# Nutrition of the cat

## 3*. Protein requirements for nitrogen equilibrium in adult cats maintained on a mixed diet

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(*Received* 5 *December* 1959—*Revised* 23 *February* 1960)

Using a mixed diet in which liver and fish were the principal sources of protein, Dickinson & Scott (1956*b*) found that growth of kittens was only satisfactory when the protein fraction (N × 6·25) exceeded 30% of the dry weight of the diet. The aim of the experiment reported here was to determine the minimum amount of protein, in a similar diet, necessary to maintain adult resting cats in nitrogen equilibrium.

### EXPERIMENTAL

*Experimental design.* The design of the experiment was based on a modification of the method of Melnick & Cowgill (1937). Six canned diets were prepared having protein concentrations ranging from 46 to 13% on the dry-weight basis. The experiment was divided into six fortnightly periods, which followed one another without interruption. During the first period each cat received the diet highest in protein, during the second period the next highest and so on. After the sixth period an attempt was made to give a N-free semi-purified diet, but it was abandoned after 6 days as the animals refused to eat. Individual N balances were done during each of the six periods.

*Management of animals.* Four adult cats were used, two males and two females, one of which (F 2) was parous. They were in excellent condition and had been maintained on the colony stock diet (Dickinson & Scott, 1956*a*) for many months. Each cat was caged separately in a rabbit metabolism cage 24 × 18 × 14 in., which gave the animal reasonable freedom of movement. The floor of the cage was a galvanized iron grid of approx. $\frac{1}{3}$ in. squares. The cage rested on a funnel of tinned copper, the whole being supported in a metal frame with the funnel dipping directly into the urine-collecting flask. The animals were exercised for about 20 min each morning while their cages were being cleaned. Adaptation to metabolism cages was achieved quickly and easily; the cats were housed in them and fed on their accustomed stock diet for a week before the experiment began. Each cat was weighed three times a week: it was found that consistent results were obtained if the weighings were carried out at about the same time each day. The cats were fed once a day without restriction of intake. Uneaten remnants of diet were dried to constant weight and weighed back at the end of each period. Tap water was provided *ad lib.*

* Paper no. 2: *Brit. J. Nutr.* (1956), **10**, 311.

*Diets*. Six diets (prepared in bulk, canned and sterilized) were compounded from the ingredients: raw liver, white fish, cut wheat, potato flake, pork dripping, ground sugar-beet residues, salt and water. Different protein levels were obtained by substituting white fish for potato flake and water. The raw liver was maintained at about 5% of the total wet mixture in all the diets. The six diets were designed to be isocaloric, to have about the same roughage content and to provide sufficient total calories. Analyses of the diets used are given in Table 1.

Table 1. *Percentage proximate composition and calorie content of diets on a dry-weight basis*

| Constituent | Diet A | B | C | D | F | H |
|---|---|---|---|---|---|---|
| Protein (N × 6·25) | 12·7 | 18·8 | 25·7 | 31·9 | 39·3 | 46·2 |
| Fat | 16·5 | 15·1 | 15·8 | 15·0 | 16·3 | 15·1 |
| Ash | 5·0 | 6·2 | 7·4 | 8·5 | 9·7 | 9·5 |
| Carbohydrate (by difference) | 65·8 | 59·9 | 51·1 | 44·6 | 34·7 | 29·2 |
| Moisture | 63·7 | 67·6 | 68·9 | 70·6 | 71·2 | 73·6 |
| Calories (kcal/g)* | 4·63 | 4·51 | 4·49 | 4·41 | 4·43 | 4·38 |

* Calculated on the basis that protein and carbohydrate provide 4, and fat 9, kcal/g.

*Balance procedure*. Preliminary experiments showed that an adjustment period of 4 days on each new diet was essential, and a collection period of 8 days desirable, to reduce errors in sampling. On the 5th morning of the 14-day period on each diet, about 0·5 g carmine was mixed with the weighed food given to each cat. All the urine, and that portion of the faeces stained red, were collected on the 6th day. Each morning subsequently, weighed food was given, and excreta collected, the last food of the 8-day balance period being given on the 12th day. Urine and faeces, representing food eaten on previous days, were collected on the 13th day when carmine was again added to the diet. Unstained faeces appearing on the 14th day were separated and included with the previous day's collection.

Urine was collected through glass-wool into a 500 ml volumetric flask. The collecting funnel under the cage was washed with distilled water each morning, washings passing directly into the flask. The volume was made up to 500 ml and a 10 ml sample transferred to a stoppered flask, containing a drop of toluene, which was stored in a refrigerator. The eight daily samples were pooled for each cat for each period.

Faeces of the cats were usually firm and easily collected, a good separation being achieved. Each day the faeces of each cat were transferred, with distilled-water washings (volume usually 100–150 ml), from the grid to a 500 ml graduated cylinder.

A glass stirrer (electrically driven) was introduced and set in motion while concentrated sulphuric acid (A.R.) was allowed to drip in slowly and continuously from a separating funnel. The faeces became hot and blackened and were reduced to a semi-fluid consistency. After cooling overnight the mixture was made up to 250 ml (ideally) and a 25 ml sample pipetted into a glass-stoppered bottle for storage. On the diets containing the smaller percentages of protein faeces were bulky and more resistant to digestion. Final volumes reached 400 ml and pipettes frequently became blocked, so that samples, constituting one-tenth of the final volume, had to be measured by means of a narrow graduated cylinder. Analyses carried out on the same material sampled by the two methods gave as good agreement as two samples measured in the same way.

At the end of each period the N content of the dried diet and the pooled samples of urine and of faeces were each estimated by a macro-Kjeldahl method with selenium dioxide as a catalyst. Duplicate analyses agreed within 1–2%. Reagent blanks were done for the whole procedure, purified glucose being used.

## RESULTS

Detailed results for each cat are given in Table 2. The N balance of each individual animal on each diet is plotted in Fig. 1, in terms of the cat's surface area (Greaves, 1957) against the percentage of protein in the diet. The mean balances for each period are also shown. There was considerable variation between the cats, particularly

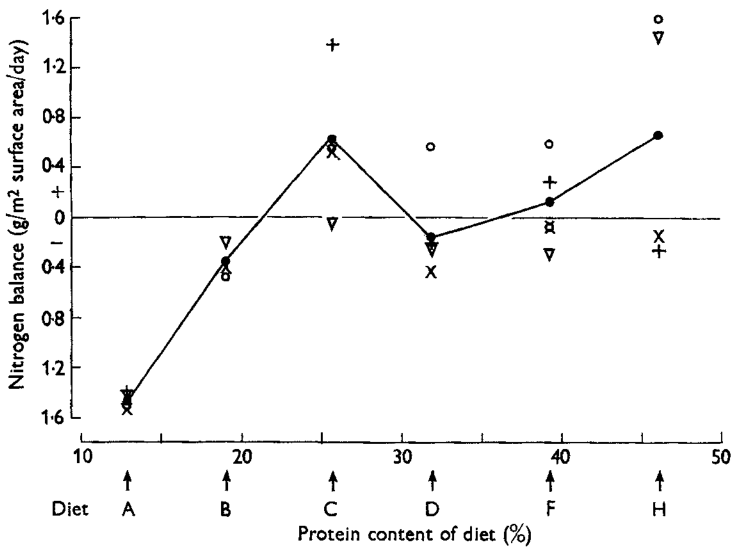


Fig. 1. Graph showing N balances of cats on diets of diminishing protein content. Closed circles show means; the other plots are individual balances. o, M 1; ×, M 2; ▽, F 1; +, F 2.

at the higher protein levels on which they began. They maintained slight positive or negative balances on all diets until the 26% protein level was reached, when one cat (F 1) was in N equilibrium and three were in positive balance. On the diets containing 19 and 13% protein all the cats were in negative balance. The mean in Fig. 1 crosses the line of zero balance at a point corresponding to a dietary protein level of 21%. This amount of dietary protein of mixed fish and liver origin would then appear to be the minimum needed to maintain adult resting cats in N equilibrium.

The weights of the animals were in general well maintained on the four diets highest in protein (Table 2), but below a dietary level of 26% protein weights began to fall.

As the protein concentration in the diet decreased the cats tended to eat more food (Table 2), their intakes rising to a maximum on the 26 or 19% protein diets and then falling as the protein concentration was further reduced. The mean daily total calorie intake rose to a maximum of 103 kcal/kg/day on the 19% protein diet, though after an

Table 2. *Mean daily values for food intake and nitrogen intake, excretion and balance for individual cats*

| Period | Protein content of diet (%)* | Cat No.† | Cat Weight (kg) | Cat Food intake (g/day)* | Nitrogen (g) Intake | Nitrogen (g) In faeces | Nitrogen (g) In urine | Nitrogen (g) Balance |
|---|---|---|---|---|---|---|---|---|
| 1 | 46·2 | M 1 | 3·75 | 63 | 4·62 | 0·73 | 3·39 | +0·50 |
| | | M 2 | 3·40 | 49 | 3·62 | 0·61 | 3·05 | −0·04 |
| | | F 1 | 2·83 | 64 | 4·70 | 0·85 | 3·47 | +0·38 |
| | | F 2 | 2·54 | 46 | 3·36 | 0·69 | 2·73 | −0·06 |
| 2 | 39·3 | M 1 | 3·79 | 59 | 3·72 | 0·81 | 2·72 | +0·19 |
| | | M 2 | 3·35 | 51 | 3·23 | 0·67 | 2·57 | −0·02 |
| | | F 1 | 2·72 | 51 | 3·21 | 0·63 | 2·66 | −0·07 |
| | | F 2 | 2·49 | 37 | 2·29 | 0·57 | 1·66 | +0·07 |
| 3 | 31·9 | M 1 | 3·80 | 68 | 3·45 | 0·94 | 2·33 | +0·18 |
| | | M 2 | 3·32 | 48 | 2·47 | 0·57 | 2·11 | −0·22 |
| | | F 1 | 2·67 | 55 | 2·81 | 0·73 | 2·13 | −0·06 |
| | | F 2 | 2·38 | 41 | 2·10 | 0·53 | 1·62 | −0·05 |
| 4 | 25·7 | M 1 | 3·87 | 79 | 3·25 | 1·15 | 1·92 | +0·18 |
| | | M 2 | 3·35 | 67 | 2·77 | 0·93 | 1·68 | +0·16 |
| | | F 1 | 2·60 | 51 | 2·08 | 0·54 | 1·55 | −0·01 |
| | | F 2 | 2·60 | 66 | 2·71 | 0·85 | 1·51 | +0·35 |
| 5 | 18·8 | M 1 | 3·83 | 78 | 2·36 | 0·92 | 1·59 | −0·15 |
| | | M 2 | 3·30 | 80 | 2·39 | 0·87 | 1·64 | −0·11 |
| | | F 1 | 2·58 | 62 | 1·85 | 0·52 | 1·38 | −0·05 |
| | | F 2 | 2·52 | — | — | 0·48 | 1·15 | — |
| 6 | 12·7 | M 1 | 3·50 | 69 | 1·41 | 0·68 | 1·18 | −0·45 |
| | | M 2 | 3·04 | 64 | 1·31 | 0·56 | 1·15 | −0·41 |
| | | F 1 | 2·36 | 42 | 0·85 | 0·34 | 0·84 | −0·33 |
| | | F 2 | 2·33 | 41 | 0·84 | 0·38 | 0·78 | −0·33 |

* Dry-weight basis.
† M, male; F, female.

Table 3. *Mean daily calorie intakes of the four cats in each period*

| Period | Protein content of diet* (%) | Calorie intake (kcal/kg body-weight) Total | From protein sources | From non-protein sources |
|---|---|---|---|---|
| 1 | 46 | 79 | 34 | 45 |
| 2 | 39 | 71 | 25 | 46 |
| 3 | 32 | 78 | 23 | 55 |
| 4 | 26 | 96 | 22 | 74 |
| 5 | 19 | 103 | 18 | 85 |
| 6 | 13 | 88 | 10 | 78 |

* Dry-weight basis (see Table 2).

initial fall the calories provided by protein alone were maintained at a fairly constant level (25–22 kcal/kg/day down to the 26% protein diet) and thereafter decreased more rapidly (Table 3); this pattern suggests that the rise in food intake was a compensatory mechanism for overcoming the diminishing protein concentration of the diet. That the non-protein part of the diet supplied adequate calories is shown by the fact that the mean daily calorie intake from non-protein sources during periods 5 and 6 was equal to or greater than the total calorie intake during periods 1 and 2, when presumably the cats were obtaining all the calories they required.

## DISCUSSION

The relation between N balance and N intake or absorbed N has been shown to be linear in the dog (Allison, Anderson & Seeley, 1946) and cat (Allison, Miller, McCoy & Brush, 1956) in regions of negative and low positive balance. The values of Table 2 are plotted in Fig. 2 to illustrate to what extent this relationship holds in the experiment described here. Individual plots for each animal are shown, as well as mean plots derived by averaging all results in each period. The broken line is drawn 'through' the mean plots to represent what might have appeared if there had been more animals contributing to the mean. In contrast to the mean curve shown in the figure which does follow the relationship described by Allison (1951), two cats (M 1 and F 1) behaved in

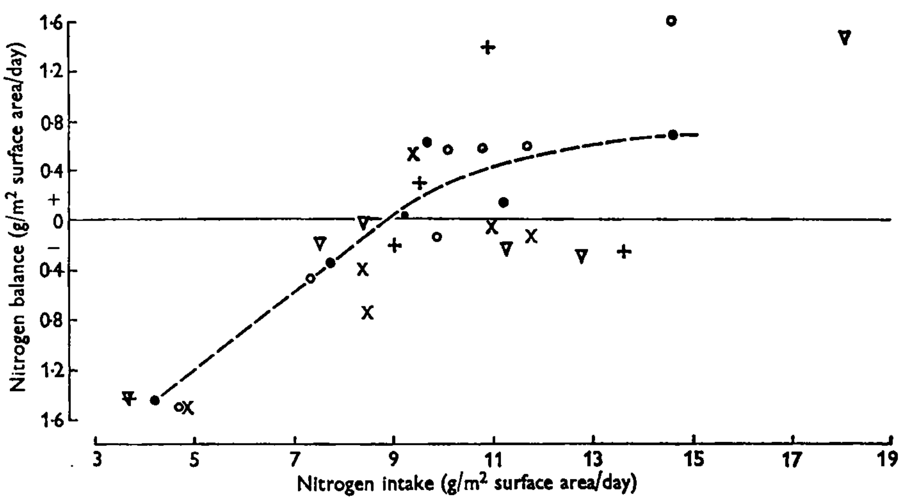


Fig. 2. Graph showing, for cats, the relation between N balance and N intake expressed in terms of surface area. Closed circles show means. ○, M 1; ×, M 2; ▽, F 1; +, F 2.

an anomalous fashion with diminishing N intake; their originally high positive balance reached a plateau at which a positive, or slightly negative, balance was maintained at a fairly constant level over a range of N intakes. The slope of the linear portion of Fig. 2 (referred to by Allison (1951) as the N balance index of N intake) is low, about 0·3, suggesting a poor biological value for the proteins of the diet. Had the diets been given in the reverse order (i.e. beginning with the lowest protein level) the slope might well have been greater, since N-balance indices tend to increase as protein stores are depleted (Allison, 1951; Greaves, 1959).

Protein requirements for maintenance vary more closely with body surface area than with body-weight (Smuts, 1935; Barnes, Bates & Maack, 1946; Mukherjee & Mitchell, 1949). Fig. 2 shows that a daily intake of from 8·4 to 9·2 g N/m$^2$ body surface area, or of about 55 g protein/m$^2$, is necessary for N equilibrium in resting adult cats, which is equivalent to a daily intake of about 0·8 g N/kg body-weight. Miller & Allison (1958) measured the negative balance on a N-free diet over a 4-day period and concluded that an adult cat with full protein stores requires daily 0·5 g N/kg for 'maintenance' and Allison *et al.* (1956) showed that 0·36 g absorbed casein N was required to maintain nitrogen equilibrium. By methods similar to that used in the experiment described here, adult rats have been shown to require from 3·6 to 7·6% dietary protein for maintaining N equilibrium (Bricker & Mitchell, 1947) and human subjects required daily 0·72 g protein/kg (Bricker, Mitchell & Kinsman, 1945) or from 18·1 to 15·0 g protein/m$^2$ (Hegsted, Tsongas, Abbott & Stare, 1946). There is no evidence to date that the high requirement for protein shown by cats is due to any specific amino-acid demand (Miller & Allison, 1958; Greaves, 1959).

To determine the true digestibility (T.D.) and biological value (B.V.) of a protein by the balance technique used in this study, it is necessary to estimate the faecal metabolic N (FMN) ($F_0$) and endogenous urinary N ($U_0$). Then, where N intake is $I$ and total N in faeces and urine are $F$ and $U$, respectively,

$$\text{T.D.} = \frac{\text{Absorbed food N}}{\text{N intake}} = \frac{I-(F-F_0)}{I} \times 100, \qquad (1)$$

$$\text{B.V.} = \frac{\text{Retained food N}}{\text{Absorbed food N}} = \frac{I-(F-F_0)-(U-U_0)}{I-(F-F_0)} \times 100. \qquad (2)$$

Since the cats would not eat a protein-free diet, the direct method (Mitchell, 1923–4; Mitchell & Carman, 1926) for measuring $F_0$ and $U_0$ could not be used. Bosshardt & Barnes (1946) suggested that $F_0$ determined in this way might differ from $F_0$ obtained on diets providing normal amounts of protein. Using young mice, they measured $F_0$ with diets adjusted to give different concentrations of protein and plotted faecal N ($F$) in g/100 g dry food consumed against percentage protein content of the diet. A line was fitted to the values at each level of protein intake and extrapolated to zero protein intake to obtain FMN at that level. This method was used for the cats and gave an estimated FMN of 0·63 g N/100 g dry food consumed. True digestibilities were then calculated for each cat for each period, giving a mean, with its standard error, of 87·2 ± 3·7% (on twenty-three values).

The apparent digestibility (A.D.) of a protein is defined as

$$\text{A.D.} = \frac{I-F}{I} \times 100, \qquad (3)$$

where $I$ is the N intake in g over a period, $F$ is the corresponding N output in the faeces. It measures the amount of N apparently absorbed, in g/100 g N intake. The

https://doi.org/10.1079/BJN19600047 Published online by Cambridge University Press

A.D. of the dietary protein N was calculated for each cat for each period and found to decrease as the percentage content of N fell (Fig. 3). If the T.D. be assumed constant for any particular protein and independent of the concentration at which it is eaten, and if the FMN is constant per unit weight of mean dry food intake (DMI) at all

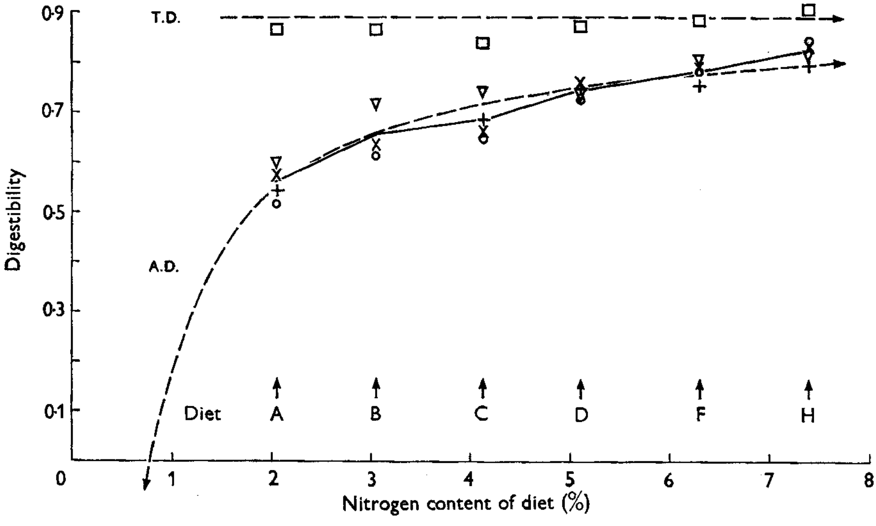


Fig. 3. Graph showing the apparent digestibility (A.D.) for cats of diets of different N content. o, M1; ×, M2; ▽, F1; +, F2. The calculated theoretical curve for apparent digestibility (broken line) is derived from the equation $y=0{\cdot}891-0{\cdot}71\ (1/x)$. Mean true digestibility (T.D.) is shown by open squares.

levels of N intake, and when $F_0$ is the amount of FMN associated with the DMI containing $I$ g N, then from equation (1)

$$F-F_0 = \left(1-\frac{\text{T.D.}}{100}\right)I,$$

$$\frac{I-F}{I} = \frac{\text{T.D.}}{100}-\frac{F_0}{I}. \tag{4}$$

If $n$ is the percentage N in the diet and $F_0'$ the FMN per 100 g DMI, then

$$\frac{I-F}{I} = \frac{\text{T.D.}}{100}-\frac{F_0'}{n}, \tag{5}$$

and from (3)

$$\text{A.D.} = \text{T.D.}-\frac{F_0'}{n}\times 100. \tag{6}$$

Equation (6) describes a rectangular hyperbola approaching asymptotically, at high values of $n$, the true digestibility. The broken curve in Fig. 3 represents the hyperbola calculated best to fit the means (shown joined by a continuous line); its equation is

$$y = 0{\cdot}89-\frac{0{\cdot}71}{x}. \tag{7}$$

Comparison of equations (6) and (7) shows that the true digestibility of the protein was 89% (shown in Fig. 3 as a broken horizontal line) and that the FMN was 0·7 g N/100 g DMI. The mean true digestibilities calculated by the method of Bosshardt & Barnes (1946) are also shown in Fig. 3 as squares; it can be seen that they closely approximate to the values given by equations (6) and (7).

The mean endogenous urinary N was estimated as 2 g N/m²/day by plotting total urinary N, expressed as g/m²/day, against N intake, in the same units, and extrapolating to zero intake. By means of this value, and for $F_0$ = 0·63, biological values were calculated from equation (2) (Bender, 1958) for each cat for each period and found to rise as the protein level of the diet fell (Fig. 4). On the three lowest protein levels the mean biological value did not vary markedly, being approximately 52. Similar observations have been reported for young rats (Henry & Kon, 1957).

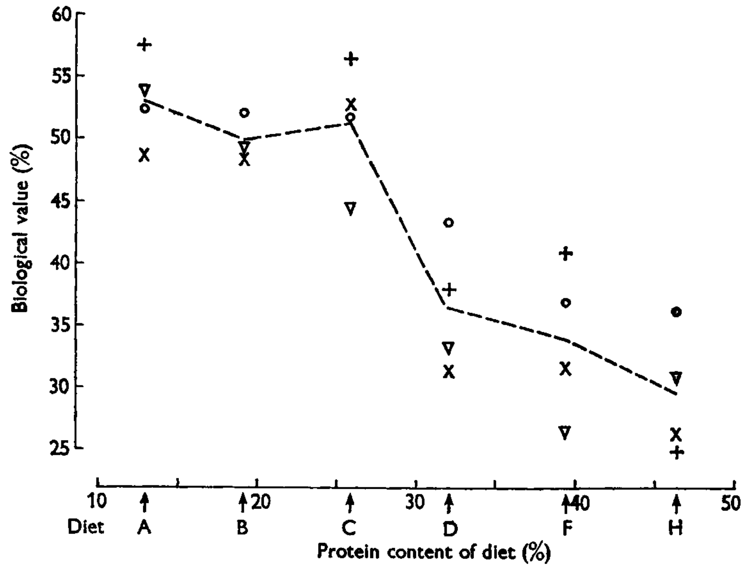


Fig. 4. Biological value for cats of mixed protein of fish and liver origin, at different levels in isocaloric diets. ○, M1; ×, M2; ▽, F1; +, F2. A broken line joins mean values.


SUMMARY

1. Nitrogen-balance measurements have been made on four adult cats to determine protein requirements for N equilibrium on a mixed diet. In six canned isocaloric diets the content of fish was adjusted to provide different levels of protein.

2. Individual 8-day balances were done in each of six consecutive fortnightly periods, during the first of which the animals received the diet highest in protein, during the second the next highest and so on.

3. On diets containing 19 and 13% protein all cats were in negative N balance and lost weight, though receiving sufficient calories.

4. N balance was linearly related to N intake in regions of negative balance; the behaviour of individual animals is discussed.

5. The apparent digestibility decreased as the N content of the diet fell; true digestibility was independent of N concentration and had a mean of $87.2 \pm 3.7$% (twenty-three observations).

6. The biological value rose to a mean of about 52 as the protein level of the diet decreased.

7. It is concluded that a minimum dietary protein ($N \times 6.25$) content of 21%, of mixed fish and liver origin, is necessary to maintain adult resting cats in N equilibrium. This is equivalent to a daily intake of 55 g protein/$m^2$ body surface area.

The authors thank Mr A. J. Harms of Messrs Petfoods Ltd for preparing the diets to their requirements, Dr J. G. Bate for helpful advice on technique, and Miss A. Elkins, Mr T. Holland and Mr D. Evans for technical assistance. The work was supported by a grant from Messrs Petfoods Ltd and formed for J.P.G. part of the requirements for the degree of Ph.D. in the University of London.

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*Printed in Great Britain*