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

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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-

labelled whole mouse brain, and the DNA was extracted and then chromatographed on hydroxy-apatite. In the preparations from scrapie-affected brain a small peak eluted at about 50°C was found. This may represent a small single-stranded specific scrapie-agent DNA.

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Deoxyglucose and Brain Glycolysis

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Injection or infusion of large doses of 2-deoxyglucose is known to cause elevated plasma concentrations of glucose and free fatty acids in man and laboratory animals, probably as a result of increased secretion of adrenaline. The deoxy sugar has also been suggested to act as an inhibitor of glycolysis in the brain. It is phosphorylated by brain hexokinase, so that both deoxyglucose and its phosphate might be expected to interact with glycolytic enzymes. Samples of guinea-pig cerebral cortex were homogenized in 0.32M-sucrose and centrifuged at 100000g for 1 h. The supernatant fraction was examined for activities of enzymes involved in the normal metabolism of glucose and glucose 6-phosphate. Enzymic specific activities ($\mu\text{mol}/\text{min per g}$) at 37°C and 2 mM substrate concentrations were: hexokinase (glucose: 5.9); glucose 6-phosphate dehydrogenase (glucose 6-phosphate: 4.1); 6-phosphogluconate dehydrogenase (6-phosphogluconate: 0.75); phosphoglucomutase (glucose 1-phosphate: 40.8); hexose phosphate isomerase (glucose 6-phosphate: 169; fructose 6-phosphate: 284). Inclusion in the reaction mixtures of 2-deoxyglucose or its phosphate at 2–4 mM had no effect on any activity except that of hexokinase. Deoxyglucose inhibits hexokinase by competition with glucose (K_i 0.25 mM; Bachelard *et al.*, 1971), but the phosphate was found to be inactive. Deoxyglucose thus has considerably less affinity for hexokinase than has glucose (K_m 0.04 mM) and also a lower affinity for the uptake process (Bachelard, 1971). Hexose phosphate isomerase is inhibited by deoxyglucose 6-phosphate in kidney and ascites-tumour cells (Wick *et al.*, 1957; Nirenberg & Hogg, 1958), but not in diaphragm (Kipnis & Cori, 1959). In the brain, only the transport and phosphorylation of

glucose seem to be affected. In order for accumulation of concentrations of deoxyglucose sufficient to cause significant inhibition of cerebral hexokinase, with resultant decreased glucose utilization and depletion of ATP, the circulating deoxyglucose concentration would have to be maintained at least one order higher than glucose. The possibility of action on enzymes acting at stages of metabolism remote from glucose 6-phosphate, although unlikely, has not been excluded. The recognition of deoxyglucose by the glucose-binding site on hexokinase, and the non-recognition of deoxyglucose 6-phosphate by the glucose 6-phosphate-binding site, provided further evidence for the allosteric properties of the enzyme.

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Increased Activity of the Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase in Rat Brain during Electrically Induced Convulsions

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Increased activity of the $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase of rat brain during convulsions induced by lethal doses of Metrazol (6,7,8,9-tetrahydro-5-azepotetrazole) has been reported by Bignami *et al.* (1966) and De Robertis *et al.* (1969). During an investigation of the effect of experimentally induced thyroid disorders on energy utilization in rat brain we have observed a similar increase in $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase activity in brain tissue from rats made to convulse by electric currents.

Male Wistar rats (100g) were stimulated through electrodes applied to the ears by monophasic square waves (85 V; 150/s; duration 1.5 ms) applied for 1 s. The animals were killed after 10 s during the tonic phase by total immersion in liquid N_2 . Homogenates (10%, w/v) of cerebral-cortex tissue and whole cerebellum were prepared in water (Samson & Quinn, 1967). $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase activity was determined in the unfractionated homogenates and in the total particulate fraction (obtained by centrifuging the homogenate at 10⁶g for 60 min) by the procedure of Rodnight & Lavin (1966).