THE CLINICAL AND BIOCHEMICAL EFFECTS OF RIBOFLAVIN AND NICOTINAMIDE SUPPLEMENTATION UPON BANTU SCHOOLCHILDREN*

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SUMMARY

Three samples of Bantu pupils selected at random were given supplements of roughly 0·5, 1 and 1·5 times the minimum daily requirements of riboflavin and nicotinamide and compared with another group, also selected at random, which received a placebo.

The results indicate a high incidence of detectable protein-calorie malnutrition as well as riboflavin and nicotinic acid deficiency. Supplementation with the minimum daily requirements of these vitamins resulted in a significant improvement when judged by clinical and biochemical criteria. A further experiment (previously published)[3] has been undertaken in which maize meal was supplemented in an attempt to confirm these preliminary results and also to test whether maize meal would be an effective vehicle for the vitamins.

Although the exact incidence of pellagra in South Africa is not known, it has been established that the disease occurs very frequently among the maize-eating Bantu population. During a recent Pretoria survey,[1] an analysis of the biochemical data indicated that there was a high incidence of subclinical deficiency of riboflavin and nicotinic acid among Bantu schoolchildren. A survey of rural and urban adult Venda males also showed biochemical evidence of subclinical nicotinic acid deficiency (unpublished data). It has, therefore, been shown that there is a need for an additional intake of riboflavin and nicotinamide.

Ideally pellagra should be prevented by improving the diet by means of natural foods. From a social, educational and economic point of view this would be virtually impossible to accomplish since this would entail drastic changes in the basic dietary habits of the Bantu population. It would, therefore, be much easier to add the two vitamins to a staple food such as maize which is known to be consumed in large quantities by the Bantu population.

In a publication *Food Enrichment in South Africa*[2] by the National Nutrition Research Institute, the basic principles which will ensure that an enrichment scheme rests on a sound scientific basis, are summarized as follows: evidence that there is a need for the nutrients to be added by enrichment; evidence that the enrichment of a staple food has advantages over the use of natural foods as a means of improving the diet; evidence that the amount of the enriching substance is sufficient to satisfy the proven need; proof that the enrichment medium is regularly consumed in significant quantities by those in need of an enriched diet; evidence that enrichment can be easily carried out in practice; evidence that the enriching substances or preparations do not appreciably alter the appearance, taste or physical characteristics of the medium.

In order to test the feasibility of supplementing maize with nicotinic acid and riboflavin in accordance with the above recommendations, two experiments were carried out.

The first experiment, described in this article, was designed to establish a level of supplementation which would effectively reduce the incidence of subclinical deficiency. It was thus necessary to find a group of children which showed a measurable degree of deficiency in respect of these two vitamins.

The second experiment, described in a previous article,[3] was designed to prove that the supplement was still effective when maize was used as the carrier and that a supplementation scheme was economically and technologically feasible.

MATERIALS AND METHODS

The experiment was carried out from September to November at a Bantu school near Pretoria. After written consent had been obtained from the parents, a random sample of 120 pupils from Standards 3 and 4 was drawn and divided into 4 groups of 30 pupils each. Group I served as a control and received a placebo, while the other three groups were given tablets of riboflavin and nicotinamide. The tablets were given every morning for 5 days each week for a total period of 12 weeks. A member of the National Nutrition Research Institute staff personally administered the tablets each day. The dosages for each group are shown in Table I. The quantities given in Table I are roughly equivalent to the minimum daily requirements suggested by a large number of workers.[4-8]

TABLE I. VITAMIN SUPPLEMENT

	Group I	*Group II* (½ *MDR**)	*Group III* (*1 MDR*)	*Group IV* (*1½ MDR*)
Riboflavin	0	0·5 mg	1 mg	1·5 mg
Nicotinamide	0	5·0 mg	10 mg	15·0 mg

*MDR = minimum daily requirement.

The pupils were clinically examined, weighed, their height measured, and a 3-hour sample of urine was collected from each. This was done before supplementation was introduced and repeated after 6 weeks and again after 12 weeks of supplementation. The second samples were collected while the pupils were still being given the supplements but the third samples were taken 10 - 14 days after the last tablets had been given. The urine specimens were examined microscopically for bilharzia and assayed biochemically for riboflavin, N^1-Me, 2-pyridone and creatinine content. The analytical methods were the same as those reported for the Pretoria Survey.[1] The weights and heights of both sexes were plotted against the Boston percentile ranges for boys.[9] The weights were also expressed as a percentage of the Boston 50th percentile values for boys and girls separately, in order to compare the percent

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of expected weight with the Goméz classification.[10] The results for the four groups were compared statistically in respect of the somatometric and biochemical measurements using the Kruskal-Wallis one-way analysis of variance followed by a multiple comparisons test.[11] Tests in respect of the clinical signs were carried out by applying the chi-square test to the appropriate contingency tables. All statistical tests were carried out at a 5% level of significance.

RESULTS

Weights and Heights for Age

Since there were no significant differences between the groups, the weights of the individual pupils in all 4 groups were plotted against the Boston percentiles as shown in Fig. 1. Sixty-six per cent fell below the 3rd percentile.

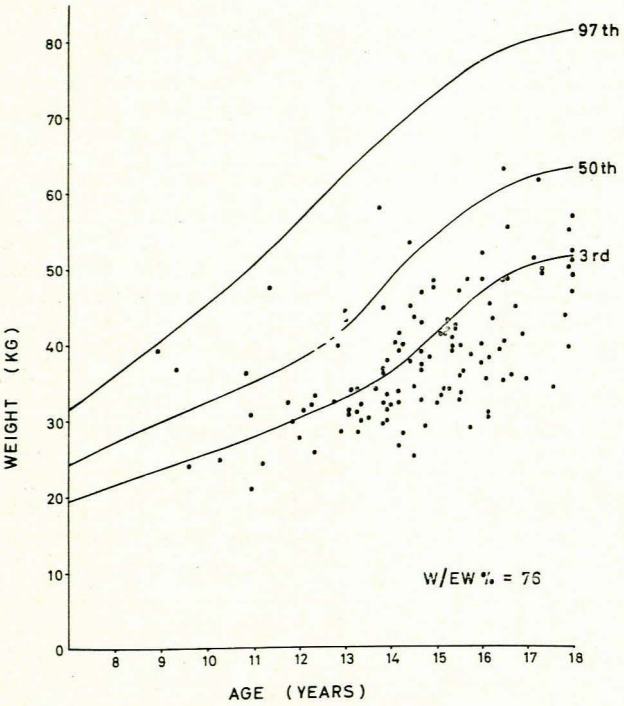

Fig. 1. Weight for age.

The mean weight was 76% of the expected. The ages of the pupils ranged from 9 - 20 years so that the sample actually consisted of children and young adults. Table II shows the degree of malnutrition by weight according to the Goméz classification. Only 14% of the children were 'normal' in weight while more than 50% had '2nd and 3rd degree' malnutrition. The height for age is demonstrated in Fig. 2. Fifty-eight per cent of the pupils were below the 3rd Boston percentile and the mean height was 92% of the expected normal. The measurements for height and weight showed very little change during the experiment and subsequent results are thus not given.

TABLE II. GOMÉZ CLASSIFICATION OF MALNUTRITION BY WEIGHT

	*W/EW %**	*Incidence*
Normal	>90	14
1st degree	90 - 76	31
2nd degree	75 - 60	44
3rd degree	<60	11

*W = actual weight; EW = expected weight according to the Boston 50th percentile.

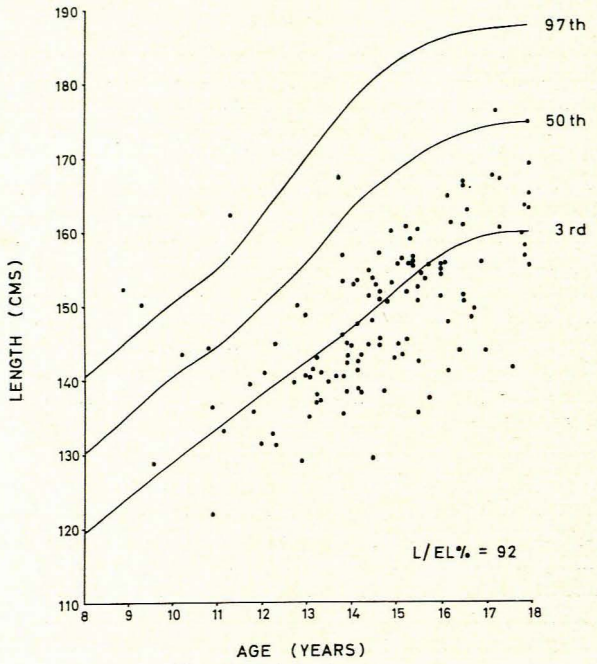

Fig. 2. Height for age.

Clinical Signs of Deficiency Disease

At the time of the first examination 4 pupils had overt clinical signs of pellagra. A further 14 had a history of pellagra, so at least 15% of the sample had suffered from pellagra at some time or other. The 4 pellagra patients were given the highest level of supplementation. After 6 weeks, skin lesions had disappeared in all of them. One month after completion of the trial, however, one had relapsed. The other 3 were not seen again.

A number of pupils showed signs compatible with a lesser degree of deficiency such as 'crazy paving' over the shins, areas of hyperpigmentation and desquamation of the skin, cheilosis and atrophy of the villi of the tongue. The incidence of these signs (present singly or in combination) and their course are demonstrated in Fig. 3. On admission and after 6 weeks the difference in incidence between the groups was not significant although there was apparently a progressive improvement from groups II - IV. After 12 weeks, however, there was a significant difference between the groups, the greatest improvement being shown by groups III and IV. Group I, which had had no dietary supplementation, showed very little change during the experimental period. One month after completion of the trial there was no clinical evidence of deterioration in the condition of any of the pupils.

For the sake of completeness it should be added that 45% of the pupils were found to be infested with bilharzia. Frank haematuria was present in 15% of the pupils with bilharzia ova in the urine and microscopic haematuria in a further 40%.

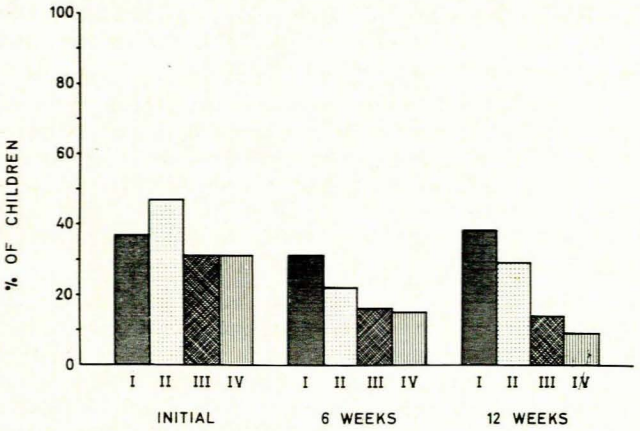

Fig. 3. Incidence of clinical signs of deficiency.

Biochemical Results

All the biochemical tests done on the urine showed essentially the same trends (Table III). The four groups were comparable before the trial. During treatment the placebo group showed very little change. In the three groups receiving vitamin supplementation, excretion of riboflavin and nicotinic acid metabolites showed varying degrees of improvement during supplementation and a marked deterioration when the supplements were no longer given.

The incidence of subnormal riboflavin excretion, as judged by the standards of Pearson,[12] is demonstrated in Fig. 4. Statistical analyses were carried out on the actual amounts found in the urine and not of the number of normal or abnormal excretions in respect of a given variable. Initially there was no significant difference between the four groups. After 6 weeks group I differed from all the other groups; group II was significantly different from group IV but not from group III; groups III and IV did not differ significantly from one another. The third analysis was done 10 - 12 days after the vitamins had been stopped and at this stage the four groups again showed no statistical differences. The marked deterioration since the second examination should be noted.

Figs. 5 - 7 illustrate the incidence of low values with regard to the excretion of nicotinic acid metabolites, namely N^1-Me, 2-pyridone and the 2-pyridone/N^1-Me ratio, each expressed per gramme of creatinine.

The following should be noted:

1. Excretions of less than 4 mg/g creatinine of N^1-Me and 2-pyridone and a ratio of less than one were considered to be below normal for the entire study.

2. The very high incidence of low values (i.e. biochemical evidence of deficiency) before supplementation.

3. In Figs. 5 and 6 there is little difference between groups III and IV after 6 weeks, while in Fig. 7 the ratio shows a higher incidence of deficiency in group IV than in group III.

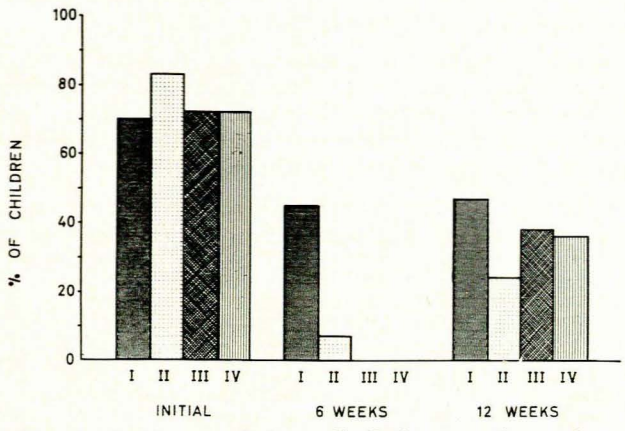

Fig. 4. Incidence of low riboflavin excretion values (<200 μg/g creatinine).

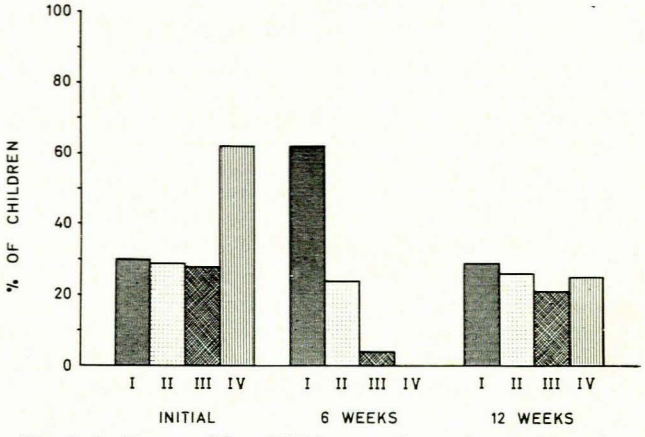

Fig. 5. Incidence of low N^1-Me excretion values (<4 mg/g creatinine).

TABLE III. MEAN VALUES FOR THE BIOCHEMICAL PARAMETERS

		Control group			*Experimental groups*								
		Group I			*Group II*			*Group III*			*Group IV*		
Variable	*Examination No.**	*Mean*	*SD*	*Sample size*	*Mean*	*SD*	*Sample size*	*Mean*	*SD*	*Sample size*	*Mean*	*SD*	*Sample size*
Riboflavin (μg/g creatinine)	1	175	176	30	172	172	30	155	100	29	178	100	29
	2	293	228	29	1 071	784	26	2 449	1 722	25	2 683	1 792	26
	3	267	243	25	304	317	22	261	149	23	275	122	22
N^1-Me (mg/g creatinine)	1	4·93	1·95	30	5·29	2·34	30	4·92	2·12	29	3·83	1·67	29
	2	4·17	1·86	29	7·53	4·39	26	10·18	4·80	25	10·72	4·49	26
	3	3·43	2·45	25	2·56	0·91	22	3·47	1·19	23	2·65	0·99	22
2-pyridone (mg/g creatinine)	1	2·71	3·21	30	2·34	2·27	30	2·05	2·48	29	1·40	1·22	29
	2	2·43	3·08	29	10·44	9·45	26	14·60	6·72	25	14·46	9·41	26
	3	1·11	2·71	25	0·82	1·94	22	0·77	1·85	23	0·25	0·69	22
2-pyridone/ N^1-Me ratio	1	0·49	9·43	30	0·43	0·40	30	0·34	0·35	29	0·35	0·29	29
	2	0·47	0·47	29	1·33	0·80	26	1·54	0·78	25	1·39	0·67	26
	3	0·21	0·42	25	0·24	0·47	22	0·16	0·38	23	0·10	0·22	22

*Examination No.: 1 = before supplementation; 2 = after 6 weeks of supplementation; 3 = 2 weeks after supplementation was stopped.

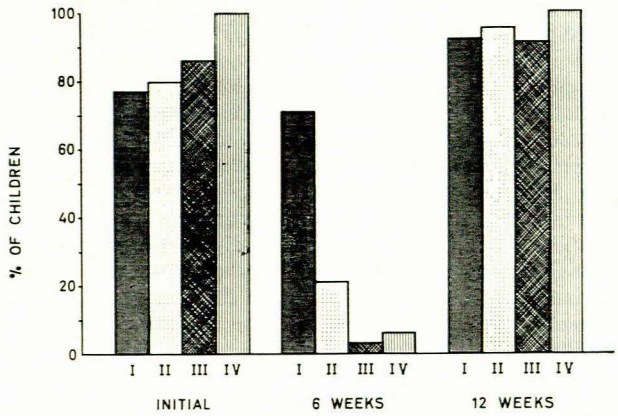

Fig. 6. Incidence of low 2-pyridone excretion values (<4 mg/g creatinine).

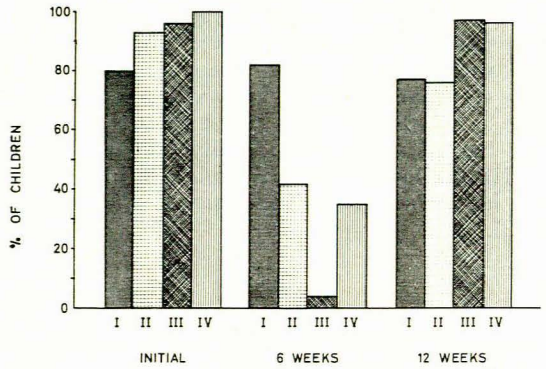

Fig. 7. Incidence of low 2-pyridone/N^1-Me ratios (ratios <1).

4. Supplementation at about the minimum daily requirement level (group III) appeared to be virtually sufficient to eradicate biochemically detectable deficiency.

5. The very marked deterioration after cessation of the supplement, i.e. at 12 weeks.

6. On statistical analysis the four groups did not differ either initially or after 12 weeks. At the 6-week stage, however, the control group was significantly different from the other three groups, which did not differ from one another.

DISCUSSION

Although the survey sample was representative of the pupils attending the school, it should not be considered representative of other schools even in the same district. This particular school was specifically selected because it was suitable for the survey.

The results indicate that the nutritional status was indeed poor. As judged by weight for age there was evidence of protein-calorie malnutrition in two-thirds or more of the children. Pellagra also was common. Although the lesions described in Fig. 3 are difficult to interpret accurately, the fact that they improved with treatment indicates that they were probably related to a deficiency of nicotinic acid and/or riboflavin. This observation is also supported by the biochemical findings. Compared with the standards of Pearson[12] and the Pretoria Survey,[1] the incidence of subnormal riboflavin and nicotinic acid status was extremely high. Some explanation is called for of the criteria used in interpreting the biochemical results.

Since existing literature does not provide a scale of interpretation of urinary excretions of N^1-Me and 2-pyridone during childhood, values below 4 mg/g creatinine for both variables were taken to be indicative of subclinical deficiency. The suggestion of De Lange and Joubert,[13] that a 2-pyridone/N^1-Me ratio of less than 1·0 is indicative of deficiency, was accepted.

It has previously been demonstrated that N^1-Me is relatively less affected than 2-pyridone excretion, even in overt pellagra.[14-16] From these results it is again suggested that 2-pyridone or the 2-pyridone/N^1-Me ratio gives a better indication of nicotinic acid status than does the N^1-Me excretion.[1] In this experiment N^1-Me showed a steeper rise during treatment than did 2-pyridone excretion. This partly explains the apparent discrepancy between groups III and IV with regard to the individual metabolites as compared with the ratio referred to in Fig. 5 - 7.

Both the clinical and biochemical results show that supplementation with riboflavin and nicotinamide at the level of the minimum daily requirement was sufficient to bring about clinical improvement and virtually to abolish biochemically detectable deficiency. In fact the children in group III were given somewhat less than the minimum daily requirements since the tablets were administered for only 5 days each week. Group IV, on a higher intake, had no measurable advantage over group III.

The very marked deterioration, even in group IV, after cessation of the supplements is not fully understood. It is possible that the period of supplementation was too short to allow for the establishment of adequate reserves in a group with fairly advanced depletion.

This study was undertaken at the request of the Secretary for Health. We are grateful for permission to publish; and we wish to thank the Department of Bantu Education for permission to work in a school under their control.

REFERENCES

1. Du Plessis, J. P. (1967): *An Evaluation of Biochemical Criteria for Use in Nutrition Status Surveys.* CSIR Research Report No. 261. Pretoria: CSIR.
2. National Nutrition Research Institute (1959): *Food Enrichment in South Africa.* CSIR Research Report No. 172. Pretoria: CSIR.
3. Du Plessis, J. P., Wittmann, W., Louw, M. E. J., Nel, A. and Laubscher, N. F. (1971): S. Afr. Med. J., **45**, 530.
4. Interdepartmental Committee on Nutrition for National Defense (1960): Publ. Hlth Rep., Wash., **75**, 687.
5. Davis, M. V., Oldham, H. G. and Roberts, L. J (1946): J. Nutr., **32**, 143.
6. Oldham, H. G., Sheft, B. B. and Porter, T. (1950): *Ibid.*, **41**, 231.
7. Horwitt, M. K., Hills, O. W., Harvey, C. C., Liebert, E. and Steinberg, D.L. (1949): *Ibid.*, **39**, 357.
8. Horwitt, M. K., Harvey, C. C., Hills, O. W. and Liebert, E. (1950): *Ibid.*, **41**, 247.
9. Nelson, W. E. (1959): In *Textbook of Pediatrics,* 7th ed., p. 50. Philadelphia: W. B. Saunders.
10. Goméz, F., Galvan, R. R., Frank, S., Cravioto, J., Chavez, M. R. and Vazquez, J. (1956): J. Trop. Pediat., **2**, 77.
11. Miller, R. G. (1966): In *Simultaneous Statistical Inference,* pp. 165 - 167. New York: McGraw-Hill.
12. Pearson, W. N. (1962): Amer. J. Clin. Nutr., **11**, 462.
13. De Lange, D. J. and Joubert, C. P. (1964): *Ibid.*, **15**, 169.
14. Goldsmith, G. A., Sarett, H. P., Register, U. D. and Gibbens, J. (1952): J. Clin. Invest., **31**, 533.
15. Horwitt, M. K., Harvey, C. C., Rothwell, W. S., Cutler, J. Z., and Haffron, D. (1956): J. Nutr., suppl. 1.
16. Walters, C. J., Brown, R. R., Kaihara, M. and Price, J. M. (1955): J. Biol. Chem., **217**, 489.