Acceleration of Vitamin E Deficiency by Torula Yeast. II. Effect of Torula Yeast Ash and Lipid. (24316)

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In previous publications from this laboratory it was shown that addition of 7.5-30% of *Torula* yeast to purified diets which were free of vit. E and selenium caused an increase in incidence and in rate of development of exudative diathesis in chicks(1,2). Part of the anti-vit. E property of *Torula* yeast was found to be due to the inorganic fraction, whereas no effect was apparent with the lipid from the yeast. Subsequently, Dam *et al.*(3) reported that samples of *Torula* yeast contained considerably more lipid than we had found, and they postulated that the unsaturated nature of the lipid could account for the anti-vit. E effect of the yeast. It is here shown that both the inorganic and lipid fractions of *Torula* yeast have anti-vit. E effects, and further, that a combination of the lipid and the ash of *Torula* yeast will duplicate the exudate-promoting properties of the whole yeast. The relatively high unsaturated fat content of the yeast reported by Dam *et al.* (3) is confirmed.

Methods. Feed grade *Torula* yeast* was used in all studies. Ashes of the yeast were prepared by incineration in a muffle furnace at 250°C for 2-3 hours, then at 550°C overnight. Average yield was 7%. When a fixative was used, magnesium carbonate equal to 8% of the weight of yeast was first mixed with the yeast. For isolation of the lipid from *Torula* yeast, 1200 g of yeast were mixed with 1200 ml of 95% ethanol and 6 l of 6N HCl. After refluxing for 1½ hours the mixture was cooled, 3 l of ethanol added, and extracted in batches with a 1:1 mixture of benzene-petroleum ether. The solvent was evaporated *in vacuo* and the dark brown oil dissolved in ethanol to facilitate mixing in the diets. To characterize the lipid fractions of yeast, 25 g samples of yeast were extracted with chloroform in a soxhlet apparatus. The material obtained was labelled "free lipid". The extracted yeast was then refluxed for 2 hours with 5 N NaOH,† cooled, neutralized with HCl and extracted successively with petroleum ether, diethyl ether and benzene. The combined extracts were washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in hexane, filtered and the solvent removed. This material was called "bound lipid." The fatty acids from the free and bound lipids were obtained after saponification and analyzed for polyunsaturation by a modification of the alkali conjugation method of Wiese and Hansen(4). In the nutritional studies, day old New Hampshire chicks, 8 per group, were fed diet C47A(2) but with an additional 0.2% DL-methionine. This diet, designated C47B, contained in %: soybean protein‡ 28, cerelose 61, vit. E-free lard§ 4, salts A 6, DL-methionine 0.8, and vitamin mix 0.2. Additions to this diet, were at the expense of cerelose. The chicks were housed in electrically heated batteries and permitted food and water *ad libitum*. After the 14th day, they were inspected daily for symptoms of exudative diathesis. Experimental periods were 28 days. In evaluating the symptoms, in addition to the incidence, average time of appearance of exudates was also calculated. Compared with the group receiving *Torula* yeast, these 2 factors indicated the exudate-promoting effect of the test substance. However, due to variation in initial stores of tocopherol by individual chicks, over-all incidence has more significance than time for appearance of symptoms.

Results. Since our previous studies(2,5) had shown that certain ashes of *Torula* yeast

* Feed grade 1N, Lake States Yeast Corp.

† Dam, *et al.*(3) obtained more lipid from alkaline than acid hydrolysis.

‡ Drackett assay protein C-1.

§ Stripped lard, Distillation Products Industries.

TABLE I. Effect of *Torula* Yeast Ash and Lipid in Promoting Exudative Diathesis.

Additions to diet C47B	Avg wt, 4 wk (g)	No. of cases*	Avg No. of days†
Exp. 1			
None	305	3/8	23.3
15% *Torula* yeast (TY)	294	6/8	16.8
1.05% TY ash‡	307	5/8	24.0
2.2% TY $MgCO_3$ ash‡	324	7/8	21.3
Exp. 2			
None	265	3/8	25.7
15% *Torula* yeast (TY)	266	6/6	18.0
1% TY lipid§	298	6/7	21.2
1% TY ash‡	310	7/8	21.6
1.8% TY $MgCO_3$ ash‡	273	6/8	22.0
1% TY lipid§ + 1.8% TY $MgCO_3$ ash‡	293	7/8	18.0

* No. of chicks with exudative diathesis/No. of chicks in the group.

† Avg No. of days in which symptoms first appeared.

‡ Prepared at 550°C. Amt of ash added was equivalent to that from 15% of yeast.

§ Equivalent to the lipid from 15% of yeast.

promoted exudates, further investigation was carried out on the possible inorganic component or components in the yeast which were responsible. Based on the mineral content of *Torula* yeast as determined from spectrographic analyses of a wet ash and from analytical data in the literature, numerous elements, as their salts, were added to the basal diet at one or more levels. Elements tested individually, and their dietary concentration in ppm were: Al, 1 and 20; As, 10, 50 and 100; Cd, 10; Mo, 10; Sb, 10; Tl, 10; Sr, 5; Si, 20 and 40; Zn, 25 and 50. A trace element mixture tested at both 5 and 10 ppm for each element contained: Al, B, Br, As, Cr, Co, F, Mo, Ni, Sr, and V. None of these additions had any effect on development of exudative diathesis.

Ashes prepared with magnesium carbonate as a fixative for possible volatile elements appeared to accelerate exudates slightly faster than did ashes without the fixative. A typical result is reported in Table I, Exp. 1. Subsequent testing, however, revealed that when magnesium oxide was added to the diet, together with the plain ash, the effect was similar to that of the ash prepared with magnesium carbonate. Addition of magnesium oxide alone to the diet, in the amount present in the ashes, did not cause formation of exudates.

To determine whether the observed effect of the ash from *Torula* yeast was restricted to this strain of yeast, the ash of brewer's yeast was also tested. In one trial, at a dietary level of 1.8%, equivalent to 30% of yeast, the ash did not produce exudates. In a second trial 1.2% of the ash, equivalent to 20% of brewer's yeast, produced exudates in 7 of 8 chicks compared with 3 of 8 on the basal diet and 7 of 7 receiving 20% of *Torula* yeast. It is known that brewer's yeast contains biologically active selenium in the form of Factor 3(6,7), and it may be that some variation in ashing conditions resulted in incomplete volatilization of the selenium in the ash used in the first trial.

In attempts to simulate the effect of *Torula* yeast ash,‖ salts of the major constituents of the ash were tested at dietary levels corresponding to 10-30% of the yeast. Compounds tested individually were 0.12 and 0.44% $CaSO_3 \cdot 2H_2O$, 0.35 and 1.3% KH_2PO_4, 1.5 and 3.0% $K_2S_2O_5$ and 1.65% $Ca_3(PO_4)_2$. None of these salts nor a 2% increase in the dietary salts A, promoted formation of exudates. A "synthetic" ash of *Torula* yeast was prepared by mixing 22.2 g of $CaSO_3 \cdot 2H_2O$, 74.0 g of KH_2PO_4 and 10.9 g of $MgCl_2 \cdot 6H_2O$ and heating at 550°C for 24 hours. The results with this material will be reported below.

When Dam *et al.*(3) reported that *Torula* yeast from the same source* as ours contained more lipid than we had previously found, it was decided to reinvestigate the effect of the lipid. The amount and fatty acid composition of the lipid obtained as described above from *Torula* yeast, as well as that from brewer's yeast, is given in Table II. *Torula* yeast contained about 6% total lipid, half of which was in the bound form, while brewer's yeast contained only 3% total lipid. Fatty acids account for one-half of the lipid in *Torula* yeast, and for about one-third of the brewer's yeast lipid. The most marked difference between the lipids from the 2 yeasts is in the content of polyunsaturated fatty acids. 45%

‖ Major cations in feed grade *Torula* yeast are: Ca 0.57, P 1.68, K 1.88, and Mg 0.13% (N.R.C. Comm. on Feed Composition, Publ. 449, 1956). Sulfur content averages about 1.2%(8).

TABLE II. Composition of Lipids Isolated from *Torula* Yeast and Dried Brewer's Yeast.*

	Torula	Brewer's
	% of whole yeast	
Free† lipid	3.01	2.03
Bound† "	2.93	.98
Total "	5.94	3.01
Free fatty acids	1.41	.75
Bound " "	1.68	.42
Total " "	3.09	1.17
	% of total fatty acids	
Unsaturated fatty acids:		
Diene	45.4	3.8
Triene	4.5	.0
Hexaene	4.7	3.5

* Pabst grade R2F.
† See text for interpretation of these terms.

of the fatty acids from *Torula* yeast are dienoic acid whereas only 3.8% of the acids from brewer's yeast contain 2 double bonds. These values agree with the analyses of Dam *et al.*(2), although the amount of *Torula* yeast total fatty acids they found was somewhat higher. Small amounts of trienoic and hexaenoic acids were present in the *Torula* yeast, but there was no significant amount of tetraenoic or pentaenoic acids.

The results of an experiment in which *Torula* yeast lipid was fed alone or with the ash are given in Table I, Exp. 2. Whereas all of the treatments increased the incidence of exudative diathesis over that in the basal group, only the combination of lipid plus $MgCO_3$ ash produced the onset of symptoms as quickly as did the intact *Torula* yeast. Since isolation of large quantities of *Torula* yeast lipid was technically impossible, and inasmuch as the lipid by analysis contained almost 50% dienoic acid, it was decided to use an oil of similar composition for further studies.

An oil which contains this amount of linoleic acid, and which also is very low in vit. E, is fractionated tall oil.¶ According to the manufacturer, this oil contains 96.8% free fatty acids, of which 48.0% is linoleic and 50.0% is oleic acid. Addition of 1% of this oil to a diet would provide 0.48% of dienoic acid. Using the value of Dam *et al.*(2) for total fatty acid content of *Torula* yeast, and the total polyunsaturated fatty acid value in Table I, 15% of *Torula* yeast in a diet would provide approximately 0.37% of these fatty acids.

The results of feeding 1% tall oil, with or without the ash of *Torula* yeast, are given in Table III. 15% of *Torula* yeast produced the usual high incidence of exudative diathesis (90%). A similar high incidence (86.5%) was produced by a combination of tall oil and synthetic ash; tall oil plus *Torula* yeast ash gave a slightly lower incidence, 80.5%. The next highest incidence was produced by *Torula* yeast ash alone (72.4%), followed by tall oil alone (57.0%). The synthetic ash (described above) by itself resulted in only 38.4% of the birds having exudates. Except for the group receiving the synthetic ash, all groups had a similar average time for appearance of symptoms.

It is apparent that the exudate-promoting action of *Torula* yeast can be duplicated by a combination of unsaturated fatty acids and minerals similar to those present in the yeast. Not only did this combination produce a high incidence of symptoms, but in one experiment it also produced a severity of hemorrhagic exudates similar only to those found in the chicks fed the diet containing *Torula* yeast. From these results, it can be concluded that the anti-vit. E property of *Torula* yeast is due predominantly to its content of (1) polyunsaturated fatty acid and (2) min-

TABLE III. Incidence of Exudative Diathesis Produced by Tall Oil and *Torula* Yeast Ash.*

Addition to diet C47B	Avg wt, 4 wk (g)	No. of cases	%	Avg No. of days
None	264	7/30	23.3	22.6
15% *Torula* yeast (TY)	312	27/30	90.0	19.8
1% TY ash†	292	21/29	72.4	21.9
1% tall oil	275	16/28	57.0	20.4
1% TY ash + 1% tall oil	314	25/31	80.5	20.6
1% synthetic TY ash‡	269	5/13	38.4	24.2
1% synthetic TY ash + 1% tall oil	304	13/15	86.5	21.9

* Data are avg of 4 separate experiments, except the last 2 groups which are from 2 experiments.
† Prepared at 550°C. Equivalent to 15% of *Torula* yeast.
‡ See text for composition.

¶ Acintol FA-2, Arizona Chemical Co., N. Y.

erals (ash). With respect to the latter, our studies suggest that it is a combination of elements rather than any single element which contributes to the pro-oxidant property of the yeast.

In other experiments, it was found that 1% of the ash of dried brewer's yeast when fed with 1% of tall oil produced a high incidence of exudative diathesis similar to that of *Torula* yeast ash plus tall oil. On the other hand, feeding an additional 2% of the salt mixture used in the C47B diet, together with 1% of tall oil, gave a low incidence of exudates. Thus, it appears that the combination of elements in *Torula* yeast, and possibly also that in brewer's yeast, has a remarkable pro-oxidative property. It is unlikely that any constituents of *Torula* yeast, other than the lipid and ash, contribute significantly to its anti-vit. E effect.

Summary. When added to a purified basal diet low in vit. E and selenium, the ash of *Torula* yeast was found to promote exudative diathesis. This effect could not be demonstrated by any of the minor elements, nor by any of the major components, of the ash. The lipid isolated from *Torula* yeast also promoted exudates; this effect could be duplicated by tall oil. A combination of lipid and ash from *Torula* yeast produced an incidence of exudative diathesis similar to that produced by an equivalent amount of the intact yeast. A combination of tall oil and a synthetic ash was just as effective. It is concluded that the anti-vit. E property of *Torula* yeast is due essentially to both its unsaturated fatty acid and mineral contents. The relatively high content of dienoic acid in the lipid of *Torula* yeast was confirmed.

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Lactic Dehydrogenase Activity in Human Semen.* (24317)

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The lactic dehydrogenase (LD) is present in normal human blood plasma and in increased concentration in myelogenous leukemia(1). The enzyme is present also in serous effusions and cerebrospinal fluid and in elevated amounts when these fluids contain or bathe growing malignant cells(2). Similarly, the proliferation of neoplastic human cells in tissue culture is accompanied by increasing lactic dehydrogenase (LD) activity of the bathing medium(2). The rapid cellular proliferation which is characteristic of human spermatogenesis, the high zinc content of human semen(3) and the presence of zinc in the molecule of LD, suggested LD activity might be present in semen. Accordingly, this possibility was investigated in the normal ejaculate and in semen derived from infertile and sterile individuals.

Methods. Lactic dehydrogenase activity of semen was estimated using 0.025 ml of a 1:10 dilution of human semen. The aliquot of semen was added to 0.6 ml 0.1 molar phosphate buffer, pH 7.4, and 0.05 ml reduced diphos-

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