The Role of Fat in the Diet of Rats*

7. The Influence on Growth of Diets Supplemented with Raw Skim Milk, Linoleic Acid or both; and of Raw Casein compared with Alcohol-extracted Casein

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(*Received* 19 *July* 1954)

In previous studies (Aaes-Jørgensen & Dam, 1954*a–e*) with rats on diets with alcohol-extracted casein and hydrogenated fat or no fat a growth-stimulating effect was found when water was replaced by raw skim milk as drinking fluid. Supplementation of the diet with linoleic acid also improved the growth rate. In the animals on diets containing hydrogenated fat or no fat the caloric intake and water consumption were high and the urine output low. The aim of the present experiments was to study in female rats the effect on growth, fluid intake and urine output of supplementing the diet with linoleic acid (20 or 100 mg linoleic acid/animal/day), of replacing alcohol-extracted casein with crude casein and of using raw skim milk as drinking fluid instead of water. The animals were fed on diets containing 28% hydrogenated peanut oil or 28% lard or no fat.

EXPERIMENTAL

Newly weaned female rats were distributed in twenty groups, each of six animals. The compositions of the diets are shown in Table 1. Food and drinking fluid were given *ad lib.* for 26 weeks. Linoleic acid was given as drops (Table 1), as were vitamins A and D. The suspension of Decamin aquosum (Ferrosan Ltd, Copenhagen) supplied 130 i.u. vitamin A, and 20 i.u. vitamin D_2/animal/week. The animals were weighed and inspected weekly, and at the end of the experiment were killed with chloroform. Food and fluid intake and urine production were measured throughout the last 16 weeks of experiment. The kidneys were weighed and examined histologically.

RESULTS AND DISCUSSION

The mean weights of the animals at the beginning and at the end of the experiment are shown in Table 2.

Effect of diets on growth. Giving 20% crude casein instead of 20% alcohol-extracted casein clearly increased the growth rate on diets with 28% hydrogenated peanut oil (groups 100 and 101), as well as on diets with 28% lard (groups 111 and 112). The diet with 28% lard and 20% extracted casein (group 111) gave a significantly

* The first five papers of this series appeared in the *British Journal of Nutrition* (Aaes-Jørgensen & Dam, 1954*a–e*) and no. 6 is that of Aaes-Jørgensen (1954).

higher growth rate than the diet with 28% hydrogenated peanut oil and 20% crude casein (group 100).

To decide whether the effect of crude casein was caused by its content of fat or of an unknown factor, the fat of crude casein was extracted by the Schmid-Bondzynski-Ratzlaff method (Fleischmann & Weigmann, 1932) and was analysed for essential fatty acids (Stillman, 1949). The total fat amounted to 1·2%, and contained: linoleic acid 1·7%, linolenic acid 0·7%, arachidonic acid 1·0%. Extracted casein contained only 0·09% fat. Each 10 g of the diets with 20% crude casein furnished the animals, therefore, with 0·4 mg linoleic acid, 0·2 mg linolenic acid, and 0·2 mg arachidonic

Table 1. *Drinking fluids and percentage composition of the diets of the rats*

Group no.	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119
Crude casein*	20	—	—	—	—	—	—	—	—	20	30	—	20	—	—	—	—	—	—	—
Alcohol-extracted casein†	—	20	20	20	20	20	20	20	20	—	—	20	—	20	20	20	20	20	20	20
Sucrose	46	46	46	46	46	45·6	45·6	46	46	46	36	46	46	46	74	74	73·6	73·6	74	74
Cystine	—	—	—	—	—	0·4	0·4	—	—	—	—	—	—	—	—	—	0·4	0·4	—	—
Hydrogenated peanut oil (m.p. 40–42°)‡	28	28	28	28	28	28	28	28	28	28	28	—	—	—	—	—	—	—	—	—
Lard	—	—	—	—	—	—	—	—	—	—	—	28	28	28	—	—	—	—	—	—
Linoleic acid (mg/animal/day)§	—	—	—	20	100	—	100	20	100	20	20	—	—	—	—	—	—	—	100	100
Drinking fluid	W	W	R	W	W	W	W	R	R	W	W	W	W	R	W	R	W	R	W	R

The diets contained 0·5% of a vitamin mixture (Aaes-Jørgensen & Dam, 1954*a*, Table 1); 5% of McCollum's salt mixture no. 185, supplemented with 13·5 mg KI, 139 mg $CuSO_4 . 5H_2O$ and 556 mg $MnSO_4 . 4H_2O$ per 100 g; and 0·5% choline chloride.
W = water; R = raw skim milk.

* Crude casein from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark. (Precipitated from skim milk by acidification with acid starter at 30–35°, heated to 60–65°, washed with water and dried.)

† Vitamin Test Casein from Genatosan, Ltd. Loughborough, England. (Precipitated from skim milk with acetic acid, washed successively with water, alcohol and ether, and dried.

‡ From Dansk Soyakagefabrik Ltd, Copenhagen.

§ From F. Hoffmann-La Roche and Co. Ltd, Basle, Switzerland.

acid/animal/day. Thus, the animals maximally got about 1 mg essential fatty acids/day from the crude casein in the diet. This is small compared with a supplement of 20 mg/day, and with the amount (about 188 mg/day) obtained by the animals on diets with 28% lard. Nevertheless, growth was better with crude casein instead of alcohol-extracted casein in the 28% hydrogenated peanut-oil diets and likewise with the linoleic-acid supplement, as well as on the lard diets. These results seem to indicate that the growth-promoting factor in crude casein is not one of the essential fatty acids. The factor is either soluble in alcohol or destroyed by treatment with it.

Raw skim milk as drinking fluid instead of water increased the growth rate significantly on the 28% hydrogenated peanut-oil diets (groups 102 and 101) and on the fat-free diet (groups 115 and 114). Change of the drinking fluid from water to raw skim milk had also a clear-cut effect on growth of the animals fed on diets with 28% lard (groups 111 and 113). It is interesting to note that the growth rate on 28% hydrogenated peanut oil with raw skim milk as drinking fluid (group 102) was only about equal to that obtained on the fat-free diet with water (group 114), and significantly lower than on the fat-free diet with raw skim milk (group 115).

Supplementation with 20 mg linoleic acid/animal/day increased the growth rate significantly on the 28% hydrogenated peanut-oil diet whether the casein (20%) was alcohol-extracted (groups 103 and 101) or crude (groups 109 and 100). Increase of the amount of crude casein from 20 to 30% in the 28% hydrogenated peanut-oil diet caused only a slight increase in growth rate (groups 110 and 109). Supplementation with 20 mg linoleic acid/animal/day increased the growth rate significantly on 28% hydrogenated peanut-oil diets also when the drinking fluid was raw skim milk (groups 107 and 102).

Table 2. *Mean weights of groups of six rats at the beginning and at the end of the experiment* (26 *weeks*)

Group no.	Diet characteristics	Weight: Initial (g)	Weight: Final with its standard error (g)
100	28% hydrogenated peanut oil, 20% crude casein, W	44·0	126 ± 4·8
101	28% hydrogenated peanut oil, 20% extracted casein, W	43·7	106 ± 6·5
102	28% hydrogenated peanut oil, 20% extracted casein, R	44·0	142 ± 5·9
103	28% hydrogenated peanut oil, 20% extracted casein, 20 mg linoleic acid, W	43·8	173 ± 6·9
104	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, W	44·0	195 ± 8·0
105	28% hydrogenated peanut oil, 20% extracted casein, cystine, W	43·7	113* ± 4·8
106	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, cystine, W	43·8	196 ± 3·7
107	28% hydrogenated peanut oil, 20% extracted casein, 20 mg linoleic acid, R	43·7	200† ± 7·0
108	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, R	44·2	220 ± 8·4
109	28% hydrogenated peanut oil, 20% crude casein, 20 mg linoleic acid, W	43·7	183 ± 7·0
110	28% hydrogenated peanut oil, 30% crude casein, 20 mg linoleic acid, W	44·0	192 ± 3·9
111	28% lard, 20% extracted casein, W	44·0	207 ± 9·7
112	28% lard, 20% crude casein, W	43·7	237 ± 4·5
113	28% lard, 20% extracted casein, R	43·8	250 ± 12·8
114	No fat, 20% extracted casein, W	44·0	140 ± 6·9
115	No fat, 20% extracted casein, R	43·8	183 ± 4·4
116	No fat, 20% extracted casein, cystine, W	44·2	140 ± 8·7
117	No fat, 20% extracted casein, cystine, R	43·5	177 ± 5·5
118	No fat, 20% extracted casein, 100 mg linoleic acid, W	42·5	201 ± 8·5
119	No fat, 20% extracted casein, 100 mg linoleic acid, R	43·5	224 ± 8·2

W = water, R = raw skim milk.
* Only five animals; one died after 23 weeks of experiment from pneumonia and pleurisy.
† Only five animals; one died after 7 weeks of experiment; cause of death unknown.

Supplementation with 100 mg linoleic acid/animal/day increased the growth rate over that obtained with the 20 mg linoleic-acid supplement of the 28% hydrogenated peanut-oil diet with water as drinking fluid (groups 104 and 103), as well as with raw skim milk as drinking fluid (groups 108 and 107). On the fat-free diet a supplement of 100 mg linoleic acid/animal/day increased the growth rate significantly (groups 118 and 114). A further increase in the growth rate was obtained with raw skim milk as drinking fluid instead of water on the linoleic acid-supplemented fat-free diet (groups 119 and 118).

Supplements of 0·4% cystine did not show any growth-stimulating effect either with the hydrogenated peanut-oil diet (groups 105 and 101) or with the hydrogenated peanut-oil diet supplemented with 100 mg linoleic acid/animal/day (groups 106 and

104). No effect of cystine supplementation was found from the fat-free diet with water (groups 116 and 114) or with raw skim milk (groups 117 and 115) as drinking fluid. Henry & Kon (1953) report a significant increase in the biological value of milk proteins (diets with 8% protein) for rats when supplemented with 0·4% cystine. They stress the findings of Riesen, Schweigert & Elvehjem (1946) that L-cystine supplementation of diets with higher proportions of casein (18–50%) did not improve the growth rate. Our diets contained 20–30% casein, which may explain the fact that we found no effect of cystine supplementation.

From Table 2 it is also seen that supplementation of 28% hydrogenated peanut-oil diets with 20 mg linoleic acid and with raw skim milk as drinking fluid (group 107) increased the average growth rate to the same level as found for the animals fed 28% lard, 20% alcohol-extracted casein and water (group 111). An almost equal effect was obtained on 28% hydrogenated peanut oil with 30% crude casein, 20 mg linoleic acid and water (group 110), or supplementation with 100 mg linoleic acid with water as drinking fluid (group 104).

Animals on the fat-free diet and water supplemented with 100 mg linoleic acid (group 118) grew at the same average rate as the animals on 28% lard and water (group 111). However, supplementation with 100 mg linoleic acid and raw skim milk as drinking fluid on 28% hydrogenated peanut-oil (group 108) or fat-free diets (group 119) did not increase the growth rate up to the level obtained with 28% lard and 20% crude casein (group 112), or 28% lard with 20% alcohol-extracted casein and raw skim milk as drinking fluid (group 113).

The growth rate on 28% hydrogenated peanut-oil diets, 20% alcohol-extracted casein and water (groups 101 and 105) was significantly lower than on the corresponding fat-free diets (groups 114 and 116). With crude casein instead of alcohol-extracted casein in the diets with 28% hydrogenated peanut oil (groups 100 and 101) the growth rate was less than that of the animals on the fat-free diet with alcohol-extracted casein (group 114). Raw skim milk as drinking fluid in place of water on the diets with 28% hydrogenated peanut oil (group 102) improved the growth rate to the same level as that found with the fat-free diet with water (group 114); it was, however, significantly lower than with raw skim milk as drinking fluid on the fat-free diet (group 115).

The animals never drank more than 50 ml. skim milk/animal/day (Table 4). Maximally 50 g of raw skim milk contain 25 mg fat, i.e. about 0·8 mg essential fatty acids. (Butterfat contains 1·9% linoleic acid, 0·7% linolenic acid and 0·5% arachidonic acid as measured by the procedure of Stillman (1949).) Again it is unlikely that the small amount of linoleic acid from raw skim milk was responsible for the relatively large effect on growth. Investigations on the effect of such small doses of essential fatty acids are in progress in this laboratory.

It should be noted that the effect of giving raw skim milk in place of water as drinking fluid was always superior to that obtained by giving crude casein instead of alcohol-extracted casein. This finding may be explained by the extra casein in the skim milk.

From Table 3 it will be seen that with 28% hydrogenated peanut oil and alcohol-extracted casein in the diet and water as drinking fluid growth stopped at about the 13th week of the experiment and from then on weight decreased (groups 101 and 105). These findings are similar to the results obtained with male rats on diets containing 20% crude casein and 28% hydrogenated whale oil, hydrogenated herring oil, hydrogenated seal oil or hydrogenated coconut oil in a 14-week experiment. These animals stopped growing about the 6th–8th week of the experiment and thereafter the weight declined (Aaes-Jørgensen, 1954). However, in the present experiment with female rats crude casein instead of alcohol-extracted casein (groups 100 and 101), with raw skim milk in place of water as drinking fluid (groups 102 and 101), caused a period, about the last 13 weeks of experiment, of nearly constant weight. On the fat-free diet (groups 114 and 116) growth stopped about the 15th week and was nearly unchanged throughout the rest of the experiment (10 weeks), whereas raw skim milk in place of water on this diet gave a rising growth curve throughout (groups 115 and 117).

As mentioned above, it seems unlikely that the growth-promoting effect of either crude casein or raw skim milk can be attributed to their content of essential fatty acids. Further, neither crude casein nor raw skim milk showed any effect in curing the skin signs of the animals fed on diets with hydrogenated peanut-oil or fat-free (Table 3). This finding is in accord with that of Brown & Burr (1936) with rats reared on fat-free diets.

In an earlier paper Aaes-Jørgensen & Dam (1954*d*) reported a significantly better growth rate of animals on 28% hydrogenated peanut-oil diets supplemented with 21·4 mg linoleic acid/animal/day than on the same diet unsupplemented. Further, the animals fed on the linoleic acid-supplemented diet grew better than animals fed on a diet with only 7% hydrogenated peanut oil and supplemented with the same amount of linoleic acid (21·4 mg/animal/day). This finding seems contrary to the common belief that increase of dietary fat increases the requirement for essential fatty acids.

These results, the present findings and the results with hydrogenated marine oils (Aaes-Jørgensen, 1954), might be explained by formation of biologically inert isomers during hydrogenation which could displace essential fatty acids in the body. Further, the composition of the diet, other than the fat is of great importance when the growth rate is used as an indication of the requirements for essential fatty acids.

Requirements for essential fatty acids. Another point of interest in connexion with the discussion of the requirements for essential fatty acids is that most experiments done in this field are (*a*) on depleted animals, and (*b*) with diets very low in or devoid of fat. The depletion is very often undertaken during the period of the animals' highest growth rate. It is difficult to know how severe the consequences of depletion are when only the growth rate, i.e. the sum of many biological processes, is used for evaluating the depletion time. Further, the ability of the animals to regain normal conditions when given linoleic acid after a depletion period has so far been studied but little. In most experiments it is found that linoleic-acid supplementation does not give a growth rate equal to that of the controls reared on diets containing, e.g., lard or vegetable oils. Secondly, the use of diets without, or with very small amounts of, fat interferes to a high degree with the calorie metabolism of these animals. Therefore, it seems to us

Table 3. *Skin signs and their duration, growth period, kidney weight and calculi content, of groups of six rats during the experimental period of 26 weeks*

Group no.	Diet characteristics	Severity* of skin signs and period (weeks) during which they were evident: Hind-legs	Fore-legs	Tail	Fur	Cessation of growth: Week	Remarks	Kidneys: Mean wet weight (g)	Mean wet weight/100 g body-weight (g)	Mean occurrence of calculi†
100	28 % hydrogenated peanut oil, 20 % crude casein, W	+ + + +, 5th–26th	+ +, 12th–26th	+ + + +, 8th–26th	Thin, dandruff	c. 15th	Maximal weight = final weight	1·80	1·43	1·6
101	28 % hydrogenated peanut oil, 20 % extracted casein, W	+ + + +, 4th–26th	+ +, 13th–26th	+ + + +, 7th–26th	Thin, greasy, dandruff	c. 13th	Maximal weight > final weight	1·66	1·57	1·0
102	28 % hydrogenated peanut oil, 20 % extracted casein, R	+ + +, 4th–26th	+ +, 11th–26th	+ + +, 6th–26th	Thin, dandruff	c. 15th	Maximal weight = final weight	1·78	1·25	1·0
103	28 % hydrogenated peanut oil, 20 % extracted casein, 20 mg linoleic acid, W	+ 14th–17th	0	+, 10th–26th	Normal	26th	Final weight highest	1·77	1·02	1·6
104	28% hydrogenated peanut oil, 20% extracted casein, 100 mg. linoleic acid, W	0	0	(+), 10th–26th	Normal	26th	Final weight highest	1·72	0·88	2·4
105	28 % hydrogenated peanut oil, 20 % extracted casein, cystine, W	+ + + +, 4th–26th	+ +, 10th–26th	+ + + +, 7th–26th	Thin, dandruff	c. 13th	Maximal weight > final weight	1·78	1·58	1·6
106	28 % hydrogenated peanut oil, 20 % extracted casein, cystine, 100 mg linoleic acid, W	0	0	(+), 10th–26th	Normal	26th	Final weight highest	1·80	0·92	1·0
107	28 % hydrogenated peanut oil, 20 % extracted casein, 20 mg linoleic acid, R	+, 11th–13th	0	+, 6th–26th	Normal	26th	Final weight highest	1·85	0·93	2·8
108	28 % hydrogenated peanut oil, 20 % extracted casein, 100 mg linoleic acid, R	0	0	+ +, 3rd–26th	Thin around the mouth periodically	26th	Final weight highest	1·82	0·83	2·5
109	28 % hydrogenated peanut oil, 20 % crude casein, 20 mg linoleic acid, W	+, 11th–18th	0	+, 8th–26th	Normal	26th	Final weight highest	1·75	0·96	4·3
110	28 % hydrogenated peanut oil, 30 % crude casein, 20 mg linoleic acid, W	+, 15th–18th	0	+ +, 9th–26th	Thin periodically, especially on the neck	26th	Final weight highest	1·98	1·03	1·6
111	28 % lard, 20 % extracted casein, W	0	0	(+), 10th–26th	Normal	26th	Final weight highest	1·87	0·90	4·2
112	28 % lard, 20 % crude casein, W	0	0	(+), 8th–26th	Normal	26th	Final weight highest	2·32	0·98	4·3
113	28 % lard, 20 % extracted casein, R	0	0	+, 3rd–26th	Normal	26th	Final weight highest	2·05	0·82	4·7
114	No fat, 20 % extracted casein, W	+ + + +, 5th–26th	+ +, 5th–26th	+ + + +, 5th–26th	Thin, dandruff	c. 15th	Maximal weight = final weight	2·00	1·43	3·0
115	No fat, 20 % extracted casein, R	+ + +, 5th–26th	+ +, 5th–26th	+ + + +, 3rd–26th	Thin, dandruff	26th	Final weight highest	1·82	1·00	3·0
116	No fat, 20 % extracted casein, cystine, W	+ + + +, 5th–26th	+, 6th–26th	+ + +, 6th–26th	Thin, dandruff	c. 15th	Maximal weight ≥ final weight	1·88	1·34	2·7
117	No fat, 20 % extracted casein, cystine, R	+ + + +, 5th–26th	+, 6th–26th	+ + + +, 3rd–26th	Thin, dandruff	26th	Final weight highest	1·97	1·11	2·5
118	No fat, 20 % extracted casein, 100 mg linoleic acid, W	0	0	+ +, 4th–26th	Thin around the mouth periodically	26th	Final weight highest	1·94	0·97	4·6
119	No fat, 20 % extracted casein, 100 mg linoleic acid, R	0	0	+ +, 2nd–26th	Normal	26th	Final weight highest	2·03	0·91	4·6

W = water, R = raw skim milk.

* Dry, no scaliness = (+); slight scaliness = +; scaliness = + +; severe scaliness = + + +; very severe scaliness = + + + +

† Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).

Table 4. *Mean values per rat for weight, daily food, calorie and fluid intake and urine output for groups of six rats during the last 8 weeks of experiment, and calorie cost of gain in weight throughout the whole experimental period of 26 weeks calculated from these values*

Group no.	Diet characteristics	Weight (g)	Surface area* (sq.cm)	Food intake (g)	Calorie intake† Cal.	Calorie intake† Cal./sq.m (*a*)	Fluid intake ml.	Fluid intake ml./sq.m (*b*)	Urine output ml.	Urine output ml./sq.m (*c*)	Difference: fluid intake − urine output ($b-c$) (ml./sq.m)	Calories used for evaporation of water‡ (measured as $b-c$) (*d*) (Cal./sq.m)	Calories available for growth ($a-d$) (Cal./sq.m)	Gain in weight throughout the 26 weeks of experiment (*e*) (g)	Calorie cost§ of weight gain $\left(\frac{a-d}{e}\right)$ (Cal./sq.m/g)
100	28% hydrogenated peanut oil, 20% crude casein, W	125	289	9·4	48·5	1678	38·1	1318	5·4	187	1131	688	990	0·45	2200
101	28% hydrogenated peanut oil, 20% extracted, casein, W	110	265	8·9	45·9	1732	34·9	1317	3·5	132	1185	721	1011	0·34	2974
102	28% hydrogenated peanut oil, 20% extracted casein, R	140	312	7·1	51·1	1638	42·6	1365	7·3	234	1131	688	950	0·54	1759
103	28% hydrogenated peanut oil, 20% extracted casein, 20 mg linoleic acid, W	167	350	9·9	51·0	1457	37·9	1083	6·1	174	909	553	904	0·71	1273
104	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, W	189	381	8·3	42·8	1123	38·2	1003	6·8	178	825	502	621	0·83	748
105	28% hydrogenated peanut oil, 20% extracted casein, cystine, W	115	273	8·6	44·4	1626	34·7	1271	4·1	150	1121	682	944	0·38	2484
106	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, cystine, W	192	385	9·1	47·0	1221	43·1	1119	6·7	174	945	575	646	0·84	769
107	28% hydrogenated peanut oil, 20% extracted casein, 20 mg linoleic acid, R	189	381	8·8	58·7	1541	39·1	1026	13·6	357	669	407	1134	0·86	1319
108	28% hydrogenated peanut oil, 20% extracted casein, 100 mg linoleic acid, R	207	405	7·6	53·1	1311	40·9	1010	15·6	385	625	380	931	0·97	960
109	28% hydrogenated peanut oil, 20% crude casein, 20 mg linoleic acid, W	176	363	11·0	56·8	1565	44·9	1237	6·5	179	1058	644	921	0·76	1212
110	28% hydrogenated peanut oil, 30% crude casein, 20 mg linoleic acid, W	187	378	9·9	51·1	1352	37·0	979	7·3	193	786	478	874	0·81	1079
111	28% lard, 20% extracted casein, W	200	395	9·1	47·0	1190	29·8	754	5·4	137	617	375	815	0·90	906
112	28% lard, 20% crude casein, W	228	432	10·0	51·6	1194	30·8	713	5·5	127	586	357	837	1·06	790
113	28% lard, 20% extracted casein, R	236	442	7·8	49·1	1111	26·2	593	7·7	174	419	255	856	1·13	758
114	No fat, 20% extracted casein, W	139	310	12·6	47·4	1529	31·9	1029	3·3	106	923	562	967	0·53	1825
115	No fat, 20% extracted casein, R	170	355	9·1	49·6	1397	45·4	1279	9·3	262	1017	619	778	0·76	1024
116	No fat, 20% extracted casein, cystine, W	141	313	12·8	48·1	1537	37·1	1185	4·1	131	1054	641	896	0·53	1691
117	No fat, 20% extracted casein, cystine, R	167	351	9·0	46·8	1333	38·2	1088	8·4	239	849	517	816	0·73	1118
118	No fat, 20% extracted casein, 100 mg linoleic acid, W	192	385	12·1	45·5	1182	39·2	1018	6·8	177	841	512	670	0·87	770
119	No fat, 20% extracted casein, 100 mg linoleic acid, R	219	420	8·8	49·5	1179	48·3	1150	25·0	595	555	338	841	0·99	849

W = water, R = raw skim milk.

* Surface area (sq.cm) = $11·36 \times \sqrt[3]{W^2}$, where *W* is weight in g (Harte, Travers & Sarich, 1948).

† Calculated by assuming that protein and carbohydrate yield 4 Cal./g and fat 9 Cal./g, and skim milk 0·34 Cal./g.

‡ 608·5 Cal./kg for evaporation of water at 29°.

§ Assuming that all the calories ($a-d$) are used for growth.

difficult to estimate the requirements for essential fatty acids from experiments with animals depleted during the period of rapid growth, or from results obtained with animals reared on fat-free diets. Apparently the discrepancy between the requirements for essential fatty acids found in our experiments and those of other workers is due to the fact that in our experiments the animals were reared on diets containing hydrogenated fats or natural fats low in essential fatty acids, whereas the other workers used depleted animals or animals reared on fat-free diets.

Efficiency of food conversion. Mean results for food and fluid intake and urine production during the last 8 weeks of experiment are presented in Table 4.

In the last column of Table 4 are shown the calculations of the calorie cost/sq.m body surface/day/g weight gain $\left(\frac{a-d}{e}\right)$, after subtraction of the estimated calories used for evaporation of water. The daily weight gain was calculated on the basis of the weight increase throughout the whole experimental period because the animals in various groups had stopped growing or even declined in weight in the last part of the experiment (Table 3).

With 28% hydrogenated peanut-oil in the diet the highest amount of calories/g weight gain was found with 20% alcohol-extracted casein, and water as drinking fluid (groups 101 and 105). A decrease in the calorie cost of weight gain was found with crude casein in place of alcohol-extracted casein (group 100), with raw skim milk as drinking fluid instead of water (group 102) and by supplementation of the diet with 20 mg linoleic acid/animal/day (group 103); a further decrease occurred with 100 mg linoleic acid/animal/day (groups 104 and 106).

On the fat-free diet raw skim milk in place of water (groups 115 and 114), as well as supplementation of the diet with 100 mg linoleic acid/animal/day (groups 117 and 114), decreased the calorie cost of weight gain.

With 28% lard in the diet the amount of calories/weight gain was decreased somewhat by substituting crude casein for alcohol-extracted casein (groups 112 and 111); a corresponding effect was obtained on giving raw skim milk instead of water as drinking fluid (groups 113 and 111).

Efficiency of food utilization was highest on diets with lard, lower on the fat-free diets and lowest on the diets with 28% hydrogenated peanut oil.

Effect of diets on the kidneys. In Table 3 are shown both the mean wet weight in g of the kidneys and the weight/100 g body-weight. The absolute weights of the kidneys of the animals on all the diets with hydrogenated peanut oil as well as on the fat-free diets were never greater than those of the lard-fed animals.

The weight of the kidneys/100 g body-weight was very high on the unsupplemented hydrogenated peanut-oil and fat-free diets, compared with that of the kidneys of the lard-fed animals. Supplementation with linoleic acid or with raw skim milk instead of water as drinking fluid decreased the relative weight of the kidneys on both types of diet. These findings are in accord with earlier results from this laboratory (Aes-Jørgensen, 1954).

On histological examination of the kidneys calculi were observed in the cortico-medullary border in all the animals. The results of these investigations are summarized in Table 3. It will be seen that the largest number of calculi was found in the groups on diets with 28% lard. The animals on the fat-free diets had more calculi than the animals fed on the diets with 28% hydrogenated peanut oil. Further, a high supplement of linoleic acid (100 mg/animal/day) seemed to increase the number of calculi on these two diets.

SUMMARY

1. Newly weaned female rats were reared on diets with 28% hydrogenated peanut oil for an experimental period of 26 weeks. Crude casein was substituted for alcohol-extracted casein, raw skim milk was given in place of water as drinking fluid, or the diets were supplemented with 20 or 100 mg linoleic acid/animal/day. The performance of these animals was compared with that of animals on fat-free diets receiving water or raw skim milk as drinking fluid, or supplemented with 100 mg linoleic acid/animal/day. Diets containing 28% lard were used for the control animals. Growth rate was recorded, calorie and fluid intake and urine output were measured, and histological studies were made on the kidneys.

2. On the diets with 28% hydrogenated peanut oil the growth rate was increased by substituting crude casein for alcohol-extracted casein, by giving raw skim milk instead of water as drinking fluid or by supplementation with linoleic acid. The effect on growth was higher with linoleic acid than with either crude casein or raw skim milk; a supplement of 100 mg linoleic acid/animal/day increased the growth somewhat more than did a supplement of 20 mg linoleic acid/animal/day. The effects of the linoleic-acid supplement and of raw skim milk seemed to be additive.

3. On the fat-free diet the growth rate was increased with raw skim milk instead of water as drinking fluid; a further increase was found on supplementation with 100 mg linoleic acid/animal/day.

4. On the diets with 28% lard the growth rate was increased by substituting crude casein for alcohol-extracted casein, and with raw skim milk in place of water as drinking fluid.

5. The growth rate of the animals fed on the 28% hydrogenated peanut-oil diet with water was significantly lower than that of the rats on the fat-free diet with water. Raw skim milk in place of water as drinking fluid increased the growth rate of the animals on the diet containing 28% hydrogenated peanut oil to the level of those on the fat-free diet with water, but it was significantly lower than that of the animals on the fat-free diet with raw skim milk.

6. The increased growth rate could not be explained by an increase of the calorie intake/sq.m, irrespective of the substitution of crude casein for alcohol-extracted casein, or of raw skim milk for water as drinking fluid, or by supplementation of the diet with linoleic acid.

7. The occurrence of calculi in the cortico-medullary border of the kidneys was more frequent on diets with 28% lard than on diets with 28% hydrogenated peanut oil.

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The Effect of Vitamin B_6 on the Growth and the Blood Picture of the Rat

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(*Received* 30 *July* 1954)

In continuing studies of rat growth on highly purified diets (Copping, Crowe & Pond, 1951) it seemed useful to test the value of the purified basal diet for biological estimation of vitamins of the B complex. A preliminary study was made with vitamin B_6. As the literature at that time contained apparently conflicting reports on the blood picture in vitamin B_6-deficient rats a haematological study was also undertaken.

EXPERIMENTAL

Animals. The 222 rats used in these experiments were of the Lister Institute black-and-white stock from a colony now maintained at Queen Elizabeth College and behaving, whenever biological tests have made comparisons possible, in the same manner as the original stock. The rats were taken at weaning at 23 days of age, weighing 35–45 g. They were placed in separate cages with open-grid floors and were given the purified basal diet with all vitamin supplements except vitamin B_6. Litters of six to eight rats were used and, when graded doses of vitamin B_6 were given, due regard was paid to the distribution of litter-mates and sexes throughout the various groups. In preliminary tests, series C_3, C_4 and C_6, it was observed that larger differences in the growth response to graded doses of vitamin B_6 were obtained if the animals were deprived of the vitamin for 3 weeks before they were dosed. On the other hand, in series C_5 the effect of this deprivation on the blood picture was found to be negligible. In this series four animals in each group were given the graded doses