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PAPER

ANAEMIA IN DAR-ES-SALAAM AND METHODS FOR ITS INVESTIGATION

BY

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Introduction

On commencing practice in Dar-es-Salaam, the medical practitioner is immediately aware that not only is anaemia a most important disease in its own right but that it complicates all other illnesses and dominates the clinical picture in the medical wards; this is no less so in the children's than in the adult wards and is a constant cause for concern in the obstetric hospital and antenatal clinics.

The great prevalence of severe anaemia in Dar-es-Salaam has been noted before (BLACKMAN, 1962; MEREDITH and EYEKUZE, 1962); these authors found malaria to be the most important cause in young children, whereas iron deficiency was incriminated in 50-70% of anaemic adults; in a majority the shortage of iron was attributed to hookworm infection. BLACKMAN (1962) found megaloblastic anaemia in only 7%, most of these patients being women and usually pregnant; this is in contrast to the 39% of 85 anaemic patients reported from Nairobi by FOY and KONDI (1960), although here again more than half were women, almost all of whom were pregnant or lactating. The anaemia responded to penicillin, oral folic acid or oral cyanocobalamin.

In East Africa and India, protein deficiency and liver disease have not been stressed as a cause of anaemia; on the other hand WOODRUFF (1955) in Nigeria found an association between macrocytic anaemia, low serum proteins and liver disease, especially in children and pregnant women. The anaemia did not respond to iron, folic acid or vitamin B12 but there was a response to milk protein.

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The patients showing macrocytic anaemia reported from Ceylon by RAJASURIYA et al. (1962) appear to have been suffering from multiple deficiencies; the authors concluded that protein shortage was not especially important. ADAMS (1954) favoured the existence of macrocytic rather than megaloblastic anaemia as a result of protein deficiency in kwashiorkor.

Incidence of anaemia in Dar-es-Salaam

This study does not provide an estimate of the incidence of anaemia in the general population, but only in those persons admitted to hospital.

Figure 1 gives the distribution of the haemoglobin (Hb) concentration of 4647 consecutive specimens of blood from persons over the age of 5 years, submitted from the

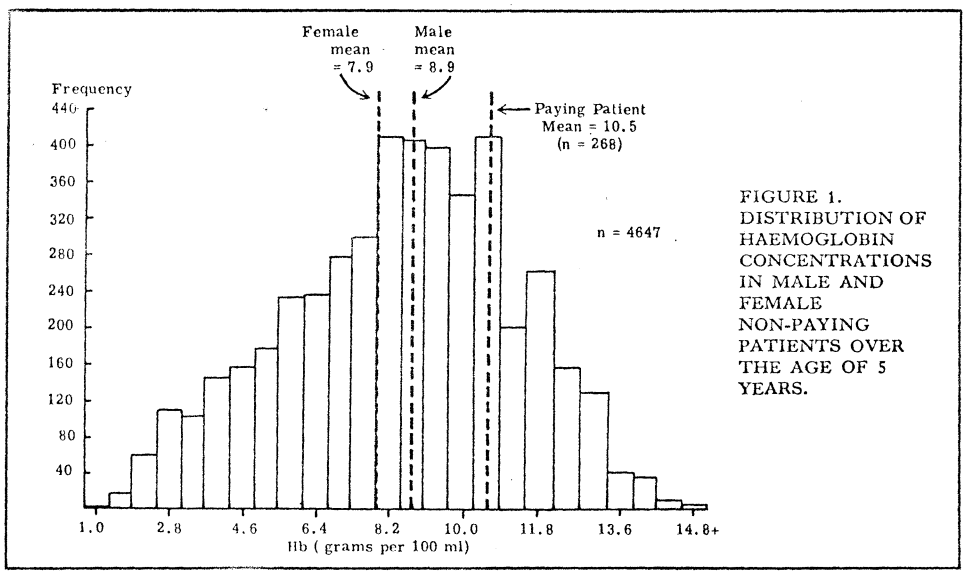


FIGURE 1. DISTRIBUTION OF HAEMOGLOBIN CONCENTRATIONS IN MALE AND FEMALE NON-PAYING PATIENTS OVER THE AGE OF 5 YEARS.

wards of the Muhimbili Hospital to its laboratory over a period in 1963; for the patients supplying these samples the male:female ratio is 1·21:1 and the respective mean Hb concentrations 8·85 and 7·86 g. per 100 ml. (SD 2·87 and 2·58). Considering both sexes together, the distribution is significantly asymmetrical and deviates significantly from normality; the presence of the longer tail towards the lower values and the rather squat appearance of the histogram can be seen in Figure 1.

Figure 2 records the distribution for the 853 specimens of blood received from children under the age of 5 years over the same period; the mean Hb concentration is 6·78 g. per 100 ml. (SD 2·32). This distribution does not deviate significantly from normality but is positively skewed, the longer tail being towards the higher values.

In 1363 instances the packed cell volume (PCV) was also measured and hence the mean corpuscular haemoglobin concentration (MCHC) was calculated. This is related to the Hb concentration in Figure 3, from which it can be seen that the points lie close to a straight line.

It may therefore be concluded that in hospital patients in Dar-es-Salaam anaemia is common, and that the MCHC behaves in a way similar to that seen in iron deficiency.

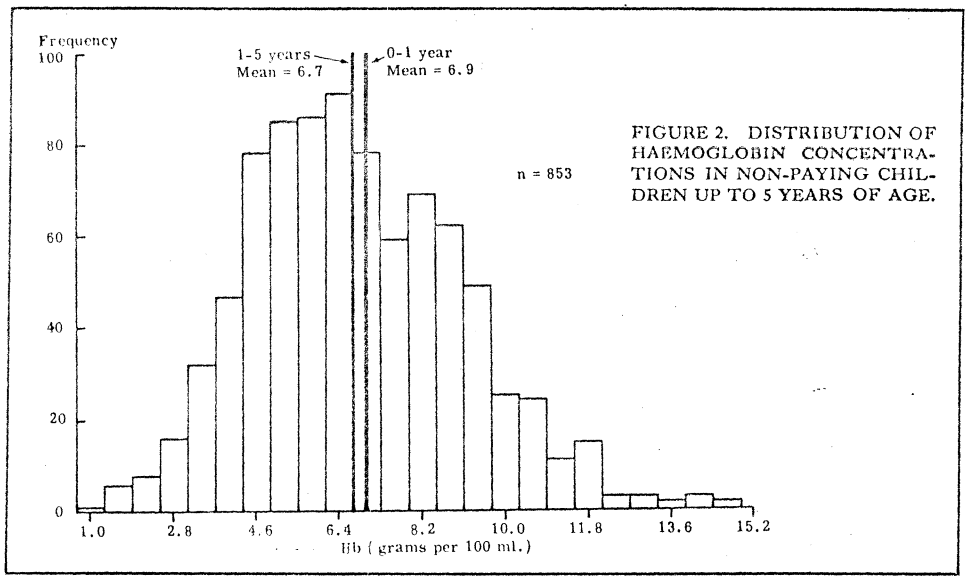


FIGURE 2. DISTRIBUTION OF HAEMOGLOBIN CONCENTRATIONS IN NON-PAYING CHILDREN UP TO 5 YEARS OF AGE.

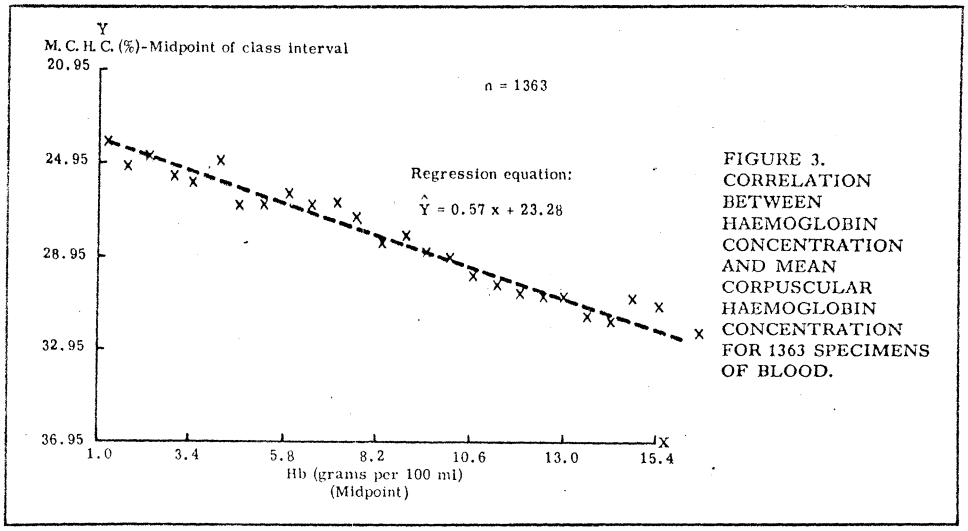


FIGURE 3. CORRELATION BETWEEN HAEMOGLOBIN CONCENTRATION AND MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION FOR 1363 SPECIMENS OF BLOOD.

Methods for the investigation of the anaemia

The investigation depends on a single measurement, the haemoglobin concentration; the conclusion regarding the part played by the lack of a substance in the aetiology of the anaemia is based on the rise in Hb level following the administration of that factor to a severely anaemic patient.

Considerable care was therefore taken in the Hb estimations. They were all carried out personally by the author, using the same pipettes for blood and diluent and the same haemoglobinometer (spectrophotometer) throughout the experiments. All hospital and antenatal specimens were venous and obtained at approximately the same time of day; no less than 2 tubes of diluted blood were examined and no less than 3 readings were taken on each.

The investigation consists of a series of experiments designed to estimate the effects of different factors administered to severely anaemic patients. The basic design is a factorial one and in Figure 4 this is shown in its simplest form, 2 factors each being employed at 2 levels. A factorial design has 3 important advantages:—(i) there is a great saving

FACTOR B	FACTOR A		TOTAL
	DOSE 1	DOSE 2	
DOSE 1			
DOSE 2			
TOTAL			

FIGURE 4.
FACTORIAL DESIGN—
2 FACTORS EACH AT
2 LEVELS.

in clinical material as each patient is used in the evaluation of the effect of every factor; as compared with designs in which each factor is tested separately, a factorial design will give estimates with greater precision for the same total number of patients, or with the same precision for a smaller number of patients: (ii) it enables estimates of the interactions between 2 or more factors to be made, an evaluation impossible by other means; (iii) the effect of a given factor is obtained over a much wider set of conditions than when factors are tested separately.

The factors examined and the doses at which they were usually applied were:—

Ferrous sulphate, 1·2 grammes
Protein, 50 grammes (as skimmed milk)
Folic acid, 15 mg.
Ascorbic acid, 100 mg.
Pyridoxine, 60 mg.
Cyanocobalamin, 100 μg.

} daily, and given orally as a single dose

In addition the effect of anthelmintic was investigated, tetrachlorethylene (TCE) and bephenium hydroxynaphthoate (Alcopar) being employed in adult doses of 4 ml. and 5 grammes respectively. The dosage schedules varied, the smallest being 2 doses of TCE and the largest consisting of 3 doses of each anthelmintic. The worms expelled were counted; they were found to be predominantly *Necator americanus*.

Although in all experiments treatments were randomly allocated to patients as they presented themselves, the groups taking part in a given comparison usually differed in respect of some feature such as age or initial Hb level. The investigation was partly concerned with the design of experiments and with means of increasing their precision, and as such features were thought to be of possible importance in the response to treatment, they were frequently used as covariates in multiple regression analyses or in analyses of covariance. These analyses will not be recorded here but where results adjusted for a particular feature are given, it can be assumed that they have been carried out.

The patients

Those patients taking part in the experiments carried out in hospital were African, were almost all adult, and were admitted because of severe anaemia. 255 were entered but 51 were withdrawn for a variety of reasons. In 15, it was thought that co-existing diseases might influence the response; these included urinary infection, high fever, carcinoma of the prostate and chronic renal disease. Other haematological disorders accounted for 14; 4 patients were found to have sickle-cell anaemia, in 5 the routine bone marrow examination showed the changes of megaloblastic anaemia. 5 others were withdrawn because they showed no response to any treatment given; the criticism that it is difficult to differentiate between these 'refractory' cases and those who responded badly, is valid. 9 patients left hospital during the experiment, in 5 a serious fall in Hb

concentration or the necessity for transfusion caused their removal; 3 patients died. In 3, such administrative accidents as failure to collect a stool and the taking of blood on the wrong day, resulted in their withdrawal.

In addition to the experiments carried out in hospital, others were conducted in a prison and at an ante-natal clinic in Dar-es-Salaam.

Most patients taking part in the hospital experiments were subsistence farmers or members of their families; their diet consisted almost wholly of maize and boiled beans. Fish was eaten occasionally but meat hardly at all; some fruit was available but little green vegetable was taken.

Experiment 1

Purpose

To investigate the relationships between the presence of hookworm infection, the level of Hb and the period of detention in prisoners living under good sanitary conditions and on a liberal diet.

Design

All prisoners were male Africans, mostly 20-40 years of age, and were investigated in 3 ways:—

(a) A specimen of finger-prick blood and one of stool were obtained from 360 prisoners and the duration of their stay in prison was recorded; the Hb concentration of the blood was determined and the stool was examined for hookworm ova.

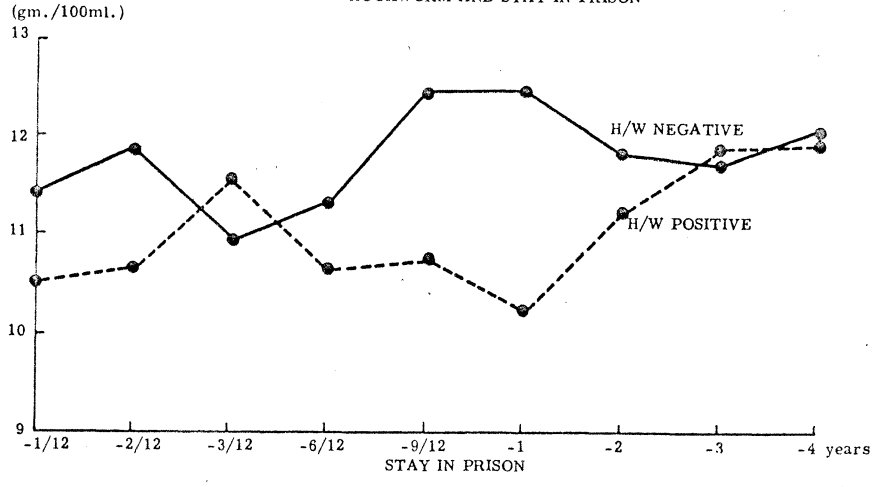
(b) In 59 men who had been in prison no longer than 8 weeks another Hb estimation was made 4 weeks later.

(c) In those who had been in prison for longer than 3 months when first seen and who were found to have hookworm ova in the stool, TCE 4 ml. was given on 2 successive mornings; Hb estimations were made on the day before the first dose and again 28 days later. In only 38 men was this investigation completed.

Results

(a) In Figure 5 are plotted the mean Hb concentrations of the prisoners according to their stay in prison and to the presence or absence of hookworm ova in the single

FIGURE 5
RELATIONSHIP BETWEEN Hb. CONCENTRATION, PRESENCE OF HOOKWORM AND STAY IN PRISON



specimen of stool examined. The mean Hb levels for those with and without ova in the stools are 10·73 and 11·66 g. per 100 ml., the mean stay in prison being 5·9 and 12·5 months respectively. When corrected for the period of detention the difference between the mean Hb values narrows slightly to 0·86 g. per 100 ml., which is highly significant. On the other hand the correlation between the Hb level and the stay in prison, suggested by inspection of Figure 5, is only just significant when the effect of the presence of hookworm is removed. These findings suggest that a stronger relationship existed between the Hb concentration and the presence of worms than between the Hb level and the stay in prison.

The proportion of prisoners in whose stools were found hookworm ova varied with the length of detention; the greatest change appeared to occur at about 3 months after admission. Up to that time 61% were found infected, whereas in those who had served a longer sentence the figure was only 31%. There was no steady decline in incidence after about 12 weeks, and 7 of 20 men who had been in prison for more than 4 years still showed ova in the stools.

(b) Those recently admitted to prison and observed over a period of 28 days showed Hb changes dependent on the presence or absence of hookworm; the mean change in Hb concentration was —0·29 and 0·43 g. per 100 ml. respectively. This difference remains highly significant after correction for initial Hb level.

(c) The effect of anthelmintic on the Hb level depended on the initial Hb concentration; where this was lower a rise was noted 28 days later, where it was higher a fall followed treatment. As a result of these observations it is suggested that (i) anthelmintic treatment is followed by the greatest rise in Hb concentration in those with the lowest initial Hb levels, and this approaches zero as the highest initial levels are reached; (ii) random (day to day) variation is greater in those with the higher Hb concentrations. The combination produces the curve fitted to the observed values and shown in Figure 6.

The findings in the prison experiment are summarized in Figure 7, from which it may be seen that the stay in prison influenced the hookworm load that itself was

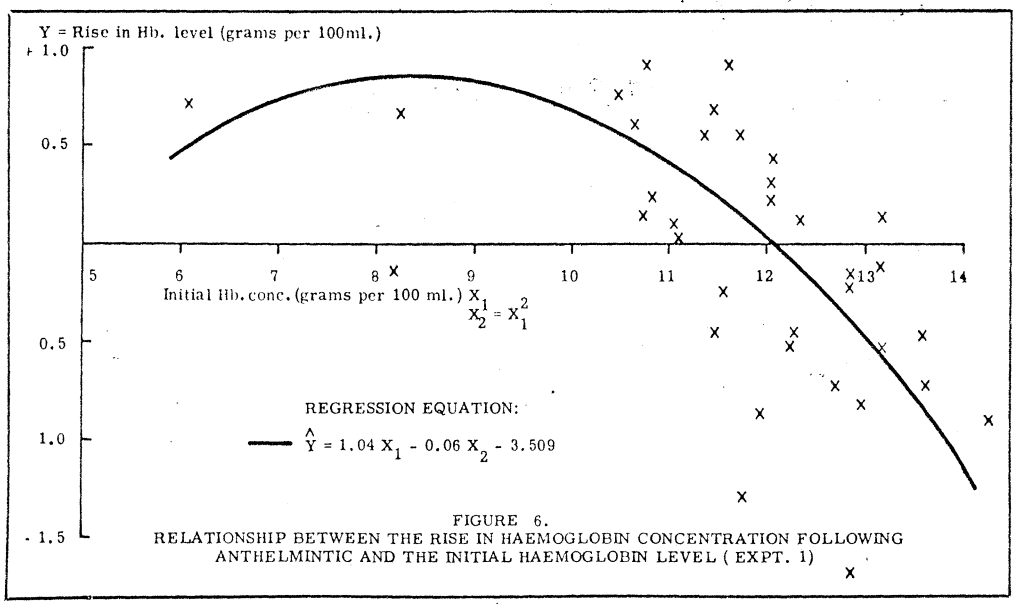


FIGURE 6.
RELATIONSHIP BETWEEN THE RISE IN HAEMOGLOBIN CONCENTRATION FOLLOWING ANTHELMINTIC AND THE INITIAL HAEMOGLOBIN LEVEL (EXPT. 1)

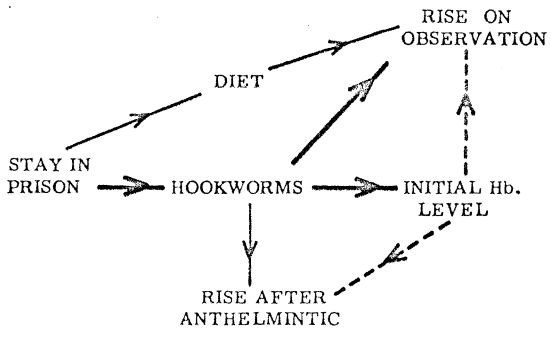


FIGURE 7 CONCLUSIONS DRAWN FROM PRISON EXPERIMENT

important in determinating the initial Hb level and the rise in Hb concentration following admission to prison; the duration of detention affected the Hb level to a smaller extent and it may perhaps be concluded that the prison diet did little to raise the concentration of haemoglobin.

Experiment 2

Purpose

To obtain information on the importance of iron, protein and anthelmintic against hookworm, in the treatment of severely anaemic patients.

Design

This consisted of a factorial experiment with the 3 factors, a = iron, b = protein, c = anthelmintic, each at 2 levels—present and absent. Only patients whose Hb concentration was greater than 4 g. per 100 ml. were included because of the 50% chance of a more anaemic patient being allocated a treatment combination containing no iron, the factor expected to be of the greatest importance; in fact, the mean initial Hb level was 5·30 g. per 100 ml.

Treatments were randomly allocated to the 24 patients taking part, and administered for a 14-day period, at the beginning and end of which Hb estimations were carried out; each group contained 3 patients, the anthelmintic was TCE, 4 ml. given on each of 2 successive mornings.

Results

The mean rise in Hb concentration for the various treatment groups is shown in Table I and the factorial effects in Table II. Only the main effect for iron is significant, those for protein and anthelmintic, and the interactions between the 3 factors, are not. Adjustment for both initial Hb concentration and age of the patient does not alter these results.

Experiment 3

Purpose

To investigate further the effects of protein and anthelmintic.

TABLE I. The mean rise in Hb concentration over 14 day period in patients on various treatment schedules (initial Hb. concentration greater than 4g./100ml.) (Expt. 2)

Anthelmintic (c)	Protein (b)	Iron (a)		Overall	
		Present	Absent	Anthelmintic	Protein
Present	Present	3·34	−0·26	1·74	1·26
	Absent	3·39	0·50		
Absent	Present	2·32	−0·35	1·49	1·97
	Absent	3·47	0·50		
Overall		3·13	0·12		

n = 3. Mean initial Hb. conc. = 5·30 g./100 ml. Total number of patients = 24

TABLE II. Factorial effects (Expt. 2)

Main Effects	A	3·03	g. per 100 ml.	Significant
	B	−0·71	,, ,, ,,	
	C	0·26	,, ,, ,,	
Interactions	AB	0·10	,, ,, ,,	
	AC	0·21	,, ,, ,,	
	BC	0·30	,, ,, ,,	
	ABC	0·25	,, ,, ,,	
A = Iron		B = Protein		C = Anthelmintic

Design

This experiment was run concurrently with Experiment 2, and the subjects included were those with an initial Hb concentration of less than 4 g. per 100 ml. Because of the very low Hb level the administration of iron was thought to be obligatory, and immediate treatment with anthelmintic inadvisable. All patients were therefore given iron by mouth for 14 days. There then followed the treatment combinations of protein and anthelmintic for a further 14 days, iron being continued in all patients. The experiment was therefore planned as a 2×2 factorial design, the 2 factors being protein and anthelmintic; no main effect for iron or its interactions with the other 2 factors could be estimated.

Hb estimations were carried out at the beginning of the preliminary period and at the beginning and end of the test period (i.e. days 0, 14, 28). 60 patients, 15 in each group, were allocated treatment combinations at random. The anthelmintic again was TCE, 4 ml. given on each of 2 successive mornings.

Results

The mean rise in Hb concentration for the 4 treatment groups is given in Table III; no significant main effect for protein or anthelmintic has been demonstrated and no interaction between the 2 factors elicited.

Adjustment for the age of the patient and for the initial Hb level does not alter these results. In addition the rise in Hb concentration during the first 14 days on iron

TABLE III. Mean rise in Hb concentration over 14 day period in patients on 4 treatment schedules. (Hb concentration on admission less than 4 g./100 ml.). (Expt. 3)

Anthelmintic (c)	Protein (b)		Overall
	Present	Absent	
Present	2·73	3·00	2·87
Absent	2·65	2·54	2·60
Overall	2·69	2·77	

n = 15
Mean initial Hb level = 6·19 g./ml.
Mean Hb level on admission = 2·68 g./ml.
Total number of patients = 60
All patients given iron

Main effects for protein and anthelmintic and protein-anthelmintic interaction all non-significant.

alone has been taken into account on the grounds that this may provide an estimate of the Hb-producing capacity of the individual patient; adjustment for this also does not alter the findings.

Experiment 4

A brisk rise in Hb concentration following the administration of one factor may conceivably result in depletion of another; a similar situation occurs in respect of folic acid in haemolytic anaemia. The interaction between 2 factors estimated by means of a factorial experiment provides information on this possibility. In view of the marked response to iron already demonstrated it was thought worth while to investigate further the iron-protein and iron-anthelmintic interactions.

Again, on the assumption that a preliminary period of treatment on iron alone might provide some information on the individual's Hb-producing capacity, oral ferrous sulphate, 1·2 grammes daily, was given to all patients for 7 days, the rise in Hb concentration being measured. There then followed a period of 7 days during which no treatment was given and this in turn was followed by the test period of 14 days during which the treatment combinations were administered. These again consisted of the groups of a factorial experiment with the 3 factors, iron, protein and anthelmintic at the 2 levels, present and absent.

When making a comparison between 2 treatments it is obviously desirable that the patients to whom they are administered have equal Hb-producing capacities. This principle was observed in the current experiment and the patients were grouped according to their performance during the preliminary 7-day period on iron. The groups can be made smaller and hence the precision made greater by means of a device known as 'confounding.' The precision of an experiment may be increased by grouping the patients according to some feature such as age, initial Hb level or Hb-producing capacity, the individuals comprising the group being similar in this respect. It may then occur that the comparison necessary to estimate a main effect or interaction consists of a comparison between groups; it is then not possible to know whether the difference observed is due to the treatment effect or to the difference between the groups. The treatment effect is then said to be confounded, or mixed up, with groups. The price paid for this is the inability to estimate certain main effects or intereactions; the particular effects so confounded may, however, be chosen.

In the current experiment there was little interest in the main effect for iron which had already been shown to exist, in the protein-anthelmintic interaction adequately

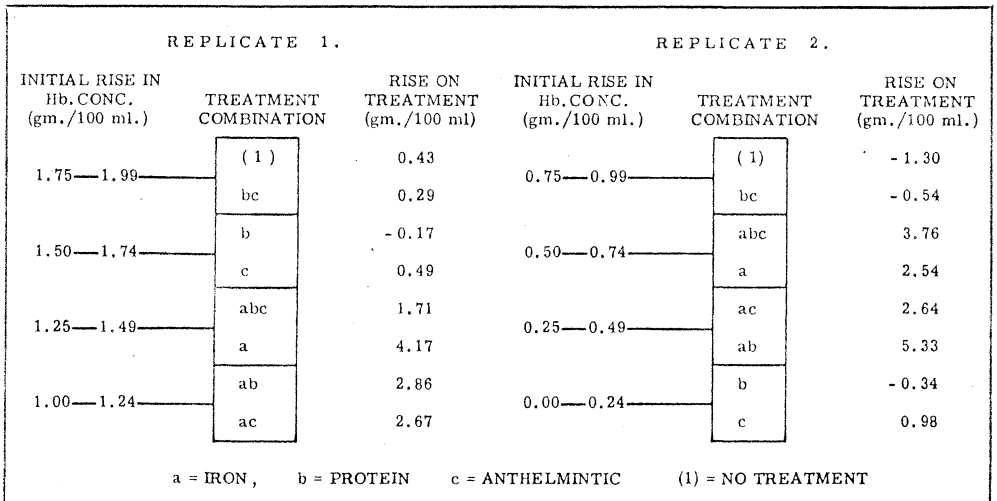


FIGURE 8 DESIGN OF 3 X 2 FACTORIAL EXPERIMENT ARRANGED IN 8 BLOCKS OF 2 UNITS EACH, THE MAIN EFFECT FOR IRON AND THE BC AND ABC INTERACTIONS BEING CONFOUNDED WITH BLOCKS (EXPT. 4)

investigated in Experiments 2 and 3, or in the iron-protein-anthelmintic interaction which was unlikely to be important. It was possible to effect a great reduction in the size of the groups or 'blocks' by sacrificing this information, and the final design is shown in Figure 8; here also is recorded the rise in Hb concentration observed over the test period of 14 days for the 16 patients taking part.

No main effect for protein or anthelmintic or interaction between iron and either protein or anthelmintic was demonstrated.

Because of great within-patient variation, no increase in precision was obtained by means of confounding carried out in this way; in addition, the age of the patient was found not to influence the response.

Experiment 5

Purpose

To investigate the main effects of iron, protein, folic acid, cyanocobalamin, ascorbic acid and pyridoxine, and certain interactions between these factors.

Design

To obtain a single replicate of an experiment involving 6 factors each at 2 levels requires 64 patients; this number may not be available, and in addition the degree of precision obtained may be more than is required. It is possible to design an experiment consisting of a fraction of a complete replicate, but, as with confounding, this is at the expense of information. It may, however, be arranged that the information sacrificed concerns some high-order interactions unlikely to be of importance.

The current experiment was designed as a $\frac{1}{4}$-replicate of a 2^6 design, a further $\frac{1}{4}$-replicate to be added if the clinical material was available; in fact the $\frac{1}{2}$-replicate was readily obtained, and in addition increased precision was attempted by means of confounding as in Experiment 4. The patients were grouped, i.e. blocks were formed according to the individual's performance over an initial 7-day period on iron alone. There followed a period of 7 days during which no treatment was given and then the test period of 14 days in which the randomly allocated treatment combination was administered.

BLOCK 1	2	3	4
abdf	ab	cd	bcdef
acf	abcdef	bce	ace
bcf	df	bdf	adf
abce	bef	abcf	ef
bde	acf	a	abef
cdef	ade	ef	abcd
(1)	bcd	acdef	de
acd	ce	abde	b

a = IRON, b = ASCORBIC ACID, c = PYRIDOXINE,
d = CYANOCOBALAMIN, e = PROTEIN,
f = FOLIC ACID

FIGURE 9.

DESIGN: A HALF REPLICATE OF A 2^3 FACTORIAL EXPERIMENT ARRANGED IN 4 BLOCKS OF 8 PATIENTS, THE BLOCKS BEING FORMED ACCORDING TO RISE IN HAEMOGLOBIN CONCENTRATION OVER PREVIOUS PERIOD ON IRON. (EXPT. 5)

In this way were formed 4 blocks of 8 patients, the final design being shown in Figure 9; as in the other designs the inclusion of a letter in a treatment combination (a, b, ...) indicates that the corresponding factor was administered, its absence signifying that it was not. Thus the patient allocated the treatment combination 'bde' received ascorbic acid, cyanocobalamin and protein, while the one allocated 'ef' received protein and folic acid only.

Results

Information was especially required about the main effects of the 6 factors, and in view of the already demonstrated marked effect of iron, about the interaction of iron and each of the other 5 factors also. It was thought that the other 2-factor interactions and all higher-order interactions would be unimportant, and these were therefore not estimated.

The required factorial effects have been obtained by the method of Yates quoted by COCHRANE and COX (1957) and are given in Table IV. It can be seen that only iron gave a significant main effect and that in no instance did the total for an interaction reach the 5% level of significance.

It should be noted that once again the formation of blocks according to the patient's performance over a preliminary period on iron alone, did not result in an increase in precision.

TABLE IV. Factorial effect totals for main effects and estimated 2—factor interactions (Expt. 5).

		Factorial Effect. Total	Significant
Main Effects	a	50·58	
	b	−6·85	
	c	−0·04	
	d	4·10	
	e	−9·59	
	f	6·52	
Estimated 2-Factor Interactions	ab	−6·75	
	ac	−6·25	
	ad	−6·61	
	ae	−4·35	
	af	0·42	

a = Iron
b = Ascorbic Acid
c = Pyridoxine
d = Cyanocobalamin
e = Protein
f = Folic Acid

Some information regarding the carry-over effect of iron administered for a period of a week can be obtained from this experiment. During the preliminary period on iron the mean Hb concentration increased by 1·42 g. per 100 ml., and during the succeeding week, during which no treatment was given, by 1·01 g. per 100 ml. To investigate any longer carry-over effect only those patients whose treatment combination during the test period did not contain iron are considered. For these patients the mean change in Hb level over the first 4 weeks of the experiment was:—

Week 1	+1·36 g. per 100 ml.	—	iron given
2	+1·20 „ „ „	—	no iron
3	−0·36 „ „ „	—	„ „
4	−0·32 „ „ „	—	„ „

It is concluded that the carry-over effect of oral iron administered for one week extends for no longer than a further 7 days.

Experiment 6

Purpose

To investigate the effect of dose of oral ferrous sulphate on the rise in Hb concentration in severely anaemic patients.

Design

Earlier experiments had shown that a substantial rise in Hb concentration followed treatment with oral ferrous sulphate for a period of a week only. In the 48 patients taking part in Experiments 4 and 5 the mean rise in Hb level over the first 7 days was 1·34 g. per 100 ml. Experiment 5 had also provided some evidence that the carry-over effect of one week's iron was restricted to a further 7 days. Previous experience had also shown that on oral iron the Hb level of severely anaemic patients continued to rise for 6 weeks.

To test the effect of differing doses of ferrous sulphate, where each patient received one level of dosage only, would require a large number of patients, and the findings of earlier experiments suggested that several different dose levels could be conveniently administered to the same patient in sequence. Regard would have to be paid to the preceding treatment and this was allowed for in the design. In view of the then unknown time-response curve for continuously administered oral iron it was thought advisable to take into consideration the position occupied by a given dose in any sequence.

It was required to include doses in common usage, for example ferrous sulphate, 1 tablet (200 mg.) or 2 tablets (400 mg.) 3 times a day, and also smaller dosages in case conventional quantities proved larger than required; it was also thought advisable to include an even larger dose than those commonly employed. In case response proved to be related to either dose or log dose, 7 levels of ferrous sulphate were included—200, 400, 600, 800, 1000, 1200 and 1600 mg. daily, corresponding to 1, 2, 3, 4, 5, 6 and 8 standard tablets of ferrous sulphate.

The original design provided for 6 treatments to be given in sequence to each patient, but for the purpose of the present account the first 3 periods only will be considered. The design is shown in Figure 10 from which it will be seen that each treatment is preceded the same number of times by every other treatment, and that this occurs at each position in the sequence. Haemoglobin estimations were carried out on days, 0, 7, 14, 21 and 28 and the appropriate dose of ferrous sulphate was given as an aqueous solution once a day.

Results and analysis

It will be seen from Figure 10 that each dose level was administered to 18 of the 42 patients taking part in the experiment. The patients receiving the dose in question and the carry-over effect from the preceding period, together with the week during which the treatment was given, are taken into account in the analysis; this provides the relative rather than the absolute rise in Hb concentration for the different dose

PATIENT	SEQUENCE →		
1	1	2	4
2	2	4	1
3	4	1	2
4	4	2	1
5	1	4	2
6	2	1	4
7	1	3	6
8	3	6	1
9	6	1	3
10	6	3	1
11	1	6	3
12	3	1	6
13	1	5	8
14	5	8	1
15	8	1	5
16	8	5	1
17	1	8	5
18	5	1	8

OTHER TREATMENT SEQUENCES ARE PERMUTATIONS OF:-

2, 3, 5 - PATIENTS 19 - 24
2, 6, 8 - PATIENTS 25 - 30
3, 4, 8 - PATIENTS 31 - 36
4, 5, 6 - PATIENTS 37 - 42

DOSES 1, 2 .....8 = 200, 400,.....1600mg
FERROUS SULPHATE ONCE DAILY

FIGURE 10 DESIGN OF CROSS OVER EXPERIMENT TO INVESTIGATE EFFECT OF DOSAGE OF ORAL FERROUS SULPHATE ON RISE IN HAEMOGLOBIN CONCENTRATION. (EXPT. 6)

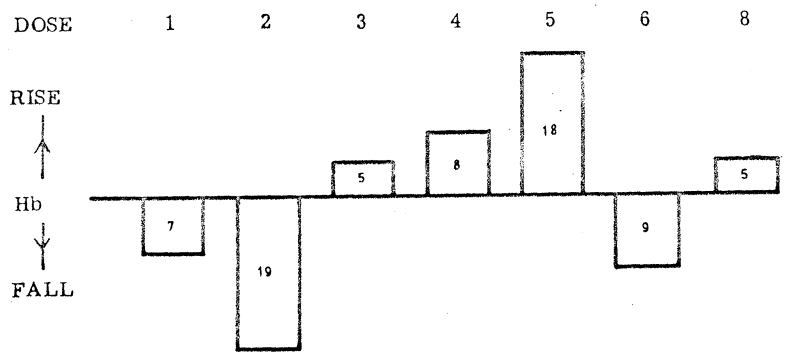


FIGURE 11.
RELATIVE RISE IN HAEMOGLOBIN CONCENTRATION FOLLOWING DIFFERENT DOSES OF ORAL FERROUS SULPHATE GIVEN DAILY FOR ONE WEEK (EXPT. 6)

levels. These are represented diagrammatically in Figure 11; there appears to be no trend of response with dosage. An analysis of variance shows that the responses to the various levels of ferrous sulphate, adjusted for the carry-over effect from one preceding week, do not differ significantly.

Assuming that iron absorbed is utilized almost immediately for haemopoiesis, any carry-over effect will depend on the interval between incorporation of iron into the developing cell and the appearance of that cell in the peripheral blood; any relationship with dose would therefore be expected to be the same for both immediate and carry-over effects. This is so; although not shown here, the carry-over effects for the different dose levels did not vary significantly among themselves.

It is concluded that the rise in Hb concentration following the administration of ferrous sulphate to these severely anaemic patients over a period of one week, does not vary within a dose range of 200 to 1600 mg. daily.

Experiment 7

Purpose

To compare the effects of oral and intravenous iron in severely iron-deficient patients.

Design

Although a self-contained experiment is always the ideal, it was felt that so much information on the response to oral iron had already been accumulated, that to allocate oral and intravenous iron therapy randomly to patients as they presented themselves would have been a waste of clinical material. It was therefore decided to treat 30 severely anaemic patients with intravenous iron and to compare the results with those of the 60 patients receiving iron alone during the first 2 weeks in Experiment 3.

In the current experiment 200 mg. of iron as saccharated oxide of iron (Ferrivenin) were given intravenously on 5 successive days; Hb estimations were carried out immediately before the first dose and again 14 days later, and the rise in Hb concentration was measured. The patients on oral iron received 1·2 grammes ferrous sulphate daily for 14 days, the increase in Hb level being estimated in the same way. On intravenous therapy the patients received 1 gramme of iron, on oral therapy 3·36 grammes. For the orally administered ferrous sulphate to provide the marrow with 1 gramme of iron, 30% would need to be absorbed, a proportion well below the maximum known to be possible in severely iron-deficient patients.

Results and analysis

Table V records the mean rise in Hb level following the 2 courses of treatment, that for oral iron being slightly greater (0·23 g. per 100 ml.) than that following intravenous iron. It will be noted that those receiving iron by mouth were younger and had a lower initial Hb level than those on intravenous therapy. As in both groups the rise in Hb concentration is age-dependent, correction has been made for this factor; the mean values adjusted in this way now differ by only 0·03 g. per 100 ml.

TABLE V. Rise in haemoglobin concentration over period of 14 days following a course of intravenous or oral iron (Expt. 7)

Mode of Administration	n	Mean Age (years)	Mean rise in Hb. Concentration (g. per 100 ml.)		Mean Initial Hb. Conc. (g. per 100 ml.)
			Unadjusted	Adjusted for age	
Intravenous	30	42·8	3·28	3·41	3·35
Oral	60	34·9	3·51	3·44	2·68
		Difference	0·23	0·03	

Dose:

Intravenous—200 mg. Ferrivenin daily for 5 days.
Oral—1·2 grammes ferrous sulphate daily for 14 days.

No correlation between the rise in Hb concentration and the weight of the patient could be demonstrated in those receiving parenteral iron, a finding that may be taken to indicate that the quantity of iron available for the marrow was as much as the marrow could use. The Hb level continued to rise for as long as 7 weeks without any further treatment.

It is concluded that intravenous iron in high dosage offers no advantage over oral iron in respect of rise in Hb concentration over a 2-week period; this is obviously important in anaemia late in pregnancy, where the greatest possible rate of increase is required. It should be remembered, however, that this lack of advantage has been here demonstrated in extremely anaemic and non-pregnant subjects and is not necessarily true in those whose anaemia is less severe and in whom a smaller proportion of orally administered iron may be absorbed.

Experiment 8

Purpose

To investigate the effect of folic acid and differing doses of ferrous sulphate administered by mouth on the Hb level of pregnant women.

Design

In 144 pregnant women attending an antenatal clinic for the first time in the current pregnancy, a venous sample of blood was examined immediately before beginning treatment, and again 14 days later. The 12 treatment schedules comprised a 2 × 6 factorial experiment, folic acid being at 2 levels, present at a daily dose of 15 mg., or absent; iron was at 6 levels, the doses being 0, 200, 400, 600, 800 and 1000 mg. of ferrous sulphate daily. The treatment combinations were allocated at random to the patients as they presented themselves, and enough tablets were given to last exactly 14 days.

Results and analysis

The mean rise in Hb concentration over the 14-day period for the 12 groups is shown diagrammatically in Figure 12; inspection suggests no marked effect for folic acid and a response to iron showing no trend with dosage. This impression is sub-

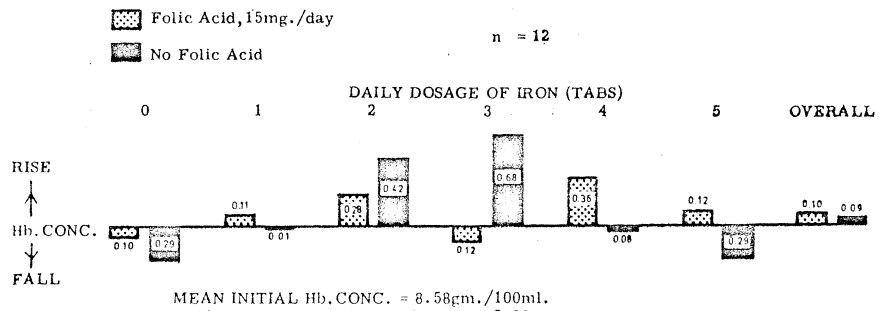


FIGURE 12.
MEAN CHANGE IN HAEMOGLOBIN CONCENTRATION OVER PERIOD OF 14 DAYS
IN 144 PREGNANT WOMEN (6 x 2 FACTORIAL EXPERIMENT)(EXPT. 8)

stantiated by an analysis of variance which shows no main effects for iron or folic acid, but a highly significant quadratic comparison for doses of iron; this merely indicates that the rises in Hb concentration may be fitted to a curve in which the smallest responses result from the lowest and highest doses of iron, and the greatest responses follow the medium doses. One explanation for this rather unexpected finding is that the rise in Hb concentration did increase with increasing dosage of iron but that the larger doses were not taken. Experience with doses of the same order administered to patients in hospital showed that unpleasant side effects were unusual.

It is interesting that the initial Hb level was negatively correlated with the duration of pregnancy and the duration of pregnancy with the number of pregnancies; it might be inferred from the latter finding that an increasing number of pregnancies reduced the urgency to attend the antenatal clinic.

Mean values for the 144 patients were:—

Initial Hb concentration	=	8·58 g. per 100 ml.
Number of pregnancies	=	2·96
Duration of current pregnancy	=	23·8 weeks

Experiment 9

Purpose

To investigate further the effect of oral iron in pregnant women.

Design

60 pregnant women attending the same antenatal clinic as those taking part in Experiment 8 were treated with folic acid 15 mg. daily and ferrous sulphate in a dose of 400, 800 or 1200 mg. per day. Venous blood was taken immediately before and after 14 days of this treatment, and the rise in Hb was calculated.

Results and analysis

Figure 13 records the mean rise in Hb concentration over the 14-day period according to the dose of ferrous sulphate administered, both unadjusted values and values adjusted

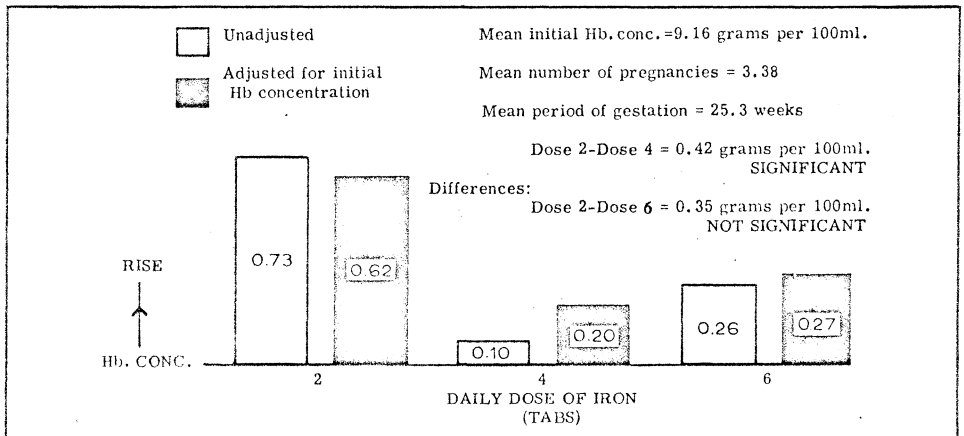


FIG. 13 MEAN RISE IN HAEMOGLOBIN CONCENTRATION OVER PERIOD OF 14 DAYS IN 60 PREGNANT WOMEN (EXPT. 9)

for initial Hb level being given. It may be seen that adjustment narrows the differences in the responses, only the difference between those receiving 400 mg. and those receiving 800 mg. each day being significant, and then only at the 5% level.

Again an unexpected result was obtained, and as before the explanation may be that the larger doses were not taken; on the other hand it may be concluded that there was no significant difference in the responses to the 3 doses of iron. The increase in the Hb concentration of 0·37 g. per 100 ml. for the 60 women considered together is unlikely to have occurred by chance ($P < 0·001$).

Some support for the conclusion that these women were iron-deficient is obtained from the examination of the relationship between Hb concentration and MCHC. The regression coefficient of 1·24% for MCHC on Hb level is highly significant (n = 38).

The correlation between rise in Hb concentration over the 2-week period on iron and the initial Hb level was highly significant, as was the correlation between the initial Hb concentration and the number of pregnancies.

The 204 women taking part in the 2 antenatal experiments were somewhat anaemic, the mean Hb concentration being 8·75 g. per 100 ml. Although there was some evidence of iron deficiency, the response to iron was small and was not related to dose in any obvious way; there was no evidence of folic acid deficiency. The relationship between initial Hb concentration and both duration of gestation and number of pregnancies suggests a lack of some haemopoietic factor, but the absence of any correlation between response and the number of pregnancies, that is a reflection of age, and between response and period of gestation, suggests that these factors did not influence the utilization of the haemopoietic substances available.

Discussion

Diet

Evidence regarding the importance of diet can be obtained from the prison experiment and from patients in hospital who were not receiving iron; in both institutions the quantity of food available was more than the inmates could eat.

Although those men recently admitted to prison and in whose stools hookworm ova were not found showed some rise in Hb concentration over a period of a month, the long-term changes in Hb level in both those with and without hookworm ova in the stools were not marked, and the general increase was certainly slow; it must be remembered, however, that this was a cross-sectional rather than a longitudinal study (Figure 5). Furthermore, those hospital patients receiving no iron showed no rise in Hb concentration. In Experiment 2, the mean rise in Hb level, over a 14-day period, in 12 patients was 0·10 g. per 100 ml.; in Experiments 4 and 5, 24 patients who had received no iron for a week showed a fall in Hb concentration of 0·46 g. per 100 ml. over a similar 14-day period.

This lack of response to an ample institutional diet may be due to the fact that it differed little in composition from that taken by the patients in their own homes; there was more meat but the bulk consisted of maize. It has been estimated that the diet of the average rural African in Tanzania contains about 27 mg. iron per day. The high phytate content of maize would appear to be less important than originally considered; TURNBULL, CLETON and FINCH (1962) showed that in normal persons phytate reduced the absorption of ferrous iron both in the presence and absence of food. This reduction was not observed when organic iron in the form of haemoglobin was investigated, and in the presence of food phytate seemed actually to enhance absorption. It was also found that ascorbic acid increased the absorption of inorganic ferrous iron but that the absorption of haemoglobin iron was unaffected; in any case oranges are plentiful in the region round Dar-es-Salaam. Folic acid deficiency might have been expected as a result of the small quantity of green vegetables eaten, and perhaps vitamin B12 deficiency because of the almost complete lack of meat in the diet.

It is concluded that the diet plays a part in the common anaemia seen in Dar-es-Salaam.

Iron

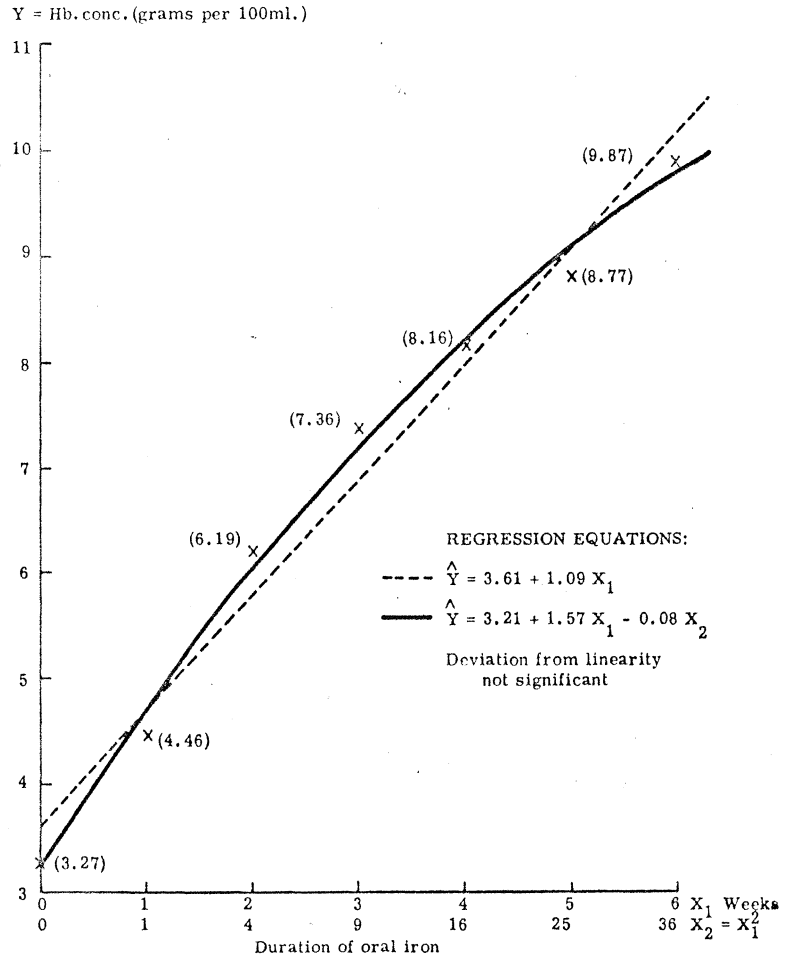
The haematological features of the anaemia were consistent with iron deficiency; especially marked was the relationship between the Hb level and the MCHC (Figure 3) and the complete absence of iron in the large number of bone marrows examined.

There is no doubt regarding the response to oral iron; in the 60 patients taking part in Experiment 3, the mean rises in Hb concentration over 2, 4 and 6 weeks were 3·51, 6·24 and 7·35 g. per 100 ml. In 42 patients receiving various doses in Experiment 6, the mean rises over 1, 2, 3, 4, 5 and 6 weeks were 1·19, 2·92, 4·10, 4·90, 5·51 and 6·42 g. per 100 ml.

There is evidence that the time-response relationship in those continuously on oral iron is linear over a large part of the range. This is illustrated in Figure 14 by the data from Experiment 6; analysis shows no deviation from linearity. A similar result is obtained by examination of the Hb levels of the 16 patients receiving oral iron continuously for 5 weeks in Experiment 4. Deviation from linearity is, however, demonstrated for the Hb levels of the 60 patients taking part in Experiment 3; from the graph this is obviously due to a single value, that for the 6th week after the beginning

FIGURE 14.

MEAN HAEMOGLOBIN LEVEL OF 42 PATIENTS TREATED WITH VARYING DOSES OF ORAL IRON OVER PERIOD OF 6 WEEKS (EXPT. 6)



of treatment. These findings would suggest that the marrow response is not a function of the existing level of haemoglobin. In 12 patients receiving ferrous sulphate in the usual dosage, daily Hb estimations were carried out for the first fortnight. Analysis of the data shows no deviation from linearity although inspection of the graph suggests that the steady rise in Hb concentration did not start until about the 3rd day of administration; this is consistent with existing knowledge on the time taken for maturation of red cells.

Conventional doses of ferrous sulphate are perhaps greater than required; in the cross-over experiment (Experiment 6) no difference in response to greatly varying doses of ferrous sulphate was demonstrated, the conclusion being (i) that 200 mg. ferrous sulphate taken by mouth daily provided the marrow with the iron it required for maximum Hb production under the circumstances prevailing at the time, or (ii) the quantity of iron absorbed did not vary with the dose of ferrous sulphate administered (Figure 11).

Assuming a blood volume of 5 litres, and that the iron content of blood with an Hb concentration of 14·8 g. per 100 ml. is 50 mg. per 100 ml., a rise in Hb concentration of 3 g. per 100 ml. would require about 500 mg. of iron. Such a rise in Hb level was common over a period of 2 weeks in the severely anaemic patients taking part in this series of experiments; this means that about 36 mg. of iron would have to be available to the marrow each day, and to provide this from 200 mg. of ferrous sulphate, the iron content of which is 20%, would necessitate 90% absorption from the gut. Studies with labelled inorganic iron have shown that, in anaemic patients, up to 94% may be absorbed and that all this may be utilized for Hb production (BEUTLER et al., 1963). This reasoning may apply only to the severely anaemic, those with a higher Hb level absorbing a smaller proportion of ingested iron; to maintain the same rate of Hb production under these circumstances a larger oral dose is perhaps required. Experiment 6 does not give information on this point.

In the pregnant women the Hb level was much higher (mean = 8·75 g. per 100 ml.), the rise in Hb concentration was much smaller, but the evidence again was that 200 mg. or 400 mg. of oral ferrous sulphate daily was adequate to provide the marrow with the iron it required to bring about this response.

That in the severely anaemic patients, absorbed iron is rapidly utilized for Hb production, is suggested by the finding that the carry-over effect of a week's iron by mouth is limited to a further 7 days.

There seems little indication for the use of intravenous iron in the treatment of very severe iron-deficiency anaemia. In those whose Hb concentration was under 2 g. per 100 ml. vomiting was a common occurrence, but even in these oral ferrous sulphate was followed by a brisk response. Experiment 7 demonstrated that the speed of response is unlikely to be increased by administering iron intravenously; it is thought improbable that a more rapid rise in Hb concentration would follow an even larger dosage of parenteral iron.

Protein

Experiments 2, 3, 4 and 5 demonstrated no main effect for protein in the 132 severely anaemic patients taking part; moreover, no interaction between iron and protein was found in the 72 patients in whom this estimate was possible. This indicates that protein supplies were adequate even for the greatly increased Hb production following treatment with iron. In view of the finding of non-significant effects for both protein and anthelmintic, it is perhaps not surprising that no interaction between these 2 factors was demonstrated.

Support for the view that protein deficiency is not important in the aetiology of the anaemia investigated here is possibly provided by serum protein estimations carried out on a number of the patients treated. In 124 of those treated in hospital the mean serum albumin concentration was 3·34 g. per 100 ml., and in 23 pregnant women seen at the antenatal clinic it was 3·32 g. per 100 ml. 39 patients taking part in Experiments 2 and 3 who received the protein supplement for 28 days and for whom the results are known, showed no significant change in the serum albumin level over this period.

It is concluded that protein deficiency played no part in the aetiology of the anaemia. This may be surprising in view of the lack of animal protein in the patients' diets. Oedema, although often present in the severely anaemic, was never gross and the skin changes associated with protein malnutrition were very rarely seen.

Other factors

In the hospital patients no main effect for folic acid, cyanocobalamin, ascorbic acid or pyridoxine was demonstrated, and no significant interaction between any one of these and iron was elicited; it is concluded that supplies were available for the greatly increased haemopoiesis following administration of iron (Experiment 5). In the 144 pregnant women taking part in Experiment 8, likewise no main effect for folic acid or interaction of the vitamin with iron was demonstrated.

Of the 255 patients entered for the experiments carried out in hospital, 5 showed megaloblastic bone marrow (2%). One man died and was found to have miliary tuberculosis; the other 4 patients were women and all were treated with folic acid. 3 responded dramatically to 15 mg. per day but the 4th only slowly, the Hb concentration later falling while she was on both folic acid and iron.

It is concluded that deficiency of these factors played no important part in the aetiology of the anaemia.

Hookworm

In the prison experiment those found to have hookworm ova in the stools showed a lower Hb concentration than those who did not, and the presence of hookworm significantly influenced the short-term rise in Hb concentration in those recently admitted. There was also the suggestion that the effect of infection diminished with increasing detention, an observation perhaps related to an ample diet consumed over a long period of time; from this experiment there is perhaps evidence for improvement in Hb concentration after an anthelmintic. It was concluded that the presence of hookworm was a more important factor than diet in determining the Hb level of prisoners.

Hookworm infection, mainly by *Necator americanus*, was extremely common and was diagnosed in 231 of 233 severely anaemic patients admitted to the experiments in hospital; they were admitted before the stools had been examined. The infection was heavy, the mean worm load for these patients being 430. The fact that in earlier experiments the dose of anthelmintic employed was certainly too low implies that this mean load has been under-estimated.

The finding of a significant correlation in Experiment 3 between initial Hb concentration and worm load must be considered as a chance finding; patients came to hospital because of symptoms and there is no reason why, for a given Hb level, symptoms should be more marked where the worm load is higher.

In the 100 patients taking part in Experiments 2, 3 and 4, no main effect for anthelmintic was demonstrated, and in Experiments 2 and 4 the response to iron was not influenced by administration of anthelmintic; furthermore, except in Experiment 5 the response to treatment was not affected by the number of worms present.

It is concluded that, in the absence of iron, the cessation of blood loss following elimination of hookworms has no effect on haemopoiesis in the already severely anaemic, and that in the presence of iron the Hb production following its administration is so much greater than the loss due to hookworms that the latter cannot be detected by the methods here employed.

Although hookworm infection probably played a part in the production of the anaemia, anthelmintic alone has no place in its immediate treatment.

Factors influencing the response to treatment

It was originally expected that the initial Hb concentration would be important in determining the response to treatment and that for this reason a suitable correction should be made in the analyses.

In Experiments 2, 8 and 9, significant correlation was found between the rise in Hb concentration over a 14-day period and the initial Hb level, a finding that might well be due to random variation; in Experiments 3, 5 and 7 no such correlation was observed. In view of the linear nature of the time-response curve over a large part of its range (Figure 14), it might be expected that the initial Hb level would not influence the response to any great extent.

The evidence for an effect of age on response to treatment was conflicting; about half the experiments showed a significant correlation while the other half showed none.

In Experiment 3 the rise in Hb concentration over a 14-day period, during which all patients were treated alike, was taken as an estimate of the individual's Hb-producing capacity and used to correct the responses in the test period. In Experiments 4 and 5 a rather similar method was used to construct 'blocks.' In all 3 experiments no increase in precision resulted from these methods and it must be inferred that the performance by an individual during one period does not indicate what it will be during another period; there is therefore great within-patient variation.

This is substantiated by evidence from Experiment 5 in which 16 patients received iron during the preliminary 7-day period. For the next 21 days they received no iron but there then followed a week during which ferrous sulphate was again administered. The rise in Hb concentration during these two 7-day periods was compared; although the mean values do not differ significantly, there is no correlation between the rise in Hb during one period and the rise during the other.

This within-patient variation obviously reduces the effect of using a previous haematological response as a means of increasing precision in an experiment where great variation also exists between patients.

It is generally concluded that the severe and prevalent anaemia in adults in Dar-es-Salaam is almost entirely due to iron deficiency, that intravenous iron offers no advantage over oral iron from the point of view of speed of response, and that the maximum response possible under the existing circumstances may be achieved with 200-400 mg. of oral ferrous sulphate daily. It is also concluded that the factorial designs employed here are suitable for this sort of investigation, that allowance for the age of the patient may result in an increase in precision, but that great within-patient variation precludes the use of previous knowledge regarding the individual's Hb-producing capacity, for this means.

Summary

The aetiology of the gross anaemia occurring in 204 patients admitted to the Muhimibil Hospital, Dar-es-Salaam, was investigated by means of a series of experiments.

In addition 204 pregnant women attending an antenatal clinic were investigated on a more limited scale.

The investigation depended on the estimation of the Hb concentration only, a rise in Hb level following the administration of a given factor being taken as an indication of deficiency in that factor.

The basic design employed was that of a factorial experiment, although such procedures as fractional replication, confounding and the formation of blocks were also employed.

Only for iron was a main effect demonstrated; no effect for protein, folic acid, cyanocobalamin, ascorbic acid or pyridoxine was elicited, and all interactions between iron and these other factors were found to be non-significant. In addition, treatment with anthelmintic showed no main effect or interaction with iron or protein.

No difference in the speed of response could be detected for oral and intravenous iron, and at the Hb levels encountered here there appeared to be no benefit in giving more than 400 mg. ferrous sulphate daily by mouth.

Investigations carried out on 360 prisoners indicated that hookworm infection was a more important predictor of Hb concentration than was diet as estimated by the stay in prison. What effect could be attributed to diet appeared to be extremely slow and this perhaps also accounted for the lack of benefit derived from the hospital diet in those admitted; hookworm infection was almost universal in the hospital patients and the load was heavy.

Factorial designs proved appropriate for this type of investigation. Within-patient variation seemed to preclude the use of designs depending on the formation of blocks based on a previously determined Hb-producing capacity. Although the response to treatment was dependent on age in some experiments, this was not always the case. A time-response curve which was linear over a large part of the range perhaps accounted for the failure to increase precision by correcting for the initial Hb level.

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DISCUSSION

Professor A. W. Woodruff: I should like to congratulate Dr. Rowland on this very fine piece of work. It forms a beautiful example of what can be done with simple apparatus and constructive thought. Virtually the only laboratory procedure essential for the study was haemoglobin estimation; others such as examination of the marrow and of the stools for parasites were incorporated and have added to the value of the work but the main results could have been obtained without them and even so the laboratory procedures required remain simple.

This work forms another chapter as it were in a series of investigations on anaemia in this geographical region carried out by personnel associated with the Department of Clinical Tropical Medicine, at the London School. I refer here especially to the work done by STOTT (1961). These studies enable us to build up a picture of the anaemias of public health importance in this part of East Africa and Mauritius. The results obtained by Rowland and by Stott agree very closely; both found a low incidence of megaloblastic anaemia, iron deficiency emerges as the predominant cause of anaemia on a public health scale in the region, factors such as administration of folic acid, vitamin B12, an anthelmintic or protein do not under the conditions of the experiments have any significantly beneficial effects.

The results which have been observed are perfectly clear and precise and the statistical design and control ensure that there is absolutely no possibility that they arose by chance. This then is what has been observed and there is no argument about it; interpretation of the results presents further problems. The experimental design and the statistical approach mean that we know exactly what each of the different factors employed will do under these given circumstances, but Dr. Rowland knows too much about this subject to infer that if the circumstances were different or if the factors were used at different levels or for different periods of time the measurable results might not be different. We can, I think, infer quite clearly from this work that folic acid deficiency and vitamin B12 deficiency do not form large problems in Dar-es-Salaam any more than they do in Mauritius, but we know that there are some cases in which these deficiencies exist even though such cases may be uncommon. Concerning the lack of any observed effect of an anthelmintic this again does not mean that the anthelmintic has had no effect which would ultimately be of benefit to the anaemia; Dr. Rowland has demonstrated a correlation between anaemia and hookworm load, significant at the 5% level. The lack of haematological response to the anthelmintic treatment surely indicates that even when one cut off the loss of iron which hookworms imposed on these infected persons they did not absorb a sufficient quantity of iron from their diet to bring about a haematological response measureable during the time interval which elapsed between administration of the anthelmintic and conclusion of the experiment. It does not mean that the anthelmintic had done the patient no good.

In the same way, concerning the lack of demonstrable response to administration of protein during the 2-and 4-week periods of observation employed in experiments 2, 3, 4 and 5, one has to recall that the work of ALLEN and the late Professor DEAN (1965) recently reported in the *Transactions* of this Society, showed that over periods of this order of duration protein administration caused an expansion of plasma volume in those who were malnourished, this increase in plasma volume naturally caused haemodilution and masked therefore any increase in haemoglobin level which might otherwise have been observed. The increased plasma volume did, however, mean that even when the haemoglobin level remained the same as before treatment, or even slightly diminished, the amount of circulating haemoglobin had nevertheless significantly increased. Even bearing this

fact in mind, however, it seems that protein deficiency is not a cause of anaemia on a large or public health scale in Dar-es-Salaam or Mauritius. Here a point is that in Dar-es-Salaam the staple diet of the poor is maize containing round about 7% protein, whereas in many parts of West Africa the staple of the poor is cassava containing less than 1% of protein.

One conclusion which, I think, emerges from this work and that of Stott, is that ways of counteracting iron deficiency are assuming increasing public health importance and that there is an urgent need for hygienists and perhaps politicians to co-operate in exploring the ways.

The final point which I would like to make is that Dr. Rowland was the precursor or prototype for the Department of Technical Co-operation (D.T.C.) lecturers and was appointed nearly a year before the Porritt scheme came into being. To use the simile of the production line I am sure that you will agree that if the later models perform as well as the prototype, the D.T.C. will have made a pretty good investment.

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Dr. S. G. Browne: To take up the question of practical measures to combat widespread hookworm anaemia, I would emphasize that it may not be necessary to await administrative or political blessing before embarking on an inexpensive scheme based on existing medical services. Faced with severe anaemia in Congolese schoolchildren in a rural area, an anaemia due principally to hookworm infection, I was able to enlist the co-operation of an efficient rural health service to give regular doses of iron to over 20,000 children. Before worming was done on a large scale, the haemoglobin rose to satisfactory levels, and thereafter anthelmintics reduced the worm load. Regular blood and stool examinations were made by the medical assistants, who also gave lessons in the local schools. Teachers reported an increase in mental alertness and in mischievousness in their pupils as they responded to these medical measures.

Dr. J. C. White: Dr. Rowland has given a wonderful demonstration of the overwhelming importance of iron deficiency in the causation of this group of anaemias in Dar-es-Salaam, and has used the most powerful statistical methods to this end. It is most interesting to find that folic acid deficiency is a relatively trivial factor in this group, as are other clearly defined haematological disorders. I would like to enquire whether the assessment of the iron status in the bone marrow samples was made both by examination of iron stores in reticulum cells and presence of stainable iron granules in the erythroblasts, and also whether re-assessment of the bone marrow iron was made after treatment. It appeared from the correlation of haemoglobin level with MCHC that the latter seldom in fact reached a normal value, and remained at 31 or below. If the MCHC values in the routine work of a haematological laboratory are plotted as a histogram there is certainly a marked left skew, but I think that only values of 32 or above can be considered to represent the normal population. It appears possible that some abnormality remained even after treatment in this series.

At Hammersmith Hospital, in collaboration with Mr. G. Dixon and Dr. G. H. Beaven, we have been interested in the anaemias of pregnancy in coloured immigrants to West London. These comprise West Indians, West Africans, Indians and Pakistanis, and Middle Asians in this order of frequency. These anaemias are usually moderate in

degree, with haemoglobin levels between 9 and 11 g. per 100 ml. Iron deficiency anaemia, with or without hookworm infection, is not such a major causative factor in this group, and genetic abnormalities of the haemoglobin molecule (traits for S, C and rarer abnormal haemoglobins) or of haemoglobin synthesis (thalassaemia-like traits) are frequently significant. I wonder to what extent the thalassaemia trait may have occurred in the present series, and limited the response to treatment, and whether the investigation of such parameters as the osmotic fragility of the red cells and electrophoretic and alkali-denaturation properties of the haemoglobin, would have detected this condition ?

Dr. C. C. Chesterman: Could Dr. Rowland suggest why the diet of his patients appears to be lacking in iron ? Is it merely the lack of green leaves, so widely used in some parts of tropical Africa ? If so, could not this be remedied rather than introducing medicinal iron into the dietary ?

Dr. Cicely Williams: Dr. Rowland is certainly to be congratulated on an important paper. For a long time we have been struggling with anaemias in Africa and to my mind the consumption of green leafy vegetables plays a major part in treatment and in prevention.

PLATT (*Chemical Analysis of Tropical Foods*) gave the following analysis, and that of soya bean is given for comparison :

TABLE

	Per 100 g. edible portion							
	Calories	Protein	Fat	CH	Ca	Fe	I.U. Vit.A	mg. ascorbic acid
Fresh dark green leaves of cassava, cowpea, spinach, sweet potato	44	4	4	6	210	3	13000	100
Sun-dried leaves	264	24	2·4	36	1260	18	7800	42
Soya bean	382	35	18	20	220	7	—	—

In many rural areas in East and in West Africa the people eat a deal of green leafy vegetables. In some areas this is limited by dry weather and in most urban areas green leaves are only obtainable in small quantities and do not compete in prestige value with white bread and Coca Cola. Some people even preserve the green leaves. The Wagogo, for instance, use over 20 varieties and in the more provident households they are kept in the form of cakes or powder and used throughout the dry season in stews or in the cereal meals. In some areas it is considered more "lucky" to use the leaves growing wild than those that have been cultivated.

The nutritionists have failed to emphasize the importance of green leaves in the diet. It is sad to hear from Dr. Rowland that with all the attention given to nutrition and to agriculture this is still ignored in the hospitals, in institutions, and in teaching.

Ten years ago we were urging that the production, the preservation and the use of green leafy vegetables should be encouraged at every level. Twice I was in Tanganiyka as a Nuffield Visitor and later to report on a famine in the Central Province (1955) and

on Child Health (1956) and suggested that more investigation was needed to prove which are the leaves with best nutritional value, what are the best methods of preserving and using them, how much of the iron and the protein, etc., are available and so forth. The use of green leaves is an admirable indigenous form of diet, surely more could have been done to insure that it is not completely abandoned ? Surely it is better to do this than to continue handing out tablets, of which only a small proportion reach those who are most in need.

One other point: anaemia is very common in young children, sometimes in a fulminating type which is often fatal. Among young children *Ascaris* is more prevalent than hookworm, and is often a major factor in malabsorption. In 1953 the out-patient figures for Tanganyika were tapeworm 9,000, hookworm nearly 30,000, and *Ascaris* 22,000.

Dr. P. J. S. Hamilton: I would like to join in congratulating Dr. Rowland.

Professor Woodruff has already mentioned the work of Dean and Allen on plasma volume in children with anaemia and kwashiorkor. Recent work in Kampala on adult patients with idiopathic splenomegaly and severe anaemia has shown that, although such patients come with very low haematocrit and haemoglobin values, their red cell mass when measured by a ^{51}Cr technique is usually in the normal range or may even be raised. Their anaemia is in fact due to markedly raised plasma volume (RICHMOND, J. H., DONALDSON, G. W. K., WILLIAMS, R., HAMILTON, P. J. S., HUTT, M. S. R. (in press)).

The mechanism of this increased plasma volume is not understood. There is early evidence that such a dilution factor may also be present in severe hookworm anaemia. I wonder if this dilution may not account for some of the anaemia in these cases and particularly in those refractory cases where it is so difficult to get the haemoglobin value above 10·0 to 12·0 g. per 100 ml. I would like to ask Dr. Rowland what the evidence of splenomegaly was among his patients and whether he has any evidence on the problem of dilution anaemia.

Dr. Rowland (in reply) : The points raised by Professor Woodruff are perfectly valid in that inferences must be drawn only within the limits of the experiments carried out; anthelmintic administered in the absence of iron might result in a rise of Hb level over a period of time longer than that used in these experiments. Regarding the possibility that the protein given resulted in an expansion of plasma volume which could mask an increase in total circulating haemoglobin, it is interesting that the factorial effect for protein shown in Table II is in fact a negative amount (B=—0·71 g. per 100 ml.); thus the administration of high doses of protein was followed by a fall in Hb level.

The assessment of iron status by bone marrow examination was based on the presence or absence of iron stores; in a large number of patients a repeat examination was made after about 6 weeks on oral iron, and little change was observed. As Dr. White inferred, the MCHC was rarely above 30% in specimens of blood from the general hospital population; many of the severely anaemic patients were followed for long periods and in them also the MCHC seldom rose above 30% despite continued iron. Hb electrophoresis and osmotic fragility tests were not carried out.

I do not know the answer to Dr. Chesterman's question regarding the apparent lack of iron in the diet. As was mentioned, the average iron intake in Tanganyika has been estimated at 27 mg. per day and it might be suggested that this is not in a form suitable for absorption. Whether the addition of green leaves to the diet would increase the quantity of iron absorbed is not known, nor is the opposition that might be encountered if this was advised. As Dr. Williams has mentioned, green leaves are used in parts of

Africa, and especially in countries such as Sierra Leone; although greens were sold in the markets of Dar-es-Salaam, they did not appear to be a regular constituent of the diet.

Dr. Browne's is an admirable suggestion, but there needs to be some administrative nucleus, either official or voluntary, to enable iron and anthelmintic to be distributed on a large scale.

Regarding Dr. Hamilton's suggestion that there may be an increased plasma volume and hence a false low Hb level in patients having an enlarged spleen, splenomegaly was not a common finding in Dar-es-Salaam and it is doubtful whether a palpable spleen was present in more than 5% of the severely anaemic patients. As no blood volume estimations were carried out it is not possible to estimate the effect of this in those patients whose Hb level did not reach 'normal' values after prolonged treatment with iron.