790. *The Alleged Occurrence of Vitamin A in Baker's Yeast.**

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The claim that vitamin A is produced in yeast after incubation in an atmosphere of oxygen has been tested but not verified. A substance with an absorption maximum at 272 mμ and an inflexion near 330 mμ has, however, been found to occur in yeast in small quantities. This strongly resembles a substance (" SA ") hitherto found in animal products only.

Ernster, Zetterström, and Lindberg [1] claimed to have discovered vitamin A in yeast which had been incubated in an atmosphere of oxygen. There does not appear to have been any other claim to have found vitamin A in yeast. In fact it is generally thought that no exception has been proved to the rule that vitamin A itself occurs only in animal tissues, although carotenoid provitamins of course occur widely in plants. The surprising claim of Ernster *et al.*[1] has neither been withdrawn nor refuted and it therefore seemed necessary to repeat the experimental work.

The results recorded below afford no support for the claim that yeast incubated in a stream of oxygen forms vitamin A. Nevertheless they demonstrate that a substance indistinguishable from " SA " can occur in an organism not belonging to the animal kingdom.

Experimental

Our first experiment was carried out according to the directions of the above authors: 1 kg. of baker's yeast (D.C.L.) suspended in distilled water (4 l.) containing glucose (50 g.), ethanol (50 ml.), ammonium sulphate (5 g.), and disodium monohydrogen phosphate (950 mg.) was cultivated in an atmosphere of oxygen produced by bubbling oxygen vigorously through the suspension. The temperature was maintained at 25—30°, and the pH was adjusted at 4·5—5·0 when necessary by the addition of a few drops of concentrated ammonia. After 2 hr., the yeast was centrifuged in a Sharples Super-Centrifuge (25,000 r.p.m.), and the solid was suspended in peroxide-free ether (700 ml.) and vigorously stirred in a Waring blendor after the addition of ethanol (700 ml.). After centrifugation at moderate speed a clear, deep yellow, protein-free extract was obtained. Two phases were obtained on adding to this extract a further 500 ml. of ether, the alcohol phase being the more deeply coloured. The alcoholic solution was washed three times with ether; the ether and part of the ethanol were then removed *in vacuo*.

This alcoholic solution possessed an absorption maximum at 259 mμ, in agreement with the band attributed mainly to adenine by Ernster *et al.*,[1] but in contrast with their experience exhibited no absorption maximum at 325—330 mμ. Moreover, when a portion of this solution was taken to dryness under reduced pressure and extracted with chloroform, the extract gave no colour or cloudiness with the antimony trichloride reagent. Examination of the ether phase by similar methods revealed the presence of ergosterol but not of vitamin A.

The negative results of the above experiment made it necessary to examine yeast by the conventional and more sensitive method of direct saponification of the material followed by examination of the unsaponifiable fraction for vitamin A. Baker's yeast (500 g.) was incubated in an atmosphere of oxygen as previously described. After centrifugation, the yeast was heated on a water-bath with 60% (w/v) potassium hydroxide (0·5 ml./g. yeast) until the mixture became homogeneous. Ethanol (2 vol.) was then added and boiling was continued under reflux for 2 hr., whereupon water was added and the mixture was extracted (five times) with ether (freshly distilled over reduced iron). The combined ether extract was washed with water until free from alkali, dried (Na_2SO_4), filtered through a sintered glass funnel, and taken to dryness under nitrogen. The absorption spectrum of the residue showed the peaks at 263, 272, 282, and 293 mμ characteristic of the 7-dehydro-steroid chromophore, but in addition an inflexion at 327 mμ.

In order to investigate the origin of this inflexion, the unsaponifiable material was subjected to chromatographic fractionation on alumina (Grade O, P. Spence & Co., Ltd., Widnes) weakened with water to Brockmann Grade III. Ether, light petroleum (" AnalaR "; b. p. 40—60°), and mixtures of these two solvents were used as eluants. Both solvents were dried over

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[1] Ernster, Zetterström, and Lindberg, *Exp. Cell. Res.*, 1950, **1**, 494.

sodium wire and redistilled before use, the ether being redistilled over reduced iron. Fractions were taken to dryness under nitrogen and later examined spectroscopically in *cyclo*hexane. 4% and 6% Ether–light petroleum mixtures eluted yellow oily fractions characterised by absorption spectra with $\lambda_{max.}$ 272—273 mμ and a step-out at 330 mμ. These fractions gave no colour with the antimony trichloride reagent, and no evidence of the presence of vitamin A was obtained either spectroscopically or by the colour reaction in any of the other chromatographic fractions. Since these fractions exhibited no selective absorption in the visible region, the presence of carotenoids is excluded.

Untreated yeast (*i.e.*, yeast *not* subjected to incubation) was then examined by chromatography of the unsaponifiable matter as described above. Once again 4% ether–light petroleum mixture eluted a yellow oil with the same characteristic absorption but showing evidence of contaminants by ergosteryl esters. In order to eliminate impurities rechromatography on alumina was necessary. This gave material with $E^{1\%}_{1\,cm.}$ 167 at 272 mμ, which also exhibited an inflexion at 330 mμ and a very weak inflexion near 410 mμ. A close estimate of the concentration of this substance in the yeast could not be made because, in the effort to characterise it, yield was sacrificed to purity.

Festenstein, Heaton, Lowe, and Morton [2] have described the properties of a yellow substance which is also eluted from alumina (weakened with water to Brockmann Grade III) by 4—6% ether–light petroleum. This material was designated " SA " by Festenstein *et al.*,[2] who also detected it in pig intestine, horse stomach and intestine, and rat liver, kidney, small intestine, and submaxillary gland. The spectroscopic properties of rat-liver SA and the purest material

Substance "SA" from rat liver and from yeast.

	Rat liver	Yeast * *a*	Yeast * *b*
In *cyclo*hexane: $E^{1\%}_{1\,cm.}$ at $\lambda_{max.}$ (272 mμ)	174	167	126
$\lambda_{min.}$ (235 mμ)	37	68·2	31·2
$\lambda_{infl.}$ (330 mμ)	12·3	15·2	8·2
$\lambda_{v.w.\ infl.}$ (410 mμ)	7·5	7·1	4·8
		a	
$\lambda_{max.}$ in: light petroleum	272	271	
$CHCl_3$	277—278	277—278	
EtOH	275	275	
HCl–EtOH	275	274—275	
KOH–EtOH	Peak disappears		
	λ (mμ) $E^{1\%}_{1\,cm.}$	λ (mμ) $E^{1\%}_{1\,cm.}$	
In conc. H_2SO_4 after 30 min. in darkness at room temp.: max.	313—315 337	311	335
infl.	335 182	355	162
	420 66·7	415—425 †	93·6
E_{315} (H_2SO_4)/E_{273} (*cyclo*hexane)	1·99	2·01	

* *a*, Most intensely absorbing prepn.; *b*, qualitatively best spectrum.
† Weak max., not shown in rat-liver prepn.

obtained from yeast are compared in the Table. The agreement is excellent except for solutions in concentrated sulphuric acid. The material from yeast exhibits a weak maximum near 420 mμ not observed with the rat-liver material. This may be due to an impurity in the yeast preparation, which had a slightly lower extinction ($E^{1\%}_{1\,cm.}$ 167 at 272 mμ) than the rat-liver material ($E^{1\%}_{1\,cm.}$ 174 at 272 mμ).

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[2] Festenstein, Heaton, Lowe, and Morton, *Biochem. J.*, 1955, **59**, 558.