

Description: One thousand sixty-four subjects from two or more villages in the following provinces, one in each of the four geographic areas of the country, were chosen for study: central plain, Pra Nakorn (Bangkok); northern, Chiangmai; north-eastern, Ubol, and southern, Songkhla. Dwellings were randomly chosen in each village, and every inhabitant of the dwelling chosen who volunteered was studied. After a general physical examination was performed, 10 ml. of blood was drawn into a syringe moistened with heparin. Blood smears for erythrocyte morphology and malaria parasites were made, 1 ml. was placed in a small bottle for hemoglobin and hematocrit determinations, and the remainder was placed in an iron-free centrifuge tube. Within 4 hours of collection the blood was taken to a "base" laboratory where a "spot" test¹ for G-6-PD deficiency was done as part of another study, and hemoglobin, and hematocrit were determined. Plasma for iron determinations was separated by centrifugation and pipetted into an iron-free storage bottle. The erythrocytes were then washed three times with normal saline and the buffy coat was removed by suction. The saline was decanted off, and the erythrocytes and plasma were stored in dry ice for shipment to Bangkok for further analyses. Stool specimens were obtained from as many subjects as the limited stay in each village would permit. Formalin - ether concentrates were examined for parasites and ova, and Stoll counts were performed on specimens containing hookworm ova.

Enzymatic spectrophotometric assays of glucose-6-phosphate dehydrogenase activity were carried out on the washed erythrocytes by the method of Kornberg and Horecker (1) using a commercially available kit. A unit of activity was defined as a change in optical density at 340 m μ in the reaction mixture of 0.001/min/gm hemoglobin. Plasma iron and iron-binding capacity were determined by the methods of Peters, et. al. (2,3). Hemoglobin electrophoresis was performed either on paper (4) or on cellulose acetate membranes (5).

Diagnoses were assigned to each subject according to the following criteria:

Anemia: Hemoglobin below the following levels:

6 months - 1 year	11.5 gm per 100 ml.
1 - 2 years	12.2 gm per 100 ml.
at 4 years	13.1 gm per 100 ml.
8 - 12 years	14.1 gm per 100 ml.
over 12 (female)	12.0 gm per 100 ml.
over 12 (male)	14.0 gm per 100 ml.

Iron deficiency: Serum iron less than 56 mcg per 100 ml.

Multiparity: Two or more pregnancies.

1. California Corporation for Biochemical Research, Los Angeles, California.

Hookworm Anemia: Anemia as defined above, with hookworm ova in the stool.

G-6-PD deficiency: No enzymatic activity in a hemolysate.

Hemoglobin E and AE: Existence of hemoglobin E on electrophoresis, alone or in combination with hemoglobin A.

Predisposing Factor: Presence of low serum iron, G6PD deficiency, abnormal hemoglobin, or hookworms, but without anemia as defined above.

An estimate of the socio-economic status of each village was formed from observations of personal possessions (radios, bicycles, motor cycles, etc.), from the general health of the population, and from discussion with local government officials.

Progress: Population characteristics. Comparison of the age and sex data with those of the 1960 Thailand Population Census revealed that the groups studied were not representative of their provinces as a whole. There were too few adult males and children under 2 years of age in the sample, and the ratio of males to females was 1:1.3. The lack of children under 2 was due largely to parental resistance or technical difficulties in blood sampling, while the abnormal sex ratio and low number of adults was the resultant of adult males being away at work at the time of examination.

The lowest economic status was found in the villagers of Ubol. The total income for the year in this area is derived from 1 crop of glutinous rice, which is barely sufficient for home consumption and barter for other necessities. The Bangkok group consisted of the families of rice farmers and day laborers, while the Chiangmai population was all rice farmers. The income on these two areas was low, but much better than Ubol. The Songkhla sample was composed of farmers and rubber plantation workers. The latter are well paid, and this relative prosperity appears to be reflected generally in that region of the country.

The villagers of Bangkok were Thai or Thai-Chinese, as were the villagers in Chiangmai, with the exception of one Shan village. The Ubol villagers were Thai-Lao, and those in Songkhla Thai-Malay. Approximately half of the Songkhla study group was Muslim.

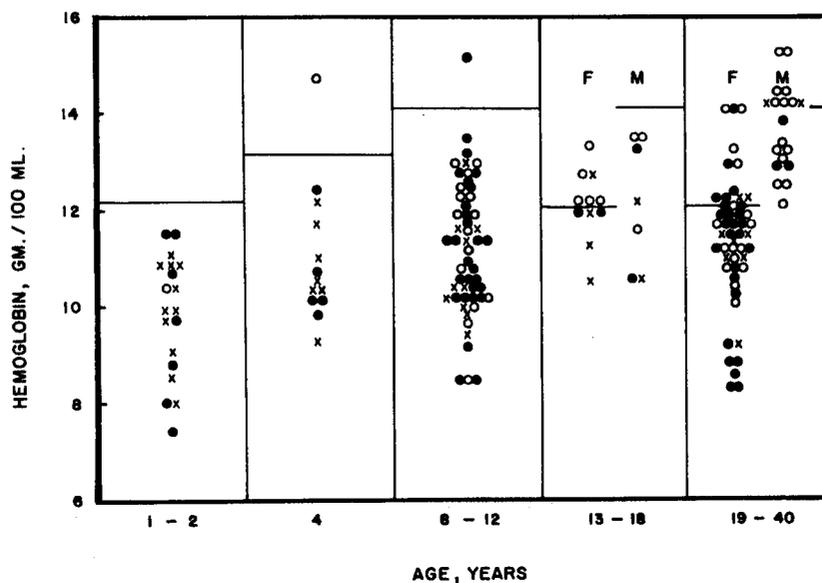
Anemia: The overall results of the survey were given in Table 1. The prevalence of anemia varied between 70 per cent in Songkhla to 89 per cent in Ubol, and was inversely proportional to the relative economic status of the region. Iron deficiency anemia was by far the most common diagnosis, accounting for an average of 55 per cent of the anemia everywhere except Songkhla, where the prevalence was 73 per cent.

The etiology of the iron deficiency varied from one area to another. Hookworm ova were found in 5 per cent of the stools in Bangkok, 20 per cent in Chiangmai,

Table 1

	Bangkok		Chiengmai		Ubol		Songkhla	
	No.	%	No.	%	No.	%	No.	%
1. ANEMIA								
a. Low serum Fe								
Hookworm	11		18		48		82	
multiparity	31		16		20		23	
other, neg stool	62		24		21		5	
other, no stool	14		15		49		28	
b. Normal serum Fe								
G6PD def.	3		13		1			
Hb AE	2		-		17		5	
neg stool	54		41		19		21	
no stool	11		22		18		12	
malaria							2	
c. Serum hemolyzed								
neg stool	36		14		14		3	
no stool	11		13		14		7	
Total	235	88.0	176	75.8	221	89.0	188	69.6
2. HEMATOLOGIC ABNORMALITY WITHOUT ANEMIA								
a. Low serum Fe	6		13		8		14	
b. G6PD def.	1		5		2			
c. Hb E or AE	0				-			
d. Hookworm	0		4		7		38	
3. NORMAL	25		34		9		30	
4. EXAMINATION ONLY NO LABORATORY DATA	11		5		16		16	
Total	278		237		263		286	
Hemoglobin E	1	3.0	0	1.8	12	22.0		5.0
AE	8		4		49		9	
G6PD def.	10	3.8	23	10.0	10	4.0	6	2.2

FIGURE I



50 per cent in Ubol, and 80 per cent in Songkhla. The fraction of the subjects with anemia due to hookworms was roughly proportional to the prevalence of infection in that area. Multiparity was a second condition frequently associated with iron deficiency. Although two pregnancies were used as the criterion for inclusion under this category, the average number of pregnancies in these women was 6, the highest number being 13.

The hemoglobin and serum iron data for selected age groups in Bangkok is shown in Figure 1. All the children 1-2 years of age were anemic, and only 1 had a normal serum iron value. All but 1 of the 4 year olds was anemic, and every anemic child in whom a serum iron determination was done had a low value. The 8-12 year olds had a somewhat higher average hemoglobin as a group, but as the normal for this age was higher, the relative degree of anemia was essentially as severe. Only about half of the adolescent females were anemic, but this apparent improvement is in part due to lowering of the normal hemoglobin value from 14.0 gm per 100 ml. to that for menstruating females, 12.0 gm per 100 ml. Iron deficiency was widespread in adult females, and was almost always associated with multiparity. About half of the adult males had normal hemoglobin values, but only 3 of the 17 in whom serum iron was measured had low values.

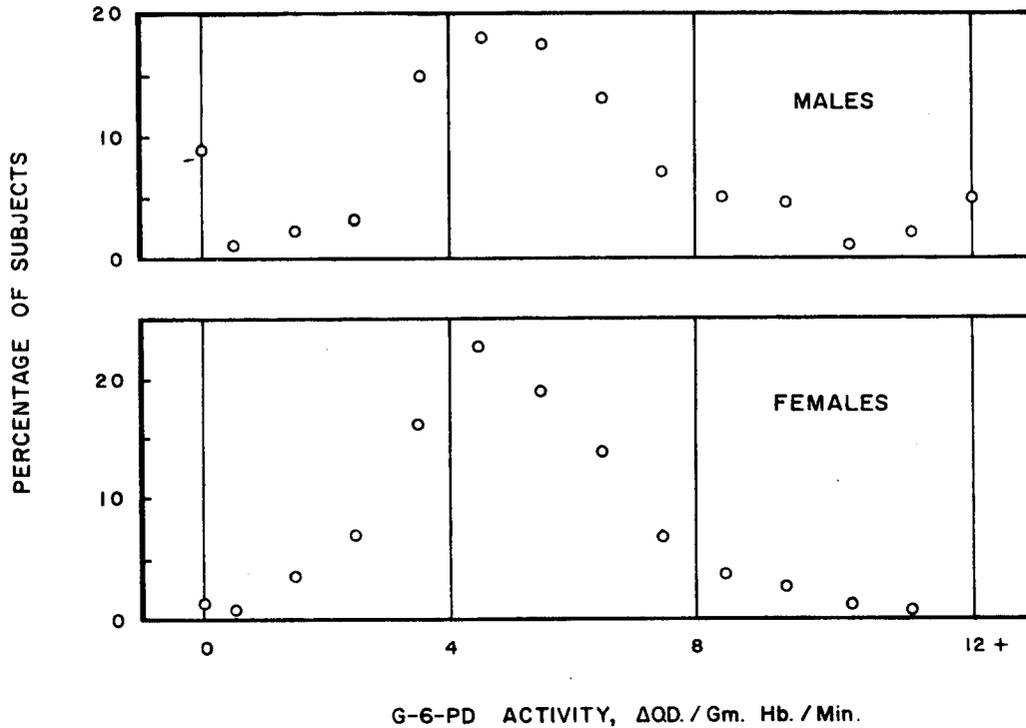
Analyses of the Chiangmai data yielded essentially the same results. This sort of analysis was not attempted on the Ubol and Songkhla data, because the high prevalence of hookworm anemia would obscure these trends.

The anemia subjects with normal serum iron values included some cases of G6PD deficiency and hemoglobin E or AE, conditions in which there is at least some potential from hemolysis. No etiologic diagnosis was apparent in the majority, however. As the unsaturated iron binding capacity was elevated and infections, particularly of the skin and respiratory tract, were seen frequently in all subjects, it is possible that these subjects represent anemia of infection. Hemoglobin F determinations currently underway will also be of value in interpreting these data.

Table 1 does not reflect the prevalence of G6PD deficiency or hemoglobin E in the subjects studied, as most of the subjects in these categories were classified under iron deficiency anemia. Figure 2 shows the distribution of G6PD values found in this study, with males and females plotted separately. With the method of biochemical analysis used, it was not possible to discriminate between normal and heterozygous females, as the "tail" of the distribution curve approached zero in both sexes. For this reason, the diagnosis of G6PD deficiency was restricted to subjects having no enzymatic activity.

The data in the table, do, however, give some indication of the relative frequency of this characteristic from one province to another. The highest prevalence, 10 per cent was in Chiangmai, while the lowest, 2.2 per cent was in Songkhla. Bangkok and Ubol had prevalences of 4 per cent.

FIGURE II



Hemoglobin E or AE was found in 22 per cent of the Ubol subjects, 3 per cent of those in Bangkok, 1.8 per cent of those in Chiangmai, and 5 per cent of those in Songkhla.

Summary and Conclusions: This survey indicates that anemia, particularly iron deficiency anemia, is an important disease problem in all parts of Thailand. In the Bangkok area, the multiparous woman and growing child were found to be particularly vulnerable. Almost half of the anemic subjects in Bangkok, however, had normal serum iron levels. Some of these undoubtedly had iron deficiency, as the serum iron concentration is a notoriously poor index of iron stores. The elevated unsaturated iron binding capacities and the frequent, almost universal, finding of skin and respiratory infections suggest that some of these subjects may represent anemia of infection (undiagnosed thalassemia, too, could explain these results).

In the Bangkok area, it was possible to demonstrate a "vicious cycle" beginning with an iron deficient mother who gives birth to iron deficient children. These children, for reasons not evident in this study, fail to achieve normal hemoglobin levels during growth; only 4 of 32 children 10-12 years of age had a hemoglobin level above 13 gm per 100 ml. The adolescent girl has a reduction in hemoglobin level with the onset of menses, and a further decrease when she marries and begins to bear children. Bangkok males appear to fare little better; all of the adolescent males were anemic and even in the prime of life, half of the men still had hemoglobin levels below 14 gm per 100 ml. This relationship became obscured as the prevalence of hookworm anemia rose in other areas of the country.

In Songkhla, which is the opposite extreme from Bangkok, fewer people were anemic, but if they were, the chances were almost 50 per cent that they had hookworms as the proximate, though perhaps not the sole cause. Hookworm anemia was given precedence in the classification, and this tends to discount the effects of other causes of anemia.

The peculiar distribution of G6PD activity values in the groups studied deserves comment. Values in this survey are generally lower than those reported elsewhere in the world, the modal values for Thai males and females in this study being between 4 and 5 units, which would be considered as "low normal" by criteria derived from other populations. This may in part be related to the general health of the group under study, for Prasertsri and Canfield, studying 100 hematologically normal Thai college students, found that G6PD activity distributed about a mode of 7 units. In spite of generally higher values, 4 per cent of their sample still had values between 2 and 4 units. In neither the study subjects nor the college students was there clear separation of normal, heterozygous females, and deficient subjects. For this reason, it was felt better to designate only subject with no demonstrable activity in their erythrocytes as deficient. In view of these difficulties, it would appear that family studies are necessary to detect heterozygous females in this population. It should also be noted that the male:female ratio was 1:1.3 in the subjects studied. The true prevalence should consequently be somewhat higher, because there were relatively too few males, who more frequently have this condition.

One very important finding that is not reflected in the data present, herein is that the anemia seen in all regions is symptomatic. Fatigue, faintness, weakness and palpitation were frequently encountered complaints. It was not difficult to equate these symptoms with the decrease in productivity and material achievement found so frequently in the more seriously affected areas.

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ADDENDUM

Abstract: Fecal iron excretion was measured in 5 adult Thai subjects, 7 females and 5 males. Excretion ranged between 3.7 and 13.6 mg per day, with an average of 8.9 mg per day. Administration of oral iron for 1 month resulted in an average rise in the hemoglobin level of 0.9 gm per 100 ml in the anemic subjects. Four anemic subjects excreted relatively large amounts of iron and had little response to iron administration, suggesting impaired absorption of this element.

Objectives: The results of the anemia survey reported above indicate that iron deficiency anemia is an important health problem throughout Thailand. Unlike other parts of the country, hookworm infection does not explain the high prevalence found in Bangkok. The evidence would suggest that dietary or metabolic factors were often responsible.

Dietary survey data indicate an adequate dietary iron intake in the Bangkok area. As the error inherent in this method of estimation is great, a pilot study utilizing a more direct approach was undertaken.

Progress: Twelve adult volunteers were chosen at random and without regard to hemoglobin level for these studies. Each subject was asked to collect all stool passed during a 5 day period in plastic bags, which were collected daily by a Public Health nurse. A small amount of stool was removed from each specimen for parasitological examination, and the remainder was frozen in dry ice until analysed.

In order to correct fecal iron data for intestinal blood loss during the collection period, 7 ml. of O negative erythrocytes labelled with Cr⁵¹ activity and hemoglobin content were determined. The amount of iron in the stool resulting from intestinal blood loss was calculated by the formula:

$$\frac{\text{Stool activity}}{\text{Blood activity}} \times \text{blood Hb content} \frac{(\text{gm Hb})}{\text{ml}} \times 3.34 \frac{(\text{mg Fe})}{\text{gm Hb}}$$

The pooled 5 day collection of stool was weighed, homogenized, and aliquots were taken for analysis. Stool iron was estimated colorimetrically with batho-phenanthroline after acid extraction of the stools by a method developed in this laboratory (Appendix). Results were expressed as average daily excretion.

In order to determine whether iron may exist in the diet in a form which cannot be assimilated or whether malabsorption of iron occurs, the subjects were treated for 1 month with 0.324 gm of ferrous sulfate daily by mouth. Hemoglobin and hematocrit were determined before and at the end of therapy, and reticulocytes were estimated before and after 1 week of iron administration.

The results of these studies are summarized in the table. The mean daily excretion of iron ranged between 3.7 and 13.6 mg, with an average in both males

Table 1

No.	Age	Sex	Parity	Stool	Hb	Serum Iron	Fecal Iron	Hb After Rx	Retic
35	38	F	7	Neg	12.1	60.6	8.5	13.7	1.0
83	44	F	10	Neg	11.8	94.9	7.2	11.3	0.3
213	37	F	7	Neg	11.2	112.9	7.0	15.4	0.5
236	48	F	10	Neg	10.8	65.8	11.9	11.9	0.5
278	26	F	2	Neg	9.3	64.9	10.0	10.0	0.9
279	26	F	4	A	11.2	108.4	11.4	11.9	0.6
SK	42	F	0	Neg	11.3		5.2	12.9	
11	30	M		Neg	14.1	95.3	3.7	14.6	0.3
53	30	M		O.V.	14.3	63.9	9.7	15.2	0.9
63H	33	M		O.V.	12.5	105.5	11.0	13.2	0.9
207	52	M		O.V.	12.8	67.8	6.4	13.7	1.1
234	38	M		H	14.2	61.1	13.6	15.1	0.8

Abbreviations used: A = Ascaris H = Hookworm O.V. = Opisthorchis viverrini

and females of 8.9 mg per day. The stools contained negligible amounts of Cr⁵¹, the maximum blood loss in any subject amounting to 3.2 cc in a 5 day period.

Six females and 2 males were anemic. Of these, 3 females and 1 male excreted over 10 mg of iron in the stool per day, while the others excreted 6-8 mg per day. Interestingly, the lowest fecal excretion, 3.2 mg per day, was in a non-anemic male.

The table also includes data on the response to oral iron administration. One female had a striking response, her hemoglobin rising from 11.2 to 15.4 gm per 100 ml. The remaining 7 anemic subjects had an average rise of 0.9 gm.

Summary and Conclusions: These preliminary studies were undertaken to determine whether the dietary intake of iron is adequate in Bangkok adults, and to determine whether they can respond to orally administered iron. These data on dietary iron intake are of course low, because they do not account for iron absorbed. Although the subjects denied discarding any stools, this is an additional source of potential error which would give low excretion values. Finally, the laboratory method used gives a measure only of iron extractable by 0.2N hydrochloric acid, and this may be less than the total stool iron. On the other hand, it was felt that such an extraction procedure might afford results which are a more reliable index of iron available for absorption.

If one assumes that the latter two errors are absent, and that the iron absorbed

is not more than 2 mg per day, these subjects have at best marginal intake. The recommended dietary allowance of the Food and Nutrition Board, NRC, are 10 mg per day for males and 12 mg per day for females. Two of the men and 5 of the women are below these levels, even when a correction is applied for absorption.

The response to oral iron was quite modest in all the anemic subjects but one, correcting the anemia in only 3 of the women, but failing to do so in either of the men.

Three of the women and one of the men (subjects, 236, 279, and 63H) had a combination of low hemoglobin values, relatively high fecal iron excretion, and very slight response to iron administration. These findings would suggest that malabsorption of iron may exist in these subjects.

APPENDIX

Estimation of Iron in Feces (Preliminary Method)

Captain Philip Z. Sobocinski, MSC
SSG (E7) Robert P. McDevitt, U.S.A.

Principle: A homogenized fecal sample is incubated at room temperature with dilute hydrochloric acid. After dilution and centrifugation at high speed, an aliquot of the supernatant is analyzed for iron content. The ferric iron is reduced to the ferrous form by means of thioglycollic acid. The ferrous iron is then treated with bathophenanthroline to form a pink complex which has maximum absorption at 535 mμ.

Method: To 0.3 to 0.5 grams of homogenized feces add 10.0 ml of 0.2 N hydrochloric acid, mix well on Vortex mixer and let stand at room temperature for thirty minutes. Centrifuge at 17,500 rpm (SS-34 Head, Serval RC-2) for thirty minutes. To 2.0 ml of the clear supernatant, add 3.0 ml of distilled water, 1 drop thioglycollic acid (80%), mix on Vortex and let stand 30 minutes at room temperature. Add 1.0 ml of 30% TCA, mix, cover, and let stand for thirty minutes. Centrifuge at 1500 rpm for ten minutes. Withdraw 3.0 ml of supernatant, add 0.5 ml of saturated sodium acetate and 2.0 ml of 0.02% Bathophenanthroline in isopropyl and isoamyl alcohol (3:1 v/v). Mix well on Vortex and let stand 15 minutes before reading at 535 mμ. A fecal blank is prepared by treating the original acid extract exactly the same as the test with the exception that, 2.0 ml of isopropyl isoamyl alcohol mixture (3:1 v/v) is substituted for bathophenanthroline solution. The spectrophotometer is set at zero O.D. with a mixture of distilled water (3.0 ml) saturated sodium acetate (0.5 ml) and Bathophenanthroline color reagent. (2.0 ml). The standard consists of pure iron wire (assay 99.9%) dissolved in 0.2 N hydrochloric acid to a concentration of 1 microgram iron per ml.

Remarks: All glassware is rendered iron free by soaking in 6 N hydrochloric acid and repeated washing with distilled water.

The concentration/optical density relation is linear up to 0.400 O.D. units which represents a standard iron concentration of 0.6 microgram iron per ml. of final solution. Determinations on samples having a final iron concentration of more than 0.6 microgram iron per ml should be repeated using proportionally less fecal specimen. Any turbidity that may develop in the final solution may be disregarded, since at the wavelength utilized there is no significant interference.

Calculations:

$$\frac{\text{O.D. Test}}{\text{Standard O.D. / per microgram Fe/ml}} \times \frac{5.5 \times 10 \times 2}{2} = \text{mgm Fe in sample aliquot}$$