preparations were however very unstable: there was a 50% loss of activity in the control sample (without octanoate) during the incubation (as compared to the original activity). When a buffer solution at pH 7.4 was added to the incubation medium, octanoate had either no effect (phosphate buffer) or five times less effect (Tris maleate buffer) on the phosphofructokinase activity (Table I). Furthermore, the loss of activity in the control incubated sample (as compare to the original activity) was negligible in the presence of phosphate buffer and about 30% in the presence of Tris maleate buffer. The pH of the supernatant prepared in isotonic KCl was followed during the incubation and found to decrease to approximately 6.9 in the controls and to pH 6.5-6.6 in the samples with octanoate (6 mM). Samples incubated in the presence of buffer at pH 6.9 and at pH 6.5 were compared (Table I). Although the activity at pH 6.5 was significantly lower than that at pH 6.9, the difference was not as great as in unbuffered samples with and without octanoate.

These results suggest that the inhibition of liver supernatant phosphofructokinase activity by octanoate is due in part to changes in the pH of the samples during the incubation - the pH decreasing more in the samples containing octanoate than in the controls. This inhibition can also be completely

Table I. EFFECT OF pH AND OCTANOATE ON LIVER SUPERNATANT PFK ACTIVITY. THE SAMPLES WERE INCUBATED 20 MINUTES AT 37°.

Incubation medium		Number of determinations	Loss of activity*
Sample 1	Sample 2		
KCl 0.5 M	KCl 0.15 M, octanoate 6 mM	6	46±3 %**
KCl 0.14 M, phosphate buffer 0.03 M, pH 7.4	KCl 0.14 M, phosphate buffer 0.03 M, pH 7.4, octanoate 6 mM	4	2±1 %
KCl 0.14 M, Tris maleate buffer, pH 7.4	KCl 0.14 M, Tris maleate buffer 0.03 M, pH 7.4, octanoate 6 mM	4	10±2 %
KCl 0.14 M, phosphate buffer 0.03 M, pH 6.9	KCl 0.14 M, phosphate buffer 0.03 M, pH 6.5	3	18±1 %
KCl 0.14 M, Tris maleate buffer 0.03 M pH 6.9	KCl 0.14 M, Tris maleate buffer 0.03 M pH 6.5	3	12±3 %

* $\frac{\text{Sample 1 - sample 2}}{\text{sample 1}} \times 100$

** ± standard error of the mean

prevented by incubating the samples in the presence of phosphate buffer at pH 7.4. Both the effect of pH and of phosphate on phosphofructokinase had been reported in the literature (Wakid and Mansour, 1965; Paetkau and Lardy, 1967). It therefore appears improbable that the rate of glycolysis could be affected by a direct action of fatty acids on phosphofructokinase (Weber *et al*, 1966). However, it could be affected indirectly by the action of fatty acids on oxidative phosphorylation, as suggested by Newsholme and Randle (1964).

REFERENCES

Lea, M.A. and Weber, G., J. Biol. Chem., 243, 1096, (1968).

Newsholme, E.A. and Randle, P.J., Biochem, J., 93, 641, (1964).

Paetkau, V. and Lardy, H.A., J. Biol. Chem., 242, 2035, (1967).

Simard-Duquesne, N., Can. J. Physiol. Pharmacol., in press, (1968).

Wakid, N. and Mansour, T.E., Mol. Pharmacol., 1, 53, (1965).

Weber, G., Convery, H.J.H., Lea, M.A. and Stamm, N.B., Science, 154, 1357, 1966.