PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 523rd. Meeting of the Society was held at the Institute of Psychiatry, De Crespigny Park, London SE5 8AF on Thursday and Friday, 17 and 18 February 1972, when the following papers were presented:

COMMUNICATIONS

A Natural Inhibitor of Liver Glutamine Aminotransferase

By P. J. BUTTERY (*Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, U.K.*) and E. V. ROWSELL (*Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.*)

Except when considerably purified glutamine aminotransferase is accompanied by a powerful ω -amidase (EC 3.5.1.3), making it possible to measure aminotransferase activity in crude liver homogenates by measuring 2-oxoglutarate formation. Several α -oxo acids are effective as amino acceptors, and we have preferred to employ 4-methyl-2-oxopentanoate (2-oxoisocaproate) (see Buttery *et al.*, 1969). During the development of this assay method it was noted, with mouse, hamster, rat or rabbit liver, that activity was proportional to tissue concentration only up to 2.5% (w/v); thereafter there was a diminishing rate increment for extra tissue added, although for all conditions leucine and 2-oxoglutarate production were equivalent. This apparent aminotransferase inhibition was not observed with dialysed liver homogenates.

A water homogenate of rat liver (20%, w/v) was heated at 100°C for 5 min and centrifuged at 100000g for 1 h. The supernatant was tested (as in all subsequent experiments) with a partially purified glutamine aminotransferase (containing ω -amidase) prepared by following the procedure of Braunstein & Hsü (1960) as far as but not including their first chromatographic step. The extract was inhibitory to an extent reflecting the self-inhibition observed with crude liver homogenates. Extracts of dialysed homogenates did not inhibit.

Passage of an inhibitory extract (20 ml) through a 5 cm $\times$ 1 cm column of charcoal-Celite 535 (1:1, w/w) did not affect its potency. When charcoal-column eluates were concentrated and run on a Sephadex G-15 column one major peak of inhibitory activity was eluted in a position distinct from that of the void volume and indicating a molecular weight of a few hundred. This inhibitory fraction was competitive with respect to 4-methyl-2-oxopentanoate and non-

competitive with respect to L-glutamine. It travelled as a discrete band when further analysed by t.l.c.

From this evidence of a naturally occurring inhibitor of glutamine aminotransferase and previous reports that glutaminase (EC 3.5.1.2) activity is modified by several common metabolites (Katunuma *et al.*, 1966), it would appear that both glutamine-degrading systems are potentially sites of metabolic control. Glutamine aminotransferase and ω -amidase are apparently in the cytosol (Meister & Tice, 1960), and glutaminase is in the mitochondria (Kalnitsky & Shepherd, 1951).

We thank Miss L. Robinson for technical assistance.

- Braunstein, A. E. & Hsü, T.-S. (1960) *Biokhimiya* **25**, 758
Buttery, P. J., Rowsell, E. V. & Carnie, J. A. (1969) *Biochem. J.* **115**, 42P
Kalnitsky, G. & Shepherd, J. A. (1951) *J. Biol. Chem.* **192**, 1
Katunuma, N., Tomino, I. Z. & Nichino, H. (1966) *Biochem. Biophys. Res. Commun.* **22**, 321
Meister, A. & Tice, S. Y. (1950) *J. Biol. Chem.* **187**, 173

Induction of Uridine Diphosphate Glucuronyltransferase: Relation to Protein and Ribonucleic Acid Synthesis

By B. BURCHELL, A. M. NEMETH and G. J. DUTTON (*Department of Biochemistry, University of Dundee, Dundee DD1 4HN, U.K., and Department of Anatomy, University of Pennsylvania, Philadelphia, Pa. 19101, U.S.A.*)

We have previously described the precocious appearance of UDP-glucuronyltransferase activity in cultured chick-embryo liver preparations (Ko *et al.*, 1967; Skea & Nemeth, 1969), initiated by removal from the embryonic environment. Phenobarbital and other compounds stimulate this precocity (Dutton *et al.*, 1970; Burchell *et al.*, 1971). We now present evidence, previously referred to, on the relationship of these effects to protein and RNA synthesis.

With [^{14}C]leucine in culture media, the radioactivity of the leucine pool and the rate of incorporation of leucine into protein remained constant during culture, as did the incorporation of added [^3H]uridine

into RNA and its relative incorporation into rRNA and tRNA. These incorporations remained constant with phenobarbital present in the medium.

After 20h of culture, the medium was 'pulsed' for 4h with various concentrations of cycloheximide. Specific UDP-glucuronyltransferase activity and protein radioactivity were measured during the next 48h. Cycloheximide clearly inhibited the development of enzyme activity. Development resumed after removal of the cycloheximide.

Enzyme inhibition by cycloheximide was exactly proportional to inhibition of total incorporation of leucine into protein, suggesting that development of UDP-glucuronyltransferase activity depends on the rate of enzyme synthesis, possibly of enzyme itself, from amino acids.

On 'pulsing' with actinomycin D, the incorporation of [³H]uridine into RNA was markedly inhibited. Large doses of actinomycin D also began to inhibit the incorporation of leucine into protein. Amounts of antibiotic that did not affect protein synthesis, but that did inhibit over 50% of the incorporation of [³H]uridine into RNA, did not alter the development of UDP-glucuronyltransferase activity. Enzyme activity fell only when exposure to actinomycin D was sufficient to inhibit protein synthesis, possibly through cytotoxicity.

Development of UDP-glucuronyltransferase activity remained unaffected by 5-fluorouridine in the medium.

UDP-glucuronyltransferase activity in chick-embryo liver homogenates was not activated by incubation or preincubation with preparations from cultured liver, or with phenobarbital or its metabolites.

Presumably therefore the observed development of UDP-glucuronyltransferase activity in culture, with or without phenobarbital, depends on protein synthesis from amino acids; this process possibly need not involve DNA-directed RNA synthesis.

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Dutton, G. J., Hanson, K. B. & Burchell, B. (1970) *Biochem. J.* **120**, 15P

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The Scrapie Agent: a Small Deoxyribonucleic Acid-Mediated Virus?

By D. H. ADAMS (*Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, U.K.*)

After the intracerebral injection of [³H]thymidine and of [¹⁴C]polysaccharide precursors into normal and scrapie-affected mice, Adams *et al.* (1969, 1970) found, by separation of heavily centrifuged brain cell-sap fractions (post-ribosomal pellets) on Sepharose 4B, a fraction having peak specific radioactivity for both labels; this specific radioactivity was two- to three-fold higher in preparations from scrapie-affected brain than in those from normal brain. When large-granule fractions of scrapie-affected mouse brain had been treated with 6M-urea and centrifuged at 164000g_{av} for 3h, the supernatant, centrifuged for a further 16h, yielded a pellet that, when chromatographed on Sepharose 4B as before, gave a similar fraction of peak specific radioactivity for ³H and ¹⁴C labels. No corresponding peak was obtained with the fraction from normal mouse brain. So far as the ³H-labelling is concerned this suggested *prima facie* the presence of a small DNA in the cell sap of normal mouse brain that linked to membrane under appropriate conditions to produce the disease of scrapie (the 'linkage-substance' hypothesis; Adams & Field, 1968).

Kimberlin & Hunter (1967), claiming that [³H]-thymidine was incorporated into DNA of scrapie-affected brain at about twice the normal rate, concluded that this increase is not directly connected with the scrapie agent. Since these authors did not isolate DNA in their experiments and such an increased incorporation into cellular DNA might obscure any specific incorporation into a scrapie-agent DNA, this present study began by preparing DNA from normal and scrapie-affected mice intracerebrally injected with [³H]thymidine and 'melting' it off hydroxyapatite columns (Miyazawa & Thomas, 1965). The label was concentrated in double-stranded DNA, and the specific radioactivity of that from scrapie-affected brain was two- to three-fold the normal value. Confirmatory evidence was obtained by CsCl-density-gradient centrifugation. The specific radioactivity of the isolated normal brain DNA was only about 4000 d.p.m./mg, suggesting a very minute turnover, either in normal or in scrapie-affected mouse brain. In further experiments large-granule fractions were prepared from 70 normal and 70 scrapie-affected mice that had each been injected with 10μCi of [³H]thymidine, and these fractions were treated with 6M-urea and centrifuged as described above. The pellets were mixed with non-