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Incorporation of [³⁵S]Sulphate into the Glycosaminoglycans of Rat Brain

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Many of the studies concerned with [³⁵S]sulphate metabolism in brain tissue have dealt mainly with the general radioautographical distribution (see, e.g., Boström & Odeblad, 1953) and with the incorporation into lipid and cerebrosides. Although [³⁵S]sulphate is known to be incorporated into 'chondroitin sulphate' (Guha, Northover & Bachhawat, 1960) and crude 'sulphomucopolysaccharide' (Robinson & Green, 1962) fractions from rat brain, there has been no attempt to analyse the distribution of radioactivity in the individual sulphated glycosaminoglycans that have been shown to be present in the tissue (Cunningham & Goldberg, 1968; Singh, Chandrasekaran, Cherian & Bachhawat, 1969).

Intraperitoneal injections of 2 μ Ci of sodium [³⁵S]sulphate/g body wt. were given to 40-day-old rats. The animals were killed 6h after the injection and the glycosaminoglycan-containing fraction was prepared from the excised brains by digesting the lipid-free material with protease followed by a precipitation with cetylpyridinium chloride and removal of nucleic acids. Resolution of the individual glycosaminoglycan components was accomplished by electrophoresis on cellulose acetate and cellulose micro-columns as described previously (Cunningham & Goldberg, 1968). Samples taken for determination of [³⁵S]sulphate were dried on planchets and their radioactivities measured by using a windowless scintillation counter. Labelled glycosaminoglycans were detected on the cellulose acetate strips by radioautography and by using a radiochromatogram scanner.

Protease treatment released 97% of the radioactivity into solution, 38% of this being precipitated from solution with cetylpyridinium chloride; the remainder was diffusible through heated dialysis tubing.

Hyaluronic acid, chondroitin sulphate, heparan sulphate and dermatan sulphate were present in the proportions 33:48:11:8 (measured as hexuronic acid). Cellulose micro-column results indicated that [³⁵S]sulphate was incorporated only into the sulphated glycosaminoglycans, namely chondroitin

sulphate (71%), heparan sulphate (14%) and dermatan sulphate (14%), one of the chondroitin sulphate fractions showing a higher rate of incorporation than the others. No radioactivity was found in regions or fractions corresponding to heparin or keratan sulphate, confirming that these glycosaminoglycans are not significant in brain tissue.

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Effect of Phenobarbital and other Compounds on Induction or Maintenance of Liver uridine Diphosphate Glucuronyltransferase during Organ Culture

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UDP-glucuronyltransferase activity towards certain substrates appears precociously, and above adult values, when early chick-embryo liver is cultured on chemically defined media; liver from older embryos develops UDP-glucuronyltransferase activity to adult values during culture (Ko, Dutton & Nemeth, 1967; Skea & Nemeth, 1969). Removal from an embryonic environment initiates this induction.

Phenobarbital, administered to chickens (I. H. Stevenson & G. J. Dutton, unpublished work) or mammals, subsequently increases liver UDP-glucuronyltransferase activity to many substrates. This communication reports that barbiturates, and possibly bilirubin, stimulate production of UDP-glucuronyltransferase activity in cultured liver when present in the culture medium (Dutton, 1970).

Phenobarbital (sodium salt) does not stimulate UDP-glucuronyltransferase activity towards o-aminophenol or p-nitrophenol *in vitro*, but added to the medium increases development, ultimate activity and persistence of the enzyme, most markedly with older-embryo liver; it prolongs survival of the enzyme in cultured hatched chick liver. High concentrations of phenobarbital are required: 1.0 mM is less effective than 10 mM, 0.1 mM being ineffective. Sodium diethylbarbiturate or sodium barbiturate, but not sodium acetate, could

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replace phenobarbital. Triton X-100, often an activator of microsomal UDP-glucuronyltransferase *in vitro*, was ineffective at 0.1% and inhibitory at 1%.

With mammalian foetal liver, phenobarbital-enriched media allowed slightly, but consistently, faster development of UDP-glucuronyltransferase activity towards both *o*-aminophenol and bilirubin. J. Fog & A. Bakken (see Bakken, 1969) suggest that bilirubin hastens development of its UDP-glucuronyltransferase activity in foetal liver; bilirubin added to media could enhance the enzyme activity of cultured foetal liver towards bilirubin (but not *o*-aminophenol), but increases so far obtained are small and poorly reproducible.

Tissues cultured with and without phenobarbital differ strikingly to the eye. Microscopically, phenobarbital delays invasion by fibroblasts and increases endoplasmic reticulum, consistent with prolonged and intensified UDP-glucuronyltransferase activity. Phenobarbital maintains protein in cultured liver, and further results indicate that induction of UDP-glucuronyltransferase in cultures requires protein synthesis. Whether barbiturates generally increase microsomal enzyme availability or produce more specific inducers of UDP-glucuronyltransferase is being investigated. The method appears convenient for such studies.

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Observations on the Biosynthesis of Thioglucuronides and Thioglucosides in Vertebrates and Molluscs

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We have previously described the biosynthesis of diethyldithiocarbamate *S*- β -glucuronide by mouse liver UDP-glucuronyltransferase and its hydrolysis by β -glucuronidase (Dutton & Illing, 1969). We now report work on another thio linkage and with further species and tissues.

The carbodithioic link in diethyldithiocarbamate *S*-glucuronide proved labile during the assay procedure. With thiophenol as substrate, an *S*-

glucuronide was formed whose thiol linkage to glucuronic acid appeared stable enough to permit more quantitative investigation.

Thiophenol was incubated with mouse liver homogenate and UDP-[¹⁴C]glucuronic acid, ethanol was added and the supernatant was chromatographed. Phenol was the substrate in parallel experiments. One rapidly moving radioactive spot was obtained from the thiophenol incubation mixtures, travelling like phenyl glucuronide in two solvent systems. The former spot, unlike phenyl glucuronide, contained organically bound bivalent or quadrivalent sulphur by the Raney nickel-lead acetate test (Feigl & Haguenaue-Castro, 1962); the spot was not due to thiophenol and appeared only when thiophenol had been incubated with UDP-glucuronyltransferase and UDP-glucuronic acid. This supposed conjugate was hydrolysed by β -glucuronidase, leaving glucuronic acid; glucarolactone prevented this hydrolysis.

Assuming negligible formation of phenol from thiophenol during 15 min incubation, comparable amounts of *S*- and *O*-glucuronides were synthesized by mouse liver homogenates. The two UDP-glucuronyltransferase activities further resemble each other in intracellular and tissue location, in pH-activity curves and in activation by UDP-*N*-acetylglucosamine. Competition studies will be reported.

S-Glucuronides are synthesized by UDP-glucuronyltransferase in vertebrate liver and kidney, but not in tissues of *Arion ater*, where the corresponding *S*-glucosides were formed from UDP-glucose; no *S*-glucosides were formed in mammalian tissues. This parallels the findings with *O*-glucosides (Dutton, 1966). After incubation of diethyldithiocarbamate and thiophenol with UDP-[¹⁴C]glucose in *Arion* gut homogenates at pH 8.5 their *S*-glucosides were identified similarly to the *S*-glucuronides. Unlike phenyl *O*-glucoside, thiophenyl *S*-glucoside resisted hydrolysis by almond emulsin β -glucosidase or by acid, confirming the results obtained by Pigman (1941); diethyldithiocarbamate *S*-glucoside was labile (see Kasander, 1966), yielding glucose on acid hydrolysis. *Arion* gut slices, but not homogenates, form *S*-glucosides from [¹⁴C]glucose.

As the *S*-glucuronides and *S*-glucosides were formed by sliced tissue the respective UDP-glycosyltransferase pathways presumably operate *in vivo*. Glucuronides were not evident with cysteine or glutathione as substrates.

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