has been shown to cause this response is 400 /ug (Herbert, 1963b) although not all patients with pernicious anaemia respond to this dose (Marshall and Jandl, 1960). The daily requirement for folic acid in pregnancy is of the order of 300 /ug, as shown by Willoughby and Jewell (1966). The results of the hospital-based study at Nqut indicate, however, that 300 /ug folic acid given daily in tablet form causes a marked rise in red cell folate concentration in the average pregnant woman, although it is inadequate for some. The regression lines suggest that even if only 300 /ug folic acid was taken daily in maize meal, there would be a slow rise in red cell folate in the average woman at the end of pregnancy. If these patients were folate replete at the beginning of pregnancy because they had been eating fortified food, an even smaller dose, sufficient to maintain folate stores, might well be adequate.

Based on the observations of Herbert (1963b) and Willoughby and Jewell (1966), the margin of safety between the requirements of pregnant women and the dose capable of causing response in vitamin B_{12} deficiency is 100 /ug folic acid daily. This margin could be increased significantly if the amount of folic acid added to maize meal was adjusted to account for increased maize consumption by pregnant women. Decreased consumption by older patients, who are more susceptible to pernicious anaemia, would thus result in the intake of folic acid being reduced to levels safely below those associated with response to folic acid in vitamin B_{12} deficiency anaemia.

V Summary and conclusions

Red cell folate levels were measured for six weeks in an index member and the oldest member of each of six families who had been given folic acid-fortified maize meal for use in the home. Five of the index patients were pregnant and one was a lactating woman. The amount of folic acid added to the maize was calculated so that each adult would receive 500 ug folic daily.

In five of the six index subjects, red cell folate levels rose significantly, and the rise was more rapid than that observed in pregnant women in the hospital-based trial who were fed, under supervision, fortified maize containing the same dose of added folic acid. In spite of the fact that their requirements are lower than those in pregnant and lactating subjects, the older members of these families showed a more sluggish rise in red cell folate concentration than the corresponding index members. One index subject, whose family ate less than was expected, showed an essentially unchanged red cell folate level after six weeks.

It is concluded that folic acid-fortified maize meal can be used effectively to supply folic acid supplements to pregnant women who store, cook and eat the meal according to local custom. The changes in red cell folate levels in the patients studied suggest that pregnant women may consume more, and elderly patients less, than the average adult in the family. This possibility would tend to increase the margin of safety when folic acid-fortified foods

are consumed by populations in which vitamin B_{12} deficient megaloblastic anaemia is found.

CHAPTER 7

DEMONSTRATION OF THE ANTIMEGALOBLASTIC EFFECT OF FOLIC ACID-FORTIFIED MAIZE MEAL AFTER COOKING

I Introduction

In previous chapters, the efficacy of fortified food as a means of correcting folate deficiency has been gauged by measuring folate concentration in serum and red cells, after administration of the food to human subjects. These measurements were made by means of assays dependent on the biological availability of folate for bacterial growth. While this technique has demonstrated that the food provides folate which is utilisable by bacteria, it has not at this stage been conclusively shown that this folate is biologically available to man. For this reason, it was considered necessary to evaluate the efficacy of folate-fortified maize meal as a therapeutic agent in patients with megaloblastic anaemia due to folate deficiency.

II Method

The antimegaloblastic effect of the cooked meal was tested by means of a therapeutic trial. This type of trial was originally devised to test the potency of liver preparations for use in pernicious anaemia (Minot et al., 1928), and was subsequently used to test whether deficiency of a specified haematinic was the cause of the anaemia in a given patient, by assessing whether an optimal response was obtained when small doses of the haematinic were given to the patient (Minot and Castle, 1935).

The three essential principles which should be followed when conducting such trials were outlined by Minot and Castle (1935) and reviewed by Herbert (1963b). These three principles and the methods used in the present study to comply with them are as follows:

1. "A control period of a few days (seven to ten days if feasible) is desirable in order to establish the constancy of the method." The control period was extended for as long as was possible in the two hospitals, which have a very rapid turnover of patients and a severe shortage of beds. By convention, the first day on which the fortified meal was given was designated day 0.

2. "Elimination of sources of active material other than the substance under test .... The diet .... should not contain such substances during observations." The diet was the usual ward diet which was free of uncooked green leafy vegetables. In previous studies by Metz (1959), Stevens (1964) and Baumslag and Metz (1964) at Baragwanath Hospital, this diet failed to induce haematological response in patients with folate-deficient megaloblastic anaemia followed for up to 30 days. This diet contains copious quantities of maize meal. The fortified meal eaten by the patients in the present trials thus merely replaced unfortified maize meal in the ward diet.

3. "It is desirable to use .... a dose just sufficient to produce a distinct reticulocyte reaction." As it was not possible to predict what a "sufficient" dose would be, the daily dose of folic acid added to the maize meal before cooking was 100/ug in the case of patient 1, 300/ug in the case of patients 2 and 3, and 500/ug in the case of patients 4 and 5. The maize meal was fortified with pteroylglutamic acid in proportions of one daily dose to each 30 g maize meal (dry weight). The mixture was cooked in boiling water for 30 to 45 minutes to form a stiff paste, and was eaten with the normal hospital meals each day.

In order to assess whether an optimal response had been achieved, an attempt was made to elicit a secondary reticulocyte response in patients 4 and 5 when the reticulocyte counts had returned to stable levels after the initial reticulocyte response. These patients were given 15 mg folic acid and 240 mg elemental iron (as ferrous sulphate) daily by mouth.

III Clinical details of patients studied

Five patients were studied, one at the Johannesburg General Hospital, and the rest at Baragwanath Hospital. All five patients were admitted to hospital with severe anaemia during the period of lactation, and continued breast-feeding throughout their hospital stay. The mean period between parturition and admission to hospital was 114 days (range 49 - 189 days). The details of the

TABLE 7.1

Therapeutic trial

Haematological investigation of patients on admission to hospital.

	Normal Range	Patient number				
		1	2	3	4	5
Haemoglobin (g%)	12.5-17.0	4.7	6.4	3.7	6.1	4.4
Red cell count million/ul	4.5-5.7	1.30	0.79	1.00	1.85	1.25
Haematocrit (%)	37-49	15.3	10.1	11.8	15.3	12.7
Mean cell volume(μ^3)	82-100	118	128	118	122	107
Mean cell haemoglobin($\mu\mu g$)	27-32	36.2	41.0	37.0	40.8	34.4
Mean cell haemoglobin concentration (%)	31-37	30.7	33.7	31.4	33.3	32.1
Leucocyte count/ul	4,000-11,000	4,500	5,000	2,750	9,500	7,400
Platelet count/ul	140,000-500,000	30,000	40,000	54,000	85,000	185,000
Reticulocyte count (%)	0-2	3.0	2.8	0.0	1.0	3.4
Blood Smear		Megaloblasts Oval macrocytes Punctate basophilia Hypersegmented polymorphs.	Megaloblasts Oval macrocytes, teardrops Punctate basophilia Hypersegmented polymorphs.	Oval macrocytes Hypersegmented polymorphs.	Megaloblasts Oval macrocytes, teardrops Punctate basophilia Hypersegmented polymorphs.	Megaloblasts Oval macrocytes Hypersegmented polymorphs.
Bone Marrow						
Cellularity		Increased	Increased	Increased	Increased	Increased
Erythropoiesis		Megaloblastic	Megaloblastic	Megaloblastic	Megaloblastic	Megaloblastic
Myelopoiesis		Megaloblastic	Megaloblastic	Megaloblastic	Megaloblastic	Megaloblastic
Myeloid/erythroid ratio		0.7:1	0.5:1	1:1	0.8:1	1:1
Megakaryocytes		Adequate	Low normal	Depressed	Depressed	Adequate
Platelets		Diminished	Diminished	Diminished	Diminished	Normal
Iron Stores		Increased	Increased	normal	Increased	Increased
Sideroblast Iron		Occasional ring form	Increased	Increased	Increased	Increased
Serum folate (ng/ml)	5-18	0.7	1.0	1.2	0.6	0.6
Red cell folate (ng/ml)	160-640	48	100	116	12	84
Serum iron (μg%)	80-180	86	114	216	145	241
Transferrin saturation(%)	16-50	40	43	79	72	89
Serum Vitamin B12 (pg/ml)	400-1020	402	505	554	1205	1068
Lactic dehydrogenase units	80-200	6130	4565	3110	2490	2630
Bilirubin(mg%) total / direct	< 1.2 / < 0.2	1.0 / 0.2	0.9 / 0.2	1.0 / 0.2	1.0 / 0.2	1.4 / 0.4

individual patients are briefly presented, and the laboratory findings on admission are summarised in Table 7.1.

Patient No. 1 23 years old. Para 3. 189 days post-partum.

Her two previous children were born 27 and ten months before the last delivery. The first infant died at three months and the second, which had a birthweight of two kg, died at two weeks, both of causes which could not be established by the author.

She attended antenatal clinic for eight weeks, but did not receive haematinics. Her infant was delivered at Newcastle Hospital, Natal. She was readmitted to the hospital 60 days post-partum with anaemia, and was transfused with three pints of blood and given pills, the nature of which is unknown to the author. She had an epistaxis 145 days post-partum and bled per vagina from day 150 to day 180 post-partum.

On admission, she was complaining of palpitations, headache and vertigo. She was noted to have scattered petechial haemorrhages and a urinary tract infection, and was treated with ampicillin by mouth from day 2 to day 12 of the therapeutic trial. On the basis of the clinical and laboratory findings, a diagnosis was made of megaloblastic anaemia due to folate deficiency.

Patient No. 2 20 years old. Para 1. 164 days post-partum.

She received no antenatal care, and delivered her infant at home in Bergville, Natal, without the assistance

of a midwife. She developed diarrhoea one week after the delivery and this persisted until her admission to hospital.

On admission to hospital, she was complaining of general lassitude and weakness. In conjunction with the laboratory findings shown in Table 7.1, it was concluded that her anaemia was megaloblastic due to folate deficiency. She was transfused with two pints of blood on the day of her hospital admission (day -6 of the therapeutic trial).

Patient No. 3 25 years old. Para 5. 49 days post-partum.

Her fourth child was born fourteen months before the fifth one, and died after six weeks from causes which could not be established by the author. After a pregnancy unassisted by antenatal care, her fifth infant was delivered at home in Carletonville, Transvaal. No midwife was present at the delivery. She bled excessively for five days post-partum, and felt weak until the date of admission.

On admission, she was complaining of reduced effort tolerance, and was noted to be pale and in congestive cardiac failure. She was given anti-failure therapy from the day of admission until day 4 of the therapeutic trial. In conjunction with the laboratory findings shown

in Table 7.1, a diagnosis of folate-deficient megaloblastic anaemia was made. She was transfused with three units of blood on the day of her hospital admission (day -8 of the therapeutic trial).

Patient No. 4 23 years old. Para 2. 88 days post-partum.

Her firstborn child was delivered 47 months before the second child, and was well. She did not seek antenatal care during the more recent pregnancy. She gave birth at home in Brits, Transvaal, without the assistance of a midwife, and passed clots of blood per vagina for ten days post-partum. She developed diarrhoea approximately 40 days post-partum and symptoms of congestive cardiac failure approximately 55 days post-partum, both of which persisted until the date of admission. Six days before admission she collapsed in the street and was taken to hospital, but she left the casualty department without seeing a doctor.

On admission to hospital, she complained of reduced effort tolerance, ankle swelling, and diarrhoea. She was noted to be pale and in biventricular congestive cardiac failure, and her temperature was measured as 39°C. The house staff made a provisional clinical diagnosis of septicaemia superimposed on an acute leukaemia and began treatment with parenteral gentamicin

sulphate and penicillin, which were continued from the day after admission (day -3) until day 9 of the therapeutic trial. Subsequently, on the basis of the laboratory findings shown in Table 7.1, the diagnosis was altered to that of folate-deficient megaloblastic anaemia.

Patient No. 5 21 years old. Para 1. 86 days post-partum.

She attended the Tladi Antenatal Clinic, Soweto, Johannesburg, from the end of the second trimester, but did not take the tablets she was given. Ankle oedema developed during the last three weeks of pregnancy, but remitted after delivery. Seventy days post-partum she developed ankle oedema, noted that she became dyspnoeic after walking twenty yards, and began to cough yellow sputum.

On admission, she was noted to be pale and in congestive cardiac failure. Prominent Roth spots were present on both fundi. On the basis of the history and a temperature of 38.2°C, the house staff considered that antibiotic therapy was indicated and penicillin was given from day -1 to day 4 of the therapeutic trial. She received antifailure therapy over the same period. With the assistance of the laboratory investigations shown in Table 7.1, a diagnosis of folate-deficient megaloblastic anaemia was made.

IV Results

The daily haemoglobin levels and reticulocyte counts of the five patients are depicted graphically in Figures 7.1 to 7.5. The significant points of these figures are summarised in Table 7.2.

None of the patients had a reticulocyte response during the control period, and neither of the two patients who received pharmacological doses of folic acid and iron had a secondary reticulocyte response.

Four patients were thrombocytopaenic at the start of the trial, and all had normal platelet counts by day 8. The single patient with a depressed white cell count on day 0 had a normal count on day 3 of the trial.

TABLE 7.2

Response of patients to fortified maize meal.

PATIENT NO.		1	2	3	4	5
Control period (days)		5	6	8	4	3
Daily dose of folic acid in meal (µg)		100	300	300	500	500
Duration of trial (days)		15	17	13	11	11
Reticulocyte peak (%)		26.8	26.4	19.4	38.5	32.2
Day of reticulocyte peak		7	7	7	5	6
Haemoglobin (g%)	Day 0	4.3	5.8	8.4	4.9	4.4
	Rise	3.1	5.0	2.9	3.0	2.7
Red Cell Count mill/µl	Day 0	1.3	1.9	2.9	1.2	1.3
	Day 7	1.5	2.6	3.4	1.9	2.4
	End of trial	2.2	3.3	3.8	2.2	2.7

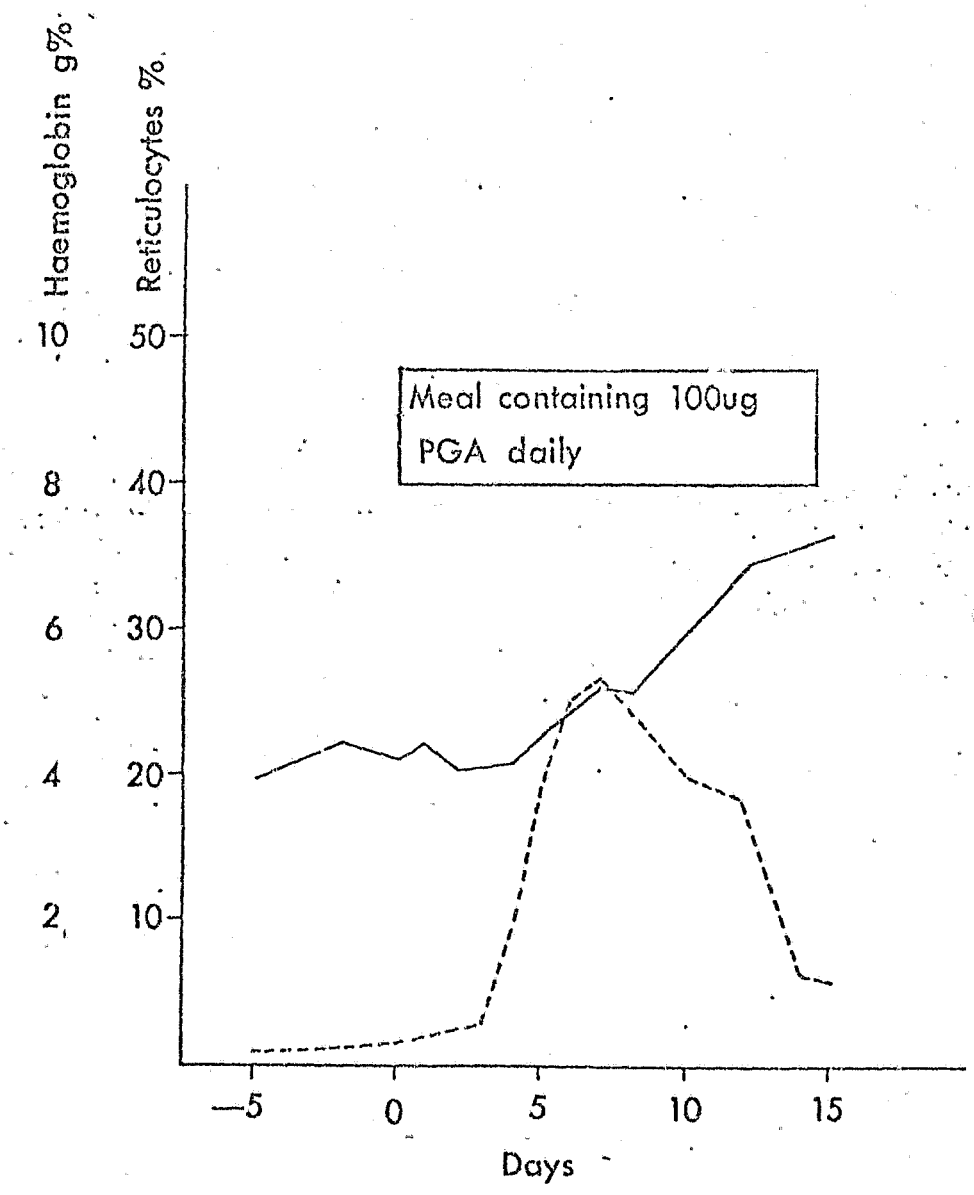

FIGURE 7.1 Effect on haemoglobin value (———) and reticulocyte count (-----) of feeding maize porridge fortified with folic acid to a lactating patient with folate-deficient megaloblastic anaemia. Patient no. 1.

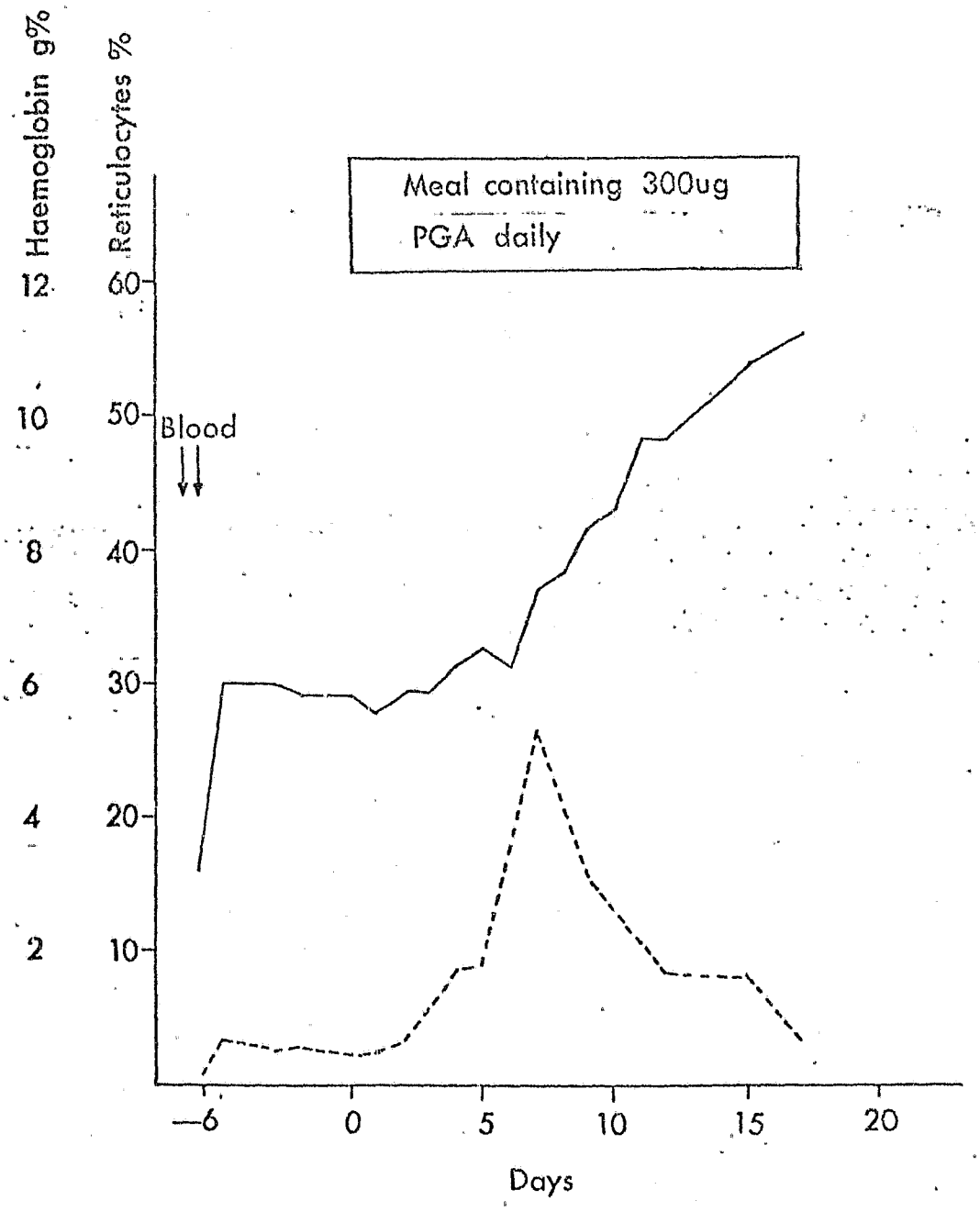

FIGURE 7.2 Effect on haemoglobin value (———) and reticulocyte count (-----) of feeding maize porridge fortified with folic acid to a lactating patient with folate-deficient megaloblastic anaemia. Patient no. 2.

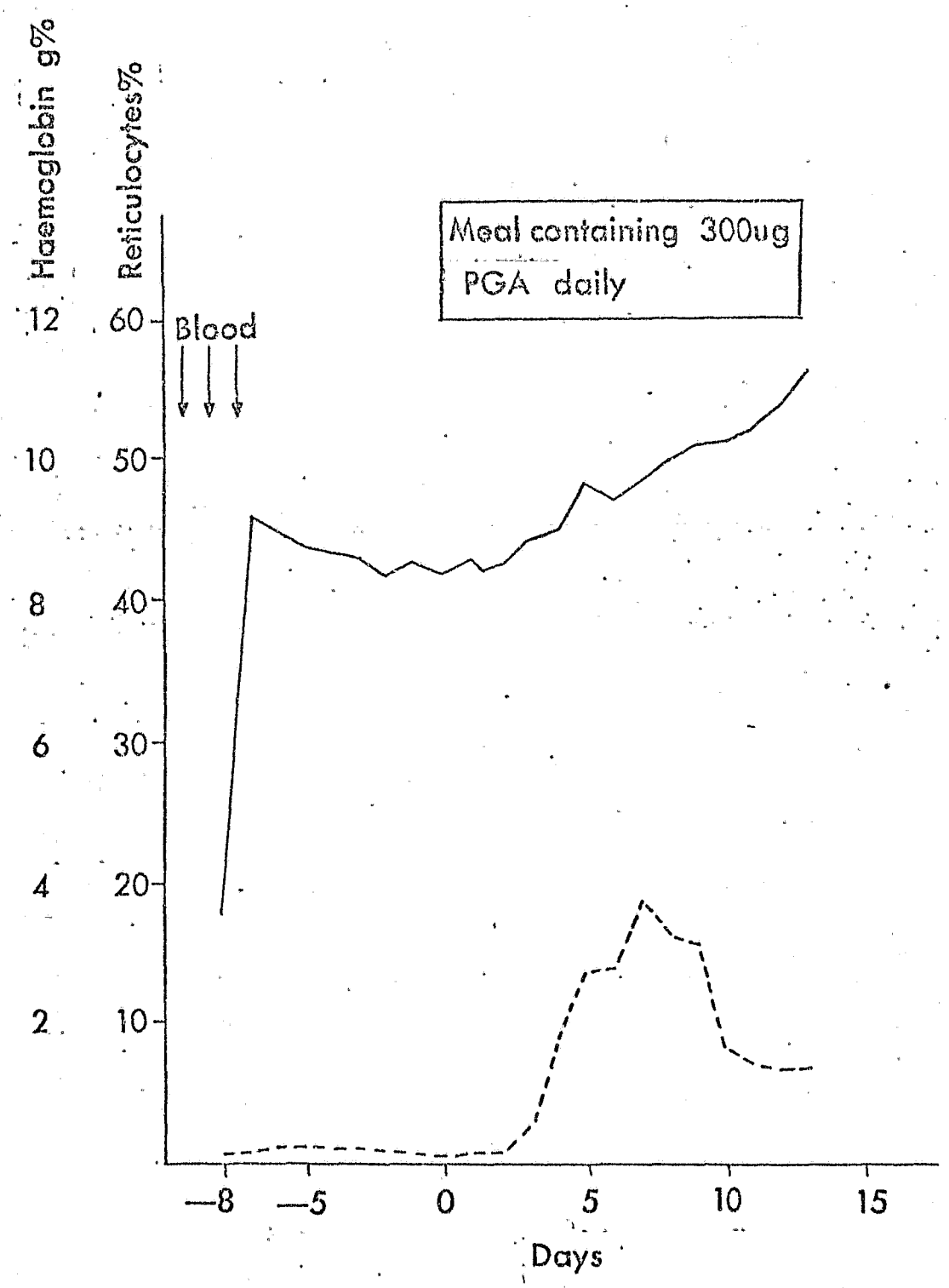

FIGURE 7.3 Effect on haemoglobin value (———) and reticulocyte count (-----) of feeding maize porridge fortified with folic acid to a lactating patient with folate-deficient megaloblastic anaemia. Patient no. 3.

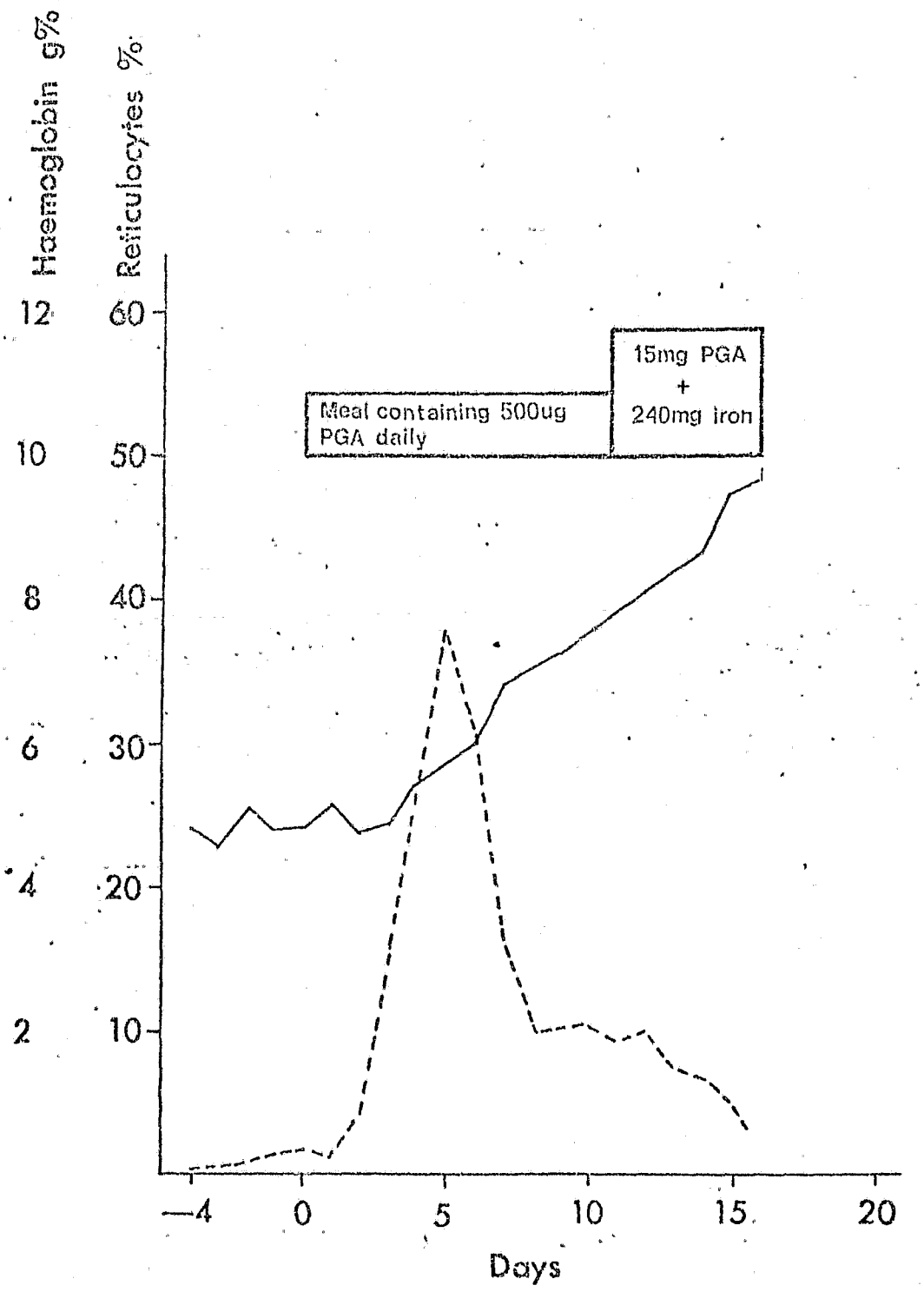

FIGURE 7.4 Effect on haemoglobin value (———) and reticulocyte count (-----) of feeding maize porridge fortified with folic acid to a lactating patient with folate-deficient megaloblastic anaemia. Patient no. 4.

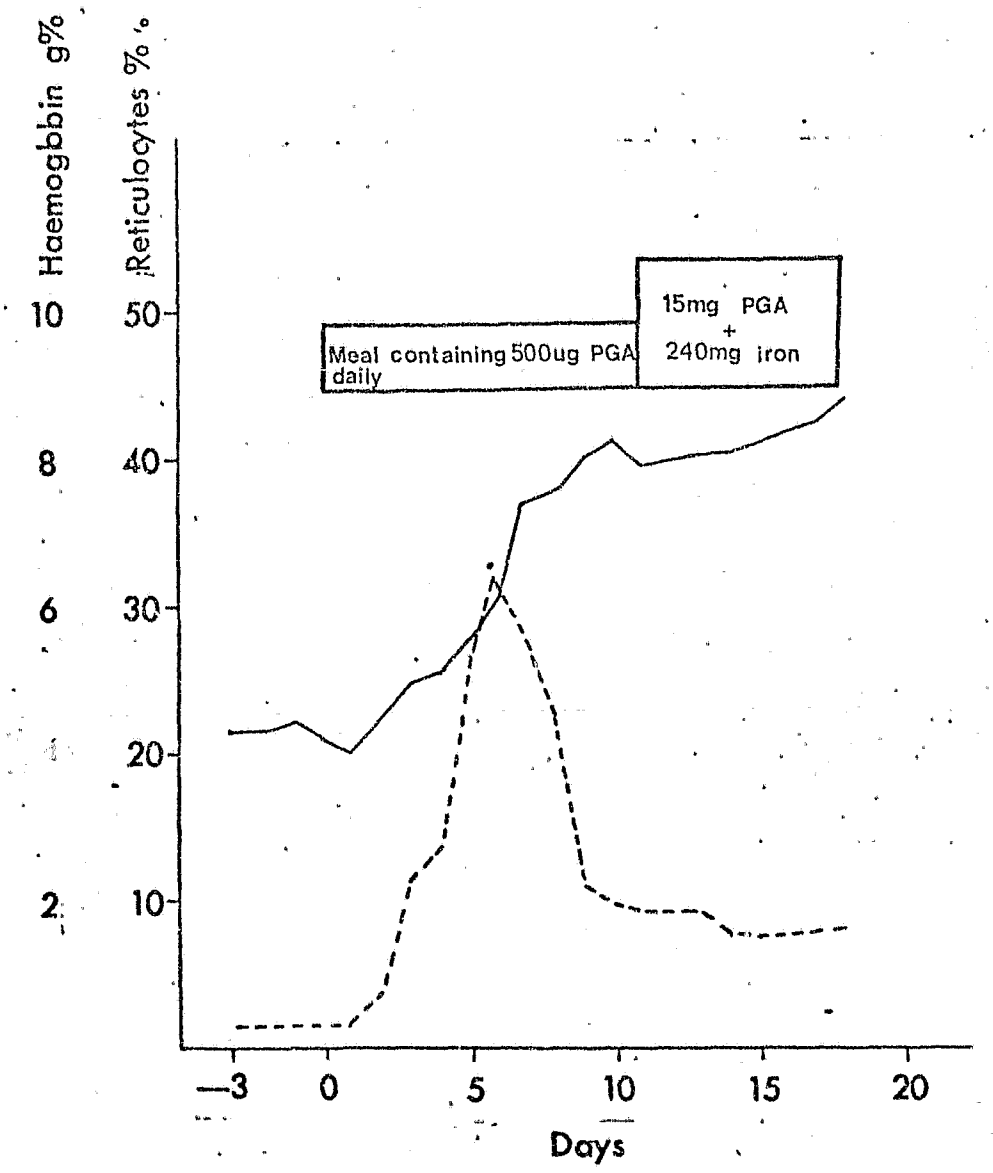

FIGURE 7.5 Effect on haemoglobin value (———) and reticulocyte count (-----) of feeding maize porridge fortified with folic acid to a lactating patient with folate-deficient megaloblastic anaemia. Patient no. 5.

V Discussion

None of the patients had a reticulocyte response during the control period. This finding is in accord with those of Metz (1959), Stevens (1964) and Baumslag and Metz (1964) at Baragwanath Hospital. It appears that the hospital diet contains insufficient folate to induce a haematological response.

The assessment of the success of a therapeutic trial is based largely on studies of patients with pernicious anaemia. In order for a haematological response to be rated as optimal, Chanarin (1969) states that the reticulocyte peak should occur between days 5 and 8. This criterion was fulfilled in all five patients. The reticulocyte count and red cell count must then be shown to reach certain specified levels.

The height of the reticulocyte peak is related to the severity of anaemia, and the expected height for a given initial red cell count has been described by Sturgis and Isaacs (1938). According to their criteria, patient no. 1 had a suboptimal reticulocyte peak, while response was optimal in the other patients.

The rise in red cell count for a given initial count was studied in a large series of cases (Bethell, 1935), and this forms a reference table for determining whether the rise in red cell count is optimal. On the basis of these figures, the rise in red cell count in patient no. 1

was suboptimal. Patients 2, 3, 4 and 5 had an optimal rise in the red cell count.

Patient 1, in whom the haematological response was suboptimal, received fortified maize meal, which contained 100/ug folic acid daily before cooking. This reflects the fact that this dose is inadequate to meet the requirements of a lactating patient, which has been established at approximately 300/ug per day (Metz, 1970). However, this patient's documented urinary tract infection may have interfered with the response (Chanarin, 1969), although this inhibition was not observed in patients 4 and 5.

An optimal haematological response was observed in patients 2, 3, 4 and 5. The failure to induce a secondary reticulocyte response with pharmacological doses of folic acid in the two patients (numbers 4 and 5) so studied is further evidence of the optimal nature of the response induced by the folic acid-fortified maize meal.

In patients 2 and 3, the therapeutic trial followed the administration of blood transfusions. Herbert (1963b) suggested that transfusion of two units of packed cells was one of the possible factors which may have caused a "spontaneous" response in a patient he studied with Zalusky. However, Stevens (1964) reported that three patients with folate-deficient megaloblastic anaemia who were transfused did not have

reticulocyte responses when followed for up to seventeen days.

Patients 1, 4 and 5 received penicillin at or before the start of the therapeutic trial. Foy et al. (1951) have reported response in megaloblastic anaemia of pregnancy in Kenya to crystalline penicillin. It is feasible that this may have contributed to the response in patients 4 and 5, but difficult to explain on this basis why patient 1 did not manifest a response. An important observation in this connection is that Cohen (1953) and Metz (personal communication) were unable to confirm the finding of Foy et al. in South African Negro patients. They treated eight patients with megaloblastic anaemia in the puerperium at Baragwanath Hospital with penicillin, none of whom manifested a haematological response. Chanarin (1969) raises the suggestion that antimalarial drugs could have contributed to the responses observed in Kenya by Foy et al., as Fleming et al. (1968) have found this factor to be of prime importance in Nigeria. This possibility would explain Cohen's and Metz's failure to demonstrate response in the Johannesburg area, where malaria is not found. Whatever the reason, it is manifest that penicillin therapy does not cause haematological response in megaloblastic anaemia of pregnancy and the puerperium in Johannesburg, where this study was undertaken.

VI Summary and conclusions

The antimegaloblastic effect of cooked folic acid-fortified maize meal was tested by feeding the meal to five lactating patients with folate-deficient megaloblastic anaemia. The dose of folic acid in the meal before cooking varied from 100 to 500 µg daily.

The one patient who received 100 µg daily had a suboptimal reticulocyte response, as would be expected in view of the requirement in the lactating subject. In addition, the response may have been inhibited by the presence of a urinary tract infection.

The meal eaten by two patients, who had been previously transfused, contained 300 µg folic acid daily, and that eaten by a further two patients contained 500 µg folic acid daily. These four patients had an optimal haematological response.

At the end of the therapeutic trial, the two patients who were given meal containing 500 µg daily were given pharmacological doses of folic acid and iron. Neither manifested a secondary reticulocyte response.

It is concluded that the fortified meal after cooking is an effective antimegaloblastic agent. The dose of folic acid in the meal necessary to induce an optimal response in lactating patients is greater than 100 µg, but less than 300 µg.

C H A P T E R 8

A POPULATION SURVEY AMONG HEALTHY INHABITANTS OF NQUTU, AND AN INVESTIGATION OF THE EFFECT OF HORMONAL CONTRACEPTIVES ON FOLATE STATUS

I Introduction

The evidence presented in previous chapters suggests that the fortification of maize with folic acid would be an effecti·e method of preventing the development of megaloblastic anaemia in pregnant and lactating women, infants, and children with kwashiorkor in maize-eating populations. The justification for such a step in terms of the FAO/WHO recommendations is that the disease is prevalent in the abovementioned sections of the population. The Joint FAO/WHO Committee recommends, however, that it should also be determined whether other segments of the population have low intakes of the nutrient in order to define the extent of the target group, and that dietary information "should be reinforced whenever possible by biochemical and other studies" (FAO/WHO, 1971).

Many surveys of nutritional status in the South African Negro population have been conducted since the National Nutrition Research Institute, in considering the indications for food enrichment in this country, concluded that "much more work will have to be carried out if a comprehensive picture of the nutritional status of the South African population is to be obtained" (C.S.I.R., 1959). For example, a report of the levels of five vitamins in 1 963 Pretoria schoolchildren refers to nine similar reports concerning children

published during the preceding decade (Louw et al., 1969). Despite this great interest and the known prevalence of folate-deficient megaloblastic anaemia in the population, there have been no reported surveys of the incidence of folate deficiency among South African Negroes. For this reason it was decided to conduct such a survey in the inhabitants of the site of the present study.

It has been mentioned previously that most authorities regard the red cell folate concentration to be the best practical measure of tissue folate stores. In accordance with this belief, the assessment of the incidence of folate deficiency was based on the number of subjects with red cell folate concentrations in the deficient range, and the use of the term "folate deficiency" in the present report refers to the results of assessment on this basis.

The survey was planned in order to assess several factors which were thought to be relevant to the present study. Firstly, serum vitamin B_{12} concentrations were measured in order to assess the magnitude of the problem of increasing the folic acid intake of people with deficiency of this vitamin. The consumption of maize meal was assessed so that the range of intake of the vehicle food could be determined. The incidence of anaemia was investigated and an attempt was made to assess the socioeconomic status of the subjects.

This chapter reports a survey of 469 healthy adult inhabitants of the Nqutu area, corresponding to one in 170 of the total population enumerated in the 1970 census and a much greater proportion of the adult population (South Africa. Department of Statistics, 1970). Children were not studied because of difficulty in obtaining consent for venipuncture and in differentiating between healthy and malnourished subjects.

In addition, it was decided to assess folate status in women receiving hormonal contraceptive agents, in view of reports that oral contraceptive pills may cause folate deficiency. These agents have recently been made available free of charge by the State Department of Health, and it is anticipated that their use will increase markedly. Because the agents are not used at present by Nqutu women, it was not possible to pursue this investigation in that area. Other rural areas were unsuitable as sites for this study for the same reasons, and it was therefore decided to investigate folate status among Negro women attending a family planning clinic in Johannesburg.

II Material and methods

(a) Subjects studied

Details of the subjects studied are listed in the Appendix.

Subjects studied at Nqutu were divided into three groups. The first (group VI) comprised 144 women in the third trimester of pregnancy, who were studied at the time of admission to the trials described in Chapter five. This was a highly selected group, in that all subjects with a haemoglobin concentration below 11 g per 100 ml were excluded from the trials, as were subjects with complications of pregnancy or medical illnesses. The first 122 subjects listed in the Appendix were those comprising groups I to V in that chapter respectively, and the remaining 22 subjects were women selected for the trials who either delivered within the first two weeks of the trial or were discharged before delivery.

The remaining subjects from Nqutu were non-pregnant women (group VII) and men (group VIII) aged at least sixteen years selected in the town and at th sites of outlying clinics, where it is usual for the local population to gather at the nearby general stores. Subjects were excluded from the study if they were seeking or receiving orthodox or traditional medical attention, if there was obvious evidence of any clinical illness or deformity, if their meals were provided by an institution, or if they had ever suffered from tuberculosis. Women were excluded if they had been pregnant or lactating within the previous six months. One hundred and forty men and 185 non-pregnant women were studied.

The subjects in whom the effects of hormonal contraceptives were investigated were healthy women attending the Johannesburg clinic of the Transvaal Family Planning Association (group IX). Many of these women were domestic servants receiving their food in White homes. Subjects were excluded from the study if they were receiving medical attention other than the provision of contraceptive agents, if there was any clinical evidence of illness or deformity, if they had had tuberculosis, or if they had been pregnant or lactated within the previous twelve months. Three subgroups were studied. The first (group IXa) comprised 74 women who were either attending the clinic for the first time to apply for contraceptive aids or else were using vaginal or intra-uterine contraceptive devices, and who had not previously used hormonal contraceptive agents. The second subgroup (group IXb) comprised 75 subjects who for a period of at least one year had received oral contraceptive pills, each containing 1 - 4 mg norethisterone acetate and 50 - 100 ug of either ethinyl oestradiol or mestranol. The third subgroup (group IXc) comprised 32 subjects who for at least one year had received 150 mg 17-hydroxy-6-methyl progesterone acetate (Depo-Provera, Upjohn) by intramuscular injection every three months. The latter subgroup was studied in an attempt to determine

whether any effect of the oral contraceptive pills was due to their progesterone component.

(b) Laboratory tests

Blood was obtained by venipuncture, and haemogram and vitamin B_{12} concentration were assessed in all samples by the methods described in Chapter 3.

As the incidence of folate deficiency rather than the mean level was the primary concern of this study, whole blood haemolysates from subjects in groups VII and VIII were initially assayed for folate, using L. casei as the test organism. The red cell folate levels were calculated using the formula:

$$\text{Red cell folate concentration (ng per ml)} = \frac{\text{whole blood folate concentration}}{\text{haematocrit}}$$

If this calculation yielded a result lower than 200 ng per ml, the whole blood haemolysate and the serum sample were subjected to the L. casei folate assay within the same batch, and the red cell folate concentration was calculated using both results as described in Chapter 3. In this way, a check was performed on all red cell folate results close to or within the deficient range of 160 ng per ml or less, and serum folate assays were not done when the red cell folate level was well above the deficient range. In the case of the pregnant subjects (group VI) and the urban women (group IX), serum folate concentrations were measured in order to assess the

effect of fortified maize meal or contraceptives on these levels.

The ranges of red cell folate, serum folate, and vitamin B_{12} concentrations which were regarded as normal, low and deficient for the purpose of this study are shown in Table 8.1. These are the ranges which have been established for the laboratory in which these assays were done, with the exception that the "low" range for red cell folate is usually regarded as normal, but was included here in order to show the number of subjects with concentrations approaching the deficient range.

TABLE 8.1

Normal, low and deficient ranges for red cell folate, serum folate, and serum vitamin B_{12} concentrations.

	Deficient	Low	Normal
Red cell folate (ng/ml)	$\leq$160	161-200	$>$200
Serum folate (ng/ml)	$\leq$3.0	3.1-5.0	$>$5.0
Vitamin B_{12} (pg/ml)	$\leq$250	251-400	$>$400

Sera which had a vitamin B_{12} concentration less than the lower limit of the normal range were tested for the presence of type I (blocking) antibodies to intrinsic factor, which are present in the sera of about 60 per cent of adult patients with pernicious anaemia (Ardeman

and Chanarin, 1963). This was done using the method of Shum et al. (1971), which tests the ability of the serum to decrease the vitamin B_{12}-binding capacity of normal human gastric juice.

(c) Assessment of maize consumption and socioeconomic status

Maize meal consumption and socioeconomic status were assessed in the 469 subjects at Nqutu.

In the assessment of family size, all people dependent on the same source of income and food as the subject studied were regarded as being part of the extended family unit. The varying needs of different family members were roughly accounted for by taking all children above the age of sixteen to be adults. Those between two and sixteen years were assumed to have half the needs of adults and those below two years of age were disregarded. The size of each family was then expressed as the number of adults in the extended unit.

Family income was calculated by adding together all moneys received by the various members of the family unit. Estimates of maize meal intake were based on the amount bought monthly by the family, which has been shown to be the most reliable method of making this assessment in South African Negroes (du Plessis et al., 1971). This method has the additional advantage of taking into account only that maize meal which was obtained from central sources and which would thus be

available for fortification.

Further information shown in the results was obtained by direct questioning. Although many of the subjects did not know their age, all were able to relate their birthdate to wars or other significant events, and the age was calculated by relating the date of birth to the date of the event stated.

III Results

The results in individual subjects in the four groups are listed in the Appendix.

(a) Pregnant women (group VI)

The mean age of the subjects seen in late pregnancy was 23.5 years (range 16 - 45), and they had had an average of 1.4 previous viable pregnancies (range 0 - 13). The distribution of haemoglobin, red cell folate, serum folate, and serum vitamin B_{12} concentrations and of mean red cell volume in the group are shown as histograms in Figure 8.1.

Red cell folate levels were in the deficient range in 63 of the 144 subjects (43.8 per cent), and in the low range in a further 22 subjects (15.3 per cent). The mean red cell folate concentration was 196.6 ng per ml with a standard deviation of 84.8 ng per ml.

Serum folate levels were in the deficient range in 40 subjects (27.2 per cent) and in the low range in a

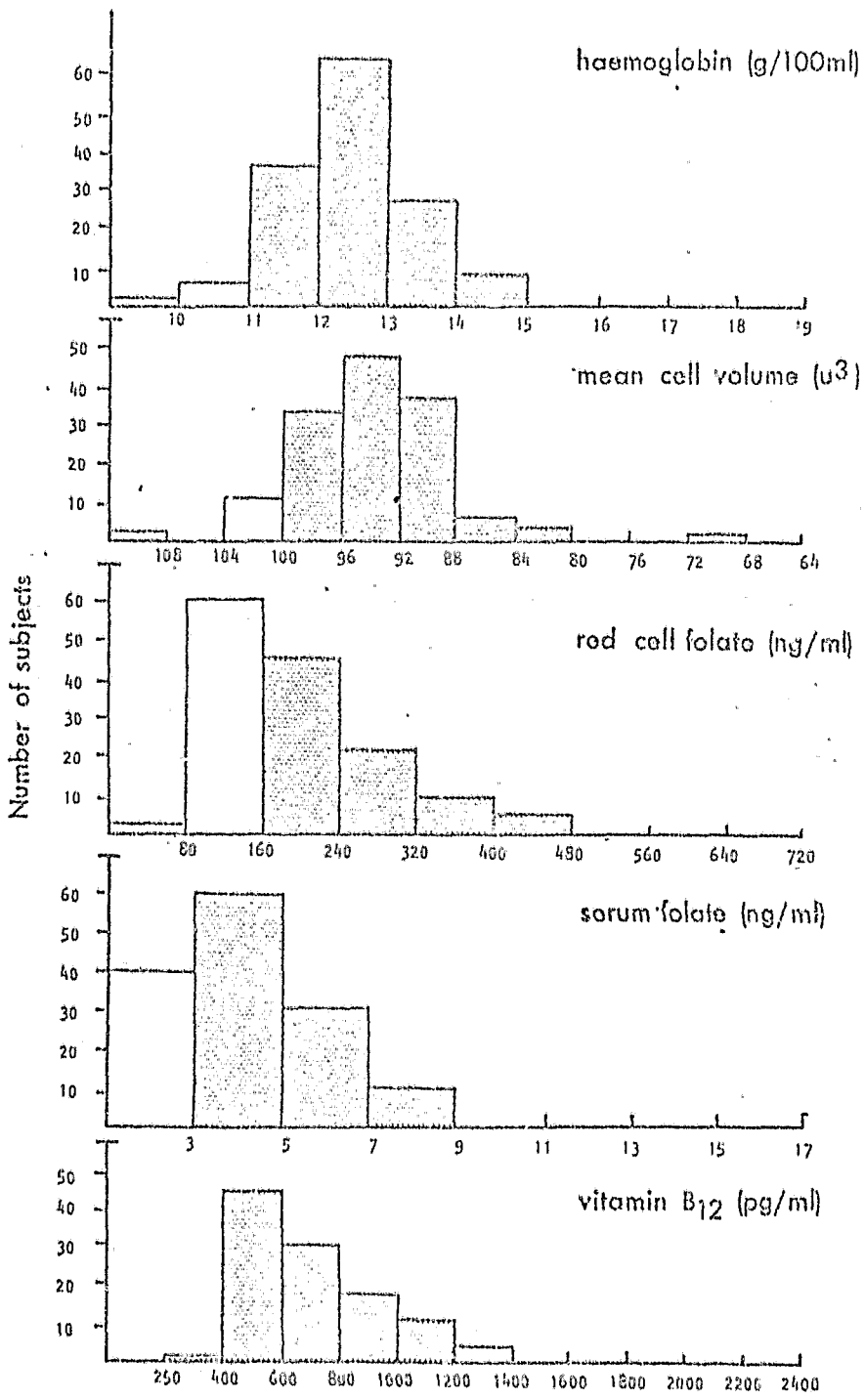

FIGURE 8.1 Results of laboratory tests in 144 pregnant women at Nqutu. All these subjects had haemoglobin levels greater than 11 g per 100 ml as measured by a field visual haemoglobinometer, but some lower concentrations were recorded on the Coulter Counter Model "S" as shown above. The shaded areas represent the respective normal ranges.

further 60 (41.7 per cent). The mean serum folate concentration was 4.43 ng per ml (standard deviation 2.12 ng per ml).

Serum vitamin B_{12} levels were measured in 106 subjects, as ascorbate had been added to the remaining sera for the reasons mentioned in Chapter five. None of the subjects had serum vitamin B_{12} levels in the deficient range, and in one serum (0.9 per cent) the concentration was 394 pg per ml, which is slightly below the lower limit of the normal range. Type I antibodies to intrinsic factor were not present in this subject's serum when tested. The mean serum vitamin B_{12} level was 710.2 pg per ml (standard deviation 223.3 pg per ml).

Despite the fact that all subjects in this group had haemoglobin levels of greater than 11 g per 100 ml as measured on a visual haemoglobinometer, the Coulter Counter recorded lower levels in eight subjects (5.6 per cent). The mean haemoglobin concentration was 12.50 g per 100 ml with a standard deviation of 1.02 g per 100 ml.

The mean cell volume was less than 82 cubic microns in two subjects (1.4 per cent) and greater than 100 cubic microns in fourteen subjects (9.7 per cent).

There was no general correlation between either haemoglobin or mean cell volume, on the one hand, and red cell folate, serum folate, or serum vitamin B_{12}

concentration on the other. However, ten of the fourteen subjects with a high mean cell volume had red cell folate levels in the deficient range (71 per cent) compared with 43.8 per cent of the whole group. The chi-square test showed that this difference was statistically significant ($p < 0.05$).

There was a very strong correlation between red cell folate and serum folate ($r = 0.5894$, $p < 0.0005$) but none of the other laboratory tests showed correlation with one another.

Haemoglobin levels and parity had a significant negative correlation ($r = -0.2246$, $p < 0.01$), and there was a positive correlation between mean cell volume and parity ($r = 0.2245$, $p < 0.01$). None of the other laboratory tests were significantly related to parity and none of the tests were related to age.

(b) Non-pregnant women at Nqutu (group VII)

The age of the women studied in this group ranged from sixteen to 79 years (mean 44.1 years), and their average parity was 4.5 pregnancies. The distributions of haemoglobin, red cell folate and serum vitamin B_{12} concentrations and of mean cell volume in the group are shown in Figure 8.2.

Red cell folate concentrations were in the deficient range in 61 of the 185 subjects (33.0 per cent) and in the low range in a further 31 subjects (16.8 per cent). The

concentration on the other. However, ten of the fourteen subjects with a high mean cell volume had red cell folate levels in the deficient range (71 per cent) compared with 43.8 per cent of the whole group. The chi-square test showed that this difference was statistically significant ($p < 0.05$).

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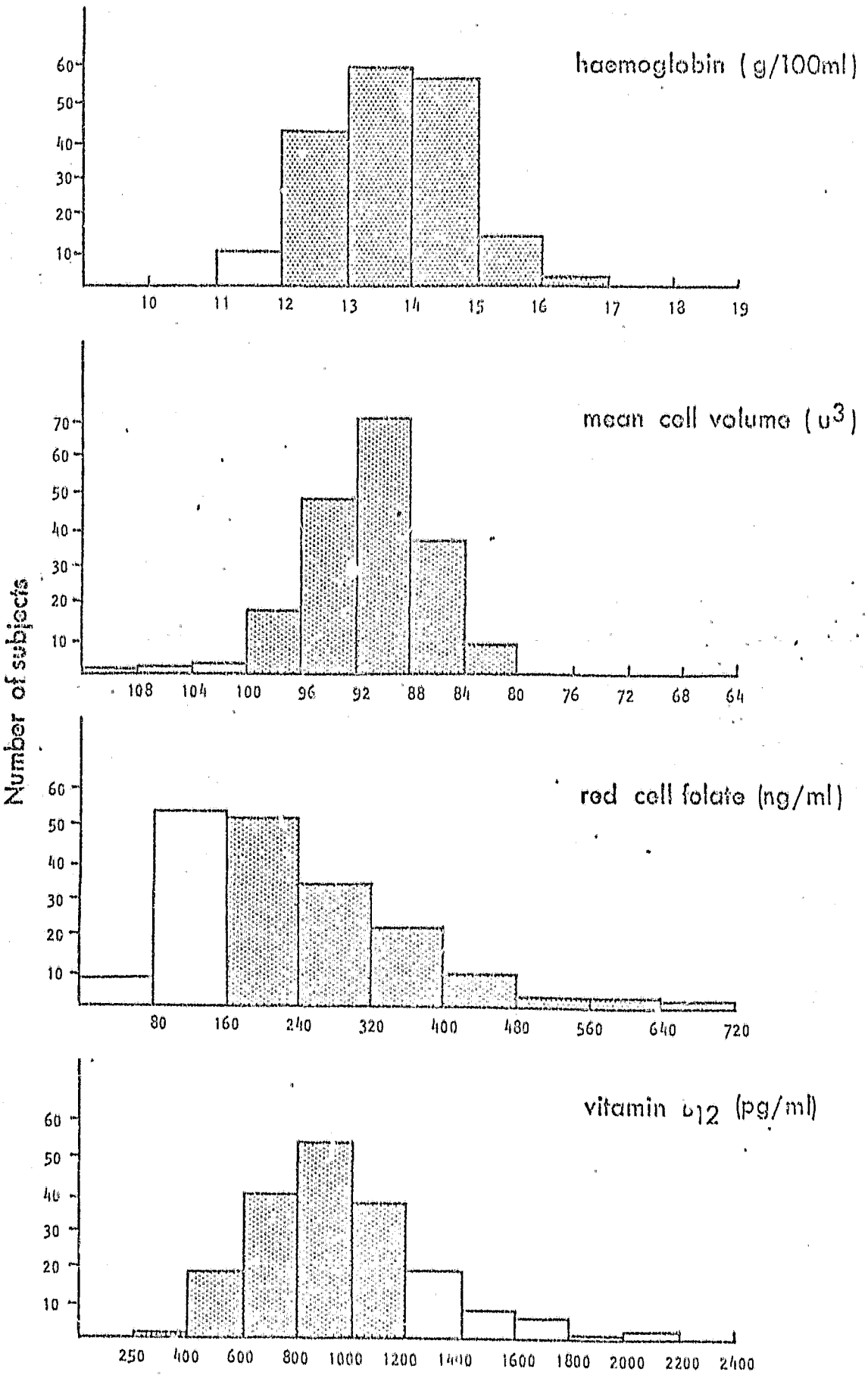

FIGURE 8.2 Results of laboratory tests in 185 non-pregnant women at Nqutu. The shaded areas represent the respective normal ranges.

mean red cell folate concentration was 234.0 ng per ml (standard deviation 123.8 ng per ml). Of the 61 subjects with a red cell folate level in the deficient range, three were nulliparous women less than 21 years old, 37 were parous women below the age of 45 years, and 21 were older women. Thirty-one of the 37 parous women under 45 had been pregnant or lactating within the previous three years, this period in the remaining six women being four, six, nine, 13, 14, and 15 years respectively. Of all women who had been pregnant or lactating within the three years prior to being studied, 47.7 per cent had red cell folate deficiency.

Table 8.2 shows the incidence of red cell folate deficiency in relation to the number of years since each subject was pregnant or lactating.

The subjects were divided into seven age groups ranging from eleven to eighty years, each age group encompassing ten years, and the incidence of red cell folate deficiency in each group was calculated. Table 8.3 shows that the incidence up to the age of forty was over 40 per cent. For the next twenty years the incidence fell sharply, but more than a third of women over the age of 60 years had red cell folate deficiency.

There were no vitamin B_{12} concentrations in the deficient range, and one subject (0.5 per cent) had a level of 363 pg per ml, which is in the low range. Blocking antibodies to intrinsic factor were not detected

TABLE 8.2

The incidence of folate deficiency in non-pregnant women of child-bearing age (16-45 years) related to pregnancy and lactation. Nqutu study.

Class	Years since pregnancy or lactation	Number of subjects	Number deficient*	Incidence of deficiency
Nulliparous	-	16	3	18.8%
Parous	$\frac{1}{2}$ - 1	43	21	48.8%
	1 - 2	16	9	56.3%
	2 - 3	6	1	16.7%
	> 3	20	6	30.0%

*Based on red cell folate concentration.

TABLE 8.3

The incidence of folate deficiency in non-pregnant women of different ages. Nqutu study.

Age (years)	Number of subjects	Number deficient*	Incidence of deficiency
16-20	24	10	41.7%
21-30	40	18	45.0%
31-40	19	9	47.4%
41-50	26	3	11.5%
51-60	32	5	15.6%
61-70	26	9	34.6%
71-80	18	7	38.9%

*Based on red cell folate concentration.

in this subject's serum. The mean serum vitamin B_{12} concentration was 972.1 pg per ml, with a standard deviation of 308.0 pg per ml.

Haemoglobin levels were below 12 g per 100 ml in 5.4 per cent of the sample (ten subjects) and 12 - 13 g per 100 ml in a further 23.2 per cent (43 subjects). The mean haemoglobin concentration was 13.75 g per 100 ml with a standard deviation of 1.04 g per 100 ml.

Mean cell volumes of 81 cubic microns were recorded in two subjects (1.1 per cent) with values greater than this being recorded in all the other subjects. Six levels greater than 100 cubic microns were recorded. The average mean cell volume and standard deviation were 91.7 ± 4.7 cubic microns.

Red cell folate deficiency was associated with a haemoglobin level below 12 g per 100 ml in four subjects and of 12 - 13 g per 100 ml in eleven subjects, and was associated with a raised mean cell volume in four subjects. The frequency of red cell folate deficiency in association with these characteristics was no higher than in the group as a whole (by chi-square for all, $p > 0.1$).

There was no direct correlation between either haemoglobin or mean cell volume on the one hand, and red cell folate or vitamin B_{12} on the other.

(c) Males (group VIII)

The results of the haemoglobin concentrations, mean cell volume, red cell folate and vitamin B_{12} concentrations in the males is shown in Figure 8.3. The age of the subjects ranged from sixteen to 82 years (mean 46.2 years).

Red cell folate levels in the deficient range were recorded in 26 subjects (18.6 per cent), and were in the low range in a further 27 subjects (19.3 per cent). The mean red cell folate concentration was 268.1 ng per ml (standard deviation 132.3 ng per ml).

The subjects were divided into seven age groups ranging from eleven to eighty years, each group encompassing ten years. The single subject more than eighty years of age had a normal red cell folate concentration.

The incidence of deficiency in the different groups is shown in Table 8.4. The incidence of deficiency was similar in all groups up to 60 years of age (13.3 per cent), but increased after the age of sixty (31.0 per cent).

There were no subjects with serum vitamin B_{12} levels in either the deficient or the low ranges. The mean serum vitamin B_{12} concentration was 1077.0 pg per ml, with a standard deviation of 335.7 pg per ml.

Haemoglobin concentration was 12 g per 100 ml or

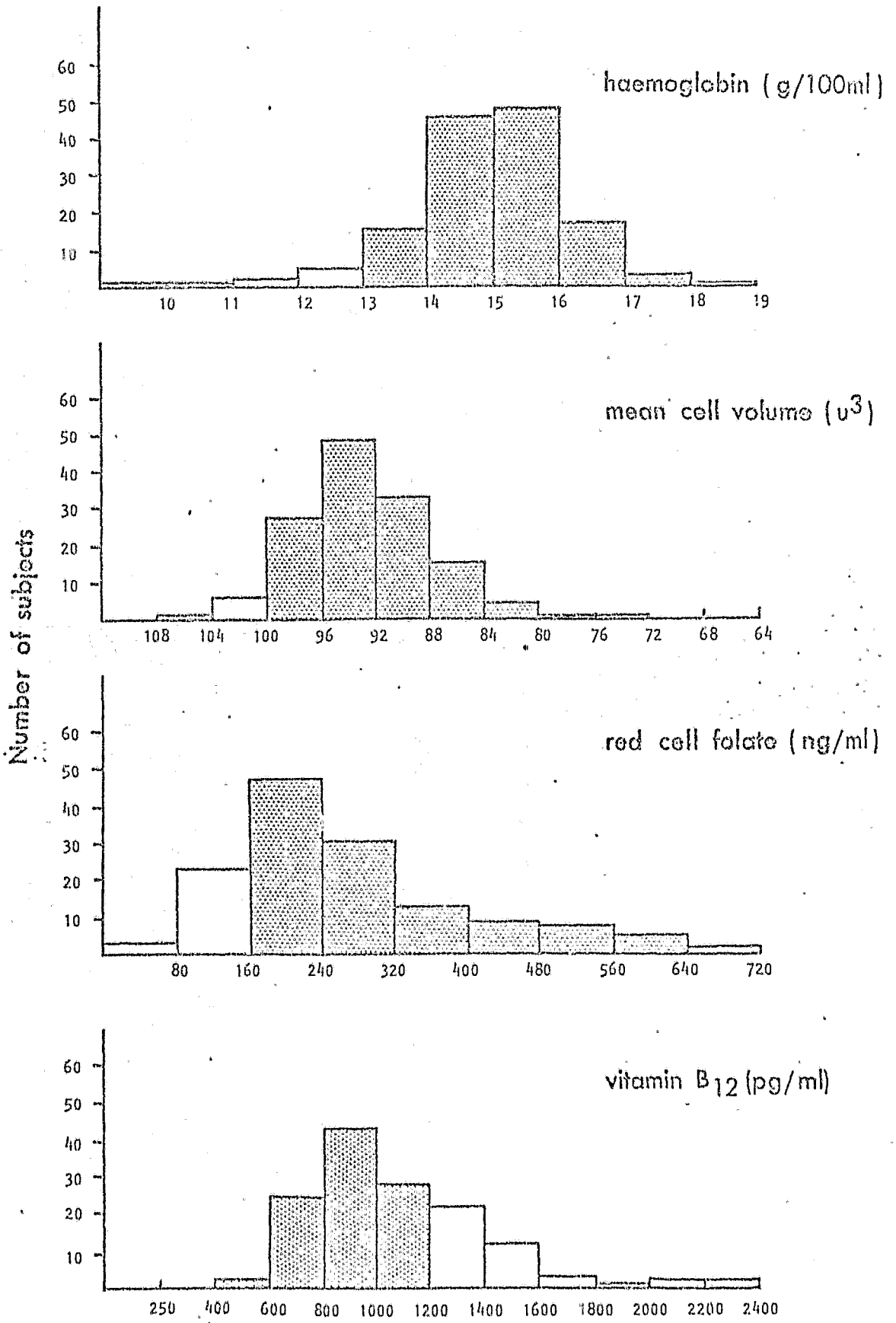

FIGURE 8.3 Results of laboratory tests in 140 males at Nqutu. The shaded areas represent the respective normal ranges.

TABLE 8.4

Incidence of folate deficiency in males according to age. Nqutu study.

Age (years)	Number of subjects	Number deficient*	Incidence of deficiency
16 - 20	16	3	18.8%
21 - 30	30	4	13.3%
31 - 40	14	2	14.3%
41 - 50	19	1	5.3%
51 - 60	19	3	15.8%
61 - 70	18	4	22.2%
71 - 80	23	9	39.1%

*Based on red cell folate concentration.

less in four subjects (2.9 per cent), 12 - 13 g per 100 ml in five subjects (3.6 per cent) and 13 - 14 g per 100 ml in a further sixteen subjects (11.4 per cent). The mean haemoglobin concentration was 15.0 g per 100 ml.

Mean cell volume was below the lower limit of normal (82 cubic microns) in two subjects (1.4 per cent) and above the upper limit (100 cubic microns) in seven (5.0 per cent), none of whom had red cell folate levels in the deficient range.

There was no correlation between any of the above-mentioned observations in this group.

(d) Urban females (group IX)

The 181 women studied at a Johannesburg family planning clinic are considered as one group initially, and differences between the subgroups are compared in the next section. The subjects ranged from twenty to 45 years of age (mean 29.6 years), and on average had not lactated or been pregnant for 3.6 years. The distribution of results of the laboratory tests performed is shown in Figure 8.4.

Red cell folate levels were in the deficient range in 37 subjects (20.4 per cent) and in the low range in a further 33 subjects (18.2 per cent). The mean red cell folate concentration was 237.4 ng per ml and the standard deviation was 97.7 ng per ml. The incidence

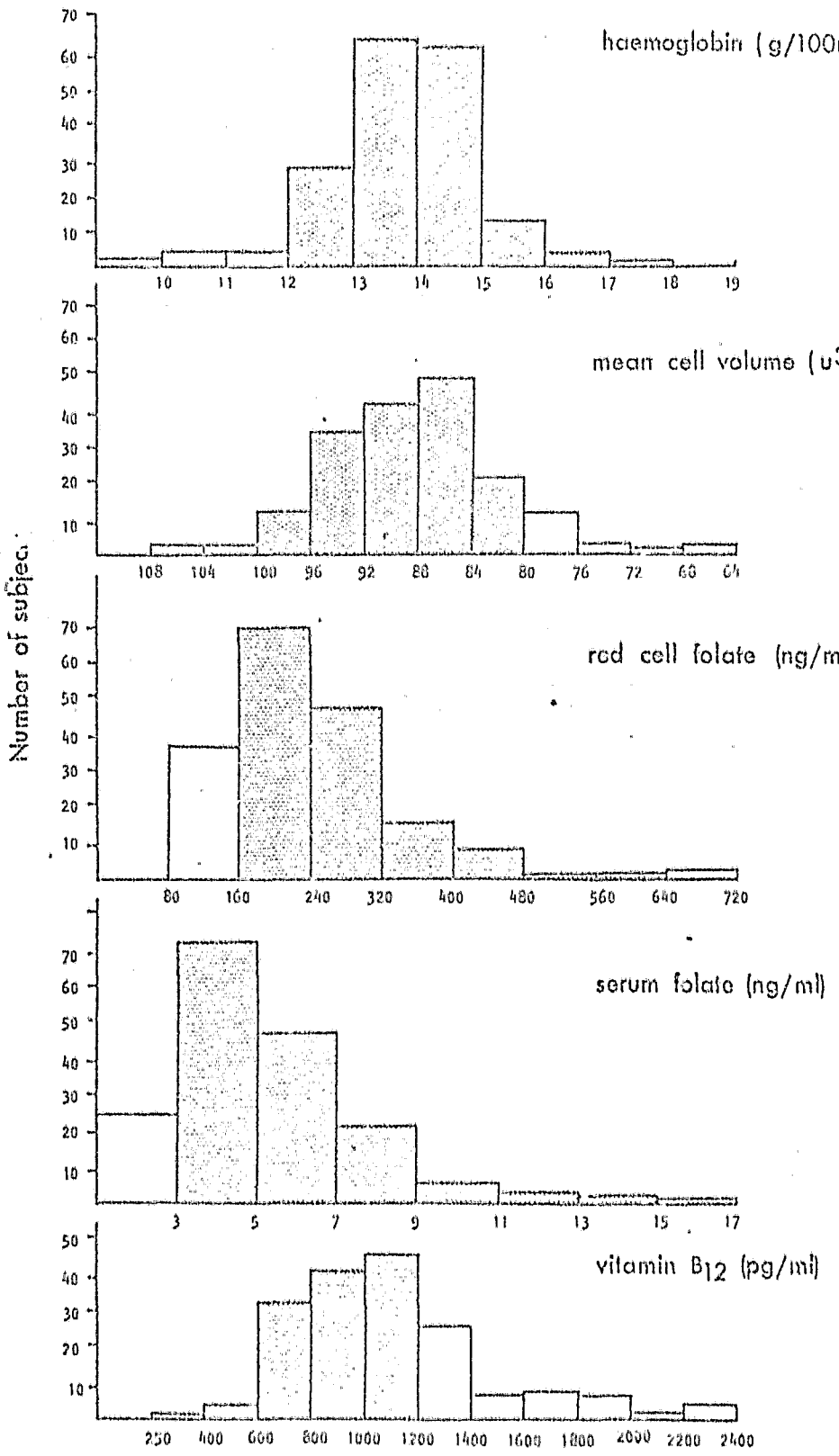

FIGURE 8.4 Results of laboratory tests in 181 non-pregnant Negro women, aged 20-45 years, attending a Johannesburg family planning clinic. The shaded areas represent the respective normal ranges.

of folate deficiency, according to the number of subjects with red cell folate levels in the deficient range, is related to the time since the last pregnancy or period of lactation in Table 8.5.

TABLE 8.5

The incidence of folate deficiency in women of child-bearing age (20-45 years) related to pregnancy and lactation. Urban study.

Years since last pregnant or lactating	Number of subjects	Number deficient	Incidence of deficiency
$\frac{1}{2}$ - 1	49	17	34.7%
1 - 2	32	7	21.9%
2 - 3	23	4	17.4%
> 3	74	8	10.8%

Serum folate levels were in the deficient range in 26 subjects (14.4 per cent) and in the low range in a further 47 subjects (26.0 per cent). The mean serum folate level, with standard deviation, was 5.56 $\pm$ 3.14 ng per ml.

The only subject with a low vitamin B_{12} level had a serum concentration of 364 pg per ml. Type I (blocking) antibodies to intrinsic factor were absent from this subject's serum. The mean vitamin B_{12} concentration was 1132.4 pg per ml (standard deviation 418.1 pg per ml).

Haemoglobin levels were less than 12 g per 100 ml in 13 subjects and 12 - 13 g per 100 ml in a further 27 subjects. The mean haemoglobin level, with standard deviation, was 13.74 $\pm$ 1.27 g per 100 ml.

Mean cell volume was greater than 100 cubic microns in three subjects, none of whom had low red cell folate levels, and less than 82 cubic microns in 22 subjects (12.2 per cent).

Apart from a strong correlation between red cell folate and serum folate levels ($r = 0.4850$, $p < 0.001$), there was no correlation between any two laboratory tests.

(e) Comparisons between and within groups

(i) Effect of hormonal contraceptives

The mean red cell folate levels were 243.1 ng per ml in group IXa (control urban subjects), 235.2 ng per ml in women receiving oral contraceptive pills (group IXb), and 229.0 ng per ml in women receiving quarterly injections of a progesterone compound (group IXc). The differences between these means were not statistically significant ($p > 0.5$).

The mean serum folate level in group IXa (4.63 ng per ml) was lower than that in group IXb (5.50 ng per ml) and group IXc (4.85 ng per ml), and these differences were statistically significant (for both, $p < 0.01$).

The number of subjects in each subgroup who had subnormal serum folate concentrations is shown in

Table 8.6. The significance of the differences between the subgroups was analysed using the chi-square test. The number of low red cell folate levels was statistically similar in each group when comparing either the three categories listed in Table 8.6 ($p > 0.4$) or when comparing the number of levels greater than 160 ng per ml with the number below this concentration. Serum folate levels below 5 ng per ml were not significantly more common in group IXa ($p > 0.5$), but when all three categories (i.e. "deficient", "low" and "normal") were considered, the differences were significant ($p < 0.01$). Thus the number of serum folate concentrations in the deficient range was greater in control subjects.

TABLE 8.6

Number of subjects with subnormal red cell folate and serum folate levels. The effect of contraceptive agents in urban women.

Group	Contraceptive	Number of subjects					
		Red cell folate (ng per ml)			Serum folate (ng per ml)		
		0-160	161-200	>200	0-3.0	3.1-5.0	>5.0
IXa	Not hormonal	19	12	43	18	22	34
IXb	"Combination" pills	13	17	45	7	20	48
IXc	Progesterone injections	5	4	23	1	15	16

The mean serum vitamin B_{12} concentrations in groups IXa, IXb and IXc were 1182.3, 1117.8, and 1051.4 pg per ml respectively. None of these differences were significant (for all, $p > 0.1$).

It was concluded that this survey provided no evidence that hormonal contraceptive agents decreased folate or vitamin B_{12} levels in urban Negro women.

(ii) Comparison between the different segments of the Nqutu population

Mean concentrations of haemoglobin, red cell folate, and serum vitamin B_{12} were all significantly lower in the non-anaemic pregnant women at Nqutu than in the non-pregnant women from that area (for all measures, $p < 0.01$), and in turn were all lower in the non-pregnant women than in the men (for all, $p < 0.02$).

Folate deficiency was much more common in the non-pregnant women at Nqutu than the men (by chi-square, $p < 0.001$). The rural women of child-bearing age had a significantly higher incidence of folate deficiency than the urban sample ($p < 0.001$).

(f) Socioeconomic status of Nqutu subjects

The average size of the family units to which the 469 subjects belonged was 6.27 adults, with two children aged 2 - 16 years being regarded as one adult. The average cash income per family was R14.46c monthly.

Because the use of the above means would bias results in favour of large families, the monthly income

per adult in each family was calculated by dividing each family income by the number of adults in the corresponding family. The mean monthly income per adult in each family was R3.15c. The distribution of this income is shown in Table 8.7.

TABLE 8.7

Monthly income of the families of 469 inhabitants of Nqutu.

Monthly income per adult* (Rands)	Number of subjects	Percentage
0 - 2.50	227	48.4
2.51 - 5.00	210	44.8
5.01 - 7.50	19	4.1
7.51 -10.00	9	1.9
10.01**-12.50	1	0.2
More than 20.00	3	0.6

* Children 2 - 16 years regarded as one half adults

** Based on State Health food charts and prices in Nqutu, R10.00 is required to buy one month's food for each adult. This excludes home produce.

The family units of the men had a higher mean monthly adult income (R3.59) than those of the pregnant women (R3.32), and those of the non-pregnant women had the lowest income (R2.69 per adult).

In order to determine the number of subjects whose families were subject to different degrees of economic deprivation, the income required for food alone was

calculated from the figures of Clarke (personal communication) which have been mentioned earlier. Clarke used food tables prepared by the South African Department of Health as a basis for her calculation that about R54 was required to buy one month's supply of food in Nqutu for a family of seven. Assuming that at least four members of this average family were under 16 years of age, it can be calculated that the income per adult required for food is R10 monthly. Based on this figure, Table 8.7 shows that less than one per cent of the sample had an income sufficient for food requirements, whereas over 90 per cent received less than half this amount. These figures do not take into account any food produced by the family.

There was no direct correlation between cash income and either red cell folate or serum vitamin B_{12} concentration in any of the three groups (for all, $p > 0.5$).

(g) Maize meal intake

The maize meal bought by each family was divided by the number of adults in the family in order to assess consumption. The average adult maize consumption was 12.12 kg monthly, corresponding to a daily intake of 399 g. The distribution of consumption is shown in Table 8.8.

From this table it can be calculated that if the level of fortification was based on an average monthly consumption of 12 kg maize meal per adult, 84.3 per cent

of the families would receive 50 - 150 per cent of the intended dose. Five per cent would receive less than half the intended dose and five per cent more than twice the dose.

TABLE 8.8

Amount of maize meal bought by the families of 469 Nqutu inhabitants.

Maize meal bought (kg /adult/month)	Number of subjects	Percentage of sample
0 - 6	23	4.9
6 - 12	232	49.5
12 - 18	163	34.8
18 - 24	27	5.7
24 - 30	17	3.6
More than 30	7	1.5

The upper limit of consumptio was 50 kg maize meal per adult monthly. However, all seven families which bought more than 30 kg per adult monthly were large tribal units which used large quantities of the meal in the brewing of traditional alcoholic beverages. The stability of folic acid in these beverages was not tested in the present study.

IV Discussion

This survey shows that folate deficiency is prevalent among South African Negroes, confirming earlier

suggestions that this might be the case (Metz et al., 1961). Based on the assumption that Nqutu is representative of South African rural areas and that folate deficiency is at least as common in children as in adults, approximately two million of the Negroes enumerated in the South African Bantu homelands in 1970 would benefit if their food was fortified with folic acid. This figure does not take account of the majority of this country's Negro population, who live in "white" rural areas and towns (South Africa. Department of Statistics, 1970).

Among women of childbearing age, folate deficiency was more common in subjects who had been pregnant or lactating within the two previous years. It has previously been said that the improvement in folate nutrition after pregnancy "is short-lived in populations whose dietary folate intake is suboptimal, as folate nutrition again tends to deteriorate as lactation progresses" (Metz, 1970). The findings of the present study suggest that it may take two or three years before folate stores in this population return to normal after cessation of lactation. Thus many women must be entering pregnancy with depleted folate stores, a situation which could be averted by food fortification.

Folate deficiency was less common in urban subjects than in rural women of comparable age. This is probably largely due to the fact that many of the urban women were

receiving food from their white employers. Although red cell folate levels did not correlate with income within the rural groups, the fact that folate deficiency was more common in rural than urban subjects can be attributed to their different socioeconomic conditions.

More than one-third of the subjects over the age of 60 in both the male and female groups were folate deficient, demonstrating that aged subjects should be regarded as a target group in a food fortification programme.

There are conflicting reports regarding the effect of oral contraceptives on folate metabolism. Megaloblastic anaemia due to folate deficiency has been reported in women taking these agents (Paton, 1969; Streiff, 1969, 1970). Mean red cell and serum folate levels were reported to be decreased in women using oral contraceptives (Shojania et al., 1968, 1969; Gaafar et al., 1973), but these findings have not been confirmed by other workers (Spray, 1968; McLean et al., 1969; Pritchard et al., 1971; Stephens et al., 1972). The findings in the present study of urban females are in accordance with the latter reports. This does not exclude the possibility that a significant effect of oral contraceptives might be demonstrated in rural Negro women, whose folate nutritional status is less satisfactory than their urban counterparts, but such a study is not feasible in present circumstances.

The small group of subjects receiving a parenterally-administered progesterone compound also had red cell folate levels no different from controls.

The mean consumption of maize meal by our subjects (399 g per adult per day) was slightly lower than the 474 g per adult per day bought by the subjects studied by du Plessis et al. (1971). It should be pointed out, moreover, that du Plessis and his coworkers regarded children over the age of ten as adults, whereas those over sixteen years of age were placed in the adult category in the present study.

In Chapters four and five, it was found that the folic acid in fortified maize meal was approximately 56 per cent as available as that in tablets and aqueous solution. In order to provide an effective dose of 200 µg folic acid in 400 g maize meal, it would be necessary to add 350 µg folic acid per 400 g maize at the time of mixing the fortified meal. At this level of fortification, the adults in 90 per cent of the families studied would have received an effective dose of 100 - 400 µg folic acid daily. 3.6 per cent would have received 400 - 500 µg daily, and five per cent would have received less than 100 µg daily. The families of 1.5 per cent of the sample bought maize in quantities such that an effective dose of up to 800 µg folic acid would have been present in the daily adult allotment if this was all used to make porridge.

However, these families used some of the maize in ways which might have resulted in destruction of the added nutrient.

Three of the 612 subjects in whom serum vitamin B_{12} concentrations were measured had equivocal levels of 360 - 400 pg per ml, the remaining levels falling within or above the normal range. None of these subjects had type I (blocking) antibodies to intrinsic factor in their sera. It seems probable that these levels reflect the presence of mild nutritional vitamin B_{12} deficiency rather than early pernicious anaemia. This low incidence of vitamin B_{12} deficiency is in accordance with the report of Brandt et al. (1963), who measured serum vitamin B_{12} concentrations in 102 Negro mine workers, using a Lactobacillus leichmannii microbiological assay. They found only one subject with a level below 200 pg per ml, the lower limit of the normal range.

The potential hazards of giving folic acid to subjects with vitamin B_{12} deficiency have been discussed in Chapter two. It was considered unlikely that deleterious effects would result from the administration of folic acid in effective doses of less than 0.4 mg daily. The administration of 0.4 - 15 mg folic acid daily would result in haematological response in some patients with undiagnosed pernicious anaemia, but according to available evidence would not have further adverse effects. The survey reported above illustrates

that the dangers are infinitesimal, as no subjects with unequivocal vitamin B_{12} deficiency were observed, and only very few subjects would have received an effective dose of folic acid greater than 400 µg daily if their maize meal had been fortified with the intention of providing an average effective daily dose of 200 µg folic acid.

C H A P T E R 9

TECHNOLOGICAL AND ECONOMIC CONSIDERATIONS

I Introduction

The investigations reported in Chapter 4 were concerned with technological factors which would be important after the vehicle food had been fortified. The folic acid in fortified maize meal was found to be stable during conventional storage and cooking procedures without conveying unwanted characteristics to the food, and its absorption from the fortified food was assessed. Subsequent chapters report confirmation of these findings and demonstrate that the added nutrient is biologically available.

It is also necessary to consider two further technological factors. These are the stage in the processing of the vehicle food at which the nutrient can be added and the cost of doing this. In this chapter, these factors will be discussed, as well as other economic considerations.

II The feasibility of fortifying maize meal in South Africa

The production, processing and marketing of maize in South Africa is controlled by law through the Maize Board, which functions in terms of the Maize and Grain Scheme (Proclamation R.113 of 1961), as amended. The annual report of this Board (Maize Board, 1973), submitted to the Minister of Agriculture in November 1973, provides

the useful information mentioned hereunder, which is relevant to the practical possibility of fortifying the maize meal consumed by South African Negroes.

"In terms of the Maize and Grain Sorghum Scheme, only persons registered as such with the Board for such purpose may grind, crush, grist or otherwise process maize ..... There are two classes of millers, namely gristing millers who only mill maize tendered by customers, and commercial millers who buy and process maize and sell the manufactured products." During the 1972-1973 season, commercial millers accounted for more than 98 per cent of all maize milled in South Africa. It is apparent that if the meal produced by commercial millers can be fortified, virtually all consumers of this food will be reached. There were 310 commercial millers registered with the Maize Board at the end of the 1973 financial year.

The Joint FAO/WHO Expert Committee has recommended that, where possible, a single centre for the fortification process should be designated within a region or country, in order to facilitate control of the programme and minimise costs. If no suitable centre is available, the nutrient may be added in a local mill, making use of pre-mixes that can be handled with moderate cost and dispensed with sufficient accuracy (FAO/WHO, 1971). In view of the considerable number of commercial millers in this country, the latter method is more feasible.

The fortified maize meal used in the present study was prepared by adding folic acid directly to the maize meal and mixing for twelve hours in a pharmaceutical blender to achieve homogeneous distribution of the nutrient in the food. However, a less intensive mixing procedure using a pre-mix produced satisfactory results i a field trial of maize meal fortified with riboflavin and nicotinamide (du Plessis et al., 1971). In this latter trial, the dose of nutrient to be added to each 180 lb. (81.54 kg) bag of maize meal was contained in two oz (56.7 g) of pre-mix. The excipient used for the pre-mix was maize meal, which was found to be more economical than starch and to possess superior free-flowing properties. Using a Miag Micro-feeder, two oz of pre-mix was delivered in 7.5 minutes with an error of approximately five per cent. Mixing was effected by means of a ten-foot-long screw conveyer which reversed the direction of mixing at its mid-point. Assay of fifteen samples of fortified maize showed that the level of fortification of the meal was reasonably homogenous.

Du Plessis and his coworkers (1971) concluded that it was technologically feasible to fortify all commercially milled maize meal in South Africa using the methods described above, and their recommendation that this be done has been accepted in principle by the Department of Health (Raymond, 1972).

III The cost of fortifying maize meal with folic acid

The cost of a food fortification programme is dependent on two factors, namely the price of the nutrient itself and the expenses incurred in carrying out the fortification programme.

Folic acid B.P. was obtainable at the time of writing this report at a price of approximately R46.00 per kg in five kg lots.

The trials reported in Chapter five suggested that folate deficiency in pregnancy could be averted by a daily intake of 300 µg added folic acid in maize meal, corresponding to an effective dose of 168 µg folic acid. The survey reported in Chapter eight showed that it would be necessary to add 350 µg folic acid to the average amount of maize meal consumed by adults in order to ensure that 90 per cent would receive an effective daily dose of 100 - 400 µg folic acid. The annual cost of a 350 µg daily supplement of folic acid would be slightly less than 0.6 cents. If this vitamin were included in the food fortification programme about to be implemented this country by adding it to the pre-mix, the cost of labour, mixing and storage would add 30 - 50 per cent to the price of the vitamin (du Plessis et al., 1971). No further expense would be entailed. The cost of fortifying maize meal in South Africa with folic acid would thus be less than 0.9 cents per adult per year. Based on an average daily consumption of 400 g maize meal per adult,

fortification with folic acid would add 0.50c to the cost of a 180 lb (81.54 kg) bag of maize meal.

In countries where fortification of food with other nutrients is not contemplated, the costs would be greater. An idea of the cost can be obtained from the report of du Plessis et al. (1971), who quoted figures calculated by Roche Products and the Maize Board. As this will give only an approximate idea of the expense entailed in countries whose wage-price structures differ from that in South Africa, no attempt has been made to adjust these estimates in order to account for inflation since May 1971. Based on the presently ruling price of folic acid, each kg of pre-mix would contain the following:

Folic acid 1.26 g at R46 per kg	=	5.8 cents
Maize meal 998.74 g at R4 per 81.54 kg	=	4.9 cents
		10.7 cents

If 30 - 50 per cent of the basic price is added to account for storage, labour and mixing costs, one kg of pre-mix would cost 13.9 - 16.1 cents and would be adequate to fortify 1438 kg maize meal. The purchase, installation, maintenance and running costs of a Miag Micro-feeder, a 10 foot mixing screw and a one-quarter horsepower motor would add an estimated 0.2 - 0.3 cents to the cost of an 81.54 kg bag of maize meal, depending on the productivity of the mill. The cost of the pre-mix added to each bag would be 0.75 - 0.92 cents. The fortification of maize

meal with folic acid alone would thus add 0.95 - 1.22 cents to the price of an 81.54 kg bag, based on the cost of labour, machinery, and maize meal in 1971. If the per capita consumption of maize in this hypothetical country was similar to that among South African Negroes, the cost of the programme would be 1.7 - 2.2 cents per adult per annum.

IV Broad economic implications of the proposed fortification programme

The costs outlined above cannot be considered in isolation. Quite apart from the humanitarian implications, the economic effects of improving the health of the population should be considered.

The relationship between health and economic benefit is complex. Economists have considered health care variously as a consumption, as an investment, or as a combination of both (Dublin and Lotka, 1946). In recent years, economists have become increasingly aware of the predominantly developmental nature of health services. "The concept of health care as an investment in 'human capital' grew out of the observation that the historical rate of economic development could not be entirely explained in terms of capital and technological investment in industry and agriculture. The central argument of the concept of health care as an investment is that returns on health expenditures - just as in the case of

expenditure on education and vocational training - raise personal and national income ..... Today the investment aspect of health care appears to be gaining acceptance" (Feldstein et al., 1973).

The economic benefits of improved health accrue mainly from increased productivity of labour, although other factors such as improved scholastic performance of children also play a role by decreasing costs in other sectors of the economy, and decreased demands on medical services may result in direct benefits to the department of health (Popkin and Lidman, 1972).

However, the techniques for evaluating these benefits are still in their infancy, and it is difficult to quantify the economic advantage which a country might expect if its population was healthy (Griffiths, 1972; Popkin and Lidman, 1972). It would be much more difficult to evaluate the benefits of a specific programme concerning a single nutrient. The fact that most economists would expect a return on this investment in "human capital" is stated here in order to reinforce the argument in favour of such a programme.

C H A P T E R 10

SUMMARY AND CONCLUSIONS

Although a large variety of morbidity has been ascribed to folate deficiency, the only unequivocal effects are megaloblastic anaemia, prematurity, and changes in epithelial surfaces, especially those of the gastrointestinal tract. The incidence of folate deficiency varies widely in different areas, and is high in a number of populations subsisting on a sub-optimal diet.

The folate requirements of pregnant women are greater than those of other groups in the population. The administration of folic acid tablets successfully prevents the development of folate deficiency and megaloblastic anaemia in pregnancy. However, large numbers of women do not attend antenatal clinics or do not take folic acid tablets when these are given to them. This is particularly true of women in under-privileged communities, in whom folate deficiency in pregnancy is of high incidence. It is therefore necessary to seek an alternative means of providing folate supplements to pregnant women in such communities.

The method most likely to succeed is fortification of a staple item of diet with folic acid. In the present study, this possibility was examined in the light of the recommendations on food fortification made by the Joint FAO/WHO Expert Committee on Nutrition.

It was found that folic acid in fortified maize meal is stable under conventional storage conditions for up to 18 months, and that the addition of the vitamin does not have undesirable effects on the colour, odour, taste, or cooking properties of the food. Folic acid in aqueous solutions of varying concentration was shown to resist destruction by usual cooking procedures for periods of at least one hour.

The effect of the presence of various vehicle foods on folic acid absorption was examined, by comparing the absorption of folic acid alone with its absorption from fortified maize, rice and bread in the same subjects. Compared with absorption of folic acid alone, the average absorption of folic acid was 56.3 per cent in the presence of maize, 54.4 per cent in the presence of rice, and 28.7 per cent in the presence of bread.

Clinical trials were carried out at Nqutu, KwaZulu, South Africa, amongst a population of rural Zulu women, whose staple item of diet is maize meal. Initial trials were carried out in pregnant women, the primary target group for folate deficiency. The women were healthy and not anaemic, and were lodging at a hospital.

Fortified maize meal was fed under supervision in such a way that each subject received 1000 µg added folic acid daily. This resulted in a marked rise in red cell folate and serum folate concentrations. In a control group, receiving unfortified maize, red cell folate

levels fell progressively.

Subsequent trials were undertaken to determine the smallest dose of folic acid in maize meal which would effectively prevent folate deficiency in pregnancy. Two groups of subjects were given fortified maize meal under supervision such that the subjects in the one group received 500 µg added folic acid daily and those in the other received 300 µg added folic acid daily. Another group received tablets containing 300 µg folic acid daily.

In subjects receiving fortified maize meal, the rate of rise in red cell folate concentration increased with increasing concentration of folic acid in the maize. The rate of rise in subjects receiving 300 µg folic acid tablets daily was slightly greater than that in subjects receiving fortified maize meal containing 500 µg added folic acid daily.

The rate of rise in red cell folate concentration in each group was corrected to account for the fall in control subjects, and the corrected rates in the groups receiving fortified maize were compared with that in the group receiving folic acid tablets. This method of calculation was used to determine the percentage of folic acid in fortified maize that was available for incorporation into red cell folate, when the availability of folic acid taken alone was regarded as 100 per cent. Fifty-five to 58 per cent of the folic acid in fortified

maize was available. This finding is in close agreement with the observation made in the current study, that 56.3 per cent of folic acid in fortified maize is absorbed, compared with the absorption of folic acid alone.

The corrected rates of rise in red cell folate concentration were proportional to the amount of the supplement in groups receiving fortified maize containing 300/ug and 500/ug folic acid daily. However, the rate in subjects receiving a single daily dose of 1000/ug added folic acid in maize suggested that a smaller proportion of this dose was available, it being likely that the excess either was not absorbed or was excreted.

Red cell folate concentration fell in a few subjects in every supplemented group, but serum folate levels rose in all of these subjects. This suggests that the total dose of folic acid administered during the few weeks of the study was occasionally insufficient to significantly improve body stores. Fortified maize meal containing 300/ug folic acid daily was as effective as folic acid tablets and more strongly fortified maize in preventing a fall in red cell folate concentration in individual subjects. This dose is equivalent to 165 - 185/ug folic acid daily in tablet form.

Mean haemoglobin levels also rose significantly in all of the groups receiving folic acid supplements, all doses being equally effective. However the regression coefficient for the group receiving 300/ug added folic

acid daily in maize was not significant, whereas it was in the other groups receiving supplements. Serum vitamin B_{12} concentrations did not change significantly in subjects receiving folic acid supplements.

In addition to the hospital-based trials, a pilot field trial was carried out to assess the effect of supplying fortified maize meal to subjects who consume it in the home environment. The results indicate that effective improvement in folate nutrition can be achieved in this way. The rise in red cell folate concentration in pregnant and lactating subjects was at least as great as that in the oldest members of their respective families. As the folate requirements of the former exceed those of the latter, this suggests that they were eating more of the fortified maize meal. This may mean that the sector of the population with the greatest folate requirement would receive the greatest folic acid supplement from fortified maize meal eaten in the home.

The biological availability of folic acid in fortified maize was demonstrated by therapeutic trials in lactating patients with folate-deficient megaloblastic anaemia. Optimal haematological responses were observed in four patients receiving 300 - 500 µg added folic acid daily in maize, but a suboptimal response occurred in one subject receiving maize containing 100 µg folic acid daily, which is equivalent to approximately 56 µg folic acid alone.

These trials showed that food fortified with folic acid can be used to increase folate stores, and so safeguard against the development of folate deficiency and megaloblastic anaemia in pregnancy.

In order to assess in greater detail the need for such supplementation in the population under study, the incidence of folate deficiency in various groups of Negroes was determined on the basis of the red cell folate concentration. At Nqutu, folate deficiency was present in 43.8 per cent of healthy non-anaemic pregnant women, 33.0 per cent of a randomly-selected sample of non-pregnant women, and 18.6 per cent of men. Among non-pregnant women, folate deficiency was more common in subjects who had been pregnant or lactating within the previous two years. Among both men and women, the incidence of folate deficiency was greater than 30 per cent in subjects over the age of 60 years. Based on these results, it is estimated that approximately two million Negroes in the "Bantu homelands" would benefit from the fortification of food with folic acid.

The incidence of folate deficiency was not as great in urban females. Twenty per cent of Negro women attending a Johannesburg family planning clinic had folate deficiency, and the incidence was not increased in those using hormonal contraceptive agents.

The only theoretical hazard of administering folic acid to a whole population is the possibility of

inducing haematological response in subjects with primary vitamin B_{12} deficiency. However, the dose of folic acid effective in preventing folate deficiency in pregnancy was found in this study to be less than the smallest dose capable of inducing such a response. Furthermore, no single instance of unequivocal vitamin B_{12} deficiency was encountered among over 600 subjects examined. Thus, the risk of the indiscriminate administration of folic acid in the population under study is infinitesimal. Western diets contain folate in amounts approximately equivalent to 300 µg folic acid daily, and the general purpose of the present proposals is to increase the folate content of the diet of impoverished people to this level.

The milling and marketing of maize meal in South Africa are controlled in such a way that its fortification would be feasible, and it is likely that this will be implemented shortly, with riboflavin and nicotinamide as the added nutrients. The inclusion of folic acid in this programme would add 0.50 cents to the cost of an R1.54 kg bag of maize meal, and would cost less than one cent per adult in the target population per annum. In maize-eating countries where food fortification is not presently contemplated, the implementation of food fortification with folic acid would raise the cost of a bag of maize meal by about one cent, and would cost about two cents per adult per year.

The results of this study provide evidence that folic acid fortification of maize meal in South Africa is indicated, and that this would fulfil the criteria laid down by the Joint FAO/WHO Expert Committee on Nutrition.

L I S T O F R E F E R E N C E S

Abramovicz, M., and Kass, E.H. (1966) : New Engl. J. Med., 275, 938.

Adams, P., Chalmers, T.M., Foulds, W.S., and Withey, J.L. (1967) : Lancet, ii, 229.

Ainley, N.J. (1961) : J. Obstet. Gynaec. Brit. Emp., 68, 254.

Alperin, J.B., Hutchinson, H.T., and Levin, W.C. (1966) : Arch. intern. Med., 117, 681.

Anand, M.P. (1964) : Scot. med. J., 9, 388.

Anderson, B.B., Belcher, E.H., Chanarin, I., and Mollin, D.L. (1960) : Brit. J. Haemat., 6, 439.

Anderson, B.B., and Cowan, J.D. (1968) : J. clin. Path., 21, 85.

Ardeman, S., and Chanarin, I. (1963) : Lancet, ii, 1350.

Armstrong, R.C., and Monie, I.W. (1966) : J. Embryol. exp. Morph., 16, 531.

Barker, E.A. (1959) : Giving and Receiving : an adventure in African medical service. London, The Faith Press.

Baumslag, N., and Metz, J. (1964) : S. Afr. med. J., 38, 611.

Baumslag, N., Edelstein, T., and Metz, J. (1970) : Brit. med. J., i, 16.

Beaton, G.H. (1972) : Proc. West. Hemisphere Nutr. Congr. III, quoted by Hegsted, D.M. (1972) : Ecol. Food Nutr. 1, 255.

Berg, A. (1973) : The Nutrition Factor : Its role in national development. Washington, D.C., Brookings Institution.

Berry, V., Booth, M.A., Chanarin, I., and Rothman, D. (1963) : Brit. med. J. ii, 1103.

Bethell, F.H. (1935) : Personal communication. Quoted by Sturgis, C.C., and Isaacs, R. (1938) : in Handbook of Haematology, ed. H. Downey, 3, 2231. London, Hamish Hamilton.

Blakley, R.L. (1969) : The Biochemistry of Folic Acid and Related Pteridines. Amsterdam, North-Holland Publishing Company.

Bobzien, W.F., and Goldman, I.D. (1972) : J. clin. Invest., 51, 1688.

Boddington, M.M., and Spriggs, A.I. (1959) : J. clin. Path. 12, 228.

Bonnar, J., Goldberg, A., and Smith, J.A. (1969) : Lancet, i, 457.

Brain, Lord, and Walton, J.N. (1969) : Brain's Diseases of the Nervous System. London, Oxford University Press.

Buetow, K.C. (1961) : Amer. J. publ. Hlth, 51, 217.

Burland, W.L., Simpson, K., and Lord, J. (1971) : Arch. Dis. Childh., 46, 189.

Butterworth, C.E., Santini, R., and Frommeyer, W.B. (1963) : J. clin. Invest., 42, 1929.

Callender, S.T.E. (1944) : Quart. J. Med., 13, 75.

Casalis, E. (1861) : The Basutos, or 23 years in South Africa. London, James Nisbet.

Cassel, R., and Metz, J. (1958) : Med. Proc., 4, 278.

Chanarin, I. (1969) : The Megaloblastic Anaemias. Oxford, Blackwell Scientific Publications.

Chanarin, I., Fenton, J.C.B., and Mollin D.L. (1957) : Brit. med. J., i, 1162.

Chanarin, I., Anderson, B.B., and Mollin, D.L. (1958) : Brit. J. Haemat. 4, 156.

Chanarin, I., MacGibbon, B.M., O'Sullivan, W.J., and Mollin, D.L. (1959) : Lancet, ii, 634.

Chanarin, I., Laidlaw, J., Loughridge, L.W., and Mollin, D.L. (1960) : Brit. med. J., i, 1099.

Chanarin, I., and Bennett, M.C. (1962) : Brit. med. J., i, 985.

Chanarin, I., Rothman, D., and Berry, V. (1965) : Brit. med. J., i, 480.

Chanarin, I., and McLean, A. (1967) : Clin. Sci., 32, 57.

Chanarin, I., Rothman, D., Ward, A., and Perry, J. (1968) : Brit. med. J., ii, 390.

Channing, W. (1842) : New Engl. Quart. J. Med. Surg., 1, 157.

Cheldelin, V.H., Woods, A.M., and William, R.J. (1943) : J. Nutr., 26, 477.

Chepenick, K.P., and Waite, B.M. (1972) : Teratology, 5, 191.

Cohen, I. (1953) : S. Afr. med. J., 27, 627.

Combrink, P.B., Turchetti, L.C., Pannel, P., and Metz, J. (1966) : S. Afr. med. J., 40, 234.

Cooper, B.A. (1973) : Clinics in Haemat. 2, 461.

Cooper, B.A., Cantlie, G.S.D., and Brunton, L. (1970) : Amer. J. clin. Nutr., 23, 848.

Council on Foods and Nutrition (1972) : J. Amer. med. Ass., 220, 855.

Cowan, J.D., Hoffbrand, A.V., and Mollin, D.L. (1966) : Lancet, i, 11.

Cox, E.V. (1961) : in Vitamin B_{12} und Instrinsic Factor, 2, ed. H.C. Heinrich, 590. Stuttgart, Enke.

Coyle, C., and Geoghegan, F. (1962) : Proc. roy. Soc. Med., 55, 764.

Crosby, W.H. (1973) : J. Amer. med. Ass., 224, 399.

C.S.I.R. (1959) : Food enrichment in South Africa; Research Report no. 172. Pretoria, C.S.I.R.

Dacie, J.V., and Lewis, S.M. (1968) : Practical Haematology; 4th ed. London, Churchill.

Darby, W.J. (1972) : Nutr. Rev., 30, 98.

Davies, A.M. (1971) : Israel J. med. Sci., 7, 753.

Dawson, D.W. (1966) : J. Obstet. Gynaec. Brit. Cwlth, 73, 44.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., and Jones, K.M. (1969) : Data for Biochemical Research. Oxford, Oxford University Press.

Diez de Ewald, M., and Molina, R. (1970) : Invest. y Clin., 36, 21.

Diez-Ewald, M., and Molina, R. (1972) : Amer. J. trop. Med. Hyg., 21, 587.

Donabedian, A., and Rosenfeld, L.S. (1961) : New Engl. J. Med., 265, 1.

Dow, W.I.M. (1971) : Brit. med. J., ii, 207.

Dublin, L.I., and Lotka, A.J. (1946) : The money value of a man. New York, Ronald Press.

Du Plessis, J.P. (1967) : An evaluation of biochemical criteria for use in nutrition status surveys; Research Report no. 261. Pretoria, C.S.I.R.

Du Plessis, J.P., Wittman, W., Louw, M.E.J., Nel, A., van Twisk, P. and Laubscher, N.F. (1971) : S. Afr. med J., 45, 530.

Edelstein, T., Stevens, K., Brandt, V., Baumslag, N., and Metz, J. (1966) : J. Obstet. Gynaec. Brit. Cwlth, 73, 197.

Elwood, P.C. (1963) : Brit. med. J., i, 224.

FAO/WHO (1970) : Wld Hlth Org. techn. Rep. Ser. no. 452.

FAO/WHO (1971) : Wld Hlth Org. techn. Rep. Ser. no. 477.

F.D.A. (1971) : Federal Register, 36, 23074.

Feldstein, M.S., Piot, M.A., and Sundaresan, T.K. (1973) : Resource allocation model for public health planning. Geneva, World Health Organisation.

Finch, C.A., and Monsen, E.R. (1972) : J. Amer. med. Ass., 219, 1462.

Fleming, A.F. (1970) : Amer. J. clin. Nutr., 23, 224.

Fleming, A.F. (1972) : J. Obstet. Gynaec. Brit. Cwlth, 79, 916.

Fleming, A.F., Knowles, J.P., and Prankerd, T.A. (1963) : Lancet, i, 606.

Fleming, A.F., Hendrickse, J.P. de V., and Allan, N.C. (1968) : J. Obstet. Gynaec. Brit. Cwlth, 75, 425.

Fleming, A.F., Allan, N.C., and Stenhouse, N.S. (1969) : Amer. J. clin. Nutr., 22, 755.

Fletcher, J. Gurr, A. Fellingham, F.R., Prankerd, T.A.J., Brant, H.A., and Menzies, D.N. (1971) : J. Obstet. Gynaec. Brit. Cwlth, 78, 781.

Forshaw, J.W.B. (1957) : Lancet, i, 1249.

Forshaw, J.W.B., Jones, A.T., Chisholm, W.N., and McGinley, W.K. (1957) J. Obstet. Gynaec. Brit. Emp., 64, 255.

Foy, H., Kondi, A., and Hargreaves, A. (1951) : Brit. med. J., i, 1108.

Fraser, J.L., and Watt, H.J. (1964) : Amer. J. Obstet. Gynec., 89, 532.

Frenkel, E.P., McCall, M.S., and White, J.D. (1970) : Amer. J. clin. Path., 53, 891.

Gaafar, A., Toppozada, H.K., and Hozayen, A. (1973) : Contraception, 8, 43.

Gallagher, E.B. (1967) : Amer. J. publ. Hlth, 57, 2127.

Gatenby, P.B.B. (1956) : Proc. Nutr. Soc., 15, 115.

Gatenby, P.B.B., and Lillie, E.W. (1960) : Brit. med. J., ii, 1111.

Gerritsen, P., and Walker, A.R.P. (1953) : S. Afr. med. J., 27, 577.

Gerson, C.D., Cohen, N., Hepner, G.W., Brown, N., Herbert, V., and Janowitz, H.D. (1971) : Gastroenterology, 61, 224.

Giles, C. (1966) : J. clin. Path., 19, 1.

Giles, C., and Shuttleworth, E.M. (1958) : Lancet, ii, 1341.

Giles, C., and Burton, H. (1960) : Brit. med. J., ii, 636.

Girdwood, R.H. (1969) : Amer. J. clin. Nutr., 22, 234.

Goetsch, C. (1962) : Amer. J. Obstet. Gynec., 83, 1474.

Gough, K.R., Read, A.E., McCarthy, C.F., and Waters, A.H. (1963) : Quart. J. Med., 32, 243.

Graham, R.M., and Rheault, M.H. (1954) : J. Lab. clin. Med., 43, 235.

Grant, H.C., Hoffbrand, A.V., and Wells, D.G. (1965) : Lancet, ii, 763.

Frenkel, E.P., McCall, M.S., and White, J.D. (1970) : Amer. J. clin. Path., 53, 891.

Gaafar, A., Toppozada, H.K., and Hozayen, A. (1973) : Contraception, 8, 43.

Gallagher, E.B. (1967) : Amer. J. publ. Hlth, 57, 2127.

Gatenby, P.B.B. (1956) : Proc. Nutr. Soc., 15, 115.

Gatenby, P.B.B., and Lillie, E.W. (1960) : Brit. med. J., ii, 1111.

Gerritsen, P., and Walker, A.R.P. (1953) : S. Afr. med. J., 27, 577.

Gerson, C.D., Cohen, N., Hepner, G.W., Brown, N., Herbert, V., and Janowitz, H.D. (1971) : Gastroenterology, 61, 224.

Giles, C. (1966) : J. clin. Path., 19, 1.

Giles, C., and Shuttleworth, E.M. (1958) : Lancet, ii, 1341.

Giles, C., and Burton, H. (1963) : Brit. med. J., ii, 636.

Girdwood, R.H. (1969) : Amer. J. clin. Nutr., 22, 234.

Goetsch, C. (1962) : Amer. J. Obstet. Gynec., 83, 1474.

Gough, K.R., Read, A.E., McCarthy, C.F., and Waters, A.H. (1963) : Quart. J. Med., 32, 243.

Graham, R.M., and Rheault, M.H. (1954) : J. Lab. clin. Med., 43, 235.

Grant, H.C., Hoffbrand, A.V., and Wells, D.G. (1965) : Lancet, ii, 763.

Grant, R.E., and Stores, O.P.R. (1970) : Brit. med. J., iv, 644.

Green, R., Musso, A.M., Mollin, D.L., and Newmark, P.A. (1974) : Brit. J. Haemat., in the press.

Greenberg, M.S., and Grace, N.D. (1970) : Arch. intern. Med., 125, 140.

Greig, H.B.W. (1959) : S. Afr. med. J., 33, 869.

Griffiths, D.A.T. (1972) : Ann. Soc. belge Méd. trop., 52, 215.

Hall, M.H. (1972a) : J. Obstet. Gynaec. Brit. Cwlth., 79, 159.

Hall, M.H. (1972b) : J. Obstet. Gynaec. Brit. Cwlth, 79, 222.

Hallberg, L., Hogdahl, A.M., Nilsson, L., and Rybo, G. (1966) : Acta obstet. gynec. scand., 45, 320.

Hansen, H.A., Nordqvist, P., and Sourander, P. (1964) : Acta med. scand., 176, 243.

Hansen, H., and Rybo, G. (1967) : Acta obstet. gynec. scand., 46, suppl. 7, 107.

Hardinge, M.G., and Crooks, H. (1961) : J. Amer. diet. Ass., 38, 240.

Hegsted, D.M. (1972) : Ecol. Food Nutr., 1, 255.

Hellström, L. (1971) : Lancet, i, 59.

Hepner, G.W., Booth, C.C., Cowan, J., Hoffbrand, A.V., and Mollin, D.L. (1968) : Lancet, ii, 302.

Herbert, V. (1961) : J. clin. Invest., 40, 81.

Herbert, V. (1962a) : Trans. Ass. Amer. Phycns, 75, 307.

Herbert, V. (1962b) : Arch. intern. Med., 110, 649.

Herbert, V. (1963a) : Amer. J. clin. Nutr., 12, 17.

Herbert, V. (1963b) : New Engl. J. Med., 268, 201.

Herbert, V. (1965) : in The Pharmacological Basis of Therapeutics; ed. L.S. Goodman and A. Gilman. New York, MacMillan.

Herbert V. (1968a) : Amer. J. clin. Nutr., 21, 743.

Herbert, V. (1968b) : Amer. J. clin. Nutr., 21, 1115.

Herbert, V. (1972a) : in Lectures, XIV International Congress of Haematology. São Paulo, Brazil.

Herbert, V. (1972b) : J. Amer. med. Ass., 222, 834.

Herbert, V., and Zalusky, R. (1961) : Fed. Proc., 20, 453.

Herbert, V., and Zalusky, R. (1962) : J. clin. Invest., 41, 1263.

Herbert, V., Spivack, M., Ocasio, E., Kimmel, K., Ghanta, V., Brenner, L., Freundlich, J., and Scott, J. (1973) : Clin. Res., 21, 538.

Hibbard, B. (1964) : J. Obstet. Gynaec. Brit. Cwlth, 71, 529.

Hibbard, B.M., and Hibbard, E.D. (1963) : Brit. med J. ii, 1430.

Hibbard, B.M., Hibbard, E.D., and Jeffcoate, T.N.A. (1965) : Acta obstet. gynec.scand., 44, 375.

Hibbard, B.M. and Jeffcoate, T.N.A. (1966) : Obstet. and Gynec., 27, 155.

Hibbard, B.M., Hibbard, E.D., Hwa, T.S., and Tan, P. (1969) : J. Obstet. Gynaec. Brit. Cwlth, 76, 1003.

Hibbard, B.M., and Hibbard, E.D. (1972) : J. Obstet. Gynaec. Brit. Cwlth, 79, 584.

Hibbard, E.D., and Smithells, R.W. (1965) : Lancet, i, 1254.

Hoel, P.G. (1963) : Introduction to Mathematical Statistics; 3rd ed. New York, Wiley.

Hoffbrand, A.V. (1970) : Arch. Dis. Child., 45, 441.

Hoffbrand, A.V. (1972) : J. clin. Path., 24, Suppl, 5, 66.

Hoffbrand, A.V., Newcombe, B.F.A., and Mollin, D.L. (1966) : J. clin. Path., 19, 17.

Hoffbrand, A.V., and Necheles, T.F. (1968) : Lancet, ii, 528.

Hoffbrand, A.V., and Peters, T.J. (1970) : Schweiz. med. Wschr., 100, 1954.

Horrell, M., Horner, D., Kane-Berman, J.S., and Margo, R.F. (1973) : A survey of race relations in South Africa 1972. Johannesburg, South African Institute for Race Relations.

Horrell, M., Horner, D., and Gordon, S. (1974) : A survey of race relations in South Africa 1973. Johannesburg, South African Institute for Race Relations.

Horwitz, S.J., Klipstein, F.A., and Lovelace, R.E. (1968) : Lancet, i, 563.

Hourihane, B., Coyle, C.V., and Drury, M.I. (1960) : J. Irish med. Ass., 47, 1.

Hunter, R., Barnes, J., Oakely, H.F., and Matthews, D.M. (1970) : Lancet, i, 61.

Hurdle, A.D.F. (1967) : M.D. thesis, University of London. Quoted by Chanarin (1969).

Hurdle, A.D.F., Barton, D., and Searles, I.H. (1968) : Amer. J. clin. Nutr., 21, 1202.

Hussain, O.A.N., Rothman, D., and Ellis, L. (1963) : J. Obstet. Gynaec. Brit. Cwlth, 70, 821.

Jelliffe, D.B.J. (1967) : J. trop. Pediat., 13, 46.

Jelliffe, D.B.J., and Jelliffe, E.F.P. (1972) : J. Pediat., 81, 829.

Jensen, O.N., and Olesen, O.V. (1970) : Arch. Neurol., 22, 181.

Johnson, E.M. (1964) : Anat. Rec., 149, 49.

Johnson, E.M., Nelson, M.M., and Monie, I.W. (1963) : Anat. Rec. 146, 215.

Kahn, S.B., Fein, S., Rigberg, S., and Brodsky, I. (1970) : Amer. J. Obstet. Gynec., 108, 931.

Kamel, K., Waslien, C.I., El-Ramly, Z., Guindy, S., Mourad, K.A., Khattab, A-K., Hashem, N., Patwardhan, V.N., and Darby, W.J. (1972) : Amer. J. clin. Nutr., 25, 152.

Karthigaini, S., Gnanasundaram, D., and Baker, S.J. (1964) : J. Obstet. Gynaec. Brit. Cwlth, 71, 115.

Killander, A. (1958) : Acta. haemat. (Basel), 19, 9.

Knowles, J.P., Shuster, S., Wells, G.C. (1963) : Lancet, i, 1138.

Lau K-S., Gottlieb, C., Wasserman, L.R., and Herbert, V. (1965) : Blood, 26, 202.

Leevy, C.M., Cardi, L., Frank, O., Gellene, R., and Baker, H. (1965) : Amer. J. clin. Nutr., 17, 259.

Lesser, A.J. (1968) : Obstet. and Gynaec., 29, 295.

Lien-Keng, K., and Odang, O. (1959) : Amer. J. Dis. Child., 97, 209.

Louw, M.E.J., du Plessïs, J.P., van den Berg, A.S., and Fellingham, S.A. (1969) : S. Afr. med. J., 43, 1516.

Lowenstein, L., Pick, C., and Philpott, N. (1955) : Amer. J. Obstet. Gynec., 70, 1309.

Lowenstein, L., Brunton, L., and Hsieh, Y-S. (1966) : Canad. med. Ass. J., 94, 636.

Luhby, A.L., Feldman, R., Salerno, L.J., and Cooperman, J.M. (1963) : Amer. J. clin. Nutr., 12, 332.

Lund, C.J. (1951) : Amer. J. Obstet. Gynec., 62, 947.

MacIver, J.E., and Back, E.H. (1960) : Arch. Dis. Childh., 35, 134.

MacKenzie, A., and Abbot, J. (1960) : Brit. med. J., ii, 1114.

Maize Board (1973) : Report on Maize for submission to the Minister of Agriculture, Pretoria, Maize Board.

Mangelsdorf, P.C. (1958) : Encyclopaedia Britannica, 14, 696.

Mannheimer, E., Pakesh, F., Reimer, E.E., and Vetter, H. (1952) : Med. Klin., 47, 1397.

Marshall, R.A., and Jandl, J.H. (1960) : Arch. intern. Med., 105, 352.

Martin, J.D., Davis, R.E., and Hähnel, R. (1963) : Lancet, ii, 716.

Martin, J.D., Davis, R.E., and Hähnel, R. (1964) : Lancet, i, 1075.

Martin, R.H., Harper, T.A., and Kelso, W. (1965) : Lancet, i, 670.

Martin, J.D., Davis, R.E., and Stenhouse, N. (1967) : J. Obstet. Gynaec. Brit. Cwlth., 74, 697.

Mati, J.K.G., Hatimy, A., and Gebbie, D.A.M. (1971) : J. trop. Med. Hyg., 74, 1.

Matoth, V., Pinkas, A., Zamir, R., Mooallem, F., and Grossowicz, N. (1964) : Pediatrics, 33, 507.

Mattson, R.H., Gallagher, B.B., Reynolds, E.H., and Glass, D. (1973) : Arch. Neurol., 29, 78.

Mayer, J. (1972) : in Human Nutrition, 274. Springfield, Illinois, Charles C. Thomas.

McKay, D.G., and Wong, T.C. (1962) : J. exp. Med., 115, 117.

McKay, D.G., Goldenberg, V., Kaunitz, H., and Csavossy, I. (1967) : Arch. Path., 84, 557.

McLean, F.W., Heine, M.W., Held, B., and Streiff, R.R. (1969) : Amer. J. Obstet. Gynec., 104, 745.

Menon, M.K., Sengupta, M., and Ramaswamy, N. (1966) : J. Obstet. Gynaec. Brit. Cwlth, 73, 49.

Metz, J. (1959) : An investigation of some aspects of anaemia and thrombocytopaenia in the South African Bantu. M.D. thesis, University of the Witwatersrand, Johannesburg.

Metz, J. (1966) : in The Proceedings of a Symposium on Folic Acid. Middlesex, Glaxo Laboratories.

Metz, J. (1970) : Amer. J. clin. Nutr., 23, 843.

Metz, J., Stevens, K., Krawitz, S., and Brandt, V. (1961) : J. clin. Path., 14, 622.

Metz, J., Brandt, V., and Stevens, K. (1962a) : Brit. med. J., i, 24.

Metz, J., Stevens, K., and Brandt, V. (1962b) : Brit. med. J. ii, 1440.

Metz, J., Stevens, K., Edelstein, T., Brandt, V., and Baumslag, N. (1963) : S. Afr. J. med. Sci., 28, 74.

Metz, J., Festenstein, H., and Welch, P. (1965) : Amer. J. clin. Nutr., 16, 472.

Metz, J., and Hackland, P. (1968) : Congress South African Society for Haematology. Cape Town.

Metz, J., Lurie, A., and Konidaris, M. (1970) : S. Afr. med. J., 44, 539.

Miller, R.G. (1966) : Simultaneous Statistical Inference. New York, McGraw-Hill.

Minot, G.R., Murphy, W.P., and Stetson, R.P. (1928) : Amer. J. med. Sci., 175, 581.

Minot, G.R., and Castle, W.B. (1935) : Lancet, ii, 319.

Monie, I.W., and Nelson, M.M. (1963) : Anat. Rec., 147, 397.

Moore, C.V., Vilter, R., Minnich, V. and Spies, T.D. (1944) : J. Lab. clin. Med., 29, 1226.

Moore, C.V., Bierbaum, O.S., Welch, A.D., and Wright, L.D. (1945) : J. Lab. clin. med., 30, 1056.

Moore, J.R., and Ball, E.W. (1972) : Arch. Dis. Childh., 47, 309.

National Academy of Sciences - National Research Council (1968) : Recommended Dietary Allowances, Revised 1968. Publ. No. 1964, Washington D.C.

National Research Council (1944) : The facts about enrichment of flour and bread. National Research Council. Washington D.C.

National Research Council (1950) : Flour and bread enrichment, 1949-50. National Research Council. Washington D.C.

Nelson, J.D., and Haltalin, K.C. (1972) : Amer. J. clin. Nutr., 25, 992.

Nelson, M.M., Asling, C.W., and Evans, H.M. (1952) : J. Nutr., 48, 61.

Neubauer, C. (1970) : Brit. med. J. ii, 759.

Newmark, P.A., Green, R., Musso, A.M., and Mollin, D.L. (1973) : Brit. J. Haemat., 25, 359.

Nomenclature (1973) : J. Nutr., 103, 159.

Osler, W. (1919) : Brit. med. J., i, 1.

Page, E.W. (1972) : J. Obstet. Gynaec. Brit. Cwlth., 79, 883.

Paton, A. (1969) : Lancet, i, 418.

Pereira, S.M. and Baker, S.J. (1966) : Amer. J. clin. Nutr., 18, 413.

Perry, J. (1971) : Brit. J. Haemat., 21, 435.

Perry, J., and Chanarin, I. (1968) : Brit. med. J., 4, 546.

Perry, J., and Chanarin, I. (1972) : Gut 13, 544.

Pfiffner, J.J., Binkley, S.B., Bloom, E.S., Brown, R.A., Bird, O.D., and Emmett, A.D. (1943) : Science, 97 404.

Pincus, J.H., Reynolds, E.H., and Glaser, G.H. (1972) : J. Amer. med. Ass., 221, 496.

Popkin, B., and Lidman, R. (1972) : Amer. J. clin. Nutr., 25, 331.

Potgieter, J.F. (1973) : The Poverty Datum Line in the major urban centres of the Republic. Institute for Planning Research, University of Port Elizabeth, Research Report No. 12. Port Elizabeth.

Pritchard, J.A., Whalley, P.J., and Scott, D.E. (1969): Amer. J. Obstet. Gynec., 104, 388.

Pritchard, J.A., Scott, D.E., Whalley, P.J., and Haling, R.F. (1970) : J. Amer. Med. Ass., 211, 1982.

Pritchard, J.A., Scott, D.F., and Whalley, P.J. (1971) : Amer. J. Obstet. Gynec., 109, 341.

Quin, P.J. (1959) : Foods and feeding habits of the Pedi. Johannesburg. Witwatersrand University Press.

Rae, P.G., and Robb, P.M. (1970) : J. clin. Path., 23, 379.

Ralston, A.J., Snaith, R.P., and Hirley, J.B. (1970) : Lancet, i, 867.

Raymond, General E. (1972) : S. Afr. med. J., 46, 1561.

Retief, F.P. (1969) : Amer. J. clin. Nutr., 22, 352.

Reynolds, E.H. (1967) : Lancet, i, 1086.

Reynolds, E.H. (1973) : Lancet, i, 1376.

Reynolds, E.H., Rothfeld, P., and Pincus, J.H. (1973) : Brit. med. J., ii, 398.

Richens, A. (1971) : Lancet, i, 912.

Roche (1963) : The present status of flour and bread enrichment. Basle. F. Hoffmann-La Roche.

Rose, J.A. (1971) : Lancet, ii, 453.

Salter, A.J. (1971) : Lancet, i, 192.

Samuel, P.D., Burland, W.L., and Simpson, K. (1973) : Brit. J. Nutr., 30, 165.

Schoeneck, F.J. (1964) : N.Y. St. J. Med., 64, 557.

Schonfield, J., Schmidt, W.M., and Sternfeld, L. (1962) : J. Pediat., 61, 430.

Schwartz, S.O., Kaplan, S.R., and Armstrong, B.E. (1950) : J. Lab. clin. Med., 35, 894.

Schweigert, B.S., Pollard, A.E., and Elvehjem, C.A. (1946) : Archs. Biochem. Biophys., 10, 107.

Scott, D.E., Whalley, P.J., and Pritchard, J.A. (1970) : Obstet. and Gynec., 36, 26.

Shapiro, J., Alberts, H.W., Welch, P., and Metz, J. (1965) : Brit. J. Haemat., 11, 498.

Shnier, M.H., and Metz, J. (1959) : S. Afr. med. J., 33, 1009.

Shojania, A.M., and Gross, S. (1964) : J. Pediat., 64, 323.

Shojania, A.M., Hornady, G. and Barnes, P.H. (1968) : Lancet, i, 1376.

Shojania, A.M., Hornady, G., Barnes, P.H. (1969) : Lancet, i, 886.

Shum, H-Y, O'Neill, B.J., and Streeter, A.M. (1971) : Pathology, 3, 311.

Smith, J.L., and Unglaub, W.G. (1972) : J. agr. food Chem., 20, 526.

South Africa. Department of Statistics (1951) : Population Census 8 May 1951. Pretoria, Government Printer.

South Africa. Department of Statistics (1970) : Population Census 6 May 1970. Pretoria, Government Printer.

South Africa. Department of Statistics (1973) : Statistical News Release, 2, Pretoria, Government Printer.

South Africa. Trigonometrical survey (1955) : 1:50,000 sheet of Nqutu. 2830 BA. Pretoria, Government Printer.

Spector, I., and Metz, J. (1966) : Brit. J. Haemat., 12, 737.

Spector, R.G. (1972) : Nature New Biology, 240, 247.

Spray, G.H. (1968) : Lancet, ii, 110.

Stamler, F.W. (1959) : Amer. J. Path., 35, 1207.

Stamp, Lord (1947) : J. gen. Microbiol., 1, 251.

Stempak, J.G. (1965) : Anat. Rec., 151, 287.

Stephens, M.E.M., Craft, I., Peters, T.J., and Hoffbrand, A.V. (1972) : Clin. Sci., 42, 405.

Stevens, K. (1964) : The demonstration of folic acid deficiency in the megaloblastic anaemia associated with pregnancy in the South African Bantu. M.D. Thesis. University of the Witwatersrand, Johannesburg.

Stevens, K., and Metz, J. (1964) : Trans. roy. Soc. trop. Med. Hyg., 58, 510.

Stokstad, E.L.R. (1943) : J. biol. Chem., 149, 573.

Stokstad, E.L.R., Fordham, D., and Grunigen, A. (1947) : J. biol. Chem., 167, 877.

Stone, M.L., Luhby, A., Feldman, R., Gordon, M., and Cooperman, J.M. (1967) : Amer. J. Obstet. Gynec., 99, 638.

Streiff, R.R. (1969) : Clin. Res., 17, 345.

Streiff, R.R. (1970) : J. Amer. m. l. Ass., 214, 105.

Streiff, R.R. (1971a) : Semin. Haemat., 7, 23.

Streiff, R.R. (1971b) : Amer. J. clin. Nutr., 24, 1390.

Streiff, R.R., and Little, A.B. (1965) : J. clin. Invest., 44, 1102.

Streiff, R.R., and Little, A.B. (1967) : New Eng. J. Med., 276, 776.

Streiff, R.K., and Rosenberg, I.H. (1967) : J. clin. Invest., 46, 1121.

Strelling, M.K., Blackledge, G.D., Goodall, H.B., and Walker, C.H.M. (1966) : Lancet, i, 898.

Sturgis, C.C., and Isaacs, R. (1938) : in Handbook of Haematology, ed. H. Downey, 3, 2231, London, Hamish Hamilton.

Sullivan, L.W., Luhby, A.L., and Streiff, R.R. (1966) : Amer. J. clin. Nutr. 18, 311.

Tamura, T., and Stokstad, E.L.R. (1973) : Brit. J. Haemat., 25, 513.

Tan, C.L., and Ong, Y.W. (1972) : Singapore med. J., 13, 208.

Tantengco, V.O., Santos, F., Monzon, A.M., Castro, C.R. de, and Cabrera, E.G. (1971) : J. Philipp. med. Ass., 47, 337.

Temperley, I.J., Meehan, M.J.M., and Gatenby, P.B.B. (1968) : Brit. J. Haemat., 14, 13.

Tepley, L.J., and Elvehjem, C.A. (1945) : J. biol. Chem., 157, 303.

Thambu, J., and Llewellyn-Jones, D. (1966) : J. Obstet. Gynaec. Brit. Cwlth, 73, 930.

Thiersch, J.B. (1952) : Amer. J. Obstet. Gynec., 63, 1298.

Thompson, R.B. (1957) : Lancet, i, 1171.

Tobias, P.V. (1971) : S. Afr. J. Sci., 67, 517.

Toepfer, E.W., Zook, E.G., Orr, M.L., and Richardson, L.R. (1951) : Folic acid content of foods. Agriculture Handbook No. 29. U.S. Department of Agriculture, Washington D.C.

Trier, J.S. (1962) : Gastroenterology, 42, 295.

Turchetti, L.C., Combrink, B., Krawitz, S., and Metz, J. (1966) : Amer. J. clin. Nutr., 18, 249.

Van Breda, W.W., and Langenhoven, H.P. (1972) : The Utilisation of Non-White Labour. Bloemfontein, University of the Orange Free State.

Vanier, T.M., and Tyas, J.F. (1967) : Arch Dis. Childh., 42, 57.

Varadi, S., and Elwis, A. (1964) : Lancet, i, 1162.

Varadi, S., Abbot, D., and Elwis, A. (1966) : J. clin. Path., 19, 33.

Veeger, W., ten Thije, O.J., Hellemans, N., Mandema, E., and Nieweg, H.O. (1965) : Acta. med. scand., 177, 493.

Velez, H., Ghitis, J., Pradilla, A. and Vitale, J.J. (1963) : Amer. J. clin. Nutr., 12, 54.

Vilter, R.W., Horrigan, D., Mueller, J.F., Jarrold, T., Vilter, C.F., Hawkins, V., and Seaman, A. (1950) : Blood, 5, 695.

Walt, F., Holman, S., and Hendrickse, R.G. (1956) : Brit. med. J., i, 1199.

Waters, A.H., and Mollin, D.L. (1961) : J. clin. Path., 14, 335.

Weckman, N., and Lehtovaara, R. (1969) : Lancet, i, 207.

Wells, D.G., Langman, M.J.S., and Wilson, J. (1972) : Brit. med. J., 4, 585.

Whalley, P.J., Scott, D.E., and Pritchard, J.A. (1969) : Amer. J. Obstet. Gynec., 105, 670.

Whiteside, M.G., Ungar, B., and Cowling, D.C. (1968) : Med. J. Austr., 1, 338.

Will, J.J., Mueller, J.F., Brodine, C., Kiely, C.E., Friedman, B., Hawkins, V.R., Dutra, J., and Vilter, R.W. (1959) : J. Lab. clin. Med., 53, 22.

Willoughby, M.L.N. (1967) : Brit. J. Haemat., 13, 503.

Willoughby, M.L.N., and Jewell, F.J. (1966) : Brit. med. J., ii, 1568.

Wills, L. (1931) : Brit. med. J. i, 1059.

Wills, L., and Mehta, M.M. (1930) : Indian J. med. Res., 17, 777.

Wilson, J., and Langman, M.J.S. (1966) : Nature, 212, 787.

Wilson, M., and Thompson, L. (1969) : The Oxford History of South Africa, I. Oxford, Clarendon Press.

Woodward, J.C., and Newberne, P.M. (1967) : J. Embryol. exp. Morph., 17, 177.

Yusufji, D., Mathan, V.I., and Baker, S.J. (1973) : Bull. Wld. Hlth. Org., 48, 15.

Zalusky, R., and Herbert, V. (1961) : New Engl. J. Med., 265, 1033.

Zuelzer, W.W., and Ogden, F.N. (1946) : Amer. J. Dis. Child., 71, 211.

A P P E N D . X

I Details of 144 pregnant women studied at Nqutu (group VI)

Results of investigations performed when subjects were first seen.

A - age (years).

B - parity.

C - monthly maize consumption per adult in family (kg).

D - monthly cash income per adult in family (Rands).

E - serum vitamin B_{12} concentration (pg per ml).

F - serum folate concentration (ng per ml).

G - red cell folate concentration (ng per ml).

H - haemoglobin concentration (g per 100 ml).

I - mean cell volume (μ^3).

A	B	C	D	F	G	H	I
25.	4.	7.69	2.31	3.70	80.	12.9	98.
18.	0.	11.11	4.44	7.40	171.	15.8	101.
18.	0.	6.60	2.00	13.00	369.	12.7	98.
26.	2.	10.00	2.40	3.70	108.	11.1	97.
16.	1.	8.33	2.50	5.50	141.	11.9	92.
36.	7.	13.33	1.07	5.20	221.	12.0	91.
29.	2.	4.40	3.33	4.40	247.	12.2	83.
32.	3.	10.00	4.00	6.80	150.	12.9	102.
30.	0.	12.00	.91	6.80	321.	13.5	96.
21.	2.	16.67	3.33	5.20	197.	11.7	99.
17.	0.	5.33	2.67	3.80	135.	13.1	96.
18.	0.	12.00	2.73	3.10	147.	12.6	94.
24.	2.	14.55	1.82	2.20	152.	11.8	99.
22.	0.	12.50	2.50	3.40	211.	11.8	94.
28.	6.	16.67	3.33	1.80	253.	12.1	104.
24.	2.	16.67	1.67	4.00	111.	11.3	93.
26.	2.	9.09	1.82	5.60	210.	11.5	99.
36.	3.	2.18	3.64	8.00	263.	13.0	95.
30.	5.	16.67	0.00	7.30	266.	12.7	90.
22.	2.	10.00	3.60	4.30	137.	12.0	95.
16.	0.	10.00	2.00	4.50	115.	14.1	95.
22.	1.	22.22	4.44	4.40	217.	11.6	97.
34.	6.	14.67	3.11	5.50	219.	11.2	100.
23.	2.	10.00	4.00	7.20	326.	14.8	91.
26.	2.	9.09	1.82	4.80	176.	13.1	97.
21.	1.	15.38	1.54	4.80	157.	12.3	95.
22.	3.	12.00	1.82	6.30	217.	11.9	95.
28.	4.	20.00	10.00	3.20	173.	11.9	99.
27.	0.	8.33	2.00	2.90	196.	12.1	91.
19.	0.	12.50	1.25	6.40	144.	13.0	94.
18.	1.	10.15	2.31	4.80	263.	13.2	102.
23.	2.	25.00	2.50	4.00	107.	11.7	109.
24.	2.	13.20	4.00	4.60	238.	12.6	97.
30.	3.	20.00	5.00	3.70	234.	13.0	97.
23.	2.	16.50	2.50	4.60	103.	11.9	99.
24.	0.	14.67	1.33	2.70	187.	14.4	97.
28.	1.	26.67	8.00	4.10	182.	14.5	97.
27.	2.	11.00	1.67	4.90	315.	12.3	92.

A	B	C	D	E	F	G	H	I
20.	0.	13.330	5.000	529.	2.7	246.	14.0	97.
45.	5.	6.286	2.857	549.	2.0	120.	12.2	93.
40.	13.	10.000	2.500	1319.	1.6	84.	11.9	110.
22.	0.	9.091	3.636	614.	3.2	90.	13.0	93.
27.	2.	11.110	2.222	529.	3.6	135.	12.2	89.
28.	3.	20.000	8.000	482.	2.6	134.	11.9	106.
23.	4.	11.000	2.000	594.	3.9	269.	12.2	94.
18.	0.	8.333	3.333	584.	4.3	162.	11.1	94.
17.	0.	8.333	3.333	827.	3.2	276.	12.0	90.
18.	0.	10.000	2.000	547.	4.4	238.	11.1	84.
25.	5.	12.500	5.000	550.	3.6	149.	12.0	98.
22.	0.	12.500	5.000	573.	4.4	179.	10.8	99.
20.	0.	11.000	3.333	732.	6.6	119.	14.1	93.
20.	1.	14.290	2.857	593.	7.0	234.	12.0	85.
20.	0.	6.250	5.000	1070.	4.4	271.	12.9	94.
19.	0.	4.000	3.636	647.	2.2	75.	13.6	98.
23.	0.	9.429	2.857	440.	5.3	149.	12.2	95.
20.	0.	14.290	1.714	552.	16.1	479.	12.3	95.
19.	0.	13.330	2.667	909.	3.4	162.	11.5	97.
25.	0.	14.290	5.714	611.	4.8	405.	12.2	93.
19.	0.	8.333	3.333	806.	2.5	155.	12.1	91.
19.	0.	10.000	4.000	621.	6.0	349.	12.5	92.
17.	0.	10.000	4.800	525.	4.8	229.	12.2	94.
18.	0.	11.110	2.222	702.	5.1	128.	12.7	93.
29.	4.	9.091	1.818	566.	10.2	404.	11.9	99.
22.	2.	12.500	5.000	685.	5.9	313.	13.5	92.
20.	0.	10.000	4.000	535.	5.2	166.	13.1	100.
23.	0.	9.429	1.429	572.	2.3	163.	14.3	94.
30.	5.	11.000	3.333	471.	2.4	202.	12.8	100.
20.	0.	7.692	1.538	524.	5.1	200.	13.8	90.
17.	0.	8.333	3.333	593.	3.7	151.	13.7	94.
21.	0.	18.180	3.636	582.	3.3	169.	13.3	93.
22.	0.	11.760	1.176	404.	3.4	113.	13.9	97.
21.	1.	16.500	5.000	527.	4.8	148.	12.6	94.
18.	0.	12.500	5.000	571.	5.4	210.	12.7	91.
17.	0.	7.692	3.077	552.	4.0	125.	12.1	93.
17.	0.	13.200	8.000	433.	5.2	177.	12.7	84.
20.	0.	14.290	2.857	608.	5.7	73.	13.1	95.
20.	1.	7.143	2.857	606.	4.0	183.	12.3	72.
38.	1.	9.429	5.714	872.	2.7	146.	12.4	91.
18.	0.	12.500	5.000	454.	4.2	130.	12.3	83.
30.	5.	7.765	2.353	790.	6.0	235.	10.4	97.
24.	1.	10.000	3.000	688.	6.2	305.	12.4	96.
23.	0.	8.800	1.333	823.	2.4	103.	12.3	93.
27.	5.	11.110	4.444	1023.	3.2	266.	9.7	95.
20.	3.	8.800	2.667	641.	4.3	247.	12.3	93.
20.	2.	9.091	3.636	745.	7.4	312.	10.8	89.
23.	0.	16.500	2.500	522.	5.6	224.	13.3	90.
39.	7.	16.500	2.500	415.	4.2	229.	13.1	91.
25.	1.	8.333	1.667	654.	8.9	265.	13.6	92.
21.	0.	13.200	4.000	745.	8.4	353.	12.7	98.
19.	0.	20.000	2.000	1006.	2.7	111.	12.6	86.
21.	0.	18.860	5.714	793.	5.1	263.	10.9	94.
21.	1.	8.250	5.000	858.	2.2	285.	12.2	91.

A	B	C	D	E	F	G	H	I
33.	7.	11.110	2.222	996.	4.0	147.	14.1	102.
30.	2.	12.500	2.500	599.	3.4	235.	12.1	92.
32.	3.	12.500	5.000	485.	3.0	100.	9.9	90.
22.	0.	16.670	3.333	836.	5.3	203.	13.3	89.
26.	0.	10.000	2.000	872.	5.0	170.	12.0	97.
18.	0.	14.290	5.714	514.	4.3	104.	12.3	95.
19.	0.	13.330	2.667	598.	4.8	396.	12.2	92.
19.	0.	12.500	5.000	729.	3.4	110.	12.6	92.
21.	0.	7.692	3.077	1055.	7.1	143.	12.5	94.
19.	0.	8.333	3.333	468.	2.0	106.	12.1	99.
24.	2.	10.000	4.000	502.	4.1	126.	12.2	95.
22.	0.	12.500	2.500	468.	3.4	160.	13.2	97.
16.	0.	5.882	1.765	460.	2.8	92.	12.4	94.
18.	1.	10.000	2.000	1028.	1.6	136.	12.1	101.
20.	0.	15.380	1.538	782.	3.0	151.	12.0	91.
26.	1.	14.290	2.857	516.	4.6	446.	12.2	94.
22.	0.	8.333	4.000	543.	6.2	231.	12.5	96.
18.	0.	10.000	4.000	394.	7.4	348.	11.3	85.
16.	0.	18.180	3.636	898.	3.6	384.	12.9	99.
25.	0.	14.290	2.857	531.	9.0	240.	12.6	94.
19.	0.	9.091	3.636	1122.	6.8	235.	12.8	92.
20.	0.	6.667	2.667	715.	4.7	245.	11.5	71.
17.	0.	8.333	3.333	851.	3.9	152.	13.4	94.
29.	1.	9.429	2.857	472.	2.6	123.	11.5	99.
25.	2.	16.670	10.000	885.	3.0	133.	11.9	90.
22.	0.	16.670	6.667	1193.	3.5	187.	13.4	89.
24.	1.	12.500	3.500	729.	2.5	218.	13.5	91.
27.	0.	14.290	2.857	731.	2.8	138.	13.2	97.
30.	1.	12.500	5.000	926.	4.5	253.	11.9	98.
31.	6.	16.670	3.333	516.	4.3	270.	11.0	86.
43.	6.	12.500	2.500	668.	2.8	152.	12.0	90.
23.	1.	20.000	5.000	1012.	2.0	146.	11.4	104.
25.	2.	12.500	2.500	716.	2.2	102.	13.8	103.
26.	1.	4.800	2.000	1077.	1.8	126.	16.3	102.
17.	0.	5.556	3.111	882.	3.0	118.	12.7	95.
28.	1.	8.800	2.667	633.	4.0	204.	14.9	95.
20.	1.	10.000	2.000	1302.	6.4	409.	12.3	91.
32.	5.	11.000	2.000	610.	5.6	177.	11.6	87.
19.	0.	16.670	5.333	524.	1.4	128.	12.8	92.
20.	0.	11.110	4.444	857.	5.0	310.	12.5	93.
26.	1.	7.692	2.308	733.	2.6	120.	13.0	94.
19.	0.	14.290	1.429	1283.	6.8	366.	13.5	90.
21.	0.	7.692	2.308	595.	6.1	183.	12.2	93.
23.	2.	20.000	6.000	732.	1.9	100.	11.2	102.
30.	6.	13.200	2.000	1146.	1.8	183.	12.7	91.
23.	0.	10.000	2.000	1324.	2.6	155.	13.0	103.
25.	0.	11.000	1.667	476.	4.0	147.	13.1	93.
18.	0.	11.110	4.444	1035.	2.2	169.	13.7	88.
27.	2.	9.429	2.857	865.	1.4	133.	13.2	89.
22.	0.	16.670	5.000	626.	2.4	131.	10.3	91.
23.	1.	14.290	2.857	717.	2.2	98.	12.1	98.
25.	1.	14.290	5.714	894.	2.6	111.	11.1	91.

II Details of 185 non-pregnant women studied at Nqutu (group VII)

A - age (years).

B - parity.

C - monthly maize meal consumption per adult in family (kg).

D - monthly cash income per adult in family (Rands).

E - serum vitamin B_{12} concentration (pg per ml).

F - red cell folate concentration (ng per ml).

G - haemoglobin concentration (g per 100 ml).

H - mean cell volume ($/u^3$).

I - years since last pregnant or lactating.

A	B	C	D	E	F	G	H	I
17.	0.	6.250	2.500	1092.	172.	14.0	87.	
41.	4.	4.167	1.667	1144.	379.	13.4	92.	6.
29.	2.	6.250	1.250	1010.	150.	13.1	93.	1.
18.	0.	14.330	6.667	748.	302.	14.0	96.	
20.	0.	5.000	2.000	1812.	329.	15.4	96.	
19.	2.	6.000	5.000	939.	185.	13.4	89.	1.
28.	1.	9.429	2.857	735.	333.	12.1	83.	2.
20.	3.	11.760	1.176	2031.	160.	14.0	99.	2.
25.	3.	25.000	2.500	512.	390.	12.9	92.	2.
40.	7.	9.091	.909	1160.	400.	15.0	93.	1.
21.	2.	7.765	4.706	1056.	131.	13.9	94.	1.
63.	6.	15.000	4.000	800.	306.	15.7	86.	25.
43.	7.	15.000	4.000	840.	379.	13.0	94.	13.
67.	5.	9.091	2.727	1196.	511.	12.0	94.	35.
45.	10.	20.000	2.000	1382.	256.	12.7	94.	8.
35.	4.	17.270	1.818	781.	210.	13.8	100.	1.
29.	6.	13.330	1.600	975.	126.	11.1	96.	1.
61.	7.	18.180	1.818	810.	156.	14.9	91.	19.
22.	2.	7.500	1.300	950.	165.	12.8	95.	2.
60.	8.	13.200	3.200	770.	300.	13.4	98.	20.
24.	4.	12.000	3.636	857.	74.	12.5	83.	1.
33.	8.	10.000	4.000	1015.	46.	12.6	92.	1.
50.	8.	16.670	3.333	938.	259.	13.2	87.	4.
22.	5.	10.000	3.000	833.	118.	12.1	87.	1.
19.	6.	11.110	1.111	797.	223.	12.3	82.	1.
20.	8.	12.000	1.818	2037.	220.	14.2	88.	1.
32.	6.	10.000	2.000	1478.	185.	13.8	89.	1.
24.	2.	28.570	1.429	899.	327.	14.9	96.	1.
67.	6.	7.143	.714	1682.	351.	14.7	94.	23.
36.	3.	12.500	2.000	1003.	139.	13.2	89.	13.
16.	0.	16.670	1.667	1230.	351.	14.2	90.	
19.	0.	12.500	1.875	1220.	320.	14.6	86.	
21.	1.	12.500	2.500	799.	81.	14.9	95.	1.
17.	1.	8.000	2.000	520.	130.	13.9	91.	1.
42.	4.	10.910	1.818	1189.	330.	15.9	93.	19.
33.	5.	8.250	3.000	561.	111.	14.7	90.	9.
46.	6.	9.524	1.429	917.	477.	15.2	86.	23.
33.	4.	6.316	2.632	998.	286.	14.4	86.	1.
23.	1.	9.091	3.636	648.	31.	11.9	95.	1.
43.	5.	14.290	2.857	1073.	335.	14.4	95.	9.
43.	4.	14.290	2.857	474.	232.	14.1	90.	2.
54.	3.	12.000	2.000	1286.	220.	13.2	88.	28.
23.	2.	14.290	1.429	1035.	155.	15.5	90.	1.
51.	9.	3.125	2.500	1081.	312.	13.9	91.	12.
17.	0.	6.250	1.250	1032.	335.	14.5	91.	
20.	1.	10.000	2.000	1156.	315.	15.4	88.	1.
32.	6.	12.000	4.000	929.	154.	14.0	92.	1.
19.	1.	12.500	5.000	506.	61.	13.0	97.	1.
30.	3.	14.290	2.857	571.	160.	13.0	88.	2.
18.	0.	9.091	3.636	1228.	232.	12.9	88.	
22.	3.	5.000	1.200	1298.	129.	13.9	85.	1.
35.	5.	15.380	1.538	1004.	102.	14.6	89.	2.
21.	1.	10.000	4.000	908.	249.	13.0	91.	3.
36.	5.	11.110	3.333	363.	446.	11.7	91.	3.

A	B	C	D	E	F	G	H	I
19.	1.	10.000	4.000	1140.	97.	13.1	88.	2.
30.	6.	6.000	5.455	1271.	216.	13.1	87.	2.
22.	1.	10.000	2.000	473.	211.	12.7	95.	3.
37.	4.	12.500	2.500	777.	154.	14.7	91.	1.
21.	2.	8.250	2.500	1428.	92.	13.2	86.	2.
44.	4.	8.333	1.667	992.	191.	13.5	86.	12.
20.	2.	7.143	4.286	784.	140.	13.6	102.	1.
67.	4.	8.333	3.333	980.	95.	14.2	89.	21.
53.	2.	6.250	2.250	737.	335.	15.3	95.	25.
45.	8.	7.143	2.857	742.	267.	12.8	94.	2.
45.	4.	9.091	3.636	706.	147.	11.7	89.	6.
45.	6.	3.571	1.571	626.	277.	12.9	86.	17.
21.	0.	9.091	1.818	1464.	179.	14.3	94.	
27.	2.	20.000	2.000	945.	298.	13.7	92.	4.
39.	2.	12.500	5.000	1728.	332.	13.4	89.	13.
24.	5.	7.692	1.538	649.	182.	13.6	89.	1.
18.	0.	11.110	2.222	874.	164.	15.5	96.	
29.	2.	10.000	3.000	869.	112.	13.9	91.	2.
23.	2.	10.000	2.000	802.	286.	13.4	89.	1.
24.	4.	8.333	3.333	726.	175.	12.7	91.	1.
30.	6.	7.692	1.231	1710.	188.	14.1	92.	1.
20.	1.	10.000	2.400	866.	114.	13.2	97.	1.
17.	0.	10.000	4.000	570.	158.	13.3	88.	
24.	2.	12.500	3.000	744.	129.	12.4	95.	1.
25.	3.	9.429	3.429	501.	159.	13.3	88.	1.
23.	4.	12.500	2.500	1386.	211.	12.4	96.	1.
25.	1.	13.330	2.667	1155.	186.	12.9	91.	1.
30.	4.	12.500	1.500	1160.	154.	16.6	93.	3.
55.	3.	7.143	1.571	1021.	319.	14.8	92.	29.
27.	2.	15.000	1.667	772.	65.	14.0	98.	1.
48.	1.	5.882	2.824	837.	198.	13.9	90.	1.
60.	2.	11.110	2.222	764.	153.	14.3	94.	36.
39.	3.	14.290	5.714	573.	114.	15.0	96.	14.
25.	1.	50.000	10.000	1553.	108.	12.5	89.	2.
38.	7.	7.692	1.538	730.	205.	12.0	96.	1.
55.	7.	11.760	1.176	1293.	426.	14.3	92.	20.
17.	0.	7.143	2.143	1068.	110.	11.8	103.	
59.	5.	11.110	2.222	689.	465.	14.1	91.	25.
59.	6.	10.000	1.000	762.	278.	14.3	91.	21.
54.	8.	8.000	1.333	1278.	320.	12.3	96.	4.
67.	11.	10.530	2.105	1233.	147.	14.3	90.	18.
72.	9.	12.500	1.250	1024.	235.	14.2	90.	30.
62.	0.	8.333	1.667	1112.	167.	14.2	83.	
53.	11.	15.380	3.077	1223.	217.	14.8	97.	15.
52.	6.	10.000	2.222	960.	170.	13.9	100.	20.
55.	7.	10.910	1.818	1721.	178.	13.6	99.	11.
53.	5.	7.692	3.077	587.	203.	11.9	89.	15.
75.	8.	8.333	1.667	566.	215.	13.4	94.	28.
57.	7.	9.091	.909	665.	174.	13.8	92.	12.
67.	5.	10.000	1.200	1040.	302.	13.6	97.	27.
54.	11.	25.000	5.000	938.	590.	13.8	90.	28.
51.	3.	11.110	4.444	1014.	128.	12.2	94.	19.
67.	0.	25.000	5.000	1203.	143.	15.9	93.	
79.	6.	16.500	2.500	1403.	86.	15.3	100.	41.
67.	2.	12.500	2.000	1532.	78.	12.5	96.	39.
67.	1.	12.500	5.000	791.	168.	12.8	91.	45.
73.	8.	12.500	7.500	639.	106.	12.9	93.	38.
78.	0.	11.000	2.000	830.	204.	14.6	90.	
55.	7.	14.290	3.429	999.	695.	14.2	88.	21.

A	B	C	D	E	F	G	H	I
75.	0.	16.500	2.500	450.	104.	13.4	92.	
78.	10.	22.000	3.333	769.	356.	16.2	92.	32.
67.	8.	14.290	1.143	1213.	332.	14.0	85.	31.
68.	5.	10.000	2.000	745.	135.	15.8	85.	33.
67.	6.	16.670	1.667	838.	607.	14.3	96.	22.
61.	12.	13.640	.909	923.	281.	14.8	89.	19.
63.	8.	7.143	1.429	901.	142.	15.0	92.	19.
29.	3.	9.091	3.636	1195.	254.	13.3	92.	1.
79.	5.	7.692	3.077	1114.	282.	12.7	93.	24.
65.	7.	10.000	2.000	550.	96.	14.2	85.	29.
70.	0.	20.000	2.000	699.	471.	14.7	92.	
46.	7.	14.290	4.286	1454.	278.	14.3	88.	15.
47.	8.	12.500	1.250	1078.	229.	13.6	95.	12.
33.	4.	13.330	2.667	855.	138.	13.5	86.	2.
66.	8.	12.500	2.500	746.	248.	14.9	95.	31.
52.	7.	4.762	1.905	762.	195.	13.8	89.	14.
77.	14.	16.670	3.333	661.	127.	12.6	90.	41.
46.	0.	25.000	2.500	1094.	187.	12.4	88.	
54.	5.	25.000	5.000	1633.	644.	15.1	105.	19.
72.	8.	6.250	1.250	941.	195.	14.6	96.	19.
50.	8.	13.200	1.000	916.	379.	14.6	86.	16.
46.	3.	9.429	.714	763.	201.	13.2	86.	13.
68.	4.	16.670	1.667	567.	137.	13.5	89.	21.
32.	2.	8.333	1.667	1199.	275.	14.2	86.	2.
20.	0.	11.110	2.222	1159.	189.	13.2	87.	
78.	4.	12.500	1.250	879.	115.	15.2	99.	37.
42.	1.	16.500	2.500	991.	149.	14.7	92.	15.
78.	6.	11.000	2.000	822.	426.	14.8	89.	28.
45.	4.	14.290	5.714	474.	244.	14.5	97.	1.
79.	10.	12.500	2.500	545.	204.	14.4	96.	29.
53.	9.	15.380	1.538	963.	195.	15.0	97.	9.
76.	9.	14.290	2.857	610.	186.	15.4	88.	30.
42.	6.	33.330	4.444	602.	174.	12.5	88.	3.
42.	6.	10.000	4.000	795.	154.	14.1	90.	2.
73.	0.	16.500	2.500	1148.	153.	14.5	96.	
60.	4.	12.500	5.000	614.	505.	13.9	96.	14.
78.	9.	22.220	4.444	942.	445.	14.4	97.	35.
68.	8.	16.670	3.333	332.	174.	13.6	96.	25.
30.	4.	12.500	3.750	881.	152.	12.4	91.	1.
65.	11.	10.000	2.800	951.	323.	14.5	90.	17.
44.	2.	11.110	1.778	1017.	170.	15.0	93.	13.
54.	6.	13.200	1.000	834.	160.	13.0	89.	8.
78.	6.	14.290	4.286	1237.	636.	14.4	93.	29.
26.	4.	9.091	1.818	1377.	174.	12.9	92.	1.
50.	7.	7.143	2.857	798.	295.	13.6	90.	4.
65.	10.	22.220	2.222	1062.	245.	12.6	95.	9.
30.	3.	13.200	4.000	924.	372.	13.6	91.	7.
67.	4.	10.000	4.000	966.	288.	16.2	92.	41.
58.	5.	18.180	3.636	960.	324.	14.0	90.	25.
18.	0.	15.380	1.538	1096.	209.	12.9	81.	
59.	4.	25.000	5.000	867.	164.	11.9	94.	31.
39.	2.	11.000	3.333	1092.	155.	13.0	100.	4.
56.	8.	25.000	2.500	1621.	331.	14.9	96.	31.
20.	0.	6.947	3.158	811.	61.	14.1	107.	
21.	0.	11.110	4.444	955.	167.	14.6	101.	
33.	4.	14.290	2.857	982.	351.	11.9	81.	4.
23.	1.	16.500	2.500	1148.	180.	14.3	92.	3.
20.	2.	14.290	2.857	847.	48.	12.2	93.	1.
28.	0.	10.000	1.000	1162.	193.	12.4	83.	

A	B	C	D	E	F	G	H	I
55.	6.	11.110	4.444	850.	112.	13.8	92.	13.
78.	7.	16.670	1.667	742.	82.	15.0	109.	41.
65.	9.	15.380	3.077	955.	320.	12.9	92.	19.
55.	6.	16.670	3.333	905.	465.	12.7	98.	7.
42.	7.	7.143	1.429	1337.	524.	13.8	92.	8.
25.	1.	7.692	3.077	785.	264.	13.8	87.	1.
40.	4.	6.250	2.500	573.	239.	13.0	90.	1.
58.	7.	4.167	.833	1438.	125.	13.5	87.	18.
42.	10.	9.091	1.818	919.	297.	13.8	87.	4.
58.	7.	9.091	3.636	904.	253.	12.7	84.	16.
55.	8.	16.670	3.333	1383.	252.	13.3	92.	11.
56.	11.	13.200	2.000	1384.	413.	12.9	92.	4.
70.	5.	18.180	3.636	704.	291.	13.3	89.	29.

III Details of 140 men studied at Nqutu (group VIII)

A - age (years).

B - monthly maize meal consumption per adult in subject's family.

C - monthly cash income per adult in subject's family.

D - serum vitamin B_{12} concentration (pg per ml).

E - red cell folate concentration (ng per ml).

F - haemoglobin concentration (g per 100 ml).

G - mean cell volume ($/u^3$).

A	B	C	D	E	F	G
24.	10.000	8.000	775.	165.	16.3	95.
20.	12.000	4.000	981.	174.	15.2	83.
16.	12.500	1.000	833.	226.	15.4	94.
35.	11.110	1.556	933.	212.	16.6	90.
38.	22.220	3.889	1424.	284.	14.9	91.
25.	22.220	3.889	1470.	293.	14.4	90.
33.	14.290	11.430	834.	219.	14.4	93.
55.	0.000	0.000	1264.	164.	15.3	97.
17.	8.333	8.000	830.	150.	15.1	89.
20.	12.500	6.000	1398.	213.	15.4	82.
22.	7.143	0.000	797.	227.	15.5	101.
22.	11.110	2.222	761.	488.	15.9	91.
52.	9.091	1.818	1472.	200.	14.7	86.
26.	14.290	22.860	734.	145.	15.8	99.
22.	12.000	56.000	999.	448.	16.9	101.
67.	12.500	4.000	773.	314.	15.3	96.
48.	16.670	6.667	1073.	205.	11.8	95.
42.	12.000	3.000	2341.	438.	14.5	99.
78.	8.333	2.000	1110.	292.	14.0	98.
79.	6.250	5.000	939.	346.	10.0	96.
76.	12.500	2.500	791.	106.	12.8	99.
33.	6.250	5.000	905.	182.	10.5	85.
41.	12.000	2.400	954.	323.	14.2	93.
45.	50.000	2.000	1214.	306.	14.6	89.
20.	20.000	4.000	1368.	284.	14.8	95.
73.	16.670	.500	779.	290.	14.9	95.
73.	12.500	1.000	690.	688.	14.3	92.
60.	25.000	2.500	892.	340.	13.2	93.
59.	14.290	5.714	672.	323.	13.8	91.
37.	14.290	1.524	1399.	356.	14.7	94.
17.	11.760	2.118	873.	391.	14.5	89.
28.	30.000	5.600	1538.	320.	16.9	97.
26.	28.570	2.857	1421.	579.	16.0	100.
23.	7.692	3.692	2404.	168.	14.3	88.
33.	15.380	2.923	1237.	371.	14.2	91.
26.	11.110	4.444	847.	315.	16.4	94.
28.	14.290	3.714	886.	192.	14.9	87.
28.	14.290	3.714	1174.	364.	15.1	91.
21.	14.290	3.714	841.	338.	15.0	92.
19.	14.290	3.714	663.	246.	12.7	82.
42.	11.110	1.556	1121.	316.	14.5	94.
55.	10.000	4.000	1111.	188.	14.2	95.
19.	10.530	1.684	1429.	135.	15.3	90.
34.	10.000	6.000	1372.	213.	14.5	108.
22.	15.380	1.846	1652.	289.	15.5	83.
21.	14.670	2.222	723.	191.	15.5	86.
41.	16.670	3.333	1353.	322.	15.2	98.
56.	12.500	5.000	805.	72.	13.7	92.
16.	7.143	2.857	925.	304.	16.7	97.
61.	16.670	4.000	1158.	320.	14.7	99.
27.	6.522	2.783	867.	291.	14.4	92.
26.	8.250	5.000	1020.	255.	15.2	89.
28.	8.333	3.333	1156.	483.	16.0	88.
32.	11.110	3.333	790.	175.	14.9	91.

A	B	C	D	E	F	G
35.	25.000	2.500	637.	178.	14.4	90.
67.	13.200	3.200	652.	373.	14.6	96.
48.	16.000	5.000	789.	190.	15.8	92.
30.	16.000	25.000	1250.	205.	16.0	95.
53.	10.000	5.000	811.	260.	14.1	98.
55.	6.667	2.667	1176.	429.	14.7	96.
67.	10.000	5.000	600.	117.	14.6	96.
46.	11.110	1.778	696.	152.	16.5	95.
41.	22.220	4.444	1418.	320.	15.7	92.
36.	10.000	4.000	1111.	188.	17.2	90.
58.	13.330	4.444	921.	627.	12.2	102.
30.	15.000	5.000	1056.	166.	15.7	93.
20.	20.000	5.000	793.	154.	15.8	95.
35.	8.333	2.667	1284.	224.	16.9	98.
67.	12.500	0.000	1453.	249.	14.9	104.
16.	12.500	5.000	1097.	161.	14.5	79.
45.	11.110	0.000	1383.	315.	14.4	98.
19.	5.882	3.529	1090.	196.	14.0	88.
73.	7.143	0.000	827.	430.	13.6	96.
23.	3.077	1.538	1263.	150.	14.6	92.
37.	50.000	4.000	954.	157.	12.5	90.
67.	6.250	.500	1041.	194.	15.0	90.
49.	10.000	3.600	1127.	315.	13.0	92.
22.	8.333	.833	1007.	164.	16.4	94.
73.	6.250	.750	686.	165.	14.5	96.
24.	8.333	1.333	1356.	150.	15.0	90.
27.	6.250	2.500	830.	229.	17.1	100.
18.	9.429	1.714	841.	255.	15.5	92.
48.	10.000	2.667	1239.	460.	15.0	96.
60.	11.110	2.222	1282.	167.	13.9	94.
32.	11.110	2.667	1820.	175.	15.3	84.
19.	6.667	2.667	1044.	2 1.	15.6	92.
19.	5.000	4.000	975.	2 8.	15.7	86.
55.	14.290	1.429	865.	5 3.	15.5	98.
55.	10.000	1.000	1449.	633.	14.3	100.
25.	16.670	1.667	1368.	481.	15.4	98.
41.	7.143	.571	2084.	574.	17.4	100.
30.	25.000	3.000	940.	338.	14.6	92.
25.	10.000	4.000	1181.	525.	15.5	96.
20.	5.556	4.444	1150.	640.	16.6	88.
60.	16.670	3.333	1162.	500.	13.5	96.
44.	10.000	4.000	897.	267.	13.4	100.
69.	8.333	2.000	1219.	273.	14.2	95.
75.	25.000	5.000	609.	165.	15.8	95.
72.	14.290	4.286	872.	124.	13.7	96.
74.	8.333	.833	1154.	68.	16.4	98.
77.	12.500	5.000	892.	71.	15.6	95.
68.	10.000	0.000	1019.	188.	16.9	96.
67.	50.000	0.000	709.	253.	15.6	93.
78.	25.000	2.500	870.	221.	15.1	96.
73.	4.000	0.000	1088.	104.	16.5	96.
67.	33.000	8.000	914.	280.	14.9	99.
78.	10.000	0.000	916.	313.	14.4	94.
65.	14.290	2.286	910.	520.	16.6	100.
79.	50.000	3.000	1263.	145.	13.7	96.
80.	10.000	0.000	564.	441.	15.2	93.
73.	13.200	4.000	1119.	120.	16.2	98.
77.	10.000	2.000	1229.	96.	15.6	97.
67.	16.670	3.333	1156.	232.	15.3	100.

A	B	C	D	E	F	G
73.	16.500	2.500	655.	184.	15.4	93.
55.	10.000	2.000	946.	204.	13.6	89.
72.	18.180	1.818	2196.	265.	15.5	91.
57.	10.530	2.105	1058.	119.	16.0	95.
78.	14.290	1.429	899.	117.	15.6	100.
68.	6.250	1.250	698.	124.	14.9	87.
60.	12.500	2.500	929.	155.	16.4	86.
32.	14.290	5.714	1199.	120.	15.5	89.
58.	10.000	1.000	1485.	483.	14.2	93.
45.	12.500	1.250	803.	209.	13.9	97.
66.	20.000	2.400	1511.	103.	15.2	93.
82.	14.290	1.429	966.	251.	15.5	91.
26.	9.091	1.455	1004.	200.	16.3	87.
45.	9.429	2.857	932.	204.	13.2	99.
52.	12.500	2.500	1768.	423.	15.3	101.
78.	10.000	4.000	1627.	463.	15.9	101.
58.	12.000	4.000	849.	179.	14.9	88.
67.	10.000	0.000	927.	128.	14.6	96.
44.	6.667	2.667	1340.	266.	15.7	95.
44.	8.333	1.667	732.	233.	13.8	86.
43.	11.110	2.222	736.	178.	15.9	85.
23.	8.250	1.250	997.	204.	15.4	87.
76.	12.500	2.500	760.	247.	14.7	93.
26.	20.000	2.000	1475.	132.	11.6	76.
67.	9.091	1.818	1360.	217.	14.3	93.
69.	7.143	3.429	802.	448.	13.6	94.
62.	13.200	2.400	1241.	363.	18.4	94.

IV Details of 181 non-pregnant women attending a Johannesburg family planning clinic (group IX)

A - age (years).

B - parity.

C - serum vitamin B_{12} concentration (pg per ml).

D - serum folate concentration (ng per ml).

E - red cell folate concentration (ng per ml).

F - haemoglobin concentration (g per 100 ml).

G - mean cell volume ($/u^3$).

H - years since last pregnant or lactating.

Group IXa - 74 subjects not receiving hormonal contraceptives

A	B	C	D	E	F	G	H
30.	2.	1368.	5.2	228.	13.4	101.	8.
39.	4.	1595.	6.0	216.	13.2	94.	6.
34.	5.	1093.	3.2	234.	13.8	88.	4.
42.	3.	1098.	6.0	347.	11.9	77.	8.
36.	2.	1093.	4.0	258.	14.2	89.	6.
24.	1.	1220.	6.8	289.	14.3	88.	6.
29.	1.	1074.	4.2	193.	12.9	84.	10.
36.	5.	1207.	2.6	304.	11.0	75.	6.
39.	3.	1270.	4.6	181.	14.5	80.	4.
27.	3.	893.	4.0	182.	15.2	88.	3.
31.	3.	1074.	4.0	154.	14.4	93.	1.
40.	4.	797.	2.0	157.	13.6	83.	12.
28.	1.	1610.	3.6	302.	10.7	67.	2.
28.	3.	694.	9.0	202.	14.5	94.	5.
40.	2.	1322.	6.0	249.	15.8	83.	7.
27.	2.	575.	2.8	151.	13.5	83.	4.
32.	4.	796.	6.2	261.	14.0	101.	7.
38.	4.	873.	5.0	229.	12.7	82.	9.
27.	2.	890.	5.6	263.	10.1	77.	3.
30.	4.	703.	3.0	100.	13.3	87.	1.
27.	3.	631.	2.0	116.	12.7	87.	1.
20.	5.	1785.	5.2	276.	13.6	88.	2.
24.	1.	1251.	5.6	169.	14.0	89.	6.
32.	6.	1032.	7.6	218.	14.8	98.	1.
20.	1.	1015.	5.4	230.	13.3	88.	1.
26.	1.	1067.	4.0	333.	15.6	90.	1.
34.	2.	500.	3.0	236.	14.0	93.	4.
22.	2.	724.	8.0	446.	13.6	80.	2.
25.	1.	1104.	4.0	264.	13.5	89.	2.
28.	2.	669.	4.8	372.	14.3	95.	3.
28.	2.	614.	4.8	276.	12.7	83.	2.
35.	6.	635.	1.8	96.	15.0	85.	1.
25.	1.	2357.	6.8	307.	14.1	89.	7.
32.	4.	653.	4.2	199.	13.9	90.	4.
20.	1.	808.	2.6	162.	13.6	80.	1.
28.	2.	910.	3.6	190.	14.5	94.	1.
29.	2.	1358.	3.2	170.	13.6	87.	3.
35.	8.	851.	2.6	147.	14.1	91.	2.
29.	4.	1877.	24.8	493.	14.7	91.	1.
32.	5.	1018.	4.6	159.	14.5	90.	1.
26.	2.	863.	8.2	388.	10.1	78.	2.
30.	9.	771.	7.6	274.	14.4	90.	1.
25.	2.	889.	13.2	426.	12.5	86.	3.
34.	5.	713.	8.4	221.	13.8	86.	1.
35.	1.	773.	3.6	210.	11.8	66.	3.
28.	3.	1939.	5.8	197.	13.9	85.	6.
22.	1.	1694.	6.4	144.	12.8	88.	3.
31.	4.	1122.	2.0	115.	12.7	81.	1.
25.	1.	1199.	5.8	148.	13.2	88.	3.
22.	0.	1439.	11.2	134.	14.9	99.	
45.	7.	1203.	2.2	135.	14.0	92.	1.
23.	2.	1133.	9.4	101.	13.1	88.	1.
28.	4.	1365.	3.6	255.	13.7	93.	2.
24.	4.	1543.	5.0	213.	13.4	92.	1.

A	B	C	D	E	F	G	H
42.	5.	817.	3.6	83.	9.8	88.	1.
30.	3.	1122.	5.8	631.	14.0	96.	1.
20.	0.	749.	8.8	421.	15.1	90.	
29.	2.	963.	24.0	271.	14.7	86.	2.
28.	3.	1667.	5.6	658.	14.4	84.	1.
37.	2.	978.	2.6	158.	9.2	83.	1.
28.	2.	1337.	2.2	187.	14.1	97.	1.
27.	2.	785.	5.6	228.	12.9	89.	1.
22.	1.	1836.	4.4	208.	14.2	94.	1.
30.	4.	1833.	2.6	151.	13.1	82.	1.
27.	2.	1926.	3.0	185.	12.8	88.	1.
44.	5.	1031.	2.4	104.	9.1	72.	1.
30.	2.	1022.	7.4	231.	12.5	92.	1.
27.	3.	1273.	2.6	170.	11.8	92.	1.
34.	3.	1658.	2.2	158.	12.2	95.	1.
27.	2.	1468.	8.6	346.	12.5	89.	7.
25.	2.	1365.	10.6	298.	12.9	93.	4.
24.	5.	2628.	10.8	667.	13.9	90.	6.
30.	2.	2367.	4.4	253.	12.3	87.	9.
31.	4.	1917.	10.6	262.	12.4	89.	1.

Group IXb - 75 subjects receiving oral contraceptives

A	B	C	D	E	F	G	H
40.	6.	1330.	2.8	240.	16.1	87.	3.
26.	1.	1425.	5.6	186.	11.8	76.	5.
28.	4.	1159.	4.4	157.	12.8	79.	3.
33.	3.	1303.	5.2	179.	13.7	84.	7.
30.	5.	902.	4.8	171.	14.9	83.	5.
25.	2.	1138.	4.6	188.	12.5	93.	1.
39.	3.	1124.	5.2	266.	14.8	85.	7.
25.	2.	1083.	4.0	118.	14.0	84.	2.
30.	2.	686.	8.6	274.	14.2	86.	3.
22.	1.	845.	3.8	274.	16.5	88.	2.
30.	2.	710.	5.0	349.	14.0	89.	7.
29.	2.	1092.	3.4	165.	14.4	98.	4.
30.	2.	1159.	3.6	254.	16.4	89.	4.
27.	1.	1391.	4.2	231.	14.6	82.	5.
43.	4.	1796.	8.0	367.	17.1	90.	6.
22.	1.	1184.	4.6	221.	15.2	92.	2.
31.	1.	766.	2.8	180.	15.6	90.	3.
38.	5.	840.	4.4	203.	14.2	87.	4.
24.	2.	725.	4.2	172.	12.8	79.	2.
25.	3.	1075.	4.8	161.	14.0	86.	2.
25.	2.	2243.	3.2	103.	14.1	95.	6.
23.	2.	1363.	8.0	157.	14.1	82.	2.
28.	2.	990.	4.2	140.	14.8	91.	1.
25.	2.	1343.	2.6	181.	16.1	95.	2.
25.	1.	796.	3.2	103.	14.9	92.	2.
28.	3.	666.	7.6	247.	14.9	93.	3.
42.	5.	868.	2.6	136.	14.8	96.	3.
27.	2.	1107.	3.6	156.	15.2	92.	1.
27.	3.	664.	8.4	287.	13.5	94.	4.
30.	5.	1085.	3.8	157.	13.3	94.	5.
30.	5.	828.	4.0	242.	13.5	95.	4.
25.	2.	950.	5.8	230.	14.3	97.	4.
25.	2.	2443.	4.6	224.	12.9	92.	4.
25.	2.	1006.	4.0	209.	13.8	93.	2.
25.	3.	1041.	5.2	233.	13.3	96.	1.
25.	3.	1301.	4.8	222.	13.4	94.	4.
28.	1.	1019.	9.6	350.	13.4	82.	9.
26.	1.	882.	6.6	361.	14.2	93.	1.
21.	0.	1185.	17.0	344.	15.0	95.	
27.	3.	1050.	3.4	200.	13.2	84.	2.
28.	3.	1358.	3.4	132.	13.5	83.	2.
38.	6.	1387.	5.6	312.	13.8	93.	1.
30.	4.	1169.	5.6	216.	14.2	85.	5.
23.	1.	990.	3.2	100.	13.4	87.	4.
24.	3.	1458.	7.0	100.	12.9	93.	5.
30.	3.	820.	4.6	257.	13.9	84.	12.
27.	1.	904.	7.4	296.	13.5	89.	10.
28.	3.	840.	8.0	341.	12.4	85.	2.
37.	2.	1067.	6.8	186.	13.8	99.	3.
40.	4.	1014.	5.2	197.	13.1	93.	11.
24.	3.	940.	7.4	193.	12.7	92.	2.
36.	5.	701.	5.0	198.	13.2	86.	6.
31.	4.	900.	5.0	198.	13.1	85.	2.
32.	1.	918.	8.0	244.	14.8	86.	6.

A	B	C	D	E	F	G	H
28.	2.	946.	4.4	184.	14.1	86.	4.
24.	1.	1133.	6.0	336.	14.2	88.	1.
24.	1.	2014.	6.8	259.	13.7	88.	4.
32.	3.	1191.	2.6	187.	13.7	80.	5.
34.	4.	941.	5.2	202.	14.5	85.	10.
26.	3.	1172.	5.2	202.	14.8	76.	10.
29.	3.	630.	4.6	296.	13.1	77.	5.
31.	2.	935.	2.2	232.	13.3	85.	13.
24.	3.	672.	4.4	273.	14.2	88.	4.
20.	1.	1228.	2.0	128.	15.0	89.	2.
23.	1.	1133.	6.2	242.	14.6	91.	3.
26.	2.	1735.	4.8	370.	13.3	88.	1.
34.	3.	1336.	4.0	274.	13.8	91.	7.
31.	2.	1073.	4.2	302.	15.0	97.	5.
21.	1.	1372.	13.4	447.	12.1	86.	6.
21.	1.	2479.	12.0	412.	12.6	91.	6.
29.	5.	999.	6.4	417.	10.4	70.	3.
30.	3.	943.	4.8	258.	13.3	90.	3.
28.	2.	777.	6.8	240.	12.7	86.	6.
30.	2.	987.	6.8	419.	13.8	98.	1.
32.	4.	1110.	11.6	355.	13.8	86.	3.

Group IXc - 32 subjects receiving "Depo-Provera"

A	B	C	D	E	F	G	H
30.	2.	1257.	4.4	137.	14.8	88.	11.
3[illegible].	2.	910.	8.0	259.	14.8	94.	5.
3[illegible].	5.	827.	8.4	265.	14.5	91.	9.
31.	4.	1193.	5.8	298.	15.1	90.	3.
29.	3.	812.	3.2	126.	14.9	84.	1.
32.	3.	716.	4.2	254.	13.8	85.	1.
30.	4.	753.	4.6	208.	14.4	93.	1.
36.	3.	778.	5.0	211.	15.5	90.	2.
33.	6.	1208.	5.6	217.	13.2	85.	3.
37.	6.	590.	6.4	113.	13.2	79.	1.
33.	2.	774.	6.8	209.	15.8	85.	2.
26.	6.	732.	4.6	213.	14.6	83.	3.
32.	5.	867.	3.6	250.	14.8	92.	4.
25.	1.	821.	3.8	148.	12.9	85.	2.
27.	2.	679.	5.6	197.	14.6	89.	1.
38.	8.	559.	3.0	201.	13.9	85.	7.
31.	6.	364.	6.4	195.	13.5	85.	2.
29.	4.	2112.	3.2	200.	14.3	91.	6.
32.	3.	1064.	7.6	241.	14.8	80.	7.
31.	6.	1877.	5.2	178.	14.1	84.	3.
34.	4.	922.	3.2	83.	15.2	97.	5.
38.	11.	873.	4.0	247.	13.1	96.	1.
30.	2.	2251.	5.2	429.	15.8	94.	3.
33.	7.	1256.	4.4	249.	14.9	96.	1.
24.	2.	995.	6.0	274.	12.3	81.	1.
29.	3.	909.	4.2	267.	13.3	95.	1.
33.	5.	1033.	7.6	254.	14.4	84.	2.
35.	6.	1156.	4.0	204.	14.1	86.	5.
31.	5.	1192.	7.0	354.	11.3	65.	2.
41.	9.	1653.	6.0	268.	14.4	83.	4.
32.	6.	1102.	5.0	240.	14.6	93.	2.
28.	1.	1[illegible] 1.	9.4	340.	14.1	*3.	8.