Some Properties of Laccase from the Latex of Lacquer Trees

WE have previously shown¹ that laccase, the oxidizing enzyme present in the latex of the Indo-Chinese lacquer tree (*Rhus succedanea*), is a copper-protein compound. Our purest preparation of this enzyme, which catalyses the oxidation of polyphenols and diamines, contained 0.154 per cent copper and 6.45 per cent nitrogen, corresponding to about 45 per cent protein. The remaining fraction of the preparation was composed of a polysaccharide. This preparation had a strong blue colour which disappeared reversibly on the addition of reducing substances or substrates and irreversibly on heating the enzyme to about 60° C. or acidifying it.

It was, however, impossible to ascertain whether this deep blue colour was due to a special combination between the copper and the enzyme protein or to a colouring substance present in our preparations.

In order to determine the relationship between the colour and the enzyme we have made an attempt to obtain and study this enzyme from other sources such as the latexes of the Japanese and Burmese lacquer trees (*Rhus vernicifera* and *Melanorrhæa usitata*). As a result of this study we are now able to state that the laccases obtained from all three sources are copper-protein compounds and that the blue colour of all these enzyme preparations is not due to the copper but to the presence in these preparations of a blue pigment, the nature of which is under investigation. Although we have been unable to obtain an active enzyme preparation free from the blue pigment, the latter can be easily obtained completely free of copper and protein but always mixed with carbohydrates. The pigment preparation separated from the enzyme can be reversibly reduced to its leuco compound and irreversibly decolorized by strong acids and by boiling. The facts that the blue pigment of an active enzyme preparation is much more sensitive to acids and to heating than the free pigment, and that a colourless enzyme preparation could not be obtained, suggest the possibility that the pigment is in some way connected with the normal activity of the enzyme. It is, however, impossible to say without further study of the problem whether the pigment is intimately bound to the enzyme molecule as an additional prosthetic group or is bound to a carbohydrate and acts as a carrier between the enzyme and certain substrates. The purest laccase preparation we have so far obtained contains 0.24 per cent copper and is still of a deep blue colour.

Although laccase shows a very wide range of activity, extending to a great variety of *ortho*- and *para*-phenols, diamines and aminophenols, it does not catalyse the oxidation of monophenols such as *p*-cresol. This peculiar property of laccase preparations suggested at first that these preparations are devoid of an additional factor essential for this reaction and present in crude extracts of mushrooms or potatoes. It was, however, a matter of some surprise to us to find that the addition of such crude extracts to laccase preparation not only fails to reactivate it in relation to *p*-cresol, but also that the crude extract itself in presence of laccase loses its ability to catalyse the oxidation of this monophenol.

This peculiar inhibitory property of laccase preparations can only be ascribed to laccase itself and not to some additional factor present in the preparation. In fact, this property is not affected by dialysis,

it is absent in blue pigment preparations devoid of laccase and it disappears from laccase preparation on boiling or after any other treatment which destroys the enzyme.

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Inactivation of Prolactin by Treatment with Phenyl Isocyanate

PHENYL ureido derivatives of serum albumin peptone¹, plastein², and native proteins³, have been prepared by treatment with phenyl isocyanate. This reaction is eminently suitable for masking the free amino groups of protein molecules without doing further damage, since the reaction conditions are very mild (0° C. and pH 8). Hopkins and Wormald³ concluded that phenyl isocyanate reacts only with the free amino groups of the lysine components of the protein molecules.

In view of reports that prolactin, the anterior pituitary hormone which stimulates the pigeon crop-gland, can be inactivated by acetylation with ketene⁴, and treatment with nitrous acid⁵, we were led to compare the crop-stimulating activity of prolactin with that of its phenyl ureido derivative.

Phenyl ureido prolactin was prepared by treating a solution of purified prolactin from ox pituitary with phenyl isocyanate at pH 8 and 0°, diphenyl urea from excess phenyl isocyanate being removed by centrifuging. The pigeon crop-gland stimulating activities of this and a control solution were estimated by Rowlands⁶ systemic method.

Test liquid	No. of birds	No. of daily injections	Volume injected (ml.)	Mean crop wt. × 100 body wt.
Prolactin	9	6	1	1.12
Phenyl ureido prolactin	10	6	1	0.45

The results in the accompanying table show that destruction of the free amino groups by reaction with phenyl isocyanate has resulted in a very marked loss of activity. The percentage inhibition (see Folley⁷) was approximately 87. The observations of Evans and his co-workers^{4,5}, that the crop-stimulating activity of prolactin depends on the presence in the molecule of free amino groups, appear therefore to be confirmed. These experiments are being continued.

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⁷ Folley, S. J., *Endocrinology*, **24**, 814 (1939).