obtained agreed with the 21-monoacetate of I by physical constants and infra-red spectrum. This result indicates that the newly introduced hydroxy group must be 14α-hydroxyl.

From these results we conclude that the structure of I is 14α-hydroxy-11-dehydrocorticosterone. This fungus, however, had no effect on the conversion of epicorticosterone and hydrocortisone. This fact shows the marked stereospecificity of this dehydrogenase, that is, it acts only on 11β-hydroxyl of corticosterone and not on that of hydrocortisone or 11α-hydroxyl of epicorticosterone.

With the other steroid substrate, this fungus also showed remarkable substrate specificity as reported previously[5]. That is, it hydroxylated progesterone or deoxycorticosterone at the 14α-position and 17α-hydroxyprogesterone or Reichstein's compound *S* at the 11α-position.

We wish to acknowledge with thanks the helpful advice of Profs. K. Tsuda and T. Asai, of the University of Tokyo.

M. Shirasaka
R. Takasaki
M. Tsuruta

Takamine Laboratory,
Sankyo Co., Ltd.,
Tokyo.

[1] Talalay, P., and Dobsen, M. M., *J. Biol. Chem.*, **205**, 823 (1953). Turfitt, G. E., *Biochem. J.*, **40**, 79 (1946), etc.
[2] Hoehn, W. M., Schmidt, L. H., and Hughes, H. B., *J. Biol. Chem.*, **152**, 59 (1944).
[3] Hanson, F. R., Mann, K. M., Nielson, E. D., Anderson, H. V., Brunner, J. N., Colingsworth, D. R., and Haines, W. J., *J. Amer. Chem. Soc.*, **75**, 5369 (1953).
[4] Meyster, Ch., Vischer, E., and Wettstein, A., *Helv. Chim. Acta*, **37**, 1548 (1954).
[5] Shirasaka, M., Hayashi, R., and Tsuruta, M., *Bull. Agric. Chem. Soc. Japan*, **23**, 244 (1959).

Enhancement of Peroxidase Action by Polysaccharides

A number of polysaccharides act as polymerization-directing matrices in peroxidase-catalysed eugenol oxidations[1]. Although deoxyribonucleic acid and cellulose increase markedly the rate of Fe(III)-catalysed pyrogallol oxidation by hydrogen peroxide[2,3], a similar test of polysaccharides has not been reported previously in peroxidase-catalysed reactions.

The present results show that the catalytic activity of peroxidase is increased appreciably by moderately low concentrations of starch and is somewhat influenced by methylcellulose as well.

Oxidations were followed at 25° C. with the Bausch and Lomb 'Spectronic 20' spectrophotometer in 10-ml. reaction mixtures containing 5 μgm. Worthington horseradish peroxidase, 10^{-2} *M* hydrogen peroxide, substrate and *M*/20 phosphate buffer, *p*H 4·5. Oxidation of 5×10^{-3} *M* pyrogallol was measured at 425 mμ; oxidation of 5×10^{-4} *M* eugenol was measured at 360 mμ.

The chemical substances used were of reagent grade when possible. Methylcellulose solutions were prepared from 'Dow Methocel *HG* 400'. Neither starch nor methylcellulose exhibited catalytic activity when tested directly with hydrogen peroxide and pyrogallol.

In ten experiments, 5×10^{-5} *M* starch increased oxidation of pyrogallol by peroxidase appreciably (Table 1). The initial rate (0·5 min.) was most affected, but considerable enhancement is still in evidence after 2–3 min., when the control reaction-rate has slowed markedly.

Table 1. Effect of Starch on Peroxidase-catalysed Pyrogallol Oxidation*

Reaction time (min.)	(a) Control Average optical density	σ	(b) Starch* Average optical density	σ	b/a (percentage control)
0	0	—	0	—	—
0·5	0·098†	± 0·018	0·153	± 0·016	156
1·0	0·254	± 0·030	0·373	± 0·019	147
1·5	0·437	± 0·036	0·597	± 0·023	137
2·0	0·635	± 0·036	0·847	± 0·023	133
3·0	0·775	± 0·062	1·033	± 0·047	133

* Reaction mixture: 10 ml. *M*/20 phosphate, *p*H 4·5, containing 5×10^{-3} *M* pyrogallol, 10^{-2} *M* hydrogen peroxide, 5 μgm. peroxidase, with or without 5×10^{-5} *M* starch. Oxidation was carried out at 25° C. and measured at 425 mμ.
† Each figure is the mean of 10 determinations.

Stimulation is not limited to the oxidation of pyrogallol, but is also obtained with eugenol as substrate (Table 2*b*).

Table 2. Effect of Several Carbohydrates on the Peroxidation of Pyrogallol and Eugenol*

Carbohydrate	Concentration (*M*)	Effect (percentage control after 1 min.)
	(a) Substrate: pyrogallol	
Glucose	5×10^{-5}	100
	10^{-3}	99
	0·5	84
Maltose	5×10^{-5}	103
	10^{-3}	100
	0·5	86
Methylcellulose	5×10^{-5}	117
Starch	5×10^{-5}	147
	(b) Substrate: eugenol	
Glucose	0·5	83
Methylcellulose	5×10^{-5}	125
Starch	5×10^{-5}	143

* Reaction conditions as given in Table 1 except that eugenol was used at 5×10^{-4} *M* and its oxidation followed at 360 mμ.

Methylcellulose is not as stimulating as starch when tested with either substrate, but the activity of these two macromolecules may be contrasted with the effects of glucose and maltose (Table 2*a*, *b*). The simple sugars have no effect in low concentrations, and they are slightly inhibitory at high concentrations. A proper comparison between simple sugars and polysaccharides cannot be made on a mole-for-mole basis, nor can they be equated in a simple way by comparing the number of monosaccharide residues present.

A macromolecular basis for the stimulatory effects of polysaccharides is suggested by the absence of enhancement of sugars over a wide range of concentrations. Possibly the polysaccharides provide a concentrating surface, hence increasing the probability of reaction, and render adsorbed peroxidase more efficient as a catalyst. These suggestions receive support from the greater effect of starch on pyrogallol oxidation at lower peroxidase concentrations. Thus, with 0·5 μgm. of enzyme, an initial simulation (0·5 min.) of 100 per cent was observed, declining to 50 per cent after 3 min.

This work was carried out at the University of Rochester with the support of Public Health Service Grant *C*–2730, National Institutes of Health.

B. Z. Siegel
4 Orchard Parkway,
White Plains, New York.

S. M. Siegel
Union Carbide Research Institute.
White Plains, New York.

[1] Siegel, S., in "Sub-cellular Particles", edit. by Hayashi, T., (Amer. Physiol. Soc., Washington, 1959).
[2] Siegel, S., and Siegel, B. Z., *Nature*, **179**, 421 (1957).
[3] Goodman, N. S., and Siegel, S., *Nature*, **184**, 53 (1959).