inhibitory concentration was less than that of androsterone ($4 \cdot 10^{-6} M$) used to mediate hydrogen transfer. An inhibitory influence was noted at a concentration as low as $3 \cdot 10^{-7} M$. In diminishing order the inhibitory potency of the Δ^4-steroids was: 3-oxo > 3α-hydroxy > 3β-hydroxy. Estradiol, 17α-ethyl-19-nortestosterone, hydrocortisone, and 11-dehydrocorticosterone were less effective inhibitors than 4-androstene-3,17-dione. The inhibitory influence produced by progesterone and 11-deoxycorticosterone was transitory with recurrence of hydrogen transfer after 10 min. The recurrence was probably due to saturation of Δ^4-bond by the Δ^4-3-ketosteroid hydrogenases in the system[4-7]. TOMKINS[8] reported that α,β-unsaturated steroids inhibited 3α-hydroxysteroid dehydrogenase of rat-liver homogenate.

The degree of inhibition of 3α-hydroxysteroid-mediated transhydrogenation correlated with the quantity of testosterone added. The extent of inhibition was greater when testosterone was added 10 min after mediation of the reaction than at the start. Testosterone inhibition of androsterone-mediated transhydrogenase depends on the relative concentration of DPN and TPN. Preliminary study indicates that a testosterone-dependent inhibitory substance may be present in the rat-liver homogenate.

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1 D. D. HAGERMAN AND C. A. VILLEE, *J. Biol. Chem.*, 234 (1959) 2031.
2 B. HURLOCK AND P. TALALAY, *J. Biol. Chem.*, 233 (1958) 886.
3 P. TALALAY, B. HURLOCK AND H. G. WILLIAMS-ASHMAN, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 862.
4 E. FORCHIELLI, K. BROWN-GRANT AND R. I. DORFMAN, *Proc. Soc. Exptl. Biol. Med.*, 99 (1958) 594.
5 E. FORCHIELLI AND R. I. DORFMAN, *J. Biol. Chem.*, 223 (1956) 443.
6 J. S. MCGUIRE AND G. M. TOMKINS, *Arch. Biochem. Biophys.*, 82 (1959) 476.
7 G. M. TOMKINS, *J. Biol. Chem.*, 225 (1957) 13.
8 G. M. TOMKINS, *Recent Progr. in Hormone Research*, 12 (1956) 125.

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Cellulose polysulfatase, an enzyme attacking cellulose polysulfate and charonin sulfate

An enzyme hydrolysing sulfuric ester bonds in cellulose polysulfate and charonin sulfate has been found in the liver extract of a marine gastropod, *Charonia lampas* (*Tritonalia sauliae*). Since chondroitin sulfate and amylose polysulfate are scarcely hydrolysed by the enzyme preparation, we propose to name the new sulfatase "cellulose polysulfatase".

The liver extract contains, besides cellulose polysulfatase, polysaccharases including cellulase and glucosulfatase, a sulfatase hydrolysing glucose sulfate and other sulfate esters of carbohydrates of low molecular weight[1]. The degradation of cellulose polysulfate by the enzyme preparation proceeds as follows:

$$\text{Cellulose polysulfate (S-rich)} \xrightarrow[\text{Cellulose polysulfatase}]{} \text{Cellulose sulfate (S-poor)}$$

$$\xrightarrow[\text{Polysaccharases}]{} \text{Glucose sulfates and glucose} \xrightarrow[\text{Glucosulfatase}]{} \text{Glucose}$$

Potassium cellulose polysulfate and potassium amylose polysulfate were prepared as usual by sulfation of cellulose and amylose respectively with chlorosulfonic acid in pyridine. (S contents: cellulose polysulfate, 17.5 %; amylose polysulfate, 15.75 %). Potassium charonin sulfate was prepared from the mucous gland of *Charonia lampas* according to EGAMI *et al.*[2] (S content, 16.2 %). It consists mainly of cellulose polysulfate. Sodium dextran polysulfate prepared as an artificial anticoagulant was kindly supplied by Dr. S. SASAKI[3] (S content, 17.7 %). Commercial sodium chondroitin sulfate (K. K. Seikagaku Kenkyusho) from shark cartilage was used (S content, 6.03 %).

The enzyme solution was prepared by homogenizing fresh liver (12 g) of *Charonia lampas* in 48 ml 0.1 *M* acetate buffer, pH 5.2, and centrifuging. The 100,000 × *g* supernatant, after dialysis, was used.

Unless otherwise mentioned, the enzyme activities were determined with a reaction mixture containing 2.5 ml enzyme solution, 15 mg polysaccharide sulfate (25 mg in the experiment of Table I), adjusted to 5 ml with 0.1 *M* acetate buffer, pH 5.2. A few drops of chloroform were added and the reaction mixture was incubated at 37°. Sulfatase activity was determined by estimating liberated inorganic sulfate by the method of DODGSON AND SPENCER[4] with the following pretreatment. To 1 ml of the reaction mixture, 2 ml of 1 % trypaflavin solution was added to precipitate polysaccharide sulfates in the reaction mixture. To 2 ml of the supernatant, ethanol, trichloroacetic acid and benzidine solution were added as described by DODGSON AND SPENCER. Polysaccharase activity was determined by estimating reducing power by the method of FOLIN AND MALMROS[5] after deproteinization with cadmium hydroxide gel.

As shown in Table I, charonin sulfate and cellulose polysulfate were rapidly desulfated, and dextran polysulfate only slowly. Amylose polysulfate and chondroitin sulfate were scarcely attacked.

TABLE I

SPECIFICITY OF CELLULOSE POLYSULFATASE

Substrates	*Liberated sulfate (%) after*		
	24 h	*72 h*	*120 h*
Charonin sulfate	38.1	74.6	92.9
Cellulose polysulfate	25.7	41.6	65.4
Dextran polysulfate	2.7	8.2	13.0
Amylose polysulfate	0	0	0.5
Chondroitin sulfate	0	0	0

As shown in Table II, 0.02 *M* sodium fluoride completely inhibited polysaccharase, but only slightly cellulose polysulfatase.

Charonin sulfate and cellulose polysulfate were digested for 3 days under the

TABLE II

EFFECT OF FLUORIDE ON POLYSACCARASES AND CELLULOSE POLYSULFATASE

Time (h)	*Liberated sulfate (%) from cellulose polysulfate*		*Liberated reducing power (% estimated as glucose for the complete hydrolysis)*	
	No inhibitor	*+ NaF*	*No inhibitor*	*+ NaF*
24	68.5	43.4	20.1	0

usual experimental conditions. 72.6 % of the sulfate and 57.2 % of the reducing power were liberated in the experiment with charonin sulfate, and 64.2 % and 29.1 %, respectively, in the case of cellulose polysulfate. For the identification of the reaction products, both reaction mixtures (each 4 ml) were boiled and centrifuged. To the supernatants, 400 mg of norit pretreated with *N* HCl were added, stirred and centrifuged. The sediments were washed 3 times with 2 ml water, then eluted several times with 5 % aq. pyridine[6]. The eluates were dried in the cold and subjected to paper chromatography with glacial acetic acid–*n*-butanol–water (15 : 50 : 35) as solvent[7], developed with aniline phthalate. Only one spot, identified with that of glucose, was obtained from both reaction mixtures. Neither disaccharides nor glucose sulfates were found to be present. On the other hand, in the presence of fluoride, S-poor cellulose sulfate was obtained. Cellulose polysulfate was incubated with the enzyme solution for 3 days as described above but with 0.02 *M* sodium fluoride. No increase of reducing power was detected during the incubation. After deproteinization, the reaction mixture was thoroughly dialyzed to remove inorganic sulfate, and ester sulfate and glucose in the non-dialyzable product were estimated. The ratio $\frac{\text{ester sulfate}}{\text{glucose}}$ was found to be 0.65. After further digestion of the product with fresh enzyme solution the ratio decreased to 0.5. Thus, the final product may be regarded as cellulose sulfate containing, on the average, one sulfate group for each cellobiose unit.

The purification of cellulose polysulfatase and the separation from polysaccharases are now in progress.

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[1] T. SODA, *J. Fac. Sci. Univ. Tokyo*, 3 (1936) 249.
[2] F. EGAMI, T. ASAHI, N. TAKAHASHI, S. SUZUKI, S. SHIKATA AND K. NISIZAWA, *Bull. Chem. Soc. Japan*, 28 (1955) 685.
[3] S. SASAKI, T. TAKEMOTO AND S. OKA, *Compt. rend. soc. biol.*, 151 (1957) 1799.
[4] K. S. DODGSON AND B. SPENCER, *Biochem. J.*, 55 (1953) 436.
[5] O. FOLIN AND H. MALMROS, *J. Biol. Chem.*, 83 (1929) 115.
[6] P. HOFFMAN, K. MEYER AND A. LINKER, *J. Biol. Chem.*, 219 (1956) 653.
[7] B. WEISSMANN, K. MEYER, P. SAMPSON AND A. LINKER, *J. Biol. Chem.*, 208 (1954) 417.

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