# The effect of riboflavin supplementation on the urinary hydroxyproline:creatinine index in a resettlement village in rural Thailand

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1. The urinary hydroxyproline:creatinine index was measured in forty-one preschool children, ranging in age from 18 months to 8 years, before and after they had received a supplement of 10 mg riboflavin/d for 7 d. It was found that the supplement increased the index in twenty-seven of the children.

2. The children were divided into two groups, those with indices $\leqslant 1.5$ and those with indices $> 1.5$ before supplementation.

3. The hydroxyproline:creatinine index was found to increase significantly ($P < 0.001$) in the group of children whose indices were $\leqslant 1.5$ before supplementation. There was no significant effect on those with indices $> 1.5$.

4. There was no significant difference between the two groups, in either riboflavin status before supplementation or mean age.

5. The part played by riboflavin in protein utilization and growth is discussed.

The urinary hydroxyproline:creatinine index was introduced by Whitehead (1965) as a criterion for use in the early detection of marginal malnutrition. It was suggested that index values below 2·0 showed marginal malnutrition and Whitehead (1965) found that subjects with clinically recognizable signs of malnutrition usually had index values below 1·5. Studies carried out in two villages in north-east Thailand in 1969 showed that index values from below 1·0 to above 5·0 were found for the infants and preschool children in both villages but not one of these children was clinically malnourished (Migasena, Thurnham, Pongpaew, Jintakanon & Harinasuta, 1971). Subclinical malnutrition, however, was possibly present among the village children since their growth rates were below those of a group of well-nourished Thai children and Western standards (Migasena *et al.* 1971). In addition, evidence of inadequate dietary intakes in civilian diets were revealed by a nutritional survey carried out by the Interdepartmental Committee on Nutrition for National Defense (1962) which showed that, although protein and calorie intakes were probably adequate, vitamin intakes were generally low.

Biochemical ariboflavinosis has been demonstrated among the preschool children in both villages referred to above (Thurnham, Migasena & Pavapootanon, 1970; Thurnham, Migasena & Vudhivai, 1971). A low dietary intake of riboflavin has been shown to reduce the growth rates of weanling rats on both high-protein and low-protein diets (Sugioka, Porta, Corey & Hartroft, 1969). Thus, it was of interest to

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determine whether the poor growth rates of the village children were connected with the ariboflavinosis. The effect of a riboflavin supplement on the urinary hydroxyproline: creatinine index was examined, therefore, to determine whether growth of preschool children, as measured by the index, might be stimulated by the supplement.

## METHODS

*Subjects.* The experiment was carried out with preschool children in a resettlement village in north-east Thailand, in the dry season between February and March 1971.

*Riboflavin supplementation.* Supplementation was considered adequate when the subjects received at least 10 mg riboflavin/d for 7 d (maximum 10 d). Full details concerning the supplementation are described by Thurnham, Migasena, Vudhivai & Supawan (1972).

*Biochemical determinations.* Random samples of urine were preserved by addition of hydrochloric acid (0·1 ml concentrated HCl in 30 ml urine). Creatinine was measured by a modification of the procedure of Folin & Wu as outlined in the Technicon AutoAnalyzer Manual (Method N-11B) (Technicon Instruments Co. Ltd, Basingstoke, Hants). In the determination of hydroxyproline, duplicate 1 ml samples of urine were hydrolysed with 1 ml 6 M-HCl as described by Prockop & Udenfriend (1960) and the neutralized hydrolysate was then analysed by the automated method of Grant (1964). Hydroxyproline: creatinine indices were calculated as described by Whitehead (1965). Erythrocyte glutathione reductase (EGR) activation coefficients were determined by the method of Glatzle, Körner, Christeller & Wiss (1970) on haemolysates collected and prepared as described by Thurnham *et al.* (1970). Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was detected by the method of Bernstein (1962). The EGR activation coefficient is not believed to be a good indication of riboflavin status in G-6-PD-deficient subjects (Thurnham, 1972), therefore such children were excluded from calculations of mean values (as in Table 2).

## RESULTS

An examination of the urinary hydroxyproline indices before and after the riboflavin supplementation showed that the index had increased in twenty-seven out of forty-one children, with two showing no change. When the children were grouped according to their hydroxyproline indices before supplementation (Table 1), there appeared to be a general trend towards higher indices after supplementation. However, the mean values of the indices for the whole group before and after supplementation were not significantly different (Table 1).

A closer examination of these results showed that two factors may have interfered: not all the children had completed the riboflavin supplementation experiment satisfactorily and there was an epidemic of chicken-pox in the village at the time of the experiment. Factors which affect growth, such as acute infections, may also influence hydroxyproline: creatinine indices (G. Arroyave, personal communication). Of the seven children whose indices were $\leqslant$ 1·0 after supplementation, two took no tablets,

Table 1. *Distribution of urinary hydroxyproline: creatinine indices* of preschool children, before and after receiving a riboflavin supplement, grouped according to their indices before supplementation*

| | Index $\leqslant$ 1·0 | | Index 1·1–2·0 | | Index > 2·0 | | Whole group | |
|---|---|---|---|---|---|---|---|---|
| | No. of subjects | % | No. of subjects | % | No. of subjects | % | Mean† | SD |
| Before supplementation | 12 | 29 | 19 | 46 | 10 | 24 | 1·7 | 1·1 |
| After supplementation | 7 | 17 | 16 | 39 | 18 | 44 | 2·0 | 1·0 |

Hydroxyproline and creatinine were measured in eighty-two random samples of urine collected from forty-one children before and after the supplementation.

* Calculated as described by Whitehead (1965).
† Difference between the means is not significant.

one took only six tablets, one child developed chicken-pox and one was in a family where chicken-pox was present. The results were regrouped, therefore, firstly to include only those for children whose riboflavin intake during the experiment was satisfactory and secondly to exclude all those for children who were infected with chicken-pox or in close contact with chicken-pox-infected children.

The children were divided into two groups according to their hydroxyproline: creatinine indices before supplementation (Table 2). Of those children who took the stipulated riboflavin course and whose indices were $\leqslant$ 1·5, fourteen out of seventeen showed an increase in the value of the index and the mean index for the whole group was significantly increased ($P < 0·001$). In contrast, of those with indices greater than 1·5 before supplementation, only nine of the eighteen indices were increased and the mean value for the index of the whole group was not significantly changed by the riboflavin supplementation.

When those children infected with chicken-pox, or in those families where chicken-pox was present, were removed (Table 2), the findings were essentially the same, particularly among those children whose indices were $\leqslant$ 1·5 before supplementation. In the group of children whose hydroxyproline: creatinine indices were greater than 1·5 before supplementation, the mean index for the group was increased both before and after supplementation, but the difference was not significant.

The index of riboflavin deficiency, the activation coefficient, has been shown to indicate ariboflavinosis when it is 1·20 or higher (Glatzle *et al.* 1970; Thurnham *et al.* 1970). The results showed that many of the children had biochemical ariboflavinosis before supplementation, but the mean values of the activation coefficients of the different subgroups, that were separated according to the hydroxyproline: creatinine indices, were not significantly different (Table 2). The effect of excluding the chicken-pox-infected children slightly increased the index for riboflavin deficiency in the group with lower hydroxyproline: creatinine indices before supplementation but the effect was not significant. Likewise there was no significant difference between the means of the ages of the children in the two groups with high and low hydroxyproline: creatinine indices.

Table 2. *Urinary hydroxyproline:creatinine index* (H:C) in preschool children, before and after receiving a riboflavin supplement, grouped according to their index before supplementation*

| | Whole group of supplemented children† | | | | Supplemented group minus 'infected'‡ children | | | |
|---|---|---|---|---|---|---|---|---|
| | H:C ⩽ 1·5 | | H:C > 1·5 | | H:C ⩽ 1·5 | | H:C > 1·5 | |
| | Before | After | Before | After | Before | After | Before | After |
| H:C index (mean ± SD) | 0·8 ± 0·5 | 1·8 ± 0·7 | 2·6 ± 1·0 | 2·3 ± 1·1 | 0·9 ± 0·6 | 2·0 ± 0·4 | 2·8 ± 1·2 | 2·8 ± 1·3 |
| Mean ± SE of the differences | 1·0 ± 0·2 | | −0·2 ± 0·9 | | 1·0 ± 0·2 | | 0·1 ± 0·4 | |
| Probability of chance difference | $P < 0·001$ | | NS | | $P < 0·001$ | | NS | |
| No. of children | 17 | | 18 | | 10 | | 13 | |
| Mean age (years) | 5·0 ± 2·0 | | 5·1 ± 1·9 | | 5·1 ± 1·8 | | 5·0 ± 1·9 | |
| Children in whom H:C increased | 14 (2 no change) | | 9 | | 9 (1 no change) | | 8 | |
| Children in whom H:C decreased | 1 | | 9 | | 0 | | 5 | |
| Activation coefficient before supplementation§ (mean ± SD) | 1·16 ± 0·18[a] (15)‖ | | 1·20 ± 0·12[b] (17)‖ | | 1·23 ± 0·18[c] (10) | | 1·19 ± 0·12[d] (13) | |

NS, not significant.
* Calculated as described by Whitehead (1965).
† All children received at least 70 mg riboflavin.
‡ Those children with chicken-pox infection were removed, including other siblings from the same families.
§ Calculated from the ratio of erythrocyte glutathione reductase activity stimulated by flavin-adenine dinucleotide over the basic EGR activity of red cell haemolysate. Differences between a and b, and between c and d were not significant.
‖ Glucose-6-phosphate dehydrogenase-deficient subjects were not included in the calculation of the mean and SD.

## DISCUSSION

The use of the hydroxyproline:creatinine index to reveal cases of marginal malnutrition is based on metabolic changes that accompany a dietary deficiency of protein or of calories or of both (Whitehead, 1965). In Thailand it has been suggested that the dietary intake of both protein and calories by the civilian population is adequate (Interdepartmental Committee on Nutrition for National Defense, 1962), although this finding has been criticized as being too sweeping by workers in northern Thailand, who considered that the ICNND survey teams did not examine a sufficient number of children below 5 years of age (Thanangkul, Whitaker & Fort, 1966). The ICNND survey also suggested that dietary intakes of vitamin A, thiamin and riboflavin were generally low throughout Thailand.

The nutritional picture among the preschool children in the village where the supplementation experiment was carried out was similar to that described in the ICNND survey except that, in addition, it was found that the dietary intake of calories may also have been low. Mean values for the calorie intake of the preschool children ranged from 55 to 65 kcal per kg body-weight; protein intake was approximately 1·7 g/kg body-weight in weaned children below 3 years of age and 1·5 g/kg in children 3–8 years of age. Protein sources were mainly rice (70%), green vegetables (5%), and meat and fish (25%) (Thurnham, Pongpaew, Migasena, Supawan and Chanbumrung, in preparation). Protein *per se* was probably adequate in the children's dietary intake but the calorie intake is low by comparison with the recommended daily energy intake of 100 kcal per kg body-weight and this would have the effect of increasing protein requirements above recommended levels, as some dietary protein would be utilized for calorie production instead of tissue formation. It is probable, therefore, that the dietary protein intake of these children was suboptimal, which may account for their poor growth rates in comparison with those of well-fed Bangkok children (Migasena *et al.* 1971).

Improper utilization of dietary proteins due to marginal or low-vitamin intakes may also be another factor contributing to poor growth rates. It has been suggested that vitamin A is required for the proper utilization of protein for growth (Arroyave, 1969) and Sugioka *et al.* (1969), in their experiments with weanling rats, presented evidence that riboflavin may have similar functions.

The results presented in this report may be evidence of an inhibitory effect of ariboflavinosis on the growth of preschool children. The effect of the riboflavin supplement was to significantly increase the hydroxyproline: creatinine index, i.e. stimulate growth (Whitehead, 1965) of those children whose indices were low before supplementation. It was not possible to demonstrate any greater deficiency in riboflavin in the group with the low hydroxyproline: creatinine indices than in the group where the indices were higher. The similarity in the riboflavin status of the two groups indicates possibly that the group of children with the low hydroxyproline: creatinine indices suffered from additional dietary deficiencies. Protein utilization and growth are not dependent on just one factor and the presence of additional dietary deficiencies would not be surprising (Interdepartmental Committee on Nutrition for National Defense, 1962). The interesting fact is that riboflavin was able to stimulate growth, as measured by the hydroxyproline: creatinine index, in the presence of the additional factors. Work is in progress to determine what this other factor(s) may be, with particular emphasis on the other vitamins that may be suboptimal in the dietary intake of the children.

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