Proteinase inhibitors and other biochemical criteria in infants and primary schoolchildren from urban and rural environments

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1. Preschool children and schoolchildren from a rural area in the northeast of Thailand were compared with children from urban areas for prealbumin, albumin, transferrin, α_1B-glycoprotein, the acute-phase reactants α_1-acid glycoprotein, α_1-antichymotrypsin, haptoglobin and the proteinase inhibitors α_1-protease inhibitor (A1PI) as well as α_2-macroglobulin (α_2M). Urinary urea nitrogen:creatinine (U–C) as well as haemoglobin were also determined. Village preschool children were grouped according to their SD score for weight-for-height based on the (US) National Center for Health Statistics (1976) standard into a normal group with SD scores of $\geqslant -1.99$ and an undernourished group with SD scores of $\leqslant -2.00$.

2. There was no significant difference between the normal and the undernourished groups of preschool children for any of the factors measured.

3. Haemoglobin and prealbumin concentrations of preschool children were lower in the rural children than in the urban preschool children from Bangkok.

4. In rural schoolchildren haemoglobin was lower but albumin, transferrin, α_1B-glycoprotein and haptoglobin were higher than in urban schoolchildren from the provincial town of Khon Kaen.

5. Serum concentrations of the proteinase inhibitors A1PI and α_2M were significantly higher in the rural children than in the urban children.

6. U–C values were lower in rural schoolchildren compared with urban schoolchildren.

For children without clinical signs of protein-energy malnutrition (PEM), but classified as malnourished by anthropometric measurements, it is difficult to relate growth retardation and disproportion of body-weight-to-length to functional deficits (Sastry & Srikantia, 1976; Richardson, 1977; Waterlow, 1978).

Children suffering from so-called subclinical PEM are obviously able to adjust their metabolism in order to maintain the levels of various biochemical factors in the same range as those of children classified as healthy. However, differences in biochemical factors might be observed when comparing groups living in a rural environment with those of an urban environment derived from a higher socio-economic class. It was demonstrated previously that serum protein patterns obtained by polyacrylamide-gel electrophoresis (PAGE) from schoolchildren and adults, and urinary urea nitrogen:creatinine (U–C) values from schoolchildren differed between urban and rural groups of individuals (Schelp *et al.* 1974; Schelp *et al.* 1976; Schreurs *et al.* 1977). Variations in individual serum protein concentrations reflect metabolic alterations even better than the serum protein pattern obtained by PAGE. Therefore several individual serum proteins have been investigated in children from urban and rural surroundings: (1) albumin, prealbumin and transferrin, suggested to be indices of the nutritional status, at least in clinical PEM (Ingenbleek *et al.* 1972; Ingenbleek *et al.* 1975; Whitehead *et al.* 1975; Reeds & Laditan, 1976); (2) the acute-phase reactants (AP) haptoglobin, α_1-acid glycoprotein, α_1-antichymotrypsin (Ach), α_1-protease inhibitor (A1PI), formerly termed α_1-antitrypsin (Koj, 1974); (3) α_2-macroglobulin (α_2M), a proteinase

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inhibitor (PI) (the AP proteins Ach and A1PI also belong to the group of PI (Heimburger *et al.* 1971); the role of PI in clinical PEM was studied recently, and it was suggested that PI may influence the mobilization of endogenous proteins (Schelp *et al.* 1977; Schelp *et al.* 1978; Schelp *et al.* 1979); (4) α_1B-glycoprotein, previously found at elevated serum levels in rural children have been studied also (Schelp *et al.* 1976).

U–C and haemoglobin were included. U–C is supposed to be an indicator of the dietary protein intake (Dugdale & Edkins, 1964; Committee on Procedures for Appraisal of Protein–Calorie Malnutrition, 1970).

EXPERIMENTAL

Subjects. The survey was done at the beginning of the rainy season (May and June in 1977 and 1978) approximately 3 months after harvesting and involved 180 preschool children in the age range 12–60 months from seven villages associated with an irrigation scheme in the Khon Kaen province and three villages nearby, approximately 450 km northeast of Bangkok, Thailand. The children were from randomly-selected families within the villages. The environment of all the villages was identical and there were no differences in any of the factors investigated between the 'irrigation' and 'non-irrigation' areas. Another group of apparently-healthy children were selected from those attending a paediatric clinic (for healthy children) attached to the Department of Paediatrics of the University Hospital, Chulalongkorn University, Bangkok. Parents of these children were regarded as belonging to the well-to-do middle class of Bangkok. Schoolchildren (134) from three different villages of Khon Kaen province were investigated together with 109 apparently-healthy schoolchildren from the University School, Khon Kaen. The parents of the urban schoolchildren belonged either to the academic staff of Khon Kaen University or to well-to-do families from Khon Kaen town. The parents of the village children were involved primarily in the farming of rice, vegetables or a cash crop. All the village children were apparently free from acute illness. Mild to moderate parasitic infection was found in approximately 20% of the preschool children and 50% of the schoolchildren; mainly hookworm and some other soil-transmitted helminths were detected.

Age was recorded from birth certificates, weight and height were measured and the SD scores for weight-for-height were calculated from a North American standard ((US) National Center for Health Statistics, 1976) according to the suggestion of Waterlow *et al.* (1977). The village preschool children were grouped according to their SD scores for weight-for-height. One group with SD scores of −1·99 or above were considered to be normal and another group with SD scores of −2·00 or lower were considered to be subclinically malnourished.

Methods. Blood was taken by finger prick and collected in heparinized capillary tubes, then plasma was separated and stored frozen at −20° before electrophoresis. The method used for quantitative measurement of serum proteins was the electroimmunoassay (rocket immunoelectrophoresis) (Laurell, 1972). Monospecific rabbit antisera for the different proteins were supplied by Behring Werke, Marburg, West Germany. Haemoglobin concentrations were determined by the cyanmethaemoglobin method (Aculute reagents; Ortho Diagnostic Corp., Raritan, New Jersey, USA) from whole blood immediately after blood collection.

Random urine samples were collected during the morning and acidified with hydrochloric acid (0·1 ml concentrated HCl in 30 ml urine). The samples were frozen before analysis. Creatinine was measured by a modification of the procedure of Folin & Wu (1919) and urea-N was determined using a modification of the method of Marsh *et al.* (1965) as described by Technicon Instruments Corp. (1972) for the AutoAnalyzer.

Results were analysed using the one-way analysis of variance (Campbell, 1967), Student's

Table 1. *Age (months), SD score for weight-for-height (US) (National Center for Health Statistics, 1976), haemoglobin, prealbumin, albumin, transferrin and urinary urea-nitrogen: creatinine for preschool children living in urban and rural areas in Thailand*

(Mean values and standard deviations; no. of children/group given in parentheses)

			Village children			
Group no. ...	Bangkok children (47) 1		SD score weight-for-height ⩾ 1·99 (145) 2		SD score weight-for-height ⩽ 2·00 (35) 3	
	Mean	SD	Mean	SD	Mean	SD
Age (months)	22·6	8·2	33·7	13·2	28·7	12·5
SD score for weight-for-height	−0·36	0·8	−0·74	0·7	−2·54	0·4
Urinary urea-N:creatinine	—	—	8·3	2·8	9·6	3·4
Haemoglobin (g/l)	131	13	118	14	116	12
Prealbumin (mg/l)	188	44	166	44	151	40
Albumin (g/l)	—	—	41	7·6	44	7·0
Transferrin (mg/l)	3206	400	3107	670	3010	431

Differences between groups were statistically significant ($P \leqslant 0·05$) when tested by analysis of variance (Campbell, 1967); age: group 1 *v.* groups 2 and 3, group 2 *v.* group 3; haemoglobin: group 1 *v.* groups 2 and 3; prealbumin: group 1 *v.* groups 2 and 3.

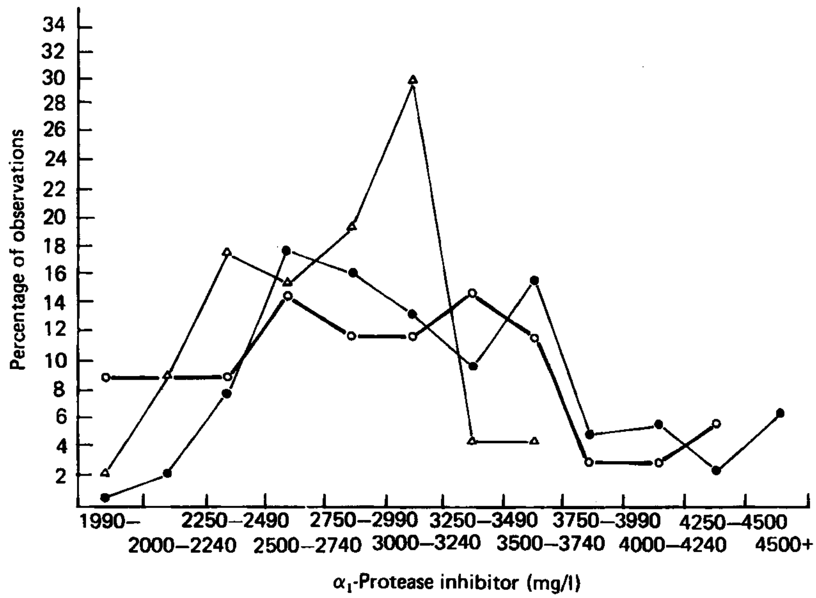

Fig. 1. α_1-Protease inhibitor in 145 preschool children from the villages with SD score weight-for-height ⩾ 1·99 ((US) National Center for Health Statistics, 1976), median of α_1-protease inhibitor 3100 mg/l (●—●), thirty-five preschool children from the villages with SD score for weight-for-height ⩽ −2·00, median 3190 mg/l (○—○), and forty-seven preschool children from Bangkok, median 2980 mg/l (△—△). Differences between the two groups of villages children and the Bangkok children were statistically significant when tested by rank sum test (Snedecor & Cochran, 1967) ($P \leqslant 0·005$).

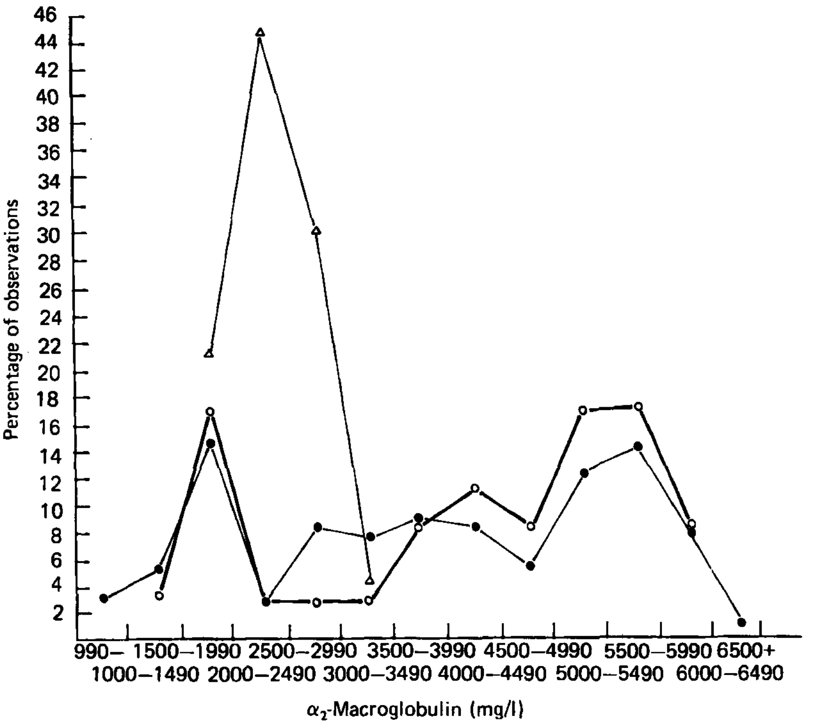

Fig. 2. α_2-Macroglobulin in 145 preschool children from the villages with SD score weight-for-height $\geqslant$ 1·99 ((US) National Center for Health Statistics, 1976), median 3960 mg/l (●—●), thirty-five preschool children from the villages with SD score for weight-for-height $\leqslant -2\cdot00$, median 4720 mg/l (○—○) and forty-seven preschool children from Bangkok, median 2340 mg/l (△—△). Differences between the two groups of village children and the Bangkok children were statistically significant when tested by rank sum test (Snedecor & Cochran, 1967) ($P \leqslant 0\cdot05$).

t test with independent samples or the rank sum test (Snedecor & Cochran, 1967). Differences between groups at $P \leqslant 0\cdot05$ were considered significant. Unfortunately urinary factors could not be assessed in Bangkok preschool children because urine collection had to be done in the afternoon, resulting in a wide variation in values. Thus a comparison with values from village preschool children, whose urine had been collected during the morning, was not possible. The volume of serum collected from Bangkok children was insufficient for the estimation of albumin.

RESULTS

Values for age, the SD score for weight-for-height, haemoglobin and factors considered to be indicative of nutritional status, U–C, prealbumin, albumin and transferrin for preschool children are given in Table 1. The preschool village children were grouped according to their SD score for weight-for-height into a well-nourished group (SD score $\geqslant -1\cdot99$) and a subclinically malnourished group (SD score $\leqslant -2\cdot00$). The age of the urban preschool children was lower than that of the village children, and those village children considered to be normal were older than the subclinically-malnourished children. Mean values for haemoglobin and prealbumin were slightly but significantly higher in the Bangkok preschool children than in the village children. Transferrin values did not differ between the groups. There was no difference between the normal and undernourished groups of preschool village children in any of the biochemical factors studied.

Table. 2. *Age (months), SD score for weight-for-height (US) (National Center for Health Statistics, 1976), haemoglobin, urinary urea-nitrogen:creatinine and various serum protein concentrations for schoolchildren living in urban and rural areas in northeast Thailand*

(Mean values and standard deviations; no. of children/group given in parentheses)

	Khon Kaen town schoolchildren		Village schoolchildren	
	Mean	SD	Mean	SD
Age (months)	92·2	7·7 (109)	100·0	10·7 (134)
SD score for weight-for-height	−0·45	0·79 (109)	−0·37	0·8 (134)
Haemoglobin (g/l)	134	12 (109)	123	13 (134)
Urinary urea-N:creatinine	9·6	2·4 (103)	5·5	1·7 (120)
Prealbumin (mg/l)	197	40 (107)	184	44 (132)
Albumin (g/l)	43	5·0 (105)	47	4·0 (56)
Transferrin (mg/l)	2424	403 (104)	2837	575 (134)
α_2-Macroglobulin (mg/l)	2804	663 (103)	3192	589 (134)
α_1B-glycoprotein (mg/l)	1237	234 (106)	1572	242 (130)
α_1-Antichymotrypsin (arbitrary units*/l)	1123	304 (107)	1234	322 (133)
α_1-Acid glycoprotein (mg/l)	763	219 (104)	835	278 (129)

* Value derived from pooled samples from 100 blood donors was set as 1000 arbitrary units/l. Differences between groups were statistically significant ($P \leqslant 0.005$) when tested by independent Student's *t* test (Snedecor & Cochran, 1967) for age, haemoglobin, urinary urea-nitrogen:creatinine, albumin, transferrin, α_2-macroglobulin, α_1B-glycoprotein.

In view of the extreme positive skew for values of A1PI (Fig. 1) and α_2M (Fig. 2) for preschool children a non-parametric significance test was used. There was a significant difference between median values for both A1PI and α_2M of the Bangkok and village children.

The results for schoolchildren are given in Table 2, Figs. 3 and 4. The age of the village schoolchildren was slightly higher than that of the Khon Kaen town schoolchildren. None of the village schoolchildren had an SD score for weight-for-height of −2·00 or lower. Serum concentrations of A1PI (Fig. 3) and haptoglobin (Fig. 4) showed non-parametric distributions. When comparing Khon Kaen schoolchildren with village schoolchildren, haemoglobin and U–C values were significantly lower but albumin, transferrin, α_2M, α_1B-glycoprotein (Table 2) A1PI (Fig. 3) and haptoglobin (Fig. 4) were significantly higher. No significant differences were observed for prealbumin, Ach, α_1-acid glycoprotein (Table 2).

Various combinations of criteria were tested for correlation by simple and multiple linear regressions but no significant correlations or very low correlation were found.

The worm-load in parasite-infected children was usually mild and all attempts to correlate one of the factors to parasite infection failed.

DISCUSSION

Albumin, prealbumin and transferrin values obtained for all groups of children were within the range for normal individuals from Western countries (Vahlquist *et al.* 1975; Hitzig, 1977). U–C values for the groups of schoolchildren in the present study were lower than those reported from Africa and Europe (Simmons, 1972).

There was no significant difference between the subclinically-undernourished group (defined by an SD score for weight-for-height of $\leqslant -2.00$) and the normal group of village preschool children in any of the biochemical factors. Besides slightly lower mean prealbumin

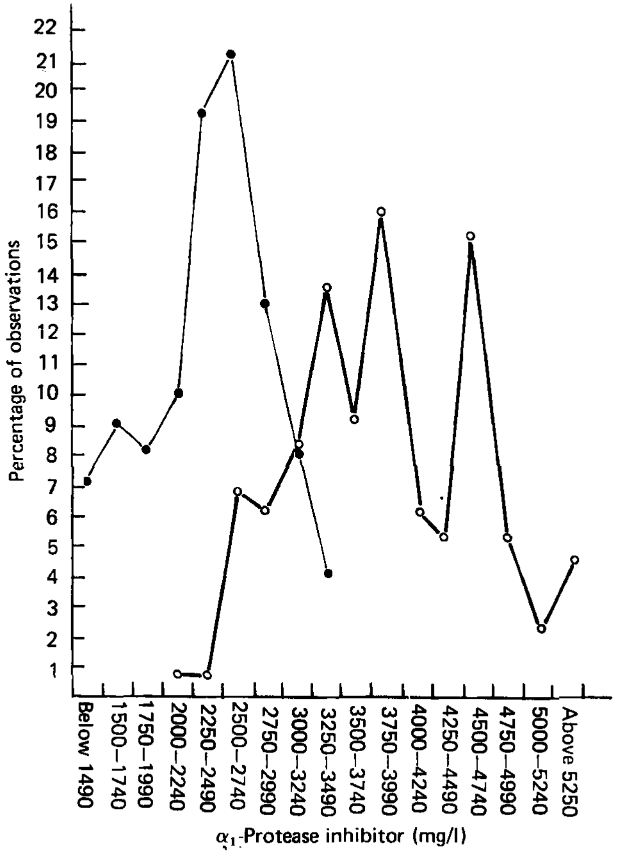

Fig. 3. α_1-Protease inhibitor in ninety-nine school children from Khon Kaen town, median 2420 mg/l (●—●), and 132 schoolchildren from villages, median 3770 mg/l (○—○). Difference between groups was statistically significant when tested by rank sum test (Snedecor & Cochran, 1967) ($P \leqslant 0{\cdot}05$).

and haemoglobin values in village preschool children compared with Bangkok children, none of the proteins indicative of nutritional status differed between the urban and the rural groups.

U–C values were significantly higher in urban schoolchildren than those in rural schoolchildren, indicating a lower protein intake in the latter group. This is in accordance with dietary surveys undertaken in the study area by other investigators (Chandrapanond *et al.* 1972; Migasena *et al.* 1974; Kumazawa *et al.* 1974). These workers found marginal intakes for energy and protein compared with requirements recommended by FAO/WHO ad hoc Expert Committee (1973). It might be assumed that in urban children from a higher socio-economic class protein and energy consumption exceeded requirements. However, the children in the rural areas obviously adapted to the marginal intakes and maintained homoeostasis comparable to that of children from well-to-do families, as suggested by the similar albumin, prealbumin and transferrin serum concentrations in all groups.

Acute infection was not apparently prevalent in a great number of schoolchildren. The percentage of schoolchildren with raised haptoglobin levels (> 2500 mg/l) was quite low (Fig. 4), and other AP proteins known to increase their concentrations rapidly in acute infection or trauma (Cleve & Strohmeyer, 1967; Wiedermann *et al.* 1970; Aronsen *et al.* 1972; Johansson *et al.* 1972; Murphy *et al.* 1972; Giblett, 1974; Powanda, 1977), e.g. α_1-acid

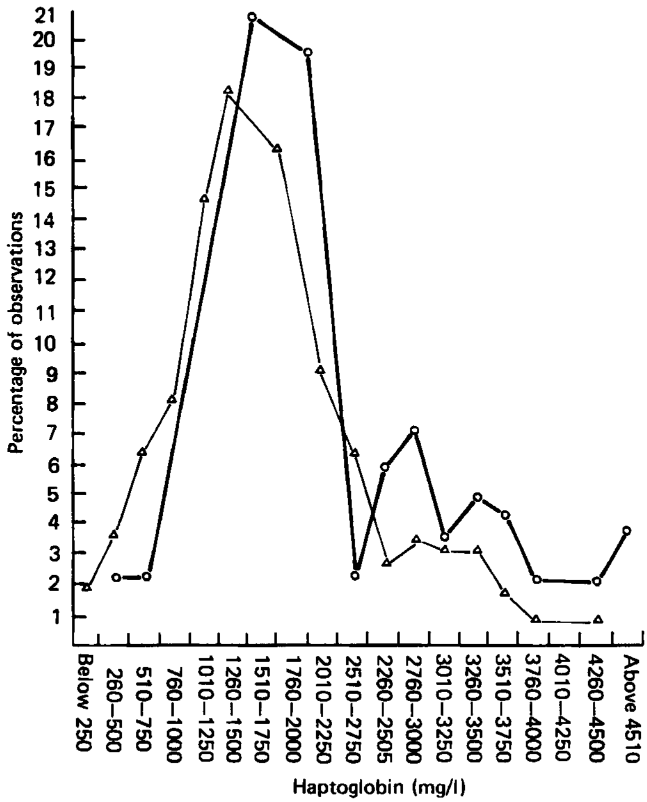

Fig. 4. Haptoglobin in 105 schoolchildren from Khon Kaen town, median 1500 mg/l (○—○), and 134 schoolchildren from villages, median 1970 mg/l (△—△). Difference between groups was statistically significant when tested by rank sum test (Snedecor & Cochran, 1967) ($P \leqslant 0{\cdot}05$).

glycoprotein and Ach, were not elevated (Table 2). Only α_1B-glycoprotein, known to increase slowly in the course of chronic infection and trauma (Clarke *et al.* 1970; Weeke & Jarnum, 1971; Aronsen *et al.* 1972), increased slightly in village schoolchildren (Table 2).

A marked significant increase in PI concentration for the village children was observed in the present study, due to elevated levels of α_2M and A1PI in the rural groups compared with the urban groups. The increased PI level was associated with an unusual non-parametric distribution of the PI in preschool children and of A1PI in schoolchildren (Figs. 1–3). Age-dependent variations were most unlikely to be the reason for these differences. Our own unpublished results (Schelp, Changbumrung, Poshakrishana, Supawan & Pongpaew) confirmed those of other investigators (Hitzig, 1977) in showing that PI and most other proteins reach serum concentration levels similar to those of adults within several months of birth. All children in the present study were more than 1 year of age. Also it was unlikely that differences in the distribution of Pi phenotypes of A1PI (Fagerhol & Braend, 1965) between the northeast region and Bangkok was the reason for higher A1PI values in the northeast. In preschool children with the most common Pi^M phenotype A1PI levels found in the northeast also exceeded those found in Bangkok (Pongpaew & Schelp, 1980).

The detailed functions of the plasma PI are far from clear (Laskowski & Kato, 1980) but it is known that A1PI and α_2M are active against a number of proteolytic enzymes

(Heimburger *et al.* 1971; Barrett *et al.* 1974; Steinbuch & Audran, 1974; Ohlsson, 1974; Ohlsson & Skude, 1976; Kress & Paroski, 1978; Miskulin *et al.* 1978; Hall & Roberts, 1978). α_2M remains unchanged or shows a slightly increased serum concentration under stress situations (Aronsen *et al.* 1972; Johansson *et al.* 1972; Lennert *et al.* 1973); however, in burned patients, a five-fold increase of extravascular α_2M had been observed although the total intravascular α_2M remained relatively normal, which indicates an increased metabolism (Farrow & Baar, 1973). In over 100000 serum samples examined, no α_2M deficiency could be detected (Ohlsson, 1974). But recently α_2M deficiency has been reported in a patient with Ehlers-Danlos syndrome (Hossein Mahour *et al.* 1978).

It is possible to speculate on a possible relationship between plasma PI levels and rates of tissue protein degradation (Schelp *et al.* 1978). In this investigation elevated PI levels found in the rural children might limit muscle protein turnover when protein intakes are low. However, this suggestion requires further information about the processes of intracellular protein degradation and the functions of extracellular proteinase inhibitors.

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