Differences in the Permeability of Bovine Erythrocytes to Fructose

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Laris (1967) showed that the rate of fructose accumulation by the erythrocytes of some cattle is low and inhibited by glucose, while with other cattle the rate is rapid and unaffected by glucose. He suggested that the cells that accumulate fructose slowly have a transfer mechanism common to both glucose and fructose, whereas the cells rapidly accumulating fructose have an additional mechanism specific for fructose. But, since he obtained blood from an abattoir, he could not establish how an animal's history influenced his results. Therefore we have studied a herd of 24 Jersey cows that is kept for research.

Erythrocytes from heparinized venous blood were washed three times with phosphate-buffered saline, pH 7.4. The rate at which they took up fructose was calculated from the fall in its extracellular concentration after incubation for 1 h with 4.2 mM-fructose at 38°C and at a haematocrit of 45%. The extracellular fructose concentration was determined by centrifuging at 38000g at 2°C for 20 min and measuring fructose in the supernatant by the automated fructosan method of Fisher & Gilbert (1970). The rate of uptake was expressed as the fraction f (mmol of fructose taken up/h per l of cells, divided by the mean concentration of extracellular fructose in mM).

The values of f fell into two distinct classes: 0.026–0.071 h⁻¹ (17 animals) and 0.113–0.162 h⁻¹ (seven animals). This classification was repeatable. We have not yet correlated an animal's class with its age, proximity to calving, diet or haemoglobin type. The effect of extracellular glucose (16.8 mM) on the uptake of fructose by the cells of four animals from each class was to decrease the value of f for the low- f class by 42.6 ± 20.7% (S.E.M.) and to increase that of the high- f class by 6.6 ± 4.3% (S.E.M.).

Our data are consistent with Laris's (1967) suggestion. We are now testing the notion that different transfer mechanisms are associated with different antigens on the cell surface.

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The Inhibition of Urea Synthesis by Leucine in the Perfused Rat Liver

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The study of the metabolic effects of dietary excesses of amino acids is often complicated by such factors as alterations in food intake. In an attempt to overcome such problems we have been studying the effects of relatively high concentrations of amino acids, especially leucine, on the metabolism of the isolated perfused liver.

The livers of starved (18 h) male Sprague-Dawley rats (body wt. 150–200 g) were perfused by using the method of Hems, Ross, Berry & Krebs (1966). In all studies, except those where glucose production was measured, 80 mg of D-glucose was added/100 ml of perfusion medium. Urea was determined by the method of Varley (1962) and glucose by a glucose oxidase method [Boehringer (London) Ltd.]. Recoveries of added urea and glucose from the perfusion medium were satisfactory.

The mean (±S.E.M.) urea production from endogenous substrates of three livers was 14.3 ± 1.5 μmol/h per g of liver; when 1.5 mmol of L-leucine was included in the perfusion medium mean urea production was 13.5 ± 1.6 μmol/h per g of liver (three livers). This result was unexpected, since Miller (1962) has shown that the perfused rat liver oxidizes leucine, albeit slowly. Further investigation of urea production was therefore undertaken.

Urea production from 2 mmol of ammonium chloride was linear for at least 2 h, but was decreased from 50.7 ± 5.3 (three livers) to 31.9 ± 2.0 (four livers) μmol/h per g of liver when 1.5 mmol of leucine was included in the perfusion medium. A decrease in activity of similar magnitude could also be demonstrated by adding leucine to a medium containing ammonium chloride after 60 min of perfusion. By using the latter method it was found that the addition of 1.5 mmol of either L-lysine or L-phenylalanine to the medium did not affect urea production; the addition of 0.53 mmol of L-ornithine, however, stimulated urea synthesis markedly in the presence or the absence of leucine in the medium. The presence of leucine in the medium did not alter the rate of glucose production from 2.0 mmol of alanine.

These results appear to conflict with the statement of Katunuma, Okada & Nishi (1964) that branched-chain amino acids stimulate urea synthesis.

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Enzymic and Electron-Microscopic Analysis of Extracellular Matrix Vesicles Associated with Calcification in Cartilage

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The biochemical sequence of events that initiates calcification in epiphyseal cartilage, and subsequently transforms it into cancellous bone, has not yet been fully elucidated. Electron microscopy has shown that the longitudinal septa of epiphyseal cartilage contain extracellular matrix vesicles that are membrane-bounded and are about $0.1\mu\text{m}$ in diameter (Anderson, 1967; Bonucci, 1967). The very first crystals of apatite that are seen in epiphyseal cartilage are found in these vesicles, which thus play an important role in calcification (Anderson, 1969; Bonucci, 1970; Bernard & Pease, 1969). Ultrastructural histochemistry has shown that alkaline phosphatase and adenosine triphosphatase are present in matrix vesicles (Matsuzawa & Anderson, 1970).

We have attempted the isolation and characterization of matrix vesicles by using a collagenase digestion method that facilitates the isolation of intact chondrocytes and lysosomes from cartilage (Ali, 1970). Bovine foetal or rabbit epiphyseal cartilage was disintegrated by incubation with collagenase for 18h at 37°C in 0.25M -sucrose, buffered at pH 7.4. After a cell count, the cartilage digest was partitioned into seven fractions by differential centrifugation (up to $300\,000g$) to obtain the extracellular particles.

Electron microscopy and DNA analysis revealed that over 80% of the chondrocytes remained intact and sedimented in fractions I and II. Matrix vesicles, resembling those found in intact tissue and often containing apatite crystals, sedimented in fraction V. Alkaline phosphatase, adenosine triphosphatase, pyrophosphatase and adenosine monophosphatase activities were mostly localized in extracellular fractions and the highest specific activity was also present in fraction V. These phosphatases therefore appear to be associated with the matrix vesicles (Ali, Sajdera & Anderson, 1970).

In contrast, acid phosphatase was mostly found in the cellular fractions, indicating that the matrix vesicles are distinct from lysosomes. β -Glucuronidase activity was too low for a satisfactory localization; glucose 6-phosphatase and an esterase were present in both the cellular and the matrix-vesicle fractions. This appears to be the first instance of isolation of extracellular membrane-bounded particles from any tissue.

The extracellular matrix vesicles could induce calcification (a) by increasing the local concentration of P_i , (b) by the hydrolysis of ATP for active transport of calcium or phosphate into the lumen of the vesicles, and (c) by the hydrolysis of PP_i , which is an inhibitor of the mineralization process.

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The Subcellular Distribution of Colchicine-Binding Protein(s) ('Microtubule Protein') in Rat Brain

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The ability of colchicine and other antimitotic alkaloids (e.g. vinblastine) to disrupt cytoplasmic microtubules in brain and other tissues appears to parallel the abundance, in these tissues, of a soluble protein that binds colchicine (Adelman, Borisy, Shelanski, Weisenberg & Taylor, 1968; Wilson & Friedkin, 1968). Much indirect evidence (for review see Schmitt & Samson, 1968) suggests that the colchicine-binding protein present in soluble extracts of brain and other tissues represents the main subunit of microtubules and that the interruption of several physiological processes in neural tissue by antimitotic alkaloids such as colchicine may be due to their interaction with microtubule protein.

More recently it was observed that at least 50% of the colchicine-binding activity in brain homogenates is associated with the crude particulate fraction obtained after high-speed centrifugation (see, e.g., Dahl, Redburn & Samson, 1970), although no intact microtubular structures could be detected in