betaine transport to give a concentration ratio of 1.09 ± 0.06 . These results suggest that betaine accumulation by kidney slices is linked to oxidative metabolism. Glycine and L-lysine were non-competitive inhibitors of betaine uptake, with glycine being the more potent inhibitor. Betaine uptake was also inhibited, in increasing order of inhibition, by: L-valine, L-hydroxyproline, L-proline.

It may be concluded that small intestine and kidney both exhibit the betaine-imino acid transport pathway *in vitro*.

We thank the Medical Research Council for a financial grant and Dr S. R. C. Hughes for radiochemical facilities.

Hagihira, H., Wilson, T. H. & Lin, E. C. C. (1962). *Am. J. Physiol.* **203**, 637.

Robinson, J. R. (1949). *Biochem. J.* **45**, 68.

Rosenberg, L. E., Downing, S. J. & Segal, S. (1962). *Am. J. Physiol.* **202**, 800.

Transport Pathway for Glycine Betaine in Rabbit Kidney *in vivo*

By C. S. FRANKLIN, D. F. EVERED* and P. B. NUNN.† (*Departments of Pharmacology and Chemistry, Chelsea College of Science and Technology, London S.W.3, U.K.*)

A transport pathway for betaine and the imino acids can be demonstrated in rat kidney-cortex slices *in vitro* (Evered & Nunn, 1970). This pathway could not readily be demonstrated *in vivo* in the rat as the urine flow is low in this species. However, the rabbit has a very similar endogenous renal plasma clearance rate for glycine (Evered, 1967) and was chosen for studies *in vivo*.

Under chloralose anaesthesia a male rabbit was infused intravenously at 2 ml/min with 5% (w/v) mannitol, to induce diuresis, and 0.5% (w/v) creatinine in 0.9% NaCl solution. Subsequently 0.25 M-betaine hydrochloride (neutralized) was infused in addition. Appropriate urine and blood plasma samples were collected for each test period. Creatinine was determined colorimetrically. Ninhydrin-reacting compounds were determined with an automatic analyser (Technicon Instruments Co. Ltd., Chertsey, Surrey, U.K.) with L-norvaline as an internal standard.

Infused betaine did not alter the glomerular filtration rate determined from plasma clearances rates of creatinine. The infusion did increase the plasma clearance rates for all amino acids deter-

mined except arginine. Most increases were modest and below 3.5 times the control value. The clearance for glutamate was raised 4.7-fold and for glycine 8-fold, but the value for proline was 190 times the control value. An effect on hydroxyproline was also expected, but, owing to a mixed peak in chromatography, no analytical result was available.

Our interpretation is that betaine infusion specifically inhibited the renal reabsorption of proline. The slight glycinuria was a secondary effect of the prolinuria induced by betaine infusion. This hypothesis is consistent with the effect seen in familial hyperprolinaemia (Seriver, Efron & Schafer 1964).

Betainuria has been observed in children (Schirmer, Tieckelmann & Guthrie, 1965). Betain-aemia leading to prolinuria could be confused with a renal hyperiminoaciduria instead of a metabolic lesion. This could occur if only ninhydrin-reacting compounds were assayed, as is common in screening for human inborn errors of metabolism.

We thank the Medical Research Council for a financial grant and for purchasing the automatic amino acid analyser.

Evered, D. F. (1967). *Comp. Biochem. Physiol.* **23**, 631.

Evered, D. F. & Nunn, P. B. (1970). *Biochem. J.* **118**, 40 P.

Schirmer, J. P., Tieckelmann, H. & Guthrie, R. (1965). *Experientia*, **21**, 448.

Seriver, C. R., Efron, M. L. & Schafer, I. A. (1964). *J. clin. Invest.* **43**, 374.

The Effect of Circulating Lactose on Brain Metabolism in the Neonatal Rat

By A. McLEAN and B. E. CLAYTON. (*Department of Chemical Pathology, The Hospital for Sick Children, Great Ormond Street, London W.C.1, U.K., and the Institute of Child Health, Guildford Street, London W.C.1, U.K.*)

Dietary lactose is normally hydrolysed to monosaccharides in the brush border of the small intestine, but the absorption of unhydrolysed lactose can occur in a variety of conditions in man (Arthur, 1966) and produces lactosuria. A number of findings have suggested that circulating lactose may have a deleterious effect in infants and young animals. The oral administration of lactose to young rats may cause retardation of growth with or without diarrhoea and also may lead to the development of cataracts (Koehler & Allen, 1934; Mitchell & Dodge, 1935). Infants with severe lactosuria may show aminoaciduria, proteinuria (Darling, Mortensen & Søndergaard, 1960) and a strong waxy pallor and are frequently irritable (Arthur, Clayton, Cottom, Seakins & Platt, 1966).

* Present address: Department of Biochemistry, Chelsea College of Science and Technology, London S.W.3, U.K.

† Present address: Department of Botany, University of Texas, Austin, Tex. 78712, U.S.A.

In addition it is not unusual to observe disacchariduria in children with non-specific mental retardation and no clinical symptoms suggestive of malabsorption, though the connection between these findings is obscure.

As part of a more extensive study on the actions of circulating lactose, the effects of this carbohydrate on the concentrations of pyruvate, glucose 6-phosphate and metabolites of the citric acid cycle were measured in the brains of rats that had received regular injections of intraperitoneal lactose from the fifth to the twentieth days of life. According to the methods described by Goldberg, Passonneau & Lowry (1966) the mean concentration of isocitrate was significantly raised whereas those of the other metabolites showed no significant change compared with controls. Studies *in vitro* with a commercial preparation of isocitrate dehydrogenase showed 16 and 23% inhibition by 1mM- and 5mM-lactose respectively. Sucrose gave 10% inhibition, whereas glucose, galactose and maltose were without effect.

These five sugars caused no inhibition of preparations of fumarase, malate dehydrogenase and citrate lyase.

As yet there is no indication of the way in which raised concentrations of isocitrate might affect the brain. Possibly it might exert a direct physiological effect analogous to that of sucrose on lysosomes (Wattiaux, Wattiaux-de Coninck, Rutgeerts & Tulkens, 1964) or cause inhibition of a key enzyme.

We are indebted to the Nuffield Foundation for financial support.

Arthur, A. B. (1966). *N.Z. med. J.* **65**, 94.

Arthur, A. B., Clayton, B. E., Cottom, D. G., Seakins, J. W. T. & Platt, J. W. (1966). *Lancet*, **i**, 172.

Darling, S., Mortensen, O. & Søndergaard, G. (1960). *Acta paediat., Stockh.*, **49**, 281.

Goldberg, N. D., Passonneau, J. V. & Lowry, O. H. (1966). *J. biol. Chem.* **241**, 3997.

Koehler, A. E. & Allen, S. E. (1934). *J. Nutr.* **8**, 377.

Mitchell, H. S. & Dodge, W. M. (1935). *J. Nutr.* **9**, 37.

Wattiaux, R., Wattiaux-de Coninck, S., Rutgeerts, M. J. & Tulkens, P. (1964). *Nature, Lond.*, **203**, 757.

DEMONSTRATION

A Flow System for the Superfusion and Stimulation of Isolated Mammalian Tissues

By H. McILWAIN and D. JONES. (*Department of Biochemistry, Institute of Psychiatry, British Post-graduate Medical Federation, University of London, London S.E.5, U.K.*)

The system is based on the quick-transfer apparatus of McIlwain & Rodnight (1962), an analytical multi-channel peristaltic pump and an incubating bath. Each tissue sample (30–100mg) is held in a defined position in relation to stimulating electrodes in beakers containing 2.5–6ml of incubating fluid. The electrodes, tubes bringing gas mixtures and tubes supplying and removing incubating fluid are mounted on an easily removable plastic frame, which fits the beaker. After flowing over the tissue, the incubating fluid is collected in a series of sample tubes, which are changed at 1 or 2min intervals.

The apparatus is demonstrated with four to six samples from the brain separately superfused with glucose-bicarbonate saline media. Chosen flow rates are maintained, e.g. 3ml/min, which implies a fluid exchange similar to that *in vivo*. Though superfused cerebral tissues have been examined in

pharmacological studies (see below) little is known about their metabolic status. Results for tissues from the piriform cortex and the neocortex of the guinea pig in the present apparatus showed the tissue content of K^+ and of creatine phosphate to be well maintained and to be responsive to electrical stimulation, as also was their formation of lactic acid. This was the case after passage of fluid up to 2000 times the volume of the tissue sample.

Uptake and release of [3H]noradrenaline, [3H]serotonin and [3H]glycine were examined in tissues from guinea-pig piriform cortex (McIlwain & Snyder, 1970). Release of the noradrenaline and serotonin was greatly increased on electrical stimulation of the samples [cf. results of Baldessarini & Kopin (1967) and Srinivasan, Neal & Mitchell, (1969) for other parts of the brain], and charts showing these results will be exhibited.

Baldessarini, R. J. & Kopin, I. J. (1967). *J. Pharmac. exp. Ther.* **156**, 31.

McIlwain, H. & Rodnight, R. (1962). *Practical Neurochemistry*, p. 145. London: J. and A. Churchill Ltd.

McIlwain, H. & Snyder, S. H. (1970). *J. Neurochem.* **17**, (in the Press).

Srinivasan, V., Neal, M. J. & Mitchell, J. F. (1969). *J. Neurochem.* **16**, 1235.