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.