Absence of toxic effects by food yeast on the rat

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There is little doubt as to the potential value of yeast in the human diet, particularly as a source of protein and B vitamins, but its use is rarely considered except in times of emergency. Two factors militate against the use of yeast: first, it appears that the consumption of amounts greater than 15 g/day can cause digestive disturbances, at least in some individuals; secondly, the material has no structure of its own and needs to be incorporated into other foods.

Our investigation was made in an attempt to resolve the conflicting reports about the toxicity of yeast to mammals. A memorandum of the Medical Research Council: Accessory Food Factors Committee (1945) concludes that some individuals cannot tolerate yeast in quantities greater than 10–15 g/day. There does not appear to be any explanation of this intolerance. One suggestion (unpublished) was that the yeast used for the work described in the Memorandum was contaminated with an anti-foaming agent (sulphonated fish oil) used in its manufacture.

In the 14 years since publication of the Memorandum improvements in methods of manufacture and the use of other anti-foaming agents yield a product that might differ from the earlier material. We have made the attempt to find out whether (*a*) food yeast produced under controlled conditions shows ill-effects in the rat, and (*b*) any differences could be shown between *Torulopsis utilis* and two other strains of food yeast. Our work was not so much concerned to confirm the beneficial properties of yeast as to determine whether any harmful effects on the rat could be detected.

EXPERIMENTAL

Yeasts

Varieties. Three varieties of yeast were used, *Torulopsis utilis*, *Zygosaccharomyces lactis* and *Candida arborea*. The *T. utilis* was derived from the strain originally used in the D.S.I.R. Chemical Research Laboratory, Teddington; the *C. arborea* from the strain used for food-yeast production in Germany during 1939–45; the *Z. lactis* from the Centraalbureau voor Schimmelcultures at Delft.

Preparation. The yeasts were produced in a pilot plant by growth on a refinery cane-molasses medium enriched with a suitable source of nitrogen. The inoculum was prepared in a small seed fermenter and was transferred at the end of the logarithmic growth phase to a larger fermenter (100 l. working capacity) in which further growth was regulated by incremental feeding. A small quantity of a silicone anti-foaming agent was added to the medium in the seed fermenter only, none being used

in the main fermenter, where reliance was placed on a mechanical foam-breaker. The amount of anti-foaming agent in the final product was consequently negligible. Operations were conducted throughout under strictly aseptic conditions, and bacteriological examination of the fermenter contents at intervals showed that the growth consisted of a pure culture of the specific yeast. Air entered the fermenter through a single orifice, at the rate of 1 l./l. liquid/min. An eight-vaned paddle, rotating at 1000 rev/min, was located centrally above the air intake. At the end of the growth period the culture was centrifuged, and the residue was washed three times with water by further centrifugation. The cream of washed cells was dried on a roller drier.

Table 1. *Mean composition on a dry-weight basis of the yeast preparations used*

Constituent*	*Candida arborea*	*Torulopsis utilis*	*Zygosaccharomyces lactis*
Crude protein (N × 6·25) (%)	55·5	58·9	58·3
Purine (%)	2·0	2·4	2·3
Ash (%)	8·5	10·0	9·2
Fat (%)	5·1	5·7	6·3
Total N (%)	8·9	9·4	9·3
Thiamine (μg/g)	15–25	15–25	25–30
Nicotinic acid (μg/g)	150–300	350–450	125–180
Riboflavin (μg/g)	30–45	45–60	50–65
Folic acid (μg/g)	10–14	6–7	6–7
Biotin (μg/g)	3	3	3

* The remainder not accounted for is usually considered to be carbohydrate of undetermined composition.

Chemical properties. Several preparations of each yeast were used during the experiment; their mean compositions are shown in Table 1.

Quantity in diet. Quantities of yeast as small as 10 g/day could make a reasonable contribution even to the British diet. This amount would add approximately 7 % to the protein, 80 % to the thiamine, 30 % to the riboflavin and 30 % to the nicotinic acid of the average diet. To allow for the differences between intakes of food by rat and man, the level of yeast in the experimental diet can be calculated on either food intake or relative body-weight. For example, to obtain the equivalent of 10 g yeast/day for a 70 kg man the rat's diet would need to contain 0·1–0·3 % yeast on a body-weight comparison or about 1·5 % on a food-intake comparison.

The lowest level used in our experiment was 3 %, as the literature reports digestive disorders after consumption of amounts greater than 15–30 g daily. The higher levels of 6, 9 and 12 % were given in the hope of emphasizing any minor effects. The 21 % level was included as possibly representing gross maltreatment.

Diet

The basic diet was that of Thomson (1936) containing 23·5 % protein, 3 % fat and 5 % fibre, together with 40 ml milk/week and 0·1 ml cod-liver oil four times a week and 0·1 ml wheat-germ oil once a week. To it the yeasts were added in the proportions stated.

Animals

One hundred and forty-four albino rats (purchased from the Mousery, Rayleigh, Essex) were used, four males and four females on each diet, together with twenty-four control animals. The animals were fed *ad lib.*

Plan of experiment

Gain in weight and efficiency of food conversion. A simple experiment was done. The animals were weighed weekly and their food consumption was measured.

Examination of the blood, tissues and urine. After 4–6 months' feeding two rats, one male and one female, on each diet were examined. They were killed with diethyl ether and weighed, and carcass length (nose to tail-root) and overall body length (nose to tail-tip) were measured.

The thorax was opened, and a sample of blood was taken from the heart for (*a*) haemoglobin estimation (20 mm^3 blood in 4·0 ml 0·04 % (v/v) conc. ammonia solution), (*b*) red-cell count (20 mm^3 blood in 4·0 ml Hayem's solution), and (*c*) white-cell count (sample diluted 1:20 in a white-cell diluting pipette with 3 % (v/v) acetic acid). Red and white cells were counted in a Neubauer counting chamber. A differential cell count was also made on a dried film of blood.

The haemoglobin was estimated by measurement of the optical density (O.D.) of the diluted blood in a Unicam SP 600 Spectrophotometer at 540 mμ; O.D. × 22·2 = haemoglobin in g/100 ml blood. This factor was checked, by means of a sample of blood distributed under the Haemoglobin Standards Scheme, by C. Davis Keeler Ltd, 47 Wigmore Street, London W. 1.

The spleen, thymus, liver, both kidneys and both adrenals were removed from the carcass, trimmed of fat and connective tissue, roughly dried on filter-paper and weighed immediately. A sample of the liver was dried at 105° overnight to determine dry weight, and the dried sample was extracted for 5 h in a Soxhlet extractor with diethyl ether to determine fat.

A sample of urine was collected in a syringe through the bladder wall and examined immediately under the microscope.

The liver and adrenal glands were examined in frozen section after staining with Sudan IV. Samples of liver, adrenals, spleen, thymus and kidneys were processed in the usual manner in paraffin wax and stained with haematoxylin and eosin.

Fertility. The seventy-two rats remaining after half of the animals had been killed for examination were mated, and the number and weight of litters were recorded.

RESULTS

Gain in weight and efficiency of food conversion

The weights of the animals after 4, 8 and 13 weeks are listed in Table 2. Statistical examination showed no significant difference between the control groups for each type of yeast and the groups on any level of yeast after 13 weeks.

The food consumption and efficiency of food conversion are shown in Table 3. No consistent differences were observed.

Examination of blood, tissues and urine

Organ weights and blood values. The mean weights of spleen, thymus, adrenals, kidneys and livers of controls and treated animals are shown in Table 4. For brevity the values for the animals given 3 and 6% yeast were combined and the values for those given 9% omitted. Carcass and overall body lengths, weight, fat and water content of liver, red-cell counts and haemoglobin are also shown. White-cell counts showed extremely wide variations within groups.

Table 2. *Mean body-weights of groups of four rats given different yeasts* (Candida arborea, Zygosaccharomyces lactis *and* Torulopsis utilis) *at various levels in the diet*

	Level of yeast in diet (%)											
	0		3		6		9		12		21	
Yeast	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	Initially											
C. arborea	54	56	54	56	54	56	55	56	55	56	54	56
Z. lactis	66	59	66	59	66	59	66	59	66	59	66	59
T. utilis	68	65	68	65	68	65	68	65	68	65	68	65
	At 4 weeks											
C. arborea	150	127	142	131	154	132	154	123	134	124	133	113
Z. lactis	171	118	167	124	162	120	165	129	165	132	166	144
T. utilis	153	130	162	129	157	124	160	128	154	126	151	127
	At 8 weeks											
C. arborea	213	163	200	174	214	170	213	154	197	151	187	149
Z. lactis	248	152	238	158	229	151	239	162	216	166	233	166
T. utilis	223	162	220	158	209	156	221	161	213	158	206	152
	At 13 weeks											
C. arborea	253	179	222	190	251	186	252	166	223	175	227	167
Z. lactis	289	162	275	170	255	166	277	178	250	182	263	185
T. utilis	255	175	261	173	237	166	250	180	249	174	249	172

None of the values differed significantly from those for the controls, with the possible exception of the liver weights of the males given 3 and 6% yeast in the diet (values combined) and 21%. These were significantly different at the 5% level only.

The values were also calculated in relation to body-weight and to body length. They showed no significant difference between the groups, and the figures are omitted.

Histological examination of tissues. In all, two male and two female rats on each of the three yeasts at each of the five levels of feeding were examined. The tissues examined (see p. 307) showed no obvious abnormalities.

Urine. The samples of urine examined showed no difference between control and treated rats. Samples from several animals of all groups contained spermatozoa and occasional nematode eggs (a few animals were heavily infested); few samples contained red blood cells.

Fertility. The fertilities are shown in Table 5. Female rats on each diet were mated with males from the same group and remated a second time as soon as the first litter

was weaned. As no difference was apparent between the different yeasts, results for all three have been combined in the table. They show a high degree of fertility with no apparent harm from the yeast.

Mortality. Of all the rats five died from unknown causes or were moribund and

Table 3. *Consumption and efficiency of conversion of food by the rats given different yeasts at various levels in the diet*

(Mean values for groups of four animals)

		Period								
		0–4 weeks			4–8 weeks			8–13 weeks		
Sex	Level of yeast in diet (%)	Food eaten (g)	Weight gain (g)	Food eaten (g)/ g weight gain	Food eaten (g)	Weight gain (g)	Food eaten (g)/ g weight gain	Food eaten (g)	Weight gain (g)	Food eaten (g)/ g weight gain
					Candida arborea					
♂♀	0	348	96	3·6	504	63	8·0	618	40	15·4
♀	0	306	72	4·3	375	35	10·7	455	16	28·4
♂♀	3	362	87	4·2	450	58	7·8	581	22*	26·4
♀	3	336	76	4·4	441	43	10·3	503	17	29·6
♂♀	6	360	100	3·6	532	60	8·9	623	37	16·8
♀	6	356	76	4·7	454	38	11·9	491	16	30·6
♂♀	9	359	100	3·6	475	59	8·1	592	39	15·2
♀	9	347	67	5·2	400	31	12·9	337	12	28·1
♂♀	12	328	80	4·1	483	63	7·7	599	26	23·0
♀	12	325	68	4·8	405	28	14·5	459	24	19·1
♂♀	21	346	78	4·4	448	55	8·1	590	39	15·1
♀	21	296	58	5·1	391	36	10·9	434	18	24·1
					Zygosaccharomyces lactis					
♂	0	347	105	3·3	574	52	11·0	640	31	20·6
♀	0	300	59	5·1	375	35	10·7	424	10	42·4
♂♀	3	398	101	3·9	537	71	7·6	605	37	16·4
♀	3	328	66	5·0	436	34	12·8	444	12	37·0
♂♀	6	389	96	4·1	554	67	8·3	602	27	22·3
♀	6	310	62	5·0	396	30	13·2	444	16	27·8
♂♀	9	425	99	4·3	497	75	6·6	613	38	16·1
♀	9	330	70	4·7	357	34	10·5	474	15	31·6
♂♀	12	388	99	3·9	471	52	9·1	586	34	17·2
♀	12	338	73	4·6	417	34	12·3	464	17	27·3
♂♀	21	383	100	3·8	488	67	7·3	670	30	22·3
♀	21	302	71	4·3	391	37	10·6	476	19	25·1
					Torulopsis utilis					
♂	0	384	85	4·5	538	70	7·7	647	32	20·2
♀	0	326	64	5·1	453	33	13·7	483	13	37·1
♂♀	3	377	94	4·0		58		657	41	16·0
♀	3	363	64	5·7	402	30	13·4	445	15	29·7
♂♀	6	402	89	4·3	512	52	9·8	580	27	21·5
♀	6	367	59	6·2	414	32	12·9	440	11	40·0
♂♀	9	420	92	4·6	555	61	9·1	628	29	21·7
♀	9	348	63	5·5	436	33	13·2	479	19	25·2
♂♀	12	377	86	4·4	568	59	9·6	633	36	17·6
♀	12	323	61	5·3	406	32	12·7	445	15	29·7
♂♀	21	388	83	4·7	558	55	10·1	628	43	14·6
♀	21	314	62	5·1	406	25	16·2	467	20	23·4

* One animal was losing weight.

Table 4. *Mean values with standard deviations for various measurements for groups of four rats given different yeasts at various levels in the diet*

	Level of yeast in diet (%)							
	Females				Males			
	0	3 and 6†	12	21	0	3 and 6†	12	21
Body-weight (g)	190 ± 13	182 ± 26	195 ± 24	189 ± 13	308 ± 39	269 ± 26	309 ± 29	269 ± 33
Total length‡ (cm)	35·4 ± 1·3	34·9 ± 1·43	35·4 ± 0·71	35·6 ± 1·1	39·8 ± 1·87	38·6 ± 1·38	39·3 ± 1·35	38·6 ± 1·74
Carcass length‡ (cm)	17·7 ± 0·8	17·5 ± 0·5	17·9 ± 0·9	18·3 ± 0·6	19·8 ± 0·7	19·3 ± 0·8	20·0 ± 0·7	19·3 ± 0·9
Spleen (g)	0·50 ± 0·10	0·44 ± 0·11	0·53 ± 0·10	0·53 ± 0·10	0·67 ± 0·06	0·61 ± 0·14	0·62 ± 0·05	0·72 ± 0·16
Thymus (g)	0·26 ± 0·08	0·28 ± 0·11	0·26 ± 0·10	0·28 ± 0·06	0·39 ± 0·06	0·33 ± 0·10	0·35 ± 0·08	0·34 ± 0·15
Kidneys (both) (g)	1·41 ± 0·17	1·35 ± 0·29	1·65 ± 0·20	1·54 ± 0·09	2·04 ± 0·41	1·96 ± 0·22	1·84 ± 0·48	2·23 ± 0·35
Adrenals (both) (mg)	48·7 ± 12·8	52·5 ± 12·9	57·3 ± 8·7	59·6 ± 16·0	40·0 ± 7·5	37·7 ± 5·9	32·9 ± 3·3	39·6 ± 4·5
Liver								
Wet weight (g)	7·27 ± 0·72	6·67 ± 1·12	7·80 ± 1·34	7·05 ± 0·68	11·44 ± 2·07	9·27 ± 1·06*	9·63 ± 1·20	8·98 ± 1·53*
Water content (%)	71·8 ± 0·88	71·55 ± 1·33	71·10 ± 1·69	71·70 ± 0·69	72·2 ± 1·14	72·1 ± 0·95	72·0 ± 2·1	72·0 ± 0·84
Fat content (%)	4·51 ± 1·52	4·85 ± 1·53	5·89 ± 1·72	4·47 ± 0·47	4·39 ± 0·97	3·94 ± 1·65	4·85 ± 0·42	5·65 ± 0·97
Red blood cells (10^{-6}/mm^3)	7·22 ± 0·63	7·27 ± 0·925	6·99 ± 0·45	7·33 ± 0·58	8·11 ± 0·69	8·24 ± 0·61	7·63 ± 0·78	7·81 ± 0·82
Differential white-cell count								
Polymorphonuclear neutrophils	17	15	11	7	16	17	16	9
Eosinophils	0	0	0	0	0	0	0	0
Basophils	0	0	0	0	0	0	0	0
Monocytes	2	4	0	1	2	4	1	2
Lymphocytes	81	81	78	92	82	79	83	89
Haemoglobin (g/100 ml)	13·2 ± 0·68	13·95 ± 0·84	13·7 ± 0·48	14·2 ± 0·57	15·20 ± 0·76	15·48 ± 1·15	14·53 ± 1·05	15·00 ± 0·60
White blood cells per mm^3	9400	7000	10700	7100	8150	7700	6300	10000

* Just significantly different from controls at the 5 % level.

† For brevity the values for the animals given 3 and 6 % yeast are combined, and the values for those given 9 % omitted.

‡ See p. 307.

therefore killed: one on the 3% *Z. lactis* diet, one on 3%, one on 12% and one on 21% *C. arborea*, and one on 21% *T. utilis*. None of the controls died, but so low a mortality during a 6-month experiment with no pattern indicating particular cause did not incriminate the yeast diet.

Table 5. *Fertility of rats given different yeasts* at various levels in the diet*

Level of yeast in diet (%)	First litter				Second litter		
	No. mated	No. giving birth	No. born	No. weaned	No. mated	No. giving birth	No. born
0	6	6	32	30	6	6	45†
3	5	5	38	29	4	4	18
6	6	6	55	47	6	5	39
9	6	6	51	45	6	5	43
12	6	6	37*	31	6	4	32
21	5	5	33	28	5	3	30

* As no difference was apparent between the different yeasts, the results for the three have been combined.

† Plus one litter killed and eaten at birth.

DISCUSSION

Toxic effects of yeast

Yeast, when given as the major source of dietary protein, has been criticized on two counts. First, as a source of protein its value is limited by its content of sulphur amino acids. Secondly, it can cause massive hepatic necrosis, which is prevented by vitamin E or sulphur amino acids (Goyco, 1956) or selenium (Kelleher, Gitler, Sunde, Johnson & Baumann, 1959).

We were not concerned with these effects as our diet contained an abundance of vitamin E and sulphur amino acids, nor was the yeast the major part of any of the diets used, but only a supplement to an already complete diet.

Digestive disturbances

There have been several reports of various digestive disturbances in human beings after the intake of more than 15 g dried yeast daily. von Loesecke (1946) summarizes ten reports that cover a diversity of opinion, ranging from a statement that 85–110 g can be consumed daily without ill-effect to another that ill-effects follow consumption of as little as 3 g/day. Not all these results can be taken at their face value. For example, Ruffin & Cayer (1944) made the following observation: three out of thirty-four subjects complained of gastro-intestinal symptoms when given placebo, six out of thirty-six complained when given liver, and nine out of thirty-nine when given yeast. The authors concluded that yeast causes gastro-intestinal symptoms. Yet in the same series of experiments they reported nausea and vomiting in eight cases out of thirty-seven fed on liver, and in only two out of thirty-eight fed on yeast.

The Memorandum of the Medical Research Council: Accessory Food Factors Committee (1945) lists four series of experiments, in three of which complaints were

made by a small number of subjects consuming 7–10 g yeast daily. Here, again, one experiment showed twenty cases of 'digestive and other upsets' in 160 children given 7 g yeast/day and seven cases in 140 controls. From the evidence available it seems that, though many people can tolerate as much as 100 g yeast without apparent ill-effect, others occasionally suffer disorders from as little as 7–10 g, but no convincing explanation has been found. The M.R.C. Report concludes that about 15 g dried yeast may be regarded as the maximum permitted daily dose.

We have here made no direct contribution to this discussion, except the negative one that we were unable to detect any ill-effects on the rat, even after giving yeast at abnormally high levels.

Uric acid

The relatively high purine content of yeast has often led to the view that it may cause ill-effects due to accumulation of uric acid. von Loesecke (1946) lists six reports of increased urinary excretion of uric acid after consumption of yeast by human subjects, two reports showing no increased excretion, one report of no effect on blood levels of uric acid and one report of an increase. However, the American Medical Association: Council on Foods (1939) concluded that 'it does not seem probable that, at least in the quantities normally consumed, it causes an increase in production of uric acid sufficiently great to exert a harmful effect on the organism or to lead to the formation of uric acid stones'. Since then Cremer & Beisiegel (1943) showed no significant effect on uric acid in blood or its excretion in urine in human subjects on the consumption of 10 g yeast/day for a long period.

SUMMARY

1. One hundred and forty-four rats, including controls, received food yeast at various levels in an attempt to discover a reason for the digestive disturbances sometimes observed in human beings consuming more than 15 g yeast/day.

2. *Candida arborea*, *Zygosaccharomyces lactis* or *Torulopsis utilis* was added to an already adequate diet, at levels of 3, 6, 9, 12 or 21 %. The animals were given the diet with the different yeast supplements for 4–6 months, when half were killed, various organs being weighed and examined histologically. The remaining animals were mated and their fertility was observed.

3. No effects were observed as judged by weight gain, efficiency of food conversion, overall body and carcass lengths, blood and urine picture, histological picture of liver, spleen, thymus, kidney and adrenals, dry weight and fat content of liver, and fertility.

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