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Metabolic Studies in Human Subjects

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It is common knowledge that food proteins differ in their physiological or nutritional value. The minimum protein requirement expressed in terms of nitrogen requirement varies with the chemical nature or composition of the proteins under study. Such differences in quality or nutritive value are attributed to differences in the essential amino acid pattern of the protein. The nutritive value of proteins may be influenced also by factors inherent to the protein such as nonessential amino acid content, balance of essential amino acids, ratio of essential to nonessential amino acids, and other factors not pertaining to protein, such as intake of calories, vitamins, and minerals. In any case, the primary function of dietary proteins is to provide a mixture of amino acids of appropriate pattern for the synthesis and maintenance of tissue proteins.

The relative adequacy of a given protein in providing the required amino acid pattern to the organism will determine the magnitude of the biological response. Accordingly, practically all methods utilized in evaluating the quality of proteins are concerned, directly or indirectly, with appraisal of the relative efficiency of different proteins in satisfying amino acid requirements.

Appraisal of the nutritive value of a protein requires the choice of an appropriate criterion, obtained under carefully controlled experimental conditions, and depends rather critically on the success in ensuring that, under the experimental conditions employed, protein is the only limiting factor for the criterion chosen.

The quality of the protein for synthesis is primarily a function of four variables: 1) the pattern of amino acids provided by the protein; 2) the intake of nitrogen; 3) the intake of calories; and 4) the physiological state of the individual. To provide some meaning to the efficiency of protein utilization, it is necessary, first, to develop it from the concepts of the dynamic state of protein metabolism.

Nitrogen Balance

The basic principle of balance studies in nutrition is no different from the idea of balance in other sciences. It is concerned with the measurement of net gains or losses in utilization of the nitrogen substances needed by the organism. Nitrogen is constantly lost from the organism and taken in, as food; determination of nitrogen in the food and excreta therefore provides a quantitative measure of protein metabolism and indicates whether the organism is losing or gaining protein under the conditions existing when the measurements are made. This dynamic catabolic and anabolic interrelation is described in Fig. 1, where nitrogen balance is defined as the amount of ingested nitrogen retained in the body. In the formula, NB stands for apparent nitrogen balance, NI for nitrogen intake, NF for fecal nitrogen, and NU for urinary nitrogen. The apparent absorbed nitrogen is equal to the difference between nitrogen intake and fecal nitrogen. The apparent nitrogen balance in the formula is positive when nitrogen intake is greater than the sum of the fecal and urinary nitrogen; in this case, an apparent gain in nitrogen occurs. If nitrogen intake is equal to the sum of the nitrogen in the feces and the urine, nitrogen balance is equal to zero, in which case the individual is in apparent equilibrium. On the other hand, if the sum of the nitrogen in the feces and urine is greater than the nitrogen intake, nitrogen balance is negative and the individual is in negative nitrogen balance.

Allison (1) pointed out that nitrogen balance is the sum of the gains and losses of all tissue proteins of the body, and it may therefore be used as a measure of dietary protein quality to the animal concerned.

However, it is possible to have a positive nitrogen balance with a loss of nitrogen occurring in some tissues; for example, under conditions of stress, although some tissues may be maintained at the expense of others, an overall positive balance is still possible. In parallel, maintenance of nitrogen equilibrium does not mean that every tissue is being maintained, just as the provision of sufficient nitrogen to maintain nitrogen equilibrium in the adult does not necessarily imply the fulfillment of nitrogen requirements. Nitrogen equilibrium can shift through metabolic adaptation, so that it is possible to maintain an individual in a depleted state.

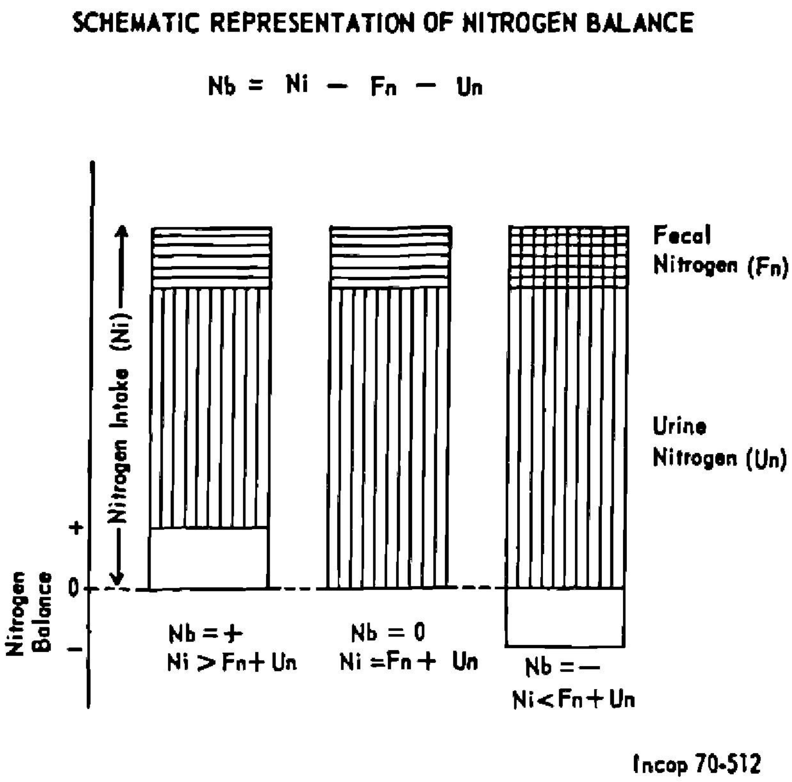


Fig. 1.

Factors Affecting Nitrogen Balance

Since nitrogen excretion can reflect so many different changes in metabolic pathways, associated with both exogenous and endogenous metabolism, nitrogen balance responds to several variables.

A) One of the most important is nitrogen intake, as seen in representative results in Table 1. These results were obtained from 4 children, 25 to 69 months old, weighing 9.9 to 18.33 Kg, fed skimmilk protein (2). It is obvious that as nitrogen intake decreased, urinary nitrogen also decreased, with a parallel decrease in nitrogen balance. This indicates, therefore, that when absolute nitrogen retention values are utilized to evaluate the quality of proteins, the study must be carried out at a fixed level of nitrogen intake.

As with other techniques of evaluating protein quality, applied either to experimental animals or to man, nitrogen balance is not capable of detecting small differences in quality, as suggested by essential amino acid content, when total nitrogen intake is high. An example is shown in Table 2. In this case, children were fed the same amount of various proteins at intakes of around 2.0 g of protein/Kg/day, at equal intake of calories of 100/Kg/day (2). In the examples shown, nitrogen balance was essentially the same for all proteins tested, whether of animal or vegetable origin, which is the case for INCAP 15 based on cottonseed, soybean, and maize flour, and INCAP 9, based on cottonseed and maize flour. The results also show that the vegetable proteins yield higher amounts of fecal nitrogen, suggesting a

TABLE 1. Effect of nitrogen intake on nitrogen balance (skimmilk) mg N/Kg/day.

NI	FN	UN	NA	NR
477	65	313	412	99
386	45	258	342	83
305	48	185	255	69
225	42	132	184	52
164	36	74	128	54
95	29	50	66	15

Caloric intake: 90–100 Kcal/Kg body wt/day.

TABLE 2. Nitrogen balance of various proteins fed at high levels of intake (mg N/Kg/day).

Protein	NI	FN	UN	NA	NR
Skimmilk	386	45	258	342	83
Egg	385	42	262	343	80
INCAP 15	398	89	216	309	93
INCAP 9	388	110	201	277	77

Caloric intake: 90–100 Kcal/Kg body wt/day.

TABLE 3. Average nitrogen balance at various levels of nitrogen intake (children).

Protein foods tested	Nitrogen intake range mg/Kg/day			
	310–360	230–251	156–174	81–114
	Nitrogen balance mg/Kg/day			
Milk	62	50	54	8
Cotton-seed-corn mix	64	40	26	–4
Soybean-corn mix	86	65	38	0
CSF-SBF-corn mix	59	51	24	–5
Soybean-tex food	82	49	9	–10
Whole egg	80	80	49	—
F	1.51	5.85**	11.06**	1.84
LSD (0.01)[a]	45	22	21	20
No. observations	31	31	32	22

**Highly significant.

[a]LSD: least significant difference.

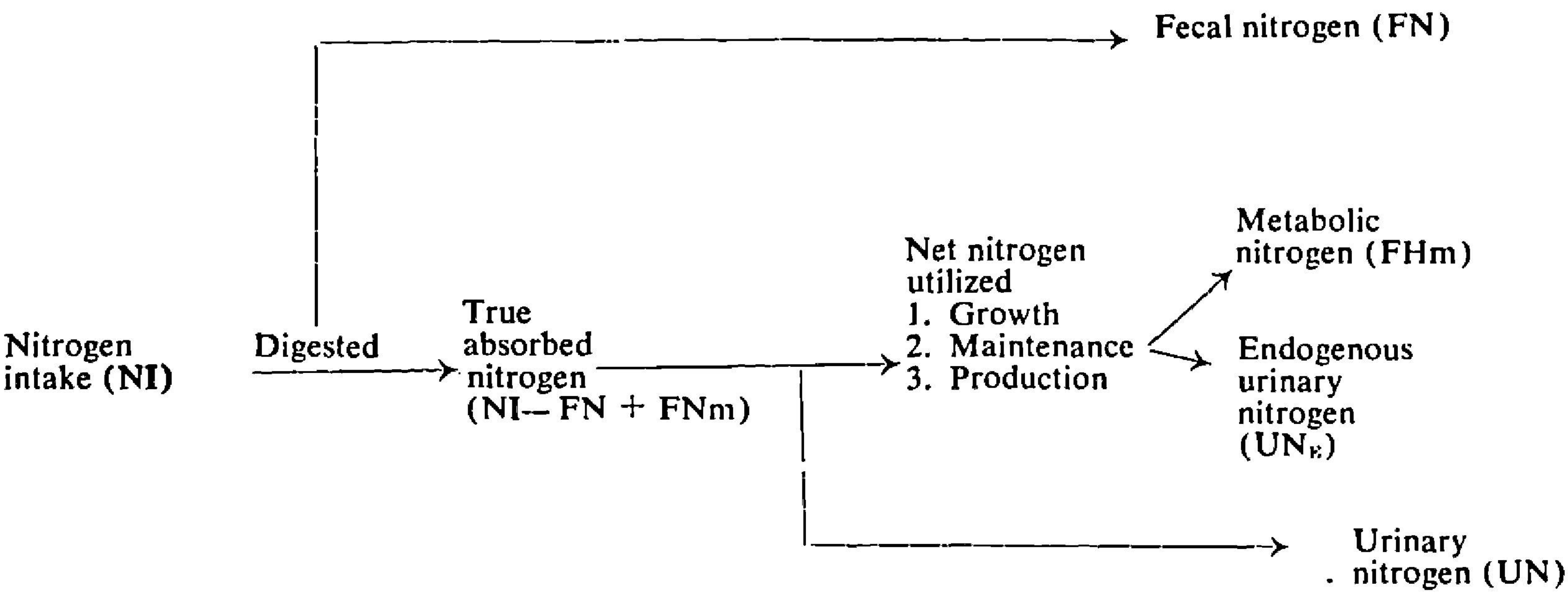


FIG. 2. Nitrogen Utilization Scheme

lower digestibility than for the animal proteins. What is of interest is that, of the nitrogen absorbed, similar amounts of retained nitrogen were obtained from all proteins, suggesting that the amino acid balance of this fraction was as good as that from the proteins of animal origin.

Therefore, when the nitrogen-balance method is utilized to evaluate protein quality, not only is it essential to keep nitrogen intake constant but it is also necessary to carry out the tests at nitrogen intakes which will maximize the differences in quality. In animal testing, 10% protein diets are used since it was found that at this level quality differences become more evident. Little work has been done on this point in children; however, results of some studies (2) are described in Table 3. To determine the level of nitrogen intake at which larger differences in nitrogen balance are obtained, the nitrogen-balance values observed at intakes between 360 and 81 mg of nitrogen were grouped as shown in the table and were studied statistically.

The analysis indicated that highly significant differences in nitrogen balance values can be detected among different proteins and in children at intakes between 156 and 251 mg of nitrogen per kilo body weight per day.

The methodology of the nitrogen-balance technique is different in children from that in adults. The basis for this difference is seen in the nitrogen-utilization scheme shown in Fig. 2. A young organism has larger needs for nitro-

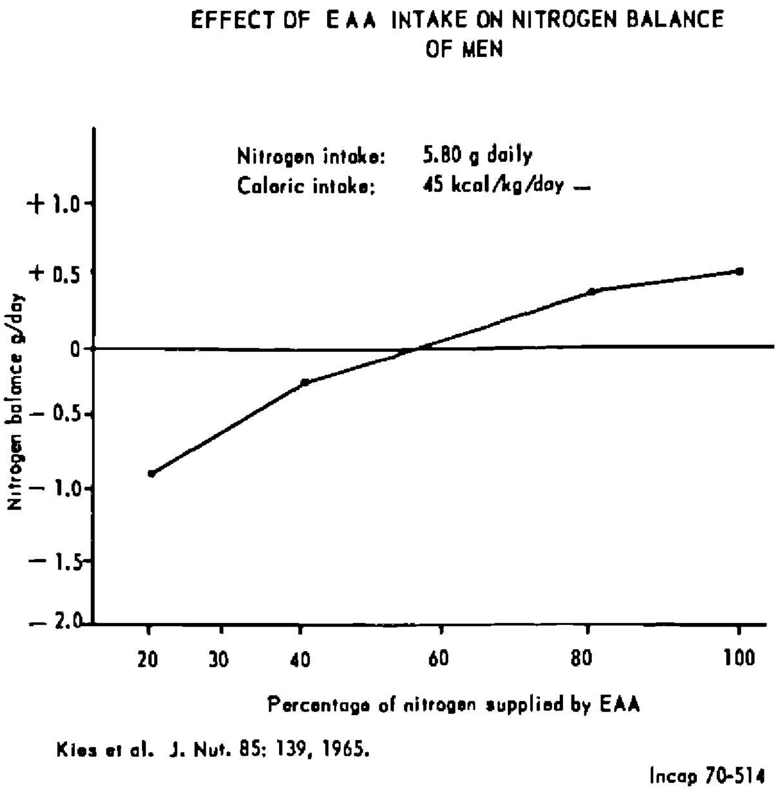


Fig. 3.

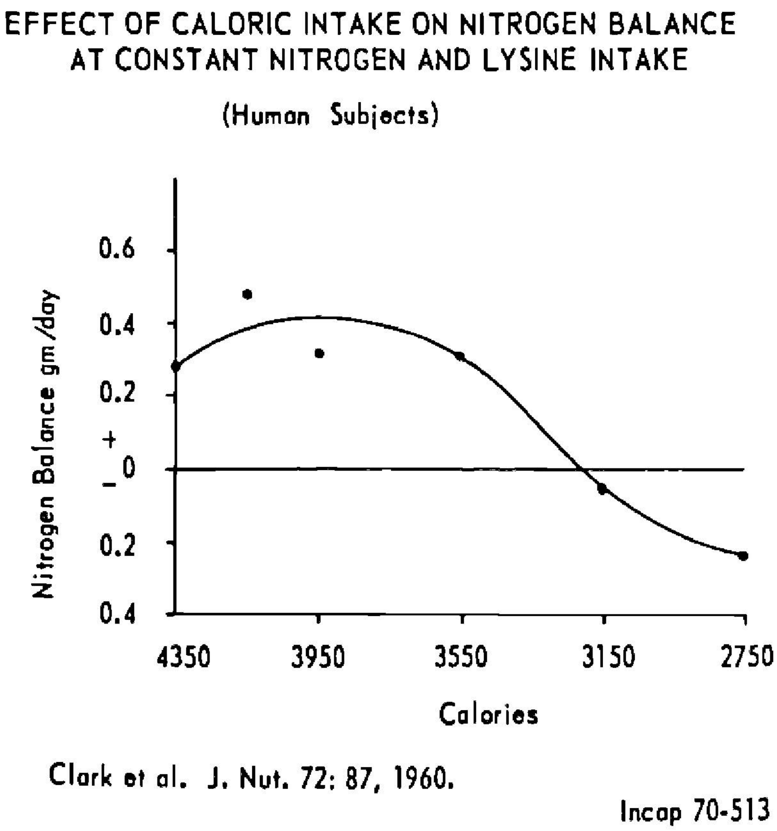


Fig. 4.

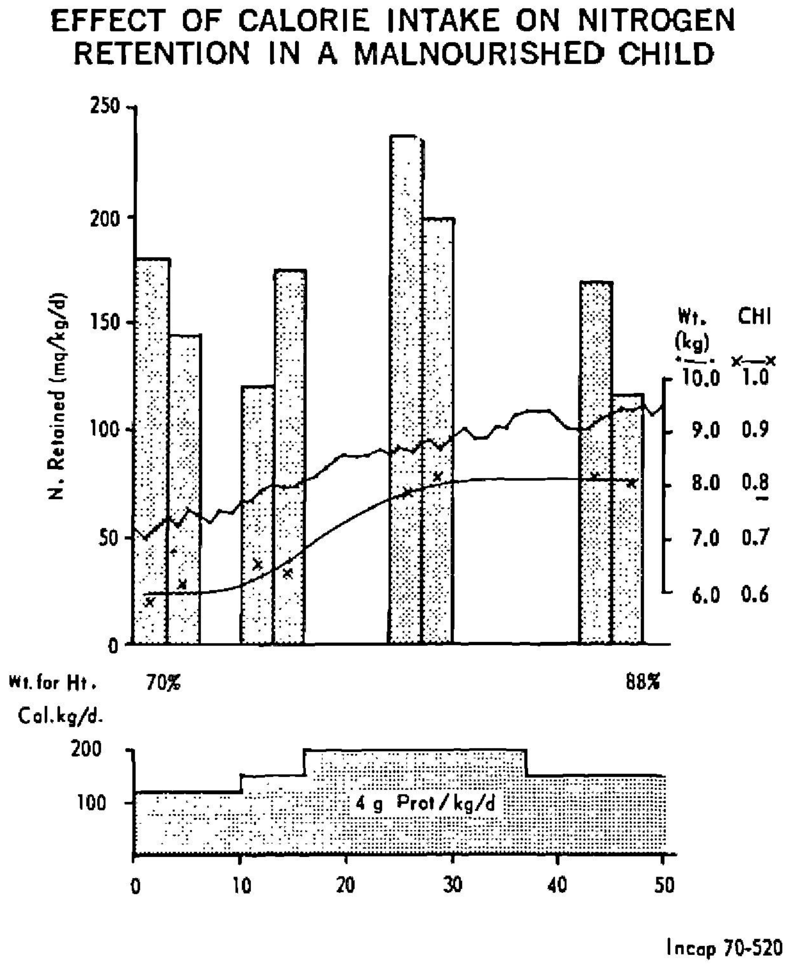


Fig. 5.

gen, mainly for growth and maintenance purposes. On the other hand, an adult subject who is not in production state or any unusual stress condition requires only enough for maintenance purposes. Therefore, when the nitrogen-balance method is used in adult subjects to measure the protein quality of different sources, the subjects must first be placed in a state of negative nitrogen balance. Once this has been achieved, the proteins under study are fed to the individual to provide a small amount of protein, usually not more than 10 g per day. Following this method, Kies *et al.* (3), for example, showed (Fig. 3) the effect on nitrogen balance of altering the intake of essential amino acid while total nitrogen was held constant. The data indicate that nitrogen retention was affected significantly by the amount of essential amino acids ingested when the intake of protein was kept at a constant value of 5.8 g/day at an intake of calories of 45/Kcal/Kg body weight per day.

B) An example of the importance of calories on nitrogen balance is shown in Fig. 4, taken from work of Clark *et al.* (16). The subject in this study received 1500 mg of lysine and 9 g of nitrogen per day. There was no apparent difference in nitrogen retention as calories decreased from 4350 to 3550 per day, but further restriction caused a downward trend, reaching a negative balance when the subject received 2750 cal/day.

Fig. 5 depicts the effect of increased caloric intake on nitrogen retention in a malnourished child.

This child received a constant protein intake on a kilogram basis during the experimental period. Consequently, as he was rebuilding his protein stores his nitrogen retention should have been decreasing. This fact is observed routinely in our patients kept on constant nitrogen and calorie intakes (48). However, the increment of caloric intake to 200 cal/Kg/day produced a marked rise in nitrogen retention even above the levels observed at the initiation of therapy. This rise in nitrogen retention was not the consequence of increased nitrogen absorption, which was, as a matter of fact, constant.

Results of studies in experimental animals (5) and men (4, 6) have clearly indicated that, on a constant and adequate intake of nitrogen, nitrogen balance is dependent on the intake of calories. Likewise, protein intake is the determinant factor when intake of calories is constant and adequate. It follows that, when protein intake is not adequate, there is a level of calorie intake beyond which, increasing it without protein or increasing protein without calories, no changes or benefits take place.

This point need not be stressed further, since nitrogen retention depends greatly on the adequacy of caloric supply (35), with a caloric deficit producing a decrease in adiposity, lean body mass (36), and nitrogen retention (16, 35). The problem arises, however, on the effect of caloric intakes above those required on nitrogen retention. Theoretically, the growth of children who are well nourished and between the ages of 1 and 4 years should require less than 25 mg/Kg/day of nitrogen from an ideal protein, which is about 12% of the total protein requirement. Children who are fully recovered from malnutrition reach the expected creatinine height index of about 1.0 with adequate therapy and do not exceed it if kept on a high protein intake. This fact indicates a limit to their protein mass imposed by their height, strongly suggesting that protein apposition in lean tissue follows their growth in length (32). Contrary to this, recovered children often become obese if a high caloric intake is maintained. Consequently, it can be postulated that any nitrogen retained as a consequence of a high caloric intake, above that required for maintenance and growth, will either be lost through insensible losses (tegumentary losses) or will become part of the body protein, most probably "labile protein," and/or of adipose-tissue protein.

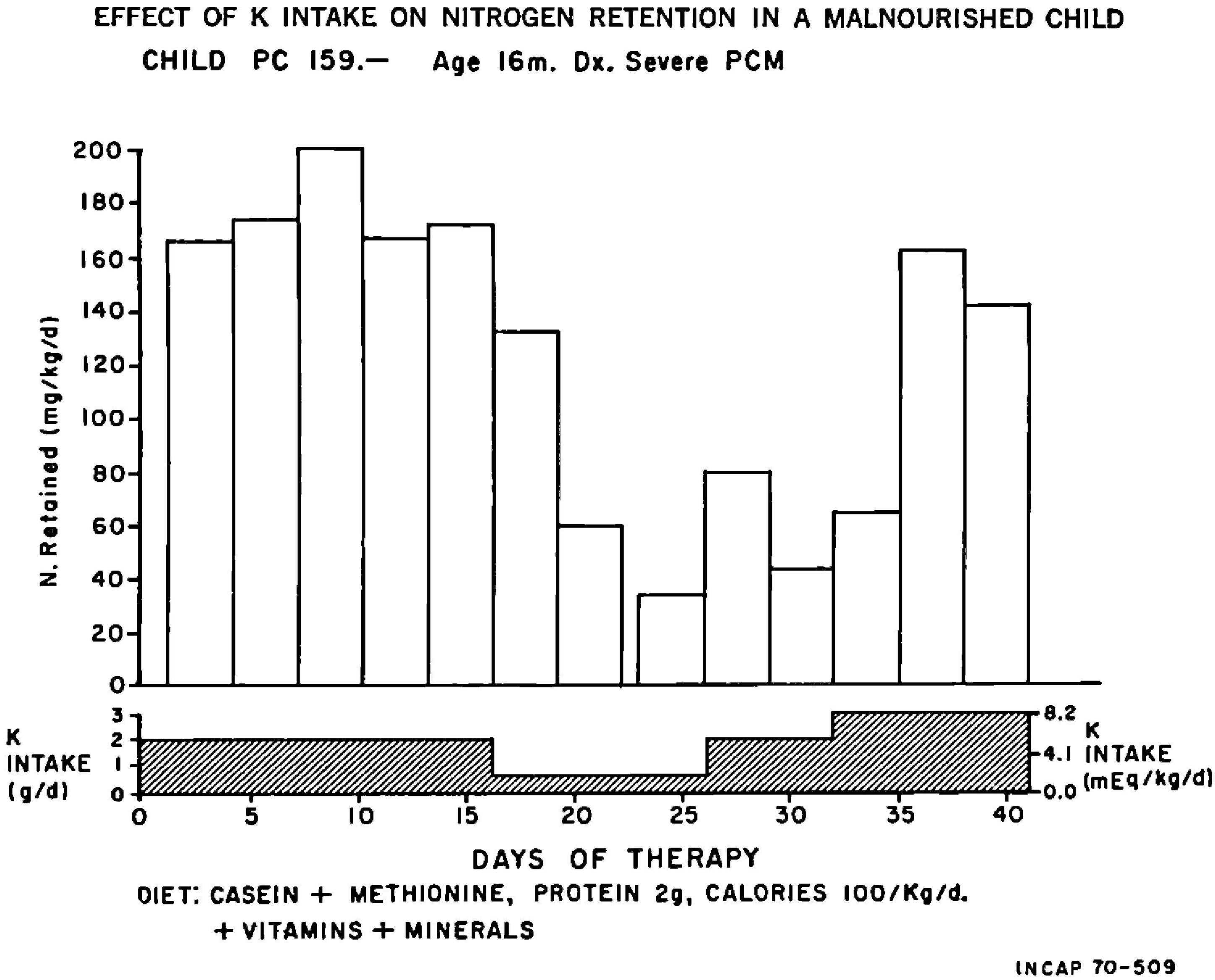


Fig. 6.

Tegumentary losses in children are unknown, but, various studies in adults, particularly when sweating occurs, indicate that the tegumentary and sweat losses can amount to significant figures and that they vary in direct proportion to nitrogen intake (38). If the tegumentary losses of a child with minimal sweating are considered similar to those of adult women (39), a child with a surface area of 0.3 m^2 can lose around 13 mg of nitrogen per kilo per day through the skin.

Sweating could easily increase these losses to twice the amount (40). In normal conditions, caloric expenditure and insensible losses tend to be higher with higher caloric intake.

The "labile protein pool" is a body compartment into which excess protein can be deposited as a consequence of excessive caloric intake, if protein intake is above requirements (41, 50). In children, this probably amounts to between 2 and 3% of the total body proteins (42). This, however, should not occur if the child is fully protein repleted and has been receiving an adequate diet prior to the tests designed to evaluate protein quality. Furthermore, since protein quality should be studied with a limited protein intake, the "labile protein pool" should not become significantly larger during the study.

Analyses of adipose tissue reveal a nitrogen content of around 1 g/100 g (37). Therefore, if a one-year-old child weighing 10 Kg is gaining weight rapidly (i.e., 500 g) during a study lasting 1 month, the nitrogen deposited in adipose tissue can amount to 26 mg/Kg/day, or essentially the same as that required for normal growth for a child that age.

The fact still remains that more nitrogen is retained in both humans and animals when protein and/or caloric intakes are increased above requirements (43, 44). In some cases (45), the excess retained nitrogen can be accounted for by increases in adiposity. In others, it can become part of the "labile protein pool" (46) or other nitrogen compartments (47).

These considerations should be kept in mind if one is to understand fully the significance of nitrogen balance studies in nonsteady-state situations.

Regardless of the magnitude and fate of the excess nitrogen retained under the rigorously standardized experimental conditions proposed, the results obtained by techniques employing nitrogen balance have yielded very reproducible and highly precise results which permit the de-

RELATIONSHIP BETWEEN THE CREATININE HEIGHT INDEX AND NITROGEN RETENTION

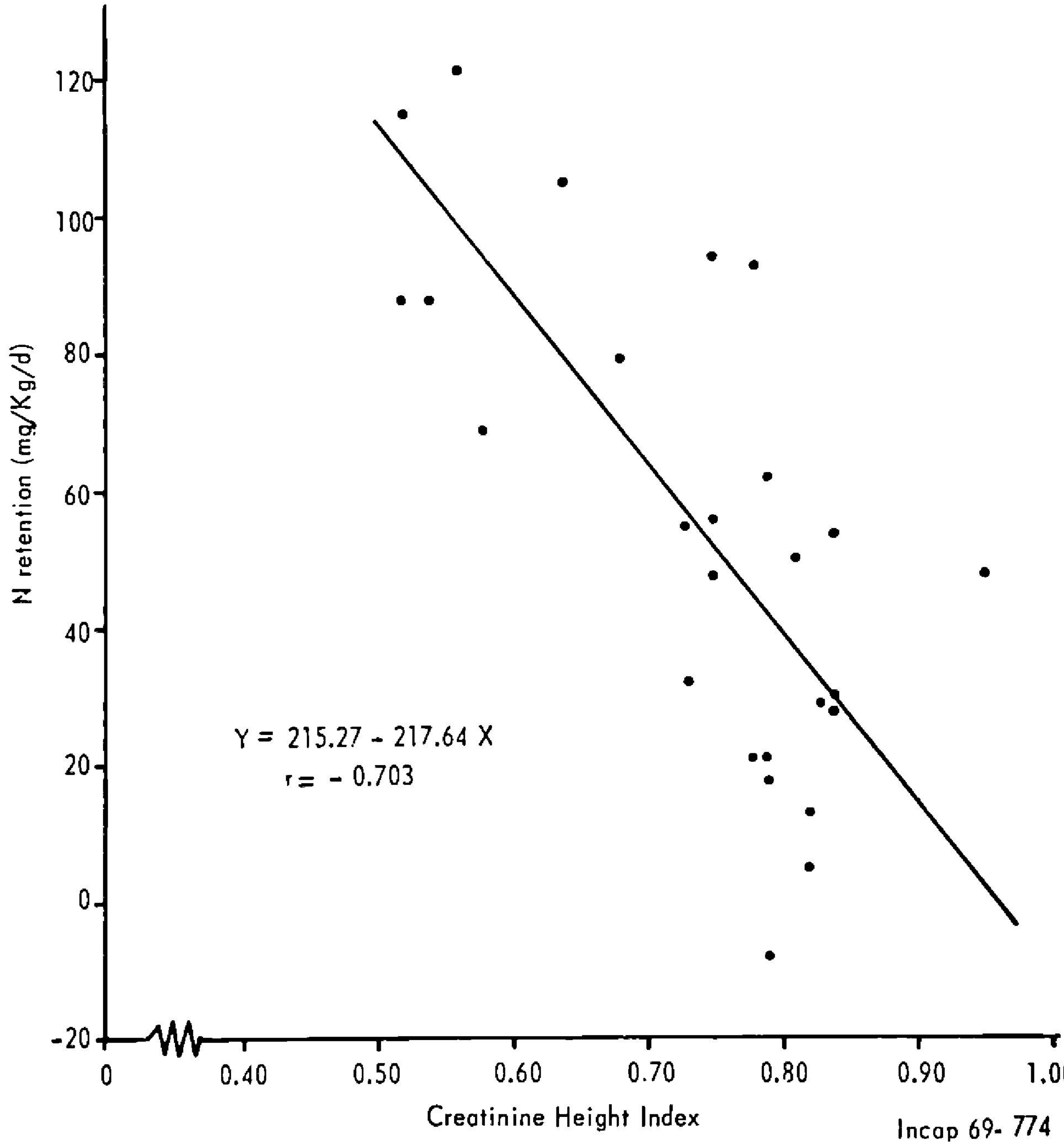


Fig. 7.

termination of protein quality.

C) As stated before, success in appraising the nutritive value of a protein depends on the basic condition that all other nutrients are supplied in adequate amounts, and the only limiting variable is protein.

Fig. 6 is a dramatic example of the importance to nitrogen retention of adequate potassium intake in a protein-calorie-malnourished child, who, because of a mistake on our part, received only a limited amount of potassium in one phase of the initiation of dietary therapy. When on a low potassium intake, nitrogen retention dropped significantly, reaching the expected retention of nitrogen again only when K supplementation was restarted at high levels and K repletion was attained. This case confirms the well established fact that K is retained in a constant ratio to nitrogen, in the absence of K deficiency, and that the retention on any one of these nutrients can limit retention of the other (33, 34).

Importance of the Nutritional and Health Status of the Subjects

That nitrogen retention is greater in protein-depleted subjects is amply substantiated from nitrogen-balance studies in protein-calorie-malnourished children. This fact assumes particular importance when the children on whom evaluation of protein quality is carried out by means of techniques involving nitrogen balance.

Fig. 7 shows that protein-calorie-malnourished children receiving essentially isonitrogenous diets retain greater amounts of nitrogen when their body protein mass, estimated by the creatinine height index (32), is low, and that nitrogen retention falls as the body protein mass recovers and finally reaches normality. Consequently, in children, it is highly desirable and,

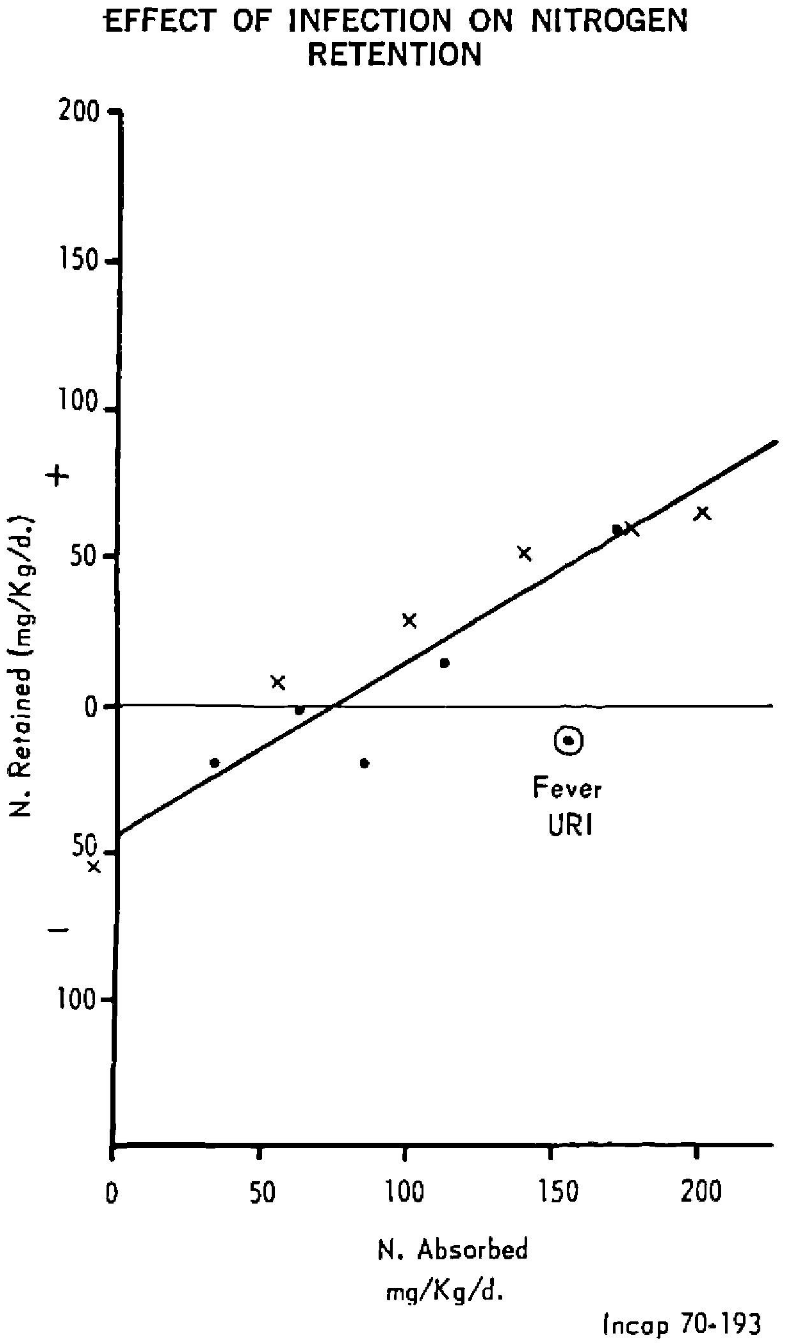


Fig. 8.

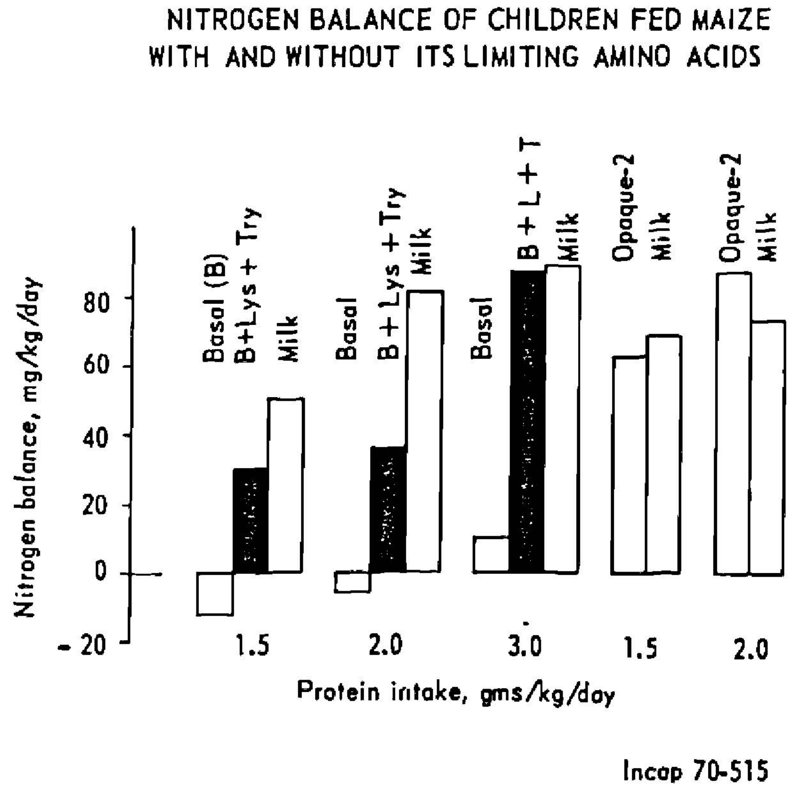


Fig. 9.

we would say, essential that proteins be tested only in fully protein-repleted children, particularly when the subjects have had severe protein depletion, as in our case.

Another important characteristic of the subjects is that they all maintain a healthy condition during the tests, since even a small upper-respiratory infection with mild fever can give faulty results. This is illustrated in Fig. 8.

Normally, following the infectious episode there is a period during which nitrogen retention is higher than normal. Therefore, studies should not be started immediately after the disease process, but a prudent period of one week should elapse before the study is reinitiated. Diarrhea may also alter the results, particularly if accompanied by fever, and not only as a consequence of decreased nitrogen absorption.

Application of Nitrogen-Balance Method

With all its limitations, the nitrogen-balance method has provided useful information in protein metabolism. There are, of course, many examples, and only a few will be given here.

One of the most usual applications is that concerned with the assessment of protein quality. A representative example is shown in Table 4, for various protein sources fed to young children (2). The results show differences in quality as indicated by the nitrogen retention values. Figures were higher for animal sources, some vegetable protein mixtures, and opaque-2 corn than for the other sources fed.

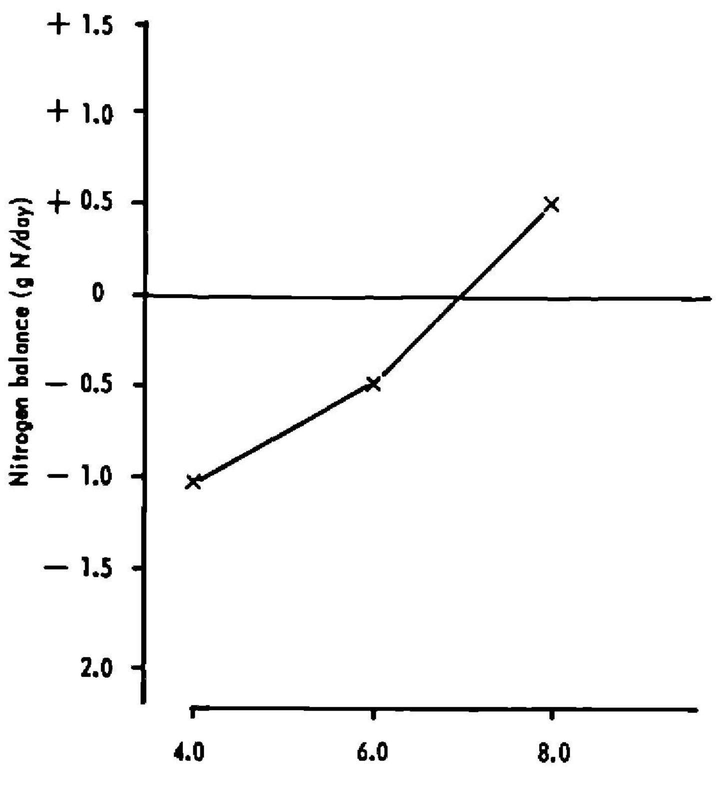
A second application is shown in Fig. 9, for the amino acid supplementation of corn (7, 8, 9). The results show the poor quality of common corn proteins even at high nitrogen intakes, in comparison with opaque-2 corn or milk.

TABLE 4. Nitrogen balance of children fed various protein sources.

Protein source	NI	FN	UN	NA	NR
	mg/Kg/day				
Milk	225	42	132	184	52
Egg	239	27	142	212	69
Cottonseed-corn	251	81	127	170	43
Soybean-corn	230	69	96	161	65
Cottonseed-soybean corn	234	54	131	178	48
Opaque-2 corn	238	68	107	170	63
Rolled oats	251	49	164	202	38
White rice	235	38	157	197	40

Intake of calories: 90–100 Kcal/Kg/day.

AVERAGE NITROGEN BALANCE OF SUBJECTS FED AT 3 LEVELS OF NITROGEN FROM WHITE DEGERMINATED CORN MEAL

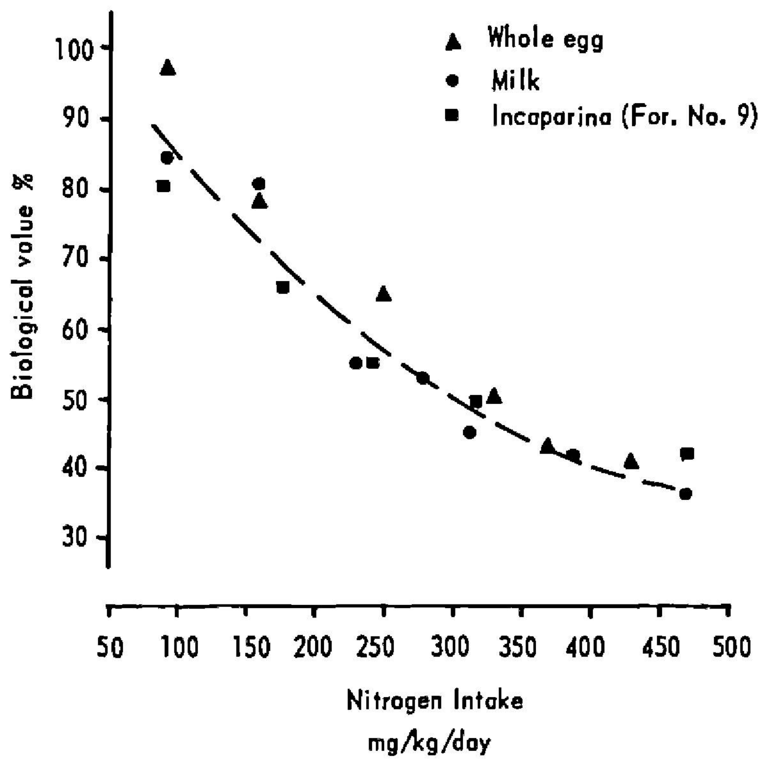


Kies et al. J. Nut. 86: 350, 1965.

Incap 70-516

Fig. 10.

EFFECT OF NITROGEN INTAKE ON BIOLOGICAL VALUE (CHILDREN)



Bressani, Viteri, Wilson y Alvarado (INCAP)

Incap 70-517

Fig. 12.

The data also show the improvement obtained when common corn proteins are supplemented with lysine and tryptophan at various levels of nitrogen intake, the benefits being higher at higher intakes of corn protein nitrogen.

Similar applications, as well as others, have been given to the method with adult human subjects. For example, Kies *et al.* (10, 11) used the nitrogen-balance method to study the effect of nonspecific nitrogen supplementation on minimum corn protein requirement and first-limiting nitrogenous factor for maintenance of nitrogen equilibrium of human adults. Representative results in Fig. 10 indicate that nitrogen retention of adult men fed isocaloric diets was significantly greater when white corn meal provided 8.0 g of N/person/day than when either 4.0 or 6.0 g of nitrogen were supplied from corn protein. The researchers also found that when 6 g of nitrogen from corn meal was supplemented with 2 g of nonspecific nitrogen, giving a total of 8 g/subject/day, optimal nitrogen retention levels could be re-established. The conclusion was reached that, for adult men, the first-limiting nitrogenous factor in corn protein is nonspecific nitrogen.

$$\text{B.V.} = \frac{\text{Ni} - (\text{Nf} - \text{Nm}) - (\text{Nu} - \text{Ne})}{\text{Ni} - (\text{Nf} - \text{Nm})} \times 100$$

Ni = Nitrogen intake (test protein consumed)
Nf = Fecal nitrogen (test protein)
Nm = Metabolic fecal nitrogen (protein-free diet consumed)
Nu = Endogenous urinary nitrogen (protein-free diet consumed)

$$\text{True protein digest} = \frac{\text{Ni} - (\text{Nf} - \text{Nm})}{\text{Ni}}$$

FIG. 11. Biological Value.

The method has also been used to study the minimum ratio of dietary essential amino acid to total nitrogen in various proteins (12, 13), amino acid supplementation and requirements (7,8,9) (14,15,16), stress and diet (17), nonspecific nitrogen intake on the adequacy of cereal proteins (10,11), evaluation of amino acid patterns (18,19), and others (20).

Biological Value

A fundamental and less variable measure of the nutritive value of a dietary protein was formulated in 1909 by Thomas, who proposed a method of utilizing information derived from nitrogen-balance studies for assessment of the quality of proteins. The biological value was defined as percentage of absorbed nitrogen retained in the body. This value can be expressed mathematically, as shown in Fig. 11. In 1924, Mitchell applied this principle in growing rats, and the procedure gave rise to what has since been called the Thomas-Mitchell Biological Value. Following this procedure, the biological value of the protein is defined as the percentage of the true dietary absorbed nitrogen which is retained in the organism. The biological-value determination takes into consideration the contributions of the metabolic and the endogenous nitrogen to total nitrogen, respectively measured in the feces and in the urine. Therefore, in the equation shown in Fig. 11, NI stands for nitrogen intake; NF fecal nitrogen; Nm fecal metabolic nitrogen; Nu urine nitrogen; and Ne endogenous urine nitrogen.

Theoretically, all the quantities in the equation should be measured in a single trial, and simultaneously. In practice, however, this is not possible, and the metabolic and endogenous fractions of the nitrogen metabolism are measured in separate trials in which experimental animals are fed nitrogen-free diets. This has been one of the serious problems in application of the biological value method, since obtaining good endogenous nitrogen values requires that experimental subjects be maintained on the protein-free diets for at least 8 days, a practice which should not be done routinely with children.

An alternative is to feed less than required levels of highly digested and utilized protein nitrogen, so as to obtain maximum utilization of the protein under study.

Since the method is based on nitrogen-balance techniques, the values obtained are affected by the same variables discussed previously. Fig. 12 shows an example of protein intake on biological value as determined in children for various proteins (2). As can be seen, biological value increased as nitrogen intake decreased. Proteins of higher quality, such as milk and eggs, had lower biological values at higher nitrogen intakes, with the value apparently increasing more as nitrogen intake decreased.

The method has been used to measure the biological value of protein foods for men by various workers. Representative values are shown in Table 5, for both animal and vegetable proteins (21,22,23,24,25). Obviously, the method separates proteins in relation to their essential amino acid content and balance.

In the biological-value method of assessing protein quality, as defined by Thomas and Mitchell, it is necessary to separate food from body nitrogen, which is probably the most important disadvantage in application of this method. The body nitrogen excretion, endogenous excretion, must be obtained without altering the physiological state of the animal or human subject. Various methods have been proposed, most of them involving short-time measurement of nitrogen excretion on a protein-free or low-nitrogen diet containing a protein of excellent quality and digestibility. If the nitrogen in the protein used is completely retained to maintain the nitrogen integrity of the animal, the excretion of urinary nitrogen represents the minimum protein requirement for maintenance, a requirement considered equal to the excretion of body nitrogen.

Murlin *et al.* (22), in 1946, found that body nitrogen excretion varied in man with: 1) the level of protein in the pre-experimental diets; 2) the position of the protein-free diet feeding in the series of periods; 3) the nature of the protein immediately preceding the protein-free diet and its level of intake; and 4) the conditions before feeding the protein tested, which

TABLE 5. Biological value of various proteins fed to human adults.

Protein source	BV (%)	Protein source	BV (%)
Milk	74	Egg albumen	91
White flour	41	Whole egg	94
Soy flour	65	Wheat gluten	42
Casein	69	Peanut flour	56
		Beef powder	67

Bricker *et al., J. Nut.* 30:269, 1945.
Hawley *et al., J. Nut.* 36:153, 1948.

TABLE 6. Endogenous urinary and metabolic fecal nitrogen excretion of nitrogen.

Subjects	Nitrogen (mg/Kg/day) Urine	Feces	Reference
Young adults	36.6	9.0	Young & Scrimshaw
Children, 39 m	57	24	INCAP
Infants	37	20	Fomon *et al.*
Children	48	25	Fomon *et al.*
Adults	29	11	Hawley *et al.*
Adults	31	—	Murlin *et al.*
Adults	46	13	Mueller & Cox

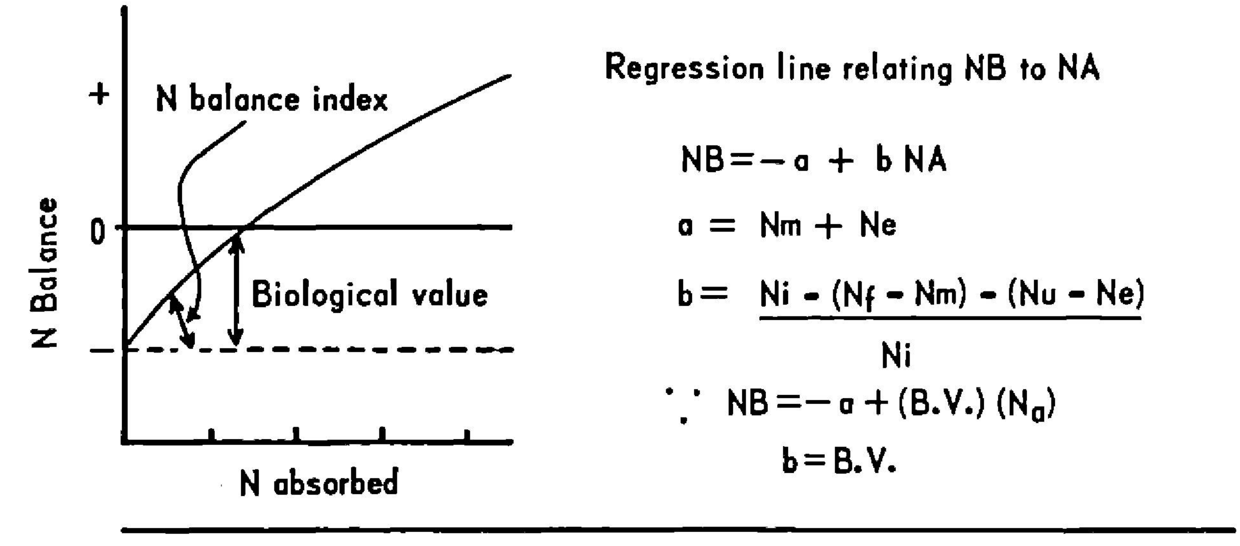


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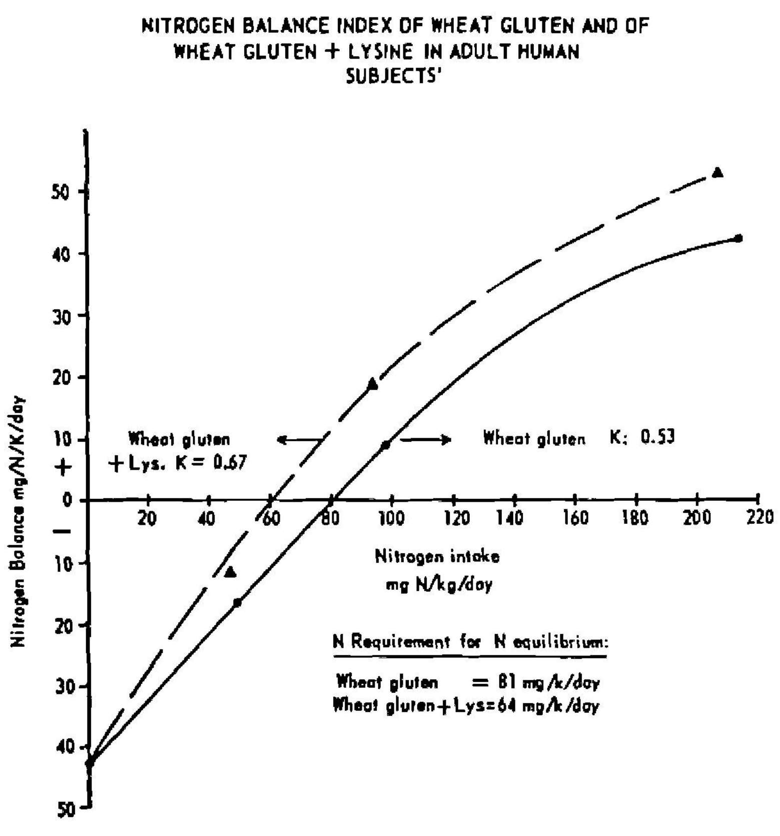
Fig. 13.

could alter the deficit of nitrogen before feeding the nitrogen-free diet. To these, must be added other factors such as the length of time of nitrogen-free feeding and the adequacy of calorie intake.

Body nitrogen excretion values for man are shown in Table 6 as determined by various workers (22,26,27,28).

These values should be interpreted with caution, however, since endogenous nitrogen excretions are probably less by subjects receiving protein-deficient diets than by subjects receiving adequate intakes of protein. The results differ importantly with the conditions of the study, particularly the amount of protein included in the protein-deficient diet, the duration of administration of the diet, and the prior intake of protein.



Hoffman & NcNeil (J. Nut. 38: 331, 1949.)

Incap 70-519

Fig. 14.

Nitrogen-Balance Index

Mitchell, in 1944 (24), and Allison, in 1949 (29), recommended the use of biological value as a useful assessment of the quality of different nitrogen sources. However, the method requires the estimation of metabolic fecal nitrogen and endogenous urinary nitrogen, which is not practical because the animal must exist on a nitrogen-free diet for a long period, which would render this condition nonphysiological.

Allison and co-workers (30) showed that, with highly standardized adult dogs, it was possible to plot absorbed nitrogen versus nitrogen balance to obtain a straight line. The relationship between nitrogen absorbed and nitrogen balance is linear in the region of negative nitrogen balance, the linearity extending over into the positive side but becoming obviously curvilinear well on the positive side of nitrogen balance. Fig. 13 gives the empirical equation for the linear portion of the relationship between NA and NB.

NB stands for nitrogen balance, NA for absorbed nitrogen, *a* for the total excretion of nitrogen on a protein-free diet (endogenous

RELATIONSHIP BETWEEN NITROGEN INTAKE AND NITROGEN ABSORBED TO NITROGEN RETENTION IN CHILDREN

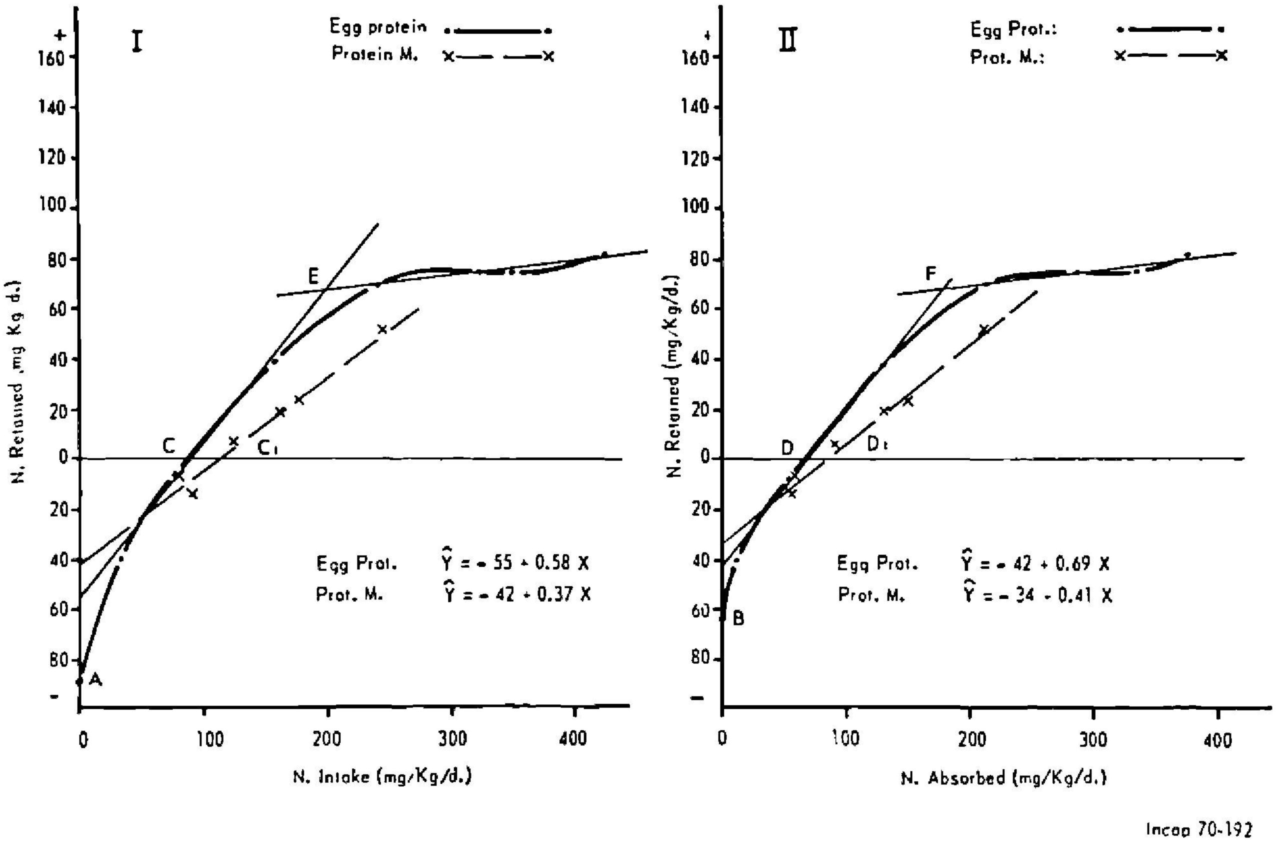


Fig. 15.

urinary and metabolic fecal), and *b* for the slope of the line or the rate of change of NB with respect to absorbed nitrogen, this being one measure of BV. The relationship between the slope *b* and BV can be derived from accepted equations defining nitrogen balance, absorbed nitrogen, and biological value.

Hoffman and McNeil have used this method

REPRODUCIBILITY OF THE NITROGEN BALANCE INDEX IN CHILDREN

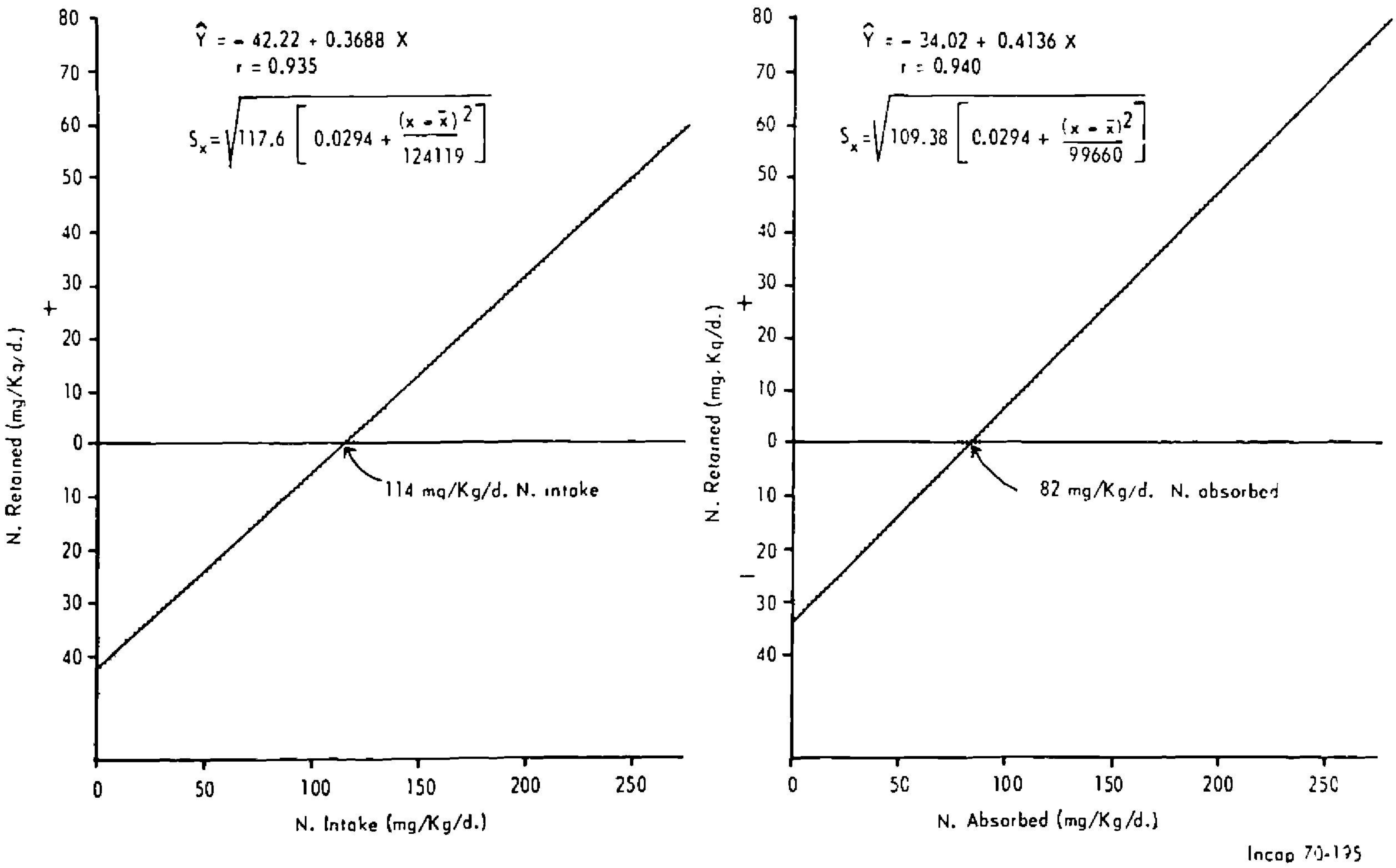


Fig. 16.

with adult human subjects, as shown in Fig. 14 (31). It can be seen that, in the region of negative and slightly positive NB, the relation between NA and NB is a straight line but that intakes of nitrogen above 100 mg/Kg/day give a curvilinear response. The nitrogen-balance index is 0.53 for wheat gluten, and 0.67 for wheat gluten supplemented with lysine. The respective nitrogen requirements for nitrogen equilibrium for wheat gluten and wheat gluten plus lysine are 81 and 64 mg/Kg/day.

The technique has also been applied to young children. An example is shown in Fig. 15 (49). This figure plots nitrogen retention values against nitrogen intake and nitrogen absorbed for two experiments in which children who were fully protein repleted, after having had severe protein malnutrition, received whole egg or a vegetable protein mixture as the only source of nitrogen. It is apparent that nitrogen retention in the case of egg protein breaks from a steep slope to a flat one at about 200 mg/Kg/day intake, and at about 180 mg N/Kg/day of absorbed nitrogen (points E and F). Furthermore, the slope at low intakes is steeper for this protein than for the vegetable protein mixture (protein M). As a consequence, nitrogen equilibrium is reached with lower levels of nitrogen intakes and nitrogen absorbed with whole egg than with protein M (points C and C_1, and D and D_1). The slopes of nitrogen retained vs. nitrogen absorbed indicate the biological value and differentiate the two proteins tested. Fig. 16 shows the high reproducibility of this method in fully recovered children under strict experimental conditions. The standard deviation around the calculated regression line is no greater than 3 mg of nitrogen retained/Kg/day at intakes between 50 and 200 mg/Kg/day.

The nitrogen-balance index method probably represents one of the best techniques available to determine the nutritive value of proteins. To be able to calculate the regression equations, the protein under study must be fed at levels of intake below and slightly above those resulting in nitrogen equilibrium, where there is a straight-line relationship between nitrogen absorbed and nitrogen balance. It is not necessary to feed nitrogen-free diets. One disadvantage is the time needed to obtain nitrogen retention values at three or four levels of nitrogen intake.

If the technique were applied under equal conditions to men and experimental animals, agreement in values between species would be better. Results of the method with rats, dogs, and children in our laboratory suggest very good agreement.

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