substrate is obtained (see Dodgson, 1961). The method would also appear to be of doubtful value for use with crude mammalian-tissue preparations in view of the need to add relatively large amounts of extra SO_4^{2-} ion to tests and controls before good recoveries of SO_4^{2-} ion can be obtained.

The method has also been successfully used to follow the alkaline- and acid-hydrolysis rates of the O-sulphates of serine and threonine (K.S. Dodgson, A. G. Lloyd & N. Tudball, to be published) and to measure the ester sulphate content of chondroitin sulphate preparations after hydrolysis with hydrochloric acid (R. G. Price, unpublished results).

SUMMARY

1. Three variations of a turbidimetric method for the determination of small amounts of inorganic sulphate have been described.

2. One variation, covering the range 0–200 μg. of SO_4^{2-} ion, is suitable for following the non-enzymic hydrolysis of monosaccharide sulphates by hydrazine; a second variation, covering the range 0–40 μg. of SO_4^{2-} ion, has been developed for the assay of purified glycosulphatase preparations; the other variation, covering the range 0–12 μg. of SO_4^{2-} ion, has been successfully used for the microanalysis of various ester sulphates.

3. The method can be used in the presence of hydrazine, imidazole, various buffers, anions, cations, monosaccharide mono- and di-sulphate and other sulphate esters. SO_3^{2-}, Ba^{2+} and

$P_2O_7^{4-}$ ions (in concentrations greater than 0·01 M), and a large number of polysaccharide sulphate esters which were tested, interfered with the method. Other limitations of the method are described.

REFERENCES

Berglund, F. & Sörbo, B. (1959). *Acta chem. scand.* **13**, 2121.

Cope, C. L. (1931). *Biochem. J.* **25**, 1183.

Dodgson, K. S. (1961). *Biochem. J.* **78**, 324.

Dodgson, K.'S. & Lloyd, A. G. (1961). *Biochem. J.* **78**, 319.

Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **55**, 436.

Dodgson, K. S. & Spencer, B. (1954). *Biochem. J.* **57**, 310.

Dodgson, K. S. & Spencer, B. (1957). *Meth. biochem. Anal.* **4**, 211.

Gassner, K. & Friedel, H. (1956). *Z. anal. Chem.* **152**, 420.

Lloyd, A. G. (1959). *Biochem. J.* **72**, 133.

Lloyd, A. G., Dodgson, K. S., Price, R. G. & Rose, F. A. (1961). *Biochim. biophys. Acta*, **46**, 108.

Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L. & Farr, A. L. (1954). *J. biol. Chem.* **207**, 1.

Nigrelli, R. F., Chanley, J. D., Kohn, S. K. & Sobotka, H. (1955). *Zoologica, N.Y.*, **40**, 47.

Spencer, B. (1960). *Biochem. J.* **75**, 435.

Vlitos, A. J. (1953). *Contr. Boyce Thompson Inst.* **17**, 127.

Potassium Glucose 6-O-Sulphate as a Substrate for Glycosulphatase

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During a study of the glycosulphatase of *Littorina littorea*, Dodgson & Spencer (1954) observed anomalies in the behaviour of the enzyme towards increasing concentrations of potassium glucose monosulphate (prepared by direct sulphation of glucose and presumed to be glucose 6-O-sulphate), which they suggested might be due to the heterogeneity of the substrate preparation. Evidence for the presence of more than one sulphate ester in the preparation was obtained by paper chromatography and from experiments in which it was shown that about 25 % of the material could be desulphated by treatment with 0·33 M-

hydrazine at pH 5·3 and 38° (see also Egami, 1938, 1940, 1942). Further work on the enzyme was deferred until pure potassium glucose 6-O-sulphate could be obtained.

Recent work from these laboratories (Lloyd, 1960) has shown that glucose, potassium glucose 6-O-sulphate and dipotassium glucose disulphate are present in preparations of potassium glucose monosulphate obtained by direct sulphation of glucose. The present study shows that the partial hydrolysis of such preparations by hydrazine can be attributed to the hydrolysis of one of the sulphate groups present in potassium glucose

disulphate. It has also been demonstrated that heterogeneity of substrate is not responsible for the anomalous behaviour of *Littorina* glycosulphatase. Attempts to detect carbohydrate sulphatases in mammalian tissues are also described.

MATERIALS AND METHODS

Carbohydrate sulphates. Potassium glucose 6-*O*-sulphate, dipotassium glucose disulphate and the potassium salts of *N*-acetylglucosamine sulphate and *N*-acetylgalactosamine 6-*O*-sulphate were prepared by the method of Lloyd (1960). Potassium glucose 3-*O*-sulphate was obtained from di*iso*-propylidene glucose as described by Dodgson & Spencer (1954). Potassium galactose 6-*O*-sulphate was prepared by definitive synthesis (Lloyd, 1960), by column electro-phoresis of the products of the direct sulphation of galactose at 37° (Lloyd, 1960) and by a similar method after direct sulphation at 60–70° (cf. Turvey & Clancy, 1959). Di-potassium galactose disulphate was separated by column electrophoresis from the products of the direct sulphation of galactose at 60–70° (cf. Lloyd, 1960). Potassium chondroitin sulphate A and C were prepared from cartilage chondroitin sulphate (Dodgson, Lloyd & Spencer, 1957) by the fractionation procedure of Meyer, Davidson, Linker & Hoffman (1956). Potassium *N*-acetylchondrosine sulphate was prepared by enzymic degradation of chondroitin sul-phate A according to Dodgson & Lloyd (1958), and a crude mixture of the corresponding higher oligosaccharides (tetra-, hexa- and octa-saccharides) was obtained by degrading chondroitin sulphate A with testicular hyaluronid-ase (cf. Hoffman, Meyer & Linker, 1956).

Enzyme preparations. A partially purified preparation of glycosulphatase was prepared from the visceral region of *Littorina* according to the procedure of Dodgson (1961*b*). Homogenates of mammalian tissues were prepared as described by Dodgson, Spencer & Thomas (1953).

Determination of enzyme activity. The activity of the *Littorina* preparation was followed by determining liber-ated SO_4^{2-} ions by the method of Dodgson (1961*a*; method B). In following the activity of other enzyme pre-parations the benzidine micromethod (Dodgson & Spencer 1953) or the chloranilate procedure of Lloyd (1959) was used. An incubation temperature of 38° was used in all enzyme experiments.

Determination of rates of hydrolysis of carbohydrate sulphates by hydrazine. The non-enzymic hydrolysis of the mono- and di-sulphate esters of glucose and galactose was followed by estimating liberated SO_4^{2-} ions by the method of Dodgson (1961*a*). The following general procedure was adopted. A portion (1 ml.) of a solution (0·05M) of the sulphate ester in 0·5M-sodium acetate–acetic acid buffer, pH 5·3, was incubated at 38° with 1 ml. of a 0·66M-hydrazine hydrate solution which was prepared by ad-justing the requisite amount of the base to pH 5·3 with 0·5M-acetic acid and diluting to the appropriate volume with 0·5M-sodium acetate–acetic acid buffer, pH 5·3. At suitable time intervals, 0·2 ml. samples were withdrawn and added to 3·8 ml. of 4% trichloroacetic acid. Thereafter the method was as described by Dodgson (1961*a*; method A). Control determinations were made in which substrate and hydrazine were incubated separately, 0·1 ml. samples of each being withdrawn at the appropriate time and added

to 3·8 ml. of 4% trichloroacetic acid. When the amount of SO_4^{2-} ion liberated in the test solutions was greater than that covered by the calibration curve, then smaller samples (0·1 or 0·05 ml.) were withdrawn and, after mixing with the trichloroacetic acid, the necessary volume of water (0·1 or 0·15 ml.) was added before proceeding.

The effect of hydrazine on chondroitin sulphates A and C was examined as follows. A portion (2 ml.) of a 2% (w/v) solution of the sulphate ester in 0·5M-barium acetate–acetic acid buffer, pH 5·3, was incubated for 48 hr. at 38° with an equal volume of a 0·66M-hydrazine hydrate solution prepared as described above. The incubation mixture was acidified with a drop of conc. HCl and the precipitated $BaSO_4$ determined gravimetrically.

RESULTS

Hydrolysis of carbohydrate sulphates by hydrazine

Sulphate esters of glucose. Fig. 1 shows the effects of hydrazine on the potassium salts of the 3-*O*-, 6-*O*- and di-sulphates of glucose. The 3-*O*- and 6-*O*-sulphates were hydrolysed only slowly by hydrazine at 38°, whereas the corresponding di-sulphate was relatively rapidly hydrolysed under the same conditions. The amount of SO_4^{2-} ion liberated after 3 days was equivalent to approx. 31% of the total available in the substrate. Addition of extra hydrazine at this point does not significantly affect the rate of hydrolysis. The shape of the curve suggests that one sulphate group only is readily hydrolysed by hydrazine. Separate experiments in which the 3-*O*-, 6-*O*- and di-sulphates were incubated with hydrazine in the
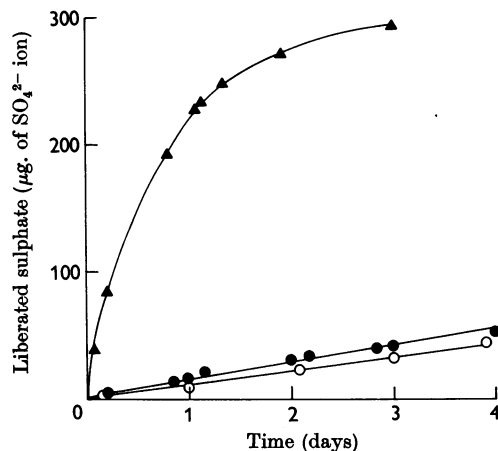

Fig. 1. Curves showing the rates of hydrolysis of 25 mM-solutions of potassium glucose 6-*O*-sulphate, potassium glucose 3-*O*-sulphate and dipotassium glucose disulphate by 0·33M-hydrazine at 38° in the presence of 0·5M-sodium acetate–acetic acid buffer, pH 5·3. Experimental points are calculated for a volume of 0·2 ml. of incubation mixture. ○, Potassium glucose 3-*O*-sulphate; ●, potassium glucose 6-*O*-sulphate; ▲, dipotassium glucose disulphate.

presence of glucose (final concn. 0·1M) showed that glucose did not significantly affect the rates of hydrolysis of the three substrates.

Sulphate esters of galactose. Dipotassium galactose disulphate was relatively rapidly hydrolysed by hydrazine (Fig. 2), the amount of SO_4^{2-} ion liberated after 3 days being equivalent to approx. 41 % of the total available in the substrate. Addition of extra hydrazine at this point does not significantly increase the rate of hydrolysis. The shape of the curve suggests that one sulphate group only is readily hydrolysed. The two different curves shown for galactose 6-O-sulphate are of some interest. The lower curve was obtained with substrate prepared by definitive synthesis and with substrate obtained by sulphation of galactose at 37°. The upper curve was obtained with substrate prepared by sulphation of galactose at 60–70°. No disulphate could be detected in this preparation and the curve may possibly reflect the presence of small amounts of an isomeric monosulphate in the preparation. Rees (1960 and personal communication) has indicated that crude glucose monosulphate and galactose monosulphate preparations obtained by direct esterification at high temperatures contain, in addition to the 6-O-sulphate and disulphate, small amounts of isomeric monosulphates.

Effect of pH. According to Egami (1938) the liberation of SO_4^{2-} ion from crude preparations of glucose monosulphate proceeded optimally at pH 5·2 and this led him to suggest that hydrazine could be regarded as a model for the enzyme glycosulphatase, a view which he subsequently modified. The effect of pH on the hydrolysis of the disulphates of glucose and galactose by hydrazine over a 90-min. period at 38° is shown in Fig. 3. Between the values 4·8 and 6·9, pH had relatively little effect on the rate of hydrolysis of either compound. Below pH 4·8 the rate of hydrolysis increased significantly. The curves were remarkably similar although galactose disulphate was desulphated at approximately four times the rate of that for the corresponding glucose derivative over this particular incubation period. These results differ from those obtained by Egami (1938) with crude glucose monosulphate preparations, although a strict comparison is not valid since Egami had to use long incubation periods and other experimental conditions were not identical.

Chondroitin sulphates A and C. Hydrazine was without effect on either compound over a period of 48 hr.

Effect of imidazole on carbohydrate sulphates

Imidazole is capable of hydrolysing various esters (see Bruice & Schmir, 1956, 1957), including some sulphate esters (K. S. Dodgson, unpublished results), and there is evidence that the active
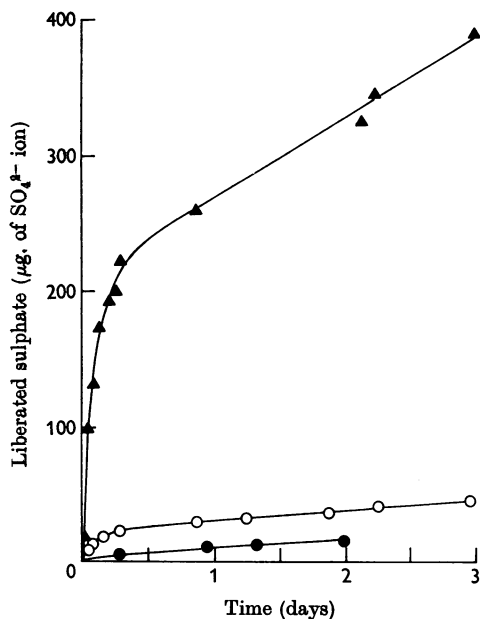

Fig. 2. Curves showing the rates of hydrolysis of 25 mM-solutions of potassium galactose 6-O-sulphate and dipotassium galactose disulphate by 0·33M-hydrazine at 38° in the presence of 0·5M-sodium acetate–acetic acid buffer, pH 5·3. Experimental points are calculated for a volume of 0·2 ml. of incubation mixture. ●, Potassium galactose 6-O-sulphate prepared by direct sulphation at 37° and by definitive synthesis (see text); ○, potassium galactose 6-O-sulphate prepared by direct sulphation at 60–70°; ▲, dipotassium galactose disulphate.
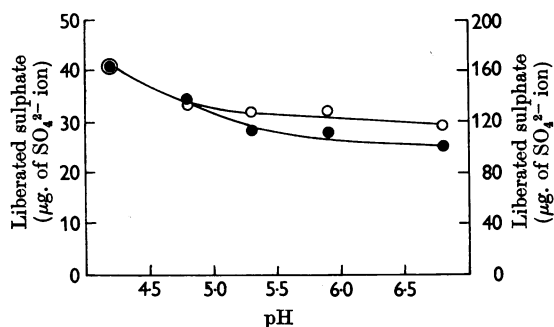

Fig. 3. Effect of pH on the hydrolysis of 25 mM-solutions of dipotassium glucose disulphate and dipotassium galactose disulphate by 0·33M-hydrazine in the presence of 0·5M-sodium acetate–acetic acid buffer. Incubation was for 90 min., at 38°, and experimental points are calculated for a volume of 0·2 ml. of incubation mixture. ○, Dipotassium glucose disulphate (left-hand ordinate scale); ●, dipotassium galactose disulphate (right-hand ordinate scale).

centres of several hydrolytic enzymes are functionally dependent upon imidazole groupings. It was therefore of interest to check the possibility that imidazole could also catalyse the hydrolysis of carbohydrate sulphate esters in a manner similar to that of hydrazine. The esters (0·1 ml. of a 0·05 M-solution in 0·5 M-sodium acetate–acetic acid buffer) was incubated with 0·1 ml. of a 0·4 M-solution of imidazole in the same buffer over a period of 18 hr. at 38°. Three different conditions of pH were used (pH 5·0, 6·5 and 7·5) with each substrate. Liberated SO_4^{2-} ion was determined according to Dodgson (1961a; method A). The 3-O-, 6-O- and di-sulphates of glucose and the 6-O-sulphate and disulphate of galactose were not affected by imidazole, irrespective of the pH of the incubation mixture.

Anomalous substrate concentration–activity curve of glycosulphatase

The substrate concentration–activity curve obtained by Dodgson & Spencer (1954) during the original studies on the enzyme is reproduced in
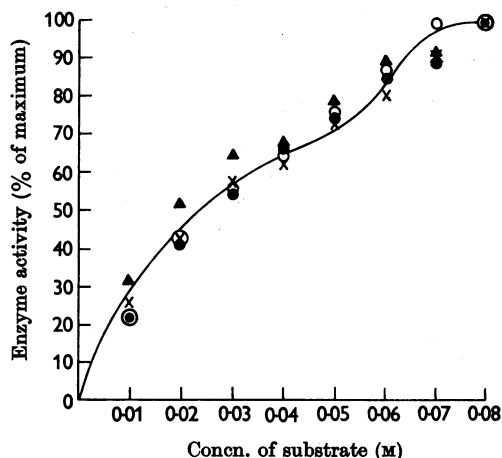

Fig. 4. Experimental points showing the effect of substrate concentration on the activity of partially purified preparations of *Littorina* glycosulphatase acting, in the presence of 0·5 M-sodium acetate–acetic acid buffer, pH 5·8, on the following substrates: O and ×, two separate preparations of potassium glucose 6-O-sulphate; ▲, potassium glucose 6-O-sulphate and dipotassium glucose disulphate mixture (4:1, w/w); ●, potassium glucose 6-O-sulphate and dipotassium glucose disulphate mixture (3:1, w/w). Where mixed substrates were used, calculations of molarity of substrate were made as though the 6-O-sulphate only was present. The continuous line is a reproduction, on the same scale, of the curve originally obtained by Dodgson & Spencer (1954) with crude preparations of potassium glucose monosulphate.

part (as the continuous line) in Fig. 4. Also reproduced in this Figure are the experimental points for a more highly purified preparation of the enzyme when pure potassium glucose 6-O-sulphate was used under the same conditions of buffer and pH. The experimental points fit closely to the original curve. Also shown are the curves obtained when pure samples of potassium glucose 6-O-sulphate and dipotassium glucose disulphate were mixed together in the proportions 3:1 and 4:1 and the mixtures then used as assay substrates. Again the experimental points fit reasonably well on the original curve. Clearly the anomalous nature of the original curve cannot be attributed to the presence of more than one sulphate ester in the preparations of crude glucose monosulphate as originally suggested by Dodgson & Spencer (1954). It will be shown later (Dodgson, 1961b) that no anomalies are present in substrate concentration–activity curves which are determined in the presence of 2-amino-2-hydroxymethylpropane-1:3-diol (tris)–acetic acid buffer.

The optimum pH of the *Littorina* enzyme when acting on 0·04 M-potassium glucose 6-O-sulphate in the presence of 0·5 M-sodium acetate–acetic acid buffer was in the region of 5·8 (Fig. 5). This agrees closely with the value of 5·85 obtained by Dodgson & Spencer (1954) with crude preparations of potassium glucose monosulphate.

Examination of mammalian tissues for carbohydrate sulphatases

Previous attempts to detect enzymes capable of liberating SO_4^{2-} ion from mono- or poly-saccharide sulphates have resulted in conflicting findings. Thus K. S. Dodgson & B. Spencer (unpublished results) failed to detect glycosulphatase in rat liver whereas T. Soda (personal communication) claimed
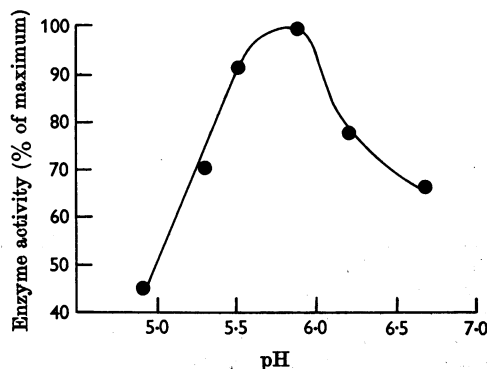

Fig. 5. Effect of pH on the activity of partially purified preparations of *Littorina* glycosulphatase towards 0·04 M-potassium glucose 6-O-sulphate in the presence of 0·5 M-sodium acetate–acetic acid buffer.

that the enzyme was present in whale and rabbit livers. Neuberg & Cahill (1936) and Dohlman & Friedenwald (1955) failed to observe liberation of sulphate from chondroitin sulphate by various tissue preparations. In contrast, the work of Dziewiatkowski (1956) and Dohlman (1956) suggested that enzymic liberation of sulphate from this compound did occur *in vivo*, and the results of Hall & Gardiner (1955) indicated that enzymic desulphation of endogenous polysaccharide sulphate occurred in pig-pancreas preparations.

Two types of substrate were used during the present attempts to detect mammalian carbohydrate sulphatases, namely simple monosaccharide sulphates (substrates for glycosulphatase) and chondroitin sulphate and the sulphated oligosaccharides derived therefrom (substrates for chondrosulphatase). The following tissues were examined: (a) fresh and acetone-dried pancreas, lung, kidney, liver, spleen, testis, ovary, brain and costal cartilage from M.R.C. hooded rats, age 3 and 12 months; (b) fresh and acetone-dried heart, knee cartilage, costal cartilage, pancreas, lung, kidney, liver, spleen, testis, ovary and brain of 4-day-old pigs; (c) acetone-dried post-mortem samples of pancreas, lung, kidney, liver, spleen and brain from a human male, age 58 years. All tissues were examined as 2 % (w/v) suspensions in 0·2 M-sodium acetate–acetic acid, tris–acetic acid and glycine–HCl buffers at several pH values within the range 3·5–8·5.

The potassium salts of the 6-*O*-sulphates of glucose, galactose, *N*-acetylglucosamine and *N*-acetylgalactosamine were used as substrates at a concentration of 25 mM. Potassium chondroitin sulphate A and the unfractionated mixture of higher oligosaccharides obtained by degradation of chondroitin sulphate with testicular hyaluronidase were used at a concentration of 0·5 %, whereas the concentration of *N*-acetylchondrosine sulphate was 0·05 %. Incubation of enzyme preparation and substrate was for 4, 12, 24 and 48 hr. at 38°.

In no case was it possible to detect liberation of SO_4^{2-} ion by the various tissue preparations.

DISCUSSION

It is now clear that the liberation of SO_4^{2-} ion from preparations of crude glucose monosulphate by hydrazine reflects the presence of glucose disulphate in such preparations. It has further been established that the anomalous substrate concentration–activity obtained when crude glucose monosulphate preparations were used as a substrate for *Littorina* glycosulphatase (Dodgson & Spencer, 1954) cannot be attributed to the presence in the preparation of sulphate esters other than the 6-*O*-sulphate.

The behaviour of hydrazine towards carbohydrate sulphate esters has been studied at some length by Egami (1938, 1940, 1942), who concluded that one of the prerequisites for the reaction was the presence, in the ester, of a potential reducing group, although hydrazine could be recovered unchanged at the end of the reaction. On these grounds chondroitin sulphates A and C would not be expected to undergo desulphation by hydrazine. The structure of glucose disulphate and galactose disulphate is clearly of some interest since in each case one of the two sulphate groups appears to be particularly labile to hydrazine. It is probably reasonable, in view of the high yields of the 6-*O*-sulphates by direct sulphation of glucose or galactose, to assume that one of the sulphate groups in the corresponding disulphates occupies position 6 of the hexose residue. The finding that potassium glucose 3-*O*-sulphate is desulphated at about the same rate as the corresponding 6-*O*-sulphate may further suggest that position 3 in the disulphate is not esterified. The principal difference between glucose and galactose lies in the relative spatial distribution of the hydroxyl group at C-4. It may therefore be of some significance that galactose disulphate is desulphated at about four times the rate of that for glucose disulphate over short incubation periods. However, examination of the infrared spectrum of galactose disulphate (Lloyd & Dodgson, 1961) has not provided any evidence for the presence of an ester sulphate grouping at C-4. Further progress now depends on the preparation of hexose monosulphates of known constitution.

Attempts to detect carbohydrate sulphatases in mammalian tissues have been unsuccessful, and it seems clear that such enzymes are confined to relatively simple organisms. Dodgson & Lloyd (1957) recently established that the chondrosulphatase of *Proteus vulgaris* was without action on polymer chondroitin sulphate. The enzyme was associated with an aminopolysaccharase which is able to depolymerize chondroitin sulphate. The sulphated oligosaccharides resulting from depolymerization were readily desulphated by chondrosulphatase. Similar conclusions have since been made for the enzyme desulphation of heparin by enzyme preparations from *Flavobacterium heparinum* (Meyer, Linker, Hoffman & Korn, 1957). For these reasons, sulphated oligosaccharides were tested as substrates during the present work. However, no enzyme capable of desulphating these substrates could be detected.

Potassium glucose 6-*O*-sulphate, prepared by the method of Lloyd (1960), is clearly a suitable substrate for the assay of glycosulphatase. Further support for this conclusion is presented in the next paper (Dodgson, 1961 b).

SUMMARY

1. Solutions of hydrazine catalyse the partial desulphation of the disulphate esters of glucose and galactose. Quantitative results show that, under standard conditions (pH 5·3 in 0·5 M-sodium acetate–acetic acid buffer at 38°), galactose disulphate is desulphated more rapidly than the corresponding glucose derivative and suggest that in each case one ester sulphate group only is readily removed.

2. The 6-O-monosulphate esters of glucose and galactose and the 3-O-monosulphate of glucose are not readily desulphated by hydrazine.

3. Unsuccessful attempts have been made to detect the presence in mammalian tissues of enzymes capable of liberating sulphate from monosaccharide sulphate esters, chondroitin sulphates A and C and from various sulphated oligosaccharides derived from chondroitin sulphate A.

REFERENCES

Bruice, T. C. & Schmir, G. L. (1956). *Arch. Biochem. Biophys.* **63**, 484.

Bruice, T. C. & Schmir, G. L. (1957). *J. Amer. chem. Soc.* **79**, 1663.

Dodgson, K. S. (1961a). *Biochem. J.* **78**, 312.

Dodgson, K. S. (1961b). *Biochem. J.* **78**, 324.

Dodgson, K. S. & Lloyd, A. G. (1957). *Biochem. J.* **66**, 532.

Dodgson, K. S. & Lloyd, A. G. (1958). *Biochem. J.* **68**, 88.

Dodgson, K. S., Lloyd, A. G. & Spencer, B. (1957). *Biochem. J.* **65**, 131.

Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **55**, 436.

Dodgson, K. S. & Spencer, B. (1954). *Biochem. J.* **57**, 310.

Dodgson, K. S., Spencer, B. & Thomas, J. (1953). *Biochem. J.* **53**, 452.

Dohlman, C. H. (1956). *Acta physiol. scand.* **37**, 220.

Dohlman, C. H. & Friedenwald, J. S. (1955). *J. Histochem. Cytochem.* **3**, 492.

Dziewiatkowski, D. D. (1956). *J. biol. Chem.* **223**, 239.

Egami, F. (1938). *J. chem. Soc. Japan*, **59**, 1034.

Egami, F. (1940). *J. chem. Soc. Japan*, **61**, 592.

Egami, F. (1942). *J. chem. Soc. Japan*, **63**, 763.

Hall, D. A. & Gardiner, J. E. (1955). *Biochem. J.* **59**, 465.

Hoffman, P., Meyer, K. & Linker, A. (1956). *J. biol. Chem.* **219**, 653.

Lloyd, A. G. (1959). *Biochem. J.* **72**, 133.

Lloyd, A. G. (1960). *Biochem. J.* **75**, 478.

Lloyd, A. G. & Dodgson, K. S. (1961). *Biochim. biophys. Acta*, **46**, 116.

Meyer, K., Davidson, E. A., Linker, A. & Hoffman, P. (1956). *Biochim. biophys. Acta*, **21**, 506.

Meyer, K., Linker, A., Hoffman, P. & Korn, E. D. (1957). In *Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto*, p. 132. London: Pergamon Press Ltd.

Neuberg, C. & Cahill, W. L. (1936). *Enzymologia*, **1**, 82.

Rees, D. (1960). *Nature, Lond.*, **185**, 309.

Turvey, J. R. & Clancy, M. J. (1959). *Nature, Lond.*, **183**, 537.

Glycosulphatase: Observations on the Activity of Partially Purified Preparations towards the Sulphate Esters of Certain Monosaccharides and Steroids

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The existence of glycosulphatase in the digestive organs of certain tropical marine molluscs was first noted by Soda & Hattori (1931). Between 1931 and 1948, in a series of publications (see Dodgson & Spencer, 1957a), Soda and his co-workers described various properties of the enzyme, although much of the quantitative work must be regarded with caution because of the impure nature of the enzyme substrate (potassium glucose monosulphate, see Dodgson & Lloyd, 1961). More recently Dodgson & Spencer (1954) showed the enzyme to be present in extracts of the digestive organs of some marine molluscs from British waters, including the common limpet (*Patella vulgata*) and the large periwinkle (*Littorina littorea*). Subsequently Roy (1956), during a study of the 3β-steroid sulphatase of *Patella*, noted that sodium cortisone 21-sulphate was desulphated by his enzyme preparations (see also Savard, Bagnoli & Dorfman, 1954) and suggested that glycosulphatase was the enzyme responsible.

In the present paper the properties of partially purified preparations of *Littorina* glycosulphatase are described. Evidence is also presented which