- Marshall, B. J. & Tibbs, J. (1969). *Biochem. J.* **115**, 10P.
 Tibbs, J. & Marshall, B. J. (1969). *Abstr. FEBS 6th Meet., Madrid*, p. 63.
 Tibbs, J. & Marshall, B. J. (1970). *J. Protozool.* **17**, 125.

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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- Boström, H. & Odeblad, E. (1953). *Acta psych. neurol. scand.* **28**, 1.
 Cunningham, W. L. & Goldberg, J. M. (1968). *Biochem. J.* **110**, 35P.
 Guha, A., Northover, B. J. & Bachhawat, B. K. (1960). *J. scient. ind. Res.* **19C**, 287.
 Robinson, J. D. & Green, J. P. (1962). *Yale J. Biol. Med.* **35**, 248.
 Singh, M., Chandrasekaran, E. V., Cherian, R. & Bachhawat, B. K. (1969). *J. Neurochem.* **16**, 1157.

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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