Effect of Enrichment of Maize Meal with Nicotinic Acid and Riboflavin upon the Vitamin and Protein Nutritional Status of Young School-Going and Pre-School Children

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SUMMARY

A series of experiments was undertaken to investigate the feasibility of enriching maize meal with nicotinic acid and riboflavin. First the necessary level of enrichment was established and then the efficacy of such enrichment of maize was tested in the field on children and young adults.

This report deals with the results of an experiment designed to establish whether such an enrichment scheme would have any detrimental effects on young growing children with marginal protein calorie malnutrition.

As in the previous experiments, a marked improvement in the nicotinic acid and riboflavin status was found in the experimental group. These biochemical findings were in conformity with the improvement in clinical status found. Moreover, such enrichment did not have any noticeable adverse effects. The hypothesis that vitamin enrichment could cause growth stimulation, and thus aggravate an existing marginal protein deficiency state, could not in any way be substantiated.

The enrichment of maize meal with riboflavin and nicotinic acid during milling has been shown conclusively to be highly effective in improving the vitamin nutritional status while at the same time not harming the protein nutritional status of young children. Since the previous experiments proved it to be economically and technologically feasible, it is now strongly recommended that such an enrichment scheme be introduced on a national basis with the least possible delay.

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At the request of the Department of Health of the Republic of South Africa, a series of experiments was undertaken to investigate the feasibility of enriching maize meal with nicotinic acid and riboflavin. The motivation for such enrichment has been dealt with in two previous publications.[1,2] In short, it was decided that an attempt had to be made to improve the nutritional status, with regard to the two most commonly occurring vitamin deficiencies (nicotinic acid and riboflavin), of a predominantly maize-eating community.

In the first experiment[1] the necessary level of supplementation was established and in the second experiment[2] the effect of such supplementation of maize was tested on children and young adults between the ages of 8 and 20 years. Although the results were clear-cut and encouraging, it was decided that a third experiment was necessary to investigate the effects of supplementation on younger children.

It is well known that there is a high incidence of protein calorie malnutrition in the South African Blacks and that both the incidence and severity of the deficiency is highest in young growing children. It has been suggested that vitamin supplementation alone could aggravate any existing protein deficiency by stimulating growth, especially in the young.[3]

This report deals with the results of an experiment designed to investigate the effects of the proposed supplementation on the vitamin and protein nutritional status of young children at school and children of pre-school age, since these were not covered in the previous experiments.

MATERIALS AND METHODS

In order to enrich maize meal for the experimental area, the Boyne Roller Mills (owned by the Zion Christian Church and entirely managed and operated by Blacks) were re-equipped with a microfeeder as was done in the

previous experiment.[2] The entire production of the mill was enriched at a rate of 1 mg riboflavin and 10 mg nicotinic acid per 400 g maize meal throughout the duration of the enrichment period (9 months). A township roughly 48 km north of Boyne was chosen as the control area since its inhabitants never obtained meal from the Boyne mills.

At schools in both the control and experimental areas a random sample of 70 children was drawn from all those in their first year at school. At the time of the first examination all children with a weight-for-age of more than 90% of the expected weight were discarded from the sample. This was done to obtain a sample of about 50 relatively underweight children in each group.

To obtain a random sample of pre-school children presented considerably more difficulties. Firstly, an enumeration was made of 200 - 300 households with children in the age group 2 - 6 years. Subsequently, a random sample of 120 children, not more than 1 per household, was drawn in both the experimental and control areas. Once again all children with a weight-for-age of more than 90% of the expected weight were discarded from the sample.

The procedure of obtaining permission to carry out the experiments and the enrolment of those investigated was the same as previously described.[2]

All children were examined clinically, weighed, and blood and urine samples were taken for analysis before the enrichment of the maize (August 1971), twice during enrichment (February and May 1972), and once again 3 months after enrichment was stopped (August 1972). It was originally intended to do a 3-monthly investigation during one calendar year but practical difficulties encountered in the field precluded the examination scheduled for November 1971.

In all instances blood was taken from the external jugular vein with the child restrained on a table in a supine position on a foam rubber mattress. Clinical data were recorded on a schedule precoded for direct transcription onto computer punchcards. On completion, biochemical data were entered on the same schedule.

The urine samples were preserved with oxalic acid and assayed for riboflavin, N^1-methylnicotinamide (N^1-Me), N^1-methyl-2-pyridone-5-carboxylamide (2-pyridone) and creatinine content. The analytical methods were the same as those reported for the Pretoria surveys.[4] The blood samples were allowed to coagulate, then centrifuged and the serum separated and frozen. Total protein was determined using the method of Weichselbaum,[5] and the protein fractions were separated by microzone electrophoresis and read on the Spinco-Beckman Analytrol.

The percentage of expected weight was obtained by expressing the weights as a percentage of the Boston 50th percentile for boys and girls separately.[6]

In addition to the above, a dietetic study was undertaken of subsamples of the experimental and control groups. An attempt was made to establish the eating habits and approximate intake of maize meal by means of a meal plan and recall questionnaire at a personal interview with the person concerned with the preparation of food for each household. A total number of 36 in the experimental group and 41 in the control group were investigated in this way, the investigation being based on availability of the responsible person and not on random sampling.

STATISTICAL ANALYSIS

The planning and design of this experiment and the data obtained from it are formally identical with those described by du Plessis *et al.*[2] In the present instance the main interest was focused on the protein levels occurring in the samples, and changes in these protein levels with time in both the experimental and control groups.

The procedure for statistical analysis is basically the same as described in the article quoted. In the current experiment, samples were obtained from two subsections of the population, namely the pre-school and school children. Results for these two groups are here treated separately.

Biochemical Measurements

Although, as in most biological experiments, a considerable number of concomitant variables were recorded in this survey, our attention was focused mainly on the four biochemical parameters: albumin, riboflavin (μg/g creatinine), N^1-Me (mg/g creatinine) and 2-pyridone (mg/g creatinine). We were mainly interested in simultaneous comparisons of these four variables in time. Hence, for the comparison of the control with the experimental groups, the significance level for individual tests was taken to be $0{,}05/16 = 0{,}003125$ in order to provide an over-all 5% protection against type I errors (i.e. of wrongly rejecting true hypotheses). In the case of pair-wise comparisons of surveys with one another, we have chosen $0{,}05/24 = 0{,}00208$ as the individual significance level, since we are doing 24 individual comparisons (keeping results for pre-school and schoolchildren and for experimental and control groups separate).

For the first-mentioned type of comparison we have used the Wilcoxon two-sample test for independent observations,[7] and for the comparison of surveys, the Wilcoxon matched pairs signed ranks test.[7]

Clinical Observations

The absence or presence of clinical signs was recorded and hence the data are available in the form of contingency tables. A test for differences in proportion was used to ascertain whether the incidence of clinical signs found during the various examinations for the experimental and control groups differed significantly. If individual significance levels were to be selected to provide 5% over-all protection against type I error, then, in view of the numerous signs considered, no significant differences would be revealed. Thus we have marked the relatively few cases where significant differences were obtained at individual 5% and 1% levels, in order to give some indication of the general pattern.

RESULTS AND DISCUSSION

It should be emphasised that the samples studied are not representative of the population of the areas concerned since children of normal weight were deliberately discarded from the random sample in order to obtain random samples of relatively underweight children in both areas. Children of normal weight were excluded because it was considered unlikely that vitamin supplementation of this order would have any measurable effect on their growth or protein status.

The enrichment technique was identical to the experiment previously reported and once again no technical problems were encountered.

Clinical Evaluation

No cases of overt kwashiorkor or pellagra were found in any of the groups. At the time of the first examination one child from the control group, who was suffering from gross marasmus and measles was referred to hospital and eliminated from the study. Some children had clinical signs compatible with early pellagra or a state of pre-kwashiorkor. These children were not, however, treated as a separate group, since the diagnosis could not be established beyond reasonable doubt. Children with mild to moderate forms of protein calorie malnutrition were deliberately included in the sample but, because of the sampling procedure, the incidence of this could not be evaluated.

The ages of the pre-school and schoolchildren were comparable for the experimental and control groups and no significant differences were found.

Lesions such as angular stomatitis, follicular hyperkeratosis and, to a lesser extent, cheilosis were relatively

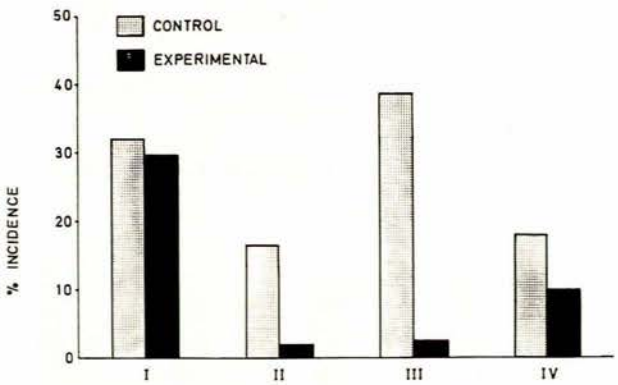

Fig. 1. Percentage incidence of cheilosis and/or angular stomatitis in pre-school children.

clear-cut. The frequency of angular stomatitis and cheilosis occurring in the groups was combined and the results are shown in Figs 1 and 2. There was a considerable fluctuation in the incidence of these lesions in the control group, presumably due to seasonal effects. In both pre-school and school-age children, the experimental and

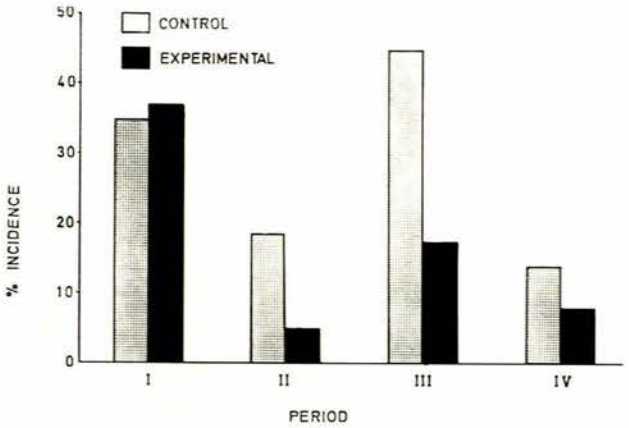

Fig. 2. Percentage incidence of cheilosis and/or angular stomatitis in schoolchildren.

control groups were comparable before enrichment was introduced (period I). During the period of enrichment (periods II and III) the decrease in incidence in the experimental group compared with the control group is obvious in both age groups. During the third period the difference in incidence between the control and experimental groups was significant at a 1% level. After withdrawal of the supplement (period IV), the results of the two groups were again comparable, although the change occurring in the control group was much more marked.

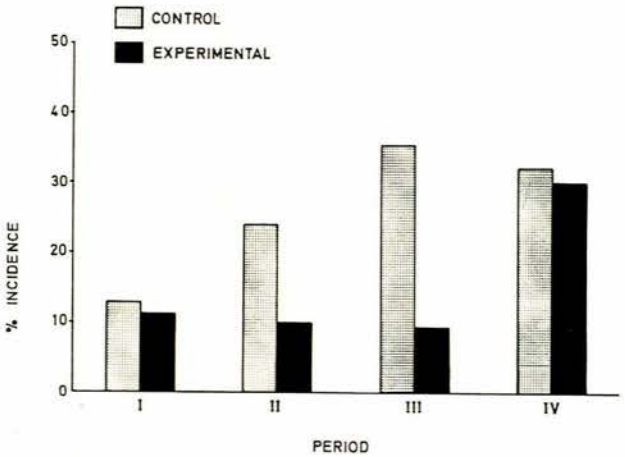

Fig. 3. Percentage incidence of follicular hyperkeratosis in pre-school children.

With regard to follicular hyperkeratosis, (Figs 3 and 4) the results were somewhat different in the school-age children but similar to the previous results obtained from the pre-school group. In the school group there was little fluctuation in the control subjects but a considerable fluctuation was observed in the experimental group, which showed an increasing incidence in the third and fourth periods compared with the second. There is no obvious explanation for the differences observed in the third

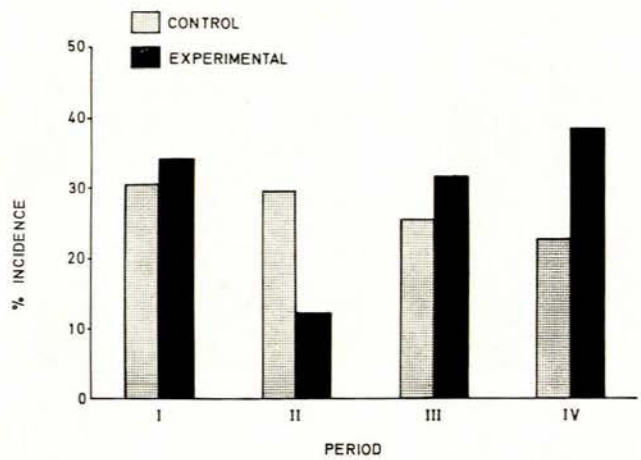

Fig. 4. Percentage incidence of follicular hyperkeratosis in schoolchildren.

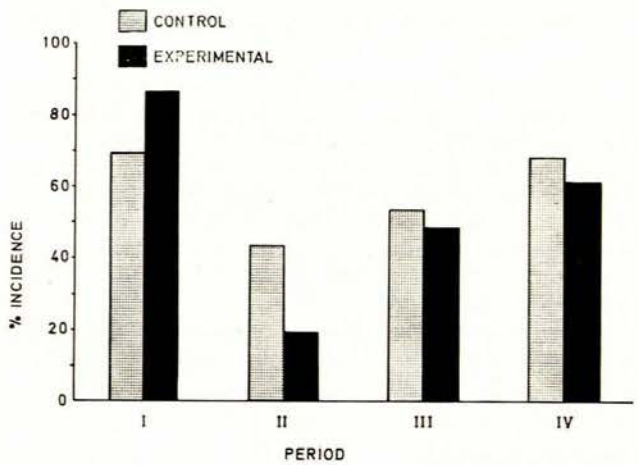

Fig. 6. Percentage incidence of skin lesions on arms and legs in schoolchildren.

period. In the third period, however, a significant difference at a 1% level in favour of the experimental group was found in the pre-school groups.

Lesser degrees of pigmentary changes, so-called crazy-paving and scaling of the epithelium, are much less easily detected and interpreted. There was a fairly high incidence of these lesions either singly or in combination. Although they could be compatible with a deficiency of either riboflavin or nicotinic acid, it must be remembered that the children are exposed to widely varying weather conditions, dust, sunshine and open fires which could, to some extent, influence the picture. These skin lesions occurring on the arms and legs were grouped together and the results are given in Figs 5 and 6. During the enrichment phase the experimental group appeared to be slightly favoured, while the incidence was more comparable between the groups both before and after supplementation. At no stage were the differences significant between the groups.

Enrichment had no apparent effect on mass for age (Tables I and II). Throughout the experiment, the mass/expected mass per cent of the control group was higher than for the experimental group. These differences were significant at a 5% level during periods I, II and IV, although there was hardly any change observed within the groups during the experiment. Accelerated growth would have been reflected by an increase in the mass/expected mass per cent. From this it is clear that no measurable growth stimulus in the experimental group was introduced through enrichment of the maize meal.

Biochemical Evaluation

Although less sensitive than mass for age, serum albumin levels remain a more specific measure of protein status. The mean serum albumin levels of both the control and experimental groups were well within the normal range throughout the experiment and showed very little fluctuation (Figs 7 and 8, and see Tables I and II).

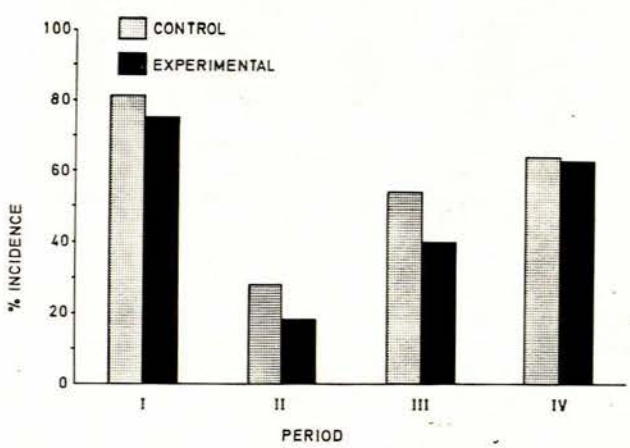

Fig 5. Percentage incidence of skin lesions on arms and legs in pre-school children.

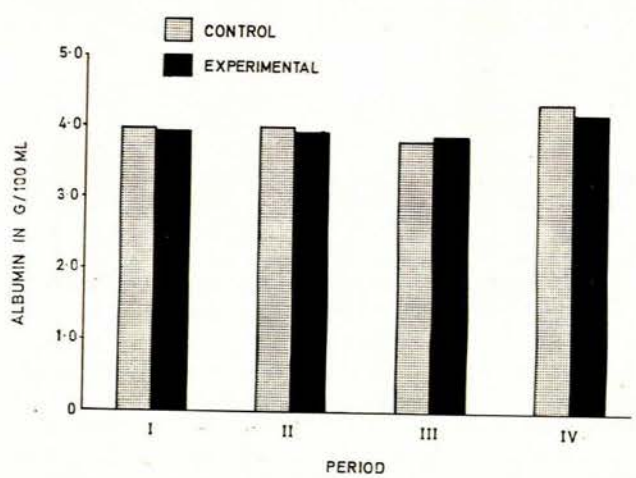

Fig. 7. Mean serum albumin values in pre-school children.

TABLE I. COMPARATIVE DATA FOR EXPERIMENTAL AND CONTROL GROUPS (PRE-SCHOOL) DURING FOUR PERIODS OF ANALYSIS

Variable		Period I		Period II		Period III		Period IV	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Mass (kg)	Mean	14,7	13,0	16,1	13,7	16,3	14,8	17,0	15,6
	SD	3,73	2,25	3,67	2,45	3,68	2,59	3,75	2,89
	N	78	81	67	50	65	43	56	40
Mass/expected mass %	Mean	82*	77	83†	77	83	78	83*	79
	SD	14	8	13	9	14	9	14	8
	N	77	81	64	50	64	43	54	40
Albumin (g/100 ml)	Mean	3,97	3,94	3,98	3,93	3,79	3,86	4,32*	4,17
	SD	0,39	0,38	0,45	0,30	0,38	0,32	0,29	0,35
	N	77	74	67	50	65	43	56	40
Riboflavin μg/g creatinine	Mean	546‡	435	480‡	910	476*	853	642	393
	SD	499	490	318	698	343	650	707	581
	N	75	73	65	48	63	42	56	39
N^1-Me mg/g creatinine	Mean	8,3*	6,8	5,2‡	7,4	7,8‡	9,9	6,3	7,0
	SD	3,2	2,6	1,8	2,9	3,4	3,3	3,5	4,4
	N	75	73	65	48	63	42	56	39
Pyridone mg/g creatinine	Mean	7,50‡	11,11	6,54‡	14,59	8,55‡	18,52	8,9	8,8
	SD	5,15	10,32	4,34	8,87	5,67	11,29	8,2	6,4
	N	75	73	65	48	63	42	56	39

* Significant at a 5% level.
† Significant at a 1% level.
‡ Significant at a 0,3125% level.

TABLE II. COMPARATIVE DATA FOR EXPERIMENTAL AND CONTROL GROUPS (SCHOOL-AGE) DURING FOUR PERIODS OF ANALYSIS

		Period I		Period II		Period III		Period IV	
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Mass (kg)	Mean	20,9	20,2	21,4	20,7	22,4	18,8	23,0	22,0
	SD	2,32	2,58	2,39	2,76	2,63	2,76	2,79	2,94
	N	46	38	44	41	47	41	44	39
Mass/expected mass %	Mean	77	77	76	76	77	78	78	77
	SD	8	9	8	9	8	9	8	9
	N	46	37	44	41	47	41	42	39
Riboflavin μg/g creatinine	Mean	279‡	312	289‡	603	264‡	584	356	305
	SD	246	215	168	392	154	504	248	240
	N	46	38	44	41	47	41	44	39
N^1-Me mg/g creatinine	Mean	6,9*	8,3	4,0‡	6,6	4,3	7,7	4,9	5,3
	SD	2,3	3,3	1,4	2,2	2,5	2,3	2,5	2,4
	N	46	38	44	41	47	41	44	39
Pyridone mg/g creatinine	Mean	5,53‡	8,29	5,15‡	12,72	7,29	12,61	7,70*	8,64
	SD	3,80	4,33	2,52	6,37	3,20	5,94	4,94	6,87
	N	46	38	44	41	47	41	44	39

* Significant at a 5% level.
† Significant at a 5% level.
‡ Significant at a 0,3125% level.

In addition to testing for differences between the control and experimental groups, the biochemical data were also analysed to establish any differences occurring within the groups during the duration of the experiment.

There was a greater tendency towards fluctuation within the control groups than within the experimental groups (see Tables I and II). The significant differences between the control and experimental groups shown in Tables I and II are thus due mainly to changes occurring in the control group. This is supported by the fact that during supplementation only the control group showed significant changes (Table III). The practical

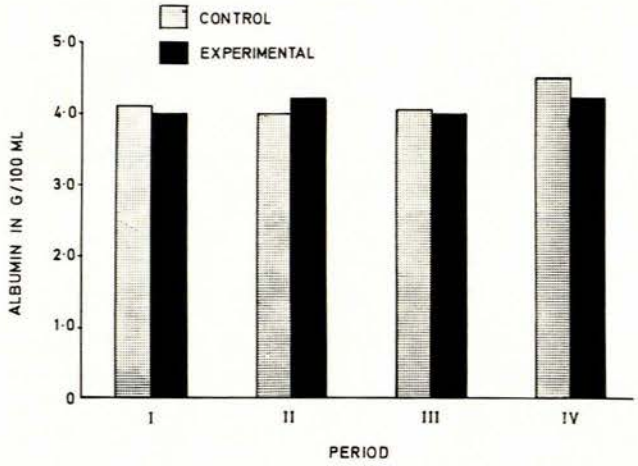

Fig. 8. Mean serum albumin values in schoolchildren.

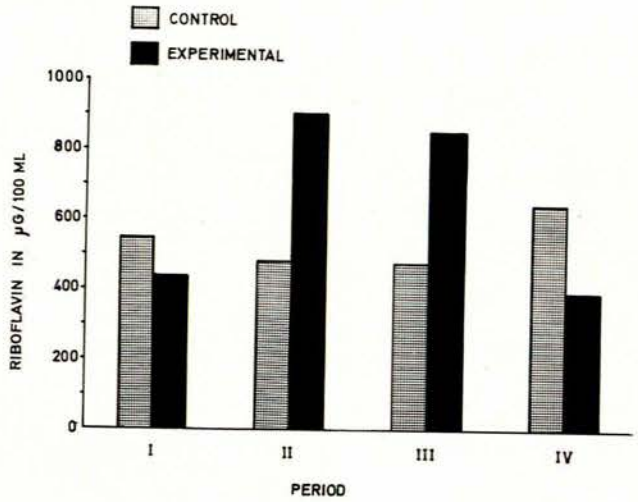

Fig. 9. Mean urinary riboflavin excretion values in pre-school children.

importance of these changes and differences observed in the albumin levels is probably negligible, since the variations were relatively small and occurred well within the normal range. It is, however, obvious that the enrichment procedure did not tend to induce hypo-albuminaemia in the experimental groups.

The urinary measurements of riboflavin and nicotinic acid metabolites are more specific and/or more sensitive than the variables previously discussed and, at the same time, would provide a better indication as to whether the supplement does in fact reach the experimental subjects. A certain margin of error could, however, be introduced by the fact that the measurements had to be made on spot urine specimens, since timed specimens at this age and under the circumstances are not possible to obtain.

The results of the riboflavin analyses are shown in Figs 9 and 10 and statistical analyses in Tables I—III. These results are clear-cut and significantly in favour of the experimental group. Note the marked deterioration in the experimental group in relation to the control group after withdrawal of the supplement (period IV). Because

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Variable	Age groups	Groups	Periods compared 1:2	1:3	1:4	2:3	2:4	3:4
Mass/expected mass	School	Control	—	—	—	*	*	—
		Experimental	*	—	—	*	*	—
	Pre-school	Control	—	—	—	—	*	—
		Experimental	—	—	—	—	—	—
Albumin g/100 ml	School	Control	—	—	*	—	*	*
		Experimental	—	—	—	—	—	*
	Pre-school	Control	—	—	*	—	*	*
		Experimental	—	—	—	—	—	—
Riboflavin µg/g creatinine	School	Control	—	—	—	—	—	—
		Experimental	*	—	—	—	*	—
	Pre-school	Control	—	—	—	—	—	—
		Experimental	*	*	—	—	*	*
N^1-Me mg/g creatinine	School	Control	*	*	*	—	—	—
		Experimental	—	—	*	—	—	*
	Pre-school	Control	*	—	*	*	—	—
		Experimental	—	*	—	*	—	—
Pyridone mg/g creatinine	School	Control	—	—	—	*	—	—
		Experimental	—	*	—	—	—	—
	Pre-school	Control	—	—	—	*	—	—
		Experimental	—	—	—	—	—	*

* Significant at a 5/24% = 0,2% level.

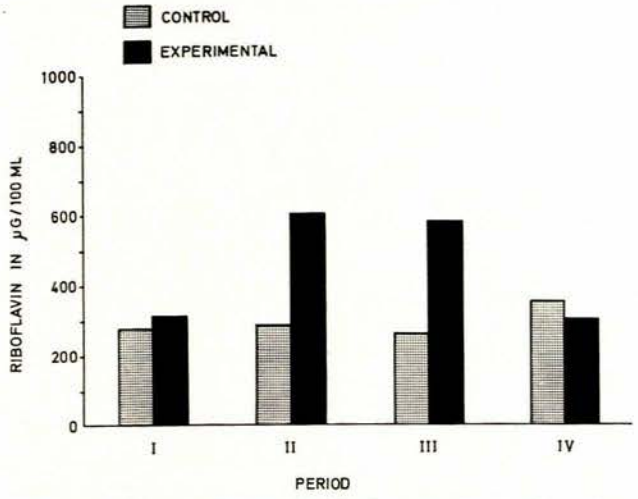

Fig. 10. Mean urinary riboflavin excretion values in schoolchildren.

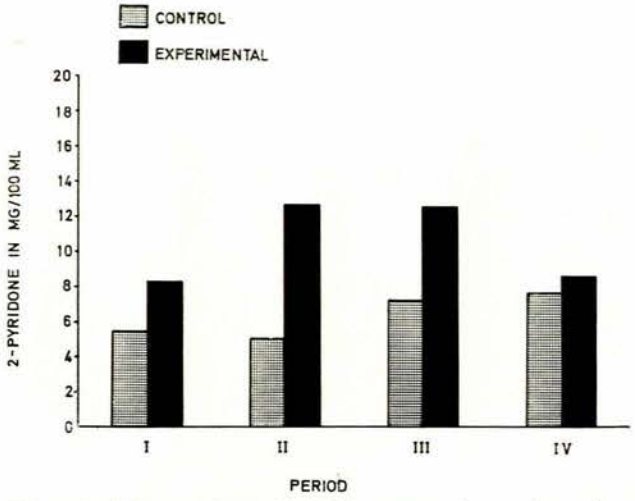

Fig. 12. Mean urinary 2-pyridone excretion values in schoolchildren.

of the influence of age, especially in the very young, no attempt was made to differentiate between normal and abnormal excretion rates as was done in the previous experiments. The absolute values, as given in Figs 9 and 10, however, showed that, during enrichment, the excretions were roughly doubled.

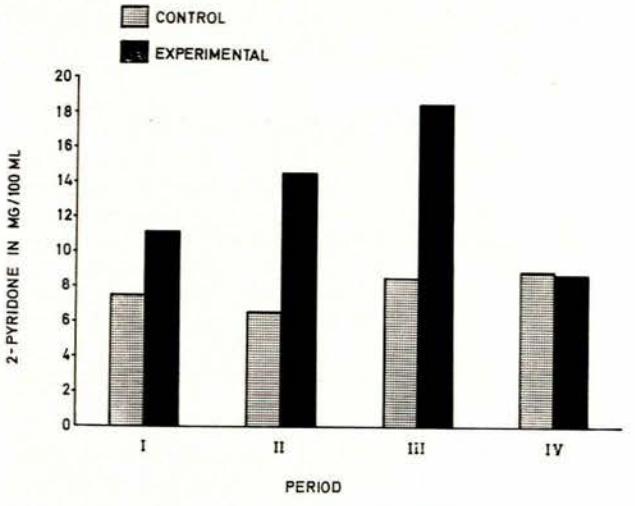

Fig. 11. Mean urinary 2-pyridone excretion values in pre-school children.

The results obtained for 2-pyridone excretions are given in Figs 11 and 12 and the statistical results in Tables I—III. These results are equally dramatic and as clear-cut as was the case for riboflavin, although there was a greater variation in the control group, probably related to seasonal differences. Once again there was a marked deterioration in the experimental group after withdrawal of the supplement.

The results of the N^1-methylnicotinamide analyses are shown in Figs 13 and 14 and the statistical data in Tables

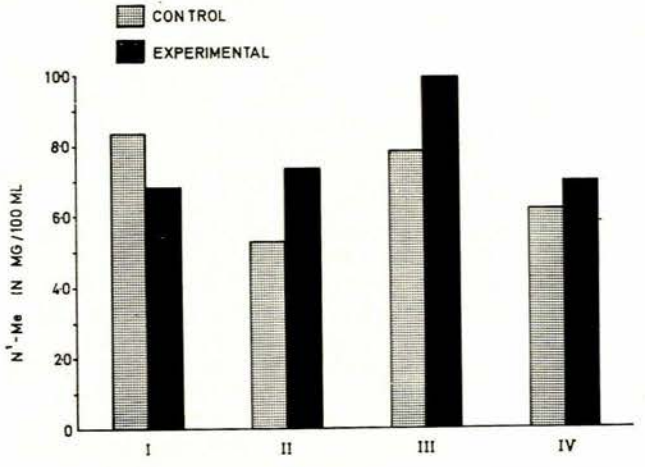

Fig. 13. Mean urinary N^1-methylnicotinamide excretion values in pre-school children.

I—III. Once again there is considerable fluctuation in the control group from periods I to IV. The supplementary effect is comparable to the variables, previously discussed, in the pre-school children but not in those at school. The difference between the two age groups is not easily explained. It must be noted, however, that during supplementation, the experimental group maintained a fairly high excretion of the metabolite while the control group deteriorated when compared with the first measurement. Furthermore, it should be pointed out that N^1-Me excretion in the two previous studies[1,2] also appeared to be a less sensitive indicator of nicotinic acid status than 2-pyridone excretion.

Dietetic Results

Maize meal is undoubtedly the staple food of the Blacks in both areas and is usually bought once a month.

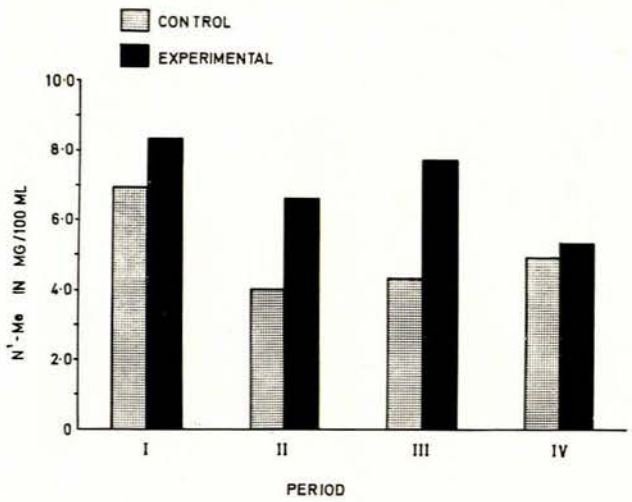

Fig. 14. Mean urinary N^1-methylnicotinamide excretion values in schoolchildren.

In order to estimate the amount of maize meal eaten, all children below the age of 3 years were disregarded and those above 3 years were counted as adults. The total amount of maize meal bought per month was equally divided between the number of adults. From this the approximate intake of maize meal per head per day was calculated.

The intakes were 395 and 472 g per head per day in the experimental and control areas respectively, consumed mainly in the form of porridge, *mahewu* and beer.

In both areas the majority of families consumed a large midday and evening meal, with a small breakfast in the morning. In the experimental area, 81% of families had bread at breakfast, usually with butter, peanut butter or jam. About half the families had tea at breakfast, usually with sugar and sometimes with milk. About 25% of families had porridge. In the control area, fewer families (44%) had bread at breakfast, usually without butter or jam. The majority had tea mostly without milk but with sugar. Porridge was more regularly consumed (40%) at breakfast.

There was no clear-cut preference for white or brown bread and both were bought with equal frequency in both areas.

In the experimental area, the midday and evening meal usually consisted of maize porridge and a variety of vegetables, including those collected in the veld or bought at the local shop. A large proportion (64%) had meat at least once a week. A fair proportion of families (47%) used eggs fairly regularly. Some fresh milk was used daily by about 60% of families, whereas powdered milk is used only for the babies. Fruit was eaten at least once a week by about half the families, the most popular being bananas, oranges and mangoes. Beer is prohibited by religious belief and very few families admitted to brewing their own beer. Although the consumption of sugar could not be determined, it is a food consumed by the majority of people with great regularity. Condensed milk, sweets, biscuits and jam are also frequently bought.

In the control area, the midday and evening meals usually consisted of porridge with vegetables collected from the veld (fresh or dried, depending on the season). Vegetables were seldom bought but occasionally eaten when home-grown. Only 9% of families ate meat at least once a week and eggs were rarely used. This was partly due to a recent outbreak of Newcastle disease. Some fresh milk was used daily by 49% of families and powdered milk by babies only. Fresh fruit was used about once a week by fewer people (40%) than in the experimental area, bananas, oranges and apples being preferred. About one-quarter of the families admitted to brewing their own beer. Sugar was less frequently used in the control area, while condensed milk, sweets, biscuits and jam were seldom bought.

Breast-feeding of babies was the rule in both areas and well over 90% continue for at least 6 months, more than two-thirds to 1 year, and about a third to 2 years. About 8% of mothers claimed to have continued breast-feeding to the age of three. Porridge was introduced into the diet almost from birth.

Although the consumption of maize meal was high in both areas, the diet in the experimental area was more varied and the intake of meat, vegetables and sugar was noticeably greater than in the control area, although a quantitative assessment was not possible. The control group followed traditional pattern more closely than the experimental group. From this point of view, the groups were thus not well matched, but such matching cannot be obtained under the circumstances prevailing.

Some of the unexplained results here reported could in part be due to differences in dietary intake.

GENERAL DISCUSSION

Since repeated measurements were made on the same children, the results obtained during the four examinations were obviously not independent. This created considerable difficulties in selecting statistical testing procedures. Concerning the biochemical parameters in particular, a multivariate analysis is obviously indicated. In this respect more stringent procedures might have been employed. For example, we are able to write down a multivariate linear model which may give a better mathematical explanation of the experiment as a whole, on the lines suggested by Potthoff and Roy.[8] Unfortunately the mathematics of this model still have to be developed theoretically for the specific covariance structure which exists in our data. This type of experiment seems to be used frequently in the biological and medical sciences and would thus warrant theoretical investigation. (Such a study is currently being undertaken by one of us—N.F.L.)

It was, therefore, decided to use relatively simple statistical techniques and to ensure that the bounds of statistical propriety are not over-stepped, deductions were made conservatively and a very high level of significance was applied.

As in the past it was not possible to guarantee that all children were getting the supplement all the time. In addition, it needs to be pointed out that this experiment was done during a period of good rains and hence there

were good crops and an abundance of veld foods during some parts of the year. These factors could in part explain the fluctuations that occurred, but the differences between the experimental and control groups were such that there can be no doubt about the beneficial effect of enrichment.

The original hypothesis[3] that vitamin supplementation in the presence of marginal protein deficiency could be harmful, was not substantiated by this study. There was no evidence of accelerated growth in the experimental group, nor was there any disadvantage with regard to the serum albumin levels when they were compared with those of the control group; there was in fact evidence of some clinical improvement during supplementation.

Once again, there was, with regard to some of the clinical and biochemical variables, a considerable deterioration after withdrawal of the supplements. Both previous experiments showed the same trend.

It is suggested that supplementation for a limited period of time in populations used to a habitually low intake inhibits the conservatory mechanisms of the individual to some extent. Withdrawal of supplementation, theoretically at least, puts such individuals at a disadvantage compared with those who have had no supplementation. Although such an effect has been described, with experiments, with respect to vitamin C intake,[9] it has not, to our knowledge, been previously found with riboflavin and nicotinic acid supplementation.

The original choice of 400 g of maize meal as a daily intake[2] was based on an estimate of caloric intake for Black children found in the Pretoria survey. The present dietary survey proved the estimate to be remarkably accurate.

As in the previous experiment, a premix with maize meal was used at a rate of 56 g per bag (82 kg) of meal. This was considered an essential procedure, since the amount of vitamins alone mixed into the millstream is too small to ensure adequate mixing. Should a national enrichment scheme be introduced, a single premix plant would have the advantage of easier quality control, better labour utilisation, a smaller outlay and better capital utilisation than if this procedure were to be undertaken by individual mills.

CONCLUSIONS

The results of the previous experiments were confirmed. Furthermore, this study provided no evidence that there was any harmful effect, in supplementing maize meal, on young growing children with mild to moderate protein calorie malnutrition, but rather suggested that there was a beneficial effect.

Since the principle of such enrichment has already been accepted,[10] it is now strongly recommended that the scheme be introduced with the least possible delay.

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