BIOCHEMICAL EVALUATION OF THE NUTRITION STATUS OF URBAN PRIMARY SCHOOL CHILDREN : RIBOFLAVIN STATUS

J. P. DU PLESSIS, M.Sc., D. J. DE LANGE, M.Sc., *National Nutrition Research Institute*; AND S. A. FELLINGHAM, M.Sc., *National Research Institute for Mathematical Sciences, Pretoria*

Riboflavin was long known as vitamin G, the so-called growth factor, and has proved to be one of the most important members of the vitamin B complex. Riboflavin deficiencies very seldom occur alone, but are often found in conjunction with deficiency syndromes such as pellagra and kwashiorkor. A combined deficiency of riboflavin and protein is very often found in overpopulated countries, for example those of the Far East, and in underdeveloped countries such as exist in Africa and South America, where the intake of milk and other high-quality protein foods is low.

Although the Republic of South Africa is a highly developed country according to African standards, the indications are that a considerable part of the population is adapted to sub-optimal protein intakes. Latent riboflavin deficiency may therefore be fairly widespread, especially among the Bantu, and it is desirable to be able to demonstrate the existence and extent of such deficiencies so that remedial measures can be applied where necessary.

The riboflavin determinations done in the nutrition status survey on primary school children in Pretoria had two objects, namely: (*a*) to evaluate the suitability of various biochemical methods for providing criteria of riboflavin nutrition status, and (*b*) to assess the riboflavin nutrition status of the 4 main racial groups.

MATERIALS AND METHODS

The nutrition status survey on White children aged 7 - 11 years was carried out during the period April - June 1962, on a statistically representative sample drawn at random from a population of 17,980. For various reasons, including non-response, only 464 of the 600 children drawn were

actually included in the survey. The number of children finally included in each age-sex group varied from 42 to 54 of the 60 actually drawn.

The survey on Bantu children done from April to June 1963 was based on a statistically representative sample of 400 children drawn at random from a population of 18,098. Only 322 children were actually included in this survey, the number in each age-sex group varying from 27 to 38 of the 40 actually drawn.

The surveys on Coloured and Indian children, completed during April to June 1964, were based on 2 randomly drawn samples of 250 children each, representing 1,153 Coloured and 1,224 Indian children respectively. There were 228 Coloured children and 206 Indian children actually included in these surveys. The number of children in each age-sex group varied from 21 to 25 in the case of the Coloured children and from 17 to 25 in the case of the Indian children (30 actually drawn in each group).

The following biochemical determinations were carried out for the assessment of riboflavin nutrition status:

1. Urinary riboflavin excretion was determined by a composite method derived from that of the Association of Official Agricultural Chemists[1] and that of Jansen.[2] This composite method, extensively modified in our laboratory, has proved to be very reliable. It is based on the conversion of riboflavin into lumiflavin by ultraviolet irradiation in an alkaline medium. The lumiflavin is measured fluorimetrically. A description of the method will be published shortly.

2. The determination of red blood cell riboflavin, total serum riboflavin and serum flavin adenine dinucleotide (FAD) was done according to the method of Burch *et al.*[3]

3. Urinary creatinine was determined according to the alkaline picrate method of Peters.[4]

The purpose of the statistical analysis which was employed was to test whether children of different age, sex and racial groups came from the same statistical population in respect of the biochemical entities recorded and, if not, to demonstrate between which age, sex and racial groups the differences lay.

For this purpose, a 3-way analysis of variance, without the assumption of additivity, was applied to the data. This analysis tests the null-hypothesis of zero main effects, i.e. the hypothesis that all age, sex and racial groups are similar, without making the assumption that there is no interaction between the main effects of age, sex and race. This form of analysis presents special problems which are discussed by Scheffé[5] for the 2-way lay-out. The extension to the 4-way lay-out was contributed by Laubscher.[6]

Comparisons between combinations of all possible pairs of groups (each group consisting of children of the same age, sex and race) were further carried out according to the multiple comparison technique as discussed by Scheffé.[5]

Calculations were done on the IBM 704 computer of the National Research Institute for Mathematical Sciences of the Council for Scientific and Industrial Research.

RESULTS AND DISCUSSION

The biochemical results are given in the form of frequency distribution curves (Figs. 1, 2, 3 and 5). The 5th, 10th, 90th and 95th percentiles are also given. This type of presentation gives much more information than the means and ranges alone. The results of the analysis of variance tests in respect of race, age and sex and the multiple comparisons in respect of race are given in Table I. A 5% level of significance was applied in all tests.

TABLE I. THREE-WAY ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON TEST FOR DIFFERENCE DUE TO RACE, SEX AND AGE*

Variable	*Race*		*Sex*	*Age*
	Analysis of variance	*Multiple† comparisons*	*Analysis of variance*	*Analysis of variance*
Riboflavin/G creatinine	$P < 0{\cdot}1\%$	W B C I	$P > 5\%$	$P < 0{\cdot}1\%$
Red blood cell riboflavin	$P > 5\%$	W B C I	$P > 5\%$	$P < 0{\cdot}1\%$
Total serum riboflavin	$P < 0{\cdot}1\%$	W B C I	$P < 0{\cdot}1\%$	$P > 5\%$
FAD	$P < 0{\cdot}1\%$	W B C I	$P < 0{\cdot}1\%$	$P > 5\%$

* P values of 5% or less indicate a significant difference.
† W = White, B = Bantu, I = Indian, C = Coloured.
For the multiple comparisons tests, the convention of underlining all groups which did not differ significantly with a common line was followed.

In the early work on the riboflavin content of blood there was either no variation found, owing to the use of insensitive methods,[7] or the changes found could not be related to the riboflavin intake.[8] The development of methods which could differentiate between free riboflavin, riboflavin mononucleotide (FMN) and riboflavin adenine dinucleotide (FAD) opened up a new field of investigation. According to Burch *et al.*[3] the total riboflavin content of the red blood cells is the most sensitive criterion of the riboflavin nutrition status of the individual.

Red Blood Cell Riboflavin

The interpretation of the red blood cell riboflavin values obtained in these surveys is rendered difficult by the fact that so little work has been done in this field, especially on children.

A fairly extensive survey of red blood cell riboflavin content has been carried out by Beal and Van Buskirk[9] on children ranging in age from 3 days to 17 years. The mean values ranged from 34 to 21 μg./100 ml. red blood cells. These figures are open to criticism in so far as the 618 determinations treated as independent observations were carried out on only 68 children. Replicate determinations on the same child cannot be treated as independent observations.

The values obtained in the Pretoria surveys (Fig. 1) are much lower than those obtained by Beal and Van Buskirk,[9] the grand mean for these surveys being 13·16 μg./100 ml. red blood cells. At this stage no explanation can be found for this difference.

The analysis of variance showed a significant influence of age, but not of sex or race, on the red blood cell riboflavin values. There is reason to believe, e.g. on the basis of dietary evidence,[10,11] that a considerable difference exists between the riboflavin nutrition status of the White children and that of the other 3 racial groups. The fact that the red blood cell riboflavin values did not serve to show up any differences between the 4 racial groups suggests that this variable is not suitable for use as a criterion of riboflavin nutrition status.

Total Serum Riboflavin, Serum FAD and Serum Free Riboflavin plus FMN

At this stage the available evidence on the value of total serum riboflavin, serum FAD and the serum-free riboflavin plus FMN as criteria of riboflavin nutrition is very contradictory. According to Suvarnakich *et al.*,[12] the FAD values provide the best criterion of human riboflavin nutrition status. The serum FAD and the serum-free riboflavin plus FMN values are unaffected by recent riboflavin intake and by the nitrogen balance of the individual, which do affect the urinary riboflavin excretion. Suvarnakich *et al.* do not consider free riboflavin plus FMN to be a practical criterion of riboflavin nutrition status as the determination is difficult to perform, and the results show a skew distribution. This skew distribution of the free riboflavin plus FMN values also affects the total serum riboflavin values as the variations in the total serum riboflavin concentration are mainly due to variations in the serum-free riboflavin fraction.[3]

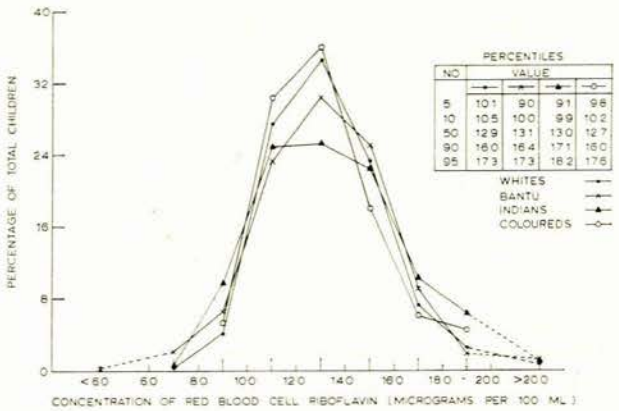

Fig. 1. Frequency distribution of red blood cell riboflavin values in Pretoria children of 7 - 11 years.

The work of Burch *et al.*[3] has shown that the serum FAD concentrations remain fairly constant, the differences due to a decreased riboflavin intake being not very pronounced.

In view of the disagreement among various authors on the merits of the different serum riboflavin fractions as criteria for riboflavin nutrition status, it was decided to determine total serum riboflavin and serum FAD in the Pretoria surveys with the intention of assessing the relative merit of each fraction. The results are given in Figs. 2 and 3.

The results of the statistical tests are exactly the same for the total serum riboflavin as for the serum FAD values. The analysis of variance showed a significant effect ($P < 0{\cdot}1\%$) of race and sex, but not of age ($P > 5\%$) in the case of both variables. The multiple comparison test in respect of the 4 races showed a significant difference (at the 5% level) between all combinations of pairs except Coloured and Indian children.

From the percentiles on the frequency distribution curves, the ranges between which 80% of the values lie can easily be determined. The ranges both for total serum riboflavin and for serum FAD agree very well with the ranges for normal adults found by Burch *et al.*[3] and Suvarnakich *et al.*[12] The ranges for the Bantu children are higher than those for any other racial group, although

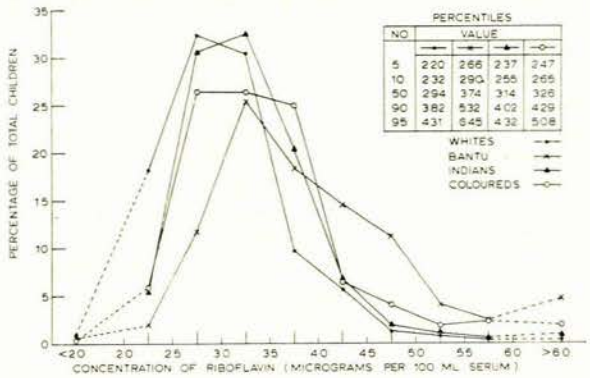

Fig. 2. Frequency distribution of the total serum riboflavin values in Pretoria children of 7 - 11 years.

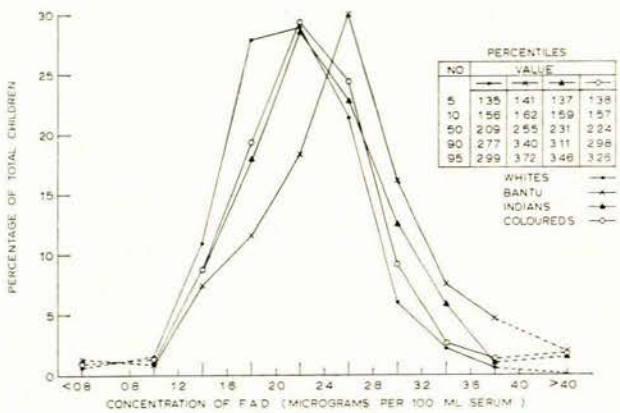

Fig. 3. Frequency distribution of serum FAD values in Pretoria children of 7 - 11 years.

still within the ranges found by Suvarnakich *et al.*[12] This anomaly, that the Bantu children with a lower riboflavin intake than the White children have the higher total serum riboflavin and serum FAD values, disqualifies these two variables as criteria of riboflavin nutrition status. There does not seem to be any relationship between riboflavin intake and the serum concentrations of these two variables.

Urinary Riboflavin

The urinary excretion of riboflavin was determined by means of fluorimetric techniques on 2-hour urine specimens taken from 9 to 11 a.m.—a diuresis having been induced with water. According to Tucker *et al.*,[13] diuresis does not influence urinary riboflavin excretion. We tested the relationship between urinary riboflavin excretion in 2 hours and that in 24 hours in 12 individuals, the results in both cases being expressed as μg. riboflavin/G creatinine. No significant difference was found between the two sets of results (unpublished data). The employment of such a short collection period is open to criticism,[14,15] but from the work of Hegsted *et al.*[15] it would appear that 2-hour urine specimens serve a useful purpose provided that the sample

size is large enough. This view is supported by our own work on 2- and 24-hour urine specimens. Plough and Consolazio[16] found that in large surveys the determination of riboflavin excretion per gram of creatinine in random urine specimens gave a satisfactory indication of riboflavin nutrition status.

As already indicated, the method we used for the determination of urinary riboflavin was a combination of those of the Association of Official Agricultural Chemists[1] and of Jansen.[2] This composite method (method I) was used for the survey on the White children in 1962. It was subsequently modified (method II) for the surveys on the Bantu, Coloured and Indian children. The query then arose whether the results obtained by these 2 methods were comparable. To investigate this aspect, the riboflavin contents of 31 two-hour urine specimens were determined according to both methods.

A linear regression line was fitted to the data. The values obtained with method II were taken as the independent variable and those obtained with method I as the dependent variable. The regression line is shown in Fig. 4

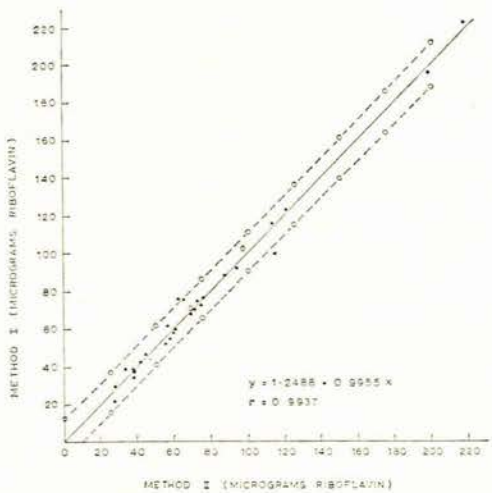

Fig. 4. Comparison of two methods for riboflavin determination.

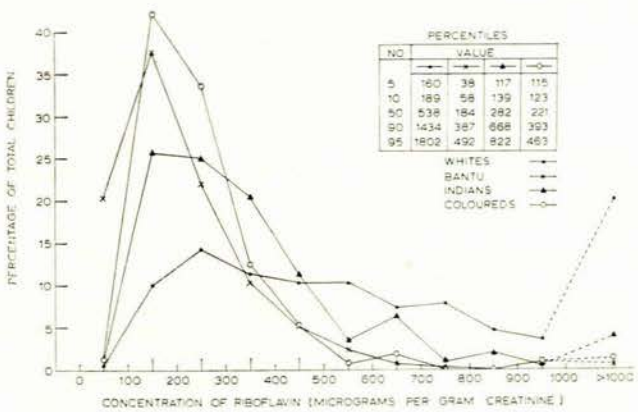

Fig. 5. Frequency distribution of urinary riboflavin values in Pretoria children of 7 - 11 years.

together with the 95% confidence limits. If the 2 methods were identical, one would expect the values to be scattered about the line $y = x$. The regression line which best fitted the data was $y = 1{\cdot}2488 + 0{\cdot}9955x$.

On application of the t-test, the constant term (1·2488) did not differ significantly from zero ($P>30\%$), nor did the regression coefficient (0·9955) differ significantly from 1 ($P>90\%$). The regression line is almost identical with the expected one and provides no justification for concluding that the two methods give different results.

When urinary riboflavin excretion is used as a criterion of riboflavin nutrition status, great caution must be exercised in the interpretation of the results, as there are various factors which influence the extent of urinary excretion. The state of nitrogen balance is one of the most important. Oldham *et al.*[17,18] found that in experiments with adult women, a highly significant negative correlation between riboflavin excretion and daily nitrogen balance could be demonstrated. Pollack and Bookman[19] found that a positive nitrogen balance was associated with an excretion of less than half, and a negative balance with an excretion of more than half of the riboflavin intake. In the interpretation of the riboflavin status of adults, the state of nitrogen balance should therefore be considered, as this may change from day to day. In the case of children, however, the effect of the nitrogen balance can safely be discounted, as only under exceptional circumstances will a negative nitrogen balance be found in healthy children.

Tucker *et al.*[13] found that sleep and short periods of heavy physical work decreased riboflavin excretion. In contrast, starvation for short periods (7 days), heat stress and enforced bed rest increased riboflavin excretion. These factors are, however, unlikely to have been operative in the Pretoria surveys.

In a very well-controlled study, Horwitt *et al.*[20] found that for subjects on a riboflavin depletion diet, the amount excreted in the urine was related to the riboflavin content of the diet and to the length of time that the diet had been taken. The amount of riboflavin excreted in the urine by individuals on diets containing riboflavin surpluses of varying extent differed considerably, but when the riboflavin intake was decreased these variations became smaller, and at very low levels of intake excretions were uniform. They found that no clinical signs of riboflavin deficiency occurred in male adults when the urinary riboflavin excretion was more than 40 μg./24 hours, corresponding to 20 - 30 μg./G creatinine.

It can be seen from the frequency distribution curves in Fig. 5 that the riboflavin excretions of the White children studied in the Pretoria surveys were considerably higher than those of the other 3 races. This finding is confirmed by the data for the dietary intake of riboflavin on the different racial groups.

The analysis of variance showed a significant influence of race and age ($P<0{\cdot}1\%$) but not of sex ($P>5\%$) on the urinary riboflavin values. The multiple comparisons tests in respect of race showed a significant difference between all combinations of pairs except Coloureds and Indians.

The Interdepartmental Committee on Nutrition for National Defense (ICNND)[21] has used riboflavin excretion/G creatinine as a means of judging riboflavin nutrition

status in many populations. Unfortunately the standards used by the ICNND are applicable to adults only, and Pearson[22] has therefore suggested a tentative guide for the interpretation of urinary riboflavin excretions of children in μg./G creatinine, which takes the age of the child into account (Table II).

TABLE II. A TENTATIVE GUIDE SUGGESTED BY PEARSON FOR THE INTERPRETATION OF RIBOFLAVIN EXCRETION IN μg./GRAM CREATININE BY CHILDREN

Age group (yrs.)	*Deficient*	*Low*	*Acceptable*	*High*
7–9	<85	85–269	270–500	>500
10–15	<70	70–199	200–400	>400

Multiple comparison tests in respect of age were applied to the data obtained in the Pretoria surveys in order to discover between which age groups the differences lay. These tests showed that no significant differences existed between the 7-, 8- and 9-year-old children nor between the 10- and 11-year-old children. A significant difference (at a 5% level) was found when values for 7-year-old children were compared with those for children of 10 and 11 years of age. These findings therefore confirm the validity of the age grouping used by Pearson[22] in his tentative guide.

Pearson's tentative guide was applied to the urinary riboflavin values obtained in the Pretoria surveys, and the results are shown in Table III. It would appear that low

TABLE III. DISTRIBUTION OF URINARY RIBOFLAVIN VALUES ACCORDING TO THE STANDARDS SUGGESTED BY PEARSON[22]

Category	*% of total White children*	*% of total Bantu children*	*% of total Indian children*	*% of total Coloured children*
Deficient	0·2	15·2	0·5	0·0
Low	16·2	52·4	35·9	57·1
Acceptable	25·2	25·8	44·6	37·3
High	58·5	6·5	18·9	5·7

riboflavin intakes occur in all 4 races, but are particularly prevalent in the non-White children.

CONCLUSIONS

In this study it was confirmed that riboflavin excretion/G of creatinine in 2-hour urine specimens provides a good criterion of riboflavin nutrition status in a population group. Red blood cell and serum riboflavin contents and serum FAD content proved unsatisfactory as the results obtained for these variables bore no relation to the riboflavin intakes of the different population groups.

As far as the riboflavin nutrition status of the 4 racial groups is concerned, it can be concluded on the basis of Table II that respectively 0·2, 15·2 and 0·5% of the White, Bantu and Indian children were in a deficient range, but no Coloured children. Among the Coloured children 57·1% fell in a low range, however, as did 16·2, 52·4 and 35·9% respectively of the White, Bantu and Indian children. It is therefore probable that in the age group 7 - 11 years between 16 and 68% of Pretoria children in the various racial groups would benefit from a higher riboflavin intake. This unsatisfactory riboflavin nutrition status is, in the non-White groups at any rate, probably due to a very low intake of milk in particular as well as of other high-quality protein foods.

SUMMARY

In nutrition status surveys conducted on Pretoria primary school children of 7 - 11 years it was found that of the different methods tested only riboflavin excretion per gram of creatinine in 2-hour urine specimens provided a satisfactory criterion of the riboflavin nutrition status of the different population groups.

Only in Bantu children did a significant proportion (15·2%) of the children have excretions falling into a deficient range. However, in 16·2% of the White children and 35·9 - 57·1% of the non-White children the excretions fell into a low range, and it is concluded that a substantial proportion of Pretoria children from all racial groups, but particularly the non-White groups, would benefit from a higher riboflavin intake.

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