effects (Wessels *et al.*, 1971), although this has been questioned by others (Turner & Carter, 1972; Haslam, 1972). We propose a model in which cytochalasin B induces exocytosis by interference with the contractile microfilaments of polymorphonuclear leucocytes; filaments normally restrict lysosome contact with the plasma membrane, but, when filament function is disturbed by cytochalasin, contact of granules with membrane takes place, and this results in release of lysosomal enzymes.

- Allison, A. C., Davies, P. & de Petris, S. (1971) *Nature New Biol. (London)* **232**, 153
 Davis, A. T., Estensen, R. & Quie, P. G. (1971) *Proc. Soc. Exp. Biol. Med.* **137**, 161
 Haslam, R. J. (1972) *Biochem. J.* **127**, 34P
 Turner, N. B. & Carter, S. B. (1972) *Biochem. J.* **127**, 1P
 Weissmann, G., Dukor, P. & Zurier, R. B. (1971) *Nature New Biol. (London)* **231**, 131
 Wessels, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. & Yamada, K. M. (1971) *Science* **171**, 135
 Woodin, A. M. & Wieneke, A. A. (1964) *Biochem. J.* **90**, 498

The Role of Ketone Bodies in the Metabolism of the Adult Desert Locust

By E. BAILEY and J. A. HORNE (*Department of Biochemistry, University of Sheffield, Sheffield S10 2TN, U.K.*) and M. E. G. IZATT and L. HILL (*Department of Zoology, University of Sheffield, Sheffield S10 2TN, U.K.*)

Bailey *et al.* (1971) demonstrated the occurrence of the ketone bodies acetoacetate and D-3-hydroxybutyrate in the blood of the adult pigeon (*Columba livia*) and haemolymph of the adult desert locust (*Schistocerca gregaria*). In the pigeon the ketone bodies probably play a similar role in metabolism to that in mammalian systems (Bailey & Horne, 1972). The present study was undertaken to try to elucidate the role of ketone bodies in the metabolism of the desert locust.

Acetoacetate and D-3-hydroxybutyrate are found in the haemolymph, fat-body and flight muscle of adult locusts. Acetoacetate is the major ketone body in haemolymph and flight muscle, whereas D-3-hydroxybutyrate often predominates in fat-body. The haemolymph concentration of acetoacetate varies with age (being highest at ages when the fat-body lipid reserves are being broken down), and is increased by flight, starvation and injection of corpus cardiacum extracts but is unaffected by stress or sex difference. Ketone bodies are formed in the fat-body although rates of formation of acetoacetate and D-3-hydroxybutyrate *in vitro* are low. However, the addition of haemolymph from starved insects to

incubation mixtures containing fat-body and potassium palmitate causes a marked stimulation of ketone-body formation. Both [3-¹⁴C]acetoacetate and D-3-hydroxy[3-¹⁴C]butyrate are oxidized by fat-body, flight muscle and testes of adult male locusts, [3-¹⁴C]acetoacetate being much more readily oxidized than D-3-hydroxy[3-¹⁴C]butyrate [only low activities of D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) have been found in the tissues]. Flight muscle is tenfold more active than testis or fat-body in oxidizing [3-¹⁴C]acetoacetate. Starvation causes a greatly decreased rate of oxidation of ketone bodies by fat-body and flight muscle, but has no effect on the ability of testis to degrade those compounds. It appears that in the fed insects flight muscle is the major user of ketone bodies, whereas in the starved male locust testis is a major user of these compounds.

The results obtained suggest that ketone bodies are formed in fat-body during mobilization of triglyceride food reserves and are then either oxidized by the fat-body or transported (mainly as acetoacetate) in the haemolymph to the flight muscle and other tissues to serve as a respiratory fuel.

- Bailey, E. & Horne, J. A. (1972) *Biochem. J.* **128**, 108P
 Bailey, E., Horne, J. A., Izatt, M. E. G. & Hill, L. (1971) *Life Sci.* **10**, 1415-1419

Measurement of the Specific Radioactivity of Glutamine and Glutamate in Plasma

By J. COSTELLO, DEIRDRE M. KEANE and E. BOURKE (*Department of Clinical Medicine, Trinity College, Dublin 2, Irish Republic*)

Studies on the kinetics of glutamine and glutamate metabolism both *in vivo* and *in vitro* are frequently complicated by the fact that under many conditions simultaneous synthesis and utilization occur. Thus measurement of the specific radioactivity of glutamine and glutamate in plasma and other biological fluids is of value in studies of turnover rates and turnover times of these amino acids, and also for investigation of control factors in their synthesis and degradation. A method is described for the simultaneous measurement of specific radioactivity of both amino acids in plasma and other biological fluids.

Total glutamate is measured enzymically by the method of Bernt & Bergmeyer (1968) and total glutamine by a method based on that of Hoek *et al.* (1969).

For the radioassay of glutamate and glutamine the following procedure is used. First, total [¹⁴C]glutamate and [¹⁴C]glutamine are measured as follows: glutamine is converted into glutamate and ammonia by incubation for 1 h at pH 5.0 with *Escherichia coli*

glutaminase. Total glutamate is then decarboxylated by the addition of glutamate decarboxylase. This procedure results in a loss of one-fifth of the total radioactivity of glutamate and glutamine as $^{14}\text{CO}_2$. Secondly, [^{14}C]glutamine is estimated thus: glutamate is completely converted into α -oxoglutarate by preincubation with glutamate dehydrogenase at pH 8.0 for 1 h. The sample is then adjusted to pH 5.0 and treated successively with glutaminase and with glutamate decarboxylase as in the first procedure. This results in a loss of one-fifth of the radioactivity of glutamine. The radioactivity of a control sample is counted directly to measure total radioactivity. Thus the radioactivity of glutamine is calculated directly and that of glutamate is calculated by subtraction. The entire reaction is carried out in counting vials, is reproducible and gives recoveries of over 96% even in the presence of other ^{14}C -labelled compounds.

The method is readily adaptable for measurement of the specific radioactivity of α -oxoglutarate.

We thank the Medical Research Council of Ireland and the Biomedical Trust, Dublin, for financial support.

Bernt, E. & Bergmeyer, H. U. (1968) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 384, Academic Press, New York
 Hoek, J. B., Charles, R., De Hann, E. J. & Tager, J. M. (1969) *Biochim. Biophys. Acta* 172, 407

Cholesterol Metabolism in Rabbits with Dietary-Induced Gall-Stones

By P. A. KYD and I. A. D. BOUCHIER (introduced by BARBARA H. BILLING) (*Department of Medicine, Royal Free Hospital, London WC1X 8LF, U.K.*)

An experimental model of cholelithiasis was studied in rabbits fed on a diet of 40% (w/w) casein, 15% oleic acid and 45% laboratory pellets. The gall-stones contained 80% (w/w) bile acids, a mixture of glycodeoxycholic acid and glycoallodeoxycholic acid, and only 1% cholesterol, which is the major constituent of human gall-stones. Nevertheless, compared with control animals fed on a laboratory-pellet diet, both hepatic and gall-bladder bile from lithogenic rabbits had increased concentrations of cholesterol relative to bile salts and phospholipids. There were striking increases in plasma cholesterol concentrations (mean 212 ± 15 S.E.M. mg/100ml compared with 54 ± 9 mg/100ml for control rabbits). Liver cholesteryl ester concentrations were markedly increased (6.08 ± 1.04 mg/g wet wt. compared with 0.57 ± 0.14 mg/g for control rabbits), whereas liver free cholesterol concentrations were only slightly raised.

The source of the increased plasma and liver chole-

sterol concentrations was investigated. Analysis of the diets showed that rabbits fed on the lithogenic diet received less cholesterol per day than did control rabbits. Cholesterol synthesis by liver and intestine was measured by incubating tissue slices 0.5 mm thick in Krebs bicarbonate buffer, pH 7.4, containing 2.5 mM-[2- ^{14}C]acetate (1 μCi) for 2 h at 37°C and determining the incorporation of labelled acetate into cholesterol, fatty acid and CO_2 . The livers of rabbits fed on the lithogenic diet showed marked inhibition of both cholesterol synthesis (11% of control values) and fatty acids synthesis (4% of control values). No difference in $^{14}\text{CO}_2$ production was observed. The inhibition of fatty acid synthesis is due probably to feedback inhibition by the 15% oleic acid in the lithogenic diet. It is known that hepatic cholesterol synthesis is depressed by cholesterol from the intestine that has been transported via the chylomicrons (Weis & Dietschy, 1969). The possibility that the lithogenic diet might have caused increased intestinal cholesterol synthesis was investigated. However, cholesterol synthesis was found to be within the control range at most points along the ileum. Oleic acid is known to increase cholesterol absorption to the maximal rate (Vahouny & Treadwell, 1959). A more likely explanation for the raised plasma, biliary and liver cholesterol concentrations is therefore that of increased absorption of dietary and endogenous cholesterol, due to the high concentration of oleic acid in the lithogenic diet.

Vahouny, G. V. & Treadwell, C. R. (1959) *Amer. J. Physiol.* 196, 881
 Weis, H. J. & Dietschy, J. M. (1969) *J. Clin. Invest.* 48, 2398

Studies on Glycolysis in the Dog Kidney *in vivo*

By J. COSTELLO, J. M. SCOTT, P. WILSON and E. BOURKE (*Department of Clinical Medicine and Biochemistry, Trinity College, Dublin 2, Irish Republic*)

Net gluconeogenesis in dog kidney *in vivo* is less than would be expected from studies with renal-cortical slices *in vitro* (Churchill & Malvin, 1970). A possible explanation is the role of renal medullary glycolysis in decreasing net glucose synthesis. The possible role of glycolysis was investigated under conditions of acidosis and alkalosis in ten male mongrel dogs.

Chronic metabolic acidosis or alkalosis was induced by daily administration of 180 mmol of NH_4Cl or NaHCO_3 respectively for 6 consecutive days. The dogs were starved for 24 h before surgery. Under anaesthesia the abdominal aorta, a renal vein and the ipsilateral ureter were catheterized and both renal plasma flow and glomerular filtration rate were