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# A study on the Mitchell method for determination of the biological value of protein

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## PREFACE

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*Leif Rein Njaa.*

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List of abbreviations

(Certain terms which are defined in connexion with their use, are not included).

| | |
|---|---|
| Bal % | Nitrogen balance (%) |
| BV | Biological value (%) |
| C | Nitrogen content of diet (mg/g) |
| $C_m$ | Nitrogen content of maintenance diet (mg/g) |
| D | True digestibility (%) |
| $D_a$ | Apparent digestibility (%) |
| E | Food intake (g) |
| F | Faecal nitrogen (mg) |
| F' | Metabolic faecal nitrogen (mg) |
| I | Nitrogen intake (mg) |
| $k_1 - k_5$ | Proportionality constants |
| NPR | Net protein ratio (g weight gain/g protein eaten) |
| P | Protein content of diet (g/100g) |
| $P_m$ | Protein content of maintenance diet (g/100g) |
| PER | Protein efficiency ratio (g weight gain/g protein eaten) |
| Pr | Protein intake |
| q | Exponent to which W is taken |
| U | Urinary nitrogen (mg) |
| U' | Endogenous urinary nitrogen (mg) |
| W | Body-weight (g) |
| $\Delta W$ | Growth rate (g/day) |

## I. INTRODUCTION

The present investigations were begun in 1954 as a study of the factors influencing the biological quality of herringmeal protein. Biological values (BV) as determined by the Mitchell method (MICHELL 1923—4 a, MITCHELL & CARMAN 1926 a) were chosen as a measure of protein quality. The reasons for this choice were that the method had been applied to a great variety of related problems and the belief that the results obtained by it possessed 'absolute significance' (MITCHELL 1943). The modifications of the method as employed in the early stages of this work were described by NJAA (1959 a, b).

The results obtained varied so much that it was decided to study the reasons for this in some detail. It was not possible, however, to perform experiments covering all the factors considered to be of interest so the discussion is, therefore, based on data taken from the literature as well as on data obtained in my own experiments.

It was hoped that the study might give clues as to how to modify the method to the various problems encountered when biological qualities of food proteins are compared.

## II. HISTORICAL

The concept 'biological value' as a measure of protein quality was introduced by THOMAS (1909). It was defined as the grams of body protein spared by 100 g of food protein. The 'biological value' so defined was for maintenance. Thomas experimented on himself and the experiments were consequently limited in extent and duration. Three equations were suggested for the calculation of the biological value, of which one is given here:

$$\text{Biological value} = \text{BV} = 100\ \frac{\text{I} - \text{F} - (\text{U}-\text{U}')}{\text{I} - \text{F}} \qquad (1)$$

(I, nitrogen intake; F, faecal nitrogen; U, urinary nitrogen; U', urinary nitrogen on a nitrogen-free diet).

The biological value defined by equation 1 is the nitrogen balance (I—F—U) corrected for unavoidable nitrogen loss in the urine (U') expressed as a percentage of the apparently absorbed food nitrogen. Thus the total nitrogen content of the faeces was assumed to be derived from the nitrogen intake. Thomas realized that this was a weakness of the method and in one of the equations for BV he introduced a correction of 1 g nitrogen per day to account for metabolic loss of nitrogen in the faeces. The correction term U' (equation 1) is equivalent to FOLIN's (1905) 'endogenous nitrogen'.

The method of Thomas was taken up by many investigators. MARTIN & ROBISON (1922), who also experimented on themselves, obtained results which indicated that the biological value of a food protein was independent of the amount of protein eaten. They drew this conclusion reluctantly and mentioned it as an argument for the usefulness of the method. They also introduced the principle for the determination of protein or nitrogen balance indices which was later used by Allison and his co-workers (ALLISON 1949). It appears that Allison was unaware of this fact.

MITCHELL (1923—4 a) introduced a routine laboratory method for the determination of biological values using young growing rats. This method is generally referred to either as the Thomas—Mitchell method or as the Mitchell method. MITCHELL (1923—4 a) described it by these words: "The method is based upon nitrogen balance data obtained under definite experimental conditions, and involves direct determinations of the amount of nitrogen in the feces and in the urine and indirect determinations of the fractions of the fecal nitrogen and of the urinary nitrogen that were of dietary origin. The biological value of the protein is taken as the percentage of the absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) that is not eliminated in the urine."
Equation 2 is a formulation of this definition:

$$BV = 100 \frac{I - (F-F') - (U-U')}{I - (F-F')} \qquad (2)$$

(The designations are the same as in equation 1; F', faecal nitrogen of metabolic origin).

Thus, historically, protein quality as measured by nitrogen balance techniques refers to the apparently (THOMAS 1909) or truly (MITCHELL 1923—4 a) absorbed portion of the protein under test. True digestibility (D) and BV are considered the main characteristics of a food protein, and the net protein utilization (NPU) is considered to be a derived quantity (BLOCK & MITCHELL 1946—7). The equations 3—5 a give the percentage true digestibility, net protein utilization and apparent digestibility ($D_a$).

$$D = 100 \frac{I-(F-F')}{I} \quad (3)$$

$$NPU = \frac{BV \times D}{100} \quad (4\,a)$$

$$D_a = 100 \frac{I-F}{I} \quad (5\,a)$$

The biological value measured with growing rats is that for maintenance and growth. It was found to be independent of the amount of food eaten, but it decreased when the protein content of the diet was increased (MITCHELL 1923—4 b). On the grounds of this latter finding MITCHELL (1924) critisized the conclusion of MARTIN & ROBISON (1922) that the BV was independent of the amount of protein eaten. In later work, however, (ARMSTRONG & MITCHELL 1955; MITCHELL 1955) it was implied that within a certain range BV is in fact independent of the protein content of the diet.

The critical requirement in the Mitchell method is to devise adequate methods for the estimation of the terms F' and U' in equation 2. MITCHELL (1923—4 a) first chose to estimate both quantities from data obtained when the experimental animals were given a protein-free diet. The method was later so modified that F' and U' were estimated from data obtained when the rats were given a diet containing whole egg-protein at a low concentration (MITCHELL & CARMAN 1926 a). It was assumed that the egg-protein was completely digested and utilized by the growing rat so that faecal and urinary nitrogen excretions represented unavoidable metabolic and endogenous losses. The metabolic nitrogen in the faeces was related to the intake of dry food and the endogenous urinary nitrogen either to the body-weight (MITCHELL 1923—4 a) or to a logarithmic function of the body-weight (SMUTS 1935, ASHWORTH 1935 b).

The criticism of the Mitchell method has been concerned mainly with the question of the validity of the assumption that a relatively constant endogenous urinary nitrogen excretion is related in some way to the body-weight (ASHWORTH & BRODY 1933 a, b; ASHWORTH 1935 a, b; SCHOENHEIMER 1942; FROST 1950; ZIMMERMANN 1952). BOAS FIXSEN (1930) and CHICK, HUTCHINSON & JACKSON (1935) accepted the concept of a constant endogenous excretion of nitrogen in the urine, but not that it was a function of the body-weight. They used adult rats and the method of MARTIN & ROBISON (1922) for the calculation of their results.

An argument against the constancy of the endogenous urinary nitrogen excretion is that occasionally biological values higher than 100 are

calculated from the experimental data, (ALLISON, SEELEY, BROWN & ANDERSON 1946 a; BARNES & BOSSHARDT 1946; BRUSH, WILLMAN & SWANSON 1947; ANDERSON & NASSET 1948). This has been taken to indicate a depression of the endogenous nitrogen metabolism by feeding a diet containing certain proteins or amino acids. The concept of a relatively constant endogenous urinary nitrogen excretion was, however, strongly defended by Mitchell and his co-workers (BURROUGHS, BURROUGHS & MITCHELL 1940, MITCHELL 1955).

Allison and co-workers (ALLISON 1949) tried to circumvent the difficulty by introducing the term 'nitrogen balance index' instead of the term 'biological value'. In the definition of nitrogen balance indices the question of the constancy of the endogenous urinary nitrogen excretion is left out of consideration. It was pointed out by ZIMMERMANN (1952) that the change of name does not solve the underlying problem.

The direct estimation of metabolic faecal nitrogen (F') has also been criticized. BOSSHARDT & BARNES (1946) advocated an extrapolation technique instead of the direct method, but MITCHELL & BERT (1954) claimed that with young rats the two methods gave identical results and that the latter involved less work than the former.

The joint determination of the biological value for maintenace and growth has been used as an argument against the Mitchell method (BARNES, BATES & MAACK 1946).

The method has been modified not so much to meet the objections mentioned as to improve its reproducibility and to simplify the procedures involved.

MITCHELL & BEADLES (1930) introduced the paired feeding technique and MITCHELL (1943) took a very strong stand against the use of the *ad lib.* feeding technique in studies on the biological quality of food proteins. Some later workers went a step further by using equalized feeding to all rats within an experiment (CAMA & MORTON 1950; SURE & EASTERLING 1952; NEHRING & HAESLER 1954; FORBES & YOHE 1955 b). This practice was followed in the present study (NJAA 1959 a).

In early comments on the method of determination of biological values Mitchell seemed to be of the opinion that only relative values could be obtained by it: "The best that can be hoped for is the determination of values representing fairly the comparative worth of different proteins under certain controlled conditions —" (MITCHELL 1924). Later his attitude changed slightly: "It has been emphasized that the biological value of protein or protein mixtures possesses the unique distinction, among other proposed measures of protein utilization, of possessing an absolute significance since in itself, and apart from other similar values, it is a quantitative measure of the extent to which the digestible portion

of a given source of dietary protein is utilized in the animal function to which protein alone contributes for the condition under which it was obtained. However, the securing of relative values not possessing this characteristic, if they are secured under properly controlled conditions, is a worthwhile scientific achievement" (MITCHELL 1943). The latter statement was, however, qualified by the stress laid on keeping the food intake and the protein content of the diet constant and on selecting experimental animals of similar age and body-weight. Even so unexpected variations between rats were observed: "Any method of biological assay of food products may thus go 'haywire' on occasion" (MITCHELL 1944).

The tendency towards regarding the biological value as a characteristic of a food protein became evident in review articles (BLOCK & MITCHELL 1946—7; MITCHELL 1948). From the data compiled it seems that the protein efficiency ratios, which assumedly possess less "absolute significance", are as characteristic of the protein sources as are the biological values. Nevertheless it is the biological values of food proteins which are given in popularized tabulations (Protein requirements, FAO Nutritional Studies No. 16 1957; Nutritional Data, H. J. Heinz Co. 1959). Another indication of the belief that the biological value or the net protein utilization (BLOCK & MITCHELL 1946—7) is characteristic of a food protein is found occasionally when new methods for protein evaluation are introduced. In some such cases the methods are standardized against values for BV or NPU taken from the literature (FINLAYSON & BAUMANN 1956; SHEFFNER, ECKFELDT & SPECTOR 1956).

After Allison and co-workers introduced the nitrogen balance index of absorbed or of ingested nitrogen as a measure of protein quality (ALLISON 1949) the views of Mitchell on the question of the absolute significance of biological values became more confused (MITCHELL 1955). On the one hand the concept of a relatively constant endogenous urinary nitrogen excretion was vigorously defended (MITCHELL 1955), on the other hand GRIFFITH & SWENDSEID (1956) pointed out the inconsistency in defending the concept and at the same time accepting the view that the endogenous excretion is correlated with the body's stores of reserve protein (ALLISON *et al.* 1946 a).

The views of those doubting that biological value should be considered to be more than a relative measure of protein quality were expressed by ZIMMERMANN (1952) when he regarded biological value as "ein Ausdruck für den prozentischen Anteil der Differenz zwischen der Stickstoff-Bilanz während der Eiweissgabe und der jenigen während des Eiweisshungers bezogen auf die Menge des wirklich absorbierten Futterstickstoffs".

The reason for the sometimes heated discussion on these points was given clearly in an early review article by MITCHELL (1926): "It is no

more than prudent, however, in considering whether to continue the use of a research method, to assure oneself that the value of the results secured is sufficient compensation for the laborious procedures undertaken."

It was because of doubts as to this latter point that the present attempt at an evaluation of the Mitchell method was made.

## III. PLAN OF THE INVESTIGATION

The composite nature of BV and of NPU is evident from equations 2—4 a. For the study of the variations in BV and NPU it is of interest to obtain information on the relative importance of the terms constituting them. An attempt at procuring such information is made in chapter V. For the sake of convenience the discussion is restricted at that stage to the variation in NPU.

In chapters VI—VIII the single terms constituting NPU are discussed. Chapter VI concerns the terms related to the faecal nitrogen excretion, chapter VII the terms related to the urinary excretion. The excretions are discussed in relation to their dependence on the body-weight and the growth rate of the experimental rats and to the relative importance of the intakes of nitrogen and of food *per se*. In this connexion the subdivision of faecal nitrogen excretion into undigested food nitrogen and metabolic nitrogen, and the similar subdivision of the urinary excretion into exogenous and endogenous portions are discussed.

In chapter VIII is discussed the general significance of BV and NPU as measures of protein utilization and whether the directly determined faecal and urinary excretions may form the basis for comparison of protein qualities.

The relationship between the methods based on the measurement of nitrogen balance and other current methods is discussed in chapter IX.

The experiments performed are described in chapter IV.

## IV. EXPERIMENTAL

### *A. Rats*

Albino rats from our own colony were used throughout. They were weaned at 21 days of age and given the experimental diets when they were between 28 and 35 days old. At this time they usually weighed between 50 and 100 g.

Litter-mate control was employed in nearly all experiments. In the few cases where this was not the case it is especially mentioned in the description of the experiment in question. Groups of two, three or four rats of the same sex were taken from each litter, one rat from each litter being allotted to each treatment. The mean body-weights were equalized as far as possible between the groups compared. Each such group consisted of from four to twelve rats. In most experiments groups of six rats were used.

### *B. Feeding of the rats*

The percentage composition of the diets is given in Table 1.

When an experiment lasted for more than 10 days vitamins A and D were given by dropper once weekly, about 1000 i. u. vitamin A and 100 i. u. vitamin D/rat.

All rats within an experimental group, and in most instances also within an experiment, were offered the same amount of food daily, usually 8 or 10 g. If a rat failed to consume its daily ration completely, or if samples of faeces or urine were lost, the results for it and its litter-mates were omitted from the calculations. In the early experiments (Expts 3—27 a) the daily ration for each rat was taken from a jar weighed before and after the experimental period; in later experiments it was weighed directly into the feeding cup, the weighing being accurate to within 0.1 g. Spilling was reduced by mixing the food with water. The vitamin solution, and eventual amino acid supplements, were pipetted into the daily ration and mixed with it.

Table 1. *Percentage composition of experimental diets.*

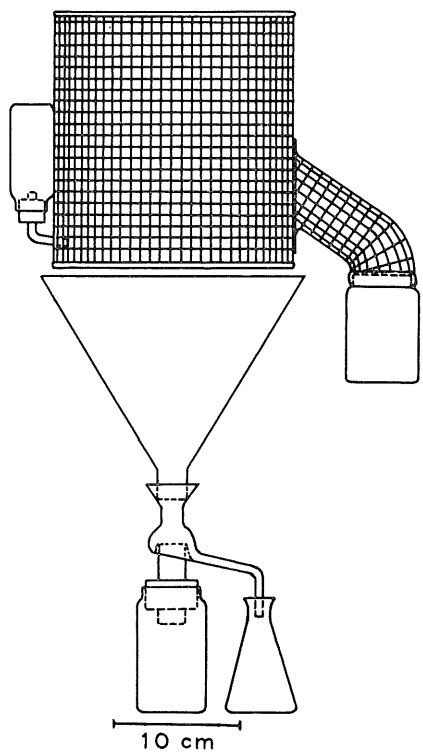
| Constituent | |
|---|---|
| Sucrose | 20.0 |
| Arachis oil | 5.0 |
| Salts (SURE 1941) | 4.0 |
| Protein source (corresponding to from 0 to 10 % protein in the diet) | 0—13.6 |
| Partly dextrinized potato stach | 71.0—57.4 |

Vitamins of the B complex were mixed into the diet daily. Each rat was given 1.0 —1.5 ml of a solution containing per 1 000 ml 20 mg each of thiamine, riboflavin, pyridoxine and nicotinic acid, 120 mg calcium pantothenate, 0.8 g inositol, 2.4 g p-aminobenzoic acid and 7.2 g choline chloride. Vitamins A and D were given by dropper once weekly about 1 000 i.u. vitamin A and 100 i.u. vitamin D/rat.

*C. Cages, and collection of faeces and urine*

The rats were kept in cylindrical wire cages with wire bottoms. The bottoms consisted of a flat spiral of stainless steel welded to a steel cross. The distance between the wires in the spiral was about 1 cm. The cages were suspended above a funnel a little wider than the cage. Below the funnel was an apparatus for separation of faeces and urine which worked on the principle that the urine ran along the funnel wall over to the wall of the apparatus and was led into a collection flask, while the faeces dropped through into another flask (Fig. 1).

Fig.1.
Type of cage and apparatus for separation of faeces and urine used in the balance experiments.




The food was given in jars joined to a slanting wire tunnel at the side of the cage. Thus the food was given slightly below the level of the bottom of the cage and the rat reached it with the mouth while the hind part of it was over the funnel.

Each feeding period lasted 9 or 10 days, faeces and urine being collected during the last 5 or 6 days. When 6-day collection periods were

used (Expts 3—9 b) carmine was used as a faeces marker; when 5-day periods were used both faeces and urine were collected from a given time on the first day of the experimental period to the same time five days later (Expts 11—3/61).

The funnel and the separation apparatus were washed daily with about 50 ml lukewarm 1 N sulphuric acid and the urine was collected separately for each rat. The total amount of urine was diluted to 500 ml. Faeces were collected separately for each rat and kept in 1 N sulphuric acid.

In some experiments the rats were used in more than one experimental period. The collection periods were then either consecutive, or separated by a time gap. In the latter case the rats were given the experimental diets at the same daily rate as in the experimental periods.

### *D. Analytical methods*

Nitrogen was determined in food, faeces and urine by the method of MA & ZUAZAGA (1942) after digestion with concentrated sulphuric acid containing anhydrous potassium sulphate and copper sulphate. 10 g samples of food were digested with 50 ml sulphuric acid, 6 g potassium sulphate and 0.5 g copper sulphate. Faeces from each rat were digested in bulk using 75 ml sulphuric acid for 5 and 6-day collections and corresponding amounts of the sulphates. Portions of the diluted urine were taken for digestion. In most experiments (Expts 3—27 b) 20 ml samples were taken, but in later experiments 2 ml samples were used. The 20 ml samples required further dilution before distillation and titration with 0.01 N hydrochloric acid; the 2 ml samples were distilled directly from the digestion flasks.

Titanium dioxide contents in food and faeces were determined by the method given by NJAA (1961 b).

### *E. Statistical methods*

In experiments where treatment differences were compared the results were subject to an analysis of variance of the type described in most elementary books on statistical analysis. The reference books used in this investigation were SCHNEDECOR (1946), MATHER (1949) and COCHRAN & COX (1950). Subdivision of treatment sum of squares was done as described by the latter authors. Calculations of correlation coefficients and regressions were done by methods described in the reference books mentioned.

### *F. Description of the experiments*

The experiments are described in sequential order and they are given the original numbering, together with reference to the experiment number used in published investigations of which the experiment in question formed part. The protein sources tested are listed in Table 2 together with their protein contents (Nx 6.25) and the protein contents of the diets used. Because diverse problems were discussed on the basis of results taken from various experiments the latter are not characterized by titles.

*Expt 3* (Shortened version of the Mitchell method (B)). (NJAA 1959 a).

Eight rat triads weighing from 70 to 80 g were used. One rat in each triad was given a low egg-protein diet and each of the two others a different herring meal diet (Herring meals nos 1 and 2). The experiment was first done with four triads (period 1) and than repeated with four new triads (period 2). Each period lasted 10 days, and faeces and urine were collected in the last 6. All rats ate 10 g food daily. The intended protein contents were 4.5 % for the low egg-protein diet and 10 % for the herring meal diets. Results obtained using the low egg-protein diet were used for calculation of metabolic and endogenous nitrogen losses (Mitchell & Carman 1926 a).

*Expt 4* (Method of Mitchell (MITCHELL 1923—4 a; MITCHELL & CARMAN 1926 a (A)) (NJAA 1959 a).

Six pairs of rats were used in four feeding periods, each of 10 days. Faeces and urine were collected during the last 6 days of each period. In the first and fourth periods a low egg-protein diet was given to all rats. Values obtained in these periods were used for calculation of metabolic and endogenous nitrogen losses (MITCHELL & CARMAN 1926 a). In the second period one rat from each pair was given one of the two herring meal diets and its litter-mate the other; in the third period the diets were reversed. The herring meals and the intended protein contents as well as the daily food intakes were the same as in Expt. 3. The rats weighed 60—75 g at the beginning and 125—135 g at the end of the experiment.

*Expt 5* (Expt 1, NJAA 1959 b).

The same procedure was used as in Expt 4 except that all rats ate 8 g per day in the first low egg-protein period and in the second period. Herring meals nos 3 and 4 were compared at the 10 % protein level. The rats weighed 54—73 g at the beginning and 96—138 g at the end of the experiment.

*Expt 6* (Expt 2, NJAA 1959 b).

The procedure was the same as that described in Expt 3 except that four diets were compared using six litters of four rats. The diets contained herring meal no 4 at the 6, 8 and 10 % protein levels and whole egg-protein at the 4.5 % level. The rats were used in two periods 3 weeks apart. Each rat received the same diet throughout the experiment including the gap between the periods, at the uniform rate of 10 g/rat/day. The rats weighed 55—67 g at the beginning and 97—139 g at the end of the experiment.

*Expt 8* Two pairs of unrelated adult rats with different dietary histories between pairs were given a protein-free diet at the uniform rate of 10 g/rat/day for 16 days. One pair was taken directly from our stock diet (about 22 % protein). The other had been given a diet containing about 4 % protein from herring meal no 4 at the uniform daily rate of 10 g/rat for 83 days before the experiment was started. The former pair weighed about 255 g, the latter about 230 g at the beginning of the experiment.

*Expt 9 a* (Expt 3, NJAA 1959 b).

The procedure was the same as that described for Expts 3 and 6 except that the low egg-protein diet was omitted. A diet containing 10 % protein from herring meal no 4 was given at the daily rates of 8.33, 10 and 12 g/rat. Heavy rats had to be chosen for this experiment, otherwise the largest ration would have been beyond the capacity of some of the rats. They were used in two experimental periods 3 weeks apart and they weighed 93—125 g at the beginning and 117—188 g at the end of the experiment. Each rat received the same amount of food throughout the experiment including the gap between the experimental periods.

*Expt 9 b* (Expt 4, NJAA 1959 b).

The procedure was the same as in Expt 9 a except that the rats were used in only one period of 10 days. Herring meal no 4 was given at the 10 % protein level at 8 and 10 g daily food intakes to two groups and at the 8 % level at 10 g daily food intake. The rats weighed 71—110 g at the beginning of the experiment.

In the preceding experiments the collection period was 6 days, in the following 5-day collection period were employed.

*Expt 11 a* (Expt 5, NJAA 1961 a; Expt 4, NJAA 1961 b).

A diet containing herring meal no 11 at the 10 % protein level was supplemented with methionine, cysteine hydrochloride, lysine hydrochloride and glycine. The supplements were equivalent to 1 g methionine per

Table 2. *Protein contents of the protein sources used, and the actual contents in the diets.*

| Expt no | Protein source | % Protein in | | | | | Expt no | Protein source | % Protein in | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Protein source | Diet | | | | | | Protein source | Diet | | |
| | | | Period (or sub-expt) | | | | | | | Sub-expt | | |
| | | | 1 (a) | 2 (b) | 3 (c) | 4 (d) | | | | (a) | (b) | (c) |
| | H—m 1 | 73.3 | 10.2 | 10.3 | | | | H—m 30 | 75.5 | 7.84 | 7.93 | 7.96 |
| 3 | H—m 2 | 74.1 | 9.78 | 10.1 | | | 18 | H—m 31 | 76.3 | 7.90 | 8.06 | 8.02 |
| | Egg | 76.2 | 4.72 | 4.49 | | | | H—m 32 | 72.5 | 7.74 | 7.98 | 7.93 |
| | | | | | | | | H—m 33 | 73.3 | 7.76 | 7.88 | 8.06 |
| | H—m 1 | 73.3 | | 9.85 | 9.88 | | | | | | | |
| 4 | H—m 2 | 74.1 | | 9.57 | 9.79 | | | H—m 38 | 73.1 | 7.84 | 7.93 | 7.85 |
| | Egg | 76.2 | 4.72 | | | 4.46 | 20 | H—m 39 | 74.4 | 7.85 | 8.01 | 7.98 |
| | | | | | | | | H—m 40 | 74.3 | 7.83 | 7.87 | 7.62 |
| | H—m 3 | 73.1 | | 10.1 | 10.1 | | | H—m 41 | 73.2 | 7.94 | 7.97 | 7.91 |
| 5 | H—m 4 | 73.3 | | 9.81 | 10.1 | | | | | | | |
| | Egg | 67.3 | 4.42 | | | 4.48 | | H—m 52 | 73.8 | 7.88 | | |
| | | | | | | | 23 | H—m 53 | 78.4 | 7.76 | | |
| | H—m 4 | 73.3 | 5.98 | 6.20 | | | | F—m 54 | 63.7 | 7.68 | | |
| 6 | — | — | 8.09 | 8.42 | | | | F—m 55 | 67.4 | 7.83 | | |
| | — | — | 10.1 | 10.4 | | | | | | | | |
| | Egg | 67.3 | 4.56 | 4.58 | | | 27 | H—m 52 | 73.8 | 8.05 | | |
| 9a | H—m 4 | 73.3 | 9.93 | 9.61 | | | 42 | H—m 107 | 72.3 | 7.81 | | |
| 9b | H—m 4 | 73.3 | 7.94 | | | | 43 | H—m 107 | 72.3 | 7.79 | | |
| | — | — | 9.75 | | | | | | — | 9.96 | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11a | H—m 11 | 75.5 | 9.80 | 9.61 | | | 2/61 | S—m 111 | 47.8 | 7.93 | | |
| 11b | H—m 11 | 75.5 | 9.75 | 9.76 | 9.76 | | 3/61 | S—m 111 | 47.8 | 8.17 | | |
| | H—m 7 | 73.9 | 7.94 | | | | 5/61 | S—M 111 | 47.8 | 8.08 | | |
| 12 | H—m 15 | 75.5 | 8.04 | | | | | | | | | |
| | H—m 16 | 81.5 | 7.91 | | | | 6/61 | S—m 111 | 47.8 | 8.48 | | |
| | E—a | 83.0 | 7.94 | | | | | | | | | |
| | | | | | | | M 1 | E—a | 81.6 | 1.02 | | |
| 13 | H—m 7 | 73.9 | 7.94 | 7.97 | | | | — | — | 2.01 | | |
| | H—m 11 | 75.5 | 7.93 | 7.97 | | | | — | — | 2.93 | | |
| | | | | | | | | — | — | 3.91 | | |
| 14 | H—m 6 | 72.9 | 7.69 | 7.86 | | | | | | | | |
| | | | | | | | M 2 | H—m 114 | 72.8 | 1.12 | | |
| | H—m 18 | 66.4 | 7.86 | | 8.18 | | | — | — | 2.16 | | |
| | H—m 19 | 68.9 | 7.81 | | | 8.07 | | — | — | 2.89 | | |
| | H—m 20 | 72.0 | 7.86 | | 7.98 | 8.25 | | — | — | 4.29 | | |
| 15 | H—m 21 | 66.2 | | | 7.25 | 8.16 | | | | | | |
| | H—m 22 | 67.6 | | | | | M 3 | L—s 115 | 33.7 | 2.07 | | |
| | H—m 23 | 67.6 | | | | | | — | — | 3.16 | | |
| | E—a | 84.8 | 7.84 | | 7.81 | 7.94 | | — | — | 4.26 | | |
| | | | | | | | | — | — | 5.08 | | |

H—m = herring meal. Egg = acetone dried whole egg. E—a = egg albumen. F—m = fish meal. S—m = soya bean meal. L—s = linseed cake meal. Under the heading % protein in diet it is referred to the protein content in either the indicated experimental period or sub-experiment.

100 g protein. The four diets were given in two experimental periods 16 days apart at the constant rate of 10 g/rat/day. The rats weighed 62—107 g at the beginning and 114—141 g at the end of the experiment.

*Expt 11 b* (Expt 1, NJAA 1961 a; Expt 5, NJAA 1961 b).

The diet used was the same as that used in Expt 11 a. The supplements given were three levels of methionine 0.5, 1.0 and 2.0 g per 100 g protein and glycine equivalent to 2 g methionine per 100 g protein. The nitrogen contents of the supplements were equalized by use of glycine. The four diets were given in three experimental periods with one-week gaps between them. The rats weighed 58—85 g at the beginning and 116—140 g at the end of the experiment.

*Expt 12*

Two herring meals (Nos. 7 and 15), an acetone dried meal prepared from herring fillets (No. 16) and egg albumen were compared at the 8 % protein level. The rats weighed 55—75 g at the beginning of the experiment and were given 10 g food daily.

*Expt 13 a and b* (Expt 6 and 7, NJAA 1961 a).

Herring meals nos 7 and 11 given at the 10 % level were supplemented with methionine and glycine. In Expt 13 a the supplements were equivalent to 1.25 g methionine per 100 g protein, in Expt 13 b to 2.5 g. The rats weighed 62—72 g (Expt 13 a) and 69—100 g (Expt 13 b) at the beginning of the experiments, and were given 10 g food daily.

*Expt 14 a and b* (Expts 8 and 9, NJAA 1961 a).

A diet containing herring meal no 6 at the 8 % protein level was supplemented per 100 g protein (1) with 1.25 g methionine, (2) with 1.5 g lysine hydrochloride and (3) with these supplements together (Expt 14 a) and with (1) 1.25 g methionine, (2) with 2.0 g valine and (3) with these two together (Expt 14 b). In both experiments the negative control group was given glycine. All supplements were made isonitrogenous by use of glycine. The rats weighed 46—73 g at the beginning of the experiment, and were given 10 g food daily.

*Expt 15 a, c and d.*

These experiments formed part of an investigation where the effect of the antioxidant BHT (butylated hydroxy toluene) on the protein quality of herring meal was studied. The meals tested were compared with egg albumen at the 8 % protein level. In Expt 15 a the herring meals compared were nos 18, 19 and 20, in Expt 15 c nos 20, 22 and 23, and in

Expt 15 d nos 19, 20 and 21. The meals were newly produced in Expt 15 a, and about 5 and 25 weeks old in Expts 15 c and d, respectively. The rats weighed 55—90 g at the beginning of the experiments, and were given 10 g food daily. The effect of BHT is not considered here.

*Expt 18 a, b and c*

These experiments also formed part of the investigation on the effect of BHT. Four herring meals (Nos 30—33) two with and two without BHT were compared at the 8 % protein level when the meals were newly produced and when they were 9 and 18 weeks old. The rats weighed 50—79 g at the beginning of the experiments, and were given 10 g food daily.

*Expt 20 a, b and c*

The experiments were similar to Expt 18. The meals tested at the 8 % protein level were nos 38—41, newly produced in Expt 20 a, 11 and 18 weeks old in Expts 20 b and c respectively. The rats weighed 54—86 at the beginning of the experiments, and were given 10 g food daily.

*Expt 23*

Two herring meals (Nos 52 and 53) and two fish meals (Nos 54 and 55) were compared at the 8 % protein level. The rats weighed 57—77 g at the beginning of the experiment, and were given 10 g food daily.

*Expt 27 a* (Expt 3, NJAA 1961 a).

This experiment was similar to Expt 11 b. A diet containing herring meal no 52 was supplemented with methionine at the levels 0.94, 1.88, 2.81 and 3.75 g per 100 g protein. The protein content of the diet was 8 % and the supplements were made isonitrogenous by use of glycine. The rats were used in only one period and weighed 56—84 g at the beginning of the experiment. They were given 10 g food daily.

In the preceding experiments the daily food ration was taken from a jar for each rat which was weighed before and after the collection period. In the following experiments the ration was weighed directly into the feeding cup.

*Expt 27 b* (Expt 2, NJAA 1961 a).

This experiment was similar to Expt 27 a. The levels of methionine tested were 0, 0.94, 1.88 and 2.81 g per 100 g protein. The diet contained 8 % protein from herring meal no 52. The rats weighed 62—72 g at the beginning of the experiment, and they were given 10 g food daily.

*Expt 42* (Expt 13, NJAA 1961 a; Expt 10, NJAA 1961 b).

Herring meal no 107 at the 8 % protein level was supplemented with cysteine hydrochloride, cysteine arabinose, cysteine glucose and glycine in amounts corresponding to 1.6 g methionine per 100 g protein. The rats weighed 48—70 g at the beginning of the experiment, and they were given 8 g food daily.

*Expt 43*

This experiment was similar to Expt 9 a, except that the collection period was 5 days and each group comprised 8 rats. Herring meal no 107 was used. The rats weighed 61—84 g at the beginning of the experiment.

*Expts 2, 3, 5 and 6/61* (Expt 4, NJAA 1961 a; Expts 1—3, NJAA 1962 b).

A soya-bean meal (no 111) was the protein source in these experiments the protein level was 8 %. The diet was supplemented with methionine corresponding to 0, 1.0, 2.0 and 3.0 g per 100 g protein (Expt 2/61). In Expt 3/61 the supplements were DL-methionine, DL-methionine sulphoxide, a 1:1 mixture of these and glycine. The supplements were equivalent to 1.5 g methionine per 100 g protein. The two other experiments were similar to Expt. 3/61 The supplements were DL-methionine, L-methionine sulphoxide, DL-methionine sulphoxide and glycine (Expts 5 and 6/61). The rats weighed 54—85 g at the beginning of the experiments. In Expts 2, 3 and 6 they were given 10 g food daily, in Expt 5/61 8 g. In all experiments the supplements were made isonitrogenous by the use of glycine.

*Expts M1—M3*

The experiments were designed to evaluate the protein level required for the maintenance of body-weight in young rats weighing 50—60 g and given food *ad lib.* The protein sources used were egg-albumen, herring meal no 116 and linseed cake meal, one protein source being tested in each experiment at four protein levels. The two former were given at the 1, 2, 3 and 4 % levels, the latter at 2, 3, 4 and 5 %. The treatment groups of 5 or 6 rats were of about equal mean body-weight within each experiment and the sexes were evenly distributed. Litter-mate control was not employed in these experiments. The experiments lasted for 12 to 14 days, the rats were weighed daily except on sundays and the food consumption of each rat was determined for the entire period.

# V. THE RELATIVE IMPORTANCE OF THE CONSTITUENT TERMS IN THE VARIATION OF THE NET PROTEIN UTILIZATION

Historically the true digestibility (D) and the biological value (BV) were considered the primary characteristics of a food protein; net protein utilization was derived from them by use of equation 4 a (p. 11) (BLOCK & MITCHELL 1946—7). It was pointed out previously (NJAA 1959 b) that NPU is more conveniently discussed than BV and that conclusions arrived at for NPU are valid for BV if D is constant. The question of the constancy of D will be discussed in chapter VI.

NPU may be written in one of the following forms derived from equations 2—4 a:

$$NPU = 100\,\frac{I-(F-F')-(U-U')}{I} = 100\left(1-\frac{F}{I}+\frac{F'}{I}-\frac{U}{I}+\frac{U'}{I}\right) \quad (4\,b)$$

$$NPU = D - 100\left(\frac{U}{I}-\frac{U'}{I}\right) \quad (4\,c)$$

$$NPU = Bal\,\% + 100\left(\frac{F'}{I}+\frac{U'}{I}\right) \quad (4\,d)$$

Bal % in equation 4 d is the percentage nitrogen balance:

$$Bal\,\% = 100\,\frac{I-F-U}{I} \quad (5\,b)$$

Equation 4 d shows that the percentage of the nitrogen intake utilized may be subdivided into one part utilized for growth (Bal %) and one part utilized for maintenance. This corresponds to the similar factorization of the protein requirement advocated by BLAXTER & MITCHELL (1948).

Equations 4 b—d are identities and nothing can be gained by calculating the partial correlation coefficient between NPU on the one hand and each of the terms constituting it on the other. The result would only be that when all other sources of variation are eliminated the variable in question would be responsible for the residual variance. Equations 4 b—d may be changed formally from identities to statistical relationships by excluding from them one term at the time. Multiple correlation coefficients (R) between NPU on the one hand and the remaining quantities on the other can then be calculated. $100\,(1-R^2)$ is taken as a measure of the percentage of the variance of NPU which can be accounted for by the quantities excluded.

The results obtained with herring meal no 2 by versions A and B of the Mitchell method (NJAA 1959 a) were treated as outlined above. The results of the calculations are given in Table 3.

Table 3. *Relative importance of single and composite terms of NPU for its variation.*

| Reference to equation | Variable | Version A (NJAA 1959 a) | | | Version B (NJAA 1959 a) | | | Diet III (MACRAE *et al.* 1943) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Variance (5 d.f.) | 100 $(1-R^2)$ | Mean | Variance (7 d.f.) | 100 $(1-R^2)$ | Mean | Variance (11 d.f.) | 100 $(1-R^2)$ |
| | NPU | 63.5 | 6.00 | | 63.2 | 5.97 | | 67.5 | 19.79 | |
| 4b | 100 F/I | 18.7 | 1.14 | 3.5 | 17.4 | 1.50 | 5.9 | 31.7 | 9.84 | 42.3 |
| | 100 F'/I | 11.6 | 0.34 | 1.6 | 11.1 | 0.62 | 2.8 | 18.5 | 2.44 | 14.4 |
| | 100 U/I | 43.3 | 9.94 | 22.1 | 42.7 | 2.82 | 28.6 | 32.7 | 15.52 | 31.7 |
| | 100 U'/I | 13.9 | 1.93 | 3.8 | 12.2 | 2.52 | 41.3 | 13.4 | 4.46 | 7.5 |
| 4c | D | 92.9 | 0.56 | 3.5 | 93.7 | 3.46 | 34.9 | 86.8 | 9.69 | 49.4 |
| | 100 (U−U')/I | 29.4 | 4.69 | 48.8 | 30.5 | 4.11 | 68.4 | 19.3 | 8.05 | 36.5 |
| 4d | Bal % | 38.0 | 12.69 | 57.6 | 39.9 | 1.81 | 27.1 | 35.6 | 30.61 | 87.3 |
| | 100 (F'+U')/I | 25.5 | 2.66 | 8.6 | 23.3 | 4.08 | 44.4 | 31.9 | 7.77 | 21.7 |

R, multiple correlation coefficient between NPU and the terms constituting it when the effect of the term given on the same line as 100 $(1-R^2)$ is eliminated.

Table 3 also shows results from a similar treatment of one set of values obtained by calculation from the primary data given by MACRAE, HENRY & KON (1943) for their diet III. They used the Mitchell method in its original form (MITCHELL 1923—4 a, MITCHELL & CARMAN 1926 a). NPU of diet III was about the same as for herring meal no 2, the protein content was 8 % compared with 10 % in versions A and B.

In the three sets of values the major part of the variation in NPU was due to a different term in each case. In version A 100 U/I was the most important term, in version B 100 U'/I was of first importance followed by 100 U/I and in the data of MACRAE *et al.* (1943) the most important single term was 100 F/I followed by 100 U/I. Of the composite terms Bal % was most important in version A, followed by 100 (U—U')/I, in version B 100 (U—U')/I was most important, followed by Bal %, and in the data of MACRAE *et al.* (1943) Bal % was most important, followed by D. Even for the least important single term no general rule can be deduced from the data collected in Table 3. It thus seems that under different experimental conditions different single terms, and different composite terms may be the most important factor in the variation of NPU. The factors influencing the single and composite terms will be discussed in later sections.

It is obvious from equations 4 b—d that apart from determining the variation in NPU the single terms will also greatly influence its absolute magnitude. This is of special importance when the two indirectly determined terms 100 F'/I and 100 U'/I are considered. In my experience the nitrogen balance determined for herring meal proteins has been approximately 40 % and the NPU approximately 70 %. Thus about 40 % of the determined NPUs are accounted for by the two indirectly determined quantities. This points to the importance of studying not only the sources of their variation, but also the methods by which their magnitudes are assessed.

## VI. THE FACTORS INFLUENCING FAECAL NITROGEN EXCRETION

### *A. General*

Faecal nitrogen in the rat is of food and metabolic origins (MITCHELL 1948). The former part is considered to depend only upon the nitrogen intake whereas the latter is composed of one part related to the food intake *per se*, one part related to the intake of crude fibre and one relatively constant part depending to some extent on the body-weight of the rat (SCHNEIDER 1934, 1935). Prevalent assumptions about the relationships

between faecal nitrogen on the one hand and the intakes of nitrogen and of food are summarized in equations 6 a, b. The effect of crude fibre is left out of consideration here.

$$F = k_1 I + k_2 E + k_3 \qquad (6\ a)$$

$$F = k_1 EC + k_2 E + k_3 \qquad (6\ b)$$

(F, faecal nitrogen; I, nitrogen intake; E, food intake; C, nitrogen content of diet; $k_1$ and $k_2$, proportionality constants, $k_3$ the relatively constant part of faecal nitrogen).

If F and I are given in mg/rat/day and E in g/rat/day the dimension of C is mg N/g food.

In the following sections an attempt is made to evaluate the validity of equations 6 a and b, and the assumptions on which they are based.

### *B. The effect of body-weight*

Possible effects of food intake and protein content of diet on the faecal nitrogen excretion cannot be studied in comparable groups of young rats without bringing about body-weight differences between the groups. The effect of body-weight on the faecal nitrogen excretion is therefore discussed first. This discussion will, however, be extended in some of the following sections.

In Table 4 are collected data from six experiments (Expts 4, 6, 9 a, 9 b, 11 a and 11 b) in which the term 100 F/I was determined for the same rats during two or three periods. For each rat the experimental conditions, except the body-weight, were kept constant as far as possible in the experimental periods, and except for Expt 4 also in the gaps between the periods. In Expts 6, 9 a, 9 b, 11 a and 11 b four treatments were compared, but the treament effect was eliminated in the analyses of variance.

In Table 4 mean values are given for 100 F/I over two periods together with the differences between periods, with their standard errors (Expts 4, 6, 9 a, 9 b and 11 a), and mean 100 F/I values for each of three periods with their standard errors (Expt 11 b). The quantity 100 F/I and the body-weights were greater in the later periods than in the earlier. The results may, therefore, be taken to indicate that the heavier rats excreted more nitrogen in the faeces than the lighter rats. A similar result would, however, be obtained also if the heavier rats had more rapid passage of faeces than the lighter. If this were so, it would be expected that the differences between 100 F/I values would be eliminated if they were corrected to equal recoveries of an indigestible indicator substance in the experimental periods compared. In all experiments in Table 4 except in Expt 6 the recovery in the faeces of ingested $TiO_2$ was determined in parallel

Table 4. *Apparent faecal recoveries of ingested nitrogen (100 F/I) as determined in two or three experimental periods.* (Means over one or two periods and mean differences (period 2 — period 1)).

| Expt no | Period no | Protein source | P (%) | Gap between periods (days) | n | Body-weight (g) | | 100 F/I | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mean | Difference | Mean | Difference | S. E. | Corrected Mean | Corrected Difference | Corrected S. E. |
| 4 | 1 and 2 | Egg | 4.5 | 20 | 11 | 103.6 | +50.1 | 24.8 | +2.3 | ±0.52 (10 df) | | +1.2 | ±0.35 (10 df) |
| 6 | 1 and 2 | Egg and H-m 4 | 4.5—10 | 21 | 20 | 93.3 | +34.9 | 21.7 | +1.3 | ±0.50 (12 df) | | | |
| 9a | 1 and 2 | H—m 4 | 10 | 21 | 15 | 131.6 | +25.8 | 20.1 | +1.6 | ±0.43 (8 df) | | +1.2 | ±0.38 (8 df) |
| 11a | 1 and 2 | H—m 11 | 10 | 16 | 16 | 107.3 | +37.9 | 21.7 | +3.6 | ±0.38 (9 df) | | −0.2 | ±0.13 (9 df) |
| 11b | 1 | H—m 11 | 10 | | 20 | 78.4 | | 22.2 | | | 22.6 | | |
| — | 2 | — | 10 | 7 | 20 | 105.8 | | 22.3 | | ±0.29 (24 df) | 22.3 | | ±0.22 (24 df) |
| — | 3 | — | 10 | | 20 | 127.7 | | 23.8 | | | 23.4 | | |

P, protein content of diet; n, number of observations.

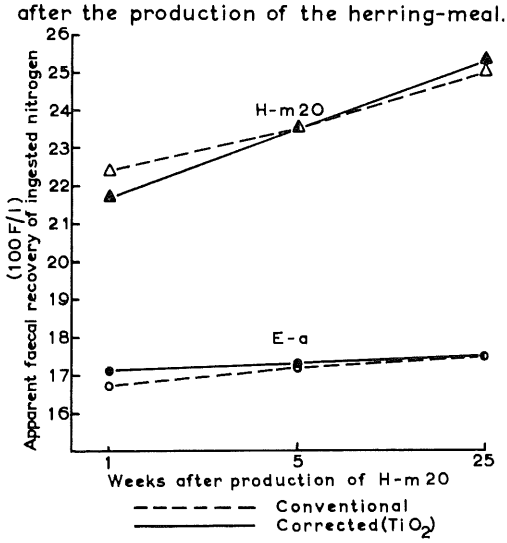
with the apparent recovery of ingested nitrogen (NJAA 1961 b). Because water was used as a blank in the colourimetric determinations of $TiO_2$ the recovery of this substance was less than 100 %. The observed values for 100 F/I were, however, corrected to an equal recovery of $TiO_2$ by multiplying by the mean recovery for the experiment. In this way the mean of the corrected 100 F/I values in each experiment was the same as the mean uncorrected value, but the differences between the periods and their standard errors were affected. The corrected differences are given in Table 4. It is seen that in each experiment the correction procedure resulted in less difference between periods. In Expts 4, 9 a, 9 b and 11 b the differences were still in the same direction as before the correction was applied and they were still significant. In Expt 11 a the difference between periods was virtually eliminated by the correction procedure. This is worthy of note because it was in this experiment that the greatest difference between periods was observed before the correction was applied. In this experiment, and also in Expt 11 b significant correlations between individual values for 100 F/I and ART (apparent faecal recovery of ingested $TiO_2$) were previously demonstrated (NJAA 1961 b, Table 5, Expts 4 and 5). The results in Table 4 indicate that part of this parallel variation in 100 F/I and ART may probably be ascribed to a tendency for the heavier rats to excrete more faeces in relation to the food intake than the lighter rats.

In four of five experiments in Table 4 there remained a difference between periods after the correction had been applied which probably may be ascribed to body-weight differences. Before accepting the significance of this inference it should be considered whether a reduced digestibility of the herring meal due to the meal being older in the later periods could explain the results. The question cannot be answered on the basis of the results in Table 4 because the body-weight differences are involved. It is, however, suggestive that the mean 100 F/I value was lower in Expt 11 b than in Expt 11 a. The question was studied more thoroughly in Expt 15 a, c, d. One of the herring meals, H-m 20, was used in all these experiments when it was about 1, 5 and 25 weeks old. The results are plotted in Fig. 2 against the logaritm of the age of the meal together with the results obtained concurrently with a spray-dried egg albumen. It is seen that using a logarithmic scale 100 F/I increased approximately linearly. The age of the meal may therefore influence the differences between 100 F/I values especially when the experimental periods follow shortly after the production of the meal. The exact ages of the meals used in the experiments shown in Table 4 were not known. They were, however, more than 2 months old in Expts 6 and 11 a so the reduction in apparent digestibility with time would be expected to be slow. Therefore, most of

the differences in 100 F/I between the periods remaining after the correction procedure was applied, were probably due to the body-weight differences. The corrected results indicated that the faecal nitrogen excretion increased by between 0.04 and 0.08 mg/g rat/day. This may be negligible when the body-weight differences are small, but may be of importance when greater differences are involved. This point will be discussed more fully in later sections. (Sections D, E and G).

Fig. 2.

Apparent faecal recoveries of ingested herring-meal (H-m 20) and egg-albumen (E-a) nitrogen at three time intervals after the production of the herring-meal.




*C. The effect of growth rate*

A relationship was demonstrated to exist between the urinary nitrogen excretion and the growth rate (Njaa 1959 b and chapter VII). It was therefore thought to be of interest to examine whether a similar relationship could be demonstrated between faecal nitrogen excretion and the growth rate. Correlation coefficients, total, partial and multiple, were calculated between 100 F/I on the one hand and the body-weight (W) and the growth rate ($\Delta$ W) on the other for values taken from ten experiments in which spray dried egg-albumen was given at the 8 % protein level to groups of 6 young rats. The body-weight (W) varied but little (coefficient of variation about 7 %) so that little effect was expected from this source of variation. The results are given in Table 5, both for directly determined 100 F/I values and for corrected values. The results with the

Table 5. *Correlation coefficients between (1) apparent faecal recovery of ingested nitrogen (100 F/I), (2) body-weight (W) and (3) growth rate ($\Delta W$) in 60 young rats given spray dried egg albumen at the 8 % protein level.*

| | | Mean | ± coefficient of variation | Correlation coefficients | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | | | Partial | | Multiple |
| | | | | $r_{12}$ | $r_{13}$ | $r_{23}$ | $r_{12\cdot3}$ | $r_{13\cdot2}$ | |
| 100 F/I | conventional | 16.7 | ± 6.4 % | −0.190 | −0.431*** | −0.261* | −0.347** | −0.508*** | 0.533 |
| | corrected ($TiO_2$) | | ±11.4 % | −0.228 | −0.164 | | −0.285* | −0.238* | 0.325 |
| W (g) | | 81.8 | ± 6.8 % | | | | | | |
| $\Delta$W (g/day) | | 1.43 | ±26.4 % | | | | | | |

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level.

uncorrected values indicated that high growth rate was associated with low values for 100 F/I, but the effect was greatly reduced when the corrected values were used. Because high growth rate at a constant food intake is usually associated with low body-weight the results may be taken to indicate that the faecal nitrogen excretion increased slightly with increasing body-weight. This is in agreement with the results discussed in section B. It is not possible to draw any definite conclusion as to whether the body-weight or the growth rate is the more important factor in this connexion, and whether the two factors influence faecal nitrogen excretion independent of one another.

### *D. The effect of food intake*

The assumed relationship between the faecal nitrogen excretion and the food intake is formulated in equation 6 b, a linear relationship being assumed when the protein content in the diet is kept constant. Generally this is found to hold true (see for instance Table 7). The ratio faecal nitrogen/food intake, or F/E, is of interest because this variable is considered when the metabolic faecal nitrogen excretion is determined by extrapolation. Equation 6 c gives the assumed relationship between F/E on the one hand and C and E on the other as derived from equation 6 b.

$$F/E = k_1 C + k_2 + \frac{k_3}{E} \qquad (6\,c)$$

It is seen that when F/E values determined at different values of C are extrapolated to C = O the intersection with the F/E axis gives an estimate of $k_2 + k_3/E$. When extrapolation procedures are employed it is tacitly assumed that the term $k_3/E$ is negligible. (Bosshardt & Barnes 1946; Bell, Williams, Loosli & Maynard 1950; Mitchell & Bert 1954; Armstrong & Mitchell 1955; Homb 1955).

It is of interest, therefore, to study the variation of F/E in relation to the food intake at a constant value of C to get some information as to whether this assumption is correct. From equation 6 c it is seen that F/E would be expected to decrease with increasing E if $k_3$ were positive, or to be constant if $k_3$ were zero. It is recalled that $k_3$ designates the assumedly relatively constant part of metabolic faecal nitrogen (Schneider 1935).

The ratio F/E was determined at different food intakes and at a constant protein concentration in Expts 9 a, 9 b and 43. The results are given in Table 6. The differences observed between the food intake levels 8.33, 10 and 12 g/rat/day (Expt 9 a) and 8 and 10 g (Expts 9 b and 43) were insignificant. In Expt 9 a the overall difference between periods was non-significant although the differences at the 10 and 12 g levels were

Table 6. *Faecal nitrogen/food intake ratios (F/E) at various food intakes at the 10 % protein level.* (Means over two periods (Expt 9 a) or over two food intakes (Expts 9 b, 43) and mean differences (period 2 — period 1) or (10 g — 8 g)).

| Expt no | Rats per group | Food intake (g/rat/day) | Body-weight (g) | | F/E (mg/g) | | | | E/W | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Conventional | | Corrected ($TiO_2$) | | (g/day/g body-weight) | |
| | | | Mean | Difference | Mean | Difference | Mean | Difference | Mean | Difference |
| 9a | 5 | 8.33 | 117.0 | +15.1 | 3.25 | −0.23 | 3.23 | −0.14 | 0.72 | +0.09 |
| | | 10.0 | 130.6 | +24.8 | 3.15 | +0.32 | 3.15 | +0.12 | 0.77 | +0.14 |
| | | 12.0 | 147.0 | +37.9 | 3.02 | +0.35 | 3.04 | +0.27 | 0.83 | +0.22 |
| | | Standard error (8 d.f.) | | | ±0.07 | ±0.11 | ±0.06 | ±0.11 | | |
| 9b | 5 | 8 and 10 | 87.6 | + 8.1 | 2.81 | −0.10 | | −0.01 | 1.03 | +0.13 |
| | | Standard error (4 d.f.) | | | | ±0.08 | | +0.140 | | |
| 43 | 8 | 8 and 10 | 75.1 | + 6.8 | 3.57 | −0.206 | | −0.086 | 1.19 | +0.16 |
| | | Standard error (7 d.f.) | | | | ±0.092 | | ±0.051 | | |

significant for the uncorrected values. The correction procedure reduced both the differences between food levels and the differences between periods. At the 8.33 g level the difference between periods maintained an opposite sign compared to the 10 and 12 g levels even after applying the correction procedure. At face value the results indicated that $k_3$ in equations 6 a-c was zero, or that $k_3/E$ was negligible. In Expts 9 a and 43 there was a tendency for uncorrected and corrected F/E values to decrease with increasing E, but the tendency was non-significant. The interpretation of the results is, however, complicated by the possible effect on the faecal nitrogen excretion of the differences in body-weight between treatment groups (section B). If the body-weight increased with the food intake it would be expected that the effect of the increased food intake *per se* would be masked. If it is accepted that $k_3$ represents a real faecal excretion of nitrogen independent of the food intake but dependent on the body-weight, the combined effects of food intake and of body-weight (W) on the F/E ratio must be considered. The two variables would excert their effects on the term $k_3/E$ if $k_1$ and $k_2$ in equation 6 c are assumed to be independent of them.

As a first approximation, $k_3$ and W may be considered to be proportional (see chart 3 of SCHNEIDER 1935). Equation 6 c then assumes the form:

$$F/E = k_1C + k_2 + k_4\,1/(E/W) \qquad (6\,d)$$

where $k_4$ is the proportionality constant relating $k_3$ to W. Thus, the ratio E/W (g food intake/g body-weight) and its relation to E becomes of deciding importance for the variation of F/E with E.

In Expts 9 a, 9 b, 43 (Table 6) E/W was higher at the higher food intakes than at the lower. The combined effect of E and W on F/E would then be expected to bring about decreasing F/E with increasing E. This was actually what was observed in Expts 9 a, 43, whereas in Expt 9 b F/E was unaffected by E. The results in Expt 9 a are the most interesting in this connexion: In period 1 E/W was about 25 % higher at the 12 g level than at 10 g, in period 2 the corresponding value was only about 7 %. In period 1 F/E showed a clear tendency to decrease with increasing E, in period 2 F/E was virtually unaffected by E. The indication seem to be, then, that the assumption of a positive value of $k_3$ dependent on the body-weight may be correct.

It was decided to try to test the assumption with data taken from the literature also. To this end linear regression equations and correlation coefficients between faecal nitrogen excretion F on the one hand, and either the nitrogen intake I or the food intake E on the other were calculated from data taken from the literature. The results are given in

Table 7. *Regression of daily faecal nitrogen excretion (F, mg) on either the nitrogen intake (I, mg) or the food intake (E, g) calculated from literature data.*

| Protein source | Protein content of diet % | No. of obs. | Regression equation | Reference |
|---|---|---|---|---|
| Corn Protein | 5 | 23 | F = 0.37 I − 4.66 | MITCHELL (1923—4 b) |
| ,, ,, | 10 | 21 | F = 0.17 I + 2.23 | —,,— |
| Casein | 5 | 16 | F = 0.31 I − 3.89 | —,,— |
| White bread (A) | 8.5 | 12 | F = 0.25 I − 6.3 | HENRY & KON (1946) |
| Cheddar cheese (B) | ,, | 12 | F = 0.18 I − 0.8 | —,,— |
| A + B | ,, | 12 | F = 0.26 I − 8.8 | —,,— |
| Potato (E) | 8 | 12 | F = 0.48 I − 16.6 | —,,— |
| Dried skim milk (F) | ,, | 12 | F = 0.40 I − 17.5 | —,,— |
| E + F | ,, | 12 | F = 0.49 I − 19.1 | —,,— |
| Soya flour | ,, | 12 | F = 0.29 I − 2.7 | —,,— |
| Diet I | 8 | 12 | F = 0.30 I + 4.5 | MACRAE *et al.* (1943) |
| Diet II | ,, | 12 | F = 0.43 I − 17.0 | —,,— |
| Diet III | ,, | 12 | F = 0.42 I − 16.3 | —,,— |
| Diet IV | ,, | 12 | F = 0.39 I − 14.5 | —,,— |
| Low egg | not | 69 | F = 2.31 E − 1.57 | HENRY & KON (1946) |
| ,, ,, | stated | 24 | F = 3.12 E − 5.76 | MACRAE *et al.* (1943) |
| Protein free | | 43 | F = 2.39 E − 2.93 | MITCHELL (1923—4 a) |

Table 7. Only results from experiments in which the correlation was high ($r > 0.8$) and highly significant ($p < 0.001$) are included. If equations 6 a and b are valid and the term $k_3$ is independent of the body-weight, the constant term in the linear regressions would be expected to be positive in the majority of the cases if $k_3$ is in fact a positive quantity, or to be positive and negative about equally often if $k_3$ is in fact zero. In contrast to these expectations the constant term in the regression equations was negative in 15 of the 17 cases tabulated. This may be explained if $k_3$ is proportional to the body-weight and if it is assumed that the food intake (E) and the body-weight (W) is positively correlated in such a manner that the linear regression of E on W contains a positive constant term, or that the regression of W on E contains a negative constant term. The term $k_3$ in equations 6 a and b may then be expressed by a sum of two new terms of which one is proportional to E and the other is a constant with negative sign. The assumption made is usually fulfilled inasmuch as heavier rats generally eat more food though less per g body-weight than lighter rats. In the five sets of values taken from MACRAE *et al.* (1943) it was found that the assumption was correct, in the other cases referred to in Table 7 its correctness was taken for granted. The assumption of a faecal nitrogen excretion independent of the food intake but dependent on the body-weight is, therefore, neither inconsistent with my own results nor with the literature data considered. It must be noted, however, that other explanations of the results are possible. It does not seem possible to prove or disprove any explanation consistent with the results. Therefore the explanation suggested by SCHNEIDER (1935) and adopted by MITCHELL (1948) may also serve as a means of describing the results.

In this section the term F/E has been dicussed although it is the term 100 F/I which enters into the equations for nitrogen balance (Bal %) and net protein utilization (NPU) (equations 5 b and 4 b). The two terms are, however, interrelated by the proportionality constant 100/C where C is the nitrogen content of the diet in mg N/g food. Thus, the difference of about 0.2 between the mean F/E values at the 8.33 and 12 g levels in Expt 9 a would represent a difference of about 1.25 between the corresponding 100 F/I values. The effect of varying food intake must therefore be reckoned with as a source of variation in 100 F/I in experiments where *ad lib.* feeding techniques are employed. The extent of the expected variation, and its direction, seems to some extent to be dependent on the ratio between the food intake and the body-weight.

*E. The effect of the nitrogen content of diet*

Equation 6 b (p. 28) describes the assumed relationship between the faecal nitrogen excretion and the protein content of the diet. Likewise, equations 6 c (p. 33) and 6 d (p. 35) describe the expected variation in F/E, the former referring to conditions when the body-weight of the rats may be left out of consideration, the latter to the condition when $k_3$ is directly proportional to the body-weight. The variable F/E is studied because of its use in the extrapolation procedures as outlined in section D. In a similar way to the effect of the food intake, which was studied at a constant protein content of the diet, the effect of the nitrogen content was studied at a constant food intake. The results obtained in Expts 6, 9b and 43 when the food intake was 10 g/rat/day at the 6, 8 and 10 % levels are given in Table 8. In all experiments the F/E value increased with the protein content of the diet, but the effect was not significant in Expt 9 b. The correction procedure based on the faecal recovery of $TiO_2$ reduced the differences between protein levels in Expts 9 b and 43, but in Expt 6 the correction was not applied because $TiO_2$ was not determined in period 2. The linear, but not the quadratic, component of the treatment sum of squares was significant in Expt 6. Thus the tendency observed for F/E to increase more from the 8 to the 10 % level than from the 6 to the 8 % was not significant. This tendency may, however, be seen as an effect of the increase in the mean body-weight due to the increase in the protein content. According to equation 6 d (p. 35) F/E would increase linearly with W when E is constant, and the effects of C and W on F/E would be superimposed. W increased more rapidly than C in Expt 6 and F/E would therefore be expected to increase likewise.

Table 8. *Faecal nitrogen/food intake ratios (F/E) at various protein levels at the daily food intake of 10 g/rat.* (Mean over two periods (Expt 6) or over two protein levels (Expts 9 b, 43) and mean differences (period 2 — period 1) or (10 % — 8 %).

| Expt no | Rats per group | Protein content of diet (%) | Body-weight (g) | | F/E (mg/g) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Conventional | | Corrected ($TiO_2$) |
| | | | Mean | Difference | Mean | Difference | Difference |
| 6 | 5 | 6 | 89.1 | + 31.2 | 2.28 | +0.25 | |
| | | 8 | 94.6 | + 37.3 | 2.47 | +0.13 | |
| | | 10 | 103.8 | + 45.4 | 2.78 | +0.10 | |
| | | Standard error (8 d.f.) | | | ±0.051 | ±0.115 | |
| 9 b | 5 | 8 and 10 | 84.4 | + 4.6 | 2.72 | +0.08 | + 0.01 |
| | | Standard error (4 d.f.) | | | | ±0.093 | ±0.120 |
| 43 | 8 | 8 and 10 | 77.2 | + 2.6 | 3.26 | +0.42 | + 0.24 |
| | | Standard error (7 d.f.) | | | | ±0.081 | ±0.042 |

The differences between periods for W and F/E indicated that the latter increased on an average about 0.004 mg N/g food/g increased body-weight. Assuming that the effect of body-weight on the differences between treatments was of the same magnitude as that between periods the difference in F/E between the 6 % level and 10 % levels would be reduced by about 20 %. The differences in protein contents may, therefore, be held responsible for most of the differences between levels in the F/E values. The results in Expt 43 point in the same direction assuming that the small body-weight difference between protein levels is negligible. The observation in Expt 9 b that changing the protein content from 8 to 10 % had virtually no effect on F/E was due to two rats excreting slightly more nitrogen in the faeces at the 8 % level than their litter-mates at 10 %. Differences of this magnitude in the protein content may therefore occasionally have no distinguishable effect on F/E.

It was pointed out (section D) that 100 F/I and F/E are inter-related by the factor 100/C. Assuming equations 6 c, d to be valid (p. 33 and 35) the variation in 100 F/I due to variation in the protein content is, therefore, determined by the variation of the term 100/C ($k_2 + k_3/E$) or 100/C ($k_2 + k_4/(E/W)$) when $k_1$ is assumed to be constant. At a constant food intake E, 100 F/I would be expected to increase linearly with increasing 1/C if $k_3$ is a constant or if $k_3$ is proportional to C (see also p. 50). 100 F/I would increase at a higher rate than 1/C if $k_3$ increased at a higher rate than C, and *vice versa*. In Expt 6 the mean body-weight increased at a higher rate than C, the term $k_3$, therefore, presumably increased likewise. According to expectation 100 F/I also increased at a higher rate than 1/C (Fig. 3). Disregarding the effect of the body-weight, 100 F/I decreased by about 1.5 units for each unit increase in the protein content of the diet (Cx 6.25). It is obvious, therefore, that 100 F/I values, or the complementary term the apparent digestibility, should not be compared unless they are obtained at the same protein content of the diet.

However, comparison of 100 F/I values at intendedly equal protein levels may refer to actually different levels. Examples of differences between intended and actual protein levels are found for instance in the data given by HENRY & KON (1957) for 28 diets used by them. The actual values ranged from 82.5 to 125 % of the intended values, and the difference between intended and actual values ranged from 1.6 to —2.2 protein percentage units. In my own experiments differences between actual and intended values were also observed. The mean analysed protein content of 50 diets intended to contain 8 % protein was 7.90 % with a standard deviation of 0.156, or a coefficient of variation of about 2%. The range was 7.25—8.27. In the most unfortunate case diets differing by about 1 % in their protein content might have been compared. According to the

considerations discussed in the next section such a difference at the 8 % level would be expected to result in a difference in the 100 F/I value of about one unit. This should be taken into account when apparent digestibilities of proteins are compared. This point is also discussed in section H 4. In Expts 15 a, c, d the protein content of the herring meal diet used was about 0.35 percentage units higher in Expts 15 c, d than in Expt 15 a. By taking this into account the line representing the results with H-m 20 in Fig. 2 should have been slightly steeper (See also p. 54).

### *F. The relative effects of food intake and protein content*

From the results obtained in Expts 6, 9 a, 9 b and 43 it is possible to discuss the relative importance for the variation in the faecal nitrogen excretion of varying the nitrogen intake by way of changing the food intake or by way of changing the protein content of the diet.

In Table 9 are summarized the results from Expts 6, 9 b and 43, the variables given besides the food intake and the protein contents of the diets are the daily faecal nitrogen excretion (F) and values for 100 F/I. Both the directly determined values and those obtained after correction based on the faecal recovery of ingested $TiO_2$ are given. The results are discussed in relation to equations 6 a and b (p. 28). From this it is seen that a greater effect on F would be expected from changing the food intake $E_1$ by $\Delta$ E at a constant nitrogen content of the diet $C_1$ than from an equivalent change in $C_1$ by $\Delta$ C at a constant food intake $E_1$. Conversely, 100 F/I would be more affected by the change in $C_1$ than in $E_1$. The equivalence of $\Delta$E and $\Delta$C refers to the changes in the nitrogen intake corresponding to them. By solving the equations under the conditions specified it is found that the quantity of faecal nitrogen ($\Delta$F) representing the difference between the two ways of changing the nitrogen intake is given by $k_2 \Delta E$, and the corresponding value for $\Delta$ 100 F/I values by

$$100\, k_2 \frac{\Delta C}{C_1 (C_1 + \Delta C)} \text{ or } 62.5\, k_2 \frac{\Delta P}{P_1 (P_1 + \Delta P} \text{ where P is the protein content}$$

of the diet (g/100 g). It should be kept in mind that in the equations and in the terms derived from them C is given in mg N/g food (see Appendix A).

Qualitatively the results obtained in Expts 9 b and 43 agreed with the predictions based on equations 6 a and b. The faecal nitrogen excretion was reduced more by reducing the food intake from 10 to 8 g/rat/day when the protein content of the diet was 10 %, than by reducing the protein content from 10 to 8 % when the food intake was 10 g/rat/day. Conversely, 100 F/I was increased more by reducing the protein content than by reducing the food intake. The question whether the observed changes in

Table 9. *Faecal nitrogen (F) and apparent faecal and urinary recoveries of ingested nitrogen (100 F/I and 100 U/I) and percentage nitrogen balances (Bal %) at various food intakes and protein levels.* (Means over one or two periods and mean differences (Δ) (period 2 — period 1)).

| Expt no. | n | P (%) | E (g) | Body-weight (g) | | Weight-gain (g/day) | | F (mg/day) | | | | 100 F/I | | | | 100 U/I | | Bal % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Conventional | | Corrected ($TiO_2$) | | Conventional | | Corrected ($TiO_2$) | | | | | |
| | | | | Mean | Δ | Mean | Δ | Mean | Δ | Mean | Δ | Mean | Δ | Mean | Δ | Mean | Δ | Mean | Δ |
| | | 6 | 10 | 89.1 | +31.2 | 1.4 | −0.1 | 22.8 | +2.5 | | | 23.4 | +1.9 | | | 35.6 | −1.7 | 41.1 | − 0.2 |
| 6 | 5 | 8 | 10 | 94.6 | +37.3 | 2.0 | −0.1 | 24.7 | +1.3 | | | 18.7 | +0.2 | | | 35.8 | −2.3 | 45.4 | + 2.2 |
| | | 10 | 10 | 103.8 | +45.5 | 2.2 | 0.0 | 27.8 | +1.1 | | | 16.9 | +0.3 | | | 36.6 | −1.9 | 46.4 | + 1.8 |
| S.E. | (8 df) | | | | | ±0.10 | ±0.14 | ±0.51 | ±1.15 | | | ±0.39 | ±1.40 | | | ±1.03 | ±2.16 | ±1.02 | ± 2.31 |
| | | 10 | 8.33 | 117.0 | +15.1 | 0.8 | +0.4 | 27.1 | −1.9 | 26.9 | −1.3 | 20.8 | −0.7 | 20.7 | −0.2 | 69.2 | +8.1 | 10.1 | − 7.3 |
| 9a | 5 | 10 | 10.0 | 130.6 | +24.8 | 1.4 | −0.3 | 31.5 | +3.2 | 31.4 | +1.3 | 20.2 | +2.8 | 20.2 | +1.6 | 58.7 | +5.2 | 21.1 | − 7.9 |
| | | 10 | 12.0 | 147.0 | +37.9 | 2.1 | −1.2 | 36.3 | +4.1 | 34.4 | +2.9 | 19.3 | +2.7 | 19.4 | +2.1 | 52.8 | +7.3 | 27.9 | −10.1 |
| S.E. | (8 df) | | | | | ±0.11 | ±0.27 | ±0.67 | ±1.00 | ±0.54 | ±1.10 | ±0.44 | ±0.74 | ±0.37 | ±0.67 | ±2.29 | ±1.82 | ±2.16 | ± 1.87 |
| | | 8 | 10 | 87.1 | | 1.8 | | 26.8 | | 27.5 | | 21.1 | | 21.7 | | 41.3 | | 37.6 | |
| 9b | 5 | 10 | 10 | 91.7 | | 2.2 | | 27.5 | | 27.5 | | 17.7 | | 17.7 | | 45.0 | | 37.3 | |
| | | 10 | 8 | 83.6 | | 1.2 | | 22.8 | | 22.1 | | 18.3 | | 17.7 | | 51.4 | | 30.2 | |
| S.E. | (8 df) | | | | | ±0.09 | | ±0.52 | | ±0.80 | | ±0.31 | | ±0.45 | | ±1.57 | | ±1.57 | |
| | | 8 | 10 | 75.9 | | 0.7 | | 30.3 | | 32.0 | | 24.5 | | 25.7 | | 41.3 | | 34.1 | |
| 43 | 8 | 10 | 10 | 78.5 | | 0.8 | | 34.7 | | 34.4 | | 22.4 | | 22.3 | | 43.5 | | 34.0 | |
| | | 10 | 8 | 71.7 | | 0.0 | | 29.4 | | 28.1 | | 23.8 | | 22.8 | | 50.7 | | 25.5 | |
| S.E. | (14 df) | | | | | ±0.05 | | ±0.58 | | ±0.33 | | ±0.44 | | ±0.27 | | ±0.93 | | ±1.17 | |

P, protein content of diet. E, daily food intake per rat. n, rats per group. S.E. Standard error.

F and 100 F/I agree quantitatively with the terms derived from equations 6 a and b above cannot be answered with certainty. The terms derived apply to the condition of a constant value of $k_3$ in the equations. However, because it is assumed that $k_3$ varies with the body-weight of the rats, and because the latter was affected more by a change in the food intake than by a change in the protein content of the diet, the difference between values for $\Delta F$ resulting from the two ways of changing the nitrogen intake would be numerically greater than $k_2 \Delta E$ by the difference between $k_3$ values corresponding to the 8 % 10 g and the 10 % 8 g levels respectively. In the case of 100 F/I it would be less than the expected value.

The results obtained in Expts 6 and 9 a were also in agreement with expectations on a qualitative basis: The faecal nitrogen excretion was more affected by the variations in the food intake in Expt 9 a than by the variations in the protein content of the diet in Expt 6, whereas with the term 100 F/I the opposite was true.

Only few experiments reported in the literature were designed for the study of the relative effects of the food intake and the protein content of the diet on the faecal nitrogen excretion. CRAMPTON & RUTHERFORD (1954) concluded from an extensive experiment that the apparent protein digestibility was influenced greatly by the protein intake and but little by the intakes of food *per se* and crude fibre. Unfortunately, the analysis of covariance on which these conclusions were based may be criticized on several points. This objection to the treatment of the results needs to be justified: Instead of accepting equation 6 a with an additional term accounting for the variation in crude fibre (M) they treated the results on the assumption that the apparent protein digestibility ($D_a$) is a linear function of the intakes of nitrogen, food and crude fibre and of the quantity of nitrogen in the faeces. As $D_a$ and 100 F/I are complementary terms this is equivalent to a description of 100 F/I by the same variables. A formulation of this would be:

$$100\ F/I = mI + nE + pM + qF + s \qquad (6\ e)$$

(I, E and M are the intakes of nitrogen, food and crude fibre respectively; F is faecal nitrogen; m, n, p and q are proportionality constants and s is the constant term in the regression equation).

The formulation given in equation 6 e does not seem meaningful for the following reasons: (1) The protein content of the diet, which seems to be the most important factor in determing the magnitude of $D_a$ or 100 F/I (Fig. 1 of CRAMPTON & RUTHERFORD 1954) does not appear in the equation, (2) I and E, and E and M, which appear together on the righthand side of the equation are not independent of one another, and (3) F and I appear on both sides of the equation, F taken to the same,

but I taken to a different power on each side. Therefore, whereas Fig. 1 of CRAMPTON & RUTHERFORD (1954) clearly demonstrates the importance of the protein content of the diet in the variation in the apparent protein digestibility, no conclusion may be drawn from their results concerning the effect of the food intake. If the relative importance of these variables is studied the variations in the nitrogen intake brought about by varying them should probably be of comparable magnitudes.

### *G. The estimation of metabolic faecal nitrogen*

#### 1. General

The sources of variation discussed in the preceding sections all in some way concern the term 100 F/I. This term includes exogenous and endogenous faecal nitrogen. However, for the determination of BV or NPU (equations 2, 4 b and d, p. 10 and 25) a knowledge is required of the part of the faecal nitrogen which is not directly derived from the ingested protein. In the equations this part of the faecal nitrogen excretion is designated by F'. It comprises in principle the nitrogen excretion which is assumed to be proportional to the food intake and the endogenous nitrogen which is relatively constant (SCHNEIDER 1935), these fractions taken together are termed the metabolic faecal nitrogen.

The methods in general use for the estimation of metabolic faecal nitrogen are based on the assumption that the portion due to the constant endogenous excretion is negligible.

Metabolic faecal nitrogen per gram of ingested food (F'/E) is determined by two basically different methods: (1) Directly, by determing the ratio on a protein-free diet or on a low egg-protein diet (MITCHELL 1923—4 a; MITCHELL & CARMAN 1926 a); (2) Indirectly, by extrapolating the F/E values obtained at different chosen protein levels to the F/E value corresponding to a protein-free diet (BOSSHARDT & BARNES 1946; BELL *et al.* 1950; MITCHELL & BERT 1954; ARMSTRONG & MITCHELL 1955).

The two methods will be discussed separately:

#### 2. The direct estimation of metabolic faecal nitrogen

In these experiments metabolic faecal nitrogen excretion was determined using a low egg-protein diet containing about 4.5 % protein (MITCHELL & CARMAN 1926 a).

In Table 10 the results obtained in Expts 4, 5 and 6 are given. The F'/E values were determined on the same rats in two experimental periods; in Expts 4 and 5 these were before and after two herring meal periods,

in Expt 6 the low egg-protein diet was used throughout. For comparison are given similar results calculated from data taken from publications by Henry & Kon and co-workers (Bartlett, Henry, Kon, Osborne, Thompson & Tinsley 1938; Macrae *et al.* 1943; Henry & Kon 1946) who used the original Mitchell method (Mitchell 1923—4 a; Mitchell & Carman 1926 a). In all experiments included in Table 10 the rats were heavier in the last egg-protein period than in the first, and in all but one the mean F/E values varied similarly. This would be expected if $k_3$ in equation 6 a was proportional to the body-weight. However, in Expt 5 and in all the experiments quoted from the literature the food intake was also higher in the last egg-protein period than in the first. This would tend to mask the effect of the body-weight if equation 6 c (p. 33) applies. Opposing effects of food intake and body-weight on F/E are indicated by the results from the literature: The ratio between $\Delta$F/E and $\Delta W_M$ was high when $\Delta$E was low and *vice versa* (Table 10); $\Delta$F/E, $\Delta$W and $\Delta$E refer to the differences between periods. The results from Expts 5 and 6 but not those from Expt 4 fit into this picture. The fact that the protein content of the diet was about 0.25 percentage units higher in the first than in the last egg-protein period in Expt 4 may be suggested as a partial explanation of this.

The results taken together indicate that F/E determined on a low egg-protein diet is influenced by the body-weight of the rats, and probably also by the food intake. Thus, the results tally with those discussed in sections B (p. 28) and D (p. 35). In the original Mitchell method (Mitchell 1923—4 a; Mitchell & Carman 1926 a) variation in F/E on the protein-free diet or on the low egg-protein diet from one standardizing period to another is taken into account by assuming linear variation of the ratio with time. It is inherent in this assumption that the combined effects of body-weight and food intake on F/E varies linearly with time. It must be considered rather doubtful that this condition is fulfilled because body-weights and food intakes are often higher when the test proteins are given to the rats than in the standardizing periods. In many experiments reported in the literature BV and NPU are calculated by use of values of F/E obtained in only one standardizing period located before, between or after the test periods (e. g. Mitchell, Hamilton & Beadles 1952; Sure & Easterling 1952; Forbes & Yohe 1954; Metta & Mitchell 1956; Forbes, Vaughan & Yohe 1958; Rippon 1959). In such modifications of the Mitchell method no account is taken of the possibility that F/E may vary from period to period.

It is highly probable that the absolute magnitude of metabolic faecal nitrogen is slightly overestimated on the low egg-protein diet because Bosshardt & Barnes (1946) working with mice, and Mitchell & Bert

Table 10. *Faecal nitrogen/food intake ratios (F/E) on low egg-protein diets. Data from own experiments and from the literature.* (Means over two periods and mean difference (final period — initial period)).

| Expt no | No. of observations | Food intake (g/day) | | Body-weight (g) | | F/E (mg/g food) | | | $(\Delta F/E)/\Delta W$ |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Difference | Mean | Difference | Mean | Difference ± S.E. | | |
| 4 | 11 | 10.0 | 0 | 100.8 | + 57.4 | 1.82 | +0.07 ± 0.036 | (10 d.f.) | 0.0012 |
| 5 | 9 | 9.0 | +2.0 | 94.7 | + 52.9 | 1.84 | +0.24 ± 0.061 | ( 8 d.f.) | 0.0045 |
| 6 | 6 | 10.0 | 0 | 85.5 | + 26.0 | 2.02 | +0.24 ± 0.057 | ( 5 d.f.) | 0.0092 |
| a) | 12 | 7.3 | +0.6 | 76.4 | + 24.0 | 2.38 | +0.25 ± 0.10 | (11 d.f.) | 0.0104 |
| b) | 12 | 8.6 | +1.9 | 114.5 | +100.0 | 2.43 | +0.52 ± 0.12 | (11 d.f.) | 0.0052 |
| c) | 12 | 8.7 | +2.7 | 96.5 | + 75.8 | 2.08 | +0.24 ± 0.061 | (11 d.f.) | 0.0032 |
| c) | 12 | 9.8 | +3.6 | 100.0 | + 80.0 | 2.16 | +0.18 ± 0.066 | (11 d.f.) | 0.0023 |
| c) | 9 | 8.1 | +4.0 | 81.8 | + 48.9 | 2.12 | −0.05 ± 0.10 | ( 8 d.f.) | 0.0010 |

a) Data from Bartlett *et al.* (1938). b) Data from Macrae *et al.* (1943). c) Data from Henry & Kon (1946).

(1954) and NEHRING & HAESLER (1954) working with rats showed that whole egg-protein is not 100 % digestible. The evidence on this point is, however, conflicting. MITCHELL & CARMAN (1926 a) who introduced the low egg-protein diet as a standardizing diet instead of the N-free diet, concluded that there was practically no difference between faecal nitrogen excretion per gram of ingested food on the two types of diet. On the other hand MITCHELL (1948) gave the mean of 156 determinations on the low egg-protein diet as 1.164 mg per gram dry food consumed with the following comment: "The fact that these measurements were made with diets containing about 4 per cent of whole egg-protein, instead of with a diet practically free of nitrogen, may account for the fact that the above average is considerably less than the one previously cited of 1.9 mg." This statement seems odd unless it is assumed either that considerably more was consumed of the low egg-protein diet than of the N-free diet, or that the mean body-weight was considerably higher on the latter diet than on the former. METTA & MITCHELL (1956) explained the difference between F/E values observed in young rats of about 120 g body-weight on high and low fat egg-protein diets by assuming that they were due to differences in the food intake. In this case it may be calculated on the basis of equation 6 c (p. 33), ignoring the body-weight difference, that a value of $k_3$ of about 6.7 mg N/rat/day may account for the F/E difference observed in young growing rats. This value for $k_3$ lies within the range given by SCHNEIDER (1935) for rats weighing about 120 g.

It was of interest because of the above mentioned results to retest with their own data the conclusion of MITCHELL & CARMAN (1926 a) that practically no difference existed between F/E values determined on a N-free diet and a low egg-protein diet. The results are given in Table 11: The nitrogen excretion per g of food eaten was about 0.4 mg N/g food higher on the N-free diet than on the low-egg protein diet, the difference being significant. The corresponding differences between the body-weights and the food intakes were 14.8 g and 1.33 g/day respectively. Ignoring again the body-weight difference it may calculated on the basis of equation 6 c (p. 33) that a value of $k_3$ of about 15 mg N/rat/day is required to explain the F/E difference. This is higher than the mean total excretions of 13 and 12 mg/rat/day on the two diets respectively. It seems doubtful, therefore, that the greater F/E difference discussed by MITCHELL (1948) could be due to different mean food intakes on the two types of diet. The difference remains, therefore, unexplained.

It was of interest to study some other results obtained on N-free diets from the point of view of their possible interrelationship with the body-weight and food intake. In Table 11 are collected some such results taken from the literature together with the results taken from MITCHELL & CAR-

Table 11. *Faecal nitrogen/food intake ratios (F/E) on N-free diets and low egg protein diets. Data from the literature.*

| Type of diet | No. of observations | F/E (mg/g food) | Food intake (g/day) | Body-weight (g) | Reference |
|---|---|---|---|---|---|
| N-free ..... | 53 | 1.9 ±0.05 | 6.62 | 129.5 | MITCHELL & CARMAN (1926 a) |
| Low-egg ..... | 68 | 1.5 ±0.05 | 7.95 | 114.7 | —,,— |
| Low-egg (Low fat) ..... | 7 | 1.57 | 9.23 | 114 | METTA & MITCHELL (1956) |
| — (High fat) ..... | 7 | 1.77 | 7.21 | 120 | —,,— |
| — (Low fat) ..... | (7) | (1.56) | equal | not stated | |
| — (High fat) ..... | (7) | (1.56) | | | |
| N-free ..... | 23 | 2.01 | 8.5 | 40—100 | COLUMBUS (1954) |
| — ..... | 25 | 2.34 | 12.1 | 100—150 | —,,— |
| — ..... | 33 | 2.35 | 13.6 | 150—180 | —,,— |
| — ..... | 25 | 2.66 | 15.3 | 180—250 | —,,— |
| N-free ..... | 6 | 2.14 | 6.78 | 65.0 | BEHM (1955) |
| — ..... | 6 | 1.94 | 4.69 | 87.5 | —,,— |
| — ..... | 6 | 2.17 | 7.69 | 102.3 | —,,— |
| — ..... | 6 | 2.62 | 2.95 | 110.6 | —,,— |
| N-free ..... | 6 | 2.50 | 12.75 | 169.6 | —,,— |
| — ..... | 6 | 2.29 | 9.38 | 184.5 | —,,— |
| — ..... | 6 | 2.15 | 14.66 | 194.4 | —,,— |
| — ..... | 6 | 2.90 | 5.90 | 202.5 | —,,— |

MAN (1926 a) and METTA & MITCHELL (1956) which have already been discussed. The results credited to COLUMBUS (1954) are weighted means of his values for F/E for arbitrarily chosen body-weight intervals, and those of BEHM (1955) are from experiments with two groups of six rats each which were given different amounts of food and whose body-weights changed from period to period.

The results of COLUMBUS (1954) indicated a clear tendency for F/E to increase with increasing body-weight although the food intake varied in the same direction. From the previous discussion, and from that in section D (p. 33) the variation in the food intake would have been expected partly to mask the effect of the body-weight. The results of BEHM (1955) indicated only little effect of the body-weight within the narrow ranges tested and the effect of the food intake seemed to have been significant only when it was quite drastically reduced.

The F/E values given by COLUMBUS (1954) and those calculated from the data of BEHM (1955) tend to be higher than the mean obtained from the data of MITCHELL & CARMAN (1926 a) referring to the N-free diet and they are higher than the values obtained with egg-protein diets by MITCHELL & CARMAN (1926 a) and by METTA & MITCHELL (1956). On the other hand they agree fairly well with my own results and those taken from Henry, Kon and co-workers (Table 10).

However, the tendency of F/E as measured on N-free and low egg-protein diets to vary both with the body-weight of the rats and with the food intake indicates that it is doubtful whether a reliable value can be determined for F/E from which the true value of the metabolic faecal nitrogen excretion in the experimental periods can be estimated. When the NPU of a protein source is determined at a low protein level and at a moderate level of food intake, the question whether the metabolic faecal nitrogen is 1.5 or 2.0 mg/g food is of importance and the magnitude of NPU may be affected appreciably by the choice. This aspect is discussed in section H 5.

### 3. The estimation of metabolic faecal nitrogen excretion by extrapolation

To my knowledge no version of the Mitchell method using rats as experimental animals prescribes estimation of metabolic faecal nitrogen excretion by extrapolation. It has been suggested, however, to use the method in experiments with pigs (BELL *et al.* 1950; ARMSTRONG & MITCHELL 1955; HOMB 1962). The reason for dicussing the method in the present communication lies in the fact that its validity explicitely rests on the assumption that the term $k_3/E$ in equation 6 c (p. 33) is zero or

negligible. BOSSHARDT & BARNES (1946), BLAXTER & MITCHELL (1948), BELL *et al.* (1950), MITCHELL & BERT (1954), CRAMPTON & RUTHERFORD (1954) and ARMSTRONG & MITCHELL (1955) seem tacitly to accept that the above-mentioned condition is fulfilled for mice, rats, pigs and cattle.

However, if it is accepted that faecal nitrogen excretion is influenced by variations in the body-weight and in the food intake, the exptrapolation technique is open to criticism. Usually, when the protein content of the diet is not too high, the rats tend to be heavier at the higher protein levels than at the lower, and often the food intake may vary likewise. Since the two variables influence F/E in opposite directions (sections D, E and G 2) the effect of one or the other may dominate the picture, or their effects may cancel out.

In Expt 6 the linear component of the treatment sum of squares for F/E was highly significant. In both experimental periods values about 1.5 mg N/g food were obtained for "metabolic" F/E by extrapolation whereas direct estimation on the low egg-protein diet gave values about 2 mg N/g food. Because the food intake was kept constant, only the body-weight differences may have influenced the results obtained by extrapolation. It was mentioned previously (p. 39) that the body-weight increased at a slightly more rapid rate than did the protein content of the diet, and that F/E seemed to vary in the same direction as the body-weight. This tends to underestimation of metabolic F/E.

The true metabolic faecal nitrogen excretion presumably lies somewhere between the value directly determined on the low egg-protein diet and the value obtained by extrapolation. The former might be corrected for nitrogen of dietary origin if the value of $k_1$ in equations 6 a—c were known. This would require a knowledge of the true digestibility of the egg-protein: MITCHELL & BERT (1954) estimated the true digestibility of their sample of egg-protein from the slope of the regression line relating F/E to the protein content of the diet (BLAXTER & MITCHELL 1948). However, this slope would not be determined only by the digestibility of the protein but would also include variation in F/E due to varying food intake and body-weight of the experimental animals. Thus, correction of F/E obtained on the low egg-protein diet by taking into account faecal nitrogen of dietary origin requires that the true digestibility of the protein be postulated. In the communication of MITCHELL & BERT (1954) it is stated that the paired feeding technique was used, but no information is given on the point of the relationship between the protein content of the diet and body-weight.

It is of interest to examine whether literature data appear to agree with equations 6 a—c when it is assumed that the term $k_3/E$ is either zero or negligible. If they do not agree with the equations the indication is

that the extrapolation technique for estimation of metabolic faecal nitrogen is not tenable. On the other hand, agreement of such data with the equations does not necessarily indicate that the technique is based upon sound assumptions: the effect of food intake and body-weight may have cancelled out.

It follows from accepting the equations 6 a—c under the conditions mentioned that 100 F/I would increase linearly with 1/C (see also p. 39). Not all available literature data lend themselves to a test of the validity of this inference because of an obviously irregular interrelationship between C and 100 F/I. This applies for instance to the results of BARNES *et al.* (1946). In Fig. 3 graphs are given drawn on the basis of results taken from four literature sources. The results taken from FORBES *et al.* (1935) are typical of many similar results reported by the same group in other communications. In the graph drawn from results taken from HAMILTON (1939) three apparently erroneous results obtained at 12, 16 and 54 % protein levels were left out of consideration. The values in the graph based upon results from CRAMPTON & RUTHERFORD (1954) were read from their Fig. 1; similarly the values from MITCHELL & BERT (1954) were obtained by reading the F/E values from their Fig. 1 and by calculating 100 F/I values from them.

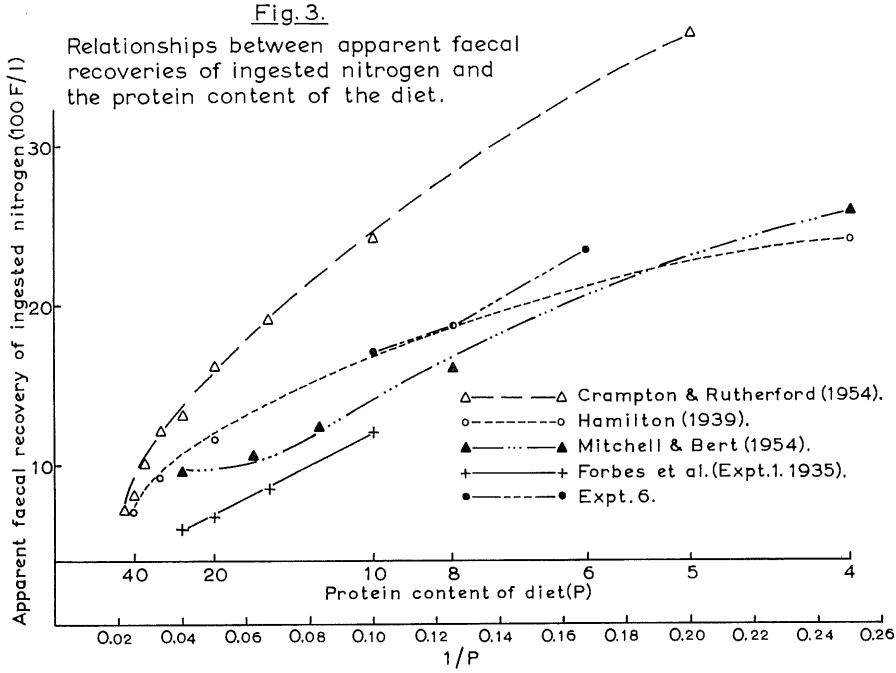
It is seen from Fig. 3 that the results taken from HAMILTON (1939) and those taken from CRAMPTON & RUTHERFORD (1954) do not indicate a linear interrelationship between 100 F/I and 1/C whereas such interrelationship is indicated by the results taken from FORBES et al. (1935), and probably by those from MITCHELL & BERT (1954). Thus in the two former cases the extrapolation technique for estimation of metabolic faecal nitrogen excretion would not be valid. In the two cases in which equations 6 a—c do apparently agree with the experimental results when it is assumed that $k_3/E$ is zero or negligible, the possibility that the combined effects of varying food intake and body-weight on faecal nitrogen excretion may have cancelled out cannot be excluded.

## *H. A general discussion of the relationship between variations in faecal nitrogen excretion and in net protein utilization*

### 1. Net protein utilization and true digestibility

One of the objects of this investigation was to study the causes of variation affecting biological values (BV) and net protein utilization (NPU) as determined by the Mitchell method (p. 14). In the seven preceding sections the various factors thought to be of importance in determining the variation in faecal nitrogen excretion have been discussed. In

Fig. 3.
Relationships between apparent faecal recoveries of ingested nitrogen and the protein content of the diet.




this section an attempt is made to discuss the importance of these factors in the variation of BV and NPU.

The interrelationship between BV and NPU was given in equation 4 a, in which D denotes the true digestibility of the protein source, as defined in equation 3. In equation 4 c the interrelationship between NPU and D was formulated. (For the equations see p. 11 and p. 25). It can be seen that NPU is linearly related to D and that BV is linearly related to 1/D when all other factors are kept or assumed to be constant. Thus, disregarding for the present discussion the probability that D may be significantly correlated with the urinary nitrogen excretion (equation 4 c), variations in NPU and BV due to the terms related to the faecal excretion may be discussed by considering the corresponding variation in D. The possible relationship between faecal and urinary nitrogen is discussed in section VIII I (p. 99).

## 2. The significance of the term 'true digestibility of protein'

In contrast to the apparent digestibility, the true digestibility of a protein source is generally considered to be one of its characteristics. It is assumed to be independent of the protein content of the diet, of the food

intake and of the body-weight of the experimental animals (ALLISON, ANDERSON & SEELEY 1946 b; MITCHELL 1948; FORBES *et al.* 1958; NJAA 1959 b). These assumptions do not lend themselves to direct verification for the reason that D cannot be directly determined. As an example of the confusion reigning in this field may be mentioned that MITCHELL & BERT (1954) estimated lower true digestibility for whole egg-protein (94.6 %) than did BRICKER & MITCHELL (1947) and MITCHELL & BEADLES (1950) who found 100 % and 97.5 % respectively. MITCHELL & BERT (1954) suggested that the differences observed were due to possible treatment differences during the preparation of the egg-powder. They did not consider, or even mention, that the digestibilities were obtained by three different methods each resting upon its own set of assumptions. Similarly, the mean "true" digestibility for the herring-meal protein used in Expt 6 was found to be about 97 % when metabolic faecal nitrogen was estimated on the low egg-protein diet (NJAA 1959 b; Expt 6 Table 11), and 92 % when D was estimated from the slope of the straight line relating F/E to C.

It is important, therefore, to distinguish between the *conception* of a constant true digestibility characteristic of the protein source under test, and *estimates* formed for it. The conception may well be retained while differences between estimates obtained by different methods may be ascribed to the experimental techniques. In the case of a lower value for D being obtained from the slope of the regression line than by use of a conventional technique, this may be explained by assuming a gradual increase in F/E due to the body-weight increasing with the protein content of the diet. This increase in F/E would be superimposed on that due to the increase in the protein content *per se*.

In the present dicussion it is chosen to regard the true digestibility of a protein source as a characteristic constant at a given time. The latter condition is included to account for the possibility that the digestibility may change as a result of time-dependent reactions (Fig. 2). Variations in D are therefore ascribed to errors in the estimations of I, F and F'. When the latter quantity is concerned errors due to faulty, or erroneous, assumptions about the factors influencing the metabolic faecal nitrogen excretion must also be considered. It is realized that the variations in D may also be otherwise explained, but a choice must be made between different possible explanations which cannot be verified experimentally. The choice is in agreement with the view generally taken in this connexion (MITCHELL 1948).

### 3. The components of the true digestibility

The two variable components of D are 100 F/I and 100 F'/I (equation 3), which together determine the variation in D. In the original Mitchell method (MITCHELL 1923—4 a) and in my own versions of it (NJAA 1959 a) 100 F'/I values are calculated for individual rats with data obtained with each rat or with one of its littermates. The procedures imply that values for D so calculated will vary less than the apparent digestibility (equation 5 a). From the data in Table 3 it is seen that this was the case with version A, with version B the reverse was true, and in the results obtained from values given by MACRAE *et al.* (1943) the variances of 100 F/I and of D were about equal.

This indicates that calculation of D may not always remove any significant amount of the error inherent in the determination of $D_a$. This is confirmed by literature data: In six determinations of $D_a$ and D for casein recorded by METTA & MITCHELL (1956) the standard error of D was greater than that of $D_a$ in three cases, in two cases the reverse was true and in one case the standard errors were equal. PUJOL (1958) reported $D_a$ and D for 11 species of edible fish, for 3 the standard error of D was greater than that for $D_a$, for 8 the reverse was true. ZIMMERMANN (1952) determined $D_a$ and D for 10 samples of grass meal; the standard error of the means were calculated from his data: In 7 cases the standard error was greatest for D, in three cases for $D_a$. Which of the two measures for protein digestibility varies the less seems, therefore, fortuitous. Thus, the chance of correcting for variation in $D_a$ due to variations in body-weight, growth-rate and probably food intake (the term $k_3/E$) and the chance of exaggerating the variation observed in $D_a$ by introducing the term 100 F'/I seem to be about equal. This suggests the use of a procedure for the calculation of D based upon the adoption of a fixed value for metabolic faecal nitrogen excretion per gram of food eaten and to use the variance of $D_a$ as representing the variance of D. In order to keep the variance of $D_a$ at a low level it would then be necessary to use rats of about equal body-weight and to keep the food intake constant.

Alternatively the metabolic faecal nitrogen may be determined with a large number of rats (COLUMBUS 1954; NEHRING & HAESLER 1954; BOCK 1958) and the value corresponding to a given body-weight read from a curve or calculated from a regression equation. It is my opinion that when this procedure is chosen, the variance calculated for $D_a$ (or 100 F/I) should be retained for D.

The implication of accepting the variance of $D_a$ as representative for that of D is to accept that the variation in metabolic faecal nitrogen cannot be adequately measured. It is believed, however, that the order of

magnitude of this faecal excretion of nitrogen can be estimated by the procedures suggested and that an approximate estimate may be formed of the mean true digestibility of the protein source under test.

It is fully realized that a sharp distinction should be drawn between the two concepts of true and apparent protein digestibilities. The latter concept is meaningful only under strictly standardized conditions whereas the former can be regarded as a characteristic of the particular protein source regardless of the dietary conditions under which it is given. Since the metabolic loss of faecal nitrogen is largely determined by the amount of food consumed, BLAXTER & MITCHELL (1948) argued that rather than regarding this loss as a variable factor in the animals requirement of protein it should be considered a tax upon the particular food given. This view seems to be in good agreement with the experimental evidence. However, in the equations for NPU the metabolic loss of nitrogen in the faeces is formally regarded as a factor in the animals requirement to be covered from the protein intake. It is important, however, to realize that the two parts of the maintenance requirement, 100 F'/I and 100 U'/I, are physiologically of different significance.

## 4. The protein concentration as a source of error

In the discussions above it has been assumed that the protein content of the diet may be regarded as constant within an experiment although it was mentioned that variations in the protein content should be considered and taken into account (p. 40). An experiment by the original Mitchell method usually extends over periods of time ranging from 3 to 10 weeks. It may be necessary, therefore, to prepare new batches of the diets used, and likewise in other experiments extending over some period of time. In such cases the variation in 100 F/I due to small, unintended differences between the protein contents of various batches of diet should be considered (sections E and F). In versions A and B of the Mitchell method referred to in Table 3 this source of error was of little importance because the protein content of the diet used varied but little between periods. It was mentioned in section E (p. 39) that 100 F/I decreased by about 1.5 unit for each unit increase in the protein content between 6 and 10 % (Expt 6). It was obvious, however, that the rate of change was greater the lower the protein content. According to equations 6 a and b the rate of change (d (100 F/I)/dC) is given by $-1/C^2 (k_2 + k_3/E)$. Assuming the sum $k_2 + k_3/E$ to be practically constant and equal to 2 mg N/g food (Tables 10 and 11) the rate of change would be given by $-2/C^2$. Since most experiments were done at the intended protein concentrations 8 and 10 %, the rates of change in 100 F/I about these contents are those

of interest. For the protein contents 7.5, 8.0 and 8.5, and 9.5, 10.0 and 10.5 % they were calculated to be 1.39, 1.22 and 1.08, and 0.87, 0.78 and 0.71 units per unit change in the protein content, respectively. It seems safe, therefore, to assume that variation in 100 F/I due to small variation in the protein content between different batches of diet will be of the magnitude of about one unit per unit change in the protein concentration about the intended value of 8 %, and about 0.8 units about the intended value of 10 %.

The greatest differences between batches of diet used in the study of the factors influencing the faecal nitrogen excretion were observed in Expts 6, 9 a and 15 (Table 2). At the 8 % level the greatest difference between diets was 0.39 units (Expt 15) and at the 10 % level 0.32 units (Expt 9 a). In Expt 15 the difference between 100 F/I values for H-m 20 in sub-experiments a and d would thus probably have been underestimated by about 0.4 units (Fig. 2) and in Expt 9 the difference between periods 2 and 1 was probably over-estimated by about 0.3 units (Tables 4 and 9). In the treatment of the results given in Tables 3—10 the probable effect of small variations in the protein contents of comparable diets was not taken into account. It is believed that the conclusions drawn from the results presented in them would not have been materially altered if such corrections were applied. However, this source of variation should be kept in mind especially when digestibilities of different protein sources are compared at the same intended protein content of the diet. It should be considered whether small, but consistent, differences may have been due to small unintended differences in the protein contents of the diets compared.

From these considerations it follows that protein digestibility should preferably be determined at a relatively high protein content in the diet. The effects of variations in the protein content between diets, and of variations due to the metabolic part of the faecal nitrogen excretion would then be minimized. On the other hand, the nitrogen excretion in urine is preferably studied at relatively low protein concentrations. Because the latter excretion is more intimately related to the protein metabolism than the faecal excretion, evaluation of protein quality, including the determination of its digestibility, is usually done at relatively low protein levels.

### 5. The magnitude of the metabolic faecal nitrogen excretion

Strictly speaking the question of the absolute magnitude of the metabolic faecal nitrogen excretion does not belong in a discussion of the factors affecting the variation of D, NPU and BV if the variation of the term

100 F/I or $D_a$ is taken as a measure of the variation of D (see section 3 p. 53). However, comparisons of values for D, NPU and BV determined for the same protein sources in different laboratories will be greatly affected by the estimates chosen for metabolic faecal nitrogen excretion. Such comparisons would be easier if D and $D_a$ (or 100 F/I) were reported separately so that it may be seen how great a part of D actually refers to the correction term 100 F'/I. For instance, at the 8 % protein level and at the daily food intake level of 10 g/rat D would be estimated about 3 units higher if F' were 2.4 mg N/g food (BARTLETT *et al.* 1938; MACRAE *et al.* 1943) than if it were 2.0 mg/g food (Expts 4—6, Table 11). The latter estimate would in turn give values of D about 7 units higher than when estimates around 1.0 mg/g food (MITCHELL 1948) were used. Examples of very low faecal excretion values were reported at the 10 % level for various protein sources by ARNRICH, HUNT, AXELROD & MORGAN (1951), and BRICKER & MITCHELL (1947) reported estimates for metabolic faecal nitrogen excretion ranging from 1.4 to 2.1 mg/g food for young rats and values about 1.2 mg/g food for adult rats. The low values for metabolic faecal nitrogen excretion indicated by the results given by ARNRICH *et al.* (1951) may probably be explained by assuming that some strains of rats may show very low metabolic excretions, but this explanation does not hold for the values reported by BRICKER & MITCHELL (1947) nor for those calculated from the data given by MITCHELL & CARMAN (1926 a) (Table 11). In the latter case the difference in mean body-weight and mean food intake could hardly explain why significantly different estimates were formed for the metabolic faecal nitrogen excretion on a protein free diet and a low egg-protein diet.

It seems safe to conclude that because of the difficulties inherent in the estimation of the metabolic faecal nitrogen excretion, the estimates formed for true digestibilities of different protein sources possess relative significance only within the laboratory in which they were determined. Even so, the concept of an intrinsically absolute value for the true digestibility of each protein source should be retained with the reservation that it is not possible to determine it adequately. As far as NPU and BV depend upon D, this conclusion is valid also for these quantities.

# VII. THE FACTORS INFLUENCING URINARY NITROGEN EXCRETION

## *A. General*

The subdivision of urinary nitrogen excretion into endogenous and exogenous parts (FOLIN 1905; MITCHELL 1955) is accepted by most investigators concerned with protein utilization, but the questions of the constancy of the endogenous part during periods of protein feeding (MITCHELL 1948) and its magnitude are still under discussion (SCHOENHEIMER 1942; FROST 1950; BIGWOOD 1952).

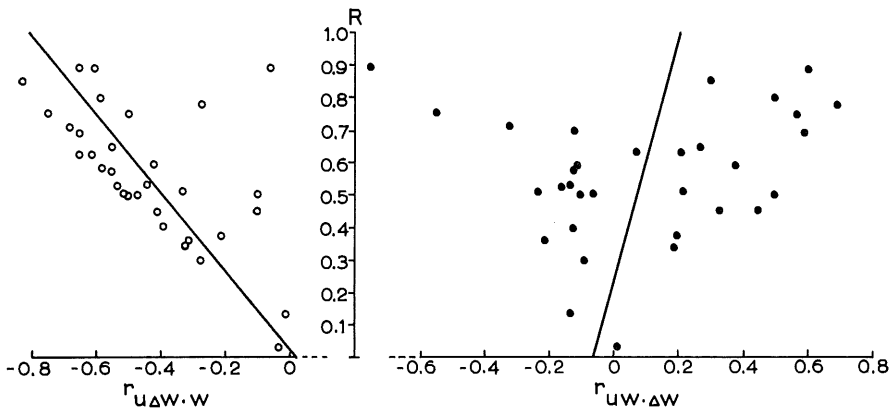
The importance of the maintenance requirement as a factor in the total protein requirement was realized due to the extensive work of Mitchell and his co-workers which also led to general acceptance of the endogenous urinary nitrogen excretion as a measure of the maintenance requirement. In the case of cattle BLAXTER & MITCHELL (1948) gave a thorough treatment of the factorial system for the evaluation of the total protein requirement. The system was found to be theoretically valid (LOFGREEN, LOOSLI & MAYNARD 1951) but it was pointed out that important sources of error were inherent in the evaluation of the maintenance factor and also in the estimation of the requirement for growth.

Determination of NPU or BV with young rats by the Mitchell method (MITCHELL 1923—4 a; MITCHELL & CARMAN 1926 a) is based on the factorial system. The factors related to the digestion of the protein source are considered in chapter VI and in the present chapter the factors related to the metabolism of the digested protein are considered.

The metabolism of protein is reflected in the urinary nitrogen excretion, maintenance and growth metabolisms being reflected by variations in the endogenous and exogenous parts of the urinary nitrogen, respectively. Only the total urinary excretion may be observed, and it is thought that the relationships between the factors influencing this excretion are more complex than the factors influencing the faecal excretion. The significant factors are believed to be the body-weight, the growth rate and probably also the age of the rats (NJAA 1959 b) as well as the protein intake, the latter being the product of the food intake and the protein content of the diet. The many possible interactions between the factors mentioned made it impossible to design experiments in which the effect of each factor might be studied separately. An attempt is made to evaluate the possible effects of other factors when the effect one of the factors is discussed.

Fig. 4.

Relationships between the partial ($r_{uw \cdot \Delta w}$ and $r_{u\Delta w \cdot w}$) and the multiple (R) correlation coefficients between urinary nitrogen excretion (U), mean body-weight (W) and growth rate ($\Delta$W) in 32 experiments with young rats.




*B. The effects of body-weight and growth rate*

The urinary nitrogen cannot any more than the faecal excretion (chapter VI B) be studied in relation to the food intake and the protein content of the diet without bringing about differences in growth-rate and body-weight between the groups of rats compared. The effect of the latter variables must, therefore, be studied first.

It was concluded previously (NJAA 1959 a and b) that the growth-rate influenced the urinary nitrogen excretion more than did the body-weight. The conclusion was based upon few experiments, and was arrived at by evaluating the partial correlation coefficients between the urinary excretion on the one hand and either the body-weight (W) or the growth-rate ($\Delta$W) on the other, and the relation of the partial correlation coefficients to the multiple correlation coefficient (R). This conclusion has now been substantiated with a total of 32 sets of values taken from my current experiments. Each set included from 11 to 24 observations on young rats which within experiments were given a diet of a constant composition at a constant daily rate. In all cases $r_{U\Delta W.W}$ had negative values, whereas $r_{UW.\Delta W}$ had positive values in 16 cases and negative in 16. The results are plotted in Fig. 4 with the multiple and the two partial correlation coefficients as coordinates. It is seen that high values of R are associated with high values of $r_{U\Delta W.W}$. Only in four cases does this rule not hold true. On the other hand, no similar trend was obvious for R and $r_{UW.\Delta W}$.

The results indicate that in the type of experiment considered the growth rate influences the urinary nitrogen excretion more than does the body-weight. This might be an effect of the body-weight varying less within an experiment than the growth-rate. In an attempt to decide whether this is the explanation of the relative importance of the two variables, results from four experiments which were also included in Fig. 4 are given in Table 12. Besides the partial correlation coefficients, the coefficients of variation for W and $\Delta$W are given. The experiments were of two general types: In Expt 6 the urinary nitrogen excretion was determined in two periods using the same rats (two samples were lost), in Expts 18 and 20 it was determined in groups of six rats at three different times using new rats each time. In Expts 3 and 4 both procedures were employed (NJAA 1959 a). In Expts 3 and 4, and 6 the variations of W and $\Delta$W as measured by the coefficients of variation were of the same magnitude, in Expt 20 $\Delta$W varied about three times as much as W and in Expt 18 about seven times as much. Even when W and $\Delta$W varied to about the same relative extent (Expts 3 and 4, and 6) $r_{UW \cdot \Delta W}$ was not consistent with respect to the sign, in contrast to $r_{U\Delta W \cdot W}$. On the other hand, the latter correlation was not any more obvious when $\Delta$W varied relatively three and seven times as much as W, than when they varied to about the same extent.

It is reasonable to assume, therefore, that there are sources of error in the determination of W and $\Delta$W which are not related to the urinary nitrogen excretion. Differences between and within experimental periods in the span of time between weighing on the one hand and the last intakes of food and water and of the last defaecation and urination on the other may be mentioned in this connexion. Likewise differences in body composition between rats may be of importance. It may be assumed that these sources of error are of less importance within an experimental period than when more than one period are involved. The correlation coefficients were, therefore, calculated for groups of 24 young rats of which groups of six were given small amino acid additions to an otherwise constant diet. Some of these experiments were described by NJAA (1961 a) (Expts 11 a and b, 14 a and b, 27 a and b) and one by NJAA (1962 b) (Expt 3/61). Because the rats were given the same amount of food within an experiment it was assumed that the growth differences were due mainly to accumulation of body-protein. The results of the calculations from the seven experiments are given in Table 12. Besides the partial correlation coefficients, the means for U, W and $\Delta$W are given with their coefficients of variation.

In all cases $r_{U\Delta W \cdot W}$ was negative, and in six of the seven cases it was significant. The magnitude of the correlation coefficient seemed to be

Table 12. *Mean values for U, W and ΔW and the partial correlations between them in current experiments.*

| Expt no | Protein source | Number of observations | U (mg N/day) | W (g) | ΔW (g/day) | $r_{U.\Delta W}$ | $r_{U\Delta W.W}$ |
|---|---|---|---|---|---|---|---|
| 3, 4 | H—m 1 (10) | 20 | 69.2 ±13.2 % | 96.5 ±17.7 % | 1.72 ±25.0 % | +0.597** | −0.866*** |
| ,, | H—m 2 (10) | 20 | 62.1 ±14.2 % | 97.3 ±17.8 % | 1.81 ±25.6 % | +0.304 | −0.830*** |
| 6 | H—m 4 (6) | 11 | 34.5 ±13.0 % | 90.5 ±18.0 % | 1.23 ±14.9 % | −0.159 | −0.527 |
| | H—m 4 (8) | 11 | 47.4 ± 8.0 % | 96.7 ±20.8 % | 1.60 ±22.2 % | −0.554 | −0.745* |
| | H—m 4 (10) | 12 | 59.7 ± 6.8 % | 103.3 ±23.1 % | 1.88 ±16.1 % | −0.206 | −0.314 |
| 18 | H—m 30 (8) | 18 | 34.6 ±17.1 % | 76.6 ±17.1 % | 0.83 ±71.1 % | +0.222 | −0.331 |
| | H—m 31 (8) | 18 | 37.3 ±18.7 % | 75.2 ±11.9 % | 0.74 ±77.2 % | −0.225 | −0.509* |
| | H—m 32 (8) | 18 | 34.5 ±18.3 % | 75.7 ±11.0 % | 0.74 ±85.1 % | −0.086 | −0.273 |
| | H—m 33 (8) | 18 | 36.2 ±18.6 % | 75.9 ± 9.9 % | 0.78 ±67.7 % | +0.449 | −0.410 |
| 20 | H—m 38 (8) | 24 | 36.4 ±19.4 % | 79.4 ± 9.2 % | 1.15 ±37.4 % | +0.270 | −0.553** |
| | H—m 39 (8) | 18 | 37.0 ±19.8 % | 79.7 ± 9.7 % | 1.24 ±28.2 % | −0.117 | −0.545* |
| | H—m 40 (8) | 18 | 38.4 ±21.9 % | 79.5 ± 9.2 % | 1.32 ±35.6 % | −0.122 | −0.645** |
| | H—m 41 (8) | 18 | 39.5 ±21.9 % | 78.9 ± 9.5 % | 1.20 ±27.5 % | +0.066 | −0.606** |
| 11a | H—m 11 (10) | 24 | 58.5 ±15.0 % | 84.4 ±14.2 % | 1.72 ±16.8 % | +0.280 | −0.439* |
| 11b | — | 24 | 45.9 ±25.2 % | 78.9 ± 6.5 % | 2.06 ±23.8 % | +0.627** | −0.804*** |
| 14a | H—m 6 (8) | 24 | 46.0 ±20.0 % | 75.0 ± 5.0 % | 1.22 ±32.2 % | +0.117 | −0.603** |
| 14b | — (8) | 24 | 48.6 ±22.0 % | 78.8 ± 7.5 % | 1.38 ±27.0 % | −0.131 | −0.642** |
| 27a | H—m 52 (8) | 24 | 35.7 ±13.2 % | 74.5 ± 7.6 % | 1.54 ±21.9 % | +0.123 | −0.391 |
| 27b | — (8) | 24 | 41.1 ±18.1 % | 74.6 ± 4.2 % | 0.95 ±37.3 % | −0.318 | −0.739*** |
| 3/61 | S—M (8) | 24 | 51.9 ±22.9 % | 79.29 ±11.0 % | 1.154 ±34.7 % | +0.509* | −0.769*** |
| | | | | 79.4 ±10.9 % | 1.12 ±37.1 % | +0.569** | −0.777*** |

U, urinary nitrogen; W, body-weight; ΔW, growth rate. * Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level. Numbers in parantheses are the protein contents of the diets.

roughly proportional to the coefficient of variation of $\Delta W$. Up to about 60 % of the variation in U was accounted for by the variation in $\Delta W$ ($100\ r^2_{U\Delta W \cdot W}$). For $r_{UW \cdot \Delta W}$ no general trend was obvious, of the seven values 5 were positive and 2 negative. However, with the exception of one experiment (Expt 11 b) $r_{UW \cdot \Delta W}$ varied roughly in parallel with the coefficient of variation of W. Usually W varied less than $\Delta W$, but in one case (Expt 11 a) the coefficients of variation were of the same magnitude. Thus, it is concluded that the variation in W observed in my current experiments, in which only one experimental period is generally employed (NJAA 1959 a), is of little importance for the variation of the urinary nitrogen excretion. The effect of variation in $\Delta W$ is clear also in these experiments, but it is not any more obvious than in the other experiments referred to in Fig. 4 and Table 12.

According to the factorial system of BLAXTER & MITCHELL (1948) the variations in U related to W and $\Delta W$ would be due to variations in the endogenous and exogenous parts of U respectively. Thus, the experiments referred to in Table 12 indicate that the variation in the endogenous part of the urinary nitrogen excretion was such that it could not be corrected for by a procedure based on the observed differences in the body-weight (MITCHELL 1923—4 a).

### *C. The accuracy of weighing rats*

The rats used in the experiments referred to in Fig. 4 and Table 12 grew at a daily rate of between 1 and 2 g, or between 5 and 12 g in the 5 or 6 day periods. Up to the time of Expt 27 a they were weighed to the nearest g at the start and at the conclusion of the experiment; in later experiments the weighings were to the nearest 0.1 g. An improved accurancy of weighing would be expected to affect mainly $\Delta W$ and only to a small extent W. This is substantiated by comparing the coefficients of variation in the two lower lines in Table 12. In the upper line the weighings were to the nearest 0.1 g, in the lower line the weighings were rounded to the nearest g. The improved accuracy of weighing did not appreciably influence the magnitude of the correlation coefficients calculated.

### *D. The effect of nitrogen intake*

The prevalent assumptions about the relationships between urinary nitrogen excretion and nitrogen intake are not so easily formulated as in the case of the faecal excretion (chapter VI). On the one hand it is assumed that BV and NPU of a protein source are independent of the food intake at a constant protein level, and that they decrease when the protein content of the diet is increased (MITCHELL 1923—4 b; BENDER 1956;

NJAA 1959 b). On the other hand it is assumed that within certain limits a constant percentage ($k_5$) of the truly absorbed nitrogen (A) is utilized for growth and maintenance in the young rat (MITCHELL 1955) and in the growing pig (ARMSTRONG & MITCHELL 1955). If the true digestibility (D) of the protein source is a constant quantity this means that a constant percentage ($k_5D/100$) of the nitrogen intake (I) is utilized.

It is generally assumed that the part of the truly absorbed nitrogen which is not utilized, is excreted in the urine together with the endogenous urinary nitrogen (U'). When the amount of truly absorbed nitrogen is A mg the total amount appearing in the urine (U mg) may thus be given by equation 7 a:

$$U = A\,(1 - k_5/100) + U' = I\,(1 - k_5/100)\,D/100 + U' \qquad (7\,a)$$

It is obvious that the terms $k_5$ and $k_5D/100$ are respectively equivalent to the terms BV and NPU (equations 2 and 4 a, p. 10 and 11).

One consequence of accepting the validity of equation 7 a within some limited range of the nitrogen intake, is that U' may be estimated by extrapolation from excretion data obtained within these limits, to zero nitrogen intake. MITCHELL (1955) and ARMSTRONG & MITCHELL (1955) estimated U' by this procedure.

Another consequence of accepting equation 7 a is that BV and NPU cannot decrease with increasing protein content of the diet within the limits where it is valid. ARMSTRONG & MITCHELL (1955) disposed of this problem by stating that the lower BVs obtained by MITCHELL (1923—4 b) and MITCHELL & BEADLES (1927) at the higher protein levels were probably determined when "current requirements" for protein were more than satisfied. They also implied that the differences observed between protein levels were not statistically significant.

Equation 7 a is assumed by MITCHELL (1955) and ARMSTRONG & MITCHELL (1955) to be valid within a more narrow range the better the quality of the protein source. However, constant BVs at different protein levels were demonstrated almost exclusively with protein sources of very high biological quality (BARNES *et al.* 1946; HENRY & KON 1952 and 1957; FORBES *et al.* 1958) and only occasionally with a protein source of poor quality (e. g. MITCHELL 1923—4 b, for potato protein). BVs and NPUs of the less good protein sources practically always decreased with increasing protein content in the diet, even when "current requirements" were obviously not fully satisfied (BARNES *et al.* 1946; FORBES *et al.* 1958; MILLER & PAYNE 1961 a).

It was pointed out previously (NJAA 1959 b) that in the few cases where equation 7 a seems to apply, the effect of the growth-rate may incidentally exactly have balanced the effect of the increased protein

content. Another important factor is that increased nitrogen intake due to increased food intake and that due to increased protein content seem to influence the urinary nitrogen excretion in opposite directions (NJAA 1959 b). According to equation 7 a written in the form of equation 7 b the value of 100 U/I varies to the same extent as a result of equivalent changes in I whether the change is brought about by changing the food intake or whether it is brought about by changing the protein content of the diet (P)

$$100\ U/I = (1-k_5/100)\ D + 100\ U'/I = (1-k_5/100)\ D + 100\ U'/EC$$
$$= (1-k_5/100)\ D + 62.5\ U'/EP \qquad (7\ b)$$

The results given in Table 9 (p. 41) do not substantiate this: The value of 100 U/I increased slightly, but insignificantly when P was changed from 6 to 8 % and from 8 to 10 % (Expts 6, 9 b and 43), but it decreased significantly when E was changed from 8.33 to 10 and from 10 to 12 g/rat/day (Expt 9 a) and from 8 to 10 g (Expts 9 b and 43). In the two latter experiments where the two ways of changing the nitrogen intake were simultaneously tested, the different effects of changing P and E on 100 U/I are clearly seen.

The results obtained in Expts 9 a and 9 b were interpreted to indicate that NPU tended to increase when the food intake was increased and to decrease when the protein content in the diet was increased (NJAA 1959 b). Very similar results were obtained in Expts 9 b and 43 and they may be similarly interpreted.

The reasoning was as follows (NJAA 1959 b): Assume that D and U' are independent of the nitrogen intake within the limits tested. From the observed values for 100 U/I it is then possible to calculate from equation 4 c (p. 25) the values of U' consistent with constant NPUs between the different levels of protein intake. The values so calculated for U' may be compared with prevalently accepted values for the endogenous nitrogen excretion. If the calculated values are decidedly higher than the normally accepted values, a relationship between U and I resulting in a smaller increase in U than expected from equation 7 a would be indicated. This in turn would indicate that NPU or $k_5D/100$ tends to increase with increasing I. Similarly, if the calculated values for U' are decidedly lower than the normally accepted values, it would indicate that NPU tends to decrease with increasing I. The former condition was found to apply when the food intake was increased, the latter when the protein content of the diet was increased (NJAA 1959 b). The results obtained at different food intakes gave values for U' ranging from 40—60 mg N/rat/day, whereas the results obtained at different protein levels gave negative values for U' (Expts 6, 9 a, 9 b and 43).

It may be seen from the data given in Table 9 (p.41) for W and $\Delta$W that 100 U/I varied in the expected direction as judged by the changes observed in $\Delta$W, whereas no consistent relationship was obvious between 100 U/I and W. Thus variations in the endogenous part of the urinary nitrogen excretion do not seem to be involved. The differing effects of changing the food intake or the protein content of the diet on 100 U/I may be explained by assuming a different effect on the growth rate of the two ways of changing the protein intake. Since equations 7 a and b apparently apply only at a low protein level when the protein content is changed and, almost exclusively, when the protein source is of very good quality, it appears that only under these conditions can a constant portion of an increased protein intake due to a change in the protein content of the diet be utilized for growth. It is inferred that when equations 7 a and b seem to apply the opposite effects on the urinary nitrogen excretion of increasing the food intake and of increasing the protein content may, for a great part, be held responsible. It is believed that the balancing effect of the two ways of changing the protein intake is mediated mainly through variations in the growth-rate. This point has also been argued previously (NJAA 1959 b).

When the protein intake becomes so high that the increment in the growth rate, or body protein gain, lags behind the intake increment, a progressively greater part of the latter is catabolized and excreted as nitrogen in the urine. When the growth rate increment becomes negligible practically the whole increment in truly absorbed nitrogen appears in the urine. This is demonstrated by the results of Forbes and co-workers (FORBES *et al.* 1935, see also NJAA 1962 a).

This is in agreement with the reasoning of ARMSTRONG & MITCHELL (1955) in the discussion of the urinary nitrogen excretion when "current requirements" have been satisfied.

Because a portion of the protein intake always seems to be utilized for growth when the amino acid composition of the protein source permits growth, the value of 100 U/I approaches, but never reaches 100 %. This point is of importance in the evaluation of the new theory for protein metabolism recently advanced by MILLER & PAYNE (1961 b). This theory requires that at some given protein level the total of the truly absorbed nitrogen is excreted in the urine. The theory is based on the assumption that NPU of a protein source always decreases linearly when P is increased and that the regression of NPU on P extrapolates to zero at the same value of P for all protein sources. The validity of this assumption was challenged by NJAA (1962 a) and by MORRISON, SABRY, GRIDGEMAN & CAMPBELL (1963).

When discussing variations in BV and NPU resulting from variations

in the urinary nitrogen excretion due to changes in the protein content of the diet, it should be noted that decreasing values may be obtained from calculations based on equations 2 (p. 10) and 4 c (p. 25) even if equation 7 a were actually valid. The decreasing values may, namely, be a result of chosing a higher value for U' when BV and NPU are calculated than the value of the constant term in the regression of U on I. This source of variation is analogous with that discussed for D in section VI H 5 (p. 55). Because U' is usually not estimated by the extrapolation procedure, this possibility must be taken into account. However, this requires a knowledge of the magnitude of the true endogenous urinary nitrogen excretion, and of the factors influencing it. An attempt at discussing these points is made in the following sections.

## *E. The endogenous urinary nitrogen*

### 1. Definition

In the present discussion the term endogenous urinary nitrogen excretion is used with the conventional meaning as introduced by MITCHELL (1923—4 a) and MITCHELL & CARMAN (1926 a) and of which BRODY (1945) gave the following definition: "Endogenous nitrogen ..... is defined, empirically, as the lowest level of nitrogen excretion attained after an empirically defined time interval on a low-nitrogen but otherwise complete diet." The definition is manifestly vague, but it seems to be the best there is. The directions on how this excretion shall be adequately measured is even vaguer: An estimate is obtained "with animals that have been so prepared that the urinary nitrogen is all of endogenous origin, including no nitrogen of immediate dietary origin nor any originating from the labile stores of protein in the body, sometimes referred to as deposit protein" (BURROUGHS *et al.* 1940).

The definition of endogenous nitrogen is discussed in relation to the discussion of the factors influencing it. In the three following sections the effect of the body-weight, the growth-rate and the food intake are examined. Then the importance of the length of the "empirically defined time interval" included in the definition referred to above (BRODY 1945), is discussed in two sections. The method of calculating the endogenous excretions from the observed excretions and body-weights are considered in six sections. On the basis of these considerations the importance of the endogenous nitrogen excretion for the variation of BV and NPU is discussed.

## 2. The effect of body-weight

When NPU and BV are calculated the endogenous urinary nitrogen excretion is considered to be proportional to the body-weight or to the latter taken to some power less than unity (MITCHELL 1923—4 a; SMUTS 1935; ASHWORTH 1935 a). The power 0.75 has been widely used by American investigators: (ALLISON *et al.* 1946 a; BRICKER & MITCHELL 1947; NASSET 1957; FORBES *et al.* 1958) but the body surface (BARNES *et al.* 1946; MITCHELL 1955) and body-weight (GOYCO & ASENJO 1947) have also been used. The use of the body-weight taken to some power less than unity as a reference basis has been rationalized by the assumption that the endogenous urinary nitrogen excretion like the basal heat production is proportional either to the body-surface (SMUTS 1935) or to the active body mass (BRODY 1945). In chosing between the body-weight and the body-surface as reference bases for the basal heat production MITCHELL & CARMAN (1926 b) preferred the latter because the ratio basal heat/body-surface had a smaller coefficient of variation than the ratio basal heat/body weight.

In Table 13 results are given calculated from 61 determinations of endogenous urinary nitrogen excretion with the low egg-protein diet at the daily rate of 10 g/rat and 19 determinations at 8 g/rat. The mean excretions are given as mg/g body-weight, mg/g (body-weight) $^{0.75}$ and mg/g (body-weight) $^{0.67}$. The latter ratio was calculated because the body-surface is often assumed to be proportional to $W^{0.67}$ (BRODY 1945). It is seen that for the 61 observations U'/W had the greatest and $U'/W^{0.67}$ the smallest coefficient of variation; for the 19 observations, those for U'/W and $U'/W^{0.75}$ were about equal whereas that for $U'/W^{0.67}$ was slightly greater.

In the lower section of Table 13 are given the correlation coefficients between the numerator and the denominator in the ratios as well as the correlation coefficient between log U' and log W. Beside the latter are given in parentheses the regression coefficients in the regression equations. These are the exponents by which W is transformed to active body mass (BRODY 1945).

The correlation coefficients related to the three ratios in the upper part of Table 13 were very similar within each set of data. On the basis of these results it is not possible to say that the endogenous urinary nitrogen excretion is more closely related to the body-weight taken to one of the chosen powers than to the body-weight itself. In relation to the problem whether the coefficients of variation of the ratios may form the basis of this choice it is pointed out that the coefficients of variation of the ratios in Table 13 were smaller the smaller the difference between the

Table 13. *Endogenous urinary nitrogen excretion in relation to body-weight or to quantities derived from it, together with correlation coefficients. Data from current experiments.*

| Number of observations | Food intake (g/day) | Means ± coefficients of variation (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | U' (mg/day) | U'/W (mg/g) | U'/$W^{0.75}$ (mg/$g^{0.75}$) | U'/$W^{0.67}$ (mg/$g^{0.67}$) | W (g) | ⊿W (g/day) |
| 61 | 10 | 19.7 ±19.2 | 0.213 ±19.7 | 0.657 ±16.2 | 0.955 ±15.3 | 95.5 ±25.8 | 0.91 ± 27.0 |
| 19 | 8 | 16.6 ±19.8 | 0.236 ±16.5 | 0.683 ±16.5 | 0.975 ±16.8 | 70.2 ±13.3 | 0.46 ±161.0 |

| | | Correlation coefficients | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total | | | | Partial | | Multiple |
| | | U'W | U'$W^{0.75}$ | U'$W^{0.67}$ | log U'log W | U'W.⊿W | U'⊿W.W | R |
| 61 | 10 | +0.610*** | +0.612*** | +0.613*** | +0.634*** (0.45)§ | +0.643*** | −0.316* | 0.675 |
| 19 | 8 | +0.569*** | +0.566*** | +0.560*** | +0.544*** (0.88) | +0.561*** | −0.733*** | 0.829 |

* Significant at the 5 % level. *** Significant at the 0.1 % level. § The numbers in parantheses are the regression coefficients in the log, log regression equations.

Table 14. *Correlation coefficients between endogenous urinary nitrogen excretion (U') and body-weight (W) on linear and on log, log bases calculated from literature data.*

| Type of diet | No. of observations | W (g) | | U' (mg/day) | | $r_{UW}$ | $r_{\log U'\log W}$ | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Mean | ± Coeff. of variation (%) | Mean | ± Coeff. of variation (%) | | | |
| | 68 | 126.6 | ±70.5 | 28.5 | ±50.8 | +0.811*** | +0.895*** | BRODY (1945 Missouri data) |
| N-free ..... | 77 | 132.2 | ±34.4 | 24.9 | ±26.0 | +0.538*** | +0.458*** | MITCHELL (1923—4 a) |
| | 140 | 75.5 | ±21.6 | 13.6 | ±18.8 | +0.627*** | +0.590*** | OLSON & PALMER (1940) |
| Low egg ... | 70 | 117.0 | ±31.3 | 24.8 | ±35.1 | +0.635*** | +0.593*** | MITCHELL & CARMAN (1926a) |
| | | | | | | ($r_{UW\cdot\Delta W}$ = | +0.537***) | |
| | | | | | | ($r_{U\Delta W\cdot W}$ = | −0.537***) | |

*** Significant at the 0.1 % level.

exponent used for W and the regression coefficient in the regression equation between log U' and log W. This is a result of the fact that the logarithmic equation is that of best fit to the data of all equations of the form $U' = kW^p$. This is, however, no proof that the relationship between U' and W is best described by an equation of this form.

The finding that no definite improvement in the correlation between U' and W was obtained either by calculating the correlation coefficients related to the ratios given in Table 13 or by calculating on the log, log basis, is in agreement with reports by NEHRING & HAESLER (1954) and BOCK (1958). Confirmation of the latter point was also obtained by calculation with several sets of values taken from the literature of which three examples are given in Table 14. BRODY (1945 p. 373) objected to the direct linear description of metabolism data (LEE 1939) because extrapolation gave a positive value for the metabolism at zero body-weight. This argument is, however, irrelevant: Although the log, log interrelationship is so chosen as to give zero for the other variable at zero body-weight, this does not warrant the conclusion that the equations are valid beyond the limits within which they were calculated. In another connexion BRODY (1945 p. 508) takes the latter view.

The results indicate that the endogenous urinary nitrogen excretion is in some way related to the body-weight. However, it does not seem possible to say with any degree of certainty that one particular reference basis should be chosen because it is biologically the most meaningful. Thus the impatience which has been expressed for instance by TREICHLER & MITCHELL (1941) who prefer to use the body-surface, or some other transformed quantity derived from the body-weight, with those authors who prefer to use body-weight itself seems unwarranted. (See also section F 1).

### 3. The combined effects of body-weight and growth rate

The partial and multiple correlation coefficients between U' on the one hand, and W and $\Delta W$ on the other for the 61 and 19 sets of values referred to above are given in Table 13. The partial correlation coefficients $r_{UW \cdot \Delta W}$ and $r_{U \Delta W \cdot W}$ were significant in both sets. This indicates that the endogenous urinary excretion varies both with the body-weight and the growth rate of the rats. Similar results were obtained with the set of values taken from MITCHELL & CARMAN (1926 a) (Table 14). Some workers did not find a significant relationship between endogenous urinary nitrogen excretion and the body-weight (CHICK *et al.* 1935, ZIMMERMANN 1952). With the data of the latter it could be demonstrated that $r_{U \Delta W \cdot W}$

was significant and of negative sign whereas $r_{UW \cdot \Delta W}$ was insignificant. The reason for the negligible effect of W as compared with that of $\Delta W$ was probably that W varied much less than $\Delta W$ (coefficients of variation about 6 % and about 36 % respectively).

## 4. The combined effects of body-weight, growth rate and food intake

In mature animals ranging from mice to cattle there seems to exist a universal relationship between the daily endogenous urinary nitrogen excretion and the body-weight. BRODY (1945) expressed this relationship by the equation $U' = 146M^{0.72}$ where U' is given in mg and the body-weight M in kg. When the body-weight W is given in g the equation becomes $U' = 1.01\ W^{0.72}$.

Although this general relationship seems to be valid for the rat it does not necessarily have any real biological significance. It was pointed out above that both my own data, and the results of NEHRING & HAESLER (1954) and BOCK (1958) indicate that there is no better correlation between U' and W in the log, log scale than in the arithmetic scale. Moreover, reported values for the coefficient of regression between U' and W on the log, log scale are not very uniform. ASHWORTH (1935 b) found the value 0.726 by calculation from data taken from Mitchell and his co-workers. OLSON & PALMER (1940) reported the value 0.531, COLUMBUS (1954) 0.601 and BOCK (1958) 0.867. The prevalent use of $W^{0.75}$ as a reference basis for the endogenous urinary nitrogen excretion seems to derive from the value of the coefficient of regression calculated by ASHWORTH (1935 b). I have calculated the coefficient of regression in the log, log scale from several sets of values taken from the literature. The values obtained ranged from 0.3 to 0.9, from my own data the values 0.45 and 0.88 were obtained (Table 13).

The great variation in the values found for the coefficient of regression between U' and W in the log, log scale may be accounted for by the fact that U' is related to $\Delta W$ as well as to W (sections 2 and 3 p. 66 and 69). Because $\Delta W$ is greatly influenced by environmental and dietary conditions, this may be reflected in great variations in the observed relationship between U' and W. An important factor influencing the growth rate is the food intake. As this varied in the literature data considered they are not in a strict sense comparable with my own results. Therefore, partial and multiple correlation coefficients between U' on the one hand and W, $\Delta W$ and the food intake E on the other were calculated for three sets of literature data (Table 15). The results calculated from the data of MITCHELL & CARMAN (1926 a) obtained with a N-free diet and from those of MIT-

Table 15. *Partial and multiple correlation coefficients between endogenous urinary nitrogen excretion on the one hand, and body-weight, growth rate and food intake on the other calculated from literature data.*

| Number of observations | Type of diet | Correlation coefficients | | | | Reference |
|---|---|---|---|---|---|---|
| | | Partial | | | Multiple R | |
| | | $r_{UW \cdot \Delta WE}$ | $r_{U\Delta W \cdot WE}$ | $r_{UE \cdot W\Delta W}$ | | |
| 58 | N-free | +0.500*** | −0.354** | +0.003 | 0.634 | MITCHELL & CARMAN (1926 a) |
| 50 | Low egg-protein | +0.386** | −0.831*** | +0.690*** | 0.830 | MITCHELL & BEADLES (1927) |
| 141 | — | +0.874*** | +0.210* | −0.319*** | 0.870 | Henry, Kon and coworkers**** |

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level. **** (BARTLETT *et al.* 1938; MACRAE *et al.* 1943; HENRY, KON & THOMPSON 1940; HENRY & KON 1946).

CHELL & BEADLES (1927) obtained with a low egg-protein diet were in accord with my own results in indicating positive partial correlation between U' and W and negative between U' and $\Delta$W. Variation in the food intake seemed to be of no importance on the N-free diet whereas on the low egg-protein diet significant positive correlation was observed between U' and E. The results calculated from the data taken from various communications of Henry & Kon and their co-workers differed from this easily comprehensible picture. Here only the positive partial correlation between U' and W remained of the above mentioned features, whereas U' and $\Delta$W were slightly positively correlated.

The following explanation is suggested: Endogenous urinary nitrogen excretion, as measured on the two types of diet used for this purpose, is generally higher at a higher body-weight than at a lower. When the measurement is uncomplicated by the intake of food protein, as it is on the N-free diet, U' is negatively correlated with $\Delta$W; that is to say, the more the rats lose in weight during the period in which the excretion is measured, the more nitrogen is excreted in the urine. On the other hand, when protein is ingested on the low egg-protein diet, increased food intake may tend to improve the utilization of the ingested protein (NJAA 1959 b), while at the same time the higher amount of absorbed nitrogen may result in a greater loss in the urine due to an increased metabolism. Conceivably the two tendencies may be of different relative importance in different experiments and the effect of the growth-rate may be obscured by the effects due to the food intake. Thus, in the experiments of

MITCHELL & BEADLES (1927) high protein intake was associated with a high urinary nitrogen loss, whereas in the experiments of Henry, Kon and co-workers the opposite was true. It is highly probably, therefore, that the great variation observed in the coefficients of regression between log U' and log W in literature data may be due to variations both in the growth rate and in the food intake. BRODY (1945 p. 443) termed endogenous urinary nitrogen excretion as determined in young rats 'apparent' because it was affected by many factors besides the body-weight. The present results emphasize this and indicate that two additional important factors are the growth rate and food intake of the rats. The latter factor is probably of importance only when the endogenous urinary nitrogen excretion is determined on a low egg-protein diet.

### 5. The effect of the time interval on low-nitrogen diet

The magnitude of the apparent endogenous excretion is highly dependent on the length of time during which the animals are given the low-nitrogen diet previous to the measurement (BRODY 1945 p. 353). The importance of the dietary history of the animals for the time required to attain endogenous (minimum) levels (ASHWORTH & BRODY 1933 a and b; ASHWORTH 1935 a; FRENCH, ROUTH & MATTILL 1941; MURLIN, EDWARDS, HAWLEY & CLARK 1946) seems to be a related problem. MITCHELL (1948 and 1955) was strongly opposed to relating the low nitrogen levels found after long continued subsistence upon nitrogen-free diets to normal maintenance requirements for protein. This view was reluctantly accepted by ASHWORTH & COWGILL (1938). However, it is not evident that the levels found after four (ASHWORTH & COWGILL 1938) or after seven days (MITCHELL 1948) are representative of normal conditions.

The data of ASHWORTH & BRODY (1933 a and b), SEEGERS (1938) and SWANSON (1959) indicated a gradual decline with time of the urinary nitrogen excretion in rats on protein-free diets; a similar gradual decline was observed in the body-weight. This suggests that the rate of weight loss during the period in which the endogenous excretion is measured is an important factor determining its magnitude. Another important factor seems to be the amount of body-nitrogen previously lost or gained.

In Expt 8 two pairs of adult rats of different dietary histories were given a protein-free diet for 16 days. One pair was taken directly from our stock colony diet (about 22 % protein), the other had been given a herring-meal diet (4 % protein) for 83 days before the experiment was started. During this time the latter pair lost about 20 % of the initial mean body-weight. The protein-free diet was given at the daily rate of

Table 16. *Expt 8. Daily urinary nitrogen excretion (U) and the corresponding change in body-weight (ΔW) in two pairs of adult rats of different dietary histories during 16 days on a protein-free diet.* (Means over pairs and mean differences (rat 1 — rat 2) within pairs).

| Day (t) | Rats from stock colony diet (Body weight 256.5 +1) | | | | Rats from 4 % herring-meal diet (Body weight 231.0 + 1) | | | |
|---|---|---|---|---|---|---|---|---|
| | U (mg) | | ΔW (g) | | U (mg) | | ΔW (g) | |
| | Mean | Difference | Mean | Difference | Mean | Difference | Mean | Difference |
| 1 | 114.0 | −24.9 | −12.5 | −3 | 34.4 | −2.4 | −2.0 | 0 |
| 2 | 64.2 | + 6.9 | − 6.0 | 0 | 28.5 | +4.3 | −2.0 | 0 |
| 3 | 46.3 | − 0.5 | − 4.5 | +1 | 27.2 | +2.4 | −1.0 | 0 |
| 4 | 52.7 | − 3.8 | − 3.5 | −1 | 28.6 | +0.6 | −1.0 | 0 |
| 5 | 53.4 | + 3.3 | − 4.0 | 0 | 27.0 | 0.0 | −1.0 | 0 |
| 6 | 48.5 | +12.9 | − 2.5 | +1 | 27.7 | +1.3 | −0.5 | +1 |
| 7 | 51.5 | + 8.8 | − 2.5 | −1 | 27.4 | +1.5 | +0.5 | −1 |
| 8 | 45.3 | + 1.1 | − 5.5 | +3 | 21.4 | −3.2 | −4.0 | −2 |
| 9 | 51.5 | + 5.4 | − 2.5 | +1 | 24.1 | −0.9 | −2.0 | 0 |
| 10 | 41.6 | + 2.6 | − 0.5 | +3 | 23.8 | −0.9 | +1.0 | +2 |
| 11 | 35.3 | + 4.2 | − 1.5 | −1 | 19.5 | −1.9 | 0.0 | 0 |
| 12 | 38.1 | 0.0 | − 3.0 | 0 | 18.9 | +0.5 | −1.5 | −1 |
| 13 | 35.4 | + 1.1 | − 1.5 | +1 | 20.5 | −0.4 | −1.5 | +1 |
| 14 | 36.1 | + 1.9 | − 2.5 | +1 | 21.0 | −0.4 | −0.5 | −1 |
| 15 | 35.9 | + 2.1 | − 2.5 | −1 | 17.2 | −1.1 | −1.0 | −2 |
| 16 | 36.5 | + 5.0 | − 2.0 | 0 | 22.4 | −1.2 | −1.0 | +1 |

$\Sigma U = 107.6\ t^{0.71}$ $\Sigma\Delta W = 12.68\ t^{0.54}$ $\Sigma U = 35.08\ t^{0.88}$ $\Sigma\Delta W = 2.11\ t^{0.75}$

(A) $\Sigma U/\Sigma\Delta W = 8.49\ t^{0.17}$ (B) $\Sigma U/\Sigma\Delta W = 16.6\ t^{0.13}$

$B/A = 1.95\ t^{-0.04}$

10 g/rat; the food offered was eaten completely. The 4 % protein rats weighed about 230 g at the start of the experiment and lost about 7 % during the 16 days. The corresponding data for the 22 % protein rats were 255 g and 22 %. In Table 16 are given the mean daily urinary nitrogen excretions and the mean weight gains for each pair, and the differences between the rats within pairs. It is seen that the responses within pairs were very similar.

The 22 % rats excreted more nitrogen in the urine than the 4 % rats throughout the experiment. This could not be accounted for by different mean body-weights: After the 6th day of the experiment the latter pair was the heavier. The relationships between on the one hand the time on the protein-free diet (t) and on the other either the cumulative excretion ($\Sigma U$) or the cumulative weight gain ($\Sigma\Delta W$) are given in the form of the log, log regressions. The correlation coefficients were about + 0.99. By

taking the ratios between the equations for $\Sigma U$ and $\Sigma \Delta W$ for each pair it is seen that the 4 % rats excreted about twice as much nitrogen per g weight loss as the 22 % rats.

## 6. Consideration of the definition of endogenous urinary nitrogen

The results in Expt 8, and those of ASHWORTH & BRODY (1933 a and b), ASHWORTH (1935 a), FRENCH *et al.* (1941) and MURLIN *et al.* (1946) all have a bearing on the definition of endogenous nitrogen. Protein-free feeding is in principle associated with loss of body protein and almost always also with loss of body-weight. The substitution of a low egg-protein diet for the protein-free diet (MITCHELL & CARMAN 1926 a) was justified by the empirically established fact that the urinary nitrogen excretions were practically equal on the two types of diet. This fact was reestablished for young rats by BRICKER & MITCHELL (1947), but they found that older rats excreted more nitrogen on the 4 % egg-protein diet than on the nitrogen-free diet. The latter finding was explained by assuming that the protein intake on the former diet had been in excess of the body needs.

In practice no distinction is made between endogenous nitrogen obtained with the two types of diet. The specifications for the determination of endogenous excretion during the use of both types are raised practically to the rank of definitions (MITCHELL 1948 p. 58; 1955 p. 194). In the same sentence MITCHELL (1955) refers to a "near nitrogen-free regime" and to results obtained with a low egg-protein diet by SMUTS (1935). Similary, SCHNEIDER (1935) and OLSON & PALMER (1940) refer to low egg-protein diets as protein-free. Conclusions are also drawn about the effects of stress conditions on the magnitude of the endogenous excretion from experiments with low egg-protein diets (TREICHLER & MITCHELL 1941; MUKHERJEE & MITCHELL 1949). No evidence is presented indicating that the quantitative responses would have been the same with the protein-free diet. In relation to the definition of endogenous excretion it should be borne in mind that the substitution of the low egg-protein diet for the protein-free diet rests on an empirically established equivalence of the excretions observed on the two types of diet under conditions which did not involve other physiological stress than lack of protein.

When a low egg-protein diet is substituted for a protein-free diet the obligatory loss of body-protein associated with the latter is either reduced or completely abolished. It is reasonable to assume that the portion of the urinary excretion associated with the loss of body-protein follows a similar trend. This inference is, however, at variance with the proposition

that the endogenous excretion is "unaffected in magnitude by subsequent feeding of dietary protein" (MITCHELL 1948 p. 56).

The dilemma encountered in discussing endogenous excretion is that the principle of its constancy and the question of measuring its magnitude are put forward as one problem: When lower excretions are observed in animals given diets containing some essential amino acids or some high quality proteins, than on nitrogen-free diets (BRUSH *et al.* 1947; MITCHELL 1948; WOMACK & MARSHALL 1955) this is taken as a challenge to the principle of the constant endogenous excretion (MITCHELL 1948, 1955). This point was discussed by ZIMMERMANN (1952).

The obvious solution to the problem is to accept that the endogenous urinary excretion associated with protein-free feeding comprises a constant part related to the body-weight and a variable part related to the loss of body-weight. This view implies that the constant part refers to the hypothetical state when no gain or loss of protein takes place, and it is conceivable that this part remains constant during periods of protein feeding (MITCHELL 1948). It is implied by MITCHELL (1948), however, that the maintenance requirement of digestible protein in terms of nitrogen is determined by the urinary nitrogen excretion on a nitrogen-free diet, attained after a short period during which the more labile stores of nitrogenous compounds are catabolized, and that this excretion remains constant during periods of protein feeding. It is difficult to imagine that the part of the endogenous excretion associated with loss of body-weight should remain constant during periods of protein feeding when growth takes place.

The quantity NPU, or BV, is intended to measure the portion of ingested, or truly digested, protein utilized in the protein requiring functions in the body. In the growing rat the important functions are generally considered to be that of hindering loss of body protein and that of allowing a positive growth rate. The former is usually termed the function of maintaining the nitrogenous integrity of the body, or more concisely, the function of maintenance. The function of maintaining a positive growth rate is termed the function of growth. The maintenance function predominates in the adult animal, in the young animal the relative importance of the two functions depend upon the rate of growth attained.

In terms of nitrogen the maintenance requirement of ingested protein is measured by the term F' + U', that of truly digestible protein by the term U'. The amount of protein utilized for growth is measured by Bal = I—F—U. The subdivision of the protein requirement into parts related to maintenance and growth forms the basis of the factorial system of estimating the protein requirement (BLAXTER & MITCHELL 1948). It is my opinion that the part of the maintenance requirement related to U'

in periods of protein feeding comprises a constant part related to the body-weight and a variable part related to the inherent tendency towards a weight loss. It seems reasonable to assume that this tendency will be greater the higher the positive growth rate which is maintened by the diet given. This view requires modification of the definition of endogenous excretion (p. 65) given by BRODY (1945): a tentative formulation of the modified definition is: Endogenous nitrogen is the nitrogen excretion which would result from substituting a protein-free diet for the diet under test at the time of the experiment.

The excretion so defined is not measureable without discontinuing the experiment. It is believed, however, that it may be estimated by the urinary nitrogen excretion in a period following immediately after the experimental period. This excretion would be related to the body-weight of the rat at the end of the experimental period, to the tendency towards loss of body-weight at this time, and to the saturation level of the body fluids with nitrogenous substances due to the protein source under test, to the dietary level at which it was tested and to the growth rate accomplished with it. The saturation level referred to is suggested to be a reflection of the nutritional and metabolic state of the rat at the time of the experiment, and to constitute an important part of the total maintenance requirements for protein of growing and adult rats.

In Expt 8 the excretion on the first day after instituting the nitrogen-free regime would probably be the best measure for the maintenance requirement as defined above if one could be reasonable confident that absorption of dietary protein was not taking place. However, whether this condition is fulfilled is not known. It is, therefore, tentatively suggested that the maintenance requirement should be estimated in a protein-free period of equal length to the experimental period and immediately succeeding the latter. The probably too high excretion on the first day would then to some extent balance the probably too low excretion of the following days associated with the fact that the protein-free regime now forms part of the dietary history of the rats. Examples of applying the procedure in experiments with young rats are given in section 11 (p. 80).

## 7. Estimation of the endogenous portion of urinary nitrogen in experimental periods

Several methods have been suggested for the estimation of the endogenous portion of the urinary nitrogen excretion observed during periods of protein feeding. Generally they are based on the assumption that the ratio $U'/W^q$ ($q \gtrless 1$) varies linearly with time between two standardizing N-free periods or low egg-protein periods, or that the ratio is a

constant for individual rats or for a group of rats. The types of method are characterized briefly below:

(A) Interpolation from ratios obtained in an initial and a final standardizing period (MITCHELL 1923—4 a; METTA & MITCHELL 1956).

(B) Extrapolation from ratios obtained in one standardizing period before (FORBES *et al.* 1958; RIPPON 1959) after (FORBES & YOHE 1954) or between (MITCHELL *et al.* 1952) the experimental periods (see also p. 44).

(C) Extrapolation from ratios obtained with littermates of the experimental rats run parallelly with these (NJAA 1959 a).

(D) Calculation from a predetermined $U'/W^q$ ratio (MITCHELL 1943 and 1948; METTA 1960).

(E) Calculation from a predetermined regression of U' on $W^q$ (COLUMBUS 1954; NEHRING & HAESLER 1954; BOCK 1958; DREYER 1959 cited by DREYER 1960).

(F) Determination of individual values for each rat without reference to the body-weight (BOAS FIXSEN 1930; CHICK *et al.* 1935).

(G) The method suggested in the last paragraph of the preceeding section.

(H) Besides these methods which are all based on determinations of endogenous excretions on protein-free diets or low egg-protein diets, indirect estimation of this excretion by an extrapolation technique assuming the validity of equation 7 a (p. 62) (MITCHELL 1955; ARMSTRONG & MITCHELL 1955) has been suggested.

The various methods are considered in the following five sections.

## 8. Interpolation between two standardizing periods (A)

This method was suggested by MITCHELL (1923—4 a) and formed part of the method for determination of BVs. The endogenous portion of the urinary nitrogen in experimental periods is estimated from the ratios U'/W in two standardizing periods and from W in the experimental periods. If the ratios are not equal in the standardizing periods the variation with time is assumed to be linear. In more recent investigations $W^{0.75}$ was used as reference basis instead of W (e.g. METTA & MITCHELL 1956).

The method was used in its original form for the calculations of values for U' used in Version A and Diet III referred to in Table 3. By reinspection of the data it was observed that in most cases the calculated values for U' in the third period of Version A were higher than the values observed in the final standardizing period (Table 17). This is obviously illogical because the rats increased gradually in weight throughout the

entire experiment. It is likely, therefore, that the method of calculating the values for U' introduced irrelevant variation into the values calculated for NPU.

It can be shown (see Appendix B) that the conditions required for the value calculated for U' in the last test period to be higher than the value observed in the final standardizing period ($U'_{fi}$) may be formulated as follows:

$$U'_p > U'_{fi} \text{ when}$$
$$(U'/W^q)_{in}/(U'/W^q)_{fi} > (p_t - 1)\,(W^q_{fi}/W^q_p) - (p - 1) \qquad (7\,c)$$

where $p_t$ is the total number of periods including the initial (in) and final (fi) standardizing periods; p is the number in the sequence of periods of the particular period in which the endogenous excretion $U'_p$ is calculated; $W_{in}$, $W_{fi}$ and $W_p$ are the mean body-weights in the periods indicated; $U'_{in}$ and $U'_{fi}$ are the observed endogenous excretions corresponding to the two former body-weights; q is equal to or less than unity.

In rats growing throughout the entire experiment (as was the case in Expt 4) the right-hand side of the inequality 7 c will always be greater than unity, which is only approached when $W_p$ approaches $W_{fi}$. Thus, one condition for $U'_p$ to be calculated to be higher than $U'_{fi}$ is that the ratio $(U'/W^q)_{in}$ is greater than $(U'/W^q)_{fi}$. This is often the case when $q = 1$ (MITCHELL & HAMILTON 1929; ASHWORTH & BRODY 1933 b). Therefore, when $U'/W^q$ decreases from the initial to the final standardizing period the ratio $(W_{fi}/W_p)^q$ is of deciding importance for whether $U'_p$ shall be calculated to be higher than $U'_{fi}$. Because the method of calculating $U'_p$ may easily produce illogical results in the last of the experimental periods, results obtained with it in the other periods may also be of doubtful significance.

It is concluded, therefore, that this method may introduce irrelevant variation into the values calculated for NPU. The case is not basically different if $W^{0.75}$ is used as reference basis instead of W (Table 17). The weakness of the method resides in the assumption on which it is tacitly based, namely that U' varies linearly with the product $W^q.p$ where p is the time in the unit of periods. It seems that this assumption has never been critically examined. The available data indicate that it is not valid.

### 9. Extrapolation from one standardizing period (B, C)

These methods are based upon the assumption that the ratio $U'/W^q$ determined in one standardizing period either for each rat (method B) or for a littermate tested in a parallel run (method C) is a constant for that rat or for that litter within the range of body-weights observed in the

Table 17. *Mean daily endogenous urinary nitrogen excretions (U') for the six rats given herring-meal no 2 in Expt 4. A, calculated by interpolation between U'/W or U'/$W^{0.75}$ ratios determined in the initial and final low egg-protein periods; B, calculated by extrapolation from U'/W or U'/$W^{0.75}$ ratios determined in the initial low egg-protein period; D, calculated by assuming that U'/W = 0.213 mg N/g body weight and U'/$W^{0.75}$ = 0.657 mg N/g (body-weight$^{0.75}$) (see Table 13).*

| Period | No. of rats | Mean body-weight (g) | U' (mg/day) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Determined | Method A | | Method B | | Method D | |
| | | | | W | $W^{0.75}$ | W | $W^{0.75}$ | W | $W^{0.75}$ |
| Initial low-egg | 12 | 72.3 | 17.4 | | | | | | |
| 2 | 6 | 96.2 | | 19.9 | 19.5 | 21.7 | 20.5 | 20.5 | 16.8 |
| 3 | 6 | 119.3 | | 24.3 | 23.4 | 30.8 | 26.9 | 25.4 | 19.9 |
| Final low-egg | 12 | 129.5 | 22.5 | | | | | | |

experiment. In Table 17 under heading B mean values of U' calculated from the ratios U'/W and U'/$W^{0.75}$ determined in the initial standardizing period of Version A are given. Values are given for the two experimental periods. In the second experimental period the mean of the calculated values is higher than the mean of the values actually determined in the final standardizing period. Thus this method (B) may also easily introduce irrelevant variation into the calculated NPUs.

In principle the same objection may be raised against method C. However, when the rats are only used in one period and they are distributed between the groups with due regard to the body-weights, the differences in body-weights within litters will most likely be of lesser magnitude than that observed in a rat used over several periods. It is likely, therefore, that the variation in NPU due to the error inherent in the estimation of U' will be less with method C than with method B.

## 10. Calculation from predetermined relationships between U' and W

These methods seem to have much to recommend them. If the predetermined U'/$W^q$ ratio or the predetermined regression is based on a sufficiently large number of observations over the range of body-weights used in the particular laboratory one may be reasonably confident that actually determined values for U' do not depart much from the calculated. However, the calculated values for U' will vary in close relation to the body-weights and the question may be raised whether it is warranted to include this variation into the variation of NPU. The coefficients of variation of published (MITCHELL 1948) and calculated values (Table 13)

of U'/$W^q$, and the published (COLUMBUS 1954; NEHRING & HAESLER 1954; BOCK 1958) and calculated correlation coefficients (Table 13—15) between U' and $W^q$ do not in fact indicate such close interrelation between the two variables as to justify this.

### 11. Estimation without reference to W (F, G)

Doubts regarding whether U' and W are significantly correlated in the adult rat occasioned BOAS FIXSEN (1930) and CHICK *et al.* (1935) to reject W as a reference basis for U' (method F). They used mean values for U' determined for each rat over several N-free periods, in the calculation of BV. Thus instead of regarding the variation in U' from period to period as due to variation in W, it was regarded as due to experimental error. It was noted (CHICK *et al.* 1935) that the observed excretions in the N-free periods tended to vary in parallel with the loss of body-weight. Because of the extensive work which would have been involved it has not been tested whether method F would tend to reduce, or to exaggerate, the variation in BV as compared with the method of Mitchell (method A) for which it was substituted.

Method G suggested in section 6 (p. 76) has in common with method F the fact that W is not taken into account when the values of U' in the experimental periods are estimated. In contrast to the assumption made in method F, U' is not regarded as a constant quantity for each rat, but as a quantity varying with a multitude of factors related to the body-weight and the dietary history of the animal at the time of the experimental period. The method has not been extensively tested, only preliminary experiments have been performed. Results obtained in two experiments (Expts 42 and 43) are given in Table 18. After the 5-day experimental period the rats were given the N-free diet for 5 days during which urine was collected. NPUs were calculated from the nitrogen balance data obtained in the experimental period and from the values for U' obtained in the N-free period. Metabolic faecal nitrogen was not determined but was assumed to be equal to 2 mg N per gram of food eaten (METTA 1960, and Tables 10 and 11). For comparison NPUs were also calculated with the values for U' obtained by method D assuming a value of 0.2 mg N/g body-weight (Table 13).

Values for 100 U/I were reported for Expt 42 by NJAA (1961 a, Table III, Expt 13) and for Expt 43 in Table 9. It is seen from the data given in Table 18 that compared to the corresponding sum of squares for 100 U/I the total sum of squares for Bal % and of $NPU_{\text{method D}}$ and the percentages of the total sum of squares ascribed to treatment and error (the part ascribed to litters is not tabulated) were of the same magnitude.

Table 18. *Examples of the use of methods D and G for the estimation of the endogenous urinary nitrogen excretion in the evaluation of NPU values.*

| | 100 F/I (1) | 100 U/I (2) | Bal % | NPU | | 100 U'/I | |
|---|---|---|---|---|---|---|---|
| | | | | Method D | Method G | Method D | Method G |
| | Expt 42 (6 rats per group) | | | | | | |
| Supplement | | | | | | | |
| gly ..... | 23.1 | 42.0 | 34.9 | 47.0 | 53.8 | 12.1 | 18.9 |
| cy. HCl ..... | 23.5 | 32.9 | 43.6 | 56.0 | 63.4 | 12.4 | 19.8 |
| cy. gluc. ..... | 23.2 | 34.0 | 42.8 | 55.0 | 64.9 | 12.2 | 22.1 |
| cy.arab. ..... | 22.2 | 37.7 | 40.1 | 52.5 | 59.6 | 12.4 | 19.5 |
| | n.s. | n.s. | n.s. | n.s. | ** | n.s. | n.s. |
| Sum of squares | | | | | | | |
| Total ..... | 29.24 | 2055.88 | 1912.58 | 1789.40 | 1347.38 | 15.87 | 392.35 |
| Treatment | | | | | | | |
| (% of total) ..... | 25.0 | 14.9 | 14.6 | 16.3 | 32.6 | 2.7 | 9.5 |
| Error (% of total) ... | 62.2 | 26.8 | 26.6 | 28.6 | 29.0 | 27.6 | 26.3 |
| $r_{12}$ {error | −0.216 | | | | | | |
| $r_{12}$ {treatment | — | | | | | | |
| | Expt 43 (8 rats per group) | | | | | | |
| Diet | | | | | | | |
| 10 % 10 g ..... | 22.4 | 43.5 | 34.1 | 44.3 | 48.9 | 10.2 | 14.8 |
| 8 % 10 g ..... | 24.5 | 41.3 | 34.2 | 46.4 | 49.1 | 12.2 | 16.8 |
| 10 % 8 g ..... | 23.8 | 51.6 | 25.6 | 37.2 | 42.4 | 11.6 | 16.8 |
| | * | *** | *** | *** | ** | *** | n.s. |
| Sum of squares | | | | | | | |
| Total ..... | 53.29 | 927.99 | 1021.49 | 1030.24 | 961.65 | 28.39 | 96.42 |
| Treatment | | | | | | | |
| (% of total) ..... | 33.6 | 41.4 | 38.4 | 36.7 | 24.6 | 62.6 | 21.9 |
| Error (% of total) ... | 40.6 | 10.4 | 15.1 | 14.4 | 17.9 | 21.9 | 53.7 |
| $r_{12}$ {error ..... | +0.266 | | | | | | |
| $r_{12}$ {treatment ..... | — | | | | | | |

Method D: The endogenous urinary nitrogen excretion was assumed to be 0.2 g/g body-weight

Method G: The endogenous urinary nitrogen excretion was determined in a nitrogen-free period immediately after the experimental period.

In both methods the metabolic faecal loss of nitrogen was assumed to be 2 mg/g food. n.s. not significant. * Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level.

This was also the case for the total and error sum of squares for $NPU_{method\ G}$ in Expt 42, whereas the treatment sum of squares was reduced. In Expt 43 the total sum of squares for $NPU_{method\ G}$ was appreciably less than for the three other measures of protein utilization, the percentage of the total sum of squares ascribed to treatment was about doubled and the percentage ascribed to error was practically unaffected. In Expt 42 there was a significant treatment effect on $NPU_{method\ G}$ but not on the three other measures considered, in Expt 43 the significance of the treatment effect was less pronounced on $NPU_{method\ G}$ than on the other measures.

The features mentioned seem to be promising for the eventual usefulness of method G for estimation of U': (1) In Expt 42 the difference was accentuated between the negative control and the other groups, and in a similar experiment with the same herring-meal (NJAA 1961 a, Table 13, Expt 14) the treatment effect was significant also when it was measured by 100 U/I. (2) The treatments tested in Expt 43 did not involve real quality differences because the same herring-meal was given without any supplements to the three groups of rats. Thus, the NPUs calculated with values for U' obtained by method G seem to indicate less effect from changing the protein intake by means of changing either the food intake or the protein content of the diet, than the NPUs calculated with values for U' obtained by method D. This result again points to the importance of the term 100 U'/I for the magnitude and the variation of NPU (NJAA 1959 b).

These results are, however, too few to permit any definite conclusion about the usefulness of method G for the estimation of U'. It is worthy of note that the results obtained in Expt 43 indicate that at the protein levels employed a change in the food intake seemed to be more important than an equivalent change in the protein content of the diet. This is in accord with previous observations, (NJAA 1959 b) but at variance with prevalent views on this point (MITCHELL 1923—4 b).

It is seen from Table 18 that although the total sum of squares was much greater for $100\ U'/I_{method\ G}$ than for $100\ U'/I_{method\ D}$ this did not reflect in a similar difference between the sums of squares for the corresponding NPU values. Thus not only the variances of the single terms but also the covariances between them, determine the variance of NPU. This point will be discussed in some detail in the general discussion of the significance of NPU as a measure of protein utilization (p. 100).

### 12. Estimation of U' by extrapolation of regressions (H)

The extrapolation technique for the estimation of U' is based on the assumption that equation 7 a (p. 62) is essentially valid. The discussion in section D (p. 61) indicates that the assumption is not tenable. It must thus be concluded that reliable estimates of U' may not be obtained by this method.

When the extrapolation technique is used an attempt is made to eliminate as far as possible the effect of variations in the body-weight between nitrogen intake levels by expressing the excretions in terms of $U/W^{0.75}$ instead of using the directly determined excretions (MITCHELL 1955; ARMSTRONG & MITCHELL 1955). However, as it must be assumed that U varies due to differences in the growth-rate as well as to differences in the body-weight, it is doubtful whether the regression equation used for extrapolation really contains a constant term (U' or $U'/W^{0.75}$) of biological significance. Also, proof is lacking for the underlying assumption that the total urinary nitrogen excretion observed at different dietary protein levels varies with $W^{0.75}$. The usual contention is that U' varies with $W^{0.75}$, but that U varies with a multitude of other factors.

## VIII. A GENERAL DISCUSSION OF THE SIGNIFICANCE OF THE BIOLOGICAL VALUE AND THE NET PROTEIN UTILIZATION AS MEASURES OF PROTEIN UTILIZATION

### *A. The assumed significance of the biological value and the net protein utilization*

The quantity 'the biological value of protein' was credited by MITCHELL (1943) with "the unique distinction, among other proposed measures of protein utilization, of possessing an absolute significance, since in itself, and apart from other similar values, it is a quantitative measure of the extent to which the digestible portion of a given source of dietary nitrogen is utilized in the animal functions to which protein alone contributes for the condition under which it was obtained".

The absolute quantitative measure of protein utilization referred to is the percentage of the truly absorbed nitrogen "that is not eliminated in the urine" (MITCHELL 1923—4 a). Because the true digestibility (D) of a protein source is assumed to be one of its characteristics (p. 52) NPU may also be considered to be an absolute measure of protein utilization (equation 4 a, p. 11). NPU thus measures the percentage of the nitrogen intake that is not eliminated in the faeces and in the urine. Under specified

conditions, it is implied, these percentages are characteristics of the protein source under test. The conditions concern the functions in which the protein is utilized: Different BVs and NPUs for the same protein source may be obtained when the function is mainly maintenance of bodyweight, than to when it includes both maintenance and growth, and to when other functions besides these are included (e. g. milk production). When BVs and NPUs of different protein sources are tabulated, the values given usually refer to maintenance and growth.

The questions at issue are whether BV and NPU can be credited with absolute or relative significance, and whether equivalent measures of protein value may be obtained by procedures less laborious than those involved when BV and NPU are determined. The discussion of these points is restricted to NPU because D is assumed to be constant for the protein sources under test.

### *B. The net protein utilization for maintenance and growth*

The values obtained for NPU with young growing rats are usually termed 'NPU for maintenance and growth'. According to prevalent assumptions (p. 25) this quantity may be expressed as the sum of two terms:

$$NPU = NPU_m + NPU_g \tag{8 a}$$

where the prefixes m and g denote maintenance and growth, respectively.

The requirement of protein for the maintenance of the nitrogenous integrity of the body tissues may be defined as the minimum amount of ingested nitrogen that will sustain nitrogen equilibrium, or maintain the animal without overall gain or loss of body protein (BARNES *et al.* 1946).

Nitrogen equilibrium is characterized by zero nitrogen balance. In terms of equation (4 d p. 25) $NPU_m$ is given by:

$$NPU_m = 0 + 100\,\frac{F' + U'}{I} = 100\,\frac{F' + U'}{EC} = 62.5\,\frac{F' + U'}{EP} \tag{8 b}$$

where F', U' and I are given in mg, E in g, C in mg N/g and P g protein/100 g.

Combination of equations 4 d, 8 a and 8 b gives:

$$NPU_g = \text{Bal } \% \tag{8 c}$$

Thus, the question whether NPU can be credited with absolute or relative significance becomes a question of which type of significance can be ascribed to $NPU_m$ and $NPU_g$ as they are defined by equations 8 b and 8 c.

### *C. The significance of net protein utilization for maintenance in nongrowing rats*

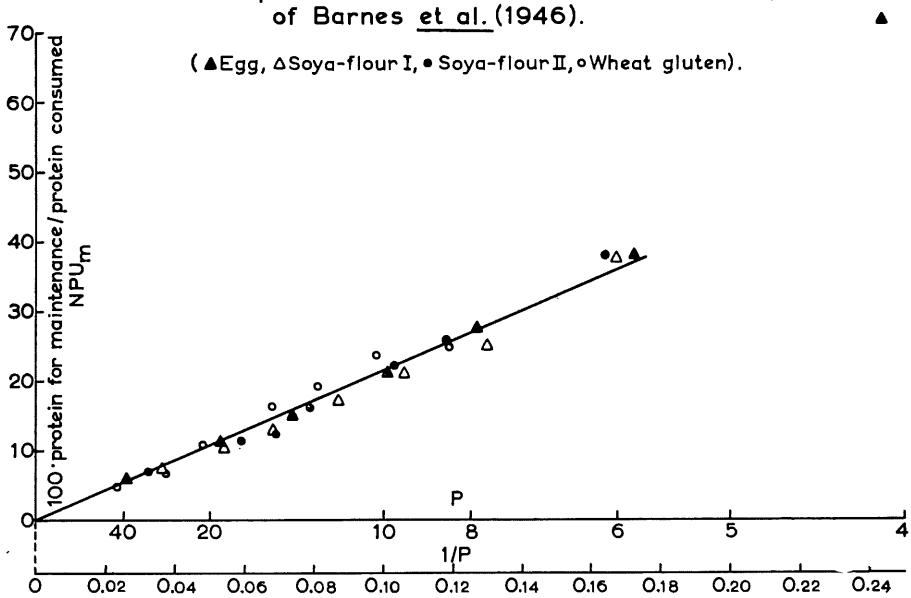
For the sake of convenience $NPU_m$ is considered for young rats of equal body-weights who consume a constant amount of food of an adequate energy content. Because maintenance alone is considered no gain or loss of body nitrogen is involved. In such rats the factors known to influence the magnitude of F' and U' are kept constant as far as possible. The two quantities may, therefore, for practical purposes be assumed to be constants. Under these conditions differences in the minimum amount of protein required for maintenance due to different protein sources must be compensated for by adjustment of the contents of nitrogen or protein in the diet. The protein content $P_m$ of a given protein source required for maintenance is obtained from equation 8 b:

$$P_m = 0.625\ C_m = \frac{1}{NPU_m}\,\frac{62.5}{E}\,(F' + U') = \frac{\text{constant}}{NPU_m} \qquad (8\ d)$$

Equation 8 d indicates that the higher the $NPU_m$, the lower is the protein content of the diet at which protein of the body is maintained without loss or gain. This is in agreement with the findings of BARNES *et al.* (1946) and MILLER & PAYNE (1961 a). It is noted, however, that the former group found less difference between the amounts of whole egg-protein and wheat gluten required for maintenance than did the latter. MILLER & PAYNE (1961 a) used an equation of the same form as equation 8 d for the estimation of the percentage of the total calories required in the form of protein calories for maintenance when the proteins derived from sources differing in their chemical scores, the latter being assumed to be equal to $NPU_m$. It was pointed out by NJAA (1962 a) that maintenance levels calculated by this equation were probably too high because the constant was chosen too high. Equation 8 d indicates that the difference between the $NPU_m$ values of two protein sources found to maintain body protein at different levels in the diet would be directly proportional to the value of the constant term. On the other hand the ratio between the two $NPU_m$ values would be independent of the constant term and equal to the inverse ratio between the corresponding values for $P_m$. Thus $NPU_m$ may primarily be credited with relative significance as long as the exact magnitude of the constant term is not known. The practical and theoretical difficulties involved in assessing correct values for F' and U' were discussed in chapters VI and VII.

It is of interest, however, to obtain information on the order of magnitude which should be ascribed to the constant term in equation 8 d. Assuming that rats weighing between 50 and 100 g will eat between 6 and 10 g food daily according to their body-weight, the discussions in chapters

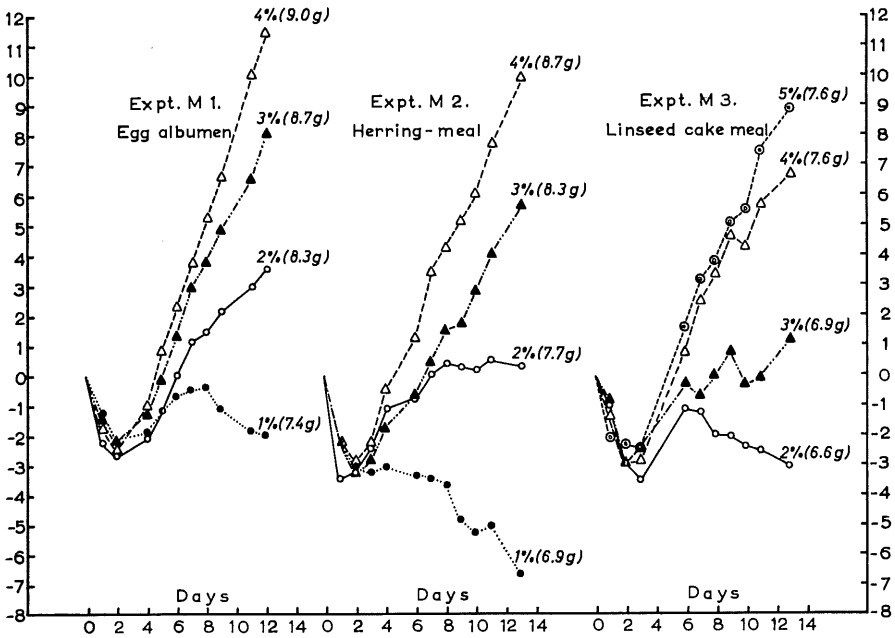
Fig.5.
Relationship between the ratio protein for maintenance / protein consumed and the protein content of the diet. Data from Table 2 of Barnes et al. (1946).




VI and VII indicate that the sum (F' + U') would probably lie between 20 and 40 mg N/rat/day and thus the constant term would be between 210 and 250. By a different approach MILLER & PAYNE (1961 a) estimated the constant term to be 400 when $P_m$ was expressed as protein calories. To obtain this value on a comparable basis with the values calcuclated above it must be multiplied by the proportionality factor relating protein content to protein calory content. Under a given set of conditions this factor was calculated to be 0.96 or higher (NJAA 1962 a). Thus, the constant term arrived at by MILLER & PAYNE (1961 a) was appreciably higher than the value calculated from assumed values of F', U' and E. BARNES *et al.* (1946) determined the maintenance requirement of protein in young rats by an extrapolation technique and checked that the values obtained agreed fairly well with values determined directly in adult rats. From their Table 2 I have calculated the part of NPU made up by $NPU_m$ by taking the ratios between the quantities 'Protein for maintenance' and 'Protein consumed'. The values obtained for the four protein sources tested are plotted against 1/P in Fig. 5. The points lie nearly on a straight line drawn through the origin and the point having the mean values of $NPU_m$ and 1/P as coordinates. The slight variation about the line may be explained by the fact that the body-weight was not constant but varied

Fig. 6.
Cumulative mean weight gains in groups of 5 or 6 young rats given diets containing low levels of protein.

(The protein sources, the protein levels and the mean daily food intakes are indicated.)




between protein sources and between protein levels. The slope of the straight line which was 212 forms an estimate of the constant term 62.5 $(F' + U')/E$ in equation 8 d. The observation at 4.1 % whole egg protein was left out of consideration because it seemed to be significantly divergent.

Maintenance protein levels calculated from equation 8d are directly proportional to the value adopted for the constant term. Indications about the magnitude of the former may, therefore, be obtained by determining the latter if the value of $NPU_m$ is known. If it is accepted that the chemical score of a protein source is an estimate of $NPU_m$ (MILLER & PAYNE 1961 a) determined values for $P_m$ may be compared with values calculated with assumed values for the constant term. Approximate values for $P_m$ were determined for egg albumen, herring meal and linseed cake meal in Expts $M_1$—$M_3$. In Fig. 6 the cumulative mean weight gains in the three experiments are plotted, together with the values for the mean daily food intakes. From a consideration of the plotted gains the approximate maintenance levels were assumed to be about 1.5, 2 and 3 % for egg albumen, herring-meal and linseed cake meal, respectively.

Using the values 400 (MILLER & PAYNE 1961 a) 210—250 (p. 86) and 212 (p. 87) for the constant term and 100, 90 and 70 for the chemical scores of the three protein sources the calculated values for $P_m$ are: 4, 4.4 and 5.7 %; 2.1—2.5, 2.3—2.8 and 3.0—3.6 %; and 2.1, 2.4 and 3 %. The chemical scores were calculated relative to the FAO (1957) provisional amino acid pattern with amino acid analyses taken from the FAO (1957) publication in the case of egg albumen, from BOGE (1960) in the case of herring-meal and from SIEMERMANN (1959) in the case of linseed cake meal. Methionine plus cystine, not tryptophane, was assumed to be the limiting entity in herring-meal (NJAA 1961 a). It is evident that the approximately determined values for $P_m$ are in better accord with those calculated with low values for the constant term in equation 8 d than with those calculated from the high values. The indications are that for protein sources with relative high chemical scores even the lower values for the constant term may be too high.

*D. The significance of net protein utilization for maintenance in growing rats*

The quantity $NPU_m$ in equation 8 a is not identical with $NPU_m$ determined under maintenance conditions except when Bal % is zero. $NPU_m$ in the growing rat is the amount of ideally composed protein ($NPU_m = 100$ at maintenance) required for maintenance expressed as a percentage of the protein intake during the particular experiment considered. Provided the maintenance requirement of ideally composed protein is essentially constant for rats of equal body-weights and independent of the amount and quality of the protein ingested, $NPU_m$ in equation 8 a decreases exponentially with increasing protein intake and along the same curve regardless of the type of protein given. In the Mitchell method for estimation of BV and NPU these conditions are accepted. The view that the maintenance and growth requirements may be considered as independent entities also forms the basis for the factorial system for calculation of the protein requirement (BLAXTER & MITCHELL 1948). Thus NPU as determined by the Mitchell method contains a term which decreases with the protein intake and which effects a tendency for NPU to decrease with increasing protein intake (BARNES *et al.* 1946).

It has been claimed that protein feeding may depress the endogenous protein metabolism and thus reduce the maintenance requirement under such conditions (p. 12). Probably the findings thus interpreted may be explained by a reduction of the loss of body protein inherent in periods of protein-free feeding. This aspect is discussed in section VII E 6 (p. 74). MITCHELL (1948) pointed out that the proposition that the endogenous protein metabolism is unaffected by protein feeding is difficult to prove

though readily conceivable. In discussions of this problem one possibility seems to have been completely ignored, namely that the maintenance requirement may be greater in a growing animal than in one just maintaining its body-weight, and greater in a fast growing animal than in a slow growing one. Synthesis of body protein must be considered a continuous process and it would seem likely that a high growth rate would require a higher metabolic activity and a greater metabolic pool of amino acids than a low growth rate. This proposition is also difficult to prove but the fact that the endogenous urinary nitrogen excretion tends to be higher when it is determined subsequent to a period of feeding a protein of high biological value than when it is determined after feeding a protein of low value (MITCHELL 1948) may be interpreted in favour of the proposition. The suggestion in section VII E 6 that the endogenous urinary nitrogen excretion should be estimated in a period after the experimental period is a consequence of this view. If the proposition is accepted that the maintenance requirement varies in magnitude in accordance with the performance of the animal, at least a partial explanation may be given for the fact that NPU usually decreases with increasing protein content in the diet even when the growth performance of the animal increases with the protein content: Part of the seemingly less economic utilization of the ingested protein at the higher levels of intake may be regarded to be due to the necessity of maintaining a higher metabolic activity the higher the growth rate. Conclusions on this question cannot be drawn on the basis of our present knowledge but must await the accumulation of more precise information about the maintenance requirement of the growing animal.

The fact that NPU calculated according to the Mitchell method contains a term related to a constant maintenance requirement has a practical bearing on the problem of the significance of differences between NPUs of different protein sources. When NPUs are compared at intendedly equal, but actually different protein levels the magnitude of the resulting differences in $NPU_m$ will depend upon the protein level. Thus, assuming the maintenance requirement of a rat weighing 100 g and eating 10 g food/day to be 40 mg N or 250 mg protein/day the rate of change in $NPU_m$ will be given by $-250/P^2$. The reasoning here is the same as that in section VI H 4 (p. 54). At the 10 % protein level the rate of change in $NPU_m$ would be about 2.5 percentage units per unit change in P and at the 8 % level about 3.9 percentage units. In section VI E (p. 39) it was mentioned that in 50 diets intended to contain 8 % protein the actual protein content varied between 7.25 and 8.27 %. Thus, in the most unfortunate case a difference between the protein contents of two diets might be about 1 percentage unit and the corresponding maximum

difference between NPUs due to difference in $NPU_m$ about 4 percentage units. It is doubtful whether any biological significance should be ascribed to a difference in NPU due to small differences in the actual protein contents of the diets used. It seems, therefore, to be preferable to standardize the conditions as far as possible as regards the age, body-weight and the food intake of the rats, as well as regards the protein content of the diets used and to base comparisons between protein sources on the observed differences in Bal % or $NPU_g$ (equations 8 a, 8 c). Such comparisons would neglect the differences due to actually different maintenance requirements between groups of rats but this error is probably small compared to the differences which may arise from erroneous estimation of this requirement and from unintended differences between the protein contents of the diets used.

### *E. The significance of the percentage nitrogen balance*

Bal % is an absolute measure of the percentage of the ingested nitrogen that is not excreted in the faeces and the urine. Generally other losses of nitrogen, e.g. through sweat and hair loss, are not considered in nitrogen balance methods. By the reasoning leading to the formulation of equations 8 a and 8 c (p. 84) Bal % is the percentage of the ingested nitrogen available for increase of body-protein. Between treatments within an experiment of the type generally employed, Bal % and the daily weight-gain ($\Delta$W) usually vary in parallel (Table 19, Expts 12, 15 d), when Bal % varies but little the same is true for $\Delta$W (Table 19, Expts 18 a, b, c). Between experiments the relationship between Bal % and $\Delta$W is not always evident: In 8 current experiments data were obtained for 27 herring-meals given at the 8 % level and at the daily rate of 10 g food/rat. Bal % varied between 31 and 45 %, $\Delta$W between 0.7 and 1.6 g/rat/day. The partial correlation coefficient $r_{Bal\,\%\,\Delta W \cdot W}$ was only + 0.42, which is just on the borderline of significance at the 5 % level. Examples of disagreement between Bal % and $\Delta$W are given in Expts 18 a, b, c (Table 19) and in Expts 9 b and 43 (Table 9 (p. 41)): Whereas Expts 18 a, b and 9 b gave values for $\Delta$W of a magnitude normally corresponding to the Bal % values observed, this was not so in Expts 18 c and 43 where relatively high values for Bal % were associated with low rates of growth.

The latter observations are difficult to explain. Several investigators have reported from experiments with various animal species that a positive nitrogen balance is not always accompanied by a corresponding increase in the body-weight (COSTA 1960) (See also p. 102). Whether these observations bear on the results in Expts 18 c and 43 cannot be decided on. It is preferred, however, to regard Bal % as the percentage of the

Table 19. *Body-weight (W), growth rate (ΔW), nitrogen balance (Bal %) and apparent urinary recovery of ingested nitrogen (100 U/I) in five current experiments.* (Means of 5 (Expt 12) or 6 (Expts 15 d, 18 a, b, c) rats).

| Expt no | Protein source | Bal % | 100 U/I | W (g) | ΔW (g/day) |
|---|---|---|---|---|---|
| 12 | H—m 7 | 37.4 | 39.8 | 77.0 | 1.2 |
| | H—m 15 | 37.9 | 40.6 | 76.0 | 1.2 |
| | H—m 16 | 49.0 | 35.0 | 79.2 | 1.6 |
| | E—a | 62.2 | 19.2 | 82.2 | 2.0 |
| 15 d | H—m 19 | 34.8 | 42.0 | 76.0 | 1.2 |
| | H—m 20 | 37.4 | 37.6 | 75.5 | 1.1 |
| | H—m 21 | 45.1 | 33.0 | 78.2 | 1.4 |
| | E—a | 64.3 | 18.3 | 84.1 | 1.7 |
| 18 a | H—m 30 | 42.6 | 34.8 | 71.2 | 1.2 |
| | H—m 31 | 38.1 | 40.7 | 69.5 | 0.9 |
| | H—m 32 | 41.7 | 38.4 | 71.1 | 1.1 |
| | H—m 33 | 42.4 | 39.2 | 71.4 | 1.1 |
| 18 b | H—m 30 | 50.2 | 28.6 | 76.1 | 1.6 |
| | H—m 31 | 47.9 | 29.5 | 74.8 | 1.5 |
| | H—m 32 | 49.4 | 27.3 | 74.7 | 1.5 |
| | H—m 33 | 50.1 | 29.3 | 76.6 | 1.5 |
| 18 c | H—m 30 | 43.2 | 33.7 | 82.3 | 0.2 |
| | H—m 31 | 43.3 | 35.3 | 80.1 | 0.2 |
| | H—m 32 | 43.5 | 33.4 | 81.3 | 0.1 |
| | H—m 33 | 44.9 | 34.0 | 79.7 | 0.2 |

ingested nitrogen which potentially may be utilized for growth. It is implied that Bal % is not always associated with growth in a measureable way, but it is believed that Bal % measures the fraction of the ingested nitrogen which is used by the animal for synthetic purposes not associated with maintenance. If these purposes are termed growth, equation 8 c states that Bal % measures the net protein utilization for growth.

### *F. The effects of food intake and protein content of diet on the percentage nitrogen balance*

In Table 9 (p. 41) the values for Bal % obtained at the food intakes and protein levels tested in Expts 6, 9 a, 9 b and 43 are given.

Between the daily rates of 8 and 12 g food/rat at the 10 % protein level Bal % increased significantly when the food intake was increased, the effect being mediated mainly through the term 100 U/I (equation

5 b, p. 25). A parallel trend was observed for $\Delta W$, but because these values are not related to the same protein intake they cannot be used directly as a measure of protein utilization. However, by dividing $\Delta W$ by E a measure equivalent with PER/10 is obtained. Beginning with the lowest food intakes these values were 0.10, 0.14 and 0.18 in Expts 9 a, 0.15 and 0.22 in Expt 9 b and 0.0 and 0.08 in Expt 43, the calculations being based on the means over periods. Thus, the two absolute measures, Bal % and $\Delta W/E$ indicated better protein utilization for growth at the higher food intakes than at the lower. If the correction term 100 (F' + U')/I were applied for the calculation of NPU (equation 4 d) the effect of the food intake on the protein utilization would be obscured: by proper choice of the values for F' and U' NPU values indicating approximately constant protein utilization independent of the food intake may be obtained. If it is accepted that NPU is independent of the food intake this seems to imply that the efficiency of protein utilization is the same for maintenance and growth (p. 96).

Between the 8 and 10 % protein levels Bal % was not significantly different (Expts 6, 9 b and 43) whereas a lower value was found at the 6 % level (Expt 6). In the latter case the effect was mediated through the term 100 F/I (equation 5 b, p. 25). A measure equivalent to PER/10 was calculated by dividing $\Delta W$ by P. Beginning with the lowest protein contents these values were 0.23, 0.25 and 0.22 in Expt 6, 0.23 and 0.22 in Expt 9 b, and 0.09 and 0.08 in Expt 43. Although Bal % and PER/10 were not completely in accord they both indicated that the protein utilization for growth was influenced only to a minor extent by the changes in the protein content of the diet.

Thus, under the standardized conditions chosen the protein utilization for growth as measured by Bal % was influenced more by changes in the food intake than by corresponding changes in the protein content of the diet. This is of importance when the merits of Bal % as a practical measure of protein utilization are considered.

### *G. The percentage nitrogen balance as a measure of protein utilization*

In view of the fact that Bal % may be regarded as an absolute measure of protein utilization for growth the suitability of this measure for practical purposes may be considered. The similarity of Bal % and PER was indicated in the preceding section. It would be expected that Bal % like PER (BARNES *et al.* 1945) would first increase up to a maximum value and then decrease with increasing protein content in the diet, when the latter is varied within wider limits than those tested in Expts 6, 9 b and 43 (Table 9 (p. 41)). This expectation was substantiated by the results of

HAMILTON (1939) obtained with diets containing from 4 to 54 % whole egg-protein: the maximum value for Bal % was at about 16 % protein. Similarly, the results of FORBES, VORIS, BRATZLER & WAINIO (1938) obtained with casein diets containing from 10 to 45 % protein indicated a maximum value of Bal % at about 10 % protein. In view of this Bal % like PER, ought to be determined at a constant protein content when comparisons are made between protein sources. In this respect I disagree with the conclusion of BARNES *et al.* (1945) who preferred to determine the maximum PER for each protein source. The results given in Table 9 indicate that 8 and 10 % protein are not far from the content giving the maximum Bal % value in the case of herring meal. In most experiments comparisons between protein sources were made at one of these protein levels.

It was pointed out above that in common with PER (MITCHELL 1944; BENDER & DOELL 1957) Bal % varied significantly with the food intake. Therefore, comparisons between protein sources based on Bal % should be made at a constant food intake. In my experiments 10 g food/day were usually taken by rats weighing from about 60 to about 100 g, but occasionally only 8 g were taken.

The most common objection to PER as a measure of protein utilization is that no allowance is made for the maintenance requirement (MITCHELL 1944; BENDER & DOELL 1957). It was argued by MITCHELL (1944) that the use of PER only credited dietary protein with the growth induced, and implied that there was no requirement of protein for the mere maintenance of life. The latter argument is considered as a provocation in a current discussion which would hardly be defended by anybody today. Therefore, it is not discussed here. The first point must, however, be examined closer because the same argument may be used against Bal % as a measure of protein utilization.

Be it accepted that the purpose of measuring protein utilization is to compare the protein sources under test, the maintenance requirement will largely cancel out of the differences when the experimental conditions are standardized as regards the protein content of the diets, the food intake and the body-weight of the rats (p. 90). In short term experiments of the type used when Bal % is determined, differences in the protein quality does not produce body-weight differences of a great magnitude, and differences in the maintenance requirement due to the small body-weight differences will probably lie well within the error inherent in its estimation. It is likely that differences in protein utilization measured by differences in Bal % will be slightly less than the corresponding NPU differences because the better protein source will always be associated with a slightly higher mean body-weight than the poorer source. On the

other hand, when the conditions of the measurements are not strictly standardized errors in the estimation of the maintenance requirement may result in NPU differences which may be either higher or lower than the corresponding Bal % differences.

In Table 20 four examples of Bal % and NPU differences between two protein sources are given. The differences between herring-meal 1 and 2 were determined by NJAA (1959 a) with two versions (A and B) of the Mitchell method. Version A was the original method (MITCHELL 1923—4 a; MITCHELL & CARMAN 1926 a) with the modification that the food intake was kept constant between rats and between experimental periods. Version B was a shortened Mitchell method in which the rats were used in only one period so that the body-weight changed but little. The food intake was kept constant. $\Delta$ Bal % was found to be slightly less than $\Delta$NPU by both versions. The differences between diets II and III (MACRAE *et al.* 1943) and between grass meals H and S (BARTLETT *et al.* 1938) were determined by the original Mitchell method in which the food intake and the body-weight of the rats changed between treatments and between periods. In both cases $\Delta$NPU was slightly less than $\Delta$ Bal %.

The important feature in these comparisons is that quality differences between protein sources seem to be as accurately demonstrated by $\Delta$ Bal % as by $\Delta$ NPU. The fact that differences estimated by the two measures may differ by about one percentage unit is of doubtful biological significance in view of the difficulties involved in the exact assessment of the maintenance requirement (p. 86). It is concluded, therefore, that under standardized conditions, differences in protein utilization may be adequately measured by $\Delta$ Bal %. Because the maintenance requirement is eliminated as a source of error, less effect due to small variations in the protein contents of the diets compared may be expected than in the case of NPU (p. 90). The objection raised against PER and which may also be raised against Bal %, that no allowance is made for the maintenance requirement, seems to be of little weight when the conditions of the measurement are standardized. Bal % may then be given the significance of measuring the protein utilization for maintenance and growth insofar as the maintenance requirement is always satisfied in growing rats. Therefore differences in protein quality between protein sources may only manifest themselves in the differences in the positive nitrogen balances induced, or in the corresponding differences in growth rates.

When differences involving negative balances are considered the significance is more difficult to interpret and it may be questioned whether comparisons between negative and positive balances are permissible. This problem is not solved by adding an estimate of the maintenance requirement to the negative balance because the directly determined

Table 20. *Comparisons between two protein sources by the quantities BV, NPU, Bal %, 100 U/I and 100 F/I. Data from own experiments and from the literature.* (Means over protein sources and differences between them in the order mentioned below).

| | Njaa (1959 a) Version A | | | Njaa (1959 a) Version B | | | Macrae *et al.* (1943) | | | Bartlett *et al.* (1938) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Herring meals nos. 1 and 2 | | | | | | RAF diets II and III | | | Grass meals H and S | | |
| | Mean | Difference | S.E. (5 df) | Mean | Difference | S.E. (6 df) | Mean | Difference | S.E. (11 df) | Mean | Difference | S.E. (11 df) |
| BV .... | 70.9 | +4.9* | ±1.76 | 69.2 | +3.5 | ±1.56 | 79.2 | +3.3* | ±1.23 | 59.5 | +15.4*** | ±2.63 |
| NPU ... | 66.2 | +5.3* | ±2.10 | 64.7 | +3.0 | ±1.31 | 69.0 | +3.3 | ±1.83 | 38.8 | +10.8*** | ±2.23 |
| Bal % .. | 40.4 | +4.7* | ±2.01 | 41.0 | +2.2 | ±1.21 | 37.4 | +3.6 | ±2.21 | 4.9 | +11.9*** | ±2.69 |
| 100 U/I | 41.4 | −3.7* | ±1.40 | 41.2 | −3.0 | ±1.45 | 31.0 | −3.5* | ±1.24 | 43.3 | −10.1** | ±2.30 |
| 100 F/I | 18.2 | −0.9 | ±0.78 | 17.8 | +0.9 | ±0.42 | 31.6 | −0.1 | ±1.53 | 51.9 | − 1.8 | ±1.55 |

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level.

balance still forms the basis for the comparison. The result taken from BARTLETT *et al.* (1938) involved a negative and a positive nitrogen balance (Table 20). For this comparison between them to be strictly valid it must be assumed that the efficiencies of utilization for maintenance and growth are equal. It is not known whether this assumption is correct, but it is inherently accepted when it is assumed that NPU is independent of the food intake (p. 61). MILLER & PAYNE (1961 b) also assumed equal efficiency of utilization of the protein used for maintenance and of that used for growth when they developed a new theory for protein metabolism. It is suggested in section D that the maintenance requirement is higher the higher the growth rate of the experimental rats. If this is accepted the concept of equal efficiency of utilization in maintenance and growth can hardly be maintained.

### *H. The apparent urinary recovery of ingested nitrogen as a measure of protein utilization*

In Table 20 the two terms constituting Bal %, namely 100 F/I and 100 U/I (equation 5 b, p. 25) are given. It is noted that in the four examples tabulated $\Delta$ 100 F/I was not significantly different from zero. The term 100 U/I, which is the percentage apparent urinary recovery of ingested nitrogen, may therefore be used to estimate $\Delta$ Bal %. In three of the four cases $\Delta$ 100 U/I gave the same information about the significance of the differences in protein utilization as did $\Delta$ Bal % and $\Delta$ NPU; in the fourth case the standard error was so much lower for $\Delta$ 100 U/I than for $\Delta$ Bal % and $\Delta$ NPU as to indicate that the difference was significantly different from zero. The fact that $\Delta$ 100 U/I was slightly lower than $\Delta$ Bal % in three cases and slightly higher than $\Delta$ Bal % in one is considered to be biologically insignificant in view of the fact that $\Delta$ 100 F/I was not significantly different from zero in any of the cases. It is concluded, therefore, that when the apparent digestibility is not significantly different between protein sources under the standardized conditions used, differences in the protein utilization may be as accurately determined with $\Delta$ 100 U/I as a measure as with $\Delta$ Bal %. Examples of this are given in Tables 18 (Expt 43) and 21. It was pointed out previously (NJAA 1962 a) that with the type of protein used the term 100 U/I had a minimum value at a protein content of the diet not far from 8—10 %. Small variations in the protein content of the type mentioned on p. 55 have, therefore, probably little effect on the magnitude of 100 U/I (Table 9).

It may be seen from Tables 18 (Expt 42) and 21 that even when there is an obvious treatment effect on the term 100 F/I, differences in the protein utilization between different sources can usually be detected by

Table 21. *Nitrogen balance and apparent faecal and urinary recoveries of ingested nitrogen in current experiments. Relationships between the sums of squares of the three variables.*

| Expt no | | | | | | Total sum of squares | Sum of squares % of total | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Groups | Error |
| | | H—m 7 | H—m 15 | H—m 16 | E—a | | | |
| | 100 F/I | 22.8 | 21.4 | 16.0 | 18.6*** | 207.16 | 66.5 | 19.6 |
| 12 | 100 U/I | 39.8 | 40.6 | 35.0 | 19.2*** | 1668.59 | 88.7 | 4.5 |
| (5) | Bal % | 37.4 | 37.9 | 49.0 | 62.2*** | 2245.05 | 91.5 | 6.7 |
| | $r_{12}$ | | | | | | | +0.316 |
| | | H—m 7+M | H—m 7—M | H—m11+M | H—m 11—M | | | |
| | 100 F/I | 22.6 | 23.0 | 23.5 | 22.7 n.s. | 43.48 | 6.7 | 59.2 |
| 13a | 100 U/I | 22.8 | 35.9 | 24.8 | 37.0*** | 1118.68 | 87.1 | 7.1 |
| (6) | Bal % | 54.7 | 41.2 | 51.7 | 40.4*** | 1116.63 | 83.8 | 12.0 |
| | $r_{12}$ | | | | | | | +0.381 |
| | | H—m 7+M | H—m 7—M | H—m11+M | H—m 11—M | | | |
| | 100 F/I | 23.5 | 22.0 | 24.3 | 25.0* | 102.03 | 29.9 | 44.5 |
| 13b | 100 U/I | 31.1 | 47.4 | 30.0 | 44.9*** | 1844.94 | 80.5 | 4.4 |
| (6) | Bal % | 45.4 | 30.6 | 45.8 | 30.1*** | 1769.01 | 78.9 | 8.4 |
| | $r_{12}$ | | | | | | | +0.171 |
| | | Gly | Gly+Lys | Gly+M | Lys+M | | | |
| | 100 F/I | 19.6 | 19.3 | 19.5 | 20.4 n.s. | 74.09 | 5.5 | 35.2 |
| 14a | 100 U/I | 41.2 | 45.1 | 32.4 | 30.9*** | 1285.64 | 66.0 | 9.1 |
| (6) | Bal % | 39.3 | 35.5 | 48.1 | 48.7*** | 1287.32 | 60.6 | 13.3 |
| | $r_{12}$ | | | | | | | +0.259 |
| | | Gly | Gly+Val | Gly+M | Val+M | | | |
| | 100 F/I | 20.4 | 20.0 | 20.4 | 19.9 n.s. | 107.62 | 1.1 | 18.2 |
| 14b | 100 U/I | 45.8 | 44.5 | 32.2 | 32.8*** | 1688.44 | 57.4 | 8.0 |
| (6) | Bal % | 33.8 | 35.5 | 47.4 | 47.1*** | 1584.00 | 60.9 | 12.2 |
| | $r_{12}$ | | | | | | | +0.381 |
| | | H—m 20 | H—m 18 | H—m 19 | E—a | | | |
| | 100 F/I | 22.4 | 22.3 | 23.7 | 16.6*** | 253.22 | 70.6 | 19.4 |
| 15a | 100 U/I | 39.9 | 40.2 | 38.3 | 19.9*** | 2037.60 | 84.6 | 6.1 |
| (6) | Bal % | 37.7 | 37.5 | 38.0 | 63.4*** | 3337.76 | 89.0 | 4.2 |
| | $r_{12}$ | | | | | | | −0.221 |
| | | H—m 20 | H—m 22 | H—m 23 | E—a | | | |
| | 100 F/I | 23.5 | 21.1 | 23.5 | 17.2*** | 202.51 | 77.0 | 12.3 |
| 15c | 100 U/I | 35.9 | 35.5 | 31.2 | 16.0*** | 2003.81 | 78.7 | 11.7 |
| (6) | Bal % | 40.7 | 43.5 | 44.9 | 66.8*** | 3182.66 | 83.3 | 10.6 |
| | $r_{12}$ | | | | | | | +0.504 |
| | | H—m 19 | H—m 20 | H—m 21 | E—a | | | |
| | 100 F/I | 23.4 | 25.0 | 21.9 | 17.4*** | 265.04 | 71.8 | 21.7 |
| 15d | 100 U/I | 42.0 | 37.6 | 33.0 | 18.3*** | 2312.45 | 82.4 | 5.7 |
| (6) | Bal % | 34.8 | 37.4 | 45.1 | 64.3*** | 3769.11 | 79.6 | 9.4 |
| | $r_{12}$ | | | | | | | +0.947 |

Table 21. Cont.

| Expt no | | | | | | Total sum of squares | Sum of squares % of total Groups | Error |
|---|---|---|---|---|---|---|---|---|
| | | H—m 52 | H—m 53 | F—m 54 | F—m 55 | | | |
| | 100 F/I | 24.3 | 22.3 | 22.9 | 28.0*** | 153.32 | 64.4 | 16.6 |
| 23 | 100 U/I | 36.7 | 40.4 | 30.7 | 34.2** | 548.55 | 46.1 | 19.8 |
| (5) | Bal % | 39.0 | 37.3 | 46.5 | 37.8*** | 553.16 | 50.6 | 14.0 |
| | $r_{12}$ | | | | | | | −0.543 |
| | | 0 % M | 1 % M | 2 % M | 3 % M | | | |
| | 100 F/I | 22.4 | 22.6 | 20.9 | 21.9 n.s. | 37.44 | 27.6 | 44.4 |
| 2/61 | 100 U/I | 51.3 | 40.8 | 34.1 | 34.8*** | 2070.74 | 55.0 | 7.2 |
| (6) | Bal % | 26.3 | 36.6 | 45.0 | 43.3*** | 2245.65 | 57.3 | 6.1 |
| | $r_{12}$ | | | | | | | −0.279 |
| | | M | MSO | M+MSO | Gly | | | |
| | 100 F/I | 21.3 | 22.4 | 22.5 | 23.1** | 88.44 | 12.1 | 7.8 |
| 3/61 | 100 U/I | 32.3 | 38.7 | 34.6 | 53.2*** | 1901.05 | 83.6 | 3.8 |
| (6) | Bal % | 46.4 | 38.9 | 42.9 | 23.6*** | 2371.90 | 76.5 | 3.4 |
| | $r_{12}$ | | | | | | | −0.120 |
| | | DLM | LMSO | DLMSO | Gly | | | |
| | 100 F/I | 24.3 | 27.1 | 23.7 | 25.9* | 160.00 | 28.0 | 38.9 |
| 5/61 | 100 U/I | 38.4 | 36.5 | 43.1 | 55.8*** | 1574.26 | 86.6 | 5.9 |
| (6) | Bal % | 37.3 | 36.4 | 33.2 | 18.2*** | 1661.12 | 85.4 | 5.7 |
| | $r_{12}$ | | | | | | | −0.421 |
| | | DLM | LMSO | DLMSO | Gly | | | |
| | 100 F/I | 19.9 | 20.7 | 20.8 | 21.6* | 32.48 | 33.0 | 35.5 |
| 6/61 | 100 U/I | 29.7 | 27.8 | 32.8 | 44.4*** | 1323.08 | 75.4 | 5.9 |
| (6) | Bal % | 50.5 | 51.4 | 46.3 | 34.0*** | 1455.80 | 79.7 | 6.1 |
| | $r_{12}$ | | | | | | | −0.016 |

n.s. = not significant. * Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0.1 % level. Significance levels refer to group or treatment effect. The numbers in parentheses are the number of animals per treatment group used in the calculations.

The protein sources or the amino acid supplements are indicated for each experiment.

differences in the term 100 U/I. It is evident that unless there is no significant treatment effect on the term 100 F/I, can 100 U/I be used as an estimate of the corresponding values for $\Delta$ Bal % and $\Delta$ NPU. NJAA (1961 a and 1962 b) used 100 U/I as a measure of protein utilization in experiments with diets supplemented with small amounts of amino acids, the supplement being different between groups within an experiment. In these investigations it was assumed that the supplements did not influence the digestibility of the ingested nitrogen. In four of the cases where 100 F/I was also determined this assumption was verified (Table 21,

Expts 13, 14 a and b and 2/61), but in four other cases the treatment effect was significant (Table 21, 13 b, 3, 5 and 6/61). The differences observed in the latter cases were generally small and probably of little biological significance. It is believed, therefore, that in these cases the values of $\Delta$ 100 U/I were also fairly good estimates of $\Delta$ Bal % and $\Delta$ NPU.

It is suggested, therefore, that $\Delta$ 100 U/I may be used as a measure of differences in protein quality when the conditions are standardized as regards the protein content of the diets, the food intake and the bodyweight of the rats, and when it is either demonstrated or may be *a priori* assumed that there is no significant difference between the protein sources tested with respect to their digestibilities. The use of 100 U/I has the advantage that fortuitous variations in the term 100 F/I have no influence upon the quantity measured.

### *I. The correlation between the apparent faecal and urinary recoveries of ingested nitrogen*

The possibility that variations in the digestibility of the protein source under test may be correlated with variations in the urinary nitrogen excretion was mentioned (p. 51). In the preceding section it was implied that this correlation was fortuitous. Because the terms 100 F'/I and 100 U'/I are not directly measured in the experimental period when NPU is determined, the hypothetical correlation must be between 100 F/I and 100 U/I. It remains, therefore, to discuss whether there was a demonstrable correlation between these terms in the experiments as they were generally performed in this investigation.

In Tables 18 and 21 the total sums of squares calculated in 15 experiments for the terms 100 F/I, 100 U/I and Bal % together with the percentages of the totals accounted for by treatment and error are given. The percentages accounted for by litter differences, are not tabulated. Because the sums of squares for Bal % and for 100 (F + U)/I are identical, complete random association of 100 F/I and 100 U/I would be indicated if the sum of squares for Bal % were equal to the added sums of squares for 100 F/I and 100 U/I. As this was generally not the case correlation between the two terms was indicated. The correlation between (1) 100 F/I and (2) 100 U/I not related to treatment and litter differences may be estimated from the error sums of squares ($S_E^2$) for these quantities and for (3) Bal % by the following equation:

$$S_{E_3}^2 = S_{E_1}^2 + S_{E_2}^2 + 2.r_{12} \sqrt{S_{E_1}^2 \times Se_{E_2}^2} \qquad (8\,e)$$

The values for $r_{12}$ calculated from equation 8 e are given in Tables 18 and 21. In most cases they were numerically small and not consistent with

respect to the sign. The results indicate that in the type of experiment used in this investigation error variation in 100 F/I is not associated with parallel variation in 100 U/I.

On the other hand it must be assumed that differences in digestibility between protein sources (treatments) will affect the term 100 U/I. A greater influx of amino acids into the blood stream due to differences in protein digestilibity may affect the term 100 U/I in either direction (p. 63 and p. 71). In 7 of the 9 cases where a significant treatment effect on 100 F/I is recorded in Tables 18 and 21 the treatment sums of squares for 100 F/I, 100 U/I and Bal % indicated positive correlation between the two former quantities. The correlation coefficients were not calculated because their degrees of freedom were only 2. The results indicated that in most of the cases considered high faecal excretion of nitrogen was associated with high urinary excretion. The tendency was, therefore, that the effect of a low digestibility was not compensated by a more efficient utilization of the amount of nitrogen absorbed.

*K. Correlations between the constituent terms of the net protein utilization*

It was mentioned (p. 82) that the variance of NPU is determined by the variances of the single and composite terms constituting it (equations 4 b—4 d, p. 25), and by the covariances between them. It is evident from inspection of Table 3 that the variance of NPU cannot be expressed as a sum of the variances of its terms. Correlation coefficients between the various terms may be calculated similarly to those between 100 F/I and 100 U/I either by equation 8 e or by the analogous equation valid when the single terms are subtracted, the sign of $2r_{12}$ in the equation then being negative. It is contended, however, that such calculations are not worth while for other quantities than 100 F/I, 100 U/I and Bal % because the magnitude and the variation of the correction terms 100 F'/I and 100 U'/I are to a great extent determined by the assumptions made about them. Correlations between a correction term and a measured term are regarded to be fortuitous at least under standardized experimental conditions.

The important question is whether the application of the two correction terms to the directly determined Bal % may influence the treatment and error variances in a meaningful manner, biologically. This problem was discussed in section G where it was concluded that more confidence could be placed in a difference in protein utilization measured by $\Delta$ Bal % than when it was measured by $\Delta$ NPU. In my opinion this answers the question posed. Thus, if it is required to include an estimate of the maintenance requirement in the measure of protein utilization by

adding to Bal % the correction term 100 (F' + U')/I this should be done without including the variances and the covariances due to the correction term. This is the same argument as that presented in the case of the true digestibility (section VI H 3, p. 53). In line with the view taken there, it is contended that the best that can be hoped for is to account for the order of magnitude of the mean maintenance requirement, and to apply this to the mean value for Bal % to form an approximate estimate of the mean NPU for maintenance and growth. It is assumed that the variance of Bal % applies to this NPU.

### *L. The variation in biological value*

Throughout the preceding discussion it has been assumed that the conclusions arrived at for NPU were valid also for BV. The quantity D is thus considered to be inherently constant for a protein source within an experiment (equation 4 a, p. 11). The question remains which estimate should be used for the variance of BV when it is accepted that the variance of 100 F/I measures the variance of D (section VI H 3, p. 53) and that the variance of Bal % measures the variance of NPU (section K). This question cannot be answered adequately here because of lack of knowledge of how to calculate the variance of a fraction from the variance of its numerator and denominator. It is evident that if the two latter could be assumed to vary independently of one another the variance of the fraction would be greater than the variance of the numerator: BV would have a greater variance than NPU. In the case under consideration this condition is not fulfilled because the denominator is contained in the numerator as a term and, secondly, because there is generally some fortuitous correlation between 100 F/I and 100 U/I which are the two terms of Bal % in the numerator (section I).

It is contended, therefore, that BV, like D (section VI H 3, p. 53) and NPU (section G) should not be used for the purpose of directly comparing different protein sources. If a mean BV is calculated from observed values of 100 F/I and 100 U/I and from estimates of 100 F'/I and 100 U'/I, it should be given without an estimate of its variance. In this respect BV differs from D and NPU for the variances of which the variances of 100 F/I and Bal % could be used, respectively. According to this view the variation of "the percentage of the absorbed nitrogen that is not eliminated in the urine" (MITCHELL 1923—4 a), cannot be measured by the types of technique generally used.

# IX. THE RELATIONSHIPS BETWEEN NITROGEN BALANCE METHODS, AND METHODS INVOLVING MEASUREMENT OF BODY-NITROGEN GAIN, OR BODY-WEIGHT GAIN

## *A. General considerations*

It is generally assumed that nitrogen balance measures body-nitrogen gain, and that both are closely related to the body-weight gain. Measurements at variance with these assumptions have been noted (NEHRING, LAUBE, SCHWERDTFEGER, SCHIEMANN, HAESLER & HOFFMANN 1957; COSTA 1960), but by and large methods of measuring protein utilization by use of the quantities mentioned have given the same relative ranking of protein sources (BLOCK & MITCHELL 1946—7; BENDER 1956; BENDER & DOELL 1957; RIPPON 1959; HENRY & TOOTHILL 1962). It is indicated, however, that absolute values should not be compared between methods even when the quantities compared theoretically are equivalent (HENRY & TOOTHILL 1962). On the basis of the cited data, and of many similar, it is concluded that adequate comparisons between protein utilizations may be made with methods based on measurements of any of the three variables mentioned.

It is important to realize that strictly valid comparisons between the merits of different methods should be based upon quantities which really are equivalent. This requirement is often overlooked when nitrogen balance methods are compared with growth methods. In Table 22 the equivalent quantities in the three types of method are listed. It is seen that the nitrogen balance method gives more detailed information about the utilization of protein than methods measuring gain in body-nitrogen directly, or gain in body-weight. This point was recently stressed by

Table 22. *Equivalent measures of protein utilization in three types of method.*

| Method | | |
|---|---|---|
| Nitrogen balance | Body-nitrogen gain | Body-weight gain |
| $D_a$ | n.e. | n.e. |
| D | n.e. | n.e. |
| Bal % | $100\,(B-B_o)/I$ | $(W-W_o)/Pr$ = PER |
| NPU | $100\,[(B-B_o) - (B_k - B_o)]/I$ = NPU | $[(W-W_o) - (W_k-W_o)]/Pr$ = NPR |
| BV | n.e. | n.e. |

n.e., no equivalent. $B_o$ = carcass N of animals initially, B = carcass N of animals fed protein diet, $B_k$ = carcass N of animals fed protein-free diet. $W_o$, W and $W_k$ are the corresponding body-weights. I = nitrogen intake. Pr. = protein intake.

HENRY & TOOTHILL (1962). In the following sections an attempt is made to discuss the nitrogen balance method in relation to the two other types of method.

### *B. Nitrogen balance and body-nitrogen gain*

It was mentioned above that the two types of method do not always give the same estimate of the amount of nitrogen retained in the body of the experimental rats (NEHRING *et al.* 1957). It is generally agreed that discrepancies may partly be explained by the fact that the balance method only takes into account nitrogen lost by way of faeces and urine. Differences in body composition seem to be of minor importance (BENDER & DOELL 1957; HENRY & TOOTHILL 1962). However, MÜLLER, WILMES & KNAPPEN (1960) found no consistent difference between the two methods of measuring nitrogen retention, and they concluded that the reason for different results may lie in the effectiveness of collecting the excreta quantitatively. NJAA (1961 b) pointed out that errors in determining the apparent faecal recovery of ingested nitrogen may result from small losses of both food and faeces, and the same would apply also for the urinary nitrogen. FORBES *et al.* (1938) gave data for body-nitrogen gain as well as for nitrogen intake and nitrogen excretions. Percentage balance values calculated from their results were higher than the corresponding values for body-nitrogen, but generally the two sets of values varied in parallel. In one instance the urinary nitrogen excretion was unexpectedly high (see NJAA 1962 a, curve 2). This was not reflected in a correspondingly low value for body-nitrogen gain, and the urinary nitrogen excretion was, therefore, probably erroneous.

It seems likely that estimation of nitrogen balance is inherently affected by more numerous sources of error than is the estimation of body-nitrogen gain apart from the fact that the latter estimation is complicated by the use of a zero time control group. Determination of body-nitrogen involves, however, either the "unpleasant and time consuming procedure" (RIPPON 1959) of mincing the rat carcasses for homogenizing them, or dissolving the whole rats in acids before aliquots are taken for analyses. Probably because of the difficulties involved in these procedures, methods for the measurement of protein utilization based on direct measurement of body-nitrogen contents have not been widely used in routine tests. When such methods were used (BENDER & MILLER 1953 a; FORBES & YOHE 1955 a; TOMARELLI, MINNICK, D'AMATO & BERNHART 1959; RIPPON 1959) good agreement was obtained between these and the alternative nitrogen balance method employed.

From Table 22 it is seen that Bal % and $100\,(B-B_o)/I$ are equivalents (B and $B_o$ are respectively the carcass N contents of animals fed the

protein diet and of animals initially). From these NPU are obtained by adding estimates of the maintenance requirement divided by the nitrogen intake in the test group. In the methods of measuring body-nitrogen gain the maintenance requirement is estimated by the body-nitrogen loss in a control group given a nitrogen-free diet during the same time as the test groups are given the diets containing the test proteins. A mathematical formulation of the relationship between NPUs measured by a nitrogen balance technique and by body-nitrogen gain is given by BENDER & MILLER (1953 a). Their suggestion that the body-nitrogen content may be estimated from the ratio body-nitrogen/body-water (BENDER & MILLER 1953 b) is discussed in section D.

Because the two types of method are theoretically equivalent, objections may be raised against the validity of the methods used for estimating the maintenance requirement in both. These objections were discussed for the nitrogen balance method in sections VIII C and D (p. 85 and 88), they are valid also for methods employing measurement of body-nitrogen gain with small modifications.

From a consideration of the literature data it is concluded that the two types of method considered give inherently the same information about protein utilization. It is preferred to use the balance method because the procedures involved in it are simpler, though more numerous, than those involved in the methods employing direct estimation of body-nitrogen gain. The nitrogen balance method is preferred also because it gives more detailed information about the utilization.

### *C. The net protein utilization, and the protein efficiency ratio*

In the discussion of the most efficient and convenient method for measurement of protein utilization the quantities NPU and PER are often contrasted. This is done regardless of the fact that the former quantity includes an estimate of the maintenance requirement and the latter does not. The quantity to be compared with PER is Bal % (Table 22), similarities between which were pointed out in sections VIII F and G (p. 91 and 92). BENDER & DOELL (1957) proposed the use of the net protein ratio (NPR) and pointed to the similarities between NPR and NPU. The former is obtained from PER by adding to it the weight loss of the negative control group divided by the protein intake of the test group. The added term is intended to account for the maintenance requirement. NPU values determined by the conventional balance-sheet method and NPR values obtained on a large series of protein sources were highly correlated (HENRY & TOOTHILL 1962). These authors also confirmed the findings of BENDER & DOELL (1957) that NPU values

calculated from body-water contents (BENDER & MILLER 1953 b; MILLER & BENDER 1955) were highly correlated with NPR values obtained on the same rats. BLOCK & MITCHELL (1946—7) showed that literature values for NPU and PER referring to the same protein source were highly correlated. BENDER (1956) confirmed this with values obtained on the same rats and my calculation from the results given for NPU and PER by BENDER & DOELL (1957) gave a similar result ($r = + 0.693$). The fact that the latter correlation coefficient is numerically smaller than that between NPU and NPR ($r = + 0.986$) is an obvious result of the two latter variables containing estimates of the maintenance requirement. It does not indicate that NPU and NPR are better measures of protein utilization than PER, but simply that they contain strictly comparable terms. It would seem to be improbable that PER should in any way be correlated with the nitrogen loss of the negative control group, but it would seem equally highly probable that this nitrogen loss would be correlated positively with the weight loss in this group. Thus, the available literature data indicate that PER is significantly correlated with that part of NPU which is related to growth, namely Bal %. The same objections against correcting Bal % by use of a term intended to account for the maintenance requirement are valid also against the similar correction of PER. It is believed that both Bal % and PER may be corrected to give approximate estimates of NPU and NPR, but that direct comparisons between protein sources should be done by use of the former set of values under strictly standardized conditions. It is my experience that in short term experiments differences in utilization between protein sources are more consistently detected by use of Bal % than by use of PER. Because it was chosen to rely on short-term experiments it is believed that the nitrogen balance method is the method of choice.

### *D. The significance of NPU estimated from body-water content*

In view of the very high values reported for the correlation coefficient between NPU and NPR (+ 0.986, BENDER & DOELL 1957; + 0.98, HENRY & TOOTHILL 1962) it seems warranted to examine whether these quantites, when they are obtained in the same experiment and using the same rats, are based on independent measurements. The quantities used for the calculation of NPU by the method of MILLER & BENDER (1955) are (1) a predetermined ratio between the contents of body-nitrogen and body-water, (2) the actual water contents of the rats in the test groups and in the negative control group, and (3) the body-weights of the rats in these groups at the end of the experiment. Assuming that the body-

nitrogen contents are adequately estimated from the combination of points (1) and (2) above, NPU is given by equation 9:

$$NPU = 100\,\frac{W\,\frac{[N]_W}{100} - W_o\,\frac{[N]_{W_k}}{100}}{I} = 6.25\,\frac{W\,[N]_W - W_k[N]_{W_k}}{Pr} =$$

$$6.25\left([N]_W\,\frac{W - W_k}{Pr} \mp \Delta\,[N]\,\frac{W_k}{Pr}\right) = 6.25\left([N]_W\,NPR \pm \Delta\,[N]\,\frac{W_k}{Pr}\right) \quad (9)$$

The symbols in equation 9 are: W and $W_k$, body-weights of test group and negative control group at the end of the experiment, respectively; $[N]_W$ and $[N]_{W_k}$, the percentage body-nitrogen contents corresponding to these body-weights: $\Delta\,[N]$, the difference between these body-nitrogen contents: I and Pr, nitrogen and protein intakes in the test group, respectively; $(W\text{-}W_k)/Pr = NPR$ by definition (BENDER & DOELL 1957).

According to BENDER & DOELL (1957) and HENRY & TOOTHILL (1962) the body-nitrogen content vary but little with the diet of the rats so that the dominating variable on the righthand side of equation 9 is NPR. It is thus indicated that NPU and NPR are not based on independent measurements under the conditions mentioned above. Moreover, because NPR is the dominating term in NPU as determined by the method of MILLER & BENDER (1955) this method must be classified closer to a growth method than to a nitrogen balance method. In accordance with HENRY & TOOTHILL (1962) it is therefore suggested that little is gained by determining NPU by the method of MILLER & BENDER (1955) as compared with determining NPR by the method of BENDER & DOELL (1957). As pointed out in section C it is my opinion that comparisons between protein sources should be done under strictly standardized conditions without taking the maintenance requirement into account. It is concluded, therefore, that for the comparison between protein sources the equivalents of Bal % and PER should be preferred. The method of MILLER & BENDER (1955) may be modified for this purpose. It would then give a measure equivalent to PER but which takes into account the small variation in body-composition between groups given different protein sources.

## X. GENERAL CONCLUSIONS

It is Mitchell's great merit to have made the estimations of biological values (BV) and net protein utilizations (NPU) routine laboratory procedures. In the general use of these measures of protein quality the fact that they are not actually measured as such but comprise both measured

and approximated quantities has largely been lost sight of. Because of this it is contended that the significance of protein quality differences should be judged on the basis of measured quantities alone. The quantities in question when nitrogen balance methods are concerned, are the apparent percentage faecal and urinary recoveries of ingested nitrogen (100 F/I and 100 U/I) and the percentage nitrogen balance (Bal %). Because these are affected by numerous factors not related to the protein quality (chapters VI, VII and VIII) their use as measures of the latter is permissible only under strictly standardized conditions. It is contended that under such conditions differences between NPU values may be measured either by the corresponding differences between Bal % values, or by the corresponding 100 U/I values if the digestibilities are not different (sections VIII G and H). The latter question should be answered on the basis of the 100 F/I values as determined under standardized conditions. The factors considered to be important to standardize are: the body-weight of the rats used, the daily food intake and the protein content of the diet, the duration of the experiment and the procedures used for collection and analysis of food, faeces and urine.

True digestibility, net protein utilization and biological value are composite quantities serving the important purpose of being approximate measures of the portions of ingested (D and NPU) and truly digested (BV) nitrogen actually utilized by the experimental animal. They make possible a ranking of protein sources on an assumedly absolute scale. However, the value assigned to a particular protein source may differ between theoretically equivalent scales. HENRY & TOOTHILL (1962) mentioned that one possible explanation of the fact that the balance-sheet method generally gave higher values for NPU than the body-water method may be that different estimates of endogenous nitrogen losses were used in the two methods. Another possible explanation not mentioned by them is that the true digestibility determined in the balance-sheet method, but which does not enter into the calculation of NPU in the body-water method, may have been estimated to be higher than the actual value. The two explanations have in common the fact that they concern quantities which cannot be actually determined and they therefore serve to stress the contention that the assumed absolute scales really possess only relative significance.

The great advantage of the assumedly absolute significance of D, NPU and BV is that they allow a factorization of the protein utilization. They offer the possibility of giving estimates of how great a part of the faecal nitrogen is actually derived from the food protein, and of how much of the ingested or truly digested protein is utilized for maintenance and growth, respectively.

This again makes possible the factorization of the protein requirement (BLAXTER & MITCHELL 1948). In my opinion this is the most important feature of the MITCHELL (1923—4 a ) method for estimation of biological values. The realization that the maintenance requirement forms an important part of the total protein requirement is the outstanding virtue of the theory for protein requirement and utilization evolved in Mitchell's early work and strongly defended by him and his co-workers to this day.

It is my opinion that much work remains to be done in establishing better methods for the estimation of the maintenance requirement of protein and especially regarding the question whether or not this requirement is independent of the performance of the experimental animal. The present state of our knowledge on this point is such that the assumed absolute measures of protein utilization (D, NPU and BV) possess only limited significance in the assessment of small differences between protein sources.

## XI. SUMMARY

I. The present study dates back to 1954. The biological value of herring meal protein as determined by the Mitchell method was found to vary so much within experiments that it was decided to examine the reasons for this.

II. The historical background of the Mitchell method is summarized. The fact that the quantity termed 'biological value' was considered to possess absolute significance in accounting for the utilization of the digestible portion of the ingested protein is pointed out. The various objections raised against the method and the assumptions on which it is based are mentioned. Doubts whether "the value of the results secured is sufficient compensation for the laborious procedures undertaken" are expressed.

III. The plan of the investigation is given.

IV. The experimental conditions employed are described as well as the individual experiments to which reference is made in the following chapters.

V. The composite nature of the terms true digestibility (D), net protein utilization (NPU) and biological value (BV) is pointed out. Reasons are given for discussing NPU instead of BV.

The relative importance of the terms constituting NPU for its variation was examined by use of analysis of covariance. In the three sets of data considered different terms were found to be of first importance.

VI. The faecal nitrogen excretion was studied in relation to the body-weight and the growth rate of the rats and their intakes of food and nitrogen. Faecal nitrogen is expressed as the daily amount (F), as the amount per g of ingested food (F/E) and as the percentage of the nitrogen intake (100 F/I).

The subdivision of faecal nitrogen into portions of dietary and metabolic origins and the significance of the term true digestibility are discussed.

The results examined indicated an effect of the body-weight on the faecal nitrogen excretion independent of the intakes of food and nitrogen. The heavier rats excreted more nitrogen than the lighter. This is in accordance with SCHNEIDER'S (1935) conclusions which were based on experiments in which the probable effect of the nitrogen intake was not considered. No clear effect of the growth rate could be demonstrated.

Changes in the nitrogen intake due to equivalent changes in the food intake and in the protein content of the diet affected F, F/E and 100 F/I differently. F increased more when the food intakes was increased than when equivalent changes in the nitrogen intake were due to changes in the protein content of the diet. F/E and 100 F/I were essentially unaffected by changes in the food intake whereas the former increased and the latter decreased when the protein content of the diet was increased, The results are discussed in relation to simple equations thought to describe prevalent assumptions about the relationships between the faecal nitrogen excretion and the factors influencing it. It is concluded that the assumptions are only approximately correct. Thus it is pointed out that reliable estimates of the metabolic excretion may not be obtained by extrapolation techniques as suggested by BOSSHARDT & BARNES (1946), BLAXTER & MITCHELL (1948) and MITCHELL & BERT (1954).

Direct estimation of the metabolic nitrogen excretion is considered and it is pointed out that it is difficult to obtain reliable estimates of the magnitude of this excretion. As a consequence reliable absolute values for D may not generally be obtained. It is suggested that the concept of a constant true digestibility characteristic of the protein source under test be retained, but that it be realized that the values obtained for it by the procedures generally employed are dependent on the assumptions on which the procedures are based. Further, it is suggested that the variance of 100 F/I be used as an estimate of the variance of D.

Comparisons between protein sources with respect to their diges-

tibilities may conveniently be based on measurement of the term 100 F/I under strictly standardized conditions. The problem encountered under practical conditions, that protein contents of diets intended to be equal may in fact be slightly different, is discussed. Theoretically the effect of such differences will have least importance at high protein contents. However, the urinary nitrogen excretion is usually the most important factor in methods measuring the protein utilization and as this excretion is most conveniently studied at relatively low protein levels, digestibilities will usually also be compared at such levels. In the evaluation of the results obtained the effect of unintended differences between protein levels should, therefore, be kept in mind.

The fact that NPU may be expressed as a sum of which D is a term indicates that the former will vary linearly with the latter, other conditions being constant. Thus, variations in D within an experiment with the same protein source will be reflected in the variation of NPU.

VII. The urinary nitrogen excretion was studied in relation to the body-weight and the growth rate of the rats and to their intakes of food and nitrogen. The excretion was expressed as the daily amount (U) or as the percentage of the nitrogen intake (100 U/I).

The subdivision of urinary nitrogen into exogenous and endogenous parts and the definition of the latter are discussed.

The results examined indicated that the growth rate was of greater importance than the body-weight for the variation in the excretion in current experiments. The effects of the two variables were in opposite directions. The body-weight is assumed to affect only the endogenous, the growth rate both the exogenous and the endogenous nitrogen.

Changes in the nitrogen intake due to equivalent changes in the food intake and the protein content of the diet affected 100 U/I differently. Within the narrow range of nitrogen intake levels tested, 100 U/I decreased when the food intake was increased whereas it increased slightly when the protein content of the diet was increased. This may be explained by the different effects that the two ways of changing the protein intake have on the growth rate. When the body's need of protein is covered, practically the total intake increment of digestible protein is excreted as nitrogen in the urine regardless of how the change in the intake is brought about. The results are discussed in relation to simple equations describing some prevalent assumptions about the relationships between urinary nitrogen and nitrogen intake. It is concluded that the assumptions do not adequately account for the observations. A consequence of this is that the endogenous urinary nitrogen excretion may not be estimated by the extrapolation technique (MITCHELL 1955).

The definition of the endogenous excretion is considered and the choice of a reference basis is discussed. It is concluded that it is not possible to assign to any particular reference basis related to the body-weight the attribute of being biologically the most meaningful. On account of this the definition of the endogenous excretion is reconsidered and a tentative reformulation is suggested. According to this the endogenous excretion consists of two parts, one relatively constant and in some way related to the body-weight and one variable related to the inherent tendency towards weight loss. The latter part is suggested to be the greater the higher the performance of the experimental animal. A tentative method of measuring the endogenous excretion is suggested and used in two experiments. Several methods suggested in the literature for the estimation of endogenous urinary nitrogen are considered and their relative merits evaluated. Under certain conditions the method originally used by MITCHELL (1923—4 a) may introduce irrelevant variation into the estimation of NPU.

VIII. The significance of BV and NPU as measures of protein utilization and the conventional subdivision of the latter into a sum of two terms accounting respectively for maintenance ($NPU_m$) and growth ($NPU_g$) are considered.

It is inferred that values of $NPU_m$ as determined under maintenance conditions may only be credited with relative significance as long as the requirement of ideally composed protein under these conditions is not known exactly. Preliminary experiments with three protein sources indicated a lower maintenance requirement of protein than would be expected on the basis of prevalent assumptions.

Under conditions of growth $NPU_m$ is inversely proportional to the protein content of the diet. Thus, unintended differences between the protein contents of diets compared may result in NPU differences thought to be of doubtful biological significance.

The maintenance requirement of protein and consequently $NPU_m$ is generally considered to be independent of the performance of the experimental animal. In contrast to this view it is tentatively suggested that under otherwise constant conditions $NPU_m$ increases with the performance. This is suggested as an alternative, or complementary, explanation of the fact that NPU is generally lower at higher protein levels than at lower.

A consequence of prevalent assumptions is that $NPU_g$ is equal to Bal %. The similarities between Bal % and PER are pointed out. Bal % is suggested as a measure of protein utilization which under strictly standardized conditions may be used to estimate NPU differences. The

suggestion is illustrated by four examples. It is indicated that when the protein sources under test are not different with respect to their digestibilities the term 100 U/I may be used as a measure of protein utilization, and to estimate NPU differences.

It is suggested that on account of the uncertainties involved in the estimation of $NPU_m$ the variance of Bal % should be used as a measure of the vaiance of NPU and that values of BV should be given without estimates of their variances. The implication is that BV and NPU are overall values accounting approximately for the total utilization of respectively digested and ingested nitrogen, but that they are as yet too roughly determined to be credited with absolute significance.

IX. The methods of evaluating protein values by use of nitrogen balance, body-nitrogen gain and body-weight gain are considered. It is stressed that comparisons of the relative merits of such methods should be based on quantities which are really equivalent. It is concluded that the methods considered generally give the same information about relative protein utilization.

The nitrogen balance method is preferred because it gives more detailed information about the protein utilization than the other methods, because it involves simpler procedures than the method based on measurement of body-nitrogen gain, and because it is better suited for short-term experiments than the method based on measurement of weight gain. It is suggested that the body-water method comes closer to being a growth method than to a nitrogen balance method.

X. Some general conclusions about the use of nitrogen balance values in the evaluation of protein quality are drawn. The necessity of strictly standardized conditions is stressed. The relationship between protein utilization and the assessment of protein requirements is indicated. It is pointed out that much work remains to be done on the assessment of maintenance requirements.

## APPENDIX

*A. Deduction of the relationships discussed in section VI F p. 40*

According to equation 6 b (p. 28) the difference between the nitrogen excretion ($\Delta F_{C_1}$) expected at a constant nitrogen content of the diet ($C_1$) when the diet is given at two food intake levels ($E_1$ and $E_2$) is given by:

$$\Delta F_{C_1} = k_1 C_1 \Delta E + k_2 \Delta E \qquad \text{(A 1)}$$

and the corresponding difference ($\Delta F_{E_1}$) expected at a constant food intake level ($E_1$) when the diet is given at two nitrogen content levels ($C_1$ and $C_2$) by:

$$\Delta F_{E_1} = k_1 E_1 \Delta C \qquad \text{(A 2)}$$

where $\Delta E = E_1 - E_2$ and $\Delta C = C_1 - C_2$. $\Delta F_{C_1}$ and $\Delta F_{E_1}$ are the differences in the faecal excretion when the excretions corresponding to the index 2 are subtracted from those corresponding to the index 1.

Assuming that the nitrogen intake $E_1C_1$ is reduced by the same amount under the two sets of conditions one has:

$$E_1/E_2 = C_1/C_2 \qquad \text{(A 3)}$$

From equation A 3 follows:

$$k_1 C_1 \Delta E = k_1 E_1 \Delta C \qquad \text{(A 4)}$$

The different effect of changing the nitrogen intake by way of changing the food intake or by way of changing the nitrogen content of the diet is expressed by:

$$\Delta F_{C_1} - \Delta F_{E_1} = k_2 \Delta E \qquad \text{(A 5)}$$

Equations A 2, A 3 and A 5 indicate that the faecal nitrogen excretion is reduced as a result of reducing either the food intake level or the nitrogen content of the diet and that the effect is greater by the amount $k_2 \Delta E$ in the former case than in the latter.

Similarly the effects of changing the food intake and the nitrogen content of the diet on the term 100 F/I or 100 F/EC are given by:

$$\Delta\,(100\ \mathrm{F/I})_{C_1} = -\,100\,\frac{k_3}{C_1}\,\frac{\Delta E}{E_1\,E_2} \qquad \text{(A 6)}$$

and

$$\Delta\,(100\ \mathrm{F/I})_{E_1} = -\,100\,k_2\,\frac{\Delta C}{C_1\,C_2} - 100\,\frac{k_3}{E}\,\frac{\Delta C}{C_1\,C_2} \qquad \text{(A 7)}$$

From equation A 3 follows:

$$\frac{\Delta C}{E_1\,C_1\,C_2} = \frac{\Delta E}{C_1\,E_1\,E_2} \qquad \text{(A 8)}$$

and thus:

$$\Delta\,(100\ \mathrm{F/I})_{C_1} - \Delta\,(100\ \mathrm{F/I})_{E_1} = 100\,k_2\,\frac{\Delta C}{C_1\,C_2} = 100\,k_2\,\frac{\Delta C}{C_1\,(C_1 + \Delta C)} \qquad \text{(A 9)}$$

Equations A 6, A 7 and A 9 indicate that when the nitrogen intake is reduced by the same amount by changing either the food intake or the nitrogen content of the diet the quantity 100 F/I increases in both cases, and that the effect is greater by the amount $100\,k_2\,\frac{\Delta C}{C_1\,(C_1 + \Delta C)}$ in the latter case than in the former.

*B. Deduction of inequality 7 c in section VII E 8 p. 78*

The designations used are the following:

$p_t$, the total number of periods in the experiment under consideration, including the initial (in) and final (fi) standardizing low egg-protein periods.

p, the number in the sequence of periods of the particular period considered.

$U'_{in}$ and $U'_{fi}$, the observed urinary nitrogen excretions in the standardizing periods indicated.

$W_{in}$, $W_{fi}$ and $W_p$, the mean body-weights of the rat considered in the periods indicated.

$U'_p$, the calculated endogenous urinary excretion of the rat considered in the particular period considered.

In the procedure for the calculation of $U'_p$ suggested by MITCHELL (1923—4 a) it is assumed that the ratio between the endogenous urinary nitrogen excretion and the mean body-weight varies linearly with the time, the latter being given in the unit of periods. Thus $U'_p/W^q_p$ would be given by:

$$U'_p/W^q_p = U'_{in}/W^q_{in} - \frac{U'_{in}/W^q_{in} - U'_{fi}/W^q_{fi}}{p_t - 1}(p - 1) \qquad \text{(B 1)}$$

and $U'_p$ by:

$$U'_p = \frac{(p_t - p)\, U'_{in}/W^q_{in} + (p - 1)\, U'_{fi}/W^q_{fi}}{p_t - 1} W^q_p \qquad \text{(B 2)}$$

The conditions for $U'_p$ being greater than either $U'_{in}$ or $U'_{fi}$ may be derived from equation B 2. Assuming that $U'_{fi} > U_{in}$ the conditions are $U'_p > U'_{fi}$ when

$$\frac{(p_t - p)\, U'_{in}/W^q_{in} + (p - 1)\, U'_{fi}/W^q_{fi}}{p_t - 1} W^q_p > U'_{fi} \qquad \text{(B 3)}$$

or by rearranging the inequality B 3:

$$(p_t - p)\,(U'/W^q)_{in}/\,(U'/W^q)_{fi} > (p_t - 1)\,(W^q_{fi}/W^q_p) - (p - 1) \quad \text{(B4)}$$

In the period preceding the final standardizing period $p_t - p = 1$ and the inequality becomes

$$(U'/W^q)_{in}/\,(U'/W^q)_{fi} > (p_t - 1)\,(W^q_{fi}/W^q_p) - (p - 1) \qquad \text{(B 5)}$$

The inequality B 5 is the same as 7 c in section VII E 8.

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