no enhancement of cytochrome P-450 with oleic acid itself, but this may not enter the yeast easily because of its acidity. If the Tween-80 is causing a substrate induction effect in our yeast, this would suggest a use for the cytochrome P-450 in the yeast on the assumption that hydroxyoleic acid was a required material, perhaps for the subsequent formation of mitochondrial membrane on return to conditions of mitochondriogenesis. However, the 17-hydroxylation of oleic acid may not occur in Saccharomyces spp., for in Torulopsis spp. this hydroxylation reaction presumably leads to degradation of substrate to yield energy for growth.


Effect of Steric Exclusion by Dextran on the Conversion of Fibrinogen into Fibrin

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Previous work (Rampling, 1974, 1976) has shown that the ability of the polysaccharide dextran to precipitate fibrinogen from solution is due solely to steric exclusion (Ogston, 1958), increasing the effective concentration of fibrinogen. The present work examines the effect of dextran on the thrombin-induced conversion of fibrinogen into fibrin.

The influence of dextran on the time of fibrin gel formation after the addition of thrombin to fibrinogen solution is illustrated in Fig. 1. Dextran has an accelerating effect on the clotting process, which increases with the molecular weight of the dextran. More particularly, Fig. 1 shows that the logarithm of the clotting time is linearly related to dextran concentration, except at high dextran concentration. It has previously been shown by Abildegaard (1966) that dextran has no significant effect on the rate of proteolysis of fibrinogen by thrombin, and so the effect on clotting time reported above must be due to its influence on fibrin polymerization alone. This was investigated directly as follows. Solutions of fibrin monomers free of other plasma proteins were produced by dissolving purified fibrin clots in NaBr/sodium acetate buffer, pH 5.3 (Latallo et al., 1962). Polymerization was induced by a tenfold dilution with phosphate buffer, pH 6.0, and followed at 350 nm in a spectrophotometer. As shown in Fig. 2 the presence of dextran increased the polymerization rate, and again the higher the molecular weight the greater the effect.

In view of the explanation of the effects of dextran on fibrinogen solubility in terms of steric exclusion, it is tempting to consider whether accelerated fibrin monomer polymerization may be similarly explained. If this concept is applied to a solution containing both dextran and fibrinogen it can be shown that the fraction of the volume, $P$, available to the fibrinogen is related to the dextran concentration $D$ by:

$$P = \exp(-KD)$$

where $K$ is a constant for a particular dextran fraction. Thus if the concentration of the
fibrinogen was originally $\phi_0$, then the concentration $\phi$ in the decreased aqueous space available to it as a result of the added dextran will be given by:

$$\log_\phi = \log_\phi_0 + KD$$  \hspace{1cm} (2)

It has been shown elsewhere (Vermylen et al., 1963) that the clotting time $C$ of a fibrinogen solution is related to the fibrinogen concentration $\phi$ by

$$\log_\phi C = A \log_\phi \phi + B$$  \hspace{1cm} (3)

where $A$ and $B$ are constants for a given thrombin concentration. Thus if steric exclusion is to explain the effect of dextran on the clotting time, the relationship between clotting time and dextran concentration must be of the form (from eqns. 2 and 3):

$$\log_\phi C = AKD + A \log_\phi \phi_0 + B$$  \hspace{1cm} (4)

That is to say, there should be a linear semi-logarithmic relationship between clotting time and dextran concentration. A further consequence of the steric-exclusion principle is that the effect should increase with increasing molecular weight of the dextran.

Fig. 1. Effect of various concentrations of the dextran fractions on the clotting time of a fibrinogen solution

The final fibrinogen concentration was 4.56 mg/ml. •, T10; ■, T20; ▲, T40; ○, T100 (fraction T10 has a mol. wt. of 10000 etc.). Final thrombin concentration was 0.8 unit/ml.
Fig. 2. Polymerization of fibrin monomers in the presence of 1% dextran

Absorbance was followed spectrophotometrically at 350 nm. The fibrin monomer concentration was 4.1 mg/ml in 1 M-bromide/0.05 M-acetate buffer, pH 5.3. Polymerization was initiated by tenfold dilution with 0.1 M-phosphate buffer, pH 6.0 (○). Control experiments were also performed by dilution with the bromide/acetate buffer containing dextran (▲), which did not cause polymerization.

(Rampling, 1974). The results presented above clearly conform to this latter requirement. They also conform to the first provided that the dextran concentration is not too high; presumably, if the dextran concentration is too high, the effective fibrinogen/fibrin concentration becomes large enough to lead to significant intermolecular interaction, which results in the breakdown of the Ogston (1958) principle presented here.

It is, therefore, concluded that the action of dextran on the rate of the fibrinogen-to-fibrin transition is due to steric exclusion by the dextran polymers, resulting in an effective increase in the concentration of the fibrinogen/fibrin in the solution.

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