α' , 130000; β , 118000; γ , 41000. The α' component, although always present, stained faintly and is tentatively assumed to be derived from the α component. Molar proportions obtained from densitometric tracings of the gels gave: ($\alpha + \alpha'$), 0.83; β , 1.00; γ , 1.06; these suggest a minimal binding weight ($\alpha\beta\gamma$) of 290000 daltons. The absorbance index, $E_{280}^{1\%}$, determined refractometrically was 12.1 ± 0.2 .

The pH 6.8/8.2 activity ratio of phosphorylase kinase rose from 0.015 to 0.38 on incubation with cyclic AMP (0.01 mM), purified cyclic-AMP-dependent protein kinase, Mg^{2+} (2 mM), [γ - ^{32}P]ATP (0.2 mM), ethanedioxybis(ethylamine)tetra-acetate (0.2 mM), EDTA (0.4 mM) and glycerophosphate (10 mM), pH 6.8. Activity and ^{32}P incorporation both reached maximum values within 20 min, but did not exactly parallel one another. It was found that 1.82 ± 0.1 mol of phosphate was incorporated/290000 daltons. Both the lack of correspondence between phosphorylation and activation and the phosphate stoichiometry disagreed with the results reported by Walsh *et al.* (1971) (0.42 mol of phosphate incorporated/100000 daltons under essentially identical experimental conditions).

Examination of the phosphorylation of each sub-unit showed that the first phosphate entered the β component, paralleling the activity increase, whereas the second phosphate entered the ($\alpha + \alpha'$) component ten times more slowly. At 1 min activation was 55%, the β component was 58% phosphorylated but the ($\alpha + \alpha'$) component was only 6% phosphorylated. After 20 min the ($\alpha + \alpha'$) and β components contained equal amounts of phosphate. No radioactivity appeared in the γ component. Phosphorylation of the ($\alpha + \alpha'$) component depended on either cyclic-AMP-dependent protein kinase or phosphorylation of the β component. Experiments are in progress to determine whether (a) cyclic-AMP-dependent protein kinase phosphorylates the α and β components at different rates or (b) phosphorylation of the β component activates phosphorylase kinase, which then autophosphorylates the α component.

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Temporal Aspects of Glucose Metabolism in the Rat at Birth

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Previous work on glucose metabolism in neonatal rats included the use of naturally delivered newborn animals, whose postnatal ages therefore varied by up to 60 min within a litter (Vernon & Walker, 1972). In the present work rats were delivered within minutes of their littermates by caesarian section in order to examine more accurately the temporal changes occurring after birth. In the first 2 h immediately after delivery there is a dramatic fall in the plasma concentrations of glucose and lactate. Specific radioactivities of plasma glucose or lactate measured at intervals after a single intraperitoneal injection of tracer amounts of [6 - ^{14}C]glucose or [U - ^{14}C]lactate were used to calculate the rates of formation and utilization of these metabolites (Wrenshall, 1955). Postnatal hypoglycaemia was due to a high rate of glucose utilization at birth that exceeded the rate of glucose formation. Hepatic glycogen concentrations, which were high at birth, remained constant for up to 2 h before any measurable decrease occurred, and during this period injection of glucagon or dibutyryl cyclic AMP (*N*-6, *O*-2'-dibutyryl adenosine 3':5'-cyclic monophosphate) were without effect, although at later ages these agents had a pronounced glycogenolytic effect. The rate of lactate utilization at birth was very high, and, in view of the delay in glycogen mobilization, it seems likely that a proportion of this lactate is being converted into glucose.

Injection of actinomycin D at birth completely blocked glucose formation, virtually abolished incorporation of [U - ^{14}C]lactate into glucose and accelerated the postnatal hypoglycaemia, but had no effect on the time-course of glycogen mobilization. The inhibitor has no effect on the postnatal development of glycogen phosphorylase (Cake & Oliver, 1969), but blocks phosphopyruvate carboxylase development (Yeung & Oliver, 1968). It thus appears that glucose formation immediately after birth is entirely dependent on gluconeogenesis.

The block in hepatic glycogen mobilization at birth may be due to a relative deficiency in the phosphorylase-activating system, as has been observed by Dawkins (1963). Schaeffer *et al.* (1969) have found that glucagon activation of phosphorylase in adult rats is dependent on the presence of glucocorticoids. We found that injection of dibutyryl cyclic AMP and cortisol together at birth had no effect on glycogen concentrations measured 1 h later; if they were injected together 1 h after birth glycogen concentrations fell markedly within 1 h, but did not if either

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

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

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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.

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Phosphoenzyme Formation Involving Serine with Phosphoglucomutase from *Micrococcus lysodeikticus*

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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