

Weight maintenance and protein intake of the young rat

By L. R. NJAA

*Government Vitamin Laboratory, Norwegian Fisheries Research Institute,
Bergen, Norway*

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Evaluation of protein quality by the criteria of net protein utilization (NPU), biological value (BV) and other similar measurements requires assumptions about the protein needs for purposes other than growth (Mitchell, 1923-4; Bender & Doell, 1957; Miller & Payne, 1961; Njaa, 1963). This portion of the total protein need is subsequently referred to as the 'maintenance requirement' of protein. The maintenance requirement is not a well-established quantity either for the rat or for other species. Barnes, Bates & Maack (1946) reported that the maintenance protein requirements of young and adult rats were nearly the same and in terms of egg protein equal per 100 cm² of body surface to about 11 mg N/day. This is equivalent to 140-150 mg N/day kg^{0.73} for rats ranging in weight from 50 to 250 g. The requirement for egg protein for maintenance of nitrogen balance found by Forbes, Vaughan & Yohe (1958) was about 0.9 mg N/day g^{0.75} which is equivalent to 160-180 mg N/day kg^{0.73} for the same body-weight range. The (USA) National Research Council's (1959) Committee on Amino Acids put the basal protein requirement at 880 mg/day kg^{0.734} (Smuts, 1935) or about 140 mg N/day kg^{0.73}, increased by 10% to allow for metabolic faecal loss of N. Recently Miller & Payne (1963, 1964) suggested that the maintenance requirement is much higher, namely 250 mg N/day kg^{0.73}. Their estimate (Miller & Payne, 1964) was based on their own results obtained with young rats and pigs (Miller & Payne, 1962), and on some results selected from the literature. Njaa (1962) pointed out that the maintenance item used by Miller & Payne (1961) was higher than generally accepted values. This communication reports experiments designed to obtain estimates of the protein levels and the corresponding protein intakes required to maintain body-weight of young rats when given eight protein sources with widely different chemical scores. By multiplying the intakes by the chemical scores estimates of the maintenance requirement of ideal protein have been obtained. The results indicate that at 22° this requirement is slightly less than 200 mg N/day kg^{0.73} and that at 28° the requirement is less than at 22°.

EXPERIMENTAL

Animals and technique

Young white rats from our own colony were weaned at 21 ± 1 days and were given a stock diet containing about 20% protein (N × 6.25) until they weighed between 50 and 60 g, usually at 25-28 days. The rats were then used in the experiments. Each experiment was with four or five groups of five or six rats, the rats being divided into

the groups according to sex and body-weight, and usually also according to litters. Only one protein source was tested in each experiment, except in Expt 9 when five protein sources were compared. The rats were kept individually in wire cages and they were usually given the experimental diets for 12–15 days. This length of period was chosen because in determinations of NPU and BV each diet is usually given for periods extending from 9 to 14 days (Mitchell, 1923–4; Miller & Bender, 1955; Njaa, 1959). In two experiments (Expts 8, 13) the experiment lasted longer (23 and 65 days). The food was given either *ad lib.* or in restricted daily amounts. The food cups were changed daily and the food residues and spillings were collected. Water was added to the diets to reduce spilling, but in one experiment (Expt 10) wet and dry diets were compared. The rats were weighed daily just before the food cups were changed, except on Sundays and other holidays.

The animal rooms were kept at $22 \pm 1^\circ$ in all experiments except in Expt 11 in which a comparison was made between this temperature and $28 \pm 1^\circ$.

The composition of the diets was as described by Njaa (1959); the protein content was varied by reciprocal variations in the contents of the protein source and partly dextrinized potato starch. In Expt 10 a mixture of raw potato starch and rice starch was compared with dextrinized potato starch.

Maintenance of body-weight was judged from the slopes of the curves of weight gain against time calculated for the period following the first 4 or 5 days (rate of weight change, g/day). In Expt 8 the slope for days 13–23 was calculated and in Expt 14 the slopes were calculated for the entire period. Maintenance of body-weight was judged to have been attained when the slope values were not significantly different from zero or were less than 0.1 g/day. The maintenance protein levels or the corresponding intakes of ideal protein were estimated by relating the slopes so obtained to the protein levels or to the intakes of ideal protein ($N \text{ intake} \times \text{chemical score}$) by fitting smooth lines to the points. In arithmetical presentation these lines were usually sigmoid. The intersections of the lines with the ordinate corresponding to zero slope were taken as estimates of the maintenance protein levels or of the maintenance requirement of ideal protein.

The criteria adopted allow only rough estimates of the maintenance protein level to be made. They do not take into consideration that the body composition of the rats may be changing during the experiment within and between groups. However, the data available in the literature indicate only small changes in the N content of rats given different types of diet (Bender & Doell, 1957; Henry & Toothill, 1962; Čabak, Dickerson & Widdowson, 1963).

Protein sources and chemical scores

The protein sources used are listed in Table 1. The egg albumin was spray-dried hen's egg white, the herring meal was a flame-dried whole meal, the casein was a rennet casein dried in a casein dryer at 60–65°, the soya-bean meal was solvent-extracted and heat-treated, the linseed meal was solvent-extracted, the pea meal was made from dried uncooked green peas, the wheat gluten was plate dried at 35–70°

and the zein was obtained from the firm Dr Th. Schuchardt, Munich, Germany. The chemical scores of egg albumin, casein, soya-bean meal, pea meal and wheat gluten were taken from the FAO (1957) publication, those of herring meal, linseed meal, and fortified zein were calculated from the FAO (1957) provisional amino acid pattern with amino acid data taken from Boge (1960), Siemermann (1959) and Block & Mitchell (1946-7), respectively. In herring-meal protein, methionine plus cystine was assumed to be the limiting entity (Njaa, 1961). The protein contents listed were not determined in this study but represent rounded values of determinations made in connexion with other experiments.

Table 1. *Protein contents ($N \times 6.25$) and chemical scores of the protein sources used*

Protein source	Protein content (%)	Chemical score (%)
Egg albumin	80	100
Herring meal	70	90
Casein	80	80
Soya-bean meal	48	70
Linseed meal	34	70
Pea meal	23	60
Wheat gluten	83	40
Zein + 1.3% L-lysine hydrochloride + 0.9% DL-tryptophan	92	30

Design of experiments

Expts 1-8. The protein sources listed in Table 1 were given at four protein levels as indicated in Table 2. At least the lowest was believed to be below the maintenance level for the particular protein source. The rats given wheat gluten were continued on new diets for an additional 11-day period after the initial 12-day period; the other experiments lasted from 12 to 14 days (Table 2).

Expt 9. Five of the protein sources used in Expts 1-8 were retested at a protein level believed to be near to the maintenance level as judged from the previous results. Egg albumin was tested at a protein level of 1.6%, herring meal at 2.0%, linseed meal at 2.9%, wheat gluten at 3.4% and pea meal at 4.9%. The experiment lasted for 12 days (Table 2).

Expt 10. Egg albumin was given at the 2.3% protein level in diets containing either partly dextrinized potato starch (67.5%) or a mixture of raw potato starch (15%) and rice starch (52.5% of the diet). The B complex vitamins which usually were pipetted as solutions into the diet (Njaa, 1959) were in this experiment given as a dry mixture in partly dextrinized potato starch of which 1% was added to the diets. Both diets were given wet and dry. The experiment lasted for 12 days (Table 3).

Expt 11. Egg albumin was given at the 2.1 and 4% protein levels; one group on each protein level was kept at $22 \pm 1^\circ$ and one group at $28 \pm 1^\circ$. The experiment lasted for 14 days (Table 4).

In Expts 1-11 food was given *ad lib*.

Expts 12 and 13. Egg albumin was given at the 0, 2, 4 and 6% protein levels (Expt 12) and at the 4.2, 6, 8.2 and 10.4% levels (Expt 13). In both experiments

Table 2. Mean daily intakes of food, calories, and nitrogen \times chemical score, in groups of six (Expts 2-5, 7-9) or five (Expts 1, 6) rats given eight protein sources at levels near to the assumed maintenance level

Expt no.	Protein source	Protein content of diet (%)	Daily intake			Mean body-weight (g)	Slope* (g/day)
			Food (g)	Calories (kcal/kg ^{0.75})	Nitrogen \times score (mg/kg ^{0.75})		
1 (12 days)	Egg albumin	1.0	7.4	236	100	53.3	(-0.19)
		2.0	8.2	257	216	55.5	+0.66
		2.9	8.7	263	320	58.2	+1.18
		3.9	9.0	242	445	68.5	+1.59
2 (14 days)	Soya-bean meal	2.1	6.2	204	126	51.7	-0.51
		3.2	7.0	223	210	54.2	-0.13
		4.5	7.5	231	307	56.8	+0.42
		5.5	8.2	246	398	59.0	+0.73
3 (13 days)	Linseed meal	2.1	6.5	208	129	53.6	-0.33
		3.1	6.9	216	196	55.9	(+0.22)
		4.0	7.6	228	269	58.8	+0.97
		5.1	7.5	219	334	59.7	+1.22
4 (13 days)	Herring meal	1.1	6.4	207	86	53.1	-0.58
		2.2	7.2	220	184	57.4	(+0.28)
		2.9	7.7	232	249	60.8	+1.09
		4.3	8.1	233	380	62.3	+1.43
5 (14 days)	Casein	1.0	5.4	180	61	51.1	-0.55
		2.0	6.2	203	137	52.3	-0.36
		3.1	7.3	224	235	56.9	+0.30
		4.2	7.2	220	312	57.4	+0.33
6 (14 days)	Fortified zein	6.8	5.4	179	153	51.3	-0.45
		7.7	5.2	173	168	51.0	-0.45
		8.9	5.5	179	187	51.5	-0.40
		10.1	5.5	183	232	51.4	-0.44
7 (12 days)	Pea meal	2.9	7.3	245	180	50.5	-0.36
		4.0	7.3	237	240	51.7	-0.32
		4.6	7.4	243	279	52.8	(+0.11)
		5.6	7.7	244	345	54.8	+0.39
8 (day 1-12)	Wheat gluten	4.4	6.7	239	160	53.6	(+0.07)
		5.5	6.6	212	195	54.1	+0.21
		6.5	6.7	238	219	53.9	+0.25
		7.8	6.7	233	274	55.8	+0.43
8 (day 13-23)	Wheat gluten	4.0	6.8	212	144	55.6	+0.24
		5.0	6.4	198	166	56.3	+0.27
		5.9	6.5	200	199	56.8	+0.31
		7.0	6.4	188	222	60.3	+0.36
9 (12 days)	Egg albumin	1.6	6.1	197	132	53.2	-0.45
	Herring meal	2.0	6.6	211	159	54.0	-0.19
	Linseed meal	2.9	6.7	211	181	54.6	(-0.03)
	Pea meal	4.9	6.4	202	251	55.0	-0.07
	Wheat gluten	3.4	6.8	213	123	55.3	-0.08

* Slope of the weight gain against time curves calculated for the period following the first 4 or 5 days. The values in parentheses were not significantly different from zero.

the daily food ration was restricted to 4.5 g/rat which was eaten completely by all the rats except those in the 0% group in Expt 12. Expt 12 lasted for 15 days and Expt 13 for 12 days (Table 5).

Expt 14. Egg albumin was given at the 1, 2, 3 and 4% protein levels for 65 days, except with the 1% group for which the period was 32 days. Restricted daily amounts of food were given for the first 46 days. The food offered was eaten completely by all the rats except those in the 1% group which refused some food from day 14 on. During the first 8 days 4.5 g/rat were given; for the next 6 days the ration was 5.5 g, for the next 11 days 6 g, for the next 7 days 5.5 g, for the next 7 days 5.0 g, and for the next 7 days 4.5 g. For the remaining 19 days food was given *ad lib.* (Table 5).

Table 3. *Expt 10. Mean daily intakes of food, calories and nitrogen by groups of six rats given egg albumin diets containing either partly dextrinized potato starch and sucrose (D) or raw potato starch, rice starch and sucrose (R) as the carbohydrate ingredients with (+) or without (-) addition of water for 12 days*

Protein content of diet (%)	Type of diet	Daily intake			Mean body-weight (g)	Slope*
		Food (g)	Calories (kcal/kg ^{0.73})	Nitrogen (mg/kg ^{0.73})		
2.3	D-	5.9	195	188	51.6	(-0.09)
2.3	D+	5.5	185	179	50.3	(-0.16)
2.3	R-	5.8	195	188	50.3	-0.16
2.3	R+	6.1	203	196	51.1	(+0.06)

* Slope of the weight gain against time curves for the period following the first 4 days. The values in parentheses were not significantly different from zero.

Table 4. *Expt 11. Mean daily intakes of food, calories and nitrogen by groups of six rats given egg albumin diets at the 2.1 and 4.0% protein levels at 22 and 28° for 14 days*

Protein content of diet (%)	Temperature (°C)	Daily intake			Mean body-weight (g)	Slope*
		Food (g)	Calories (kcal/kg ^{0.73})	Nitrogen (mg/kg ^{0.73})		
2.1	22	6.3	207	183	51.8	(0.00)
4.0	22	7.8	237	400	57.9	+0.80
2.1	28	4.6	150	132	52.6	(-0.11)
4.0	28	5.2	162	272	56.2	+0.39

* Slope of the weight gain against time curves for the period following the first 5 days. The values in parentheses were not significantly different from zero.

RESULTS

Graphs of cumulative weight gains are given in Figs. 1-6. The protein contents of the diets used, the intakes of food, calories, and N × chemical score, are given in Tables 2-5. The intakes of calories and of N are given on the basis of daily intake per kg body-weight to the power 0.73 (Miller & Payne, 1962). The slopes of the weight gain against time curves (p. 444) are also given; the values in parentheses were not significantly different from zero.

Table 5. Mean daily intakes of food, calories and nitrogen by groups of six young rats given restricted daily amounts of egg albumin diets at different protein levels

Expt no.	Protein content of diet (%)	Daily intake			Mean body-weight (g)	Slope*
		Food (g)	Calories (kcal/kg ^{0.73})	Nitrogen (mg/kg ^{0.73})		
12 (15 days)	0.1	4.1	141	—	48.8	-0.38
	2.0	4.5	152	129	50.0	-0.12
	4.0	4.5	148	248	52.0	(-0.06)
	6.0	4.5	147	372	52.3	(+0.08)
13 (12 days)	4.2	4.5	148	261	52.1	-0.10
	6.0	4.5	148	372	52.1	(0.00)
	8.2	4.5	147	505	52.8	(-0.03)
	10.4	4.5	148	640	52.6	-0.12
14 (days 1-8)	1.0	4.5	152	64	50.2	—
	2.1	4.5	151	128	50.8	—
	3.0	4.5	152	193	49.9	—
	3.9	4.5	151	249	50.7	—
14 (days 9-14)	1.0	5.5	202	86	44.7	(-0.08)
	2.1	5.5	198	167	46.0	(-0.01)
	3.0	5.5	202	254	44.8	+0.10
	3.9	5.5	198	324	46.0	(+0.07)
14 (days 15-25)	1.1	5.5	202	94	44.6	(-0.02)
	2.1	6.0	210	187	47.6	+0.32
	3.0	6.0	210	257	47.5	+0.43
	4.0	6.0	203	343	49.9	+0.60
14 (days 26-32)	1.1	3.9	147	68	43.3	-0.41
	2.1	5.5	197	167	49.3	+0.11
	3.0	5.5	188	236	50.1	+0.18
	4.0	5.5	178	296	54.0	+0.31
14 (days 33-39)	—†	—	—	—	—	—
	2.1	5.0	171	150	49.6	+0.18
	3.0	5.0	168	212	50.5	+0.15
	4.0	5.0	158	266	54.9	+0.21
14 (days 40-46)	2.1	4.5	151	134	50.4	(-0.04)
	2.9	4.5	149	200	51.6	-0.19
	4.0	4.5	142	236	56.0	-0.22
14 (days 47-65)	2.1	5.1	166	147	52.8	+0.18
	3.2	8.7	250	340	61.9	+0.97
	4.2	10.3	261	460	74.3	+1.98

* Slope of the weight gain against time curve for the period following the first 4 days (Expts 12 and 13) or for the entire period (Expt 14). The values in parentheses were not significantly different from zero.

† Discontinued.

Maintenance of body-weight under ad lib. feeding conditions

Judged by the criteria adopted (p. 444), the maintenance protein levels and the corresponding maintenance requirements of ideal protein estimated from the results obtained in Expts 1-8 (Table 2; Fig. 1) were about 1.2% (120 mg N/day kg^{0.73}) for egg albumin, about 2% (160 mg) for herring meal, about 2.7% (190 mg) for casein, about 2.8% (185 mg) for linseed meal, about 3.5% (230 mg) for soya-bean meal, about 4% and about 3.5% (150 and 130 mg) for wheat gluten, and about 4.5% (270 mg) for pea meal. Maintenance of body-weight was not attained on the diets containing zein fortified with lysine and tryptophan at protein levels below 10%.

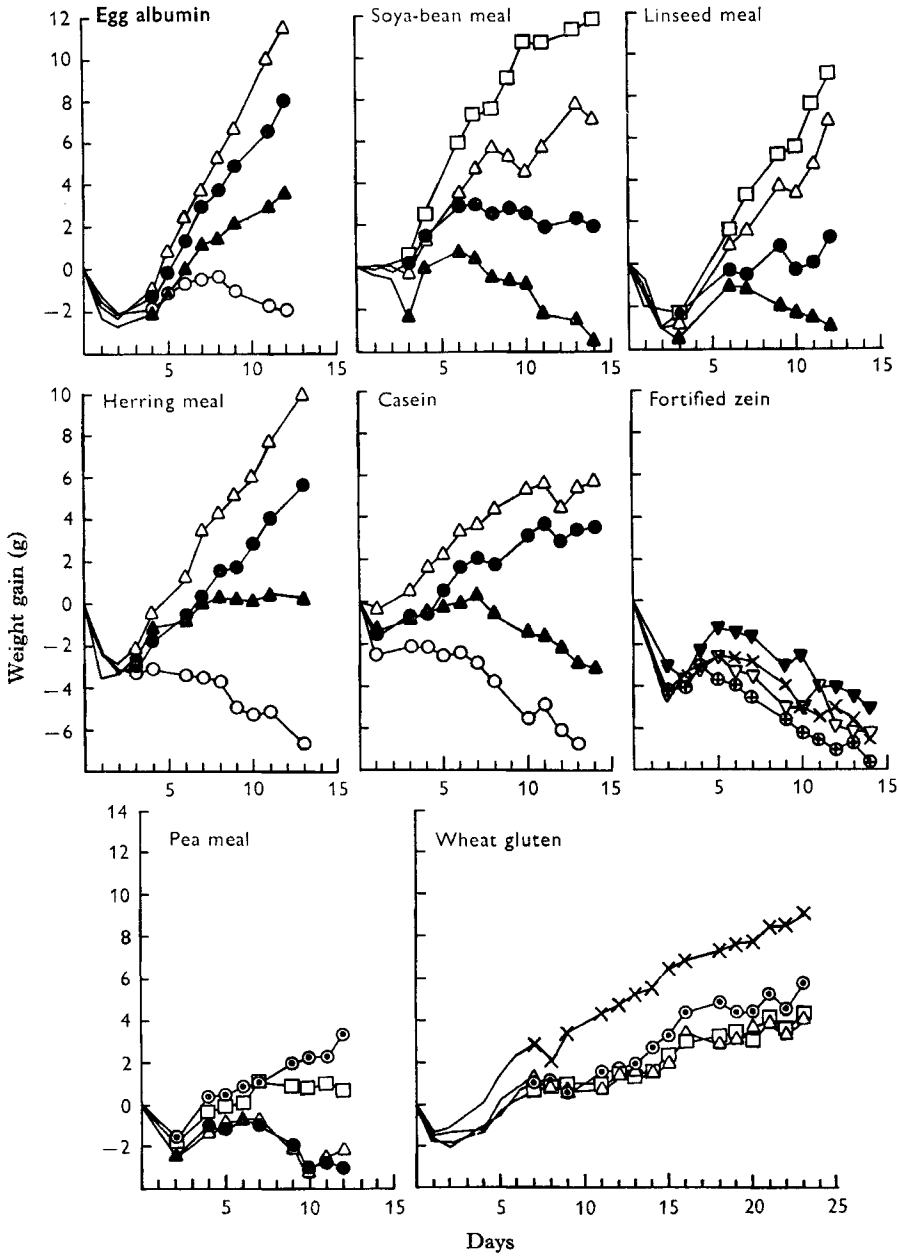


Fig. 1. Expts 1-8. Mean cumulative weight-gain curves for groups of five or six rats given eight protein sources, each at four protein levels. ○—○, 1.0% egg albumin, 1.1% herring meal, 1.0% casein; ▲—▲, 2.0% egg albumin, 2.1% soya-bean meal, 2.1% linseed meal, 2.2% herring meal, 2.0% casein; ●—●, 2.9% egg albumin, 3.2% soya-bean meal, 3.1% linseed meal, 2.9% herring meal, 3.1% casein, 2.9% pea meal; △—△, 3.9% egg albumin, 4.5% soya-bean meal, 4.0% linseed meal, 4.3% herring meal, 4.2% casein, 4.0% pea meal, 4.4 and 4.0% wheat gluten; □—□, 5.5% soya-bean meal, 5.1% linseed meal, 4.6% pea meal, 5.5 and 5.0% wheat gluten; ⊙—⊙, 5.6% pea meal, 6.5 and 5.9% wheat gluten; ×—×, 6.8% fortified zein, 7.8 and 7.0% wheat gluten; ⊕—⊕, 7.7% fortified zein; 8.9% fortified zein; ▼—▼, 10.1% fortified zein.

The results obtained in Expt 9 with herring meal, linseed meal, wheat gluten and pea meal agreed fairly well with these estimates (Table 3; Fig. 2), whereas the results with egg albumin indicated that the maintenance protein level was higher than 1.6% and that the maintenance requirement of protein was higher than 130 mg N. The results obtained in Expts 10 and 11 indicated that the maintenance protein level was about 2% and the maintenance requirement about 190 mg N. The maintenance protein level of about 2% for egg albumin was not significantly affected when the

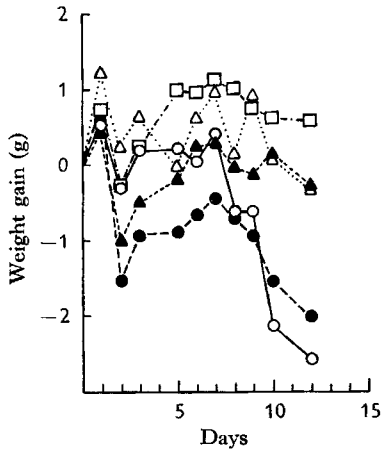


Fig. 2

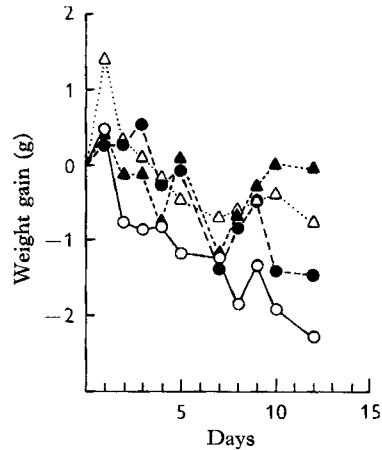


Fig. 3

Fig. 2. Expt 9. Mean cumulative weight-gain curves for groups of six rats given five protein sources at protein levels near to the maintenance levels. \circ — \circ , 1.6% egg albumin; \bullet — \bullet , 2.0% herring meal; \blacktriangle — \blacktriangle , 2.9% linseed meal; \square — \square , 3.4% wheat gluten; \triangle — \triangle , 4.9% pea meal.

Fig. 3. Expt 10. Mean cumulative weight-gain curves for groups of six rats given egg albumin as protein source at the 2.3% protein level in diets containing two types of carbohydrate ingredient with (+) and without (-) added water. \triangle — \triangle , partly dextrinized potato starch, sucrose (+); \bullet — \bullet , partly dextrinized potato starch, sucrose (-); \circ — \circ , raw potato starch, rice starch, sucrose (-); \blacktriangle — \blacktriangle , raw potato starch, rice starch, sucrose (+).

diet was given wet or dry (Expt 10; Fig. 3), by either type of carbohydrate ingredients in the diet (Expt 10; Fig. 3), or by a change of the temperature in the animal rooms from 22 to 28° (Expt 11; Fig. 4). The maintenance requirement was with egg-albumin protein lower at 28° than at 22°, about 135 and 190 mg N/day $\text{kg}^{0.73}$, respectively (Expt 11, Table 4; Fig. 4).

In the groups approximately maintaining their mean body-weight at 22° the mean daily food intake ranged from 5.4 to 7.4 g/rat (Expts 2-4, 7-11; Tables 2-4). The diet contained by calculation about 3.8 kcal/g, thus the daily calorie intake ranged from 180 to 245 kcal/ $\text{kg}^{0.73}$. The calorie intakes in growing groups were not greatly different from this (Expts 1-5, 7-9, 11; Tables 2-4). Gain in weight was observed in only one experiment on an intake of less than 200 kcal/day $\text{kg}^{0.73}$ (Expt 8, period 2; Table 2). The calorie intake on the egg-albumin diet at the 2.3% protein level was not influenced by the addition of water to the diet or by the change of carbohydrate ingredients in it (Expt 10; Table 3). At 28° the daily calorie intakes at 2.1 and 4%

protein levels were lower than those observed at 22°. The latter were within the range usually observed at this temperature (180–245 kcal/kg^{0.73}). At 28° the daily intake of the 2.1% diet was equivalent to about 150 kcal/kg^{0.73} and that of the 4% diet to about 160 kcal/kg^{0.73} (Expt 11; Table 4).

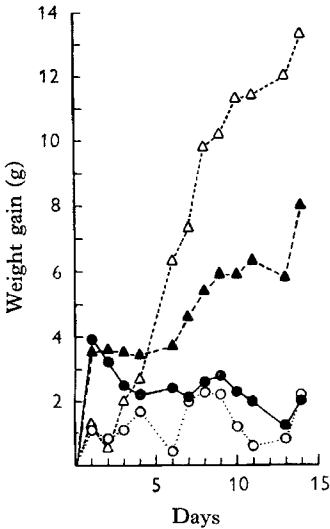


Fig. 4

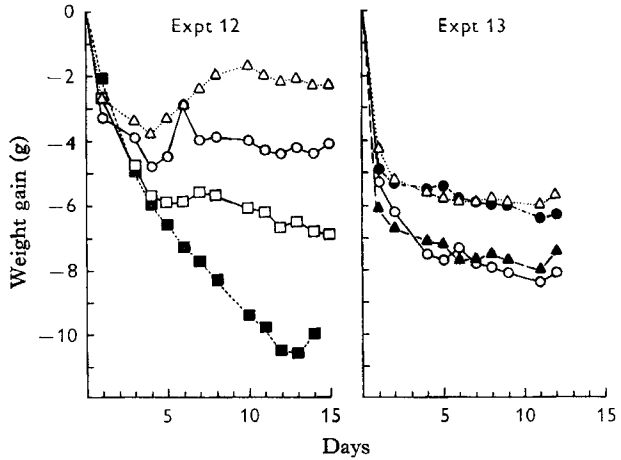


Fig. 5

Fig. 4. Expt 11. Mean cumulative weight-gain curves for groups of six rats given egg albumin as protein source at the 2.1 and 4.0% protein levels at 22° and 28° room temperatures. ○····○, 2.1%, 22°; ●—●, 2.1%, 28°; △---△, 4.0%, 22°; ▲---▲, 4.0%, 28°.

Fig. 5. Expts 12 and 13. Mean cumulative weight-gain curves for groups of six rats given diets with egg albumin as protein source at a restricted daily amount of 4.5 g/rat at different protein levels. ■—■, 0%; □—□, 2.0%; ○—○, 4.0% (Expt 12), 4.2% (Expt 13); △...△, 6%; ▲---▲, 8.2%; ●····●, 10.4%.

Maintenance of body-weight under conditions of restricted feeding

When the egg-albumin diets were offered in restricted amounts the rats apparently maintained their body-weight at the 1.0–1.1% protein level (Expt 14, days 9–14 and 15–25, Table 5; Fig. 6). However, they refused some of the food offered, and after 32 days were so emaciated that the group had to be discarded. At the 2.1% protein level the rats maintained their body-weight when the daily food intake was 5.5 g and 4.5 g (days 9–14, 26–32, and 40–46), and they grew at food intakes of 6.0 g and 5.0 g (days 15–25 and 33–39). They also grew at a slow rate when they were given free access to the food at a mean daily intake of 5.1 g (days 47–65). Thus, the maintenance protein level under conditions of restricted feeding is difficult to define but seems to be slightly less than 2.1%. In Expts 12 and 13, but not in Expt 14 (Table 5; Figs. 5 and 6), the body-weight attained after the initial rapid loss during the first 4 days was approximately maintained at protein levels ranging from 2.0 to 10.4%. The higher protein levels tended to give higher body-weights than lower levels.

The rats maintaining their body-weight, or growing slowly, at the 2.1% protein

level seemed to require less calories and protein the longer the period of restriction (Expt 14, days 9-14, 26-32, 33-39 and 40-46, Table 5). When the rats were given free access to the food they grew slowly at about 170 kcal/day $\text{kg}^{0.73}$ and about 150 mg N/day $\text{kg}^{0.73}$ (days 47-65). Under the latter condition there was also a clear tendency for the calorie intake to increase with increasing protein content of the diet, in contrast to the results obtained with unrestricted diets (Table 2).

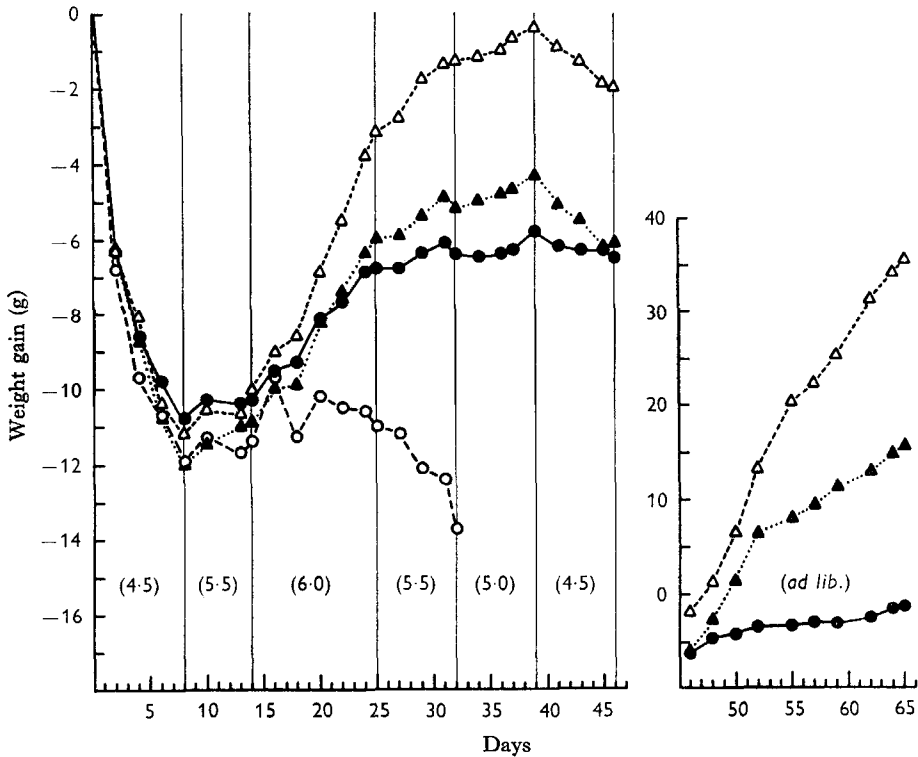


Fig. 6. Expt 14. Mean cumulative weight-gain curves for groups of six rats given diets with egg albumin as protein source at four protein levels and at various daily food intakes. \circ --- \circ , 1.0-1.1%; \bullet --- \bullet , 2.1%; \blacktriangle \blacktriangle , 2.9-3.2%; \triangle --- \triangle , 3.9-4.2%. The graphs were drawn on the basis of the weighings every 2 or 3 days. (The daily amounts (g) of food given are shown in parentheses.)

DISCUSSION

Maintenance of body-weight under ad lib. feeding conditions

The low levels of protein needed for maintenance found in these experiments confirm the suggestion made on theoretical grounds (Njaa, 1962) that maintenance levels predicted by the equation of Miller & Payne (1961) are too high. The calorie intake of the rats maintaining their body-weight was about 200 kcal/day $\text{kg}^{0.73}$, and the mean calculated maintenance requirement for ideal protein was equivalent to about 190 mg N/day $\text{kg}^{0.73}$. (The high value obtained with pea meal and the low value obtained with wheat gluten are commented upon below.) The corresponding quantities obtained by combining the equations given by Miller & Payne (1961, 1962)

were 156 kcal and 250 mg N. Thus, the higher food intakes in my experiments only partly explain the low maintenance protein levels observed. The different results could neither be explained by the practice of adding water to the diet (Njaa, 1959), as might be suggested by the findings of Keane, Smutko, Krieger & Denton (1962, 1963), nor could they be due to improved protein utilization resulting from better utilization of the carbohydrate part of the diet (Heard, 1964; Expt 10). The higher food intake resulted from keeping the rats at 22° as compared with 28° used by Miller & Payne (Miller, 1963). However, the maintenance protein level seemed to be the same at both temperatures (Expt 11) and the maintenance protein need consequently seemed to be higher at the lower temperature. The results obtained in Expt 9 with the egg-albumin diet indicate that the higher need was not merely due to the greater calorie intake at the lower temperature. The rats lost weight at a calorie intake equal to that observed for maintenance at 22° and at a N intake equal to that observed for maintenance at 28°.

The calculated maintenance needs showed divergent results when the protein sources were wheat gluten and pea meal (Table 2). This may be explained for wheat gluten by assuming that the chemical score for maintenance is appreciably higher than for growth. It has been adequately demonstrated that the lysine requirement for maintenance is much lower than that for growth (Mitchell, 1947; Henry & Kon, 1952) and the results of Barnes *et al.* (1946) indicate a nutritive value of wheat gluten for maintenance of 62 and for growth of 21. The chemical score adopted for pea meal (FAO, 1957) is probably too high. Amino acid analyses by Block & Mitchell (1946-7), Tsien & Johnson (1959) and Schuphan & Postel (1960) give chemical scores relative to the FAO (1957) amino acid pattern of 52, 45 and 33. Moreover, the chemical scores used by Carpenter (1951) and cited by Munro (1964) indicate that the score for peas is about 35% relative to egg albumin. It is probable therefore that the chemical scores of wheat gluten and pea meal as given in Table 1 should be interchanged. This would be in agreement with the growth responses observed and it would bring the calculated maintenance protein needs into accord with those found with the other protein sources.

The estimate of 190 mg N/day $\text{kg}^{0.73}$ for the maintenance protein needs agrees well with that of Forbes *et al.* (1958) but it is higher than the estimate of Barnes *et al.* (1946). However, the results agree in indicating that the maintenance protein requirement is at most about 75% of the quantity suggested by Miller & Payne (1962, 1964).

The finding, attributed to Goettsch (1951), that the maintenance protein requirement of rats equals 250 mg N/day $\text{kg}^{0.73}$ (Miller & Payne, 1964) warrants some comment. Her results recalculated to the basis of $\text{kg}^{0.73}$ gave 230 mg N/day $\text{kg}^{0.73}$ for rats weighing more than 160 g and 190 mg N for rats weighing less than this. However, it is doubtful whether it is permissible to base such estimates on experimentally determined NPU values, as was done by Goettsch (1951), because NPU already includes estimates of the two most important items of the maintenance requirement, namely metabolic faecal N and endogenous urinary N.

The sum of these inevitable N losses is often used as an estimate of the maintenance

requirement. It is of interest, therefore, to compare it with the estimates of the maintenance requirement obtained in the experiments now described and with those of Barnes *et al.* (1946) and Forbes *et al.* (1958). If the daily calorie intake at maintenance is 140 kcal/kg^{0.73} (Brody, 1945) the corresponding food intake of a diet containing 3.8 kcal/g is 37 g/kg^{0.73}. According to Mitchell (1948), the metabolic faecal N excretion is 1–2 mg/g food and the endogenous urinary excretion is 0.6 mg/g^{0.75}. Corresponding values given by Metta (1960) are 2 mg N/g food and 0.5 mg N/g^{0.75}. Thus the sum of these excretions would be about 140–175 mg N/day kg^{0.73}. In the experiments presented now, performed at a lower room temperature, the calorie intake was about 200 kcal/day kg^{0.73}, and in previous experiments the metabolic faecal excretion was about 1.9 mg N/g food and the daily endogenous urinary excretion about 0.66 mg N/kg^{0.75} (Njaa, 1963). Thus, the sum of these excretions corresponds to about 210 mg N/day kg^{0.73}. The magnitudes of the metabolic and endogenous N losses are probably overestimated in the procedures used to determine them (Njaa, 1963) but, on the other hand, estimates of the maintenance protein requirement based on them do not take into account the N lost in hair. This loss is relatively small (Miller & Payne, 1964) and may probably be more than compensated for by the overestimation of other N losses. In my experience an unusually great loss of hair was observed in one experiment with eighteen adult rats given low-protein diets. A 1-day collection of hair from these rats weighed about 0.5 g, which by calculation was found to be equivalent to a N loss of about 12 mg/day kg^{0.73}. This estimate of loss of N in hair should be regarded as an upper limit. Thus, the excretion data referred to give estimates of the maintenance protein requirement in good agreement with the estimates arrived at from the data of Barnes *et al.* (1946) and Forbes *et al.* (1958), and those obtained in the experiments now described.

The effect of environmental temperature

The temperature of the animal rooms in different laboratories varies between 20 and 30°, but it is often not stated. This was so with my own communications (Njaa, 1959, 1961, 1962, 1963) and with those of Miller & Bender (1955) and Miller & Payne (1961, 1962). Recently Miller (1963) revealed that experiments were done at 28 ± 1° and argued that this temperature range is better suited for protein utilization studies than temperatures outside the range of thermal neutrality for the rat. However, Jacob & Payne (1964) found that efficiencies of protein utilization were the same at 15 and 25° at low protein levels.

The results obtained in Expt 11 indicated that the maintenance protein requirement was higher at 22° than at 28° although the maintenance protein levels were equal. The 4% group of rats grew at a slower rate at the higher temperature. A measure of the efficiency of protein utilization of the amount consumed above the maintenance needs is the quotient between the extra weight gain and the extra protein consumed, this quantity being 4.0 g/g protein for the 28° group and 5.0 for the 22° group. There seems therefore to be no advantage in measuring the protein utilization by criteria of weight-gain measurements within the range of thermal neutrality for the rat. Tem-

peratures within this range are near to the point where temperature regulation breaks down (Swift & Forbes, 1939; Herrington, 1941) and it may be questioned whether a temperature range found to be consistent with minimum metabolism under short-term measurements on fasting rats is also the range for minimum metabolism of rats with free access to the food and kept for a longer period of time at the high temperature (Kleiber, 1961, p. 274).

For comparison of results on protein utilization between laboratories it seems to be important that the temperature of the animal rooms should be stated. Further, the effect of the temperature on growth, N balance and maintenance protein requirement should be given more attention in discussions of methods of protein evaluation.

Maintenance of body-weight under conditions of restricted feeding

The results obtained in Expt 14 showed that the rats on restricted diets were able to maintain their body-weight at lower intakes of calories than those required by rats fed *ad lib.* This is in agreement with the results of Quimby (1948), Kaunitz, Slanetz, Johnson & Guilmain (1956) and Lee & Lucia (1961) who showed that the calorie requirement of rats given sufficient food just adequate to maintain their body-weight tends to decline. This is probably a consequence of a reduced basal metabolism due to the conditions of restricted feeding. As the rats maintained their body-weight, or grew slowly, at the 2.1% protein level, the maintenance protein requirement declined in parallel with the calorie requirement. Thus, under conditions of both *ad lib.* and restricted feeding the rats maintained their body-weight on slightly less than 1 mg N/kcal. When the rats were restricted from the beginning of the experiment to the calorie intake on which they eventually maintained their body-weight in Expt 14 there was a tendency for higher protein intakes to be required in order to maintain the body-weight attained after the first rapid weight loss (Expts 12 and 13).

SUMMARY

1. The protein levels required for the maintenance of body-weight at 22° room temperature of young rats weighing 50–60 g were determined on groups of five or six rats with eight protein sources of widely differing chemical scores. Each protein source was given under *ad lib.* feeding conditions at four protein levels, of which at least the lowest was assumed to be below the maintenance level.

2. The maintenance protein level was about 2% for egg albumin and herring meal, between 2 and 3% for casein, about 3% for soya-bean meal and linseed meal, about 3.5% for wheat gluten and about 5% for pea meal. Zein fortified with lysine and tryptophan to give a chemical score of about 30 did not maintain body-weight when included at protein levels up to 10%.

3. The daily intake of ideal protein (actual intake × chemical score) was reasonably constant at about 190 mg N/kg^{0.73} when the rats maintained their body-weight on the seven protein sources. The corresponding calorie intake was about 200 kcal/kg^{0.73}; this quantity was not appreciably higher in growing rats.

4. The type of carbohydrate in the diet and the practice of giving the diet wet or dry did not significantly influence the protein utilization of the egg albumin diet at the 2.3% level.

5. When egg albumin protein was given at the 2.1 and 4% levels at 22 and 28° room temperature, the body-weight was maintained at the 2.1% level at both temperatures. The food intake at both levels was lower at 28 than at 22°. Thus the maintenance protein requirement was lowest at the former temperature. The rats on the 4% protein diet grew faster at 22 than at 28°; the protein utilization measured as g extra gain in weight per g extra protein eaten above maintenance was slightly better at 22°. The importance of stating at which temperature protein utilization studies are performed is stressed.

6. Under conditions of restricted feeding with egg-albumin diets the 2.1% protein level seemed to be near the maintenance level. The calorie intake and the amount of protein required for maintenance seemed to decline in parallel, so that the protein requirement was equal to about 1 mg N/kcal under conditions of both *ad lib.* and restricted feeding.

REFERENCES

- Barnes, R. H., Bates, M. J. & Maack, J. E. (1946). *J. Nutr.* **32**, 535.
 Bender, A. E. & Doell, B. H. (1957). *Br. J. Nutr.* **11**, 140.
 Block, R. J. & Mitchell, H. H. (1946-7). *Nutr. Abstr. Rev.* **16**, 249.
 Boge, G. (1960). *J. Sci. Fd Agric.* **11**, 362.
 Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold Publishing Corp.
 Čabak, V., Dickerson, J. W. T. & Widdowson, E. M. (1963). *Br. J. Nutr.* **17**, 601.
 Carpenter, K. J. (1951). *Br. J. Nutr.* **5**, 243.
 FAO (1957). *F.A.O. nutr. Stud.* no. 16.
 Forbes, R. M., Vaughan, L. & Yohe, M. (1958). *J. Nutr.* **64**, 291.
 Goettsch, M. (1951). *J. Nutr.* **45**, 609.
 Heard, C. R. C. (1964). *Proc. Nutr. Soc.* **23**, 110.
 Henry, K. M. & Kon, S. K. (1952). *J. Dairy Res.* **19**, 216.
 Henry, K. M. & Toothill, J. (1962). *Br. J. Nutr.* **16**, 125.
 Herrington, L. P. (1941). In *Temperature, its Measurement and Control in Science and Industry*, p. 446. [C. O. Fairchild, J. D. Hardy, R. B. Sosman and H. T. Wensel, editors.] New York: Reinhold Publishing Corp.
 Jacob, M. & Payne, P. R. (1964). *Proc. Nutr. Soc.* **23**, v.
 Kaunitz, H., Slanetz, C. A., Johnson, R. E. & Guilmain, J. (1956). *J. Nutr.* **60**, 221.
 Keane, K. W., Smutko, C. J., Krieger, C. H. & Denton, A. E. (1962). *J. Nutr.* **77**, 18.
 Keane, K. W., Smutko, C. J., Krieger, C. H. & Denton, A. E. (1963). *J. Nutr.* **81**, 87.
 Kleiber, M. (1961). *The Fire of Life*. New York: Wiley.
 Lee, M. & Lucia, S. P. (1961). *J. Nutr.* **74**, 243.
 Metta, V. C. (1960). *J. Am. diet. Ass.* **37**, 234.
 Miller, D. S. (1963). *Publs natn. Res. Coun., Wash.*, no. 1100, p. 34.
 Miller, D. S. & Bender, A. E. (1955). *Br. J. Nutr.* **9**, 382.
 Miller, D. S. & Payne, P. R. (1961). *Br. J. Nutr.* **15**, 11.
 Miller, D. S. & Payne, P. R. (1962). *J. Nutr.* **78**, 255.
 Miller, D. S. & Payne, P. R. (1963). *J. theor. Biol.* **5**, 398.
 Miller, D. S. & Payne, P. R. (1964). *Proc. Nutr. Soc.* **23**, 11.
 Mitchell, H. H. (1923-4). *J. biol. Chem.* **58**, 873.
 Mitchell, H. H. (1947). *Archs Biochem.* **12**, 293.
 Mitchell, H. H. (1948). In *Proteins and Amino Acids in Nutrition*. Ch. 2, p. 46. [M. Sahyun, editor.] New York: Reinhold Publishing Corp.
 Munro, H. N. (1964). In *Mammalian Protein Metabolism*. Vol. 2, p. 3. [H. N. Munro and J. B. Allison, editors.] New York: Academic Press Inc.
 National Research Council (1959). *Publs natn. Res. Coun., Wash.*, no. 711, p. 15.
 Njaa, L. R. (1959). *Br. J. Nutr.* **13**, 137.

Njaa, L. R. (1961). *J. Sci. Fd Agric.* **12**, 757.

Njaa, L. R. (1962). *Br. J. Nutr.* **16**, 185.

Njaa, L. R. (1963). *FiskDir. Skr.* **4**, no. 5.

Quimby, F. H. (1948). *J. Nutr.* **36**, 177.

Schuphan, W. & Postel, W. (1960). *Z. Lebensmittelunters.* **113**, 223.

Siemermann, B. (1959). Möglichkeiten zur Verbesserung der biologischen Eiweisswertigkeit durch Kombination verschiedener Eiweissfuttermittel. (Säulenchromatographische Aminosäuregehaltsbestimmungen in Soja-, Erdnuss-, Rops-, Kokos- und Palmkernschrot). Thesis, Bonn.

Smuts, D. B. (1935). *J. Nutr.* **9**, 403.

Swift, R. W. & Forbes, R. M. (1939). *J. Nutr.* **18**, 307.

Tsien, W. S. & Johnson, B. C. (1959). *J. Nutr.* **68**, 419.