PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 509th Meeting of the Society was held at the Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN, on Friday, 16 October 1970, when the following papers were presented:

COMMUNICATIONS

The Binding of Calcium Ions by Human Erythrocytes

By C. LONG and BARBARA MOUAT. (*Department of Biochemistry, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN, U.K.*)

Harrison & Long (1968) reported that washed human erythrocytes had a mean calcium content of only $16\text{ }\mu\text{mol/l}$ of packed cells. However, earlier work (Kahn, 1958; Gent, Trounce & Walser, 1964) had indicated that both intact erythrocytes and 'ghost' cells could bind considerably greater amounts of radioactive Ca^{2+} . In the present work, the binding of Ca^{2+} by intact washed erythrocytes in various suspending media has been studied by using atomic absorption spectrophotometry for calcium determination.

Maximum calcium binding by human erythrocytes occurred in a medium of low ionic concentration, such as iso-osmotic sucrose. Calcium uptake varied with Ca^{2+} concentration in the medium and graphical extrapolation indicated maximum binding of about $450\text{ }\mu\text{mol/l}$ of packed cells. With 2.5 mM-CaCl_2 , which was used in most experiments, the binding sites on the erythrocyte membrane were 90–95% saturated with calcium. When a slight excess of EDTA was present, calcium uptake by erythrocytes was prevented.

Partial replacement of sucrose by iso-osmotic KCl markedly lowered calcium uptake. The presence of 50 mM-KCl lowered the calcium uptake by 50%. Other univalent chlorides acted similarly, Rb^+ and Cs^+ being most effective. Bivalent chlorides, when present at the same concentration as calcium (2.5 mM), also lowered calcium uptake, Cd^{2+} being particularly effective. These observations suggest that all cations in suspending media compete for negatively charged groups on the outside surface of the erythrocyte.

Since the zeta potential of an erythrocyte suspension is due to the ionized carboxyl groups of sialic acid residues (Eylar, Madoff, Brody & Oncley, 1962) and since also the NANA* content of human erythrocytes is about $800\text{--}900\text{ }\mu\text{mol/l}$, these residues seemed possible binding sites. This idea received support from the approximately linear

relationship between calcium uptake and NANA content of erythrocyte samples of human and other mammalian species. Removal of NANA from human erythrocytes by neuraminidase led to a lowered calcium uptake. The plot of calcium uptake against NANA content had a slope of 0.33, suggesting that binding sites contain three NANA residues. However, at zero NANA content, the cells still bound calcium to the extent of about $136\text{ }\mu\text{mol/l}$, indicating that sites other than NANA residues must also be involved in calcium binding.

Eylar, E. H., Madoff, M. A., Brody, O. V. & Oncley, J. L. (1962). *J. biol. Chem.* **237**, 1992.

Gent, W. L. G., Trounce, J. R. & Walser, M. (1964). *Archs Biochem. Biophys.* **105**, 582.

Harrison, D. G. & Long, C. (1968). *J. Physiol., Lond.*, **199**, 367.

Kahn, J. B., jun. (1958). *J. Pharmac. exp. Ther.* **123**, 263.

Acetoacetate as a Fuel for Perfused Rat Skeletal Muscle*

By C. R. S. HOUGHTON and N. B. RUDEMAN. (*Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.*)

Utilization of acetoacetate by various animal tissues is well established (see Williamson & Hems, 1970, for review) but information on the quantitative aspects of the contribution of acetoacetate as a fuel of respiration is limited. The development of an isolated perfused hind-quarter preparation of rat muscle has made possible the accurate measurement of the removal of acetoacetate by skeletal muscle at rest and during exercise.

The net removal of acetoacetate (2 mM) was $1.86 \pm 0.59\text{ }\mu\text{mol/min}$ per 30 g of muscle (mean $\pm$ S.D.; $n = 6$) at rest; under these conditions oxygen consumption was approx. $10\text{ }\mu\text{mol/min}$ per 30 g. The removal of acetoacetate was not significantly altered by the presence of 1 mM-oleate or 5 mM-glucose in the perfusion medium. Insulin had no effect on the uptake of acetoacetate. Reduction to 3-hydroxybutyrate ($0.56 \pm 0.11\text{ }\mu\text{mol/min}$ per 30 g; $n = 6$) represented only a minor fate of acetoacetate.

* Dedicated to Professor H. A. Krebs, F.R.S., on his seventieth birthday.

* Abbreviation: NANA, N-acetylneuraminic acid.

Exercise by means of bilateral stimulation of the sciatic nerves at rates of 1/s and 5/s did not significantly increase the removal of acetoacetate nor did it increase the release of 3-hydroxybutyrate.

Exercise increased the uptake of glucose (5mM) in the absence of insulin, confirming the findings of Szabo, Mahler & Szabo (1969). In contrast with the findings with perfused rat heart (Williamson & Krebs, 1961) addition of acetoacetate to the perfusion medium containing 5mM-glucose did not inhibit the increased uptake of glucose in the presence of insulin, nor did it inhibit the increased uptake of glucose during exercise in the absence of insulin.

Although the rate of removal of acetoacetate by this preparation was less than 10% of that reported for the perfused rat heart, sufficient acetoacetate was removed to account for 70–80% of the fuel of respiration at rest.

This investigation was in part supported by the Medical Research Council and the British Diabetes Association.

Szabo, A. J., Mahler, R. J. & Szabo, O. (1969). *Hormone & metab. Res.* **1**, 156.

Williamson, D. H. & Hems, R. (1970). In *Essays in Cell Metabolism*, p. 271. Ed. by Bartley, W., Kornberg, H. L. & Quayle, J. R. London: Wiley-Interscience.

Williamson, J. R. & Krebs, H. A. (1961). *Biochem. J.* **80**, 540.

Activity of Ketone-Body Utilization Pathway in Brain of Suckling and Adult Rats*

By M. ANN PAGE and D. H. WILLIAMSON. (*Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.*)

The capacity of brain to utilize ketone bodies has been measured in suckling and adult rats by determining the activities of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), 3-oxo acid CoA-transferase (EC 2.8.3.5) and acetoacetyl-CoA thiolase (EC 2.3.1.9). The activity of 3-hydroxybutyrate dehydrogenase in brain at birth was about two-thirds that of adult brain. The activity rose throughout the suckling period and at the time of weaning (20–22 days) reached values about three times higher than those for the adult. Similar findings have been reported by Klee & Sokoloff (1967). The changes in 3-oxo acid CoA-transferase paralleled those of 3-hydroxybutyrate dehydrogenase. The transferase activity at birth was about 1 U/g fresh wt. of brain, rose to 6 U at 21 days and then gradu-

ally declined to the adult value of 2.5 U. In contrast, brain acetoacetyl-CoA thiolase remained virtually constant at 4.0 U for 30 days after birth, which is higher than the adult activity of 2.5 U. The activities of these enzymes in kidney and heart gradually increased but did not reach the adult values until 3–4 weeks after birth. Thus during the suckling period rat brain, because of its size in relation to kidney and heart, is potentially the most important site of ketone-body utilization.

This work was supported in part by the Medical Research Council.

Klee, C. B. & Sokoloff, L. (1967). *J. biol. Chem.* **242**, 3880.

Metabolic Effects of Propionate Administration to Normal and Vitamin B₁₂-Deficient Rats*

By D. L. WILLIAMS, G. H. SPRAY and D. H. WILLIAMSON. (*Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.*)

Oberholzer, Levin, Burgess & Young (1967) reported that an oral load of propionate caused hypoglycaemia in a patient with congenital deficiency of methylmalonyl-CoA mutase (EC 5.4.99.2), which interconverts methylmalonyl-CoA and succinyl-CoA. To investigate possible reasons for this effect, propionate was administered to normal and vitamin B₁₂-deficient rats. The latter are unable to utilize propionate effectively (Weidemann, Hems, Williams, Spray & Krebs, 1970) because vitamin B₁₂ is an essential cofactor for the mutase. The rats were starved for 48 h and were killed 30 min after intraperitoneal injection of either propionate (1mmol) or 0.9% NaCl. The liver was freeze-clamped and blood collected from the aorta.

Propionate caused a slight increase (about 1 μ mol/ml) in blood glucose in both normal and vitamin B₁₂-deficient rats. The concentration of lactate in blood was not significantly altered in control animals given propionate, but rose from $1.26 \pm 0.44 \mu$ mol/ml (s.d.; $n = 9$) to 3.05 ± 1.77 (10) μ mol/ml in the deficient group. The changes in blood pyruvate followed a similar pattern.

The lactate concentrations in livers from normal and vitamin B₁₂-deficient rats were the same at 0.39 μ mol/g. After propionate the lactate concentration did not change significantly in the control livers, whereas in the deficient livers it increased to 1.20 ± 0.71 (12) μ mol/g. Similarly, hepatic pyruvate concentrations did not alter in normal animals

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