lipase and diglyceride lipase of synaptosomes had apparent K_m values approx. 1.5 and 0.5mM respectively.

Triglyceride lipase activity in osmotically ruptured synaptosomes was stimulated 49% ($P < 0.001$) by 1.0mM-noradrenaline and 24% ($P < 0.001$) by 1.0mM-5-hydroxytryptamine. Noradrenaline (1.0mM) was without effect on the triglyceride of microsomal material and depressed slightly (11%; $P < 0.02$) the triglyceride lipase activity of mitochondria. Diglyceride lipase activity was stimulated 35% ($P < 0.001$) by noradrenaline (1.0mM) and 21% ($P < 0.001$) by 5-hydroxytryptamine (1.0mM). Monoglyceride lipase activity was unaffected by either noradrenaline or 5-hydroxytryptamine. Since nerve endings of brain contain noradrenaline and 5-hydroxytryptamine (Michaelson & Whittaker, 1963; Michaelson *et al.*, 1963; Zieher & De Robertis, 1963; Levi & Maynert, 1964) bound to synaptic vesicles (Michaelson *et al.*, 1963; Maynert *et al.*, 1964), similar changes may take place with endogenous glycerides *in vivo* during synaptic transmission.

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- Eichberg, J., Whittaker, V. P. & Dawson, R. M. C. (1964) *Biochem. J.* **92**, 91
 Levi, R. & Maynert, E. W. (1964) *Biochem. Pharmacol.* **13**, 615
 Maynert, E. W., Levi, R. & Lorenzo, A. J. D. (1964) *J. Pharmacol. Exp. Ther.* **144**, 385
 Michaelson, I. A. & Whittaker, V. P. (1963) *Biochem. Pharmacol.* **12**, 203
 Michaelson, I. A., Whittaker, V. P., Laverty, R. & Sharman, D. F. (1963) *Biochem. Pharmacol.* **12**, 1450
 Whittaker, V. P., Michaelson, I. A. & Kirkland, R. J. A. (1964) *Biochem. J.* **90**, 293
 Zieher, M. H. & De Robertis, E. (1963) *Biochem. Pharmacol.* **12**, 596

The Synthesis of Mercapturic Acids from Allyl Compounds in the Rat

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In previous investigations (Clapp *et al.*, 1969; Kaye & Young, 1970) the presence of mercapturic acids in the urine of rats dosed with a number of allyl compounds was demonstrated. It appeared that allyl halides and esters of allyl alcohol derived from strong acids give rise to the excretion of allylmercapturic acid and 3-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine and *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine, in the urine of the dosed animals. On the other hand, allyl alcohol and its esters with weak acids were found to give rise to

the formation of 3-hydroxypropylmercapturic acid, but not to allylmercapturic acid.

In the present work further support for this pattern in the metabolism of allyl compounds has been obtained. After the administration of allyl formate or allyl propionate to rats no allylmercapturic acid was detected in the urine, but it was possible to isolate 3-hydroxypropylmercapturic acid, as its dicyclohexylammonium salt, in amounts representing 6.8 and 9.1% respectively of the administered ester. Further, diallyl phthalate and allyl nitrite were found to give rise to the formation of 3-hydroxypropylmercapturic acid, but not to allylmercapturic acid. Administration of an ester of allyl alcohol derived from a strong acid, however, namely allyl nitrate, led to the isolation of both allylmercapturic acid and 3-hydroxypropylmercapturic acid, the latter as its dicyclohexylammonium salt, from the urine of the dosed rats in amounts corresponding to 5.9 and 2.6% respectively of the injected ester.

Two other allyl compounds, allylamine and allyl cyanide, were shown to lead to the excretion of 3-hydroxypropylmercapturic acid, but not allylmercapturic acid, in the urine of the dosed rats.

Gray & Barnsley (1971) have suggested that the metabolic formation of 3-hydroxy-1-methylpropylmercapturic acid from crotyl alcohol occurs with the formation of crotonaldehyde as an intermediate. In the present work, 3-hydroxypropylmercapturic acid, as its dicyclohexylammonium salt, was isolated in 10.5% yield from the urine of rats dosed with acrolein, and there are therefore grounds for believing that this aldehyde may be an intermediate in the metabolic conversion of allyl alcohol into a mercapturic acid. No evidence was found for the excretion of allylmercapturic acid by rats dosed with acrolein.

The metabolic pathway for the formation of mercapturic acids from allyl compounds was discussed.

Clapp, J. J., Kaye, C. M. & Young, L. (1969) *Biochem. J.* **114**, 6P

Gray, J. M. & Barnsley, E. A. (1971) *Xenobiotica* **1**, 55-67

Kaye, C. M. & Young, L. (1970) *Biochem. J.* **119**, 53P

A New Micro Method for the Determination of Acid Glycosaminoglycans in Urine

By P. D. WHITEMAN (*Department of Chemical Pathology, Institute of Child Health, London WC1N 1EH, U.K.*)

Procedures currently available for the determination of total urinary glycosaminoglycans tend to be lengthy and complicated. Methods include precipitation with quaternary ammonium salts, which is the most widely used (Di Ferrante & Rich, 1956; Teller

et al., 1962; Terry & Linker, 1964; Thompson & Castor, 1966; Manley *et al.*, 1968; Manley & Williams, 1969; Pennock *et al.*, 1971), and complexing with 9-aminoacridine hydrochloride (Muir *et al.*, 1963), but the quantitative reliability of both methods has been questioned (Muir *et al.*, 1963; Di Ferrante, 1967; Dean *et al.*, 1971).

The hexuronic acid content of the precipitated material is subsequently determined, but this does not measure keratan sulphate.

Scott & Dorling (1965) and Scott & Willett (1966) studied the interaction of polyanions and Alcian Blue 8GX under controlled conditions and extended their findings to the field of histochemistry. The method presented here depends on the formation of an insoluble complex between acid glycosaminoglycans and Alcian Blue 8GX (Imperial Chemical Industries Ltd.) under similar controlled conditions. It has been adapted to determine directly the glycosaminoglycan content of the urine of patients with a suspected diagnosis of mucopolysaccharidosis.

Maximum interaction of glycosaminoglycans in standard solutions and in urine was found to occur with 0.05% Alcian Blue 8GX in 50mm-sodium acetate (adjusted to pH 5.8 with acetic acid) containing 50mm-MgCl₂; in the standard procedure 20 μ l of glycosaminoglycan solution or centrifuged urine was mixed with 1ml of Alcian Blue reagent. After equilibration for 2h at room temperature, the glycosaminoglycan-Alcian Blue complex was centrifuged at 2000g for 15min. After the precipitate had been washed with ethanol (2ml) it was dissociated with 1ml of 40% (w/v) Manoxol-IB (dibutyl ester of sodium sulphosuccinic acid; BDH Chemicals Ltd.) in 50mm-sodium acetate buffer, pH 5.8. The extinction of the resulting clear blue solution was measured at 620nm. Blank values were low, and the whole procedure can be carried out in small disposable polystyrene tubes.

It was found convenient to standardize the method with chondroitin 4-sulphate. Calibration curves were almost linear over the range 50–500 μ g of glycosaminoglycan/ml. Sulphated glycosaminoglycans bearing two anionic groups/disaccharide unit gave similar standard curves. Hyaluronic acid and keratan sulphate gave slightly lower readings.

The procedure is relatively specific, although Tamm-Horsfall glycoprotein does react with Alcian Blue 8GX under these conditions. However, the colour development due to this glycoprotein is less than one-quarter of that from a comparable concentration (w/v) of glycosaminoglycan.

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Dean, M. F., Muir, H. & Ewins, R. J. F. (1971) *Biochem. J.* **123**, 883
Di Ferrante, N. (1967) *Anal. Biochem.* **21**, 98

Di Ferrante, N. & Rich, C. (1956) *J. Lab. Clin. Med.* **48**, 491
Manley, G. & Williams, U. (1969) *J. Clin. Pathol.* **22**, 67
Manley, G., Severn, M. & Hawksworth, J. (1968) *J. Clin. Pathol.* **21**, 339
Muir, H., Mittwoch, U. & Bitter, T. (1963) *Arch. Dis. Childhood* **38**, 358
Pennock, C. A., Wharton, B. A. & White, F. (1971) *Acta Paediat. Scand.* **60**, 299
Scott, J. E. & Dorling, J. (1965) *Histochemie* **5**, 221
Scott, J. E. & Willett, I. H. (1966) *Nature (London)* **209**, 985
Teller, W. M., Burke, E. C., Rosevear, J. W. & McKenzie, B. F. (1962) *J. Lab. Clin. Med.* **59**, 95
Terry, K. & Linker, A. (1964) *Proc. Soc. Exp. Biol. Med.* **115**, 394
Thompson, G. R. & Castor, W. C. (1966) *J. Lab. Clin. Med.* **68**, 617

Investigation of the Polysaccharides Produced by Extracellular Glycosyltransferases from *Streptococcus mutans*

By J. K. BAIRD and D. C. ELLWOOD (*Microbiological Research Establishment, Porton Down, Salisbury, U.K.*)

Large amounts of dental plaque are formed as a result of feeding on diets containing sucrose (Carlsson & Egelberg, 1965). Streptococci isolated from the human oral cavity can synthesize extracellular polysaccharides from sucrose, and the water-soluble polysaccharides have been characterized as 'dextrans' and 'levans'. A water-insoluble glucan produced from sucrose by culture filtrates of *Streptococcus mutans* strain OMZ 176 has been shown to contain almost exclusively α -(1 $\rightarrow$ 3)-linkages (Guggenheim & Schroeder, 1967).

Streptococcus mutans strain Ingbritt A was grown in batch culture (4% glucose), and the culture supernatant was incubated with sucrose. The high-molecular-weight products, which represent less than 10% by weight of the sucrose hydrolysed, were fractionated on the basis of their insolubility in water and in 55 and 75% ethanol solutions.

The water-insoluble glucan is soluble in 1 M-NaOH and has $[\alpha]_D^{21} + 203^\circ$ (c 1). The material was largely resistant to dextranase (8% hydrolysis on incubation with a commercial dextranase from Koch-Light Laboratories Ltd.).

The glucan was methylated with sodium hydride and methyl iodide, and the methylated polysaccharide was extracted with chloroform and methanolysed with methanolic 3% HCl. After neutralization with Ag₂CO₃, the methyl *O*-methylglycosides were separated by g.l.c. The only methyl ethers of methyl α -D-glucopyranoside present were identified as 2,3,4,6-tetra- (10%), 2,3,4-tri- (27%), 2,4,6-tri- (51%) and two di-*O*-methylglycosides (12%) at present un-