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.

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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.

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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

monitored by the method described by Bourke *et al.* (1971). A priming dose ($1.4 \mu\text{Ci/kg}$) and sustaining dose ($1.4 \mu\text{Ci/kg}$) of [$1\text{-}^{14}\text{C}$]glucose was commenced and, after a 40 min equilibration period, urine collections and arterial and renal-venous blood samples were withdrawn continuously for six successive clearance periods by using a modification of the technique of Churchill & Malvin (1970).

Total glucose was measured enzymically by using the hexokinase trap. Radioactive glucose was measured by its enzymic conversion into ribulose 5-phosphate and $^{14}\text{CO}_2$ by the method of Costello *et al.* (1972). Glycolysis was calculated from the arteriovenous [^{14}C]glucose difference expressed in $\mu\text{mol/min}$. Similarly net and total gluconeogenesis were calculated.

Glycolysis was demonstrated under conditions of acidosis and alkalosis. Under alkalotic conditions however, significantly more glycolysis was consistently demonstrated. Although no significant difference in net gluconeogenesis between the two groups was observed, gross gluconeogenesis was significantly greater in the chronically alkalotic dogs.

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Concentration of Oestrogens in Jugular-Venous Plasma of Cows during the Oestrous Cycle

By R. G. GLENCROSS and G. S. POPE (*National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, U.K.*) and I. B. MUNRO (*Ministry of Agriculture, Fisheries and Food Cattle Breeding Centre, Shinfield, Reading RG2 9AT, U.K.*)

Samples of jugular-venous plasma were collected daily from lactating dairy cows during three 7-day periods each including oestrus, and subsequently during five complete oestrous cycles.

Concentrations of oestradiol and oestrone were determined in samples by radioimmunoassay after extraction with ether and chromatography on ion-exchange resin and lipophilic dextran gel.

Two antiserum preparations, A and B, had been prepared from immunogens in which oestradiol-17 β was conjugated through the 17 β -oxygen, and another antiserum preparation, C, from an immunogen in which 6-oxo-oestradiol-17 β was conjugated at C-6. Antisera A and B cross-reacted considerably with oestrone and oestradiol-17 α whereas antiserum C had very little cross-reaction with oestradiol-17 α relative to the 17 β isomer.

Concentrations of oestradiol were determined in all samples, about half with antiserum A or B and half with antiserum C. Concentrations of oestrone were determined in samples from three complete cycles by means of antiserum B.

Mean concentrations of oestrone were below 1 pg/ml throughout the cycle except for a possible small rise 1–2 days before oestrus.

Oestradiol concentrations measured with antiserum A, B or C were very similar at comparable times of the cycle, and it is tentatively concluded that it was oestradiol-17 β that was being determined. Pooled oestradiol-17 β determinations show two peaks: (1) a pre-oestrus peak with concentrations above basal values for 4 days and a maximum of 5 pg/ml 1 day before oestrus; (2) a post-ovulatory peak with concentrations above basal values for 3 days and a maximum of 7 pg/ml 6 days after oestrus.

The present results are consistent with those obtained by bioassay by Pope *et al.* (1965) and with those of Echternkamp & Hansel (1971) and Wettemann *et al.* (1972), who used radioimmunoassay and uterine protein binding assay.

Whereas the physiological significance of the pre-oestrus oestradiol-17 β secretion is generally accepted, that of post-ovulatory oestradiol-17 β is relatively unknown. The possibility that it has a luteotrophic action will be discussed.

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Isolation of a Fraction from Rat Cerebellum Enriched in Nerve Terminals of One Single Type (Mossy-Fibre Terminals)

By R. BALÁZS, F. HAJÓS,* I. A. MICHAELSON, R. TAPIA† and G. WILKIN (*Medical Research Council Neuropsychiatry Unit, Woodmansterne Road, Carshalton, Surrey, U.K.*)

Isolation of synaptosomes has advanced the understanding of the processes of neurotransmission (Whittaker, 1965; De Robertis, 1967). Preparation of synaptosomes from tissue homogenates has the limitation of heterogeneity of terminal types often contaminated by other structures. An improved

* Present address: Department of Anatomy, Semmelweis Medical School, Budapest, Hungary.

† Present address: Instituto de Biología, Universidad Nacional de México, México 20, D.F., México,