NITROGEN METABOLISM AND PUBLIC HEALTH

K. C. HOLEMANS and B. J. MEYER

Department of Physiology, University of Pretoria

Today, 30 years after Williams' observations on kwashiorkor, it is unanimously believed that this disease is a variety of the 'starchy-food dystrophy' known in Europe since 1906. It has been common knowledge for many years in tropical medical circles that kwashiorkor could be cured and, even better, prevented by an adequate nitrogen intake.

When we published the first nitrogen-balance studies on kwashiorkor patients 7 years ago,[1] it became obvious that in these patients neither the ability to absorb, nor

the capacity to retain sufficient amounts of nitrogen in their bodies, was impaired to any extent. In fact, the nitrogen retention, expressed either in absolute amounts, or as a percentage of nitrogen intake, was much higher than that observed in White children consuming liberal diets. Similar observations were subsequently made in many hospitals, both in Africa and in Latin America.

On the other hand, the brilliant studies of several South African workers, directed by Prof. J. F. Brock,[2] demonstrated conclusively that protein was the factor responsible for the prevention or cure of the disease.

Thus, both observations in clinical practice and laboratory studies provided convincing evidence that, if the diet of a population exposed to the risk of kwashiorkor was 'enriched' in protein, the disease would not occur. No wonder that, in African countries ruled by responsible governments, protein fortification of the diets has become a matter of public health concern.

The public health officer, obviously, is interested in 2 main questions: (1) how much protein should be supplied, and (2) what kind of protein should be supplied.

Both these questions have from time to time been answered by laboratory workers who considered *only* the results of short-term nitrogen-balance studies. In these balance studies, only one aspect was usually taken into consideration, viz. nitrogen retention as a function of nitrogen intake. The rationale in these studies was obviously the following: the best level of nitrogen intake and the best type of protein are those which lead to the highest nitrogen retention, for the ultimate goal in the prevention or cure of nitrogen deficiency is to keep the bodily stores saturated or, if depleted, to refill them.

It is the purpose of this paper to point out why the results of nitrogen-balance studies cannot be applied to clinical practice without careful consideration of all the factors contributing to these results. It will be noticed that not all these factors can be estimated in a sufficiently quantitative manner.

NITROGEN BALANCE

As far as nitrogen balance is concerned, nitrogen retention depends, *inter alia*, upon the following 3 variables: (1) nitrogen intake, (2) biological value of the protein used as a source of nitrogen, and (3) the degree of nitrogen depletion of the subject.

We should like to stress that on a given nitrogen intake, the degree of nitrogen depletion is much more important than the biological value of the protein. In protein-depleted children, viz. kwashiorkor patients, nitrogen retentions of 700 - 800 mg. per kg. per day are found on intakes of 1,000 mg. per kg. per day. In normal American children, Macy[3] and Stearns[4] noticed mean retentions of less than 100 mg. per kg. per day on similar intakes and with the same protein. Important differences of this order have *never* been recorded in studies where retention ratios for different proteins were measured. The only possible explanation is that the degree of nitrogen depletion is much more important as a determinant of the nitrogen retention than the biological value of the protein. An immediate consequence of this statement is that balance studies, conducted to establish the biological value of a given protein, must be performed on subjects in exactly the same state of nitrogen depletion. Failure to recognize this leads to the situation where nitrogen depletion, rather than biological value or level of intake, determines differences in nitrogen retention.

Undoubtedly, information has sometimes been supplied to public health officers concerning the quality of protein suitable for rehabilitation after starvation, where the self-evident influence of nitrogen depletion on the results of nitrogen-balance studies has been ignored. We may illustrate this with typical examples of each of the problems under consideration, viz. recommended amount of protein and recommended quality of protein.

A. Recommended Amount of Protein in Rehabilitation Diets

Keys *et al.*,[5] in their monumental study on human starvation, maintained adult subjects for several months on a low-calorie (1,570), low-nitrogen (8 G.) starvation diet. This resulted in a nitrogen loss of 3·1 G. per day. In order to study the ideal level of nitrogen intake for prompt rehabilitation, the nitrogen-depleted subjects were divided into 2 groups: group U with a nitrogen intake of 11 G. per day, and group Y with a nitrogen intake of 13 G. per day. After 6 weeks of this 'rehabilitation diet', nitrogen balances showed a higher retention in group U, both as a percentage of the intake *and* in absolute amounts of nitrogen retained. During the following 6-week period, the difference in nitrogen intake of groups U and Y was increased to 6 G. per day and, at the end of this period, the nitrogen balances, once again, showed a *higher* retention in the *lower-intake* group. According to Keys *et al.*,[5] high protein intake is of little or no value for the quick restoration of the body's nitrogen stores.

However, we cannot agree with this conclusion, since in the many nitrogen-balance studies performed in Africa and elsewhere in recent years, in no instance did the nitrogen retention decrease with increasing intakes. An alternative explanation for the observations of Keys *et al.* may be that the nitrogen deficit in the body stores was more pronounced after 6 weeks in the U-group, and these patients, therefore, retained more nitrogen. The higher retention cannot be regarded as a higher efficiency of the lower-intake levels, but rather as an expression of the higher degree of depletion. The therapeutic advice given by Keys *et al.* is wrong, because the important factor of the degree of nitrogen depletion was not taken into account. In more clinical terms, it can be said that the purpose of a rehabilitation diet is to restore normal conditions, including nitrogen equilibrium or a zero retention. The members of the Y group of Keys' experiments, who retained less at the end of 6 weeks on higher intakes, were closer to the nitrogen equilibrium than were the members of group U. Therefore, the higher intakes of group Y should have been recommended.

The foregoing example shows clearly how careful public health officers must be in putting into practice the results of experimental work, clinical or otherwise.

B. Recommended Quality of Protein in Rehabilitation Diets

De Maeyer and Vanderborght[6] tested 4 different proteins to assess their value in the cure of kwashiorkor. The results of this very careful study were published in

sufficient detail to permit a statistical analysis of the data. The retention-absorption regressions for these data show the following angular coefficient values: milk — 0·709, beans + peanuts — 0·595, peanuts alone — 0·533, and soybeans — 0·528.

Since the angular coefficients of these data are close to the biological values of these proteins (as defined by Allison in 1945), the authors claimed that the proteins could be classified in the same order with regard to their value in the treatment of kwashiorkor.

Since numerous studies are at present in progress to determine the value of alimentary protein, it is important to know the limitations of such methods. In this respect, we should like to stress the following points concerning the methods used and the conclusions based on the findings.

1. *Methods*

The number of balances required to obtain statistically significant differences in angular coefficients is quite high in order to compensate for the scatter of the results. In the study under consideration, 95 balances were insufficient to obtain statistically significant differences between the angular coefficients in the case of 4 proteins (analysis of variance and application of the 't' or 'F' test on the angular coefficient values).

But even if the statistical problem were solved, we think that it is meaningless to ascribe a higher or lower alimentary value to a given protein according to a higher or lower nitrogen retention found in balance experiments.

As we have pointed out before, the degree of nitrogen depletion of the subject is the most important single factor in determining nitrogen retention. Therefore, if nitrogen retention is to measure accurately the quality of different proteins, the degree of depletion should be identical in all the experiments. It is just about impossible to evaluate this condition, for there is no known method for the estimation of the degree of nitrogen deficit. If the necessary balances are performed on the same individual, it is unlikely that his state of nitrogen depletion will remain unchanged in the course of the experiments. The importance of the degree of nitrogen deficit in the determination of nitrogen retention is illustrated by the results obtained in different countries with the same protein (milk). Macy in America[7] found that less than 10% was retained, de Maeyer[6] in the Belgian Congo found a 62% retention, and Clegg and Dean[8] a 23% retention in Kampala. Taking these differences into consideration, one cannot attach much value to nitrogen retention as a measure of the quality of a protein.

2. *Conclusions*

If the angular coefficients were statistically significant, and all the subjects depleted to the same extent, would one be justified in using the retentions of short-term nitrogen balances as a measure of the alimentary value of protein? We do not think so, since various important properties of the protein under investigation in balance experiments will not be considered, e.g. its acceptability. It is obvious that a protein of which only 30% is retained may be better than another one with a 60% retention if the acceptability of the first, for reasons of taste, habit or religious beliefs, is more than twice that observed for the second.

To summarize, it can be said that, using the present techniques, the results of nitrogen balances in man are only loosely correlated with the practical alimentary value of protein. These results cannot be used to classify different proteins in a strict order of preference.

DISCUSSION

What then should be the advice of the nutritionist to the public health officer with regard to protein supplementation?

Once it is established that protein supplementation is desirable, we think our present knowledge about nitrogen balances permits the following statements.

1. *Amount to be Supplied*

Any protein supplementation, however small, is worth while. Indeed, nitrogen balances show clearly that protein-depleted people, consuming amounts of protein close to the minimum daily requirement, can store almost 100% of the ingested protein over and above the minimal requirements, until a higher and more adequate level of nitrogen metabolism is reached. For example, if 4 G. of protein are added to a daily intake of 40 G. it should not be regarded as a mere 10% increase, but as 4 G. of protein that can be stored daily over long periods of time.

Hence, if a limited supply of protein is available for distribution, as many people as possible should be supplied. Prophylactic increase of the protein intake above 14 - 15% of the total calories has no value. In therapeutic diets, however, and especially in children, 20% and more of the calories can be supplied by protein.

2. *Quality of the Protein to be Supplied*

Under normal conditions, a balanced dietary mixture of amino acids is achieved by varying the different sources of protein. We are of the opinion, therefore, that any protein can be used effectively for enrichment, apart from the protein which constitutes the main source of nitrogen in the non-enriched diet. For instance, enrichment of cassava flour with mealies gave excellent nitrogen retention in the Belgian Congo; in South Africa, however, one can hardly expect good results with this protein, for maize is the staple food in the local diet.

SUMMARY

Certain concepts concerning nitrogen-balance studies as a criterion of protein deficiency are discussed. The degree of nitrogen depletion itself as the main determinant of nitrogen retention and the implications of this fact, are stressed.

Certain suggestions concerning protein supplementation are presented.

REFERENCES

1. Holemans, K. and Lambrechts, A. (1955): J. Nutr., **56**, 477.
2. Brock, J. F., Hansen, J. D. L., Howe, E. E., Pretorius, P. J., Davel, J. G. A. and Hendrickse, R. G. (1955): Lancet, **2**, 355.

3. Macy, I. G. (1942): *Nutrition and Chemical Growth in Childhood*, vol. I: Evaluation. Baltimore: C. C. Thomas.

4. Stearns, G. (1956): In *Infant Metabolism*: Proceedings of the WHO Seminars held at Leyden and Stockholm in October and November 1950. New York: Macmillan.

5. Keys, A., Brozek, A., Henschel, A., Mickelson, O. and Taylor, H. L. (1950): *Biology of Human Starvation*. Minneapolis: University of Minnesota.

6. de Maeyer, E. M. and Vanderborght, H. (1958): J. Nutr., **65**, 335.

7. Macy, I. G. and Kelly, H. J. (1957): *Chemical Anthropology: a New Approach*. Chicago: University of Chicago Press.

8. Clegg, K. M. and Dean, R. F. A. (1960): Amer. J. Clin. Nutr., **8**, 885.