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DIGESTION AND METABOLISM OF CARBOHYDRATES

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Problems in carbohydrate absorption and metabolism

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In recent years emphasis in carbohydrate research has shifted away from the 'test-tube' approach of 20 years ago in favour of the integrated view obtained using the whole organ or organism. For example, no longer are digestion and absorption of carbohydrates considered to be individual and separable sequential processes. Modern observations suggest a molecular integration of these processes within the brush-border membrane of the epithelial cells of the small intestine. Similarly, the major pathways of carbohydrate metabolism are no longer studied merely as a sequence of chemical reactions leading to a finite end product such as lactate or pyruvate. It is now realized that the control of the sequence is the key to the understanding of carbohydrate metabolism. The investigation of the control mechanisms has also to take into account the way in which the products of glycolysis, for example, have important implications on fat and protein metabolism in the major organs and the derangements which occur in metabolic disease.

Carbohydrate digestion

Carbohydrate digestive enzymes originate in the salivary glands, the pancreas and the mucosal lining of the small intestine. The amylolytic properties of the salivary and pancreatic secretions are attributable to the presence of α-amylases (*EC* 3.2.1.1) which have been isolated, crystallized and characterized (Fischer & Stein, 1960). The hydrolytic properties of the intestinal mucosa, on the other hand, are contributed by a multiplicity of enzymes which have only lately been subjected to intensive experimentation. They are not well characterized; none has been isolated in pure form. The efficiency of hydrolysis of dietary carbohydrate has been ascribed to the adsorption of the enzymes on to the membrane on the surface of the microvilli where the reaction occurs, a phenomenon which has been called membrane or contact digestion (Ugolev, 1965).

There has been an upsurge in research concerning the hydrolysis of disaccharides. These can either be formed from polysaccharides (maltose) or ingested as sucrose and lactose, which enter the small intestine largely unchanged. The disaccharidase activity of the intestinal juice (succus entericus) is very small (Dahlqvist & Borgström, 1961) and the evidence is now conclusive that the specific disaccharidases are located in, or closely associated with, the membranous portion of the microvilli of intestinal mucosal cells (Eichholz & Crane, 1965, 1966; Eichholz, 1967). Characterization of the specific disaccharide-splitting enzymes in man (four maltases, one or two sucrases, one trehalase and two lactases) is now well documented (Semenza, Auricchio & Rubino, 1965; Dahlqvist, Lindquist & Meeuwisse, 1968). With this important advance has come the recognition of a group of syndromes based on inadequate levels of intestinal disaccharidase activity (see reviews by Mansford, 1967; Dahlqvist *et al.* 1968).

Carbohydrate absorption

The final stage in carbohydrate absorption is that of transport of the monosaccharide across the mucosal cell. The experimental evidence indicates that this transport is brought about by mobile carrier systems situated in the plasma membrane (Crane, 1968*a*). Hydrolysis precedes absorption and is closely integrated with it (Crane, 1968*b*). Glucose and galactose have the same specific transport system, their absorption being 'active', i.e. against a concentration gradient, and dependent on the presence of Na^+ (Crane, 1968*a*). Fructose, however, is absorbed in direct proportion to its concentration (Holdsworth & Dawson, 1964), though the rapidity suggests that its diffusion is also facilitated by a carrier (Crane, 1968*b*). Although the glucose carrier has not yet been identified, the kinetics of the absorption process resembles those of an enzyme–substrate reaction. The unique position of glucose in satisfying the specificities of both the carrier and the hexokinase (*EC* 2.7.1.1) step in the metabolic pathway has been discussed by Smyth (1971).

Csáky & Zollicoffer (1960) showed the requirement for Na^+ of glucose absorption by the rat. On the basis of evidence obtained from hamster epithelial cells incubated anaerobically in Na^+ or K^+ medium, Crane, Miller & Bihler (1961) postulated the primary interaction of Na^+ with the sugar transport system to be at the brush-border membrane with two binding sites on the same mobile carrier, one of them specific for Na^+. When present, Na^+ interacts with the carrier and activates the movement of a restricted group of sugars across the mucosal membrane. In the absence of Na^+, physiologically speaking an unlikely event, the carrier is less efficient in transferring substrate (Crane, 1964). An opposing view that Na^+ influences sugar transport only by virtue of an energy-dependent translocation from the basal pole of the cell into the lamina propria without interaction with the sugar carrier (Csáky, 1963; Capraro, Bianchi & Lippe, 1963; Curran, 1965) has been disputed by Crane, Forstner & Eichholz (1965), who have shown that the higher the concentration of Na^+ the higher the affinity between sugar and carrier and vice versa.

It is important to realize that the rate of sugar transport is appreciably influenced by Na^+ only when the sugar concentration is low. At saturating sugar concentrations,

the same maximal rate of transport is attained irrespective of the Na^+ concentration.

It has been shown that monosaccharide malabsorption exists as a disease entity (Lindquist, Meeuwisse & Melin, 1962). The principal finding is that children suffering from this disease absorb neither glucose nor galactose and the presence of these sugars in the diet precipitates diarrhoea. The same children, however, thrive when given fructose. D-Xylose is less readily absorbed, its transport possibly involving, therefore, the same carrier as glucose and galactose (Alvarado, 1966).

Carbohydrate metabolism

Current interest in mammalian carbohydrate metabolism is largely centred on the operation of metabolic pathways in tissues and whole animals. Recent studies have indicated that the various pathways may be controlled by one or two key enzymes which function as pace-makers. In addition, transport systems in the plasma membranes and also in intracellular membranes, such as the mitochondrial membrane, are of increasing importance in control and hence in the operation of metabolic pathways.

A regulatory enzyme can be defined as one, the activity of which is controlled by factors other than substrate availability, and the activity of which controls the rate of flux or the concentrations of intermediates of a metabolic pathway. Thus recent investigations have shown that glycolysis may possess several regulatory enzymes, the function of which is to control the total rate of glucose degradation and to keep constant the concentration of the intermediates of the pathway.

The allosteric theory (as proposed by Monod, Changeux & Jacob, 1963) states that the substrate-binding site of an allosteric enzyme is spatially distinct from the allosteric or regulatory site, so that compounds that bind at the regulatory site and modify the catalytic activity require no structural relationship to the substrate or product of the enzyme reaction. This theory has emphasized that control of a metabolic pathway can be provided by intermediates of metabolism which may have no obvious relationship to that pathway, but which regulate the pathway in accord with the rate of another metabolic process. One of the best documented examples of this is the action of citrate as an allosteric effector of phosphofructokinase (*EC* 2.7.1.11) in the control of glycolysis (Newsholme & Randle, 1964).

Regulation of glucose uptake and glycolysis in isolated heart tissue. The perfused heart is a convenient system to illustrate the investigation of the control of glucose uptake and glycolysis.

In the study of glucose uptake by the isolated perfused rat heart, it is convenient to regard the process as consisting of three sequential steps (Mansford, 1968): (1) diffusion of glucose from the capillary to the muscle cell surface, (2) transport of glucose through the cell membrane and (3) intracellular metabolism of the glucose.

In the isolated perfused rat heart, the rate of glucose uptake from the perfusate into the heart cells plays an important role in the regulation of subsequent intracellular glucose utilization. Glucose uptake is limited by the rate of transport of glucose across the cell membrane (Morgan, Randle & Regen, 1959), a process thought to involve again a stereospecific glucose carrier (Fisher & Zachariah, 1961). The

increased glucose uptake by the perfused heart, when insulin is added to the medium, is explained by acceleration of membrane transport; and in these circumstances, the rate of membrane transport exceeds the rate of phosphorylation of intracellular glucose, and phosphorylation becomes limiting for glucose uptake (Morgan, Henderson, Regen & Park, 1961).

Because of the absence of glucose-6-phosphatase (G-6-P) (*EC* 3.1.3.9) in the heart, G-6-P must be utilized by entering pathways of either glycolysis or glycogen synthesis. When glycolysis in the perfused rat heart is increased by anoxia, the concentrations of G-6-P and fructose-6-phosphate decrease, whereas the concentration of fructose-1,6-diphosphate increases (Newsholme & Randle, 1961). This is evidence for control by phosphofructokinase because there is no detectable fructosediphosphatase (*EC* 3.1.3.11) activity in the heart muscle (Newsholme & Randle, 1962). The properties of highly purified phosphofructokinase from the guinea-pig heart have shown that, as in many other tissues and species, the enzyme is strongly inhibited by one of its substrates, ATP; the inhibition is relieved by cyclic AMP, AMP and inorganic phosphate (Mansour, 1963). Another substrate, fructose-6-phosphate, and both products of the reaction (ADP and fructose-1,6-diphosphate) also relieve ATP inhibition (Passonneau & Lowry, 1963). In isolated heart tissue deprived of oxygen, a threefold increase of AMP and inorganic phosphate concentrations can account for the increased phosphofructokinase activity. However, changes other than those in adenine nucleotides must be invoked to account for changes in the glycolytic flux in states of enhanced fatty acid oxidation (Newsholme & Randle, 1964).

During perfusion of the isolated heart with fatty acids or ketone bodies, or in the hearts of alloxan-diabetic or starved rats, the citrate concentration increases several times. Citrate in physiological concentrations inhibits phosphofructokinase in vitro and, it is thought, in the perfused rat heart. Thus, phosphofructokinase is highly sensitive to inhibition by two end-products of aerobic glycolysis, ATP and citrate.

Metabolism of fructose. Our consumption of fructose via sucrose ingestion has risen enormously in the last 20 years (Bett, Morland & Yudkin, 1967), so that it is of interest to compare the metabolism of fructose with glucose. Three organs share the 'specific' route of fructose metabolism by which more than 70% is utilized: the liver, the kidney and the intestinal mucosa. This pathway involves phosphorylation by fructokinase (*EC* 2.7.1.4) to fructose-1-phosphate. This in turn is split to give dihydroxyacetone phosphate (an intermediate of glycolysis) and glyceraldehyde. This specific fructose metabolite may be metabolized by three pathways in liver tissue of which the most important is direct phosphorylation by triokinase (*EC* 2.7.1.28) to glyceraldehydephosphate (Hers & Kusaka, 1953; Heinz & Lamprecht, 1961).

It is now possible to indicate various ways in which the controls of glucose metabolism can be evaded by fructose: (a) in yielding glyceraldehyde phosphate direct, from fructose-1-phosphate, fructose bypasses one of the major bottlenecks of glycolysis, the phosphofructokinase reaction; (b) the complete utilization of ingested fructose can proceed via the insulin-independent pathway outlined above

in the intestinal mucosa and the liver; (c) transport into adipose tissue is independent of insulin and phosphorylation by hexokinase is not inhibited in this tissue by free glucose (Froesch, 1965).

Problems concerning fructose metabolism still remain, such as the presence of fructose in the blood and foetal fluids of certain species. The list of 'fructogenic' species now includes goat, horse, pig, ox, deer and sheep, but the possible biochemical mechanisms whereby fructose can be formed in the foetus from maternal glucose (Hers, 1960*a*, *b*) have been criticized by Walker (1968). Although the mechanism by which sucrose ingestion gives rise to dental caries is not completely clarified, the fact that children and adults with hereditary fructose (and hence sucrose) intolerance show a complete absence of caries (Froesch, 1965) points to direct involvement of these sugars in the aetiology.

Carbohydrates and man

The problems of over-consumption of carbohydrate by man currently receive considerable attention because of the implications in diabetes, obesity and, albeit controversially, in ischaemic heart disease (Yudkin, 1968). It is, however, a miracle of homoeostasis that body composition is so little affected by the extreme challenges given, for example, by miners in South America on the one hand, who may consume 5000 kcal/d (21 MJ/d) mostly as carbohydrate, whereas the Eskimo may derive 80–90% of a similarly large amount of energy from lipid (Cahill & Owen, 1968). Although man as a whole shows this amazing versatility, his individual tissues and organs may have much more stringent requirements. For example, of the 16 mol oxygen consumed each day by sedentary man, about 4 mol are utilized by nervous tissue which requires, therefore, some 120 g of glucose daily. There is normally a specific dependence of the brain for glucose, although foetal and neonate brain is somewhat resistant to hypoglycaemic damage (Dawes & Shelley, 1968). Also, under conditions of prolonged starvation, some adaptation to utilization of ketone bodies is now thought to occur (Owen, Sullivan & Cahill, 1966). Total carbohydrate stores of a 70 kg man, however, represent only 600 kcal (2·5 MJ), i.e. barely 1-d supply for cerebral function. Thereafter gluconeogenesis must provide this amount but during prolonged starvation it appears to fall far short of the target. Within a few days, nitrogen excretion, resulting from the metabolism of glucogenic amino acids to provide glucose, falls to 10 g/d and if the fast is more prolonged to 3 g/d (Cahill & Owen, 1968). A further problem is the control of the release of the necessary precursors from peripheral depots. What biochemical signal informs the adipose tissue and mobilizes the quantity of free fatty acid required by the liver? What directs the mobilization of muscle protein as amino acid into the blood during starvation to sustain gluconeogenesis in liver and kidney? Because of the similarities between the metabolic pictures seen in fasting and mild diabetes, Cahill & Owen (1968) have directed attention to insulin as the most important regulator of peripheral fuel increase. Alternatively, Goodner & Tustison (1964) postulate an exquisitely sensitive centre in brain which responds to lowering of the glucose concentration and which stimulates free fatty acid release via the sympathetic nervous system.

The possibility of a hormone cascade involving both neurogenic and insulin-mediated mechanisms has been reviewed by Hales (1967).

Proponents of both hypotheses have drawn attention to the genetically obese hyperglycaemic hyperinsulinaemic mouse as a possible model of human obesity associated with mature onset non-ketotic diabetes. These mice are hyperphagic, markedly hypoactive and become significantly overweight from approximately 6 weeks of age. Christophe (1965) suggested that obesity is the prime event, carbohydrate intolerance arising as a secondary consequence. Hellman (1967), however, found that a simple intraperitoneal glucose tolerance test reveals carbohydrate intolerance as early as 23 d after birth, at a time when increased carcass lipid was not yet apparent and no excess weight could be demonstrated.

Reviewing the metabolic features of the obese-hyperglycaemic and other syndromes in rodents, Renold (1968) concluded that the primary event is probably decreased responsiveness to insulin. Studies in human obesity, however, have tended to suggest that obesity as such may be the primary event resulting, through an as yet hypothetical mechanism which could include distension of the adipose cells, in modulation of the insulin responsiveness and of the insulin-producing β-cells (Karam, Grodsky & Forsham, 1963; Bagdade, Bierman & Porte, 1967; Salans, Knittle & Hirsch, 1967). Recent results, using obese mice given a diet restricted to that of the lean controls, indicated that a decreased sensitivity to insulin at the cellular level is not the primary defect in the obese-hyperglycaemic syndrome (R. Abraham, personal communication). Similarly, using food deprivation to reduce the weight of obese mice, Chlouverakis & White (1969) showed that the insensitivity of the obese mice to insulin disappeared. In view of the fact that Rabinowitz & Zierler (1962), using the human forearm preparation, concluded that the sensitivity of the muscle tissue of obese subjects to insulin could be restored following reduction of the body-weight, the genetically obese mouse still appears to be a valid and useful model of the human condition.

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Digestion and metabolism of carbohydrates in the foetal and neonatal ruminant

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Carbohydrate metabolism during the development of the ruminant can conveniently be divided into four stages. (1) The intra-uterine stage when the foetus is supplied from the maternal circulation via the placenta with the glucose required