# Relationship between biochemical and clinical indices of B-vitamin deficiency. A study in rural school boys

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1. A study amongst schoolboys in villages around Hyderabad, India, showed that almost all the boys had riboflavin deficiency, 61 % had pyridoxine deficiency, and 9·4 % had thiamin deficiency as judged by enzymic tests.

2. The prevalence of angular stomatitis was 41·3 % and that of glossitis was 18·2 %. Biochemical deficiency of riboflavin and pyridoxine was marginally higher in children with angular stomatitis with or without associated glossitis, than in children without oral lesions.

3. Treatment with B-complex vitamins (containing 4 mg riboflavin and 10 mg pyridoxine) daily for 1 month produced significant reduction in the prevalence of glossitis but had no effect on angular stomatitis. The latter responded to topical application of gentian violet.

4. Small but significant changes in erythrocyte enzymes occurred over the period of 1 month even without vitamin supplements.

5. Results suggest that while glossitis is a relatively early manifestation of riboflavin or pyridoxine deficiency or both, angular stomatitis has a more complex aetiology perhaps associated with infection.

Lesions of the mouth such as angular stomatitis and glossitis have been shown in experimentally-induced riboflavin deficiency as well as pyridoxine deficiency. Recent reports from the National Institute of Nutrition, Hyderabad, show that in adults this condition responds to treatment with either riboflavin or pyridoxine (Krishnaswamy, 1971; Iyengar, 1973). While glossitis seems to be more common amongst the adults of our community, angular stomatitis is more common amongst the children.

A recent survey of rural schoolchildren living near Hyderabad, revealed a higher incidence of angular stomatitis amongst boys than amongst girls. The condition failed to respond to treatment with either riboflavin alone or with B-complex vitamins over a period of 3 weeks and 4 weeks respectively (K. V. R. Sarma, M. Damodaran and A. Tiar, unpublished results). The present study was carried out to investigate this observation further, by applying biochemical tests for assessing vitamin status, and examining the response to topical application of gentian violet.

## EXPERIMENTAL

The subjects, 407 boys aged 5–13 years old and attending four rural schools near Hyderabad, were examined for clinical signs of vitamin deficiency such as angular stomatitis, glossitis, cheilosis, Bitot's spots, phrynoderma and angular scars. Of these, 168 boys had active angular stomatitis with or without glossitis (group A) whereas 134 boys were completely free of all lesions of the mouth including healed scars (group B). Boys in group A were further divided randomly into three subgroups (groups A1, A2 and A3) and those in group B into two subgroups (groups B1 and B2).

These boys were treated daily with either two tablets of B-complex vitamins (containing (mg/tablet): thiamin 2, riboflavin 2, pyridoxine 5, calcium pantothenate 2, niacin 20) (groups A1 and B1) or with placebo tablet containing 100 mg lactose (groups A2 and B2) or by topical application of a solution of gentian violet (10 g/l) to the lesion at the corners

of the mouth (group A3). All the boys were re-examined after 1 month of treatment for the presence or absence of angular stomatitis and glossitis.

Samples of venous blood were taken from fourteen to fifteen boys in each sub group before and after 1 month of treatment, and analysed for erythrocyte transketolase activity (*EC* 2.2.1.1; ETK), and its in vitro stimulation with thiamin pyrophosphate TPP (ETK-AC) (Bamji, 1970) for thiamin status; erythrocyte riboflavin (Sharada & Bamji, 1972), and erythrocyte glutathione reductase activity (*EC* 1.6.4.2) (EGR) and its in vitro stimulation with FAD (EGR-AC) (Bayoumi & Rosalki, 1976) for riboflavin status; erythrocyte aspartate aminotransferase (*EC* 2.6.1.1) (EAT) and its in vitro stimulation with pyridoxal phosphate (EAT-AC) (Stanulovic *et al.* 1967) for pyridoxine status. Activation coefficient (AC) was defined as enzyme activity with added coenzyme:enzyme activity without added coenzyme.

Blood was taken at school between 09.30 and 10.30 hours. Most children had taken a light meal consisting of a cereal such as rice, wheat or Jowar (*sorghum vulgare*), occasionally some vegetable or legume, and tea to drink before going to the school. It was not possible to obtain fasting samples of blood.

The dietary history of fifty-three boys was obtained by oral questionnaire using the 24 h recall method by visiting their homes and questioning the mothers (Pasricha, 1959).

<div align="center">RESULTS</div>

<div align="center">*Clinical findings*</div>

Amongst the nutritional deficiency signs the most prevalent was angular stomatitis (41·3 %), followed by glossitis (18·2 %), and Bitot's spots (7·9 %). The prevalence of other signs was less than 5 % (Table 1). Bleeding gums were not observed. None of the children complained of signs attributable to neuropathies.

Initially sixteen of the fifty-one boys in group A1 and 28 of the 107 boys in groups A2 and A3 suffered from glossitis in addition to angular stomatitis. Treatment with B-complex vitamins produced a significant improvement in glossitis (group A1; Table 2). In fifteen boys (53·6 %) glossitis was cured even without B vitamins (groups A2, A3; Table 2). In normal boys (groups B1 and B2) treatment with B-complex vitamins protected against development of glossitis, though not in every case. Thus, while 1·6 % of the initially-normal boys in group B1 developed glossitis at the end of 1 month, 10 % in group B2 developed this condition, however the difference was observed to be statistically not significant (Table 2).

B-complex vitamins did not have a significant effect on the treatment or prevention of angular stomatitis, but gentian violet application had a marked effect (Table 2). Thus, while 23·5 % of the boys in group A1 and 12 % of those in group A2 were free of stomatitis at the end of 1 month, 81 % showed healing in group A3. Amongst normal children, 21·3 % in the vitamin-treated group (B1) and 13·3 % in the untreated group (B2) developed stomatitis at the end of 1 month (Table 2). The difference between the vitamin-treated group and the group receiving the placebo was not significant.

<div align="center">*Biochemical findings*</div>

*Riboflavin.* A direct inverse correlation between erythrocyte riboflavin and EGR-AC was not observed. Careful examination of the results showed that when the erythrocyte riboflavin levels were less than 220 $\mu$g/l, AC values were more than 1·2, and in most cases more than 1·4. However, very often despite very high erythrocyte riboflavin levels, AC was more than 1·4.

Results given in Table 3 indicated a very high prevalence of biochemical riboflavin deficiency in this community; the prevalence in group A being significantly higher than that

Table 1. *Prevalence of nutritional deficiency signs in 407 rural Indian schoolboys examined*

| | Prevalence | |
|---|---|---|
| Clinical sign | No. of boys | % total |
| Bitot's spots | 32 | 7·9 |
| Angular stomatitis | 168 | 41·3 |
| Glossitis | 74 | 18·2 |
| Cheilosis | 10 | 2·5 |
| Follicular hyperkeratosis | 5 | 1·2 |
| Angular scars | 58 | 14·3 |

Table 2. *Effect of treatment in rural Indian schoolboys‡ with oral lesions (group A) and without oral lesions (group B)*

| | | Group A | | | | Group B | |
|---|---|---|---|---|---|---|---|
| | Treatment ... | A1 B-complex vitamins | A2 Placebo | A3 Gentian violet | | B1 B-complex vitamins | B2 Placebo |
| Lesion | Response | | | | Response | | |
| Angular stomatitis | Not cured | 39 | 44 | 11* | Appeared | 13 | 8 |
| | Cured | 12 | 6 | 46* | Did not appear | 48 | 52 |
| | Total | 51 | 50 | 57 | Total | 61 | 60 |
| | | A1 | A2+A3 | | | B1 | B2 |
| Glossitis | Not cured | 1† | 13 | | Appeared | 1 | 6 |
| | Cured | 15† | 15 | | Did not appear | 60 | 54 |
| | Total | 16 | 28 | | Total | 61 | 60 |

\* Effect of treatment is significantly different from groups $A_1$ and A2 by normal proportions test ($P < 0\cdot001$).

† Effect of treatment is significantly different from groups A2+A3 by $\chi^2$-test with Yate's correction ($P < 0\cdot05$).

‡ For details, see Table 1 and p. 432.

in group B before treatment. Boys in group A also had marginally higher AC than those in group B (Table 4) indicating a slightly inferior riboflavin status in the former group. There was no difference in the riboflavin status of thirty boys who had only angular stomatitis and seven boys who had glossitis in addition to angular stomatitis.

Treatment with B-complex vitamins produced a significant increase in basal as well as stimulated EGR activity and a decrease in EGR-AC (Table 5). However, some boys who received vitamins for 1 month (groups A1 and B1) continued to have an EGR-AC value greater than 1·2 after treatment, indicating persistence of biochemical riboflavin deficiency (Table 3). A small but significant decrease in AC was observed also in boys of groups A2 and A3, though these children had not received any vitamins (Table 5). Erythrocyte ribo-flavin concentration showed a significant increase after treatment with B vitamins (Table 5).

*Pyridoxine.* Of fifty-one boys in whom EAT estimation was done initially, thirty-two had AC values greater than 1·7 initially (Table 3). AC values for boys in group A tended to be marginally higher than for those in group B, indicating a slightly inferior pyridoxine status in the former (Tables 3 and 4). Amongst the boys in group A, those who had glossitis had the lowest basal as well as stimulated EAT activity (Table 4). Basal as well as stimulated EAT activities showed a significant increase after treatment with B-complex vitamins

Table 3. *Percentage distribution (% no. of boys in the group) of biochemical riboflavin, pyridoxine and thiamin status (EGR-AC; EAT-AC; ETK-AC) of rural Indian schoolboys† before (BT) and after (AT) treatment*

| Vitamin | Group† | No. of boys | Treatment | High risk | | Medium risk | | Low risk | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | BT | AT | BT | AT | BT | AT |
| Riboflavin EGR-AC | | | | $\geqslant 1\cdot4$ | | $1\cdot2-1\cdot4$ | | $< 1\cdot2$ | |
| | A1 | 16 | B-vitamins | 94 | 31 | 6 | 38 | 0 | 31 |
| | A2 | 12 | Placebo | 100 | 75 | 0 | 17 | 0 | 8 |
| | A3 | 13 | Gentian violet | 100 | 85 | 0 | 15 | 0 | 0 |
| | B1 | 15 | B-vitamins | 80* | 33 | 20 | 20 | 0 | 47 |
| | B2 | 13 | Placebo | 77* | 85 | 23 | 15 | 0 | 0 |
| Pyridoxine EAT-AC | | | | $\geqslant 2\cdot0$ | | $1\cdot7-2\cdot0$ | | $< 1\cdot7$ | |
| | A1 | 8 | B-vitamins | 38 | 0 | 25 | 50 | 37 | 50 |
| | A2 | 9 | Placebo | 22 | 67 | 56 | 0 | 22 | 33 |
| | A3 | 12 | Gentian violet | 25 | 67 | 42 | 33 | 33 | 0 |
| | B1 | 10 | B-vitamins | 10 | 20 | 30 | 40 | 60 | 40 |
| | B2 | 12 | Placebo | 0 | 42 | 67 | 33 | 33 | 25 |
| Thiamin ETK-AC | | | | $\geqslant 1\cdot25$ | | $1\cdot15-1\cdot25$ | | $< 1\cdot15$ | |
| | A1 | 12 | B-vitamins | 8 | 0 | 8 | 8 | 84 | 92 |
| | A2 | 8 | Placebo | 12 | 12 | 12 | 25 | 76 | 63 |
| | A3 | 10 | Gentian violet | 10 | 0 | 10 | 0 | 80 | 100 |
| | B1 | 11 | B-vitamins | 0 | 18 | 9 | 0 | 91 | 82 |
| | B2 | 12 | Placebo | 8 | 0 | 25 | 8 | 67 | 92 |

EGR-AC, erythrocyte glutathione reductase activation coefficient; EAT-AC, erythrocyte aspartate aminotransferase activation coefficient; ETK-AC, erythrocyte transketolase activation coefficient.

* Value was significantly lower in group B than in group A by $\chi^2$-test with Yate's correction for continuity ($P < 0\cdot05$).

† For details, see table 1 and p. 432.

Table 4. *Erythrocyte riboflavin concentration, erythrocyte glutathione reductase activity (EC 1.6.4.2; EGR) (μmol NADPH oxidized/ml erythrocytes per h), its stimulation with FAD added in vitro (EGR-AC), erythrocyte aspartate aminotransferase activity (EC 2.6.1.4; EAT) (μmol NADH oxidised/ml erythrocytes per h) and its stimulation with PLP added in vitro (EAT-AC), in rural Indian schoolboys of groups A and B‡ before treatment*

(Mean values with their standard errors)

| Group | Erythrocyte riboflavin (μg/l) | | | EGR activity | | | | | | | EAT activity | | | | | | | |
| | n | Mean | SE | n | −FAD Mean | SE | +FAD Mean | SE | EGR-AC (+FAD:−FAD) Mean | SE | n | −PLP Mean | SE | +PLP Mean | SE | EAT-AC (+PLP:−PLP) Mean | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A Oral lesions: | 37 | 221 | 10·6 | 42 | 72·7 | 2·97 | 142·0 | 5·88 | 1·98 | 0·051* | 36 | 61·0 | 2·38 | 109·3 | 2·90 | 1·83 | 0·043* |
| Only angular stomatitis | 30 | 218 | 11·7 | 31 | 72·6 | 3·92 | 140·4 | 7·37 | 1·96 | 0·061 | 29 | 63·0 | 2·77 | 113·2 | 3·01 | 1·84 | 0·050 |
| Angular stomatitis with glossitis | 7 | 234 | 25·9 | 11 | 72·8 | 2·82 | 146·4 | 8·93 | 2·01 | 0·094 | 7 | 52·6 | 2·78* | 93·1 | 4·81*† | 1·79 | 0·084 |
| B Without oral lesions | 29 | 208 | 08·5 | 32 | 76·9 | 4·05 | 134·3 | 6·46 | 1·81 | 0·066 | 28 | 65·2 | 3·01 | 108·7 | 3·64 | 1·70 | 0·036 |

\* Significantly different from group B by Student's two tailed *t* test (*P* < 0·05).
† Significantly different from group A boys with only angular stomatitis by Student's two tailed *t* test.
‡ For details, see Table 1 and p. 434.

Table 5. *Effect of vitamin B-complex treatment on erythrocyte riboflavin and Erythrocyte Glutathione Reductase test†* of rural Indian schoolboys with oral lesions (groups A1, A2, A3) or without oral lesions (groups B1, B2)‡

(Mean values with their standard errors)

| Group | Treatment | Erythrocyte riboflavin (μg/l) | | | EGR activity | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Mean | SE | n | −FAD Mean | −FAD SE | +FAD Mean | +FAD SE | +FAD:−FAD Mean | +FAD:−FAD SE |
| **A Oral lesions** | | | | | | | | | | | |
| A1 | B-vitamins | 12 | | | 16 | | | | | | |
| | BT | | 213 | 18·0 | | 66·4 | 4·03 | 135·1 | 9·06 | 2·05 | 0·82 |
| | AT | | 291 | 13·8**** | | 126·3 | 9·91**** | 164·0 | 8·62 | 1·35 | 0·064**** |
| A2 | Placebo | 10 | | | 12 | | | | | | |
| | BT | | 231 | 17·3 | | 74·7 | 6·84 | 140·6 | 11·68 | 1·92 | 0·095 |
| | AT | | 266 | 15·2** | | 84·8 | 6·11 | 142·8 | 9·60 | 1·72 | 0·115*** |
| A3 | Gentian violet | 12 | | | 13 | | | | | | |
| | BT | | 233 | 20·8 | | 78·3 | 5·04 | 152·2 | 11·20 | 1·95 | 0·097 |
| | AT | | 205 | 16·8 | | 85·2 | 5·15 | 149·0 | 7·86 | 1·78 | 0·076* |
| **B Without oral lesions** | | | | | | | | | | | |
| B1 | B-vitamins | 11 | | | 15 | | | | | | |
| | BT | | 196 | 13·1 | | 78·9 | 5·76 | 142·0 | 8·72 | 1·89 | 0·112 |
| | AT | | 242** | 10·8** | | 125·1 | 7·70** | 160·5 | 5·69* | 1·34 | 0·088**** |
| B2 | Placebo | 12 | | | 13 | | | | | | |
| | BT | | 222 | 15·9 | | 76·2 | 7·52 | 131·2 | 12·03 | 1·76 | 0·089 |
| | AT | | 243 | 15·7 | | 92·6 | 7·49 | 157·3 | 9·00 | 1·76 | 0·094 |

AT, after treatment; BT, before treatment.
Values for AT were significantly different from values for BT by paired *t* test (two-tailed).
* P < 0·05, ** P < 0·02, *** P < 0·01, **** P < 0·001.
† For details, see Table 4.
‡ For details, see Table 1 and p. 434.

(Table 6). AC however did not show a significant change. In boys who did not receive vitamin supplements (groups A2, A3 and B2) AC values tended to increase over the 1 month period (Table 6).

*Thiamin.* Results shown in Table 3 indicate a fairly low prevalence of biochemical thiamin deficiency. None of the boys complained of neurological symptoms. Treatment with thiamin did not produce a significant change in ETK-AC (Table 6). Boys whose AC value was more than 1·15 showed a decrease at the end of 1 month regardless of the type of treatment. Despite B-complex vitamin treatment, ETK-AC value was increased in two boys.

*Dietary history.* Information on dietary history showed that the intake of vitamin A and riboflavin was less than 40 % of the recommended dietary allowance (RDA) (Nutrition Expert group, Indian Council of Medical Research, 1968) for that age. Marked deficits in the intake of energy, thiamin, iron and vitamin C were also present. Diet provided 70 % of the RDA for the other nutrients such as protein, calcium, pyridoxine and niacin (Table 7).

## DISCUSSION

Information on dietary intake revealed marked deficiency of energy and vitamins, particularly vitamin A, thiamin and riboflavin (Table 7). The RDA values of the Indian Council of Medical Research are higher than those of the World Health Organization with regard to protein, iron and Ascorbic acid. The higher Indian allowance is due to the source of these nutrients and cooking practices in India.

The results of enzymic tests showed that the prevalence of biochemical riboflavin deficiency was very high in these boys, followed by pyridoxine deficiency, whereas thiamin deficiency was not a problem (Table 3). Low prevalence of biochemical thiamin deficiency and the absence of clinical deficiency despite a low intake of the vitamin was surprising. It is possible that thiamin requirement is more closely dependent on energy than that of riboflavin, and thiamin deficiency was not seen due to an associated energy deficiency of equal magnitude.

A high prevalence of biochemical pyridoxine deficiency despite the fact that the diet provided more than 75 % of the RDA is also surprising. Since results of enzymic tests and dietary intake studies were available on only a subsample and not for the same boys, a direct comparison of the EAT test and pyridoxine intake cannot be done. Riboflavin deficiency has been reported to affect pyridoxine metabolism and increase pyridoxine requirement (Lakshmi & Bamji, 1974, 1975, 1976).

Although enzymic tests have been used for the detection of biochemical thiamin, riboflavin and pyridoxine deficiencies for several years, 'cut-off' points to distinguish between adequacy and deficiency have not been clearly established. Sauberlich *et al.* (1973) have suggested tentative guide-lines based on several reports, and these have been adopted in the present study for ETK-AC and EGR-AC. In the instance of the EAT test, AC values ranging from 1·5 to 2·0 have been used as 'cut-off' points by different workers (Salkeld *et al.* 1973; Sauberlich *et al.* 1973; Thurnham, 1977). In the present study, the value of 1·7 was used because the average stimulation after 1 month of treatment with B-complex vitamins approximated to this value.

The increase in basal and stimulated activities of EGR and EAT after B-vitamin supplements (Tables 5 and 6) suggested that relatively-high doses of these vitamins can increase the levels of apoenzymes either by synthesis of new enzyme or by stabilization of existing enzyme. This would increase the vitamin requirement for apoenzyme saturation and the values for respective AC might fail to decrease after supplementation, as was observed in the instance of the EAT test (Table 6). Interpretation of results of enzymic tests under these situations becomes difficult.

Table 6. *Effect of B-vitamins treatment on erythrocyte transketolase (EC 2.2.1.1) (ETK) activity, μmol sedoheptulose formed/ml erythrocytes per h, its stimulation with TPP added in vitro (ETK-AC) and EAT† test of rural schoolboys‡ with oral lesions (groups A1, A2, A3) or without oral lesions (groups B1, B2)*

(Mean values with their standard errors)

| Group | Treatment | n | EAT activity −PLP Mean | SE | +PLP Mean | SE | +PLP:−PLP Mean | SE | n | ETK activity −TPP Mean | SE | +TPP Mean | SE | +TPP:−TPP Mean | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A Oral lesions** | | | | | | | | | | | | | | | |
| A1 B-vitamins | BT | 8 | 50·1 | 3·12 | 103·9 | 4·61 | 1·84 | 0·073 | 12 | 7·19 | 0·527 | 7·41 | 0·401 | 1·05 | 0·035 |
| | AT | | 70·3 | 2·70*** | 118·1 | 7·14* | 1·67 | 0·060 | | 8·15 | 0·435 | 8·50 | 0·550 | 1·04 | 0·029 |
| A2 Placebo | BT | 9 | 60·1 | 5·24 | 108·9 | 3·92 | 1·88 | 0·110 | 8 | 6·82 | 0·269 | 7·59 | 0·530 | 1·11 | 0·035 |
| | AT | | 55·3 | 6·06 | 110·1 | 6·62 | 2·08* | 0·130* | | 8·17 | 0·726 | 8·54 | 0·479 | 1·07 | 0·049 |
| A3 Gentian violet | BT | 12 | 65·3 | 5·31 | 112·6 | 5·52 | 1·78 | 0·080 | 10 | 7·00 | 0·872 | 7·34 | 0·855 | 1·06 | 0·037 |
| | AT | | 54·8 | 2·82* | 114·4 | 5·40 | 2·10 | 0·047**** | | 7·83 | 0·371 | 8·09 | 0·425 | 1·03 | 0·017 |
| **B Without oral lesions** | | | | | | | | | | | | | | | |
| B1 B-vitamins | BT | 10 | 64·1 | 4·93 | 103·6 | 5·19 | 1·66 | 0·068 | 11 | 6·48 | 0·575 | 6·74 | 0·544 | 1·05 | 0·020 |
| | AT | | 74·3 | 5·09** | 126·5 | 7·07* | 1·73 | 0·072 | | 8·84 | 0·503 | 9·24 | 0·452 | 1·06 | 0·035 |
| B2 Placebo | BT | 12 | 63·2 | 3·10 | 109·8 | 4·20 | 1·75 | 0·038 | 12 | 5·69 | 0·401 | 6·16 | 0·484 | 1·08 | 0·030 |
| | AT | | 61·1 | 4·09 | 114·1 | 4·05 | 1·92 | 0·087 | | 7·53 | 0·480*** | 7·91 | 0·440 | 1·06 | 0·025 |

AT, after treatment; BT, before treatment.

Values for AT were significantly different from values for BT by paired $t$ test (two tailed).

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$.

† For details, see table 4.

‡ For details, see table 1 and p. 434.

Table 7. *Profile of nutrient intake\* in rural Indian schoolboys (n 53) (% recommended dietary allowances†, % RDA) (as recommended by the Indian Council of Medical Research, ICMR)† & by the WHO‡*

| Nutrient | % RDA | % WHO RDA |
|---|---|---|
| Protein | 86·7 | 114·5 |
| Energy | 58·3 | 47·5 |
| Calcium | 97·3 | 97·3 |
| Iron | 63·7 | 114·0 |
| Retinol | 33·5 | 33·5 |
| Thiamin | 54·6 | 56·3 |
| Riboflavin | 39·5 | 30·3 |
| Pyridoxine | 76·5§ | 98·4§ |
| Nicotinic acid | 70·8 | 59·5 |
| Ascorbic acid | 61·4 | 119·4 |

* Values for all the nutrients in foods (except pyridoxine) were obtained by referring to food tables (Gopalan *et al*. 1971). Pyridoxine content of foods was determined by referring to values given by Bapurao (1975) and FAO (1972) food tables.
† Recommended by the Nutrition Expert Group. Indian Council of Medical Research (1968).
‡ Recommended by the FAO/WHO (1973).
§ Derived arbitrarily by assuming requirement of 1·5 mg/50 g dietary protein. Estimates of protein requirement obtained from % RDA.

The changes in the enzyme activities seen in the unsupplemented groups (groups A2, A3 and B2) (Tables 3, 5 and 6) suggest that small but significant alterations may occur without any intervention, and emphasizes the need for inclusion of suitable placebo ('no treatment') groups in studies of this nature. These changes may represent intra-individual daily variations and in any evaluation of changes after intervention only changes above these should be considered as really significant. It is rather surprising that while riboflavin status in the unsupplemented children tended to improve over the 1 month period, pyridoxine status tended to decrease (Tables 3, 5 and 6). Since the dietary sources of these two vitamins are generally common, non-dietary environmental factors must also have an effect on enzymic tests.

The absence of an inverse correlation between erythrocyte riboflavin concentration and EGR-AC is surprising since EGR is the major flavoprotein in the erythrocyte. Basal and stimulated EGR activities also failed to correlate with erythrocyte riboflavin. These observations are in agreement with our earlier findings (Bamji, 1969). The results, however, suggest that while erythrocyte riboflavin values less than 220 $\mu$g/l may indicate riboflavin deficiency as judged by an EGR-AC value of 1·2 or more, high levels need not always mean sufficiency. One reason for this discrepancy might be the fact that the blood samples were not collected under fasting condition. In animals, pyridoxine deficiency has been shown to increase erythrocyte riboflavin (Sharada & Bamji, 1972). However, in the present study erythrocyte riboflavin levels failed to correlate with EAT activity or AC. This point needs further investigation. According to Sauberlich *et al*. (1973), an erythrocyte riboflavin concentration of more than 150 $\mu$g/l indicates adequacy. The present results do not support the use of this 'cut-off' point.

Results on treatment and prevention of oral lesions with B-complex vitamins (Table 2) suggest that while glossitis has a cause-effect relationship with deficiency of B-complex vitamins (riboflavin and pyridoxine) angular stomatitis may have a more complex aetiology. In the present study niacin status was not assessed. However, earlier reports on urinary excretion of N'-methylnicotinamide do not suggest niacin deficiency in Hyderabad children (Bhat & Belavady, 1970). Response to gentian violet suggests that infection might be the more immediate cause for angular stomatitis. However, gentian violet is an astringent, and

it is not specific for any infection. Response to treatment with specific anti-infective agents needs to be examined. The lower riboflavin and pyridoxine status of children with angular stomatitis (group A), (Tables 3 and 4), may simply mean that the agent that causes angular stomatitis also affects the status of these two vitamins, but there is no causal relationship between vitamin deficiency and angular stomatitis.

Thurnham *et al.* (1971) and Buzina *et al.* (1973) have also examined the correlation between angular stomatitis and riboflavin status as judged by the EGR-AC test in children. They found a higher incidence of angular stomatitis in children with AC values above 1·2 than in children with low AC values, though a direct correlation between clinical and biochemical deficiency was not always observed. Thurnham *et al.* (1971) have in fact implicated factors other than riboflavin deficiency in the development of this condition. Neither of these workers have discussed glossitis, nor have they studied pyridoxine status.

In the authors' experience subjects with EGR-AC values less than 1·2 seldom show oral lesions particularly glossitis, but the reverse is not true. Some subjects with very severe biochemical deficiency of riboflavin are also normal clinically. The two probable factors for this phenomenon are: (*a*) biochemical lesions appear and disappear faster than morphological lesions, (*b*) biochemical lesions predispose the tissue to the disease, but morphological lesions may have a more complex aetiology involving other factors (such as infection) as well.

A correlation between an abnormal EAT test and oral lesions is still more difficult to establish, though clinical studies of Krishnaswamy (1971) and Iyengar (1973) do implicate pyridoxine deficiency in the development of oral lesions, and in the present study boys in group A had higher EAT-AC than boys in group B. In group A, boys with glossitis had the lowest EAT (basal and stimulated) activity (Table 4).

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