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Studies on unidentified growth factors

1. Factor G, a growth factor for rats

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1. Weanling rats were given diets containing all the nutrients known to be required, with L-amino acids in place of protein. Dietary supplements were added isonitrogenously.

2. Torula yeast (50 g/kg diet) and dried brewers' yeast (50 g/kg) significantly improved the rats' growth rate. Part of this activity was attributed to the minerals present in the yeasts, zinc, iodine, iron, copper and manganese all being implicated.

3. Marmite (20 g/kg) and a basic fraction (6·6 g/kg) obtained from it by cation-exchange chromatography significantly improved growth. The ash component of the basic fraction was inactive.

4. Growth was increased by an extra supplement of vitamins and by treating the rats with neomycin sulphate and tetracycline. The growth stimulus due to Marmite was additive with that due to the antibiotics, but not with that due to the vitamin supplement.

5. The growth rate with torula yeast (50 g/kg) was maintained at about 30% greater than on the basal diet during a test lasting 80 d. At the end of that period, six control and five supplemented male rats sired normal litters. Post-mortem examination, including histology, of others showed no abnormalities due to factor G deficiency.

6. Fresh ox liver (100 g/kg diet) significantly improved the growth rate, but this improvement could be attributed to the effects of the water and minerals contained in the liver.

7. These results confirm the discovery by Schwarz, Smith & Oda (1966) that yeasts contain an organic growth-promoting factor (factor G) for rats receiving amino acid diets. Factor G is not likely to be one of the vitamin B group.

Schwarz, Smith & Oda (1966, 1967) and Schwarz (1970) have reported the existence of an unidentified natural factor, designated factor G, that increased the growth rate of rats receiving an amino acid diet devoid of protein. Yeasts, liver, kidney and spleen contained factor G, but many other proteinaceous materials did not. The effects of factor G, which were to improve both the growth rate and the efficiency of food conversion, were found in spite of several important changes to the basal diet, such as alterations to the amount or balance of amino acids and changes in the vitamin and mineral supplements.

We have investigated the growth-promoting properties of torula yeast and ox liver for rats receiving an amino acid diet.

EXPERIMENTAL

Fractionation of Marmite

Marmite was obtained from Marmite Ltd, Burton-on-Trent, Staffs. A column, 40 mm diam. × 290 mm long, of 400 g Dowex 50W-X8 sulphonic acid cation-exchange resin (20–50 mesh, in the H-form) was washed first with 750 ml 2 M-HCl and then

Table 1. *Composition of basal amino acid diet*

Ingredient	Dietary concentration (g/kg)
Amino acid mixture*	166·9
Lard	30·0
Salt mixture†	57·3
Vitamin mixture‡	4·2
Cellophane flakes	20·0
Sucrose	721·6

* See Table 2.

† See Table 3.

‡ Contained (mg/kg diet): thiamin 9·3, riboflavin 19, nicotinic acid 93, pyridoxine 9·3, calcium pantothenate 93, pteroylmonoglutamic acid 1·9, cyanocobalamin 0·028, *myo*-inositol 93, *p*-aminobenzoic acid 93, choline chloride 460, menaphthone sodium bisulphite 0·55, D-α-tocopheryl acetate 70 and also, in gelatin-coated beadlets, 3·4 mg retinyl acetate and 36 μg cholecalciferol.

with deionized water to pH 5. A solution of 100 g Marmite in 2 l deionized water was passed through the column and the column was washed with a further 2 l water. The eluant from this procedure was concentrated under reduced pressure to give 46·6 g 'neutral fraction'. The 'basic fraction' (32·9 g) was then obtained by washing the column with 3 l 0·5 M-NH_4OH and concentrating the eluant under reduced pressure. The ash component of the 'basic fraction' was obtained by heating it to 550° for 18 h in a muffle furnace, the yield being 0·044 g ash/g 'basic fraction'. For assays of growth factor activity, the 'neutral fraction' was added to the diet at 9·3 g/kg, the 'basic fraction' at 6·6 g/kg and the ash of the 'basic fraction' at 0·29 g/kg. These concentrations were equivalent to 20 g Marmite/kg diet.

The behaviour of biotin and inositol was examined in the chromatographic system used to fraction Marmite. A column containing 40 g resin was loaded with 10 mg D-biotin and washed with 400 ml water. Evaporation of the eluant under reduced pressure yielded 8·5 mg dry white solid. A fraction equivalent to the 'basic fraction' was then obtained by washing the column with 300 ml 0·5 M-NH_4OH. The eluant was concentrated under reduced pressure, giving 4·1 mg dry white solid. With no added biotin, a similar column yielded 1·5 mg and 1·7 mg of dry solid when washed with water and 0·5 M-NH_4OH, respectively. Thus, 70% of the added biotin appeared in the wash water.

A similar column was loaded with 30 mg inositol and washed with water and 0·5 M-NH_4OH as described above. Thin-layer chromatography on silica gel plates (0·25 mm deep) showed that inositol was present in the fraction equivalent to the 'neutral fraction' of Marmite, but not in that equivalent to the 'basic fraction'. The chromatograms were developed in the solvent system acetic acid–acetone–methanol–benzene (5:5:20:70, by volume). Inositol was identified by spraying with a solution containing 0·1 M-$AgNO_3$ and 5 M-NH_4OH (1:5, by volume) (Stahl, 1969).

Experimental diets and ingredients

The composition of the basal diet is given in Table 1. The amino acid mixture (Table 2) was the same as that used in diet D 1 of Ranhotra & Johnson (1965), except that we accepted their final suggestion of 5·0 g/kg as the optimal level for arginine hydrochloride and that we used L-alanine and L-serine instead of the DL-forms.

Table 2. *Amino acid composition of basal diet and dietary additives (g/kg diet)*

Amino acid* (L-forms except glycine)	Basal diet†	Torula yeast‡ at 50 g/kg diet	Ox liver§ at 100 g/kg diet (or 32 g dried liver‖/kg diet)
Arginine hydrochloride	5·0	1·57	1·49
Histidine hydrochloride.H_2O	4·5	0·95	0·54
Isoleucine	8·2	1·25	0·91
Leucine	11·1	1·80	1·60
Lysine hydrochloride	18·0	2·38	1·45
Methionine	8·2	0·36	0·53
Cystine	3·5	0·25	0·23
Phenylalanine	11·6	1·10	1·11
Tyrosine	3·5	1·05	0·78
Threonine	5·3	1·30	0·82
Tryptophan	1·5	0·20	0·29
Valine	8·2	1·35	1·14
Alanine	3·5	1·60	1·22
Aspartic acid	3·5	2·25	1·52
Glutamic acid	35·0	3·85	2·21
Glycine	23·3	1·20	1·30
Proline	3·5	0·90	0·95
Serine	3·5	1·40	0·99
Asparagine	6·0	ND	ND

ND, not detected.

* Amino acids were obtained from Deutsche Ajinomoto GmbH, Hamburg, Germany; Cambrian Chemicals Ltd, Croydon, and Sigma Chemical Co., London.

† See Table 1 for composition.

‡ Lake States Yeast & Chemical Division of St Regis Paper Co., Rhinelander, Wisconsin.

§ Obtained from a local butcher.

‖ Dried at 90° for 18 h in a ventilated oven.

Additions to the basal diet were made at the expense of sucrose. When adding torula yeast or ox liver (fresh or dried), the total amino acid composition of the diet was kept constant by omitting from the basal amino acid mixture amounts of amino acids equivalent to those contained in the additives. The amino acid composition of torula yeast was determined by means of a Technicon amino acid analyser after acid hydrolysis (Bunyan & Woodham, 1964). Tryptophan was determined without preliminary hydrolysis by the method of Spies & Chambers (1949, N procedure).

The results for torula yeast are shown in Table 2 together with the amino acid composition of ox liver (Block & Weiss, 1956) and the composition of the basal mixture. The amino acid contribution from the additional 20 g Marmite/kg diet was considered to be low enough for there to be little error in compensating for it by omitting a small part (9·1 g/kg) of the basal amino acid mixture, calculated on a nitrogen basis.

N contents of diet and additives were determined by the Kjeldahl method.

The composition of the basal diet salt mixture is given in Table 3, together with the composition of salt mixtures made up to imitate the mineral compositions of torula yeast and ox liver. The analysis of torula yeast was given by the manufacturer. For liver, we took the highest values quoted by Monier-Williams (1949), McCance & Widdowson (1960) and the (US) National Research Council (1959). The ash fractions

Table 3. *Composition of mineral salt mixtures (g/kg diet)*

Ingredient	Basal diet* salt mixture	Mixture equiv.† to minerals of 50 g torula yeast/kg diet	Mixture equiv.† to minerals of 100 g ox liver/kg diet
$CaCO_3$	19·46	0·835	—
$CaH_4(PO_4).H_2O$	—	0·745	1·03
KH_2PO_4	—	3·50	0·75
KCl	3·73	—	—
$NaH_2PO_4.2H_2O$	30·00	—	—
NaCl	—	0·019	0·232
Na_2CO_3	1·28	—	—
$MgSO_4.H_2O$	2·40	0·30	0·115
Ferric citrate	0·16	0·053	0·180
$MnSO_4.2H_2O$	0·21	0·0073	0·0011
$ZnSO_4.7H_2O$	0·064	0·028	—
$ZnCO_3$	—	—	0·0122
Magnesium silicate	—	0·89	—
KI	0·00032	0·00029	—
NaF	0·00027	—	—
$(NH_4)_6Mo_7O_{24}.4H_2O$	0·0021	—	—
$CoSO_4.7H_2O$	0·011	0·00001	—
$KAl(SO_4)_2.12H_2O$	0·00076	0·025	0·054
$CuSO_4.5H_2O$	0·021	0·0035	0·024
Na_2SeO_3	0·00033	—	—
Lead acetate	—	0·00033	—
$NiSO_4.7H_2O$	—	0·00033	0·00004

* See Table 1.
† See p. 169.

of torula yeast and ox liver were obtained by heating the materials to 520° for 18 h in a muffle furnace.

Experiments

Weanling male CFY rats (specified pathogen-free) were obtained from Carworth Europe, Huntingdon, at 18 or 21 d of age (see below). They were then given, for 5 d, a diet containing (g/kg): casein 100, lard 30, salt mixture (Diplock, Green, Bunyan, McHale & Muthy, 1967) 53·5, vitamin mixture (Diplock *et al.* 1967) 4·0, and glucose 812·7. The cyanocobalamin concentration was erroneously described by Diplock *et al.* (1967) as ten times higher than that actually used. Retinyl acetate was added as gelatin-coated beadlets to give 3·4 mg/kg diet. Sodium selenite was added to give 0·15 mg Se/kg, and D-α-tocopheryl acetate, 70 mg/kg, was also added.

In early experiments, some batches of rats failed to respond to any dietary supplement, including torula yeast. Invariably, the growth rate of these rats was abnormally high on the basal diet, suggesting that they had derived reserves of factor G from their dams or from the stock diet. Schwarz (1970) encountered the same difficulty. In the experiments reported here, we overcame this difficulty by taking steps to decrease the rats' access to sources of factor G. The rats were then obtained at 18 d rather than 21 d of age and they were given the basal amino acid diet until the start of the experiment.

At 23 d of age the rats were grouped at random in fives. They were then given the

experimental diets, described below, three cages of five rats to each diet. The unsupplemented basal diet was included in every experiment. Four to six other diets were compared with this negative control, one diet usually being supplemented with 50 g torula yeast/kg as a positive control. Food and water were always freely available. Food consumption was measured in some experiments. The rats were weighed individually at the beginning and the end of the experimental period of 8–10 d, except in Expt 19.

In Expt 19, the basal amino acid diet and the diet containing torula yeast were each given to three groups of five rats as usual, but the experimental period was extended to 80 d and the rats were weighed every 3 or 4 d.

At the end of the experiment, six control rats and five supplemented rats were mated, each with one normal female rat of the same strain. Each female rat received the same experimental diet as its mate during the 20 d mating period and was then returned to the stock diet. In addition, three male rats from each dietary group of Expt 19 were killed for histological examination of kidney, liver, testis, stomach, brain, small intestine, large intestine and pancreas.

Histological examinations

Tissues were fixed in buffered formol-saline, pH 7·0. Paraffin-sections were stained with haematoxylin and eosin. Sections of liver were also stained with Oil Red O.

Statistical interpretation of the results

The results were analysed, separating the variance between groups treated alike from the variance within groups. When the between-groups variance was significant, it was used to provide an estimate of error. However, in most experiments it was not significant and it was therefore pooled with the within-groups variance to provide the estimate of error variance. The significances of treatment effects were assessed by the method of least significant differences. Any individual weight gain more than 2 SD above or below the mean for the group of rats treated alike was replaced by the mean value for the remainder and the total number of degrees of freedom was decreased by one.

RESULTS

As shown in Table 4, torula yeast consistently improved the growth rate of rats receiving the amino acid diet. Improvements in feed conversion efficiency (not shown in Table 4) were about as great as the effects on growth rate. The ash of torula yeast seemed to account for about one-half of the activity of the yeast. A similar situation was found with dried brewers' yeast, the growth increments for the yeast and its ash being 42 (SEM 10) % and 18 (SEM 7) %, respectively (one experiment only, not shown in the tables). A mineral salt mixture supplying the same amounts of minerals as torula yeast increased growth rate to about the same extent as the yeast ash (Table 4).

The activity of torula yeast ash and the mineral mixture suggested that the basal diet, although formulated to satisfy estimated requirements ((US) National Research Council, 1962), might have been deficient in some mineral factor. However, it was not

Table 4. *Growth-promoting activity of torula yeast and other materials for rats receiving an amino acid diet*

(In each experiment, each diet was given to three groups of five rats; mean results with their standard errors are given)

	Growth rate (g/d)								
Addition to basal diet† (g/kg)	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7	Expt 8	Expt 9
None	2·35	2·70	2·31	2·50	2·12	2·46	2·79	2·49	3·00
Torula yeast, 50	4·30***	—	—	3·88***	—	3·01*	4·17***	4·33***	4·08**
Ash of torula yeast, 3·5	3·81***	3·22*	—	3·33*	—	—	—	3·11*	3·63*
Salt mixture‡ equivalent to the minerals of torula yeast, 5·6	3·66***	3·35**	—	3·30*	3·11***	3·39**	—	—	—
Salt mixture‡ without Pb, Si, Al and Ni, 5·6	—	3·57***	—	—	—	—	—	—	—
Other mineral additions§									
Pb, Si, Al and Ni	2·56	—	—	—	—	—	—	—	—
Fe, Zn, I, Ni and Cu	—	—	—	3·23*	—	—	—	—	—
K, Mg, Fe and Cu	—	3·53***	—	—	—	—	—	—	—
K and Mg	—	—	2·29	—	—	—	—	—	—
Ca and P	—	3·06	—	—	—	—	—	—	—
Zn	—	3·73***	3·49**	—	—	2·94	2·81	—	—
I	—	3·47**	3·43**	—	—	2·53	3·11	—	—
Cu	—	—	3·20*	—	2·49	2·40	3·07	—	—
Mn	—	3·49**	2·86	3·14	—	—	—	—	—
Fe	—	—	3·46**	—	2·32	2·30	3·27	—	—
SE of treatment means	0·18	0·17	0·22	0·25	0·19	0·27	0·19	0·18	0·25

Significantly greater than controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† See Table 1 for composition of the basal diet and p. 169 for details of dietary adjustments.

‡ For composition see Table 3.

§ Final dietary concentration (mg/kg), as also given by the salt mixture ‡: 980 P and 450 Ca (as $CaH_4(PO_4)_2.H_2O$), 1000 K (as K_2CO_3), 88 Mg (as $MgSO_4.H_2O$), 20 Si (as magnesium silicate), 8·8 Fe (as ferric citrate), 6·3 Zn (as $ZnSO_4.7H_2O$), 1·8 Mn (as $MnSO_4.4H_2O$), 1·4 Al (as $KAl(SO_4)_2.12H_2O$), 0·9 Cu (as $CuSO_4.5H_2O$), 0·23 I (as KI), 0·18 Pb (as lead acetate), 0·08 Ni (as $NiSO_4.7H_2O$).

found possible to attribute the growth effect of the mineral salt mixture to any one mineral. Omission of lead, silicon, aluminium and nickel from the mixture did not decrease its activity and a combination of these four minerals was ineffective (Table 4) Cobalt and sodium were not studied further because the amounts added were only 0·1–0·2 % of those already present in the diet. The combinations of magnesium and potassium, and calcium and phosphorus proved inactive (the latter pair added only about 10 % to the amount already in the diet). A growth stimulus occurred with a combination of iron, zinc, iodine, Ni and copper. Ni was not studied further because of its inactivity in combination with Pb, Si and Al. Five minerals, Fe, Zn, I, manganese and Cu were examined separately and all of them in various experiments gave significant growth responses. In Expts 2 and 3, several minerals separately stimulated growth. The amounts of Fe, Zn, I, Mn and Cu in torula yeast at 50 g/kg diet were equivalent respectively to 35, 45, 100, 4 and 17 % of those already present in the basal diet.

Separate, non-additive effects of more than one mineral may be explicable as a common effect, possibly on a single enzyme system or on the bacterial flora of the gut, rather than as the correction of multiple nutritional deficiencies.

Vanadium was also tested for factor G activity in view of the recent finding of Schwarz & Milne (1971) that V is an essential trace element for rats maintained in a sterile isolator, the requirement being about 0·05 mg/kg diet. No growth response was obtained in our rats maintained under conventional conditions on adding ammonium metavanadate to give dietary levels of V of 2·0 and 0·2 mg/kg (not shown in the tables).

The addition of 100 g fresh ox liver/kg diet also promoted growth, but 32 g dried liver/kg was much less active (Table 5). Addition of 70 g water/kg, equivalent to the moisture content of the raw liver, increased growth by about 42 % (Expts 13–16). Schwarz (1970) has also observed this effect of water and attributed it to the high osmolarity of the amino acid diet. He routinely used a basal diet with water added. In our experience, torula yeast improved growth regardless of the water content of the basal diet.

The activities of liver ash and a similar mineral mixture were almost equal to that of dried liver (Table 5). Combinations of water with either liver ash or its mineral salt equivalent were assayed once. The growth responses were 75 and 63 %, respectively.

From these results it seems that liver does not contain an organic growth factor for rats receiving an amino acid diet, its activity being due to minerals and water.

It seemed possible that factor G might operate by inducing some change in the gut flora and that the removal of the gut flora might, therefore, eliminate the growth effect. To test this hypothesis, we gave rats neomycin sulphate and tetracycline. This treatment, though not completely sterilizing the gut, would at least have greatly limited the growth of aerobic bacteria. Marmite, the commercial yeast extract containing about 250 g H_2O/kg, was used as the source of factor G in this experiment. In our experience, this material at 20 g/kg diet promoted growth consistently and significantly; the mean response in twenty-four successive experiments was 25·2 (SD 11·1) % of controls. As shown in Table 6, the antibiotic treatment itself significantly

Table 5. *Growth-promoting activity of ox liver and other materials for rats receiving an amino acid diet*

(In each experiment, each diet was given to three groups of five rats; mean results with their standard errors are given)

Addition to basal diet† (g/kg)	Growth rate (g/d)							
	Expt 10	Expt 11	Expt 12	Expt 13	Expt 14	Expt 15	Expt 16	Expt 17
None	2·11	2·47	2·73	3·32	1·99	2·43	3·00	3·08
Ox liver, 100	3·66***	—	3·37**	5·13***	4·71***	—	—	—
Dried ox liver, 32	—	3·07*	—	3·98	—	—	—	—
Liver ash, 1·6	—	3·31**	—	4·14	2·34	—	—	—
Salt mixture‡ equivalent to the minerals of liver, 2·4	—	3·54***	—	—	—	2·59	—	3·67
Deionized water, 70	—	—	—	4·05	3·09***	3·67***	3·67**	—
Liver ash, 1·6 + deionized water, 70	—	—	—	—	3·49***	—	—	—
Salt mixture‡, 2·4 + deionized water, 70	—	—	—	—	—	3·57***	—	—
SE of treatment means	0·19	0·19	0·15	0·30	0·20	0·17	0·17	0·22

Significantly greater than controls: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† See Table 1 for composition of the basal diet and p. 169 for details of dietary adjustments.

‡ For composition see Table 3.

Table 6. *Expt* 18. *Effects of antibiotics on the growth-promoting activity of Marmite*

(Each diet was given to two groups of five rats; mean results with their standard errors are given)

Group	Addition to basal diet‡ (g/kg)	Addition to the drinking-water (mg/l)	Growth rate (g/d)
1	None	None	2·96
2	Marmite (20)	None	4·14***
3	Neomycin sulphate (0·5)	Tetracycline (75)	3·61*
4	Marmite (20) and neomycin sulphate (0·5)	Tetracycline (75)	4·59***†
5	Vitamin mixture§	None	3·93***
6	Vitamin mixture§ and Marmite (20)	None	4·21***
7	Vitamin mixture§ and neomycin sulphate (0·5)	Tetracycline (75)	4·07***
8	Vitamin mixture§, Marmite (20) and neomycin sulphate (0·5)	Tetracycline (75)	4·65***†
	SE of treatment means		0·20

Significantly greater than group 1: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
Significantly greater than group 3: † $P < 0.01$.

Mean effects of vitamins and Marmite, averaged over the antibiotic factor (g/d)

	No Marmite	Marmite
No vitamins	3·28	4·36
Vitamins	4·00	4·46
SE of means	0·14	

Mean effect of antibiotics, averaged over the vitamin and Marmite factors (g/d)

No antibiotics	3·81
Antibiotics	4·23
SE of means	0·10

‡ See Table 1 for composition of the basal diet and p. 169 for details of dietary adjustments. Allowance was made for the water content of Marmite by making the water content of each diet up to 20 g/kg.

§ As described in Table 1, but with D-α-tocopheryl acetate and retinyl acetate omitted and biotin (60 mg/kg) added.

promoted growth. Marmite was effective both alone (cf. groups 1 and 2) and together with the antibiotics (cf. groups 3 and 4). The effect of factor G would therefore seem to be independent of the aerobic gut flora. An extra vitamin supplement was included as the third factor in this factorial experiment, because the antibiotic treatment might have diminished the synthesis of vitamins by the gut flora. As shown in Table 6, this supplement itself significantly improved growth, but its effect was not additive to that of Marmite (cf. groups 2, 5 and 6). These effects of the vitamin supplement were confirmed in other experiments (results not shown).

Marmite was fractionated as described on p. 167. The 'neutral fraction' had no growth factor activity, the mean responses in two experiments being −8 and +6% of controls (non significant). The basic fraction consistently promoted growth, the mean response in twelve experiments being 20 (SD 6)%. However, the ash component of the basic fraction was inactive (mean responses of −1 and −7% in two experiments).

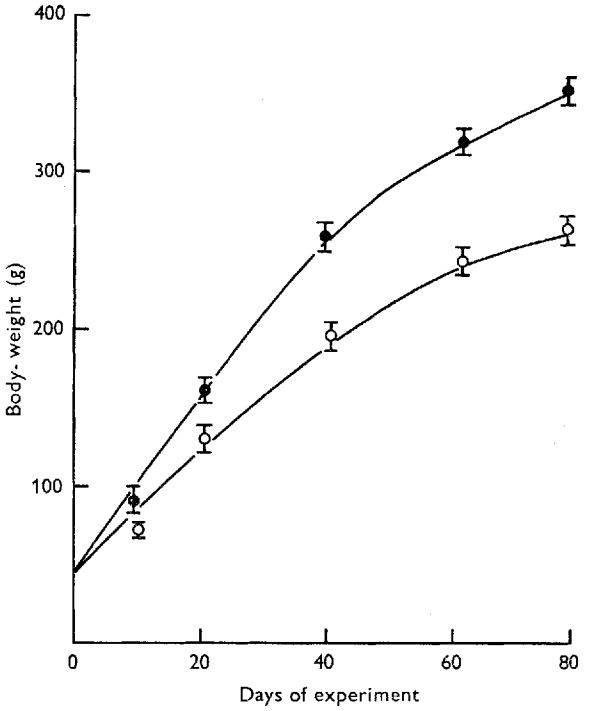

Fig. 1. Expt 19. Mean body-weights of rats given the basal amino acid diet (see Table 1) (○) or that diet supplemented with 50 g torula yeast/kg (●). The vertical bars indicate the SEM for the fifteen rats on each diet.

The long-term effects of factor G were examined separately (Expt 19). The growth response to torula yeast appeared at once and was maintained throughout (Fig. 1). After 80 d the supplemented rats weighed on average about 30% more than control rats. The control rats suffered a moderate degree of hair loss. The effect of factor G deficiency on fertility was then investigated as described on p. 171. All the female rats mated with factor G-deficient males, and all but one of those mated with control males, bore and reared normal litters. The average litter size was eight. Histological examination of other males from the two groups revealed no pathological change attributable to the dietary difference.

DISCUSSION

The methods used in our studies with rats were similar to those described by Schwarz (1970). However, we found that a supplement of amino acids equivalent to 50 g torula yeast/kg induced a significant growth increase, presumably because of the resultant increase in the level of crude protein (N × 6·25) from 135 g/kg to 160 g/kg. We therefore added the precaution of making the N content of each supplemented diet equal to that of the control diet by adjustment of the amino acids in the basal mixture. Furthermore, we found that the general health and appearance of the rats was better when the 100 g lard/kg and 50 g cottonseed oil/kg used by Schwarz (1970) were replaced with 30 g lard/kg only.

Although Schwarz (1970) found that the growth response to factor G sources was unaffected by changes in the type and amount of salt mixture included in the basal diet, he did not examine the activity of the ash components of his active natural products. We found considerable activity in the ash of torula yeast, but were unable to ascribe the effect to a single mineral deficiency. Zn, I, Fe, Cu and Mn seemed to be implicated, even though all of these minerals were present in the rat diet at the concentrations recommended by the (US) National Research Council (1962). The Laboratory Animal Science Association (1969) preferred the higher level of 50 mg Fe/kg diet, recommended by Cuthbertson (1957), to the value of 25 mg/kg diet given by the (US) National Research Council (1962). The addition of 50 g torula yeast/kg of our basal diet raised the level of Fe to 33·8 mg/kg. The basal diet B used by Schwarz (1970) contained more Zn, I and Fe, but less Cu and Mn than ours. It is possible therefore that the growth response reported by Schwarz (1970) for torula yeast could have been due partly to a mineral factor and partly to the rise in the protein level of the diet, in addition to the true response to factor G. The effects o minerals and protein level are probably of less significance when highly active fractions of torula yeast are added to the diet at low concentrations.

Apart from these unusual effects of mineral additions to the diet, we also encountered growth stimulus due to antibiotics and to an extra vitamin supplement. It might be thought that the vitamins in yeast could account for its growth-promoting capacity after the mineral effect had been allowed for. However, the results with Marmite suggested that this is unlikely. Apart from biotin and inositol, the vitamins supplied by Marmite at 20 g/kg are less than one-fifth of the amounts already present in the diet. As described on p. 168, it is likely that much more of the biotin and inositol in Marmite would have been recovered in the 'neutral fraction', which had no growth factor activity, than in the 'basic fraction', which did promote growth.

The vitamin concentrations in the basal amino acid diet all exceeded the recommendations of the (US) National Research Council (1962). The concentrations also exceeded the levels quoted by the Laboratory Animal Science Association (1969), except for choline and menaphthone which were included at 93 and 30% of the recommended levels, respectively. It is possible that the rat's requirement for vitamins may be greater with an amino acid diet than with a conventional diet.

Schwarz (1970) reported considerable factor G activity for a number of liver preparations. In our experiments, when the difference in protein level had been removed, the activity of liver for rats could be attributed to its mineral and water components.

Apart from the differences described above, our studies amply confirm the claim of Schwarz (1970) that torula yeast contains an organic growth factor for rats receiving a purified amino acid diet containing all the nutrients known to be required by the rat. We have confirmed that the effect of factor G becomes apparent within a few days and that the growth differential continues until the rats reach maturity. After 80 d depletion of factor G, the rats showed no macroscopic or microscopic abnormality, apart from some loss of hair, and were fertile. Therefore, in male rats the effect of factor G appears to be solely on growth. Schwarz (1971) has reported briefly on the

separation of factor G into two highly active forms, FG1 and FG2. The present authors hope to publish details of the fractionation of natural sources of factor G in due course.

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