agent was injected alone. The block in glycogenolysis at birth may be partly due to a relative corticosteroid insufficiency.

We are indebted to the Wellcome Trust for generous financial support.

- Cake, M. H. & Oliver, I. T. (1969) *Eur. J. Biochem.* **11**, 576–581
Dawkins, M. J. R. (1963) *Quart. J. Exp. Physiol. Cog. Med. Sci.* **48**, 265–272
Schaeffer, L. D., Chenoweth, M. & Dunn, A. (1969) *Biochim. Biophys. Acta* **192**, 292–303
Vernon, R. G. & Walker, D. G. (1972) *Biochem. J.* **127**, 521–529
Wrenshall, G. A. (1955) *Can. J. Biochem. Physiol.* **33**, 909–925
Yeung, D. & Oliver, I. T. (1968) *Biochemistry* **7**, 3231–3239

Increased Phospholipid Depletion Alters the Apparent Specificity of Phospholipid-Dependence of Glucose 6-Phosphatase Activity

By B. R. CATER and T. HALLINAN (*Department of Biochemistry, Royal Free Hospital School of Medicine, Hunter Street, London WC1N 1BP, U.K.*)

A biphasic inhibition of glucose 6-phosphatase (glucose 6-phosphohydrolase) occurs when rat liver microsomal fraction is treated with *Clostridium welchii* and/or *Bacillus cereus* phospholipase C. This results in partial (50–75%) loss of activity at 5°C. Subsequent post-incubation at 20° or 37°C in the absence of substrate causes complete and irreversible loss of the remaining activity without further detectable phospholipid hydrolysis (Cater *et al.*, 1970). With *Cl. welchii* phospholipase C the glucose 6-phosphatase activity lost at 5°C is completely restored by the addition of sonicated phosphatidylcholine (lecithin) or a 1:1 mixture of phosphatidylethanolamine+phosphatidylserine. Further, if bovine serum albumin is present throughout the phospholipase treatment, extensive protection against the loss of glucose 6-phosphatase activity at 5°C is apparent. This latter observation raised doubt as to the specific and obligatory dependence of glucose 6-phosphatase activity on phospholipids.

Marked differences occur if phospholipid depletion is carried out by using both the phospholipase C preparations together. The loss of activity at 5°C is only 0–15% greater than that observed when treatment was with *Cl. welchii* phospholipase C alone, but the glucose 6-phosphatase activity is now no longer restored by the addition of sonicated phosphatidylcholine, nor is any protection conferred at 5°C by bovine serum albumin. However, 80–100% of the glucose 6-phosphatase activity is still restored by the addition of a sonicated mixture of phosphatidylethanolamine+phosphatidylserine (1:1). Therefore

it seems that the greater and qualitatively different phospholipid removal due to the wider substrate specificity of *B. cereus* phospholipase C exerts a critical effect on the apparent phospholipid specificity for glucose 6-phosphatase reactivation. Although both phospholipases hydrolyse phosphatidylcholine completely, the *B. cereus* enzyme, unlike that from *Cl. welchii*, also hydrolyses virtually all the phosphatidylethanolamine and phosphatidylserine and partially hydrolyses phosphatidylinositides, but does not attack sphingomyelin.

We interpret these results as indicating that phosphatidylcholine alone is not able to restore glucose 6-phosphatase activity, but that the acidic phospholipids remaining after the action of *Cl. welchii* phospholipase C in some way allow it to restore glucose 6-phosphatase activity. The lack of protection by bovine serum albumin in the system extensively depleted of phospholipid would also appear to indicate a specific and obligatory phospholipid requirement for glucose 6-phosphatase activity. It would thus appear that almost complete phospholipid depletion of an enzyme system may be required before unequivocal conclusions about the specificity of its phospholipid-dependence can be drawn.

We gratefully acknowledge personal support from the Royal Free Hospital School of Medicine (to B. R. C.) and research expenses from the Wellcome Trust.

Cater, B. R., Poulter, J. & Hallinan, T. (1970) *FEBS Lett.* **10**, 346

Phosphoenzyme Formation Involving Serine with Phosphoglucomutase from *Micrococcus lysodeikticus*

By M. BIRCH, H. G. BRITTON and J. B. CLARKE (*Department of Physiology, St. Mary's Hospital Medical School, Paddington, London W.2, U.K.*)

Hanabusa *et al.* (1966) isolated a phosphoglucomutase from *Micrococcus lysodeikticus* that required glucose 1,6-diphosphate as a cofactor, but that could not be obtained in a phosphorylated form, and for which the initial-velocity patterns suggested that glucose 1,6-diphosphate and the substrates had to be present simultaneously on the enzyme molecule. Thus its mechanism appeared to be different from the phosphoenzyme mechanism of the rabbit muscle phosphoglucomutase (Ray & Roscelli, 1964; Britton & Clarke, 1968), and the active form of the enzyme appeared to be an enzyme-glucose 1,6-diphosphate complex. However, despite these findings, recent induced-transport studies have shown that it has a phosphoenzyme mechanism (Britton & Clarke, 1972). Nevertheless it was still possible that the active form of the mutase could be an enzyme-glucose