The absence of UPPGA in the Type I pneumococcus suggests that the uridine pyrophosphogalacturonic acid is derived from UPPGal by a mechanism similar to that recorded[9] for the conversion of UPPG to UPPGA, and not by a Waldenase type of enzyme acting on UPPGA.

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Received December 17th, 1956

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A lipid present in yeast with certain properties similar to those of tocopherol

The unsaponifiable fractions of the lipids present in Fleischmann Yeast 50B and *Torula* yeasts (1 N and 3 N from Lake States Yeast Corporation, Rhinelander, Wisc.) have been shown to contain a substance which gives the Emmerie-Engel test. Moreover it behaves similarly to the tocopherols when chromatographed on $ZnCO_3$-coated filter paper according to the method of GREEN *et al.*[1], and has been shown to protect the erythrocytes of vitamin E-depleted rats from haemolysis *in vitro* in the test described by CHRISTENSEN *et al.*[2].

Two methods were used to isolate the unsaponifiable matter from the yeast:

(1) The yeast was heated under reflux (in presence of pyrogallol and in an atmosphere of N_2) with methanolic KOH until attack appeared complete (1–2 h). The mixture was then diluted with water and extracted with ether. Under these conditions, particularly with Fleischmann yeast, a lipid material sparingly soluble in benzene was produced.

(2) The yeast was extracted in the Soxhlet apparatus for 24 h with absolute ethanol containing 2% (w/v) dry HCl and 2% (w/v) ascorbic acid, the ethanol extract being then diluted with an equal vol. of water and extracted with petroleum ether. The lipid from an aliquot of the petroleum ether was saponified with ethanolic alkali in presence of pyrogallol and the unsaponifiable matter extracted with ether[3]. This method yielded 5–6% of total lipid, but in the case of Fleischmann yeast the unsaponifiable matter was considerably less than that obtained by the first method. No satisfactory method has been found for fractionating the unsaponifiable matter. Direct chromatography on $ZnCO_3$-coated filter paper gave a diffuse band of reducing material, and pretreatment with Filtrol earth resulted in some adsorption of this reducing material.

The method of GREEN *et al.* for chromatography of tocopherols was modified by the use of benzene as developing solvent, since cyclohexane was found to be unsatisfactory with the zinc carbonate obtainable here. Development with this solvent for one hour gave the following R_F values for the tocopherols: α 0.74; γ 0.58; δ 0.36. A reducing substance present in the yeasts had a mean R_F value of 0.50, although this varied somewhat depending on the purity of the spotting solution. The band was rather diffuse, but was always distinguishable from the clear-cut bands given by added β- or δ-tocopherols. Fleischmann yeast contained a substance of reducing power equivalent to 50 μg α-tocopherol/g yeast. *Torula* yeast contained more than six times this amount. Some reducing material also remained on the origin of the chromatogram.

The material obtained by alkaline hydrolysis of the yeast was purified by freezing out sterols and by passing the supernatant, in benzene solution, through a column of Filtrol earth purified by treatment with hydrochloric acid and stannous chloride[3]. Some of the reducing material was adsorbed to give a purple band. Chromatography on paper of the purified material resulted in the recovery of less than 50% of the total reducing matter present in the ethanolic spotting solution. When stored at 5° C this ethanolic solution showed no loss of reducing power, but when diluted with benzene A.R. quality (Merck) the total reducing power fell by more than

50% in the course of a few days, giving a value equal to that obtained by paper chromatography with benzene as developing solvent. The cause of this was not determined, although on some chromatograms a reducing band of R_F value approximately 0.64 was obtained, suggesting molecular rearrangement or partial oxidation as possible causes.

Molecular distillation of the unsaponifiable material at 100° C for 1 hour showed that less than 1% of the reducing material passed into the distillate, whereas added α-tocopherol could be recovered quantitatively.

With the ferric chloride-$\alpha\alpha'$-dipyridyl reagents in the concentrations used by GREEN *et al.*[1], a steady spectrophotometer reading was obtained after 2 minutes with purified samples. The compound gave no colour with the diazotised di-*o*-anisidine–sodium carbonate reagent.

Purified solutions of the compound were chromatographed on paper and the reducing zone cut out and eluted with ethanol. The substance obtained in this way showed an anti-haemolytic activity comparable with that of an amount of α-tocopherol of equivalent reducing power when tested on the erythrocytes from vitamin E-depleted rats by the method of CHRISTENSEN *et al.*[2] (incubation at 42° C in physiological saline without the addition of dialuric acid). This temperature was chosen to give a convenient rate of haemolysis (Fig. 1). This agrees with the finding of GYÖRGY AND ROSE[4] that addition of yeast to the diet of E-avitaminotic rats lowers the susceptibility of their erythrocytes to haemolysis.

The nature of this compound occurring in yeast has not been determined, but is of interest owing to the striking similarity of some of its chemical and biological properties to those of the tocopherols.

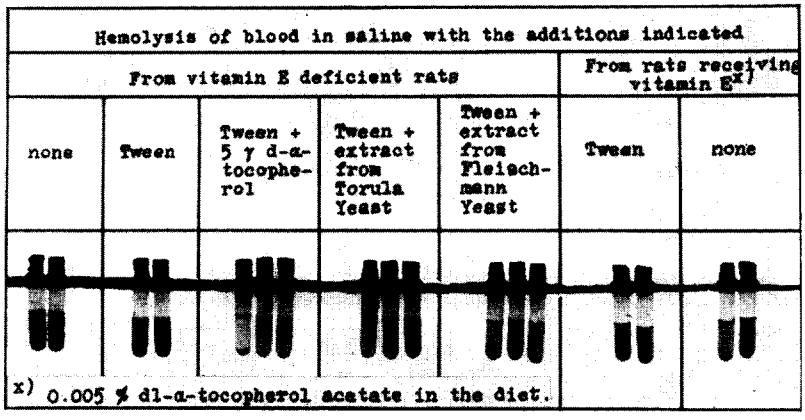

Fig. 1. The tubes on the left were prepared by incubating erythrocytes from a vitamin E-deficient rat for 30 min at 37° C with the substances shown, followed by centrifuging, resuspending in saline and maintaining at 42° C for 2.5 h. 5 μg α-tocopherol and equivalent amounts of the reducing substance from the yeasts were suspended in 0.25 ml saline by means of 20 μg Tween 80. The tubes on the right were similarly prepared from the erythrocytes from a vitamin E-supplemented rat. No haemolysis occurred with tocopherol or with the compounds from yeast, whereas without such protection complete haemolysis occurred. A similar experiment using 1 μg of tocopherol and equivalent quantities of the compounds showed partial protection against haemolysis.

Acknowledgements. The authors are grateful to Professor HENRIK DAM for helpful discussions, and to mag. sc. F. CHRISTENSEN for help concerning the haemolysis test. The work has been aided by a grant from Rask-Ørsted Fondet.

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Received December 19th, 1956