synthesis. In contrast, the primary function in muscle and brain is the production of ATP.

The phosphofructokinase (PFK) reaction has been shown to be the major site of glycolytic regulation in muscle and brain. PFK from these tissues exhibits certain kinetic properties that have been correlated with this regulatory role. These include inhibition by ATP and citrate and activation by ADP, AMP, 3',5'-(cyclic)-AMP and phosphate. In these properties, PFK from rat epididymal fat pad has been shown to be qualitatively similar to both muscle and brain PFK. Quantitatively, adipose tissue PFK is more sensitive to activation by 3',5'-(cyclic)-AMP (Denton & Randle, 1966).

Attempts have been made to define the role of PFK in the control of glycolysis in fat pads incubated *in vitro*. Glycolytic rates have been altered by anoxia or by additions of insulin or adrenaline or both to the incubation medium and the changes in rate correlated with changes in the steady-state whole-tissue concentrations of hexose phosphates, adenine nucleotides and citrate (M. L. Halperin & R. M. Denton, unpublished work). The observed mass-action ratio of PFK (calculated from the whole-tissue concentrations of products and substrates) varied between 10^{-3} and 10^{-4} of the value of the ratio at equilibrium, assuming intracellular pH to be 7.4. This suggests that PFK plays a primary role in the control of the glycolytic breakdown of hexose monophosphate.

However, a clear correlation between changes in PFK activity (as indicated by changes in mass-action ratio) and changes in glycolytic flux was seen only with adrenaline. Both activity and flux were increased by adrenaline in the presence and absence of insulin. Activation of PFK under these conditions may be the result of the changes in adenine nucleotide concentrations and the increase in 3',5'-(cyclic)-AMP concentration (Butcher, Ho, Meng & Sutherland, 1965) despite a rise in the concentration of the inhibitor, citrate. Insulin increased glycolytic flux without significantly altering the PFK mass-action ratio. The increased flow through PFK may be explained by the observed increase in the concentration of the substrate, fructose 6-phosphate. Under anaerobic conditions, glycolytic flux is decreased but this does not appear to be the result of inhibition of PFK, since the PFK mass-action ratio is greatly increased. This apparent activation of PFK may be attributed at least in part to the changes in adenine nucleotide concentrations; the decreased glycolytic flux may be secondary to the diminution in glucose uptake.

When glucose availability is limited, adipose tissue is capable of synthesizing L-glycerol 3-phosphate from pyruvate through a pathway involv-

ing phosphoenolpyruvate carboxykinase (Ballard, Hanson & Leveille, 1967). Under these conditions any pyruvate kinase (PyK) activity would serve merely to recycle pyruvate at the expense of ATP. Investigations with isolated fat-pad PyK show that the enzyme exists in two interconvertible forms, PyK-A and PyK-B. PyK-A has a high K_m for phosphoenolpyruvate and exhibits co-operativity with phosphoenolpyruvate, K^+ , NH_4^+ , Mg^{2+} and ATP (as inhibitor); fructose 1,6-diphosphate produces a marked activation. PyK-B, resembling muscle PyK, has a low K_m for phosphoenolpyruvate and exhibits co-operativity only with K^+ and NH_4^+ . PyK-B is converted *in vitro* into PyK-A on incubation with EDTA, ATP or citrate; this process is reversed by fructose 1,6-diphosphate. These properties are consistent with the hypothesis that PyK-B predominates under conditions of normal glucose availability; where the glucose concentration is insufficient to maintain the supply of L-glycerol 3-phosphate, this would be reflected in a lowered fructose 1,6-diphosphate concentration favouring the conversion into the high- K_m (relatively inactive) PyK-A (Pogson, 1968a,b).

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The Control of Carbohydrate Metabolism in Mammalian Development

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The mammalian foetus is dependent on the facilitated transfer of glucose across the placenta from the mother for its supply of energy. There is no good evidence that the placental store of glycogen that occurs in some species is concerned in the regulation of the foetal-blood glucose concentration (Walker, Lea, Addison & Rossiter, 1967; Dawes & Shelley, 1968). The maternal hormonal and hepatic homeostatic mechanisms must therefore be major controlling systems, but foetal hormones may also be involved. The transported glucose is used by the foetus to build up stores of glycogen in the liver, muscle (including the heart) and other tissues (Dawes & Shelley, 1968) and to make lipid (Ballard & Hanson, 1967). After birth the independent new-born animal utilizes its carbohydrate reserves rapidly and milk, a high-fat and low-carbohydrate food, becomes the nutritive supply.

The biochemical morphology of rat liver at various stages of development has been studied in several Laboratories recently. The rat is a particularly convenient species for such studies because birth and the commencement of weaning occur 15 days apart, thus permitting the influence of these events to be assessed independently. The marked glycolysis and lipogenesis in foetal liver is reflected by appreciable activities of enzymes (Burch *et al.* 1963) such as hexokinase, pyruvate kinase and ATP citrate lyase; the latter two activities decrease rapidly after birth and increase again with weaning (Ballard & Hanson, 1967; Taylor, Bailey & Bartley, 1967; Vernon & Walker, 1968a). Glucokinase (Walker, 1963a, 1968; Walker & Eaton, 1967) and NADP-specific malate dehydrogenase (Ballard & Hanson, 1967; Taylor *et al.* 1967; Vernon & Walker, 1968a) appear with significant activities only at weaning. Many enzymes show increases in activities a few days before birth; these include glucose 6-phosphatase, fructose 1,6-diphosphatase and pyruvate carboxylase, three enzymes concerned in the marked capacity for gluconeogenesis in the neonatal period (Dawkins, 1966; Walker, 1968). The postnatal development of gluconeogenesis is initiated by the rapid (Yeung, Stanley & Oliver, 1967; Vernon & Walker, 1968a,b) and parallel (Yeung & Oliver, 1967) appearance of phosphopyruvate carboxylase activity.

How far does this and related information help us to understand the mechanisms controlling carbohydrate metabolism during development? What are the relevant molecular processes that occur at each stage of differentiation and hepatic maturation? The results represent activities measured *in vitro* under optimum conditions. They may be partly explained by changes in cell type but, apart from the decreasing proportion of haemopoietic cells, whose contribution is unlikely in any case to be great (Oliver, Blumer & Witham, 1963), the changes in activity with age represent adaptations to environmental factors or maturation phenomena of uncertain origin. A complication with all such measurements is the possibility that the intracellular distribution of the enzyme or its binding to intracellular organelles may change during development. Whenever it has been tested in liver, synthesis of enzyme rather than activation of protein precursor appears to be involved.

It is important to enquire whether the same enzyme is being assayed at all stages of development. The existence of the high- K_m glucokinase was revealed during a comparative examination of the effects of glucose concentration on glucose-phosphorylating activity in foetal and adult liver (Walker, 1963a). The realization that many enzymes exhibit microheterogeneity, e.g. the familiar lactate dehydrogenase isoenzymes phenomenon,

should warn that caution is necessary in generalizing about the detailed nature of enzymes in a tissue under varying physiological conditions. There are indications, for example, that chick-embryo heart hexokinase is inhibited by glucose 6-phosphate (Gaja, Loreti, Ragnotti, Guidotti & Foa, 1967) as are mammalian hexokinases, and that rat foetal liver fructose 1,6-diphosphatase, like the adult liver enzyme, is inhibited by AMP and higher concentrations of its substrate (Wallace & Newsholme, 1967; Yeung *et al.* 1967). These observations, however, must be assessed in the knowledge of the existence of isoenzyme forms of hexokinase and much detailed work on the subunit structure of fructose 1,6-diphosphatase. In general it would seem that mammalian enzyme adaptations represent changes in the rate of synthesis or degradation, or both, of the same molecular form, but this may not be true for developmental changes; this requires checking in detail on many enzymes. The proportions of the isoenzyme forms of hexokinase (Katzen & Schimke, 1965; Schröter & Tillmann, 1968) and of lactate dehydrogenase (e.g. Vesell & Philip, 1963; Blatt, Blatteis & Mager, 1966) change with age. The detailed nature of hepatic pyruvate kinase (M. C. Middleton & D. G. Walker, unpublished work) and the type of hepatic aldolase change dramatically during late gestation (Rutter & Weber, 1966; Rensing, Schmid & Leuthardt, 1967).

Environmental factors such as temperature and the diet are known to influence postnatal development (e.g. Hahn & Koldovský, 1966). Hormonal changes must also be important but little detail is known. Evidence based on the study of enzymes involved in glucose utilization (glucokinase, pyruvate kinase, ATP citrate lyase, NADP-specific malate dehydrogenase) and glucose formation (glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphopyruvate carboxylase) indicates that many of the enzymic adaptations occurring in the neonatal and weanling rat can be interpreted in terms of the known dietary adaptations in adult animals (Walker & Eaton, 1967; Vernon & Walker, 1968b). On the other hand the developmental changes in activity of hepatic ketohexokinase (fructokinase; Walker, 1963b) and galactokinase (Walker & Khan, 1968) are not controlled significantly by their substrates.

Birth appears to influence the appearance of some hepatic enzymes such as rat phosphopyruvate carboxylase (Yeung & Oliver, 1967) and rabbit glucose 6-phosphatase (Dawkins, 1961). Such effects may be interpreted in terms of a de-repression of enzyme synthesis, due possibly to the loss of a maternal or placental factor or to a hormonal change acting in some way. The bacterial model for regulation of enzyme synthesis, upon which such

interpretations are based, may not, however, be wholly correct for mammalian systems. Glucose infusion prevents the postnatal increase in hepatic glucose 6-phosphatase (Dawkins, 1966) and phosphopyruvate carboxylase (Yeung & Oliver, 1968) activities, but there is no apparent normal change in glucose concentration that could account for the development of the enzymes. Hormonal effects appear to be permissive rather than direct.

A number of the characteristic enzymic control sites of the mammalian liver develop therefore at around birth or during weaning. The factors controlling these steps in maturation probably involve processes in addition to genetic control mechanisms (Reiner, 1968). Further analysis of events at the enzyme level are likely to be rewarding and to lead us closer to the nature of the triggering mechanisms.

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COMMUNICATIONS

Properties of Particles derived from Detergent - Disaggregated Erythrocyte Membranes

By R. W. BONSALE and S. HUNT. (*Department of Biological Chemistry, University of Manchester*)

Non-ionic detergents in combination with urea and mercaptoethanol have been used to extract proteins from biological membranes (Schneiderman, 1965). Certain other detergents also cause disaggregation of the membrane, and results obtained suggest that the process may be partially reversible (Green *et al.* 1967). The use of non-ionic detergents has advantages in that the solutions obtained are relatively stable and that they retain the charge characteristics of the membrane components.

Human erythrocyte membranes (prepared by a

method adapted from that of Dodge, Mitchell & Hanahan, 1963) in the presence of excess of Triton X-100 (an alkylphenylpolyoxyethylene condensate) break down into particles that give a single partially included peak on 4% agarose-gel exclusion chromatography. The membrane acetylcholinesterase activity, which has been shown to be closely associated with the structure (Mitchell, Mitchell & Hanahan, 1965), coincides with this peak. Separation of the enzyme from a single major fraction can, however, be achieved by DEAE-cellulose chromatography in detergent solution with salt-gradient elution.

When exhaustively dialysed against water, whole membranes aggregate into a heavy precipitate caused by decrease in ionic strength of the suspending medium. The phenomenon appears to