—is the result of a radical transfer mechanism while the formation of thiyl radical in the former case follows the absorption of light quanta by cystine.

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[1] Swanepoel, O. A., and Louw, D. F., *J. S. African Chem. Inst.*, **16**, 31 (1963).
[2] Swanepoel, O. A., *J. S. African Chem. Inst.*, **16**, 48 (1963).
[3] Swanepoel, O. A., and Van Rensburg, N. J. J., *Bull. Inst. Textile France* (in the press).
[4] Walling, C., and Rabinowitz, R., *J. Amer. Chem. Soc.*, **81**, 1243 (1959).
[5] Swanepoel, O. A., and Van Rensburg, N. J. J. (in preparation).
[6] Ellman, G. L., *Arch. Biochem. Biophys.*, **82**, 70 (1959).
[7] Swanepoel, O. A., and Van Rensburg, N. J. J. (to be published).
[8] Hettler, H., in *Chromatographic Reviews*, edit. by Lederer, M., **1**, 225 (Elsevier Publ. Co., Amsterdam, 1959).

Reversible Action of Pullulanase

THE enzyme pullulanase[1] is a bacterial *R*-enzyme in that it is capable of cleaving certain α 1→6 glucosidic bonds where these occur together with α 1→4 glucosidic bonds as in pullulan, starch and dextrins. The substrate specificity of this enzyme has recently been reported[2] to embrace oligosaccharides and dextrins containing as few as two 1→4 linked α-D-glucopyranoside units in the '*A*' chain and two such units in the '*B*' chain. Thus, a tetrasaccharide linked 2 on 2 is the smallest known substrate for the hydrolytic action of this enzyme[2].

A recently developed technique[3] for examining enzyme action on micro-quantities of individual compounds of a mixture is to separate the compounds in one dimension by chromatography of a single spot on a large sheet of filter paper. The resolved components are then sprayed with an appropriately buffered enzyme, dried, and the ensemble is submitted to chromatography in a direction at right angles to the original direction. Use of this technique revealed that pullulanase acts on branched oligosaccharides (from action of malt α-amylase on amylopectin) to give the expected G_2 (maltose), G_3, and higher linear oligosaccharides. Unexpectedly, a small amount of material with the R_F of a branched tetrasaccharide was also formed from maltose. Thus, there is an apparent synthetic reaction.

We have tested this synthetic action of pullulanase on a larger scale by treating carefully purified, chromatographically pure G_2 with pullulanase, and subjecting the digest to paper chromatography. A well-defined spot appeared in the region characteristic for a tetrasaccharide containing a 1→6 linkage. In addition, there was a weak spot corresponding to a hexasaccharide containing two 1→6 links. No other compounds apart from G_2 appeared. The extent of the reaction was found to be concentration-dependent, in that little tetrasaccharide formed at a G_2 concentration of under 5 per cent, and the amount of tetrasaccharide increased with increasing concentration up to the highest concentration of G_2 we tested (about 25 per cent). Absence of other compounds, specifically D-glucose, indicates that disproportionation or transfer reactions were not involved.

Using G_3 as substrate, we obtained a spot corresponding in position to a hexasaccharide containing a 1→6 link, with no other compounds (except G_3) apparent.

These observations indicate that pullulanase action is reversible through condensation reactions, for example: 2 G_2 → tetrasaccharide + H_2O. With G_2 as substrate, there are two possible 2 on 2 structures; with G_3, there are three possible 3 on 3 structures. Studies in progress should indicate the extent to which each of these structures is produced during the reaction. The interesting possibility arises that pullulanase may condense G_2 and higher oligosaccharides with relatively long amylose chains. Such reactions could occur during the action of pullulanase on amylopectin, thus preventing complete attack by β-amylase. In a test system containing G_2 plus amylose there was definite evidence for condensation.

Similar condensation reactions should be possible with amylase; and, in fact, we were able to obtain a weak G_4 spot from G_2 by action of β-amylase. The amount produced was much less than the amount of tetrasaccharide formed by pullulanase action, however, owing to the difference in free energy of hydrolysis of 1→4 and 1→6 glycosidic bonds.

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[1] Bender, H., and Wallenfels, K., *Biochem. Z.*, **334**, 79 (1961).
[2] Abdullah, M., Catley, B. J., Lee, E. T. C., Robyt, J., Wallenfels, K., and Whelan, W. J., *Cereal Chem.*, **43**, 111 (1966).
[3] French, D., Pulley, A. O., Effenberger, J. A., Rougvie, M. A., and Abdullah, M., *Arch. Biochem. Biophys.*, **111**, 153 (1965).

Influence of Cortisone on the Substrate-induction of Enzymes in Rat Liver

Civen and Knox[1,2] and Feigelson and Greengard[3,4] concluded from their experiments with tryptophan-pyrrolase and tyrosine-α-ketoglutarate-transaminase that in animal tissues a distinction can be made between the two types of induction: one caused by substrate and the other caused by hormone. While substrate-induction was found to be due to stabilization of the enzyme and saturation with coenzyme, the cortisone acted by stimulating the synthesis of *m*RNA. The latter process could be inhibited by actinomycin *D*.

Previously we showed that in intact animals actinomycin *D* significantly inhibited substrate-induction of both the enzymes mentioned heretofore[5]. Substrate-induction, therefore, might also be connected with synthesis of *m*-RNA.

We used adrenalectomized rats for our investigations. Tryptophan-pyrrolase and tyrosine-α-ketoglutarate-transaminase were induced either by substrate or small amounts of cortisone or by the combination of both. The activity of tryptophan-pyrrolase was measured by the method of Knox *et al.*[6], the one of tyrosine-α-ketoglutarate-transaminase according to Rosen *et al.*[7].

On injection of 500 mg/kg L-tryptophan the enzyme activity increased 6-fold (see Table 1). This was due, of course, to the stabilization of the enzyme and to the saturation with coenzyme. Actinomycin *D* cannot change this effect. 2·5 mg/kg cortisone instead of L-tryptophan did not bring about any increase of activity. However, in the case of substrate applied together with cortisone, the hormone in an amount which cannot induce tryptophan-pyrrolase by itself, the enzyme activity even rose ten times as much. The additional stimulation is completely prevented by actinomycin *D*.

Neither the injection of 600 mg/kg L-tyrosine nor of 2·5 mg/kg cortisone led to an increase of the enzyme activity (see Table 2). But in combining the two substances one observed a 3-fold higher activity than in the control. Again, this effect does not take place in the presence of actinomycin *D*.

Table 1. INDUCTION OF TRYPTOPHAN-PYRROLASE IN ADRENALECTOMIZED RATS

+ Cortisone	+ L-Tryptophan	+ Cortisone + L-Tryptophan	+ Cortisone + L-Tryptophan + Actinomycin *D*	+ L-Tryptophan + Actinomycin *D*
1·0 ± 0·4*	0·7 ± 0·3	6·4 ± 1·3	10·3 ± 1·3	5·9 ± 2·3	6·2 ± 2·4

* μMoles kynurenin/g liver/h.

The animals were killed 3 h after the intraperitoneal application of the substances. L-Tryptophan, 500 mg/kg: cortisone, 2·5 mg/kg; actinomycin *D*: 450 μg/kg 1 h before the other substances. Each point 6–10 animals.