

## The availability of lysine in groundnut biscuits used in the treatment of kwashiorkor

By K. MARY CLEGG\*

*Medical Research Council, Infantile Malnutrition Research Unit,  
Mulago Hospital, Kampala, Uganda*

(Received 30 October 1959—Revised 5 February 1960)

Malnourished children under treatment in the wards of the Infantile Malnutrition Research Unit, Kampala, have for several years received biscuit meals made largely from groundnuts, with smaller quantities of maize and wheat flours, cottonseed oil, sugar and skim-milk powder.

The meals have been of two chief kinds. In the first, 8 or 15% skim-milk powder was cooked with the other ingredients, but in the second the powder was added after the other ingredients had been cooked and milled. The recipes were adjusted so that the total protein of the mixtures was always about 20%.

The value of the meals has been compared with that of a diet providing the same amount of protein entirely from milk. The milk diet appeared to be excellent in every way; its ability to produce changes in clinical condition and composition of serum, and to promote nitrogen retention, provided a useful set of standards. In a long series of experiments (R. F. A. Dean, to be published) it was found that the biscuit containing 15% skim-milk powder added before cooking, although fairly satisfactory, failed to produce the standard changes. On the other hand, biscuit of the other kind—with 15% powder added after cooking—was the equal of the milk diet in almost every way.

The most likely explanation of the differences in the results was that cooking the biscuit reduced the value of the protein. It has been known for many years that proteins from vegetable and animal sources may be damaged by heat. One of the suggested causes is a change in amino acids that makes them unavailable for metabolic processes, and there is evidence that lysine is easily susceptible to the change, probably by the blocking of its  $\epsilon$ -amino groups. From a series of feeding trials with rats, Block, Cannon, Wissler, Steffee, Straube, Frazier & Woolridge (1946) reported that cooking the mixed ingredients of a cake mixture reduced the protein efficiency, which was further decreased by toasting slices of the cake. The protein efficiency was restored by supplementing the baked and toasted cake with lysine. Carpenter, Ellinger, Munro & Rolfe (1957) found that the biological values of various proteins were closely correlated with the numbers of free  $\epsilon$ -amino groups of lysine present. The possibility of damage to the lysine of our biscuit meals was of particular interest because the

\* Present address: Ministry of Agriculture, Fisheries and Food, Experimental Establishment, Greyhope Road, Aberdeen.

total amount in the meals, including that from the skim-milk powder, was rather low; it was only 3.8–4.5 g/16 g N, about half the amount (7.8 g/16 g N) in the milk diets which contained skim-milk powder and calcium caseinate.

#### EXPERIMENTAL

##### *Composition and preparation of biscuits*

The composition of the biscuits used in the clinical trials is denoted by their code names, 8C and 15C, 8U and 15U. In this coding the numbers are the percentages of skim-milk powder, and the letters refer to its state (C, cooked; U, uncooked). The percentage composition of biscuits 8C and 8U was groundnuts 48, maize flour 20, wheat flour 8, cottonseed oil 4, sucrose 12, and skim-milk powder 8; and the percentage composition of biscuits 15C and 15U was groundnuts 41, maize flour 20, wheat flour 6, cottonseed oil 6, sucrose 12 and skim-milk powder 15.

All the ingredients except the milk powder were products of Uganda. The milk powder was spray-dried in the United States and was supplied by UNICEF. The groundnuts, which were of the Valencia variety, were bought shelled. They were not roasted, and their red skins were not removed: they were ground in a simple machine with grating action. The maize and wheat flours were both about 70% extraction, the sucrose was from sugar-cane, and the cottonseed oil was extracted by pressure at low temperature.

To make the biscuit, 6 kg of the ingredients were mixed with 700 g water and rolled into slabs 1 cm thick. The slabs were cooked in an electric oven at about 200° for 15 min on one side and for 5 min on the other. They were lightly browned by the cooking. The slabs of biscuit were usually stored for from 7 to 10 days and were then broken into small pieces, which were ground to a fine meal in a hammer-mill. When the skim-milk powder had to be added to the meal, it was stirred in by an electric mixer soon after the milling. The meals, irrespective of their kind, have been kept in unsealed containers without apparent deterioration for many months, but the meals used in the experiments here described were not more than 2 or 3 weeks old.

##### *Analytical methods*

Nitrogen was estimated by the micro-Kjeldahl procedure; the factor 6.25 was used for calculating protein. Lysine was not determined directly, but its concentration was calculated from tables of Harvey (1956) for the amino acids of proteins. No method for the determination of total lysine was available in our laboratory, but absolute values were of small importance.

The free  $\epsilon$ -amino groups of the lysine were determined as 'available lysine' by the chemical method of Bruno & Carpenter (1957). The method was applied not merely to biscuit meals 8C and 8U, 15C and 15U, but also to (1) the individual protein-containing ingredients, (2) various combinations of the ingredients, (3) mixtures in which the 15% of skim-milk powder in biscuits 15C and 15U was simulated by calcium caseinate and lactose, added to the basic ingredients separately or together.

In a further experiment 4% lactose (equivalent to the lactose in 8% skim-milk powder) was added to the basic ingredients before or after cooking.

As it was theoretically possible that the sucrose of the mixtures was responsible for some of the reductions found in 'available lysine', a biscuit meal was prepared according to the 15C recipe but without sucrose, and analysed for 'available lysine'.

The results have been expressed as g 'available lysine'/16 g N, to allow for slight variations in the percentage of N found in different batches of the material analysed. Two batches were made of each of the four biscuit meals (8C, 8U, 15C and 15U); each result quoted is the mean obtained from the analyses of at least two samples from each batch. All analyses were in duplicate.

So far as it is at present developed, the chemical method is not exact; in estimations on biscuit meals made in sextuplicate we have found the standard deviation to be  $\pm 9\%$ . Carbohydrate and histidine may interfere with the colour reaction (Bruno & Carpenter, 1957; Clegg & Davies, 1958; Carpenter, Jones & Mason, 1959). No allowance was made for the interference, because it seemed reasonable to presume that it would be constant.

## RESULTS

### *Ingredients*

The determination of the 'available lysine' in the protein-containing ingredients gave the apparent values: groundnuts 2.83, the mixture of maize and wheat flours 1.89, and the skim-milk powder 6.18 g/16 g N. The values for total lysine, calculated from Harvey's (1956) tables, were 3.9, 3.0 and 7.7 g/16 g N, respectively. Hence the apparent reduction in the lysine of the proteins was greatest in the cereals, intermediate in the groundnuts and least in the skim-milk powder. The 'available lysine' in the calcium caseinate, a preparation almost entirely free from carbohydrate, was 7.50 g/16 g N, which agreed well with the expected value of 7.9 g/16 g N.

For an uncooked mixture of the 'basic' ingredients—groundnuts, the two flours, cottonseed oil and sucrose, in the proportions used in the biscuits—the 'available lysine' was found by the chemical method to be 2.63 g/16 g N, which agreed with the figure of 2.78 g/16 g N obtained by calculation from the determined values for the individual ingredients. The chemical method, applied to the same mixture after it had been cooked, gave a value of 2.48 g 'available lysine'/16 g N. The figures of 6.18 g/16 g N for skim-milk powder, 7.50 g/16 g N for calcium caseinate and 2.48 g/16 g N for the cooked 'basic' biscuit meal were used to calculate the theoretical values for 'available lysine' in the mixtures. The theoretical values have been included in Table 1.

### *Biscuit meals 8C and 8U, 15C and 15U*

The results of the analysis of four biscuit meals used in the clinical trials are given in Table 1. They show that the cooking of the skim-milk powder with the other ingredients reduced the 'available lysine' by about 37% compared with the theoretical value, and that the percentage reduction was the same whether 8 or 15% of the powder was included.

*Mixtures with calcium caseinate and lactose*

The results are given in Table 1. When the calcium caseinate was added there was a small reduction (12%) in the 'available lysine'; when the lactose was added, there was a greater reduction (26%), and when the calcium caseinate and the lactose were added together, the reduction (34%) was equal to that found with the skim-milk powder. The reduction of 22% in the 'available lysine' in the meal with 4% lactose was approximately the same as when 7.5% lactose was added (result not shown in the table).

The reduction in the 'available lysine' in the biscuit meal made without sucrose (see p. 327) was nearly the same as that in biscuit meal 15 C which includes sucrose in the mixture (Table 1).

Table 1. 'Available lysine' in biscuits made from the 'basic' ingredients—groundnuts, maize and wheat flours, cottonseed oil and sucrose—with various test materials added uncooked or cooked with the ingredients

Test material in mixture	Test material uncooked		Test material cooked		Percentage reduction found $\frac{T_c - F_c}{T_c} \times 100$
	$T_u$ (g/16 g N)	$F_u$ (g/16 g N)	$T_c$ (g/16 g N)	$F_c$ (g/16 g N)	
Skim-milk powder, 8% (biscuits 8 U and 8 C)	2.83	2.82	2.85	1.79	37
Skim-milk powder, 15% (biscuits 15 U and 15 C)	3.19	2.96	3.26	2.07	37
Calcium caseinate, 5.9% (equivalent in protein to 15% skim-milk powder)	3.79	3.70	3.84	3.38	12
Lactose, 7.5% (equivalent in lactose to 15% skim-milk powder)	2.76	2.60	2.76	2.03	26
Calcium caseinate, 5.9%, + 7.5% lactose (equivalent in protein and lactose to 15% skim-milk powder)	3.97	3.50	3.99	2.63	34
Skim-milk powder, 15% (in mixture without sucrose)	—	—	3.64	2.50	31

$T_u$  and  $T_c$ , theoretical values calculated from summation of the values for the cooked 'basic' ingredients and the test material, and adjusted for the nitrogen content of the batch of biscuit meal (see p. 327);  $F_u$  and  $F_c$ , values obtained by chemical determination.

## DISCUSSION

If the validity of the chemical method is accepted, the results must mean that the cooking of the skim-milk powder along with the other ingredients of the biscuit mixtures reduced greatly the total available lysine: the reduction was about one-third, and it was the same whether 8 or 15% of the powder was used or when 15% of the powder was simulated by a combination of milk protein and lactose.

About two-thirds of the total lysine of the mixtures was in the plant-protein moiety and the rest in the milk; a loss of one-third of the whole does not itself indicate whether the plant protein or the milk protein was more affected, or, in terms of the chemical estimation, whether the free amino groups of lysine of the one or other protein suffered most blockage. It seems likely that both proteins suffered rather than

one. The result obtained by adding lactose—at two levels—to the ‘basic’ ingredients was a 22–26% reduction in the free amino groups of lysine, and the increase of the reduction to 34% when the milk protein was added may indicate that the free amino groups of lysine in that protein had also been reduced. The failure of the higher level of lactose to produce a greater effect than the lower level might be interpreted as meaning that the effect had reached its maximum.

Chemical methods for the determination of the free  $\epsilon$ -amino groups of lysine have been applied to fish meals (Carpenter *et al.* 1957), groundnut flour (Bensabat, Frampton, Allen & Hill, 1958) and bread (Clegg & Davies, 1958), but not previously, as far as I know, to any mixtures of foodstuffs, although all practical diets (except breast milk) consist of mixtures. It is probable that amino-acids other than lysine in our biscuits were made unavailable, but it seems reasonable to suppose that the alteration of the lysine was largely responsible for the poor results obtained when children were fed with the biscuits 8C and 15C.

The findings suggest that it would be worth while to re-examine the value of cooking skim-milk powder with various food-stuffs. The remedy for the damage we caused was obvious and easy, but that remedy would not always be applicable.

#### SUMMARY

1. The method of Bruno & Carpenter (1957), which measures ‘available lysine’ by estimating free  $\epsilon$ -amino groups, was applied to biscuit mixtures that have been used in the treatment of children with kwashiorkor in Uganda.
2. It was found that the cooking of skim-milk powder with the other ingredients of the mixtures reduced the ‘available lysine’ by about one-third. The damage was probably caused chiefly by the lactose of the powder.
3. It is suggested that the results could be correlated with clinical and biochemical findings in children treated with the biscuit mixtures.

This work was aided in part by a grant from the Food and Nutrition Board, National Academy of Sciences—National Research Council of the United States.

I am grateful to Dr R. F. A. Dean for his help in the presentation of the results.

#### REFERENCES

- Bensabat, L., Frampton, V. L., Allen, L. E. & Hill, R. A. (1958). *J. agric. Fd Chem.* **6**, 778.  
 Block, R. J., Cannon, P. R., Wissler, R. W., Steffee, C. H., Straube, R. L., Frazier, L. E. & Woolridge, R. L. (1946). *Arch. Biochem.* **10**, 295.  
 Bruno, D. & Carpenter, K. J. (1957). *Biochem. J.* **67**, 13P.  
 Carpenter, K. J., Ellinger, G. M., Munro, M. I. & Rolfe, E. J. (1957). *Brit. J. Nutr.* **11**, 162.  
 Carpenter, K. J., Jones, W. L. & Mason, E. L. (1959). *Biochem. J.* **73**, 11P.  
 Clegg, K. M. & Davies, N. (1958). *Proc. Nutr. Soc.* **17**, x.  
 Harvey, D. (1956). *Tech. Commun. Bur. Anim. Nutr., Aberd.*, no. 19.