BIOCHEMICAL EVALUATION OF THE NUTRITION STATUS OF URBAN SCHOOL CHILDREN OF 12 - 15 YEARS—RIBOFLAVIN STATUS

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Riboflavin forms an integral part of various enzymes essential for the maintenance of life in man. The importance of an adequate riboflavin intake for the promotion of growth and health can therefore not be overemphasized. A low riboflavin intake is usually associated with a low protein intake, and frank or latent riboflavin deficiency can therefore be expected to be widespread in countries where large parts of the population exist on sub-optimal protein intakes. As this appears to be the situation in the Republic of South Africa, it is therefore desirable that we should be able to recognize riboflavin deficiency and gauge its extent and severity.

The riboflavin determinations done in the nutrition status surveys on Pretoria school children had two objects, namely, to arrive at suitable criteria for the biochemical evaluation of riboflavin nutrition status and to assess the riboflavin nutrition status of the 4 main racial groups.

MATERIALS AND METHODS

Details of sample sizes and the dates of execution of the surveys are given in the paper on protein nutrition status published in this issue (page 509). The statisitcal planning of the surveys has been fully considered by Fellingham.[1]

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 partly from the method of the Association of Official Agricultural Chemists[2] and partly from that of Jansen.[3] This composite method, extensively modified in our laboratory, has proved to be very reliable. It is based on the conversion of riboflavin to lumiflavin by ultraviolet irradiation in an alkaline medium. The lumiflavin is measured fluorimetrically.
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.*[4]
3. Urinary creatinine was determined according to the method of Peters.[5]

The purpose of the statistical analyses 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 test between what age, sex and racial groups the differences lay. Details of the statistical methods used were given in a previous paper.[6]

Calculations were done on the IBM 704 computer of the National Research Institute for Mathematical Sciences, 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 4). The 5th, 10th, 50th, 90th and 95th percentiles are also given. The results of the analyses of variance in respect of race, sex and age and of the multiple comparisons tests in respect of race are given in Table I. A 5% level of significance was applied in all statistical tests.

Red Blood Cell Riboflavin

The possibility that red blood cell riboflavin concentration might serve as a criterion of riboflavin nutrition status, and the problems of interpretation resulting from the paucity of information in this field were considered by Du Plessis *et al.* in a previous paper.[6] As no noteworthy differences were found between the 4 racial groups, these authors concluded on the basis of results obtained for children of 7 - 11 years that red blood cell riboflavin did not provide a suitable criterion of riboflavin nutrition status.

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

Variable	*Race*		*Sex*	*Age*
	Analysis of variance	*Multiple comparisons†*	*(analysis of variance)*	*(analysis of variance)*
Riboflavin/G creatinine	$P < 0{\cdot}1\%$	WBCI	$P > 5\%$	$P > 5\%$
Red blood cell riboflavin	$P < 0{\cdot}1\%$	WBCI	$P > 5\%$	$P < 0{\cdot}1\%$
Total serum riboflavin	$P < 0{\cdot}1\%$	BWCI	$P > 5\%$	$P > 5\%$
Serum FAD	$P < 0{\cdot}1\%$	BWCI	$P > 5\%$	$P > 5\%$

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

In the present study on children of 12 - 15 years the results obtained for the White children were higher than those for the other 3 racial groups (see percentiles in Fig. 1).

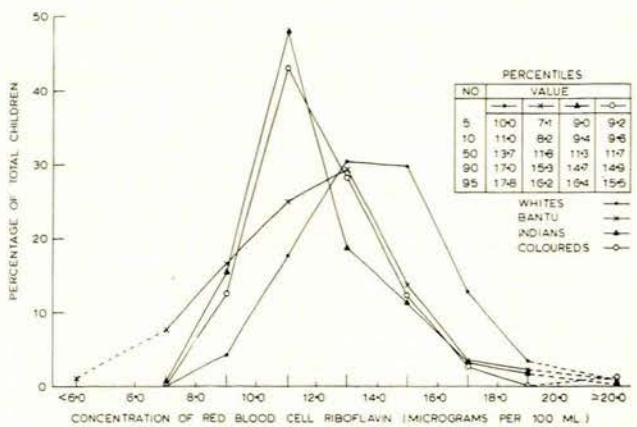

Fig. 1. Frequency distribution of red blood cell riboflavin values in Pretoria children of 12 - 15 years.

The analysis of variance in respect of race showed that the red blood cell riboflavin values of the White children differed significantly from those of the Bantu, Coloured and Indian children. No significant difference was found between the values for the 3 non-White races. Although significant, the differences between the values for the White and the 3 non-White races were small, and it would be extremely difficult to differentiate between children with deficient, low and acceptable riboflavin intakes on the basis of the present findings. The results obtained in this study were furthermore much lower than those obtained by Beal and Van Buskirk[7] for children ranging in age from 3 days to 17 years and by Burch *et al.*[4] for adults.

The results obtained in this study on the whole confirm the conclusion of Du Plessis *et al.*[6] that red blood cell

riboflavin concentration cannot be used as a criterion of riboflavin nutrition status.

Total Serum Riboflavin and Serum FAD

From Figs. 2 and 3 it is clear that the total serum riboflavin and the serum FAD values obtained for the Bantu children were higher than the values for any of the other 3 racial groups. The dietary intake studies[8,9] showed that the riboflavin intakes of the White children were appreciably higher than those of the Bantu children. The results

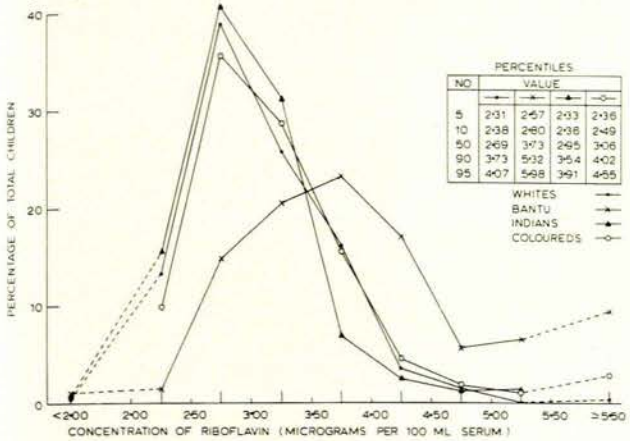

Fig. 2. Frequency distribution of serum riboflavin values in Pretoria children of 12 - 15 years.

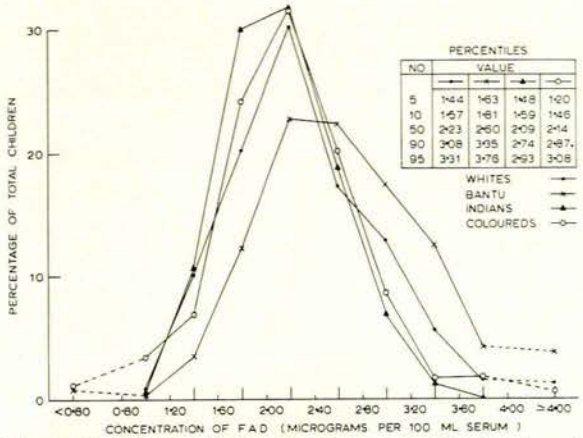

Fig. 3. Frequency distribution of serum FAD values in Pretoria children of 12 - 15 years.

for total serum riboflavin and serum FAD therefore appear to contradict the results of the dietary intake studies. This anomaly, that the Bantu children with the lower intake have higher total serum riboflavin values than the White children, was also found in the age-group 7 - 11 years.[6] The present study therefore confirms the conclusion of Du Plessis *et al.*[6] that total serum riboflavin and serum FAD are of no value in the recognition of latent riboflavin deficiency.

Urinary Riboflavin per Gram Creatinine

In a previous paper Du Plessis *et al.*[6] briefly discussed the suitability of 2-hour urine specimens for use in surveys of riboflavin nutrition status and the effect of various factors such as nitrogen balance, diuresis, sleep and work on the riboflavin excretion of the individual. The authors concluded that in the case of the Pretoria surveys these factors might safely be discounted, as they were either not likely to have been operative or else could have had no influence on riboflavin excretion (e.g. diuresis).

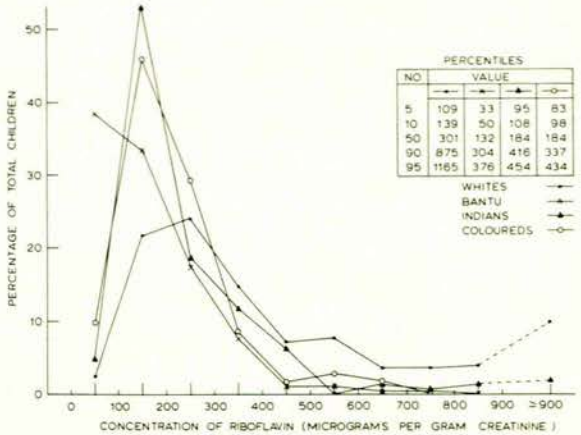

Fig. 4. Frequency distribution of urinary riboflavin values in Pretoria children of 12 - 15 years.

The frequency distribution curves of the values for urinary riboflavin per gram creatinine (Fig. 4) demonstrate the higher values found for the White children than for the children of the other 3 racial groups (see also the percentiles in Fig. 4). These results are in accordance with the dietary findings.[8,9]

The analysis of variance showed a significant influence of race ($P < 0{\cdot}1\%$), but not of sex or age, on the riboflavin excretion values. The multiple comparisons test in respect of race showed significant differences between all pairs of racial groups with the exception of (*a*) Bantu and Coloured and (*b*) Coloured and Indian.

Interpretation of the urinary riboflavin values per gram creatinine of the 4 racial groups according to the tentative scale suggested by Pearson[10] (Table II) showed that low riboflavin intakes probably occurred in all 4 racial groups, but were particularly prevalent in the non-White children.

TABLE II. DISTRIBUTION OF URINARY RIBOFLAVIN VALUES ACCORDING TO STANDARDS SUGGESTED BY PEARSON[10] FOR AGE-GROUP 10–15 YEARS

Category	*% of total White children*	*% of total Bantu children*	*% of total Indian children*	*% of total Coloured children*
Deficient				
< 70 μg./G creatinine	0·4	20·1	1·3	0·6
Low				
70–199 μg./G creatinine	24·6	51·7	56·9	55·2
Acceptable				
200–400 μg./G creatinine	39·1	25·1	30·6	37·9
High				
> 400 μg./G creatinine	35·9	3·0	11·3	6·3

This was also found to be the case in the children of 7 - 11 years.[6] Since respectively 25, 72, 56 and 58% of the White, Bantu, Indian and Coloured children could be classified as being in the low or deficient ranges, it is clear that riboflavin deficiency constitutes a problem of considerable magnitude among children of all 4 races in Pretoria.

Although no significant influence of age on urinary riboflavin excretion was found in the age-group 12 - 15

years, a significant influence was found in the children of 7 - 11 years. In Fig. 5 the influence of age on the riboflavin excretion of all children studied in both age-groups

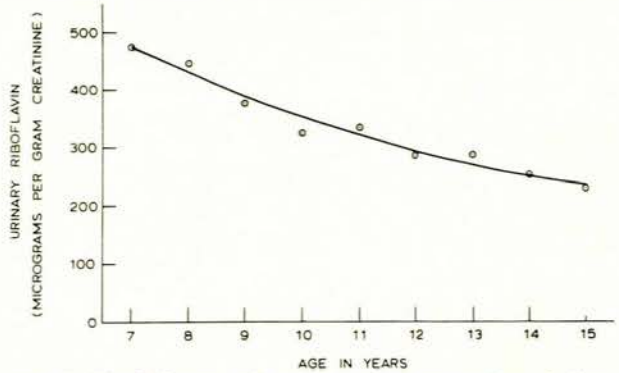

Fig. 5. The influence of age on urinary excretion of riboflavin per gram creatinine in Pretoria children of 7 - 15 years (all races).

(7 - 15 years) is illustrated. The graph represents the combined values of all 4 racial groups and thus gives a composite picture of the influence of age.

CONCLUSIONS AND SUMMARY

Red blood cell riboflavin, serum riboflavin and serum FAD all proved unsatisfactory as a means of recognizing latent deficiency in children of 12 - 15 years. The most satisfactory criterion of riboflavin nutrition status was provided by the urinary riboflavin excretions.

It can be concluded on the basis of the urinary riboflavin values (Table II) that respectively 0·4, 20·1, 0·6 and 1·3% of the White, Bantu, Coloured and Indian children were probably deficient in riboflavin. A further 24·6, 51·7, 55·2 and 56·9% of the children in the respective racial groups could be classified as falling into a 'low' range. A large proportion of children of all races, and particularly the non-White races, are thus in a sub-optimal state of riboflavin nutrition, and riboflavin deficiency is clearly one of the most important nutritional problems in Pretoria.

Our thanks are due to Dr. M. L. Neser for her assistance in the preparation of this paper.

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