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STUDIES ON IRON-DEFICIENCY ANEMIA IN CENTRAL AMERICA

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION.

IRON AND FOLATE DEFICIENCIES

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INTRODUCTION

Anemia can be defined, for practical purposes, as a percentual level of packed red cells (PCV), a concentration of hemoglobin and/or a number of red blood cells per unit volume below a certain chosen limit which is, in theory, the lower limit of normality. For physiological reasons this hematological normality varies depending on the age, the sex, the physiological condition of the individual and the altitude above sea level at which he lives. Consequently, the limit of normality must be different for each category mentioned. On the other hand, the prevalence of anemia in population groups depends on the establishment of this lower limit of normality.

In order to define the hematological status of the Central American population we decided to start precisely at defining the hematological norms for this geographical area.

A set of hematological norms was proposed (1) starting from a population which was apparently healthy, hookworm-free and upon which biochemical evidence of adequate iron, folate and vitamin B_{12} nutrition was available. Based upon these hematological norms and from distribution plots, the risk carried by various levels of hemoglobin or PCV of belonging to a subnormal (anemic) population were derived. Then, a statistically representative population in Central America (2) was analyzed in order to estimate the prevalence of individuals whose hemoglobin or PCV values carried an elevated risk of belonging to a population with subnormal levels of those variables (anemic) (3). The relative importance of hookworm infection in producing anemia was also investigated at a population level (4).

The results so far obtained can be summarized by stating that hookworm-free healthy, tropical populations from developing nations, with biochemical evidence of adequate iron, folate and vitamin B_{12} nutrition, have hematological values which do not differ from the best standards obtained in developed, non-tropical nations (5-7). However, the prevalence of individuals with low hemoglobin concentration

carrying a high risk ($>$ 75%) of being below normal (from now on in the text this population will be referred to as anemic), is high in the general population: 19.7% for altitudes between 0-750 m above sea level, and 12.6% and 11.7% for altitudes between 750-1500 and 1500-2800 m above sea level respectively. Children between 1-4 years of age are particularly vulnerable.

Hookworm infection increased the prevalence of anemia when the average egg count was above 1000/g of feces, but hookworm free populations already had an elevated prevalence of anemia in specific age and sex groups (4).

In a cooperative study on nutritional anemia, which involved Guatemala (8), it was further shown that pregnant women, selected as a nutritionally vulnerable group, had a high prevalence of anemia, that is, of hemoglobin levels below 11 g when corrected for altitude. From the biochemical evidence obtained, it was also concluded that iron deficiency was the overwhelming culprit; folate deficiency could not be associated with a prevalence of anemia of public health significance.

The work to be presented in this report includes a continuation of epidemiological studies aimed at establishing the etiology of the

frequently observed low hemoglobin values; verifying the epidemiological findings by means of responses to the administration of hematinics and collaterally confirming the proposed norms, and studying the relation between hemoglobin or PCV values and the capacity of men to perform an exercise test demanding near maximal cardio-respiratory activity.

MATERIALS AND METHODS

a) Hematological Status of the Central American Population.

The population studied was drawn from a sample representative of the Central American population, examined on occasion of the Central American Nutrition Survey. It consisted of a total of 3,804 individuals all of whom had complete hematological and biochemical studies. These studies included PCV, hemoglobin and RBC counts performed by microhematocrit, cyanmethemoglobin and Coulter Counter, respectively (9); serum iron and total iron binding capacity (TIBC), determined by the Ramsay method (10,11); serum folates determined by L. casei assay following Herbert's techniques (12); serum vitamin B_{12} determined by E. gracillis assay, following

Anderson's techniques (13). The population was catalogued by altitude above sea level, by age and sex (Table 1), and by socio-economic index, according to Mendez' criteria (14).

The majority of subjects in this sample also had stool examination for ova and parasites by the Stoll method (15)* and belonged to families in whom a detailed dietary survey was performed (2)**. In this report only the rural Guatemalan sample is considered with regard to socio-economic index and dietary survey.

b) <u>Hematological Survey of Pregnant Women at Term and of their Offspring</u>.

The population studied consisted of a total of 107 pregnant women delivering at the Maternity Ward of the Roosevelt Hospital in Guatemala. None had received pre-natal care. All of the women belonged to a low socio-economic group and lived in Guatemala City and surrounding towns. Blood was drawn from the mother

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* Stool examinations performed by the Division of Microbiology, INCAP.
** Dietary surveys performed by the Dietary Survey Section of the Division of Applied Nutrition, INCAP.

prior to delivery, and from the cord immediately upon clamping, by letting the blood flow freely into sterile, iron free tubes containing 5 mg Na_2 EDTA/ml of blood.

Besides the hematological and biochemical determinations performed in the Central American sample, total serum proteins (16) and electrophoretic fractionation (17), and red cell folates (12), were also performed in mother and cord bloods. Bone marrow aspirations were also obtained from 66 mothers.

c) Population Studies Involving Administration of Oral Ferrous Sulphate Alone or in Combination with Folic Acid.

These studies include two populations:

i) Healthy pre-school age children attending a day nursery in Guatemala City.

The subjects were 27 children who had attended the nursery regularly for at least 6 months prior to the initiation of the study. They were chosen because they were growing at an adequate rate and had a satisfactory health since attending the nursery.

These children were studied before and 3 months after the carefully supervised daily oral administration of either 60 mg of iron as ferrous sulphate (Experimental Group) or a placebo (Control Group).

Experimental and Control children were carefully matched. The hematological evaluation, which included all of the studies performed in the pregnant women previously described, was done blindly.

ii) Low-land Guatemalan populations, living near sea level.

A total of 274 individuals of both sexes and all ages were studied. After an initial evaluation, which included stool egg counts, complete hematology, serum iron, TIBC, folate and vitamin B_{12}, and red cell folates, they were divided into three groups: The control group received an iron-free, folate-free lactose tablet twice a day; the iron group received 100 mg of iron as $FeSO_4$ in identical tablets as those of the control group; the iron plus folic acid group received the same tablets as the iron group, plus a tablet containing 10 mg folic acid once a week. The

tablets were administered in a carefully supervised fashion by a member of the community who did not know their contents. Hematological evaluation was done blindly at two month intervals; the study lasted six months. At the end of the study all groups received oral iron and folic acid for an additional period of two months.

The male population comprised between 15 and 44 years was tested in their performance of the Harvard Step Test before and at the end of supplementation. The test was done in a double blind fashion by one member of the team: neither the investigator nor the subjects knew who were receiving iron, iron plus folic acid or lactose.

RESULTS

a) Central America Population

Table 2 presents the number of individuals at each altitude age-sex category and the lactating women, with the percent of them whose hemoglobin concentration carried a chance greater than 20% or 75% of belonging to an anemic population. The levels of hemoglobin concentration carrying different risks of anemia have been previously defined (1, 3). It is evident that the prevalence

of individuals of all ages and both sexes who have hemoglobin levels with a chance greater than 20% of anemia is elevated (mean 26.7%, 24.3% and 31.6% for altitudes above sea level between 0-750 m, 751-1500 m, and 1500-2800 respectively). For risks greater than 75% the corresponding mean figures are 15.9%, 12.0% and 14.9%.

The sex-age groups at greater risks are better appreciated in Figure 1. Children 1-4 years of age seem to be at greater risk. It is worth while pointing out that except for altitudes above 1500 m, where this sample is small, the prevalence of males and females with risk $>$ 75% of belonging to an anemic population is very similar, and that in all altitudes lactating women have greater risk of anemia than women of reproductive age who are neither pregnant nor lactating.

In the rural Guatemalan population, another analysis has been done: the population has been divided in three categories by socio-economic index (S.E.I.), irrespective of altitude above sea level, and in each category the prevalence of individuals with risk out

of belonging to an anemic population has been determined. It is evident in Figure 2 that the low S.E.I. group has about twice as many individuals with a risk of anemia greater than 75% than the intermediate or high S.E.I. groups. No differences could be established on the degree of hookworm infection in the S.E.I. groups.

Iron intake estimated by dietary survey (2) in the three groups with different S.E.I. showed that the low S.E.I. group contained a greater number of families whose iron intake was very low (Figure 3). A further analysis of dietary intake showed that the amount of meat-iron increased as S.E.I. improved. Figure 4 shows that the mean meat iron intake in the low S.E.I. was 0.95 mg/person/day while in the high S.E.I. group it was 1.65 mg/person/day. Furthermore, the percentual distribution of families by meat-iron intake shows clearly that over 75% of the low S.E.I. group consume less than 1 mg meat iron per day, and only 11% consume more than 1.9 mg of meat iron per day. This distribution improves as S.E.I. improves.

In the rural Guatemalan sample, the mean iron intake was determined in hookworm free individuals having different risks

of belonging to an anemic population. The results are shown in Figure 5. One fact that emerged was that iron intake at altitudes between 1500 and 2800 m was higher than at the other two altitudes. Also, at each altitude, the mean iron intake was lower in the group with lower hemoglobin (risk $>$ 75%) than in the other two groups (risks 20-75 and $<$ 20%).

Folate intake has not been investigated in such a detailed fashion. However, the L. casei-available folate in composite analysis of 49 typical central American rural diets consumed at different altitudes, and a calculation of L. casei-available folate intake in three dietary surveys in the Guatemalan low-lands comprising 60 families and performed at different seasons of the year, indicate that folate intake is inadequate, ranging from 18 to 183 ug/person/day, and from 18 to 51% in terms of adequacy of recommended intake.

The biochemical data in the Central American sample has been analyzed by dividing the population into discrete categories of serum iron, saturation of TIBC and serum folates. The limits

for the different categories are shown in Table 3.

Table 4 presents the distribution of cases in the different serum iron categories for the three altitudes above sea level and for cases with different risk of belonging to an anemic population. The mean and standard error for the percent cases in each serum iron category have been obtained by taking the percent cases for each age-sex-physiological status as follows: both sexes for ages 1-4, 5-8, 9-12 years; males and females separately, for ages 13-16, 17-49 and over 49 years, and lactating women.

The graphic representation of Table 4 (Figure 6) shows clearly that the cases with a hemoglobin level associated with a risk greater than 75% of belonging to an anemic population have a predominant percentage with serum iron concentrations below 50 ug/100 ml, and only a small percentage with concentrations above 75 ug/100 ml. As the risk of anemia decreases, the distribution of cases shifts towards a higher percentage with serum iron concentrations above 75 ug/100 ml. A similar analysis of the Central American population in terms of the % saturation of

TIBC (Table 5 and Figure 7), confirm the serum iron data and indicate that saturations of TIBC below 15% are predominant among populations with hemoglobin levels carrying $>$ 75% risk of anemia. Also as the risk decreases, the population with % saturation of TIBC above 20% becomes predominant.

The results obtained from the analysis of the serum folate concentration are different from the previous ones pertaining to iron nutrition. Table 6 and Figure 8 indicate that the population of children (1-12 years old) and of individuals over 12 years of age, differ in their serum folate concentrations.

In the children the serum folate values above 5 ng/ml are predominant, while in the subjects $>$ 12 years of age, serum folate values below 5 ng/ml predominate, there being a large proportion of cases with serum folates less than 3 ng/ml. These distributions in serum folates occur regardless of altitude or risk of anemia as determined by hemoglobin concentration. The same trend is observed when the cases are grouped by altitude above sea level and by state of iron nutrition based on saturation of TIBC, (Figure 9).

HEMATOLOGICAL STATUS OF PREGNANT WOMEN AT TERM AND OF THEIR OFFSPRING

In order to analyze the data obtained from the pregnant Guatemalan women at term it was necessary, as a first step, to derive from the population studied, the limits of normality for hemoglobin concentration. To accomplish this, a probability plot of cummulative frequency distribution of hemoglobin levels in the pregnant women was obtained in a normal distribution paper as shown in Figure 10. A straight line was drawn by the least square method taking into account all cummulative frequencies above 12 grams of hemoglobin. This straight line represents then the normally distributed segment of the pregnant population containing higher hemoglobin concentrations. It is also evident from Figure 10, that as hemoglobin concentration decreases, the cummulative frequency distribution of cases is higher than expected from the normally distributed population with higher hemoglobin levels. The shadowed area therefore, shows the percent excess of cases over those expected if the population was all normal.

A Z transformation of the normal segment of the population obtained in Figure 10, yields the normal distribution for that population (Population A in Figure 11), which has a mean of 12.8 g of hemoglobin and a standard deviation of 1.2 g/100 ml. The total

distribution of the population corresponds to population B in Figure 11. Population C is produced by subtracting from population B, population A up to the point where both populations cross which is at 12.8 grams of hemoglobin. This population can be considered as having subnormal hemoglobin concentrations, and represents the shadowed area of Figure 10. From these plots the risk of belonging to the population with subnormal hemoglobin can be derived by dividing the percent of cases in population C for different levels of hemoglobin by the percent of cases in the total population (Population B) for the same hemoglobin level. From these calculations in this sample as well as in the total Latin American sample which was corrected for altitude and reported by Cook et al. (8), the different risks of belonging to an anemic population, associated with different hemoglobin levels at two different altitudes above sea level have been derived and tabulated in Table 7. Included are also the risks carried by various hemoglobin levels in non-pregnant women at sea level and also at 850 meters above sea level, drawn from the Central America hematological norms (1). A graphic representation of the risks in Table 7 is shown in Figure 12. It is evident that in the non pregnant populations the risk increases in a sharp and parallel fashion at both altitudes, as hemoglobin

concentration decreases. The differences in hemoglobin level due to altitude is 0.8 g for the same risk, and the risk increases 10.75% for each 0.2 g decrement of hemoglobin. In the pregnant women, the risk of belonging to an anemic population also changes in a parallel fashion at both altitudes, but does not increase as sharply as in the non pregnant women: 6.8% per each 0.2 g decrement of hemoglobin. On the other hand, the difference obtained from altitude appears to increase from 0.8 to 1.3 g of hemoglobin.

From the previous results, two hemoglobin concentrations have been chosen to form three groups with different risks of anemia in the sample of pregnant women studied in Guatemala City: one carrying 50% risk, and the other carrying a 20%-risk. These values are 10.9 and 11.9 g of hemoglobin/100 ml, respectively.

Table 8 contains the distribution of cases by percent risk of belonging to an anemic population, based on the limits previously established. It is evident that 35% of cases have a risk greater than 20%, and 22% of cases have a risk greater than 50% of being anemic. The PAHO Cooperative Study on nutritional deficiency and anemia in Latin America (8), indicates a 38.5 percent prevalence of anemia among pregnant women, taking 11 grams as the limit of

normality for pregnancy at sea level (18). However, by cummulative frequency distributions, 22% of the population was comprised in a population with lower levels of hemoglobin, which corresponds to those of the risk greater than 50% in our population.

The red cell indices in the three groups according to hemoglobin concentrations are also presented in Table 8. The limits for indices are the mean ± 2 standard deviations from the norms. Results are that those cases with hemoglobin less than 11 grams/100 ml very often are microcytic and hypochromic; however, among the group with over 11.9 grams of hemoglobin/100 ml, the prevalence of macrocytosis reaches 18%.

The blood levels indicative of iron and folate nutrition for the different groups established on the basis of risks, are presented in the same table. As in the total population from Central America, the cases with lower hemoglobin concentrations also have lower serum irons and lower percent saturation of TIBC. This last biochemical indicator is also below 15% in 77% of the pregnant women with hemoglobin concentrations carrying less than 20% risk

of being anemic. This is higher than what is observed in the same risk group in the Central American population of non pregnant women of reproductive age (cases with TIBC % saturation below 15 = 14%).

The serum folate concentration as well as the red blood cell folates do not follow a clear pattern in the three populations grouped by percent risk of anemic. However, it is only in those with risks greater than 50% than 5% of cases have red blood cell folates below 70 ng/ml.

These findings which indicate primarily an iron deficiency anemia in pregnancy are further corroborated by bone marrow studies in this same population which indicated that 87% of the cases had no iron in bone marrow spicules and only 3% (two cases out of 66 adequate for interpretation), had clear megaloblastic changes. Both of these cases had a vitamin B_{12} concentration below 100 pg/ml; one of them had biochemical indications of folate deficiency without anemia nor iron deficiency; the other had anemia and biochemical evidence of iron deficiency. Twenty two percent of cases had minor changes in the white and/or red cell series suggestive of deficiency of erythrocyte maturation factors, while 73% of cases had bone marrow morphology without suggestion of megaloblastosis .

In order to gain a better insight of the relative importance of iron and folate nutrition in the Guatemalan pregnant women, they were further catalogued according to percent saturation of TIBC and red cell folate levels (Figure 13). It is evident that as the percent saturation of TIBC increases the number of cases with red cell folates below 100 ng/ml also increases and viceversa. The linear regressions and correlation coefficients between red cell folates and percent saturation of TIBC for women on different risk categories of belonging to an anemic population is presented in Table 9. The significantly negative correlations are essentially identical and with a higher correlation coefficient in the women with risks greater than 20% and 50% of belonging to an anemic population.

This negative correlation between folate and iron nutrition in the pregnant Guatemalan population, is also apparent when the cases are distributed according to mean corpuscular volume (MCV). Microcytosis is present in close to 20% of cases with biochemical evidence of iron deficiency (Figure 14), while macrocytosis occurs in about 37% of cases whose serum iron concentrations are above

75 ug/100 ml and their percent saturation of TIBC is above 15. On the other hand, macrocytosis occurs in 40% of the cases with red cell folates below 100 ng/ml or serum folates below 3 ng/ml (Figure 15). This data is suggestive of the existence of a combined iron and folate deficiency in late pregnancy. Iron deficiency apparently predominates as a limiting erythropoietic substance..

Besides the risks of anemia for the pregnant mother what repercussions do iron and folate deficiencies and anemia in the mother have on the offspring? Some of our findings are presented in the next three Figures. First, the hemoglobin concentration in cord blood apparently defines at least two populations of newborns (Figure 16): one comprising 69% of cases with a mean hemoglobin concentration of 14.7 g/100 ml and a standard deviation (S.D.) of 1 g/100 ml, and a second population comprising 31% of cases with a mean hemoglobin concentration 18.5 with a S.D. of 1.1 g/100 ml. From these two populations, and based on the values in the literature (19, 20) it appears that only a small proportion of the newborns from the pregnant women studied had normal hemoglobin concentrations (population

with a higher hemoglobin level). Also, this distribution of cases allows us to proceed to an analysis of other variables which may differ in children belonging to different populations, based on hemoglobin concentrations.

The distribution of the mothers by their hemoglobin concentration according to the risk of anemia previously mentioned for pregnant women (Figure 17), indicate that mothers with a hemoglobin of less than 11 g/100 ml (risk $>$ 50%) have 62% of children with hemoglobin concentrations below the mean of the low hemoglobin population of newborns and nearly 80% of children below 16.7 grams of hemoglobin/100 ml, which is the hemoglobin value at which the normal and the lower hemoglobin populations of newborns cross each other. Therefore, nearly 80% of children of anemic mothers are born with unsatisfactory hemoglobin concentrations. Among women with hemoglobins above 11.9 grams (risk $<$ 20%), still 21% of children are born with hemoglobin concentrations below 14.7 g of hemoglobin, and therefore, severely anemic.

Figure 18 shows that not only the hemoglobin concentration of the mother influences that of her child; a higher prevalence of lower serum iron and folates in the newborns from mothers with lower serum iron and folate is also observed. The serum levels

of these hematinics are higher in the newborns in an order of magnitude over the maternal values.

So far, we have been unable to demonstrate in the mothers any association between the number of pregnancies and the hematological status nor between this last aspect and vitamin B_{12} concentrations in serum. In the newborn no clear association has been found between the hemoglobin concentration and any other biochemical variable that we have measured (serum iron, percent saturation of transferrin, serum folates, red cell folates, serum vitamin B_{12}, total serum proteins or albumin). The weight of the term newborn ($>$ 38 weeks gestation) is also not significantly affected by the hematological status of the mother in the sample studied.

EFFECT OF THE ADMINISTRATION OF ORAL IRON ALONE OR IRON PLUS FOLIC ACID IN GUATEMALAN POPULATION GROUPS

a) Studies on pre-school age children in a day-nursery in Guatemala.

Table 10 presents the mean and standard deviations of the control and experimental children for the hematological variables

before and after 3 months of study. It is evident that the control children did not differ from the basal, while the experimental group after iron administration had a significant increase in hemoglobin, hematocrit, mean corposcular volume, mean corposcular hemoglobin, and mean corpuscular hemoglobin concentration. Table 11 shows the distribution of children before the study and three months after, by the risk of the hemoglobin levels of belonging to an anemic population and by groups of serum iron, percent saturation of TIBC, and serum, red cell and whole blood folates. The control group did not improve in hemoglobin concentration and presented only a slight shift towards higher serum and red cell folates after three months of study; in contrast, the changes in the experimental group were very clear: 81% of the children attained a hemoglobin level compatible with less than 20% risk of anemia, the number of children with serum iron below 50 micrograms/100 ml decreased and there were no children with percent saturation of TIBC below 10, indicating a clear improvement in iron nutrition. This change in iron nutrition is substantiated by bone marrow studies which revealed that spicule iron was absent in over 90% of the control

children after three months of study-which was the same as observed in the basal period-while in the experimental group, bone marrow iron was present in all cases. Folate blood values in the iron treated group remained unchanged or showed a slight tendency towards lower concentrations than the basal or control groups.

Table 12 shows the distribution of cases by the risk carried by the level of hemoglobin of being below normal, and by different categories of iron and folate blood values. In this Table, the basal values and those of the control group after three months of study have been pooled since they presented no significant differences. The children with hemoglobin values carrying greater than 75% risk of belonging to an anemic population, also showed serum iron and percent saturation of transferrin values compatible with iron deficiency. None of these children presented biochemical evidence of folate deficiency. The results of the experimental group after three months of iron administration show that the only case with a hemoglobin value carrying more than 75% risk of being below normal had biochemical evidence compatible with folate or vitamin B_{12} deficiency and moderate iron deficiency. His blood smears and bone marrow did not reveal any megaloblastic

changes. No clear evidence of iron, folate or B_{12} deficiency was obtained in the two cases with risks between 20 and 75% of being below normal. The 13 children with higher hemoglobin levels, showed a marked improvement in iron nutrition and, as stated before, only a suggestion of a slight deterioration in terms of whole blood and red cell folate levels. However, no signs of folate deficiency could be observed in these children; furthermore, the administration of folic acid, induced no further hematological changes but was accompanied by a rise in blood folate values, as expected.

The changes in hemoglobin and mean corpuscular volume (MCV) in the children are presented in Figure 19. In the lower part of this Figure, both control and experimental groups have been divided by hemoglobin levels which carry a different risk of anemia. The results already described are evident.

The upper part of Figure 19, shows the distribution of cases into three categories of MCV: those having less than the mean -2 standard deviations (72 cubic microns), those lying between 1 and 2

standard deviations below the mean, and those with a MCV greater than 79 cubic microns, which is the mean -1 standard deviation (1). At the third month of study the control group showed a slight decrease in the group falling between 1 and 2 standard deviations below the mean, with a slight increase in the group with higher MCV. The experimental group had no cases with a MCV below 72, but the number of cases between 79 and 72 was higher than expected for a normal distribution. Therefore, the red cell population of the supplemented group at 3 months still showed some microcytosis.

b) Studies in low-land population groups in Guatemala.

The effect of iron and iron plus folic acid administration on the hemoglobin level of tropical populations in Guatemala is clearly depicted in Figure 20 which shows the distribution of cases in three categories based on the risk of belonging to an anemic population according to the hematological norms for this altitude (1). It is presented in this fashion rather than by hemoglobin levels in order to be able to pool the data of individuals of both sexes and all ages. The distribution of cases in these categories is presented for the norms, for the population before iron or folate administration and also for the groups who participated in the study for 6 months. These include a control group (lactose tablets), and the two experimental groups who received iron or iron

plus folic acid. It would be expected for any normal population to show 16% of cases with a risk greater than 20%, and 7% of cases with a risk greater than 75% of belonging to an anemic population since the values carrying such risks are the mean minus 1 and the mean minus 1½ S.D.- Before iron or folate and after 6 months of no therapy, the percent of individuals with a risk greater than 20% of belonging to a subnormal population was 65%, the majority having a risk greater than 75%. Six months of iron administration markedly improved the picture, reducing those with high risks to 19% and those with a risk greater than 20% to 37% of cases. However, the administration of iron and folic acid produced a further improvement in the population, reducing to 11 the percent of individuals with risks greater than 75% and to 23 the percent of individuals with risks greater than 20%. It is important to reiterate that the population studied had hookworm infection and that the three groups studied again six months after the initiation of the program did not differ in the severity of this infection. Furthermore, in this sample a causal relationship could not be demonstrated between the severity of hookworm infection by egg counts and a hemoglobin level carrying a high risk of belonging to an anemic population in the individuals before or after receiving iron or iron plus folate.

In the iron treated group the subjects who remained with low hemoglobin levels, carrying over 75% risk of anemia, consisted of 4 children, 9 adult women, 3 of whom were lactating, and 10 adult men. In the group receiving iron plus folic acid, 4 were children, 1 was a lactating woman and 2 were adult men.

Table 13 shows the hematological characteristics of the subjects who had complete hematological and biochemical studies before and after 6 months of iron or iron plus folic acid administration.

The findings in this population before therapy are very similar to those obtained in the Central American population living between 0-750 m above sea level: the group with greater risk had a greater percentage of individuals with low serum iron and saturation of TIBC. Among the same population, those with hemoglobin levels carrying little risk of belonging to an anemic population had a better biochemical profile regarding iron nutritional status. In contrast, an elevated percent of subjects with low serum and red cell folates was present regardless of the risk of anemia carried by their hemoglobin level.

In the iron supplemented group, the biochemical indices of iron nutrition revealed an improvement since the percent of subjects with higher saturation of TIBC increased even among the group with hemoglobin levels still carrying a high risk of being below normal. On the other hand, the biochemical indices of folate nutrition suggest a deterioration among this group who was receiving no extra folic acid: a higher number of individuals had serum folates below 3 ng/ml and red cell folates below 70 ng/ml. Concomitant to this deterioration in red cell folates, the prevalence of macrocytosis (MCV $>$100 u^3) increased from 14% to 34%.

The group who received iron plus folic acid attained a hematological and biochemical status which is the closest to normal in the groups so far studied, in spite of having widespread hookworm infection. Eleven percent (7 subjects) still had hemoglobin levels compatible with a risk greater than 75% of being below normal. Their biochemical profile, however, was not suggestive of a predominant iron or folate deficiency. Their hematological indices did not clarify the picture either. The same can be stated of the 8 cases which comprised 12% of this group, whose hemoglobin levels fell in the 20 to 75% risk category.

The analysis of the results obtained in terms of distributions of hemoglobin concentrations and packed cell volumes in specific age and sex groups allows us to define further what is a normal hematological status for this population and to establish the

risks carried by different hemoglobin concentrations and PCV in these age groups. The distribution of hemoglobin concentrations in non pregnant women, 21-49 years of age in the Guatemalan lowland after 6 months of iron or iron plus folic acid administration are compared to the Central American norms and those proposed for Swedish women after iron administration (1, 7) (Figure 21). The mean hemoglobin value for the Swedish population is lower than the mean of the norms proposed by us and of the means obtained after either iron or iron plus folic acid administration. The dispersion of data around the mean is largest in the population who received iron alone, followed by the Central American norms and by both the values obtained after iron + folate administration and the Swedish norms. These last two are essentially identical. The wider distribution of the Central American norms as well as their higher mean hemoglobin can be explained on the basis of the differences in altitudes among the populations studied (0-750 m for the norms) while the supplemented population resided at 200 m above sea level. The same plots for men 21-49 years of age are presented in Figure 22. In this population, the lowest mean

hemoglobin was obtained in those individuals who received only iron supplementation. This group also showed the widest amplitude of values, many of them falling clearly below the distribution obtained from any of the other distributions. The iron plus folic acid supplemented group had a higher mean hemoglobin concentration and the distribution was narrower than that of the iron supplemented men or of the Central American norms (1). The norms proposed by Garby (7) for men have a narrower distribution and their mean falls between that of the iron plus folic acid supplemented group and of the Central American norms. Table 14 presents the means and standard deviations of all of the distributions presented in Figures 21 and 22.

Figure 23 shows the distribution of PCV in the women 21-49 years of age in the Guatemalan lowlands before and after iron or iron plus folic acid administration and also in comparison to both previously mentioned norms (1,7). The supplemented population was further divided in two groups, those who responded to iron or iron plus folic acid administration with a rise greater than 3% in PCV and those which did not respond to the administration of the supplements. As can be seen, those who responded belong to a popu-

lation whose mean PCV was 29.2 while those who did not respond had to start with, a mean PCV of 38.8. The standard deviation of this group of non responders was 1.6 which compares very favorably with the Swedish norms and with the same group after iron plus folic acid administration.

From these distributions the risks carried by different PCV of belonging to a population with subnormal PCV can be estimated. These risks are presented in Figure 24 for the population of women 21-49 years of age in the lowlands of Guatemala. The risks established on the basis of the Central American norms (1) and those from the Swedish women according to data of Garby (7) are presented together with the risks calculated from the supplemented populations, considering: a) the values prior to supplementation of the responders versus the non responders; b) the responders before supplementation versus after supplementation with iron plus folic acid, and c) the responders prior to supplementation versus the Central American norms for this age group. A risk of 50% is attained in all groups with PCVs between 35.5 and 36.8. On the other hand, at a PCV of 35.5 the maximal risk is 80%, the minimum being 50%; while at a PCV of 36.8 the minimal risk is 26%, the maximum being 50%. If the mean PCV for 50% risk

is calculated, it turns out to be 36.2%. The range of risks at this particular PCV is from 36 to 64%.

HEMATOLOGICAL STATUS OF AGRICULTURAL MALE WORKERS IN GUATEMALA AND THEIR CAPACITY TO PERFORM A NEAR MAXIMAL, SHORT-TERM EXERCISE (HARVARD STEP TEST)

In developing countries the agricultural labor force constitutes an important segment of the population, not only in numbers but also as an economic force. Work in a pre-industrialized situation demands physical effort and work output depends greatly on the physical capacity of the laborer. On the other hand the worker depends on its physical fitness for its productivity. In this population, anemia may be an important handicap in terms of productivity and health. The data presented in Figures 25 and 26, briefly summarize our findings (23, 24) and clearly point out that the physical fitness of agricultural laborers, measured by the Harvard Step Test, increases as their PCV are raised as a consequence of iron or iron plus folic acid administration. The control group, which did not change in PCV had also a stable Harvard Step Test Score throughout the study. The relationship between PCV and Harvard Step Test Score is presented in Figure 27. It can be noted that

the Harvard Step Test Score, which measures cardio-respiratory reserve mostly, increased in an exponential fashion as PCV was higher, up to a PCV greater than 40, where no further significant increments in score are obtained. Below this PCV a change between 5 and 10% in hematocrit is accompanied by a significant change in the Harvard Step Test Score (r= 0.72 between PCV and log Harvard Step Test Score). The value of 40 in PCV corresponds to that derived from the Central American norms below which a risk of anemia greater than 75% is present (1).

DISCUSSION

The different studies in this presentation will be taken as the basis for discussion of hematological norms and criteria for anemia, of the problem of iron and folate deficiency and the repercussions of anemia, and finally, of some considerations on measures for correction and prevention of nutritional deficiencies leading to anemia in Central America.

The Central American norms originally derived from a highly selected Central American population have been very useful, firstly in demonstrating that a tropical population in whom adequate iron,

folate and vitamin B_{12} nutrition has been documented and which is not infected with hookworm has a hematological status which compares favorably to that obtained from healthy non tropical highly developed populations. Secondly, the norms have allowed us to calculate for each age-sex-altitude group the risks of anemia associated to different hemoglobin concentrations or PCVs. The consideration of populations with different risks of anemia, based on hemoglobin or PCV has also permitted us to investigate, on a firmer basis, the etiology of anemia in the Central American population.

The results from the study of pregnant women and newborn children have yielded also what we consider applicable hematological norms for both groups, as well as reasonable risks of anemia associated to different hemoglobin and PCV values, although a higher number of individuals is desirable.

The results presented on the effects of iron and iron plus folic acid administration, confirm the value of the previously proposed norms and extend their validity by demonstrating that the proposed levels are reachable when iron and folate nutrition is corrected, independent of hookworm infection. Furthermore, these studies have shown that the risks of anemia derived from

different populations follow very similar trends within relatively narrow margins of hemoglobin or packed cell volumes and therefore can be applied to populations in very different geographical areas.

The estimation of the risk carried by a level of hemoglobin or PCV of belonging to an anemic population for different groups of age-sex-altitude and physiological status constitutes a sound basis for the estimation of the prevalence of anemia in a population. During the elaboration of the Central American norms it was noted that the values corresponding to the mean minus 1 standard deviation carried a risk of anemia which varied for different age-sex-altitude groups within a range of 0 to 35%, the mean being 20%. On the other hand, the values corresponding to the mean minus 1½ standard deviations carried a risk which in every group was higher than 50%, and had an average of 75% (1). Upon studying the Central American population to estimate the prevalence of anemia, cummulative distribution plots of hemoglobin or PCV levels were used. It was noted that as a rule the number of cases found with hematological values in the lower range of normal began to be higher than expected from the normal distribution, precisely at or between the levels corresponding

to the mean -1 and the mean -1½ standard deviations of the norms (Figure 28). There were only a few exceptions to this rule. This is a sound argument for the use of the levels of hemoglobin or PCV carrying 20 and 75% risks of anemia in defining populations in terms of hematological status. Subjects having hemoglobin or PCV levels at or below those associated to 75% risk of anemia can be considered anemic until proven otherwise.

This concept of risk based on distributions, on the other hand, implies that within a population group there will always be a certain proportion of non anemic subjects with hemoglobin or PCV levels carrying more than 20 or 75% risk of anemia. These would amount to 16 and 7% respectively. The definition of a level carrying as close to 100% risk of anemia is further necessary in order to catalog if the cases with more than 75% risk of anemia after a supplementation program have hemoglobin or PCV levels within 75 and 100% risk or if their risk is greater. The mean minus two standard deviations constitutes such a level.

The prevalence of anemia for the Central American population included in this report confirm that already published (3), which

indicates that anemia is commonly found at all altitudes, and affects all age and sex groups infected or non infected with hookworm (4). The prevalence of anemia in pregnant women at term, calculated on the basis of cases with risk greater than 50% agrees very closely to the prevalence of anemia in pregnancy derived from distribution plots published from the Latin American study (8). A new finding which needs intensive study is the very high prevalence of unsatisfactory hemoglobin levels found in newborn children from anemic mothers. A striking association was found between mothers with hemoglobin levels carrying a high risk of anemia and newborn children with also very low hemoglobin levels. This association is absent in other studies (23, 24).

The approach to define the etiology of anemia in the Central American population has been that of first forming groups on the basis of the risk of anemia carried by different levels of hemoglobin or packed cell volume. Then, within each group determine the prevalence of deficiency of specific hematinics, based on biochemical and morphological evidence. The most probable etiology

can be put forward if the populations with high risk of anemia have a high prevalence of deficiency of specific hematinics while populations with low risk of anemia have a low prevalence of deficiency of the same hematinics. The confirmation of the cause of anemia will be evident from the results obtained upon administration of the suspected specific hematinics. If this trend of thought is accepted, most of the nutritional anemias in Central America, regardless of altitude above sea level or of hookworm infection, are due to iron deficiency. On the other hand, serum levels of iron and percent saturation of transferrin compatible with iron deficiency are prevalent also in populations with risk of anemia between 20 and 75% and even in populations with less than 20% risk. This agrees with the concept that iron deficiency anemia occurs in populations where iron deficiency is chronic and severe. Large segments of the Central American population apparently suffer from iron deficiency but do not necessarily have anemia; they are, however, highly vulnerable to anemia.

The last statement can be applied to the state of folate nutrition: the prevalence of serum and red cell folate levels

compatible with folate deficiency is very high in the Central American population. However, there is essentially no evidence that folate deficiency is an important cause of nutritional anemias. The reasons for this are not at all clear but the serum and red cell folate levels found in pregnant women and the changes in folate levels observed in the process of iron supplementation of tropical populations help us understand the situation: a) there is a significant inverse relationship between the biochemical evidence of iron deficiency and that of folate deficiency in the Guatemalan pregnant women; b) the administration of iron to a population with a high prevalence of biochemically suspected iron and folate deficiencies resulted in an improvement in iron nutrition and in a concomitant deterioration of folate nutrition as evidenced by lower serum and red cell folates and an increase in the prevalence of macrocytosis even as late as 6 months after the initiation of supplementation.

These findings support the hypothesis that the Central American population is deficient in both iron and folate but that iron deficiency predominates establishing a limit in red cell production and therefore, diminishing the expressivity of folate

deficiency. It is only in those cases with adequate iron nutrition, which can be the consequence of iron supplementation, that folic acid deficiency becomes evident. It is important to recall that the population group who received only iron supplementation did not reach the hematological normality attained by the same population receiving iron plus folic acid. The subjects who received iron but who were still anemic had a noticeable improvement in iron nutrition but several of them had biochemical evidence of inadequate iron and folate nutrition. These subjects were carefully monitored throughout the study so that we are certain of their supplementary iron intake.

The reasons for the inadequate hematological response of the population who received only iron are not clear; but the comparison with the population who received iron plus folic acid suggests that folic acid was the main reason for the better hematological response in this last group. Folic acid administration may result in better hematological status through two mechanisms: a) the administration of folic acid improved a subclinical malabsorption which enhanced the absorption of folic acid and iron, and b) folic acid became a limiting factor in erythropoiesis. This last hypothesis receives some support

from the increase in macrocytosis observed in the population who received iron alone as a supplement, and indirectly from studies in other areas of the world where administration of iron plus folic acid has been necessary to attain a full hematological response (25-28).

We still do not know which is the basic reason for the high prevalence of iron and apparently of folate deficiency in Central America. However, the detailed intake studies of total iron, meat iron and free folates (L. casei-available) in the rural Guatemalan population provide important information. The consideration of the socio-economic level based on an index which defines categories within the socio-economic structure of each community (14), discloses that the population with a low index has a higher prevalence of anemia and a lower intake of total iron and meat iron: 65% of these families consume less than 15 mg of total iron/day, and 76% of them consume less than 1 mg of meat iron per day. Furthermore, the population with a high risk of anemia, based on its low hemoglobin values, has a lower

iron intake than populations with normal hemoglobin levels. The fact that this occurs in hookworm-negative individuals suggests that in the Central American population an inadequate iron intake in a poorly absorbable form (29), is one of the fundamental causes of iron deficiency. It is for this reason that relatively low levels of hookworm infection enhance the prevalence of iron deficiency anemia in the Central American population (4).

Unfortunately, it is impossible to carry out a careful analysis on the characteristics of folate intake. However, the data available is highly suggestive also of inadequate folate intake in Central America, as a consequence of dietary and cooking habits alone. Widespread subclinical malabsorption can still worsen the situation. This aspect is being explored.

Long discussions have taken place regarding the benefits or the lack of benefits which take place by the correction of mild anemia (30, 31). It is often difficult to justify the concern placed on improving the hematological status of individuals with mildly or moderately low hemoglobin concentrations, with the possible exception of pregnant women. Even in this population discussion exists, and few and contradictory results suggest a

benefit to the child as a consequence of an improved hematological condition of the mother (32, 33). However, the association between anemia in mothers and their offspring which is apparent from the data in this report supports the contention that anemia in pregnancy can be harmful to the child. From the association observed between serum iron and folate levels in newborns and mothers another possible harmful effect on the offspring due to nutritional deficiencies in the mother can be suspected. Longitudinal studies of the effect on the child of deficiencies of erythropoietic substances and anemia in the mother must be considered in the near future.

In the labor population of pre-industrialized regions, there is another important reason for concern of even "mild" anemia: it is the excellent correlation found between a near maximal exercise test and the packed cell volume. The consequences of anemia in terms of productivity are being explored at present, but from the findings already at hand a higher productivity in labor-intensive situations can be predicted.

Finally, it is hoped that the evidence presented is convincing to point out the urgency to improve the hematological situation

of the Central American population, and the necessity of intense efforts to find adequate preventive measures of iron deficiency and of actions aimed at improving folate nutrition. We are continuing our efforts along these lines in the hope of finding within the next few years an iron salt which will be adequate to fortify a suitable vehicle to bring an added 10-15 mg of adequately absorbable iron to the population. This level of iron must be tested, and we are in the process of doing so. It is derived from the epidemiological data available which includes level of intake and origin of food iron and probable losses from hookworm and Trichuris infection. From these data it can be estimated that an added 2.5 to 3 mg of absorbed iron would essentially eliminate iron deficiency from Central America. An iron salt that would be absorbed up to 20% in situations of high iron demand would satisfy these added requirements if administered at a dose of 15 mg/day.

TABLE 1

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION

NUMBER OF SUBJECTS IN THE SAMPLE FROM CENTRAL AMERICA

Age years	Altitude Above Sea Level 0-750 m			751-1500 m			1501-2800 m			
	Male		Female	Male		Female	Male		Females	
1-4		234			87			22		
5-8		359			124			27		
9-12		362			134			16		
13-16	154		144	41		33	7		16	
17-49	620		467	125		161	31		53	
50 and over	125		194	33		67	12		10	
Lactating	-		106	-		25	-		15	
Total	899	955	911	199	345	286	50	65	94	3,804

TABLE 2

PERCENTAGE OF INDIVIDUALS IN EACH ALTITUDE-AGE-SEX CATEGORY WITH RISK GREATER THAN 20% AND GREATER THAN 75% OF BELONGING TO A POPULATION WITH HEMOGLOBIN CONCENTRATION BELOW NORMAL

		ALTITUDE ABOVE SEA LEVEL								
		0-750 m			751-1500 m			1501-2800 m		
Age years	Sex	No.	20% Risk (% Subjects)	75% Risk (% Subjects)	No.	20% Risk (% Subjects)	75% Risk (% Subjects)	No.	20% Risk (% Subjects)	75% Risk (% Subjects
1-4	M-F	234	42	28	87	46	25	22	55	14
5-8	M-F	359	31	9	124	11	8	27	26	0
9-12	M-F	362	23	13	134	19	10	16	32	13
13-16	M	154	23	14	41	20	10	7	0	0
17-49	M	620	21	10	125	18	11	31	13	0
50+	M	125	28	19	33	3	0	12	0	0
13-16	F	144	30	22	33	36	12	16	38	25
17-49	F	467	17	12	161	22	8	53	39	24
50+	F	194	25	14	67	40	16	10	60	40
Lactating	F	106	27	18	25	28	20	15	53	33

TABLE 3

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION

LEVELS OF SERUM IRON, % SATURATION OF TOTAL IRON BINDING CAPACITY (TIBC), SERUM FOLATES AND RED CELL FOLATES USED AS LIMITS FOR DISTRIBUTION OF CASES IN THE BIOCHEMICAL EVALUATION

Serum Iron (mcg/100 ml)	% Saturation of TIBC %	Serum Folates (ng/ml)	Red Cell Folates (ng/ml)
< 50.0	< 10.0	< 3.0	< 70
50.0 - 74.9	10.0 - 14.9	3.0 - 4.9	70-99
75.0 - 99.9	15.0 - 19.9	4.9 - 6.9	100-139
> 99.9	20.0 - 24.9	> 6.9	> 139
	> 24.9		

TABLE 4

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION

DISTRIBUTION OF CASES (IN %) WITH DIFFERENT SERUM IRON CONCENTRATIONS, GROUPED BY RISK OF HAVING HEMOGLOBIN LEVELS BELOW NORMAL, AND ALTITUDE ABOVE SEA LEVEL

Chance of Hb below normal		0-750 m			751-1500 m			1500 - 2800 m		
		>75%	20-75%	< 20%	>75%	20-75	< 20%	>75%	20-75	< 20%
Number of Cases		390	276	2099	100	90	640	31	38	140
		% Cases	% Cases	% Cases	% Cases	%Cases	% Cases	% Cases	% Cases	% Cases
Serum Iron (ug/100 ml)										
< 50	$\bar{X}$	53.0	19.0	9.7	47.3	15.5	9.1	61.3	36.8	15.6
	S. E.	4.7	2.7	1.5	7.4	5.3	1.5	----	----	2.1
50.0-74.9	$\bar{X}$	24.5	29.2	24.5	28.0	28.0	24.9	19.4	26.3	30.3
	S. E.	3.4	4.6	2.0	4.6	9.5	2.9	----	----	3.7
75.0-99.9	$\bar{X}$	11.5	29.3	33.1	15.0	30.0	35.0	12.9	15.8	21.8
	S. E.	2.0	2.2	2.0	7.6	8.0	2.1	----	----	4.2
> 99.9	$\bar{X}$	12.1	19.5	32.8	9.2	21.5	30.8	6.4	21.0	32.2
	S. E.	2.6	2.7	3.8	3.6	9.5	4.2	----	----	8.2

TABLE 5

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION

DISTRIBUTION OF CASES (IN %) WITH DIFFERENT PERCENT SATURATION OF THE TOTAL IRON BINDING CAPACITY (TIBC), GROUPED BY RISK OF HAVING HEMOGLOBIN LEVELS BELOW NORMAL, AND ALTITUDE ABOVE SEA LEVEL

Chance of Hb being below normal		ALTITUDE ABOVE SEA LEVEL 0-750 m >75%	0-750 m 20-75%	0-750 m < 20%	751 - 1500 m >75%	751 - 1500 m 20-75%	751 - 1500 m < 20%	1501 - 2800 m >75%	1501 - 2800 m 20-75%	1501 - 2800 m < 20%
Number of Cases		390	276	2099	100	90	640	31	38	140
% Saturation T I B C		% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cas
< 10.0	$\bar{X}$	38.7	10.6	1.5	26.8	3.3	2.5	39	3	6.0
	S. E.	3.1	3.2	0.4	8.7	2.4	0.3	--	-	3.1
10.0-14.9	$\bar{X}$	17.4	18.9	8.7	24.3	30.0	7.4	26	29	13.3
	S. E.	2.6	2.7	1.5	2.6	12.0	1.8	--	--	3.3
15.0-19.9	$\bar{X}$	14.3	15.9	14.2	8.3	11.3	12.3	10	26	23.5
	S. E.	2.8	2.5	1.5	3.8	5.2	1.6	--	--	5.6
20.0-24.9	$\bar{X}$	10.1	20.7	18.6	16.0	13.2	24.3	13	16	11.4
	S. E.	2.0	1.6	1.1	4.5	4.1	3.1	--	--	2.2
> 24.9	$\bar{X}$	19.0	33.8	56.9	24.0	32.1	54.2	13	26	45.6
	S. E.	3.5	5.4	3.6	7.1	8.4	3.2	--	[illegible]	7.1

TABLE 6

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION

DISTRIBUTION OF CASES (IN %) WITH DIFFERENT SERUM FOLATE CONCENTRATIONS, GROUPED BY RISK OF HAVING HEMOGLOBIN LEVELS BELOW NORMAL, AND ALTITUDE ABOVE SEA LEVEL

		ALTITUDE ABOVE SEA LEVEL								
		0-750 m			751 - 1500 m			1501 - 2800 m		
Chance of Hb being below normal		>75	20-75	<20	>75	20-75	<20	>75	20-75	<20
Number of Cases		390	276	2099	100	90	640	31	38	140
Serum Folates ng/ml		% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cases	% Cases
Children 1-12 years										
<3.0	$\bar{X}$	16.0	6.7	11.7	4.7	16.0	6.3	20.0	10.5	9.8
3.0 - 4.9	$\bar{X}$	27.0	27.0	24.3	34.3	14.0	20.0	0.0	26.3	34.1
5.0 - 6.9	$\bar{X}$	18.7	26.0	23.3	16.3	15.7	16.3	40.0	15.8	22.0
6.9	$\bar{X}$	38.7	40.3	40.0	44.3	54.7	56.7	40.0	47.4	34.1
Subjects above 12 years										
3.0	$\bar{X}$	37.1	43.1	35.6	23.6	15.1	24.7	42.3	42.1	31.3
	S.E.	3.9	8.8	5.4	11.0	7.2	6.7	-	-	5.4
3.0 - 4.9	$\bar{X}$	28.6	25.7	34.6	29.1	44.6	31.0	34.6	21.0	54.6
	S.E.	2.5	2.9	2.4	8.2	15.5	1.7	-	-	6.9
5.0 - 6.9	$\bar{X}$	15.4	8.0	14.4	11.1	20.7	16.3	3.8	10.5	4.0
	S.E.	2.5	2.9	2.4	3.8	8.2	2.0	-	-	4.4
>6.9	$\bar{X}$	19.0	22.7	15.1	21.6	19.4	28.1	19.2	15.8	9.1
	S.E.	2.9	5.5	3.8	7.3	8.3	6.3	-	-	4.7

TABLE 7

HEMATOLOGICAL STATUS OF PREGNANT AND NON PREGNANT WOMEN OF REPRODUCTIVE AGE. PERCENT RISK OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION, ASSOCIATED WITH VARYING HEMOGLOBIN CONCENTRATION LEVELS AT TWO ALTITUDES ABOVE SEA LEVEL

RISK %

Hb	Sea Level		1850 m Above Sea Level	
(g/100 ml)	Non Pregnant	Pregnant*	Non Pregnant	Pregnant
14.5			0	
14.0			2.6	
13.5		0	18.8	
13.0	0	1.6	43.2	0
12.5	25.4	4.7	70.5	5.8
12.0	53.8	8.1	94.7	17.5
11.5	78.5	11.2	100.0	32.0
11.0	100.0	16.1		45.0
10.5		23.6		59.5
10.0		36.1		77.6
9.5		54.8		89.3
9.0		72.4		95.0
8.5		85.7		98.3
8.0		93.3		100.0

* Calculated from Cook et al. (1971)

TABLE 8

HEMATOLOGICAL STATUS OF PREGNANT WOMEN AT TERM.

BLOOD LEVELS INDICATIVE OF IRON AND FOLATE NUTRITION IN 100 CASES.

DISTRIBUTION BY HEMOGLOBIN LEVELS CARRYING VARIOUS RISKS OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION

% Risk		>50	50-20	< 20
Hb (g/100 ml)		<11.0	11.0 - 11.9	>11.9
			PERCENT OF CASES	
Individuals		22	13	65
M C V (u^3)	<78	43	0	3
	78-87	24	41	36
	88-97	29	50	43
	>97	5	8	18
M C H (pg)	<26	48	8	3
	26-27	10	0	13
	28-29	5	58	31
	>29	38	33	52
M C H C (%)	< 32	43	25	8
	32	17	25	14
	33	5	17	32
	> 33	38	33	45
Serum Iron (ug/100 ml)	< 50	70	54	30
	50-75	24	8	43
	>75	6	38	27
Saturation of T I B C (%)	< 10	76	27	36
	10-14.9	18	18	31
	15-19.9	6	37	24
	>19.9	0	18	9
Serum Folates (ng/ml)	< 3.0	10	35	11
	3-4.9	42	35	34
	5-6.9	16	6	16
	>6.9	32	24	39
RBC Folates (ng/ml)	<70	5	0	0
	70-139	30	33	63
	>139	65	67	37

TABLE 9

HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM. LINEAR REGRESSIONS AND CORRELATION COEFFICIENTS BETWEEN RBC FOLATES (Y) AND % SATURATION OF TIBC (X) FOR WOMEN IN DIFFERENT RISK CATEGORIES OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION

Risk (%)	$\hat{y} = a + b\,x$	r
> 50	$\hat{y} = 178 - 1.9\,x$	- 0.503
> 20	$\hat{y} = 175 - 1.9\,x$	- 0.491
< 20	$\hat{y} = 182 - 3.2\,x$	- 0.280
All Cases	$\hat{y} = 173 - 2.2\,x$	- 0.345

TABLE 10

ORAL ADMINISTRATION OF IRON TO CLINICALLY NORMAL PRE-SCHOOL AGE CHILDREN IN A DAY NURSERY IN GUATEMALA. HEMATOLOGICAL VALUES BEFORE AND AFTER THREE MONTHS OF STUDY

	B A S A L (Control and Fe Administration)		3 M O N T H S			
			Control		Fe Administration	
Number of Children	27		11		16	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Hemoglobin (g/100 ml)	11.5	1.0	10.6	1.4	12.6*°	0.8
Hematocrit (%)	35.0	2.2	34.5	3.0	38.5*°	1.9
RBC Count ($10^6/mm^3$)	4.74	0.36	4.51	0.43	4.80	0.31
MCV (u^3)	74.2	6.4	77.2	11.1	80.2*	3.1
MCH (pg)	24.5	2.8	23.7	4.5	26.3*	1.2
MCHC (%)	32.9	1.4	30.5	1.5	32.8°	1.4

* $p < 0.01$ with basal

° $p < 0.01$ with control 3 months

TABLE 11

ORAL ADMINISTRATION OF IRON TO CLINICALLY NORMAL PRE-SCHOOL AGE CHILDREN

IN A DAY NURSERY IN GUATEMALA.

EFFECTS ON IRON AND FOLATE BLOOD VALUES

(% OF CASES)

		BASAL Control and Fe Administration (N=27)	3 m Control (N=11)	3 m Fe Administration (N=16)
Risk of Hb being below normal	> 75	44.4	54.6	6.2
	20-75	11.1	36.4	12.5
	< 20	44.4	9.1	81.2
Serum Fe (mcg/100 ml)	< 50	74.1	81.8	25.0
	50-99	22.2	18.2	62.5
	>99	3.7	0.0	12.5
% Saturation of T I B C	< 10	61.5	54.6	0.0
	10-19	26.9	36.4	50.0
	> 19	11.5	9.1	50.0
Serum Folates (ng/ml)	< 3	0.0	0.0	0.0
	3-6	37.0	18.2	25.0
	> 6	63.0	81.8	75.0
R B C Folates (ng/ml)	< 70	3.7	0.0	12.5
	70-139	25.9	9.1	37.5
	>139	70.4	90.9	50.0
Whole Blood Folates (ng/ml)	< 30	7.4	9.1	18.8
	30-69	55.6	54.6	62.5
	> 69	37.0	36.4	18.8

TABLE 12

ORAL ADMINISTRATION OF IRON TO CLINICALLY NORMAL PRE-SCHOOL AGE CHILDREN IN A DAY NURSERY IN GUATEMALA. DISTRIBUTION OF CASES (IN %) BY THE RISK OF THE HEMOGLOBIN LEVEL BEING BELOW NORMAL. IRON AND FOLATE BLOOD VALUES

Risk of Hb. being below normal		Basal and Control 3 m. after >75	20-75	<20	Fe Administration for 3 m. >75	20-75	<20
Number of Children		18	7	13	1	2	13
Serum Iron (mcg/100 ml)	<50	94	71	54	100	0	23
	50-99	6	29	38	0	100	62
	>99	0	0	8	0	0	15
% Saturation of T I B C	<10	78	29	38	0	0	0
	10-19	22	29	46	100	0	54
	>19	0	43	15	0	100	46
Serum Folates (ng/ml)	<3	0	0	0	100	0	0
	3-6	56	14	8	0	50	15
	>6	44	86	92	0	50	85
R B C Folates (ng/ml)	<70	6	0	0	100	0	8
	70-139	22	29	15	0	50	38
	>139	72	71	85	0	50	54
Whole Blood Folates (ng/ml)	<30	11	14	0	100	0	15
	30-69	44	71	62	0	100	62
	>69	44	14	38	0	0	23

TABLE 13

HEMATOLOGICAL CHARACTERISTICS OF A LOW LAND GUATEMALAN POPULATION, BEFORE AND AFTER SIX MONTHS OF IRON OR IRON PLUS FOLATE ADMINISTRATION.

INDIVIDUALS ARE GROUPED BY THE RISK (%) OF BELONGING TO A SUBNORMAL POPULATION, BASED ON THEIR HEMOGLOBIN CONCENTRATIONS

		Before Therapy			Iron			Iron + Folic Acid		
Risk (%)		>75	20-75	<20	>75	20-75	<20	>75	20-75	<20
Number of Individuals		96	18	104	18	14	35	7	8	51
		PERCENT OF CASES								
Individuals (%)		44	8	48	27	21	52	11	12	77
Fe (ug/100 ml)	< 50	76	35	20	78	8	11	43	50	25
	50-74	13	24	25	11	58	26	0	25	45
	75-99	7	35	31	6	25	43	57	25	20
	>99	4	6	24	6	8	20	0	0	10
Saturation of T I B C (%)	<10	61	11	9	22	7	0	33	17	2
	10-14.9	19	37	12	33	7	9	0	33	10
	15-19.9	6	32	22	6	36	29	17	33	38
	>19.9	13	21	57	39	50	62	50	17	50
Serum Folates (ng/ml)	< 3	59	42	43	61	62	60	29	0	4
	3-4.9	22	47	26	22	31	11	43	12	6
	5-6.9	7	5	18	6	0	6	14	25	4
	>6.9	12	5	12	11	8	23	14	63	86
RBC Folates (ng/ml)	<70	44	71	64	61	83	82	42	12	25
	70-139	38	24	28	33	17	9	0	38	27
	>139	18	5	8	6	0	9	57	50	47

TABLE 14

HEMOGLOBIN CONCENTRATION OF ADULT LOWLAND GUATEMALAN MALES AND FEMALES AFTER 6 MONTHS OF IRON AND IRON PLUS FOLIC ACID ADMINISTRATION. COMPARISON WITH NORMS.

	Hemoglobin Concentration (g/100 ml)		
	Mean		S. D.
Adult Males			
Central American norms (1)	15.4	±	1.4
Norms proposed by Garby (7)	15.0	±	0.8
After iron administration	14.3	±	1.4
After iron plus folic acid administration	14.8	±	1.2
Adult Females			
Central American norms (1)	13.6	±	1.2
Norms proposed by Garby (7)	12.7	±	0.6
After iron administration	13.1	±	1.3
After iron plus folic acid administration	13.0	±	0.6

FIGURE 1

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. DISTRIBUTION OF CASES (IN %), BY RISK OF ANEMIA, SEX, AGE AND ALTITUDE ABOVE SEA LEVEL

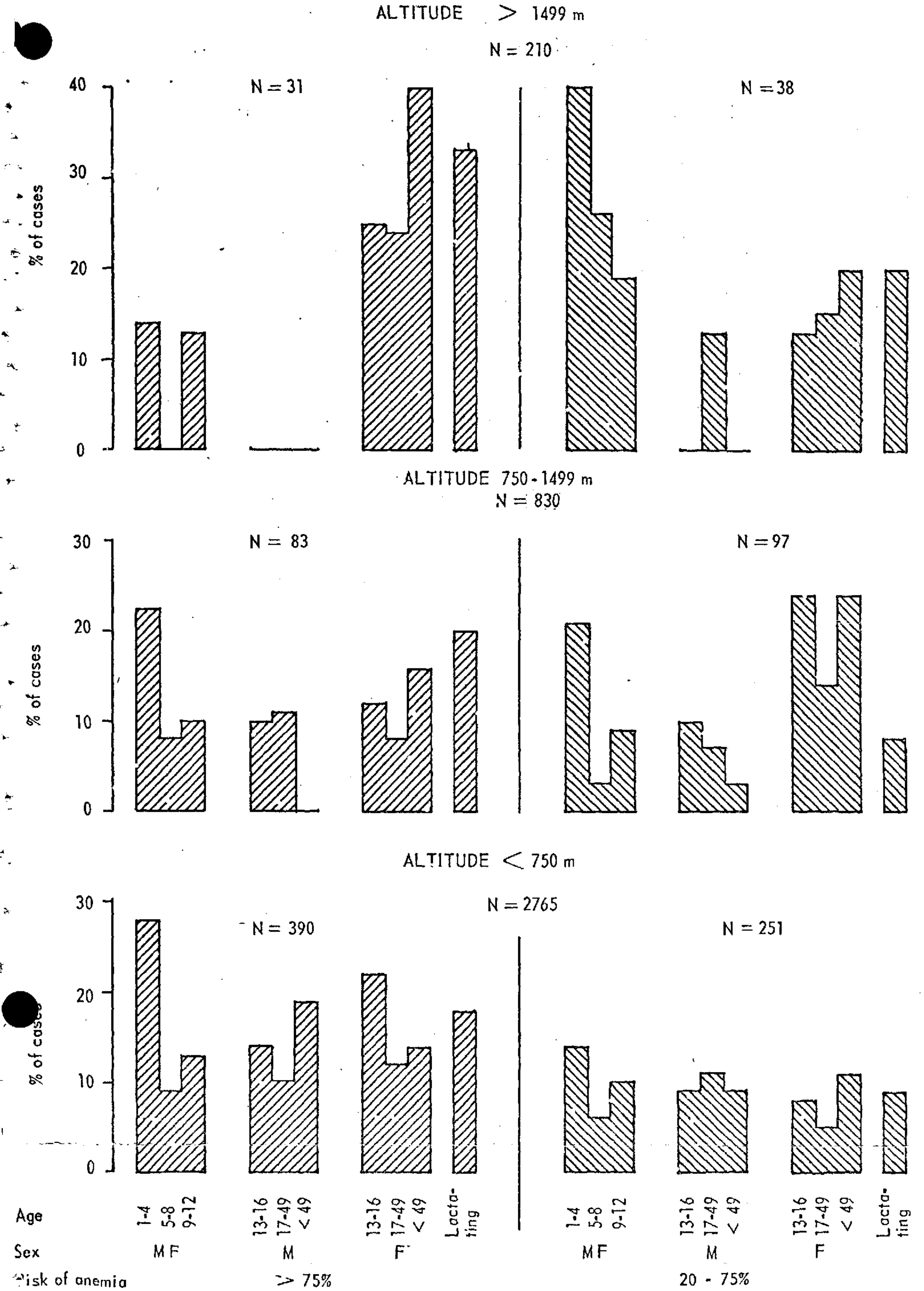

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FIGURE 2

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION.
DISTRIBUTION OF CASES BY THE RISK (%) OF BELONGING TO A POPULATION WITH LOW HEMOGLOBIN CONCENTRATION AND BY SOCIO ECONOMIC INDEX (SEI) IN RURAL GUATEMALA

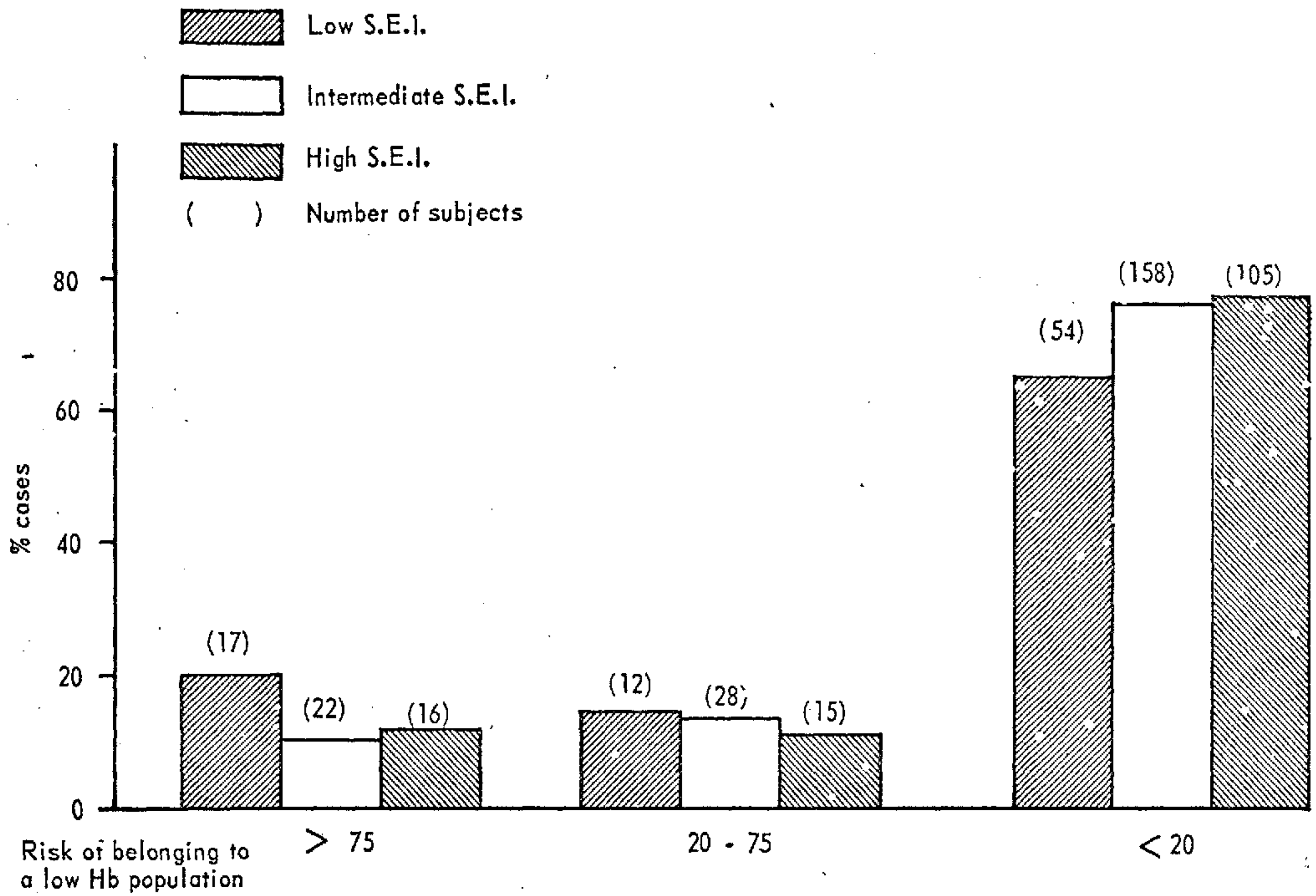

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FIGURE 3

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. IRON INTAKE IN THE RURAL GUATEMALAN POPULATION. DISTRIBUTION OF FAMILIES BY SOCIO ECONOMIC INDEX

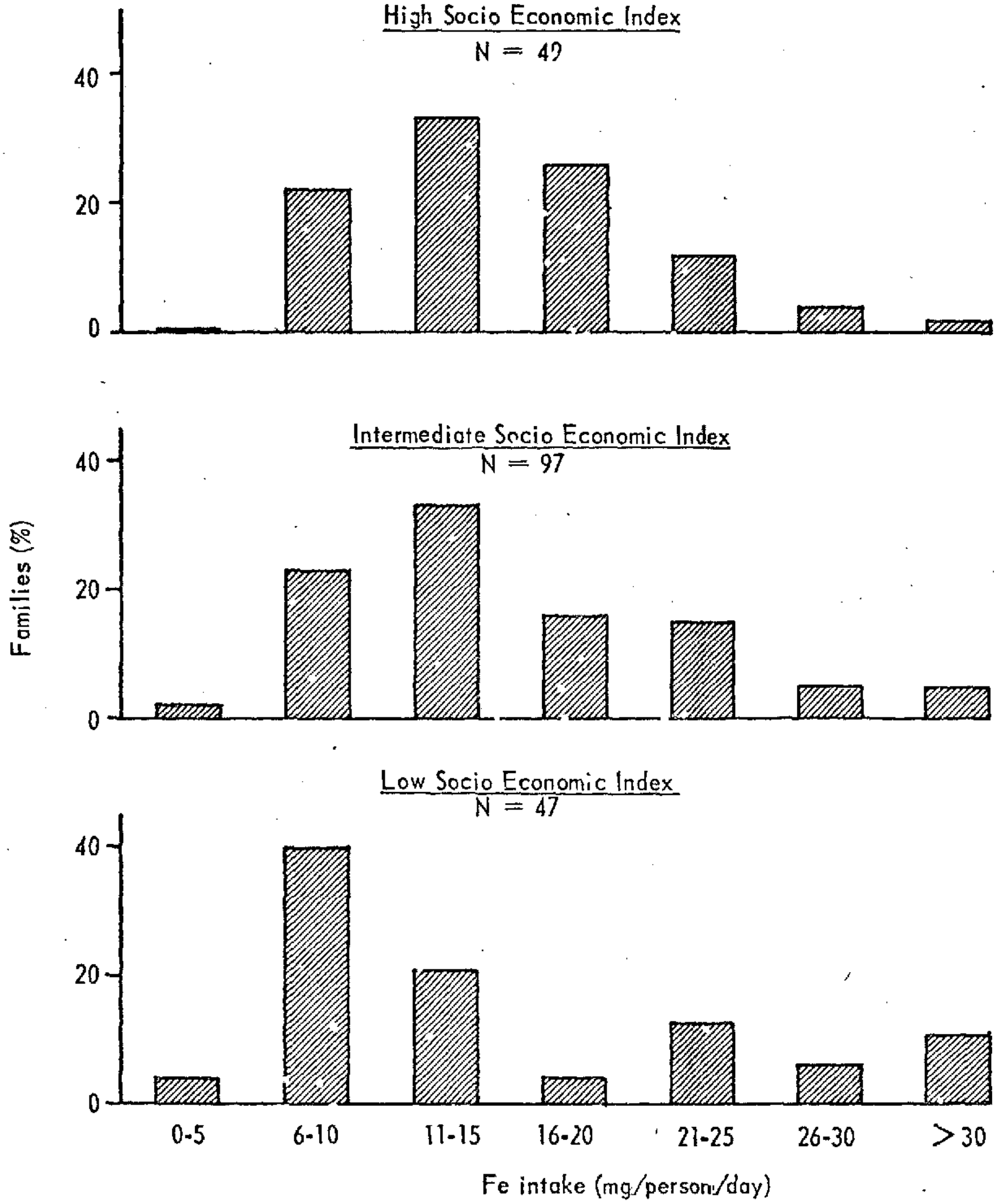

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FIGURE 4
HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION.
MEAT-IRON INTAKE IN RURAL GUATEMALA. DISTRIBUTION OF FAMILIES BY
SOCIO ECONOMIC INDEX (S.E.I.)

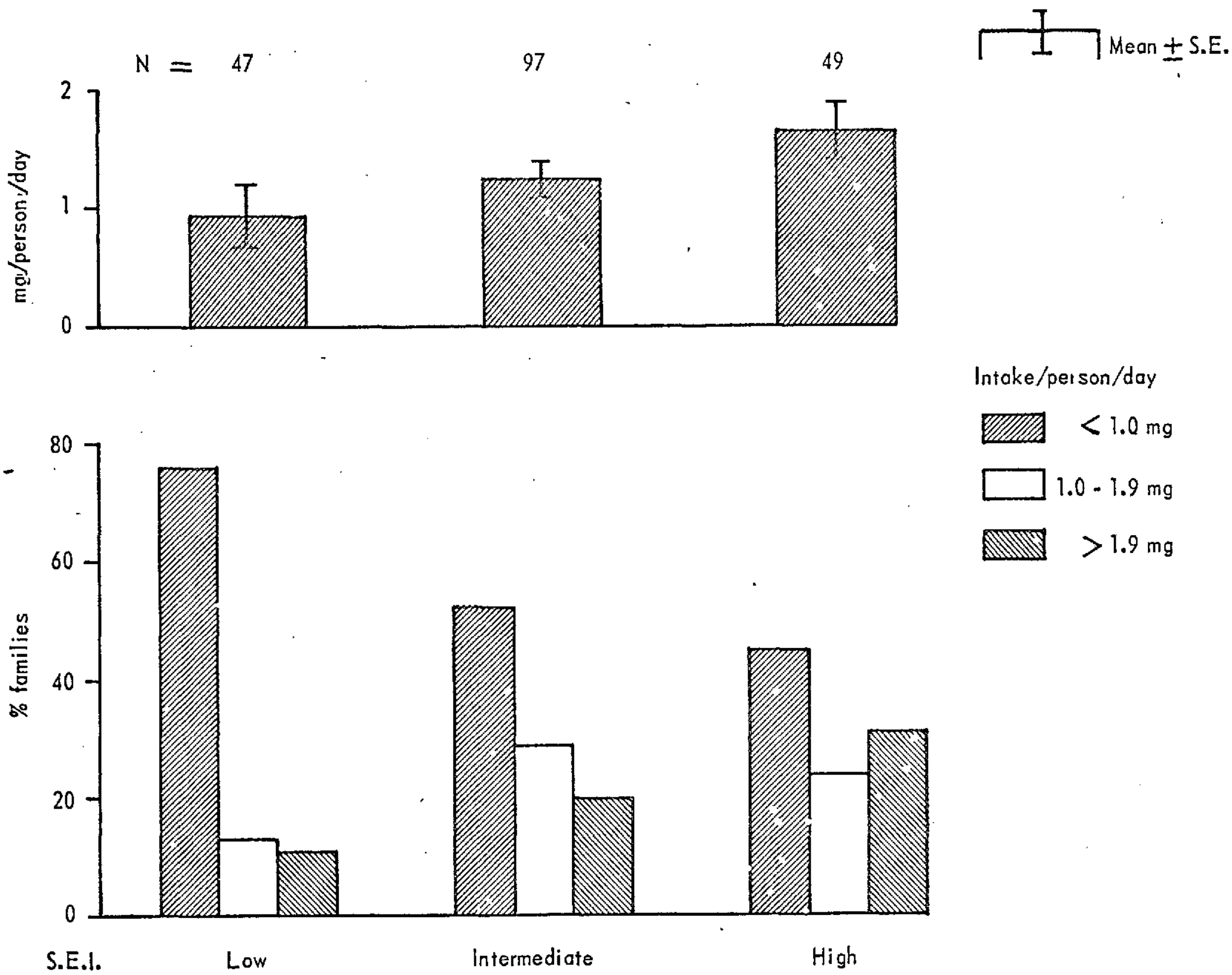

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FIGURE 5

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION.
IRON INTAKE IN HOOKWORM-FREE RURAL GUATEMALANS GROUPED BY ALTITUDE ABOVE SEA LEVEL AND BY THEIR RISK OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION

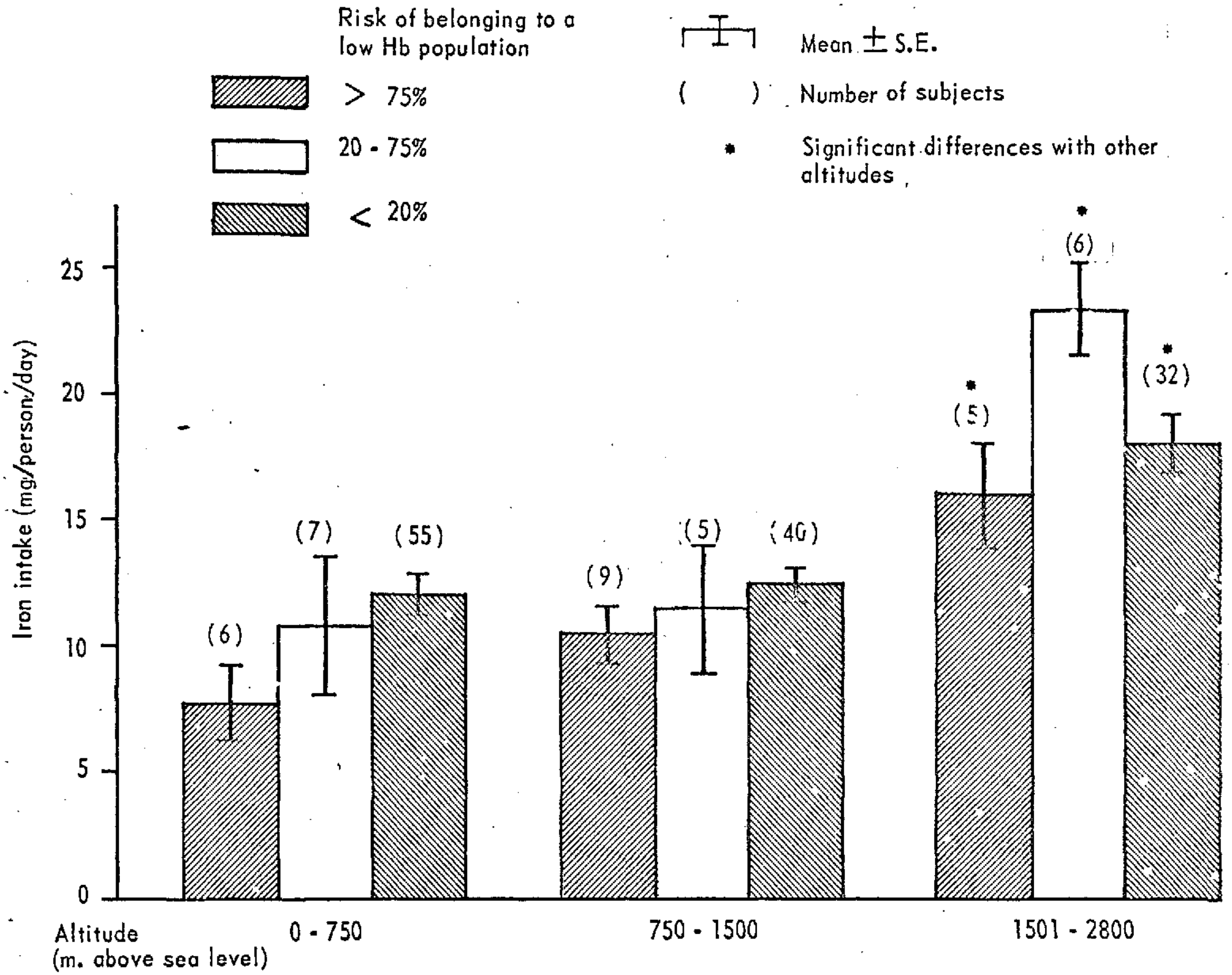

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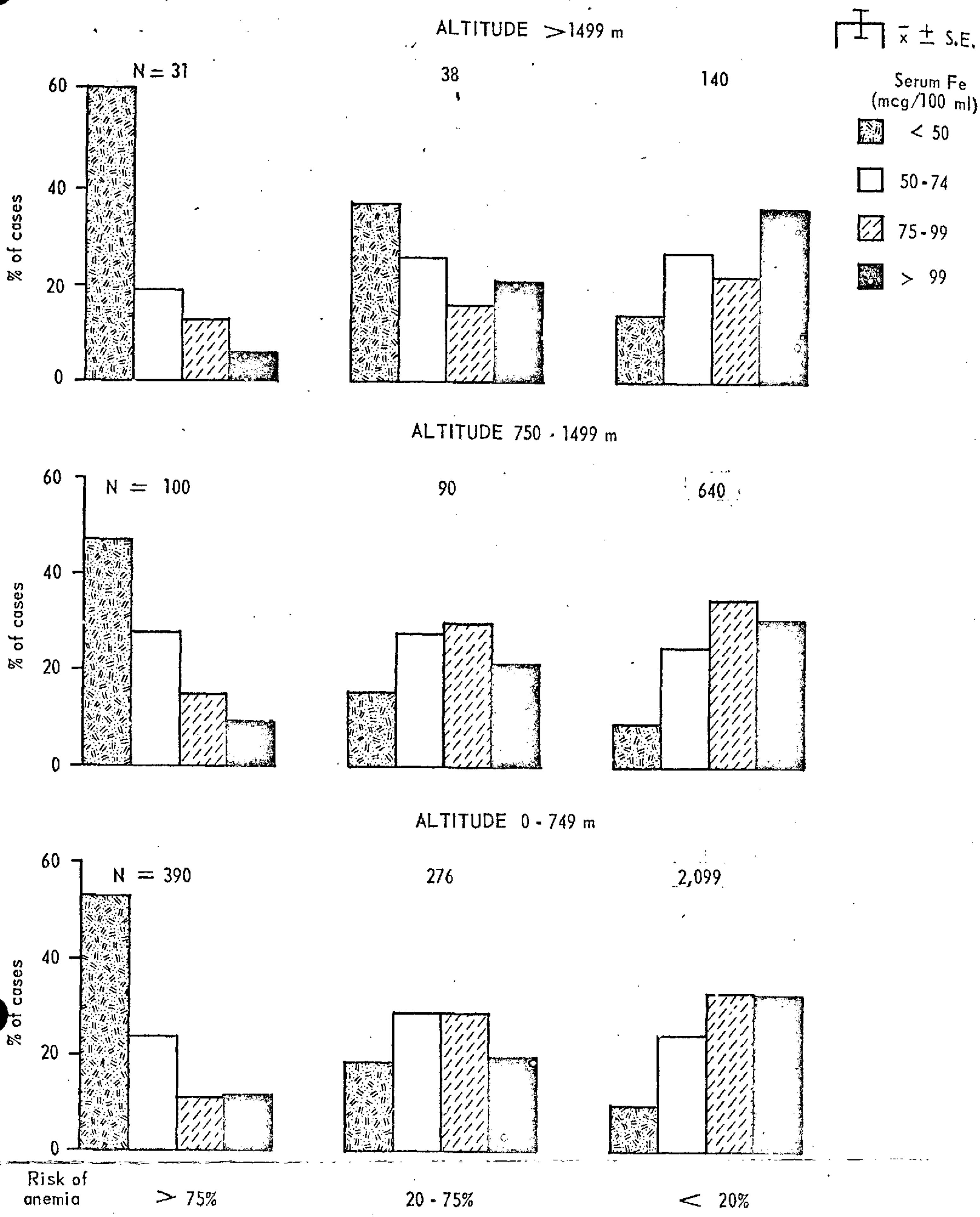
FIGURE 6

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. DISTRIBUTION OF CASES (IN %) WITH DIFFERENT SERUM IRON CONCENTRATIONS, GROUPED BY RISK OF ANEMIA AND ALTITUDE ABOVEA SEA LEVEL



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HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. DISTRIBUTION OF CASES (IN %) WITH DIFFERENT LEVELS OF SATURATION OF TOTAL IRON BINDING CAPACITY (TIBC), GROUPED BY RISK OF ANEMIA AND ALTITUDE ABOVE SEA LEVEL

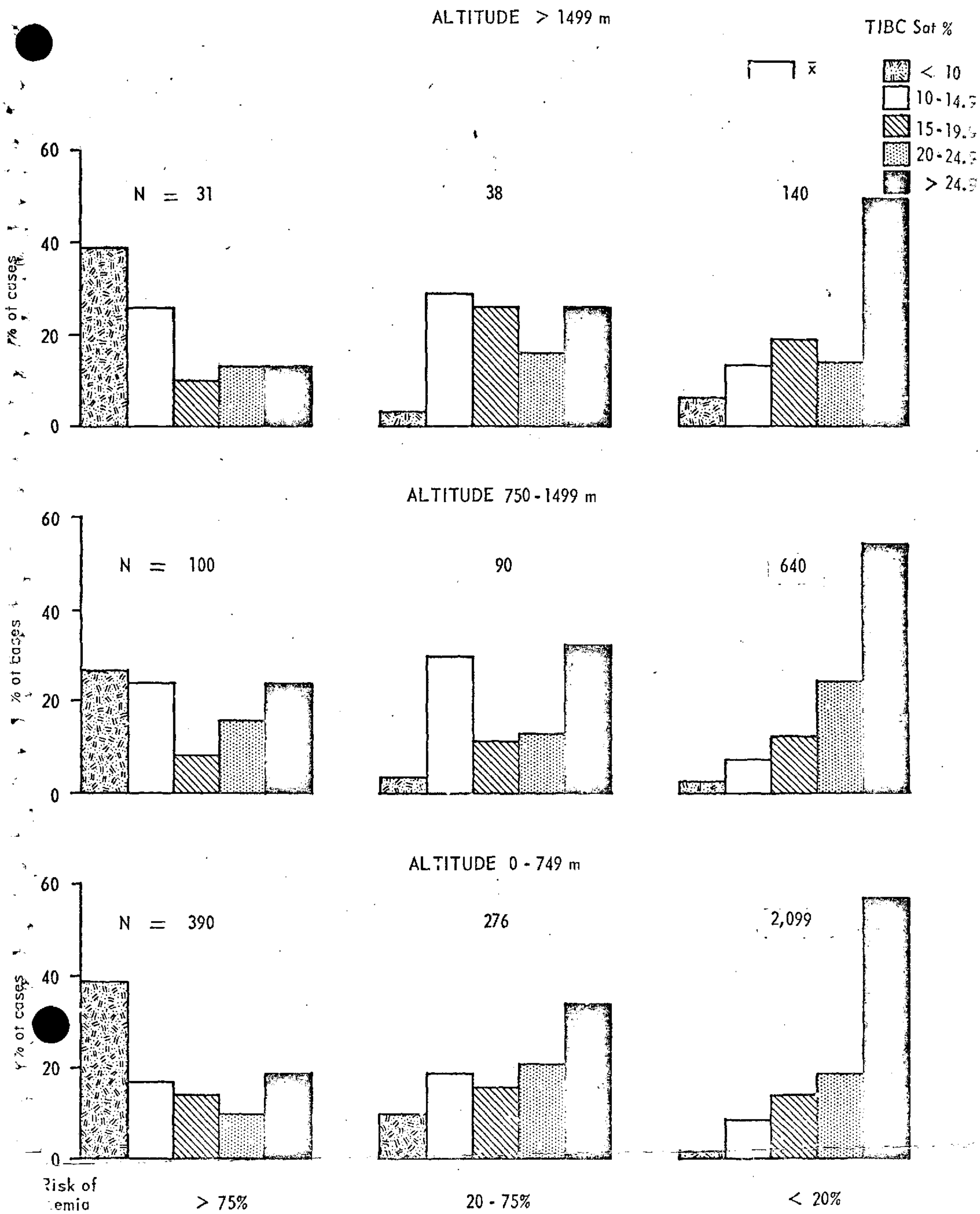

FIGURE 7

FIGURE 8

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. DISTRIBUTION OF CASES (IN %) WITH DIFFERENT SERUM FOLATE CONCENTRATIONS, GROUPED BY AGE, RISK OF ANEMIA AND ALTITUDE ABOVE SEA LEVEL

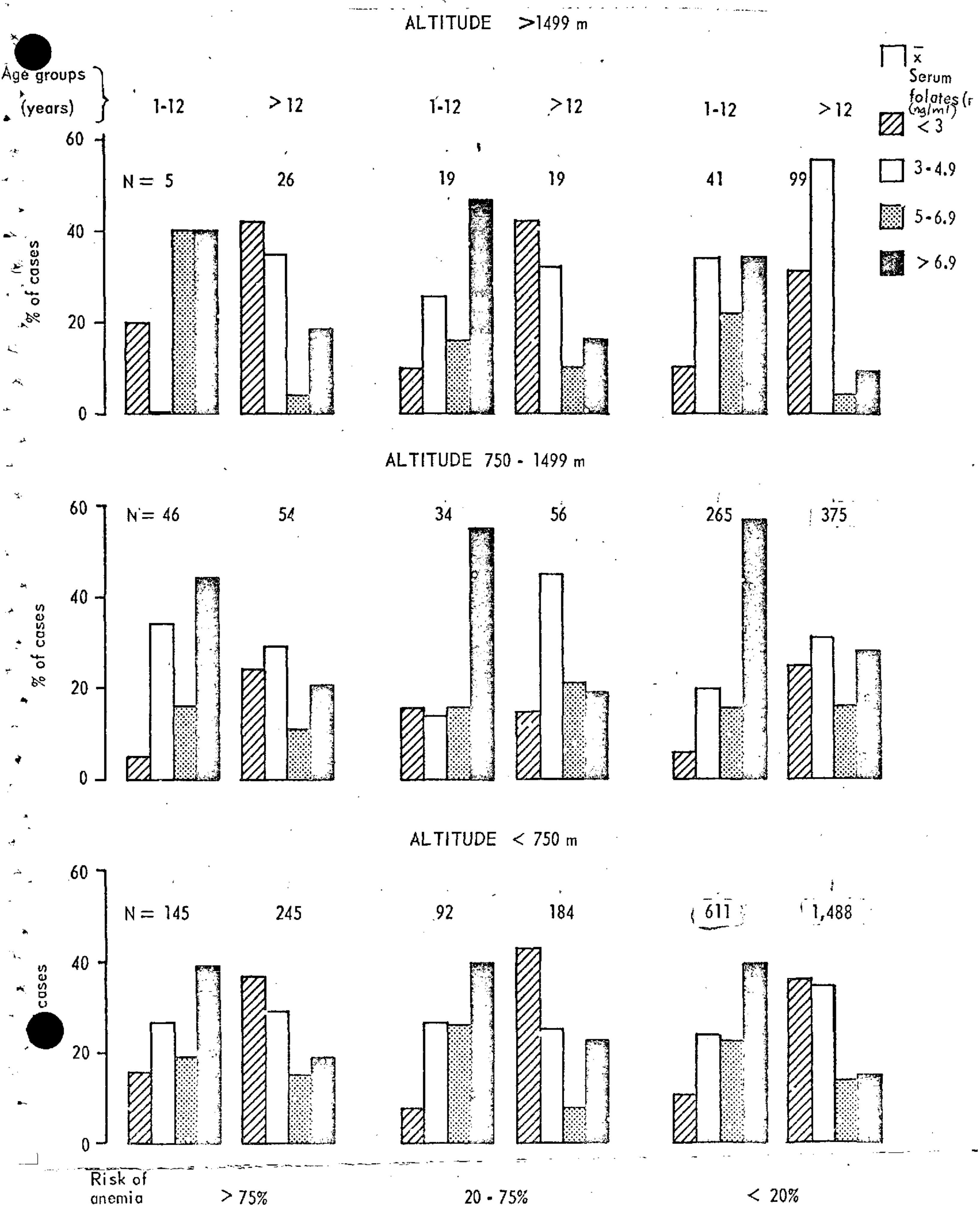

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FIGURE 9

HEMATOLOGICAL STATUS OF THE CENTRAL AMERICAN POPULATION. DISTRIBUTION OF CASES (IN %) WITH DIFFERENT SERUM FOLATE CONCENTRATIONS, GROUPED BY AGE, % SATURATION OF TIBC AND ALTITUDE ABOVE SEA LEVEL

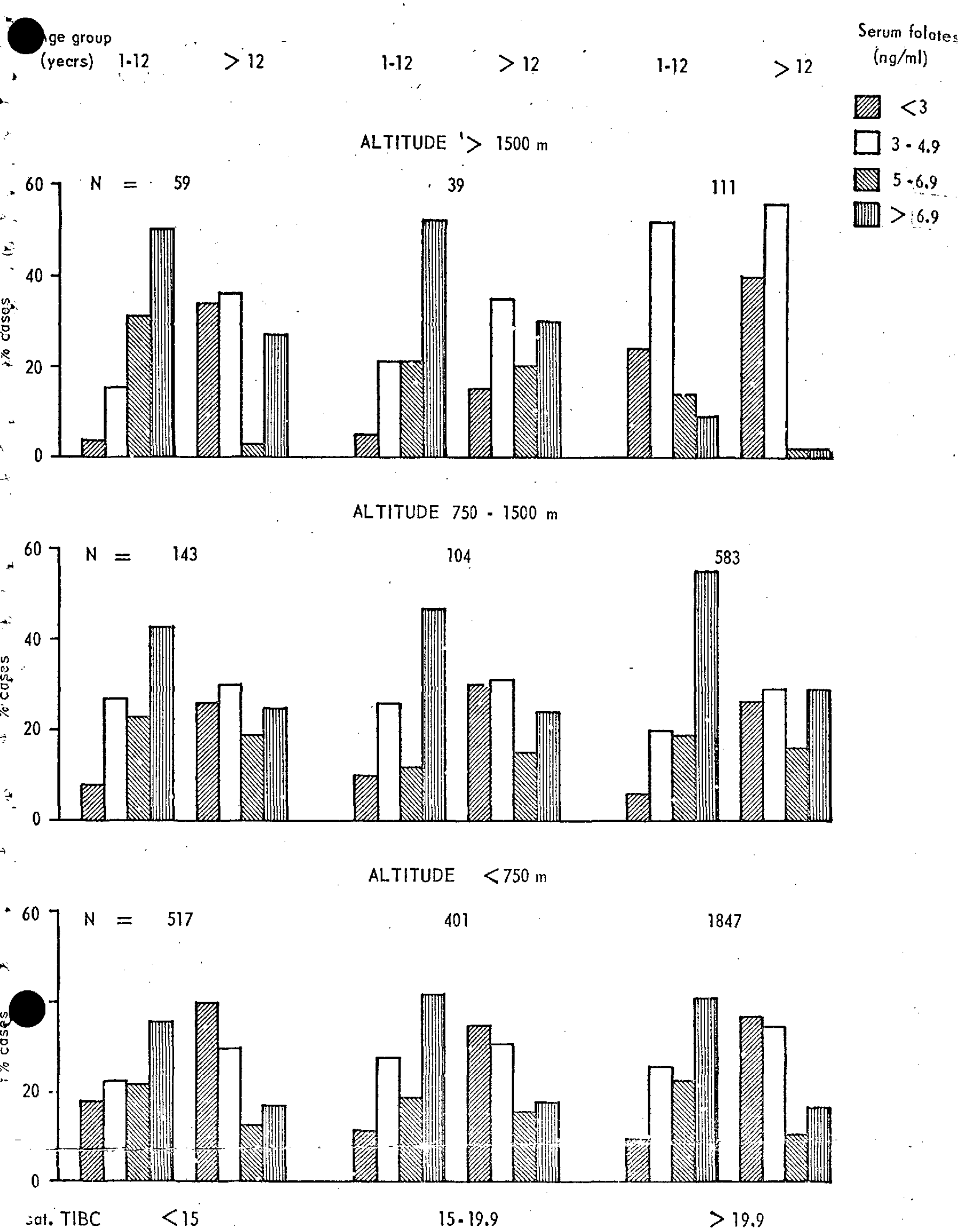

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FIGURE 10
PROBABILITY PLOT OF CUMMULATIVE FREQUENCY DISTRIBUTION FOR HEMOGLOBIN LEVELS IN PREGNANT WOMEN
(GUATEMALA CITY, MEAN ALTITUDE 1850 m)

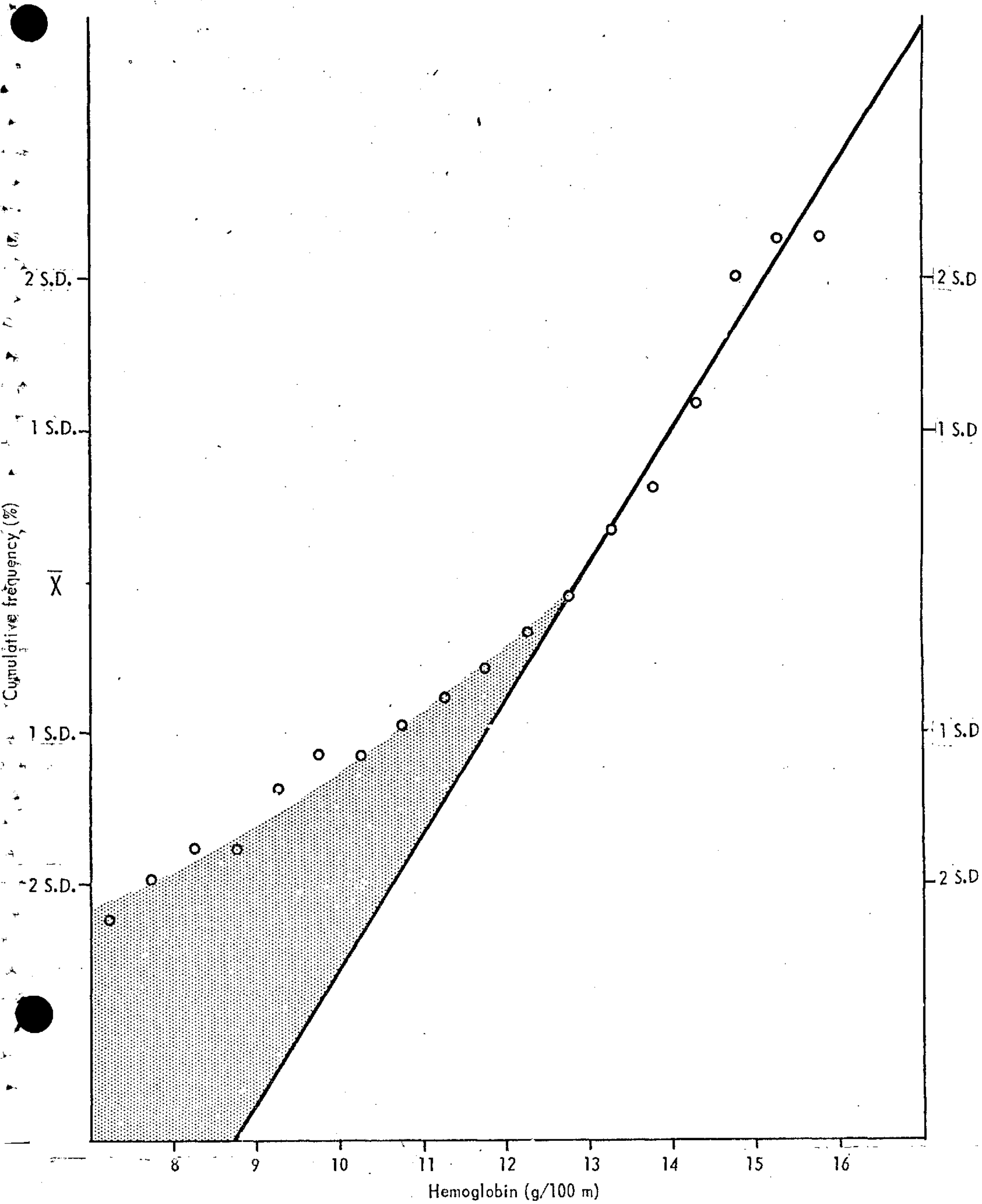

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FIGURE 11 . DISTRIBUTION OF HEMOGLOBIN CONCENTRATION IN PREGNANT GUATEMALAN WOMEN AT TERM (ALTITUDE: 1850 m)

DISTRIBUTION (A) WAS OBTAINED FROM THE NORMALLY DISTRIBUTED SEGMENT OF THE FROBABILITY PLOT OF CUMMULATIVE FREQUENCY DISTRIBUTION

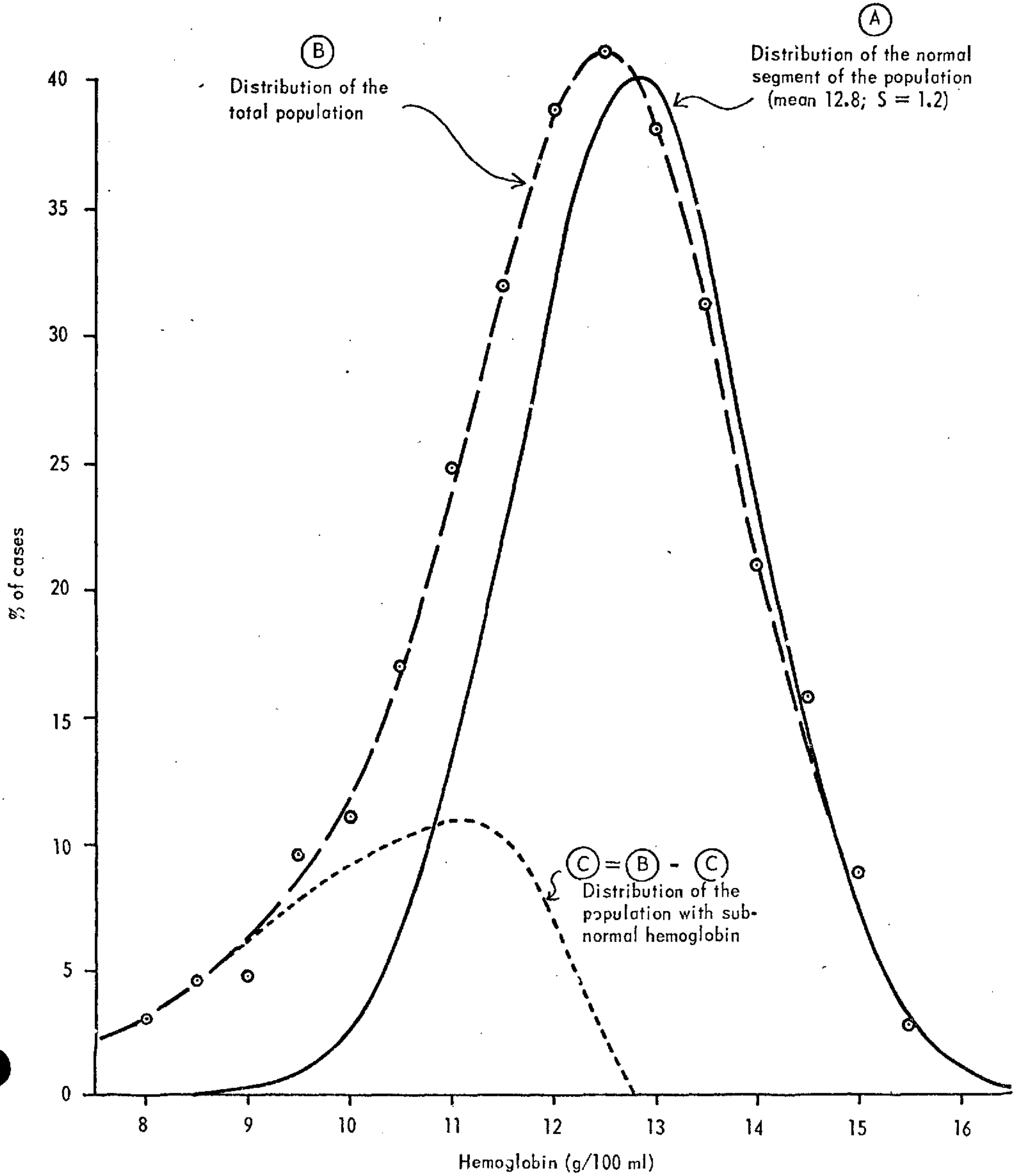

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FIGURE 12 HEMATOLOGICAL STATUS OF PREGNANT AND NON PREGNANT WOMEN OF REPRODUCTIVE AGE. GRAPHIC REPRESENTATION OF THE RISK (%) OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION, ASSOCIATED WITH VARYING HEMOGLOBIN CONCENTRATION LEVELS AT TWO ALTITUDES ABOVE SEA LEVEL

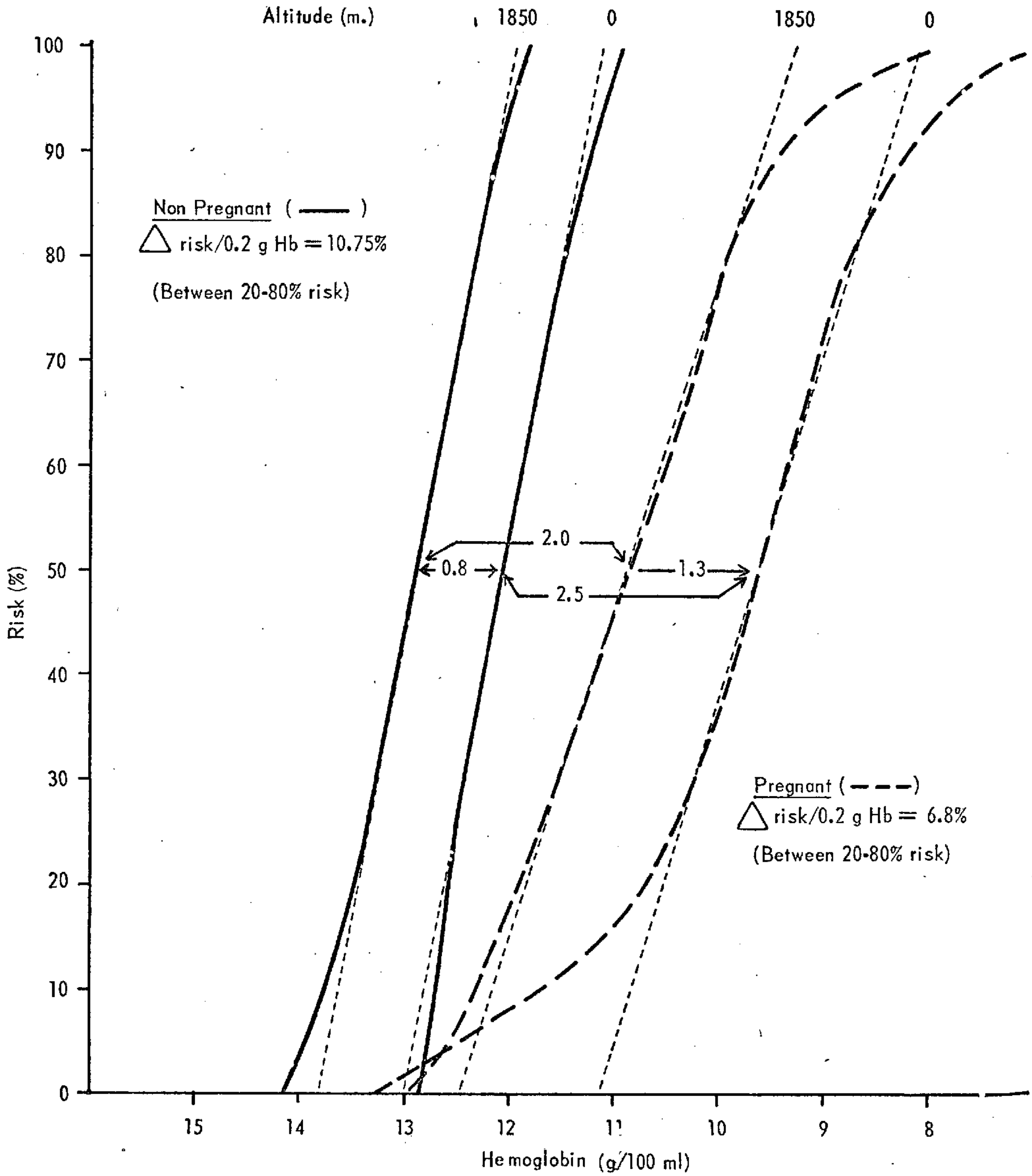

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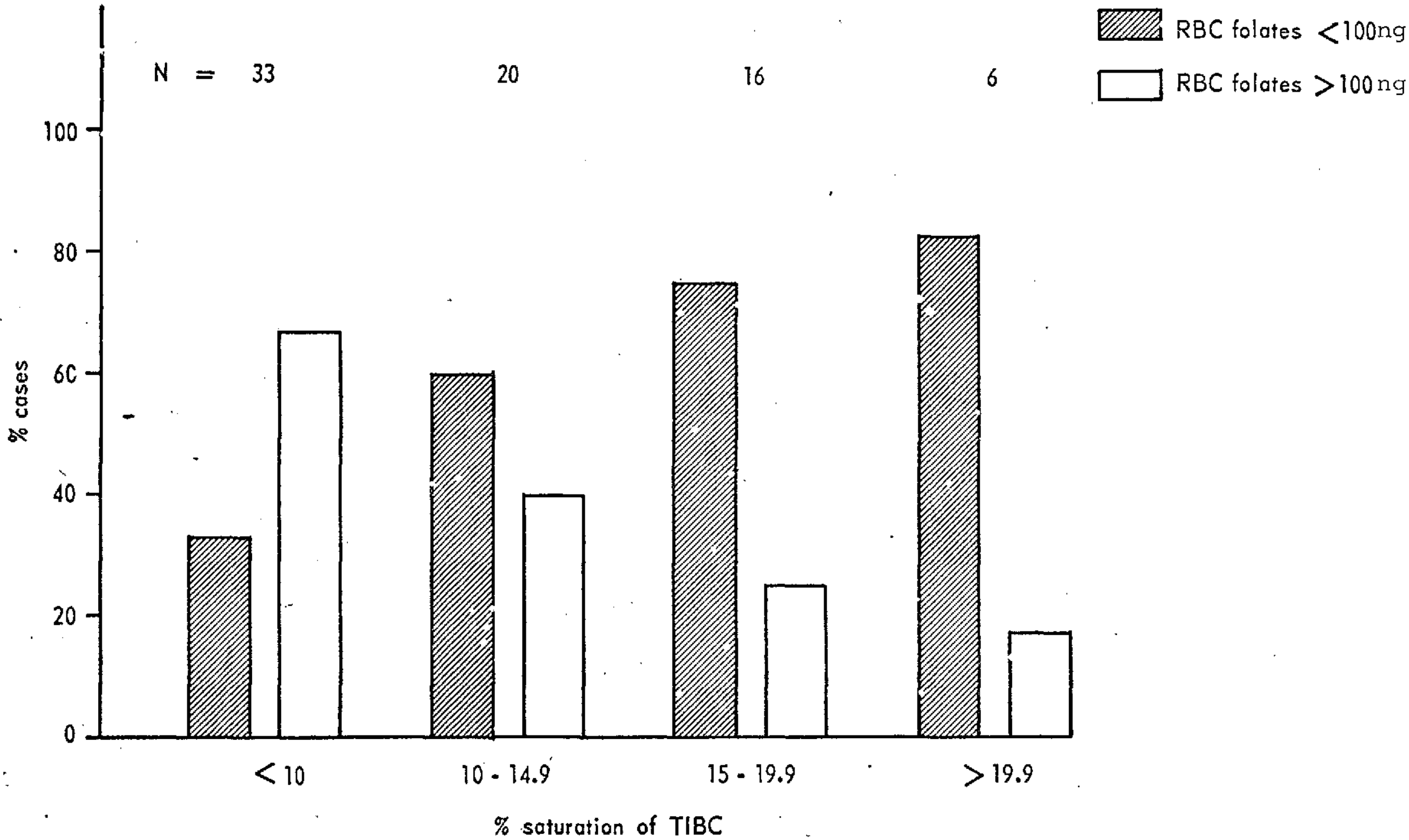
FIGURE 13

HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM.
PERCENT OF CASES WITH LOW AND HIGH RBC FOLATE CONCENTRATIONS
FOR VARYING SATURATION OF TIBC (%)



Incap 73-710

FIGURE 14 HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM. DISTRIBUTION OF CASES (IN %), BY SERUM IRON OR % SATURATION OF TIBC, AND BY MEAN CORPUSCULAR VOLUME (MCV)

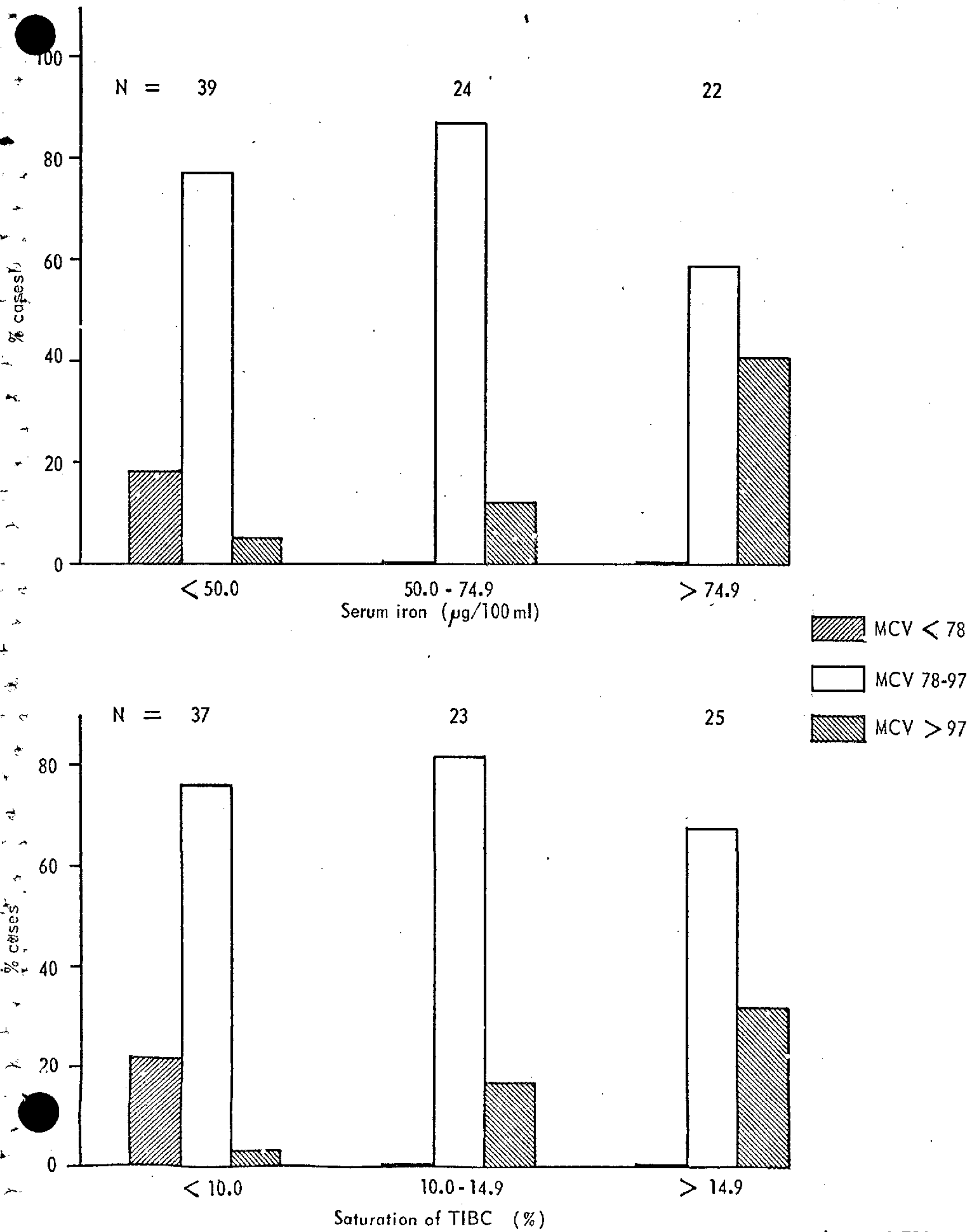


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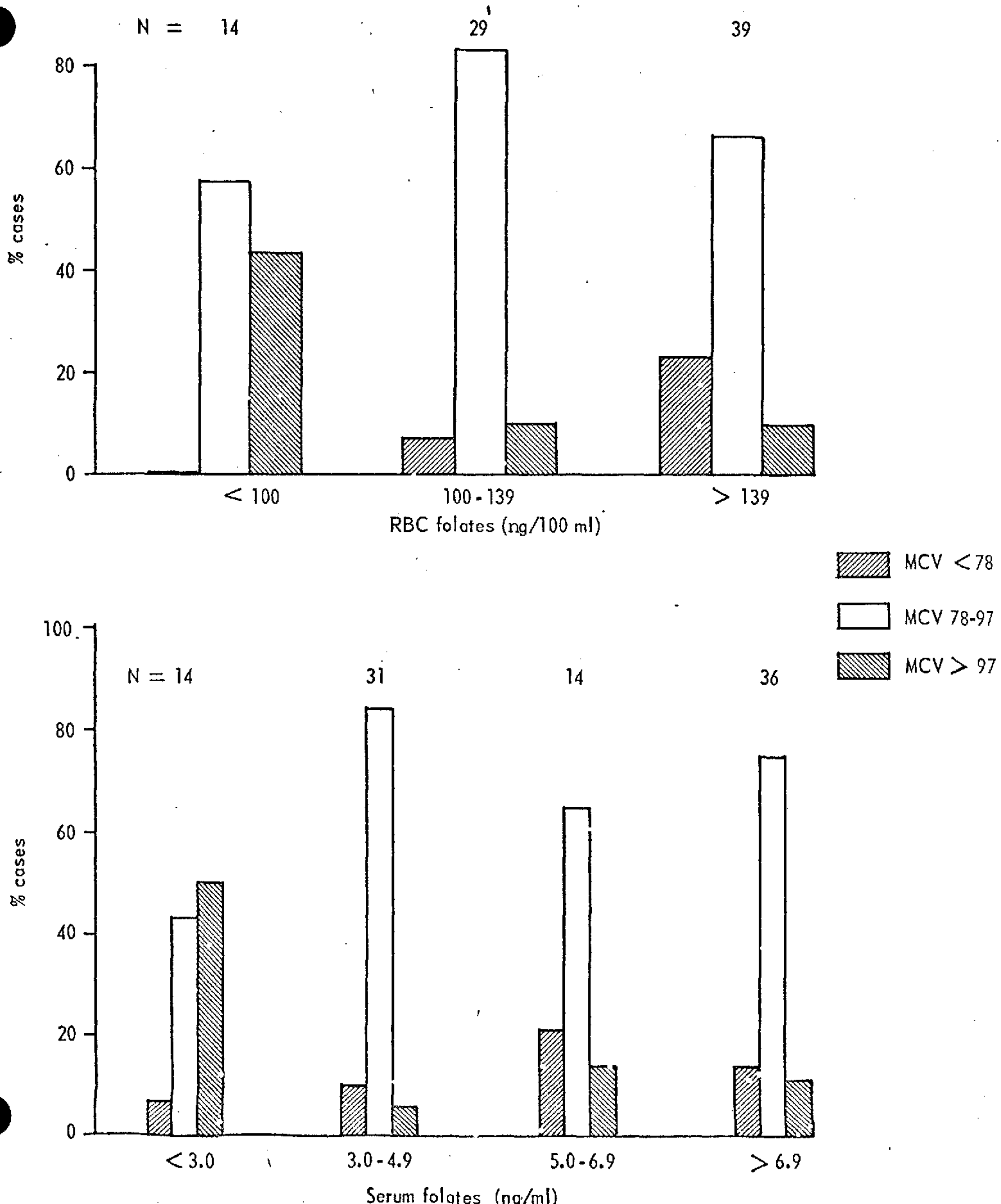
FIGURE 15 HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM. DISTRIBUTION OF CASES (IN %), BY RBC OR SERUM FOLATE LEVELS, AND BY MEAN CORPUSCULAR VOLUME (MCV)



Incap 73-711

FIGURE 16

HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM AND OF THEIR OFFSPRING. DISTRIBUTION OF CASES ACCORDING TO CORD BLOOD HEMOGLOBIN CONCENTRATION IN 80 GUATEMALAN NEWBORNS AT TERM

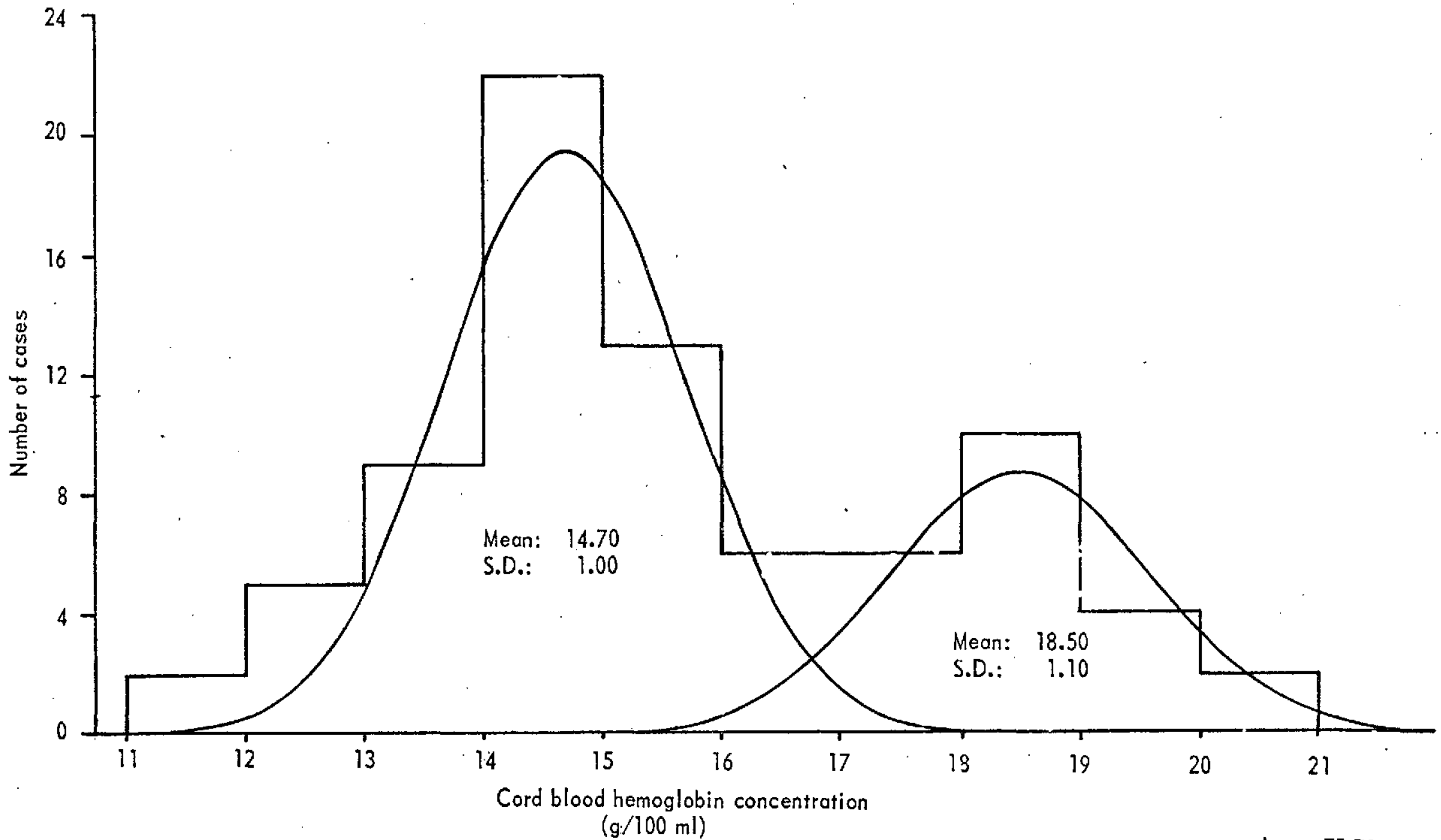


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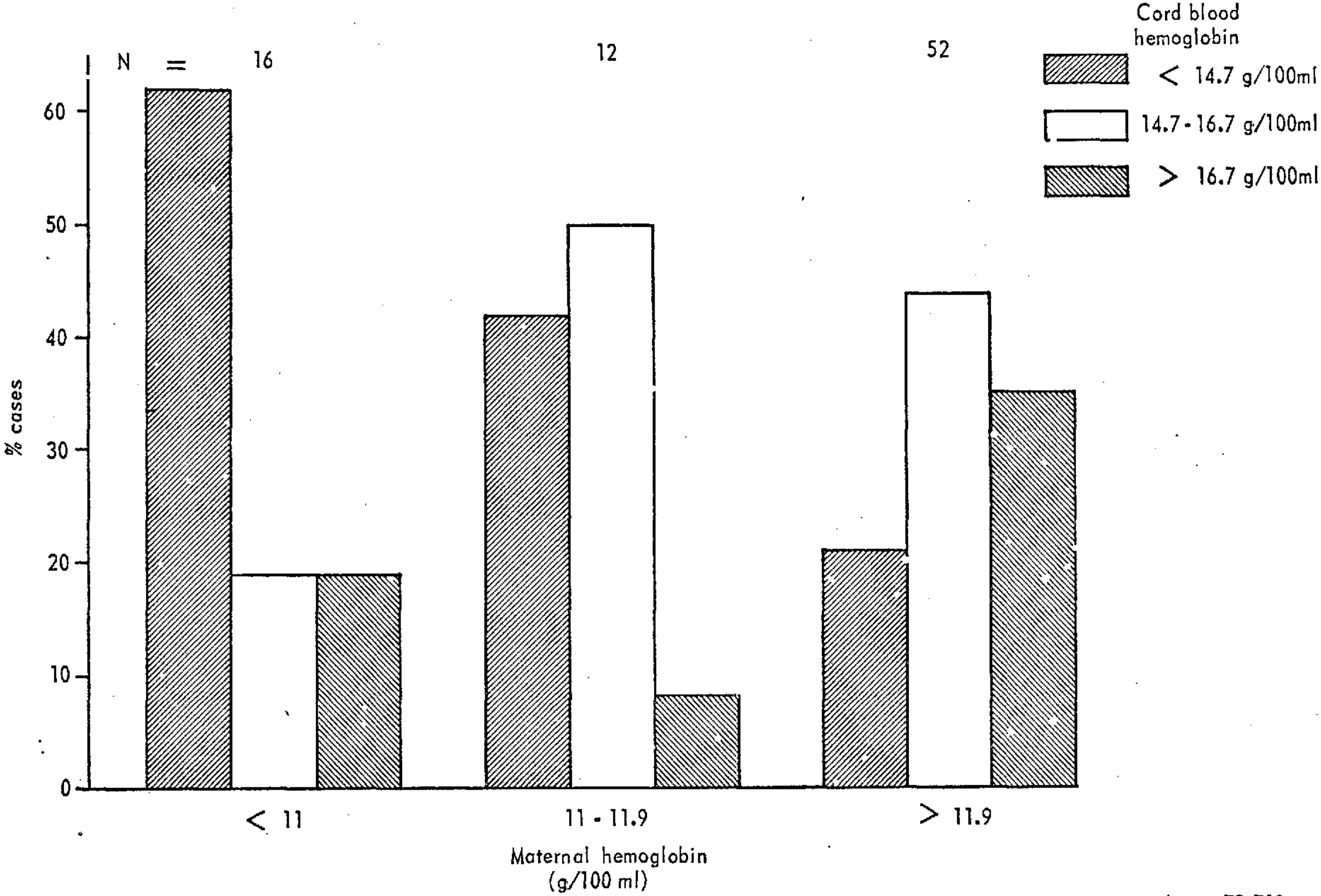
FIGURE 17

HEMATOLOGICAL STATUS OF GUATEMALAN PREGNANT WOMEN AT TERM AND OF THEIR OFFSPRING. DISTRIBUTION OF CORD BLOOD HEMOGLOBIN CONCENTRATION INTO THREE CATEGORIES FOR THREE MATERNAL HEMOGLOBIN CONCENTRATIONS CARRYING DIFFERENT LEVELS OF RISK OF BEING BELOW NORMAL



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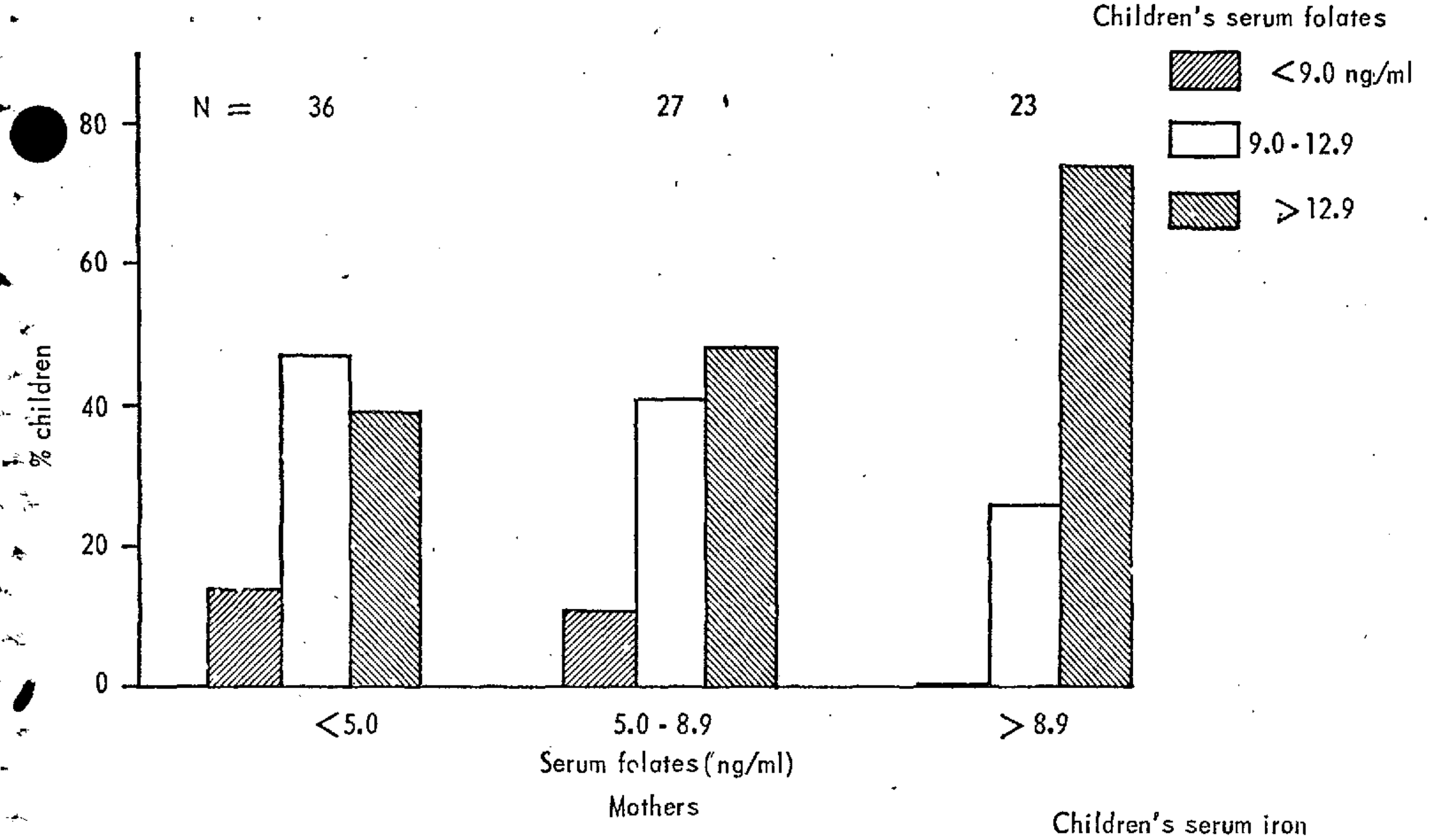
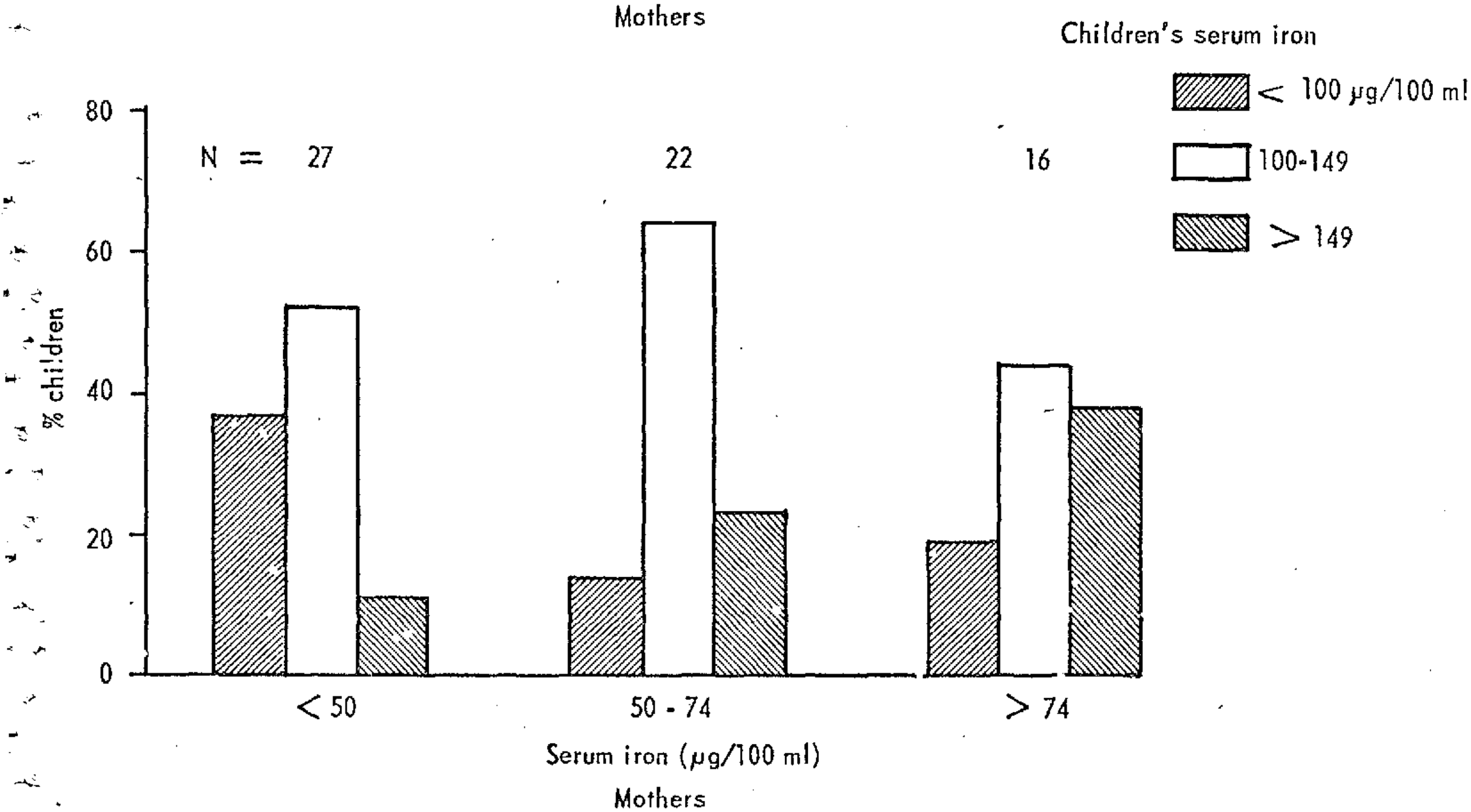
HEMATOLOGICAL STATUS OF PREGNANT GUATEMALAN WOMEN AT TERM AND OF THEIR OFFSPRING. SERUM IRON AND FOLATE CONCENTRATIONS IN THE BLOOD FROM THE MOTHERS AND FROM THE CORD





Incap 73-713

FIGURE 18

FIGURE 19

ORAL IRON ADMINISTRATION TO CLINICALLY NORMAL PRESCHOOL AGE CHILDREN IN A DAY NURSERY IN GUATEMALA

EFFECTS ON HEMOGLOBIN CONCENTRATION AND ON MEAN CORPUSCULAR VOLUME

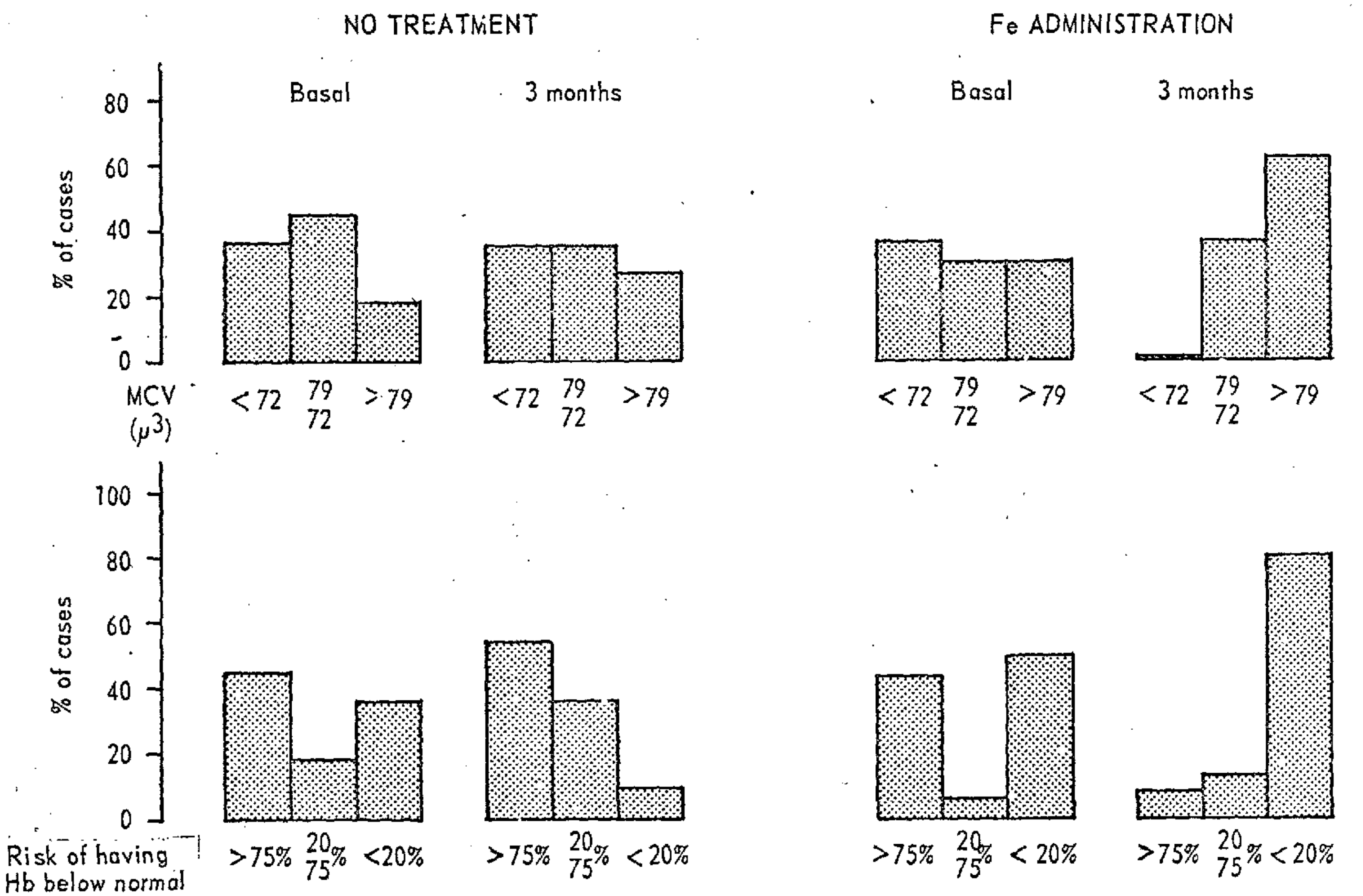


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FIGURE 20

DISTRIBUTION OF CASES IN A LOW LAND GUATEMALAN POPULATION IN THREE CATEGORIES BASED ON THE RISK OF BELONGING TO A POPULATION WITH SUBNORMAL HEMOGLOBIN CONCENTRATION. EFFECT OF IRON AND OF IRON + FOLIC ACID ADMINISTRATION FOR 6 MONTHS

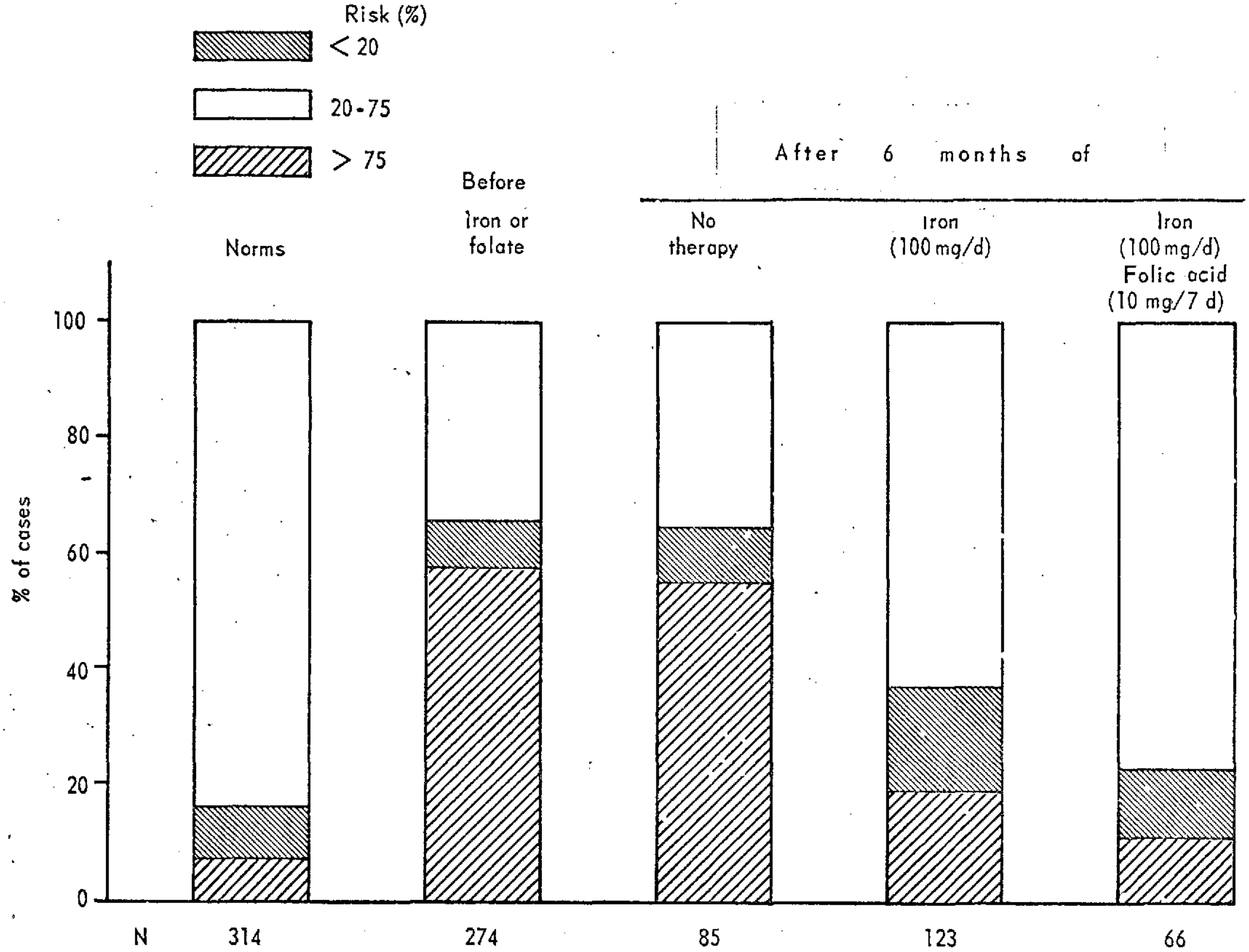

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FIGURE 21

DISTRIBUTION OF HEMOGLOBIN CONCENTRATIONS IN WOMEN 21-49 YEARS OF AGE IN THE GUATEMALAN LOWLANDS. COMPARISON OF NORMALIZED POPULATIONS AFTER IRON OR IRON AND FOLATE ADMINISTRATION

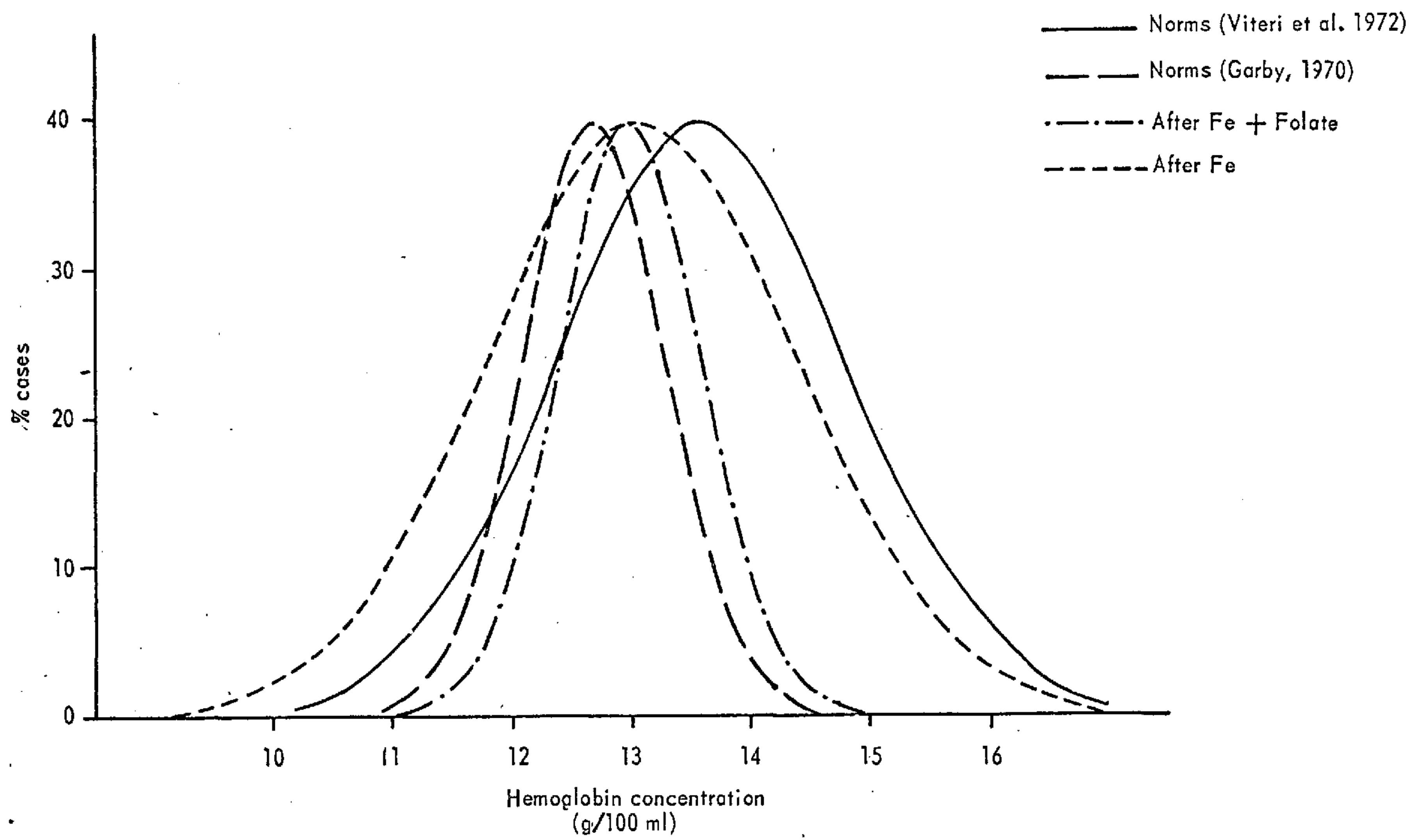

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FIGURE 22

DISTRIBUTION OF HEMOGLOBIN CONCENTRATIONS IN MEN 21-49 YEARS OF AGE IN THE GUATEMALAN LOWLANDS. COMPARISON OF NORMALIZED POPULATIONS AFTER IRON OR IRON AND FOLATE ADMINISTRATION

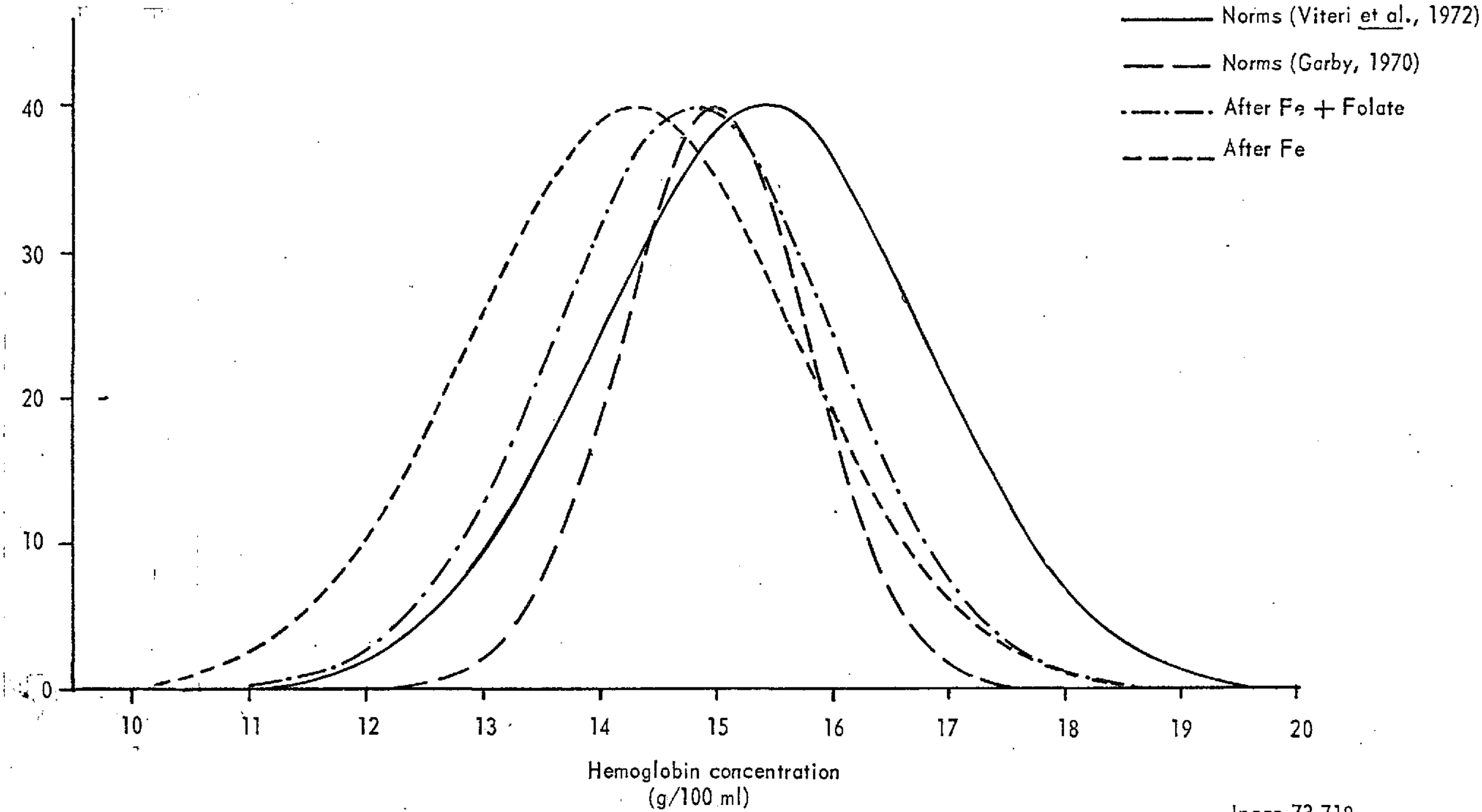

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FIGURE 23

DISTRIBUTION OF PACKED CELL VOLUMES IN WOMEN 21-49 YEARS OF AGE IN THE GUATEMALAN LOWLANDS. COMPARISONS OF NORMALIZED POPULATIONS BEFORE AND AFTER IRON OR IRON AND FOLATE ADMINISTRATION

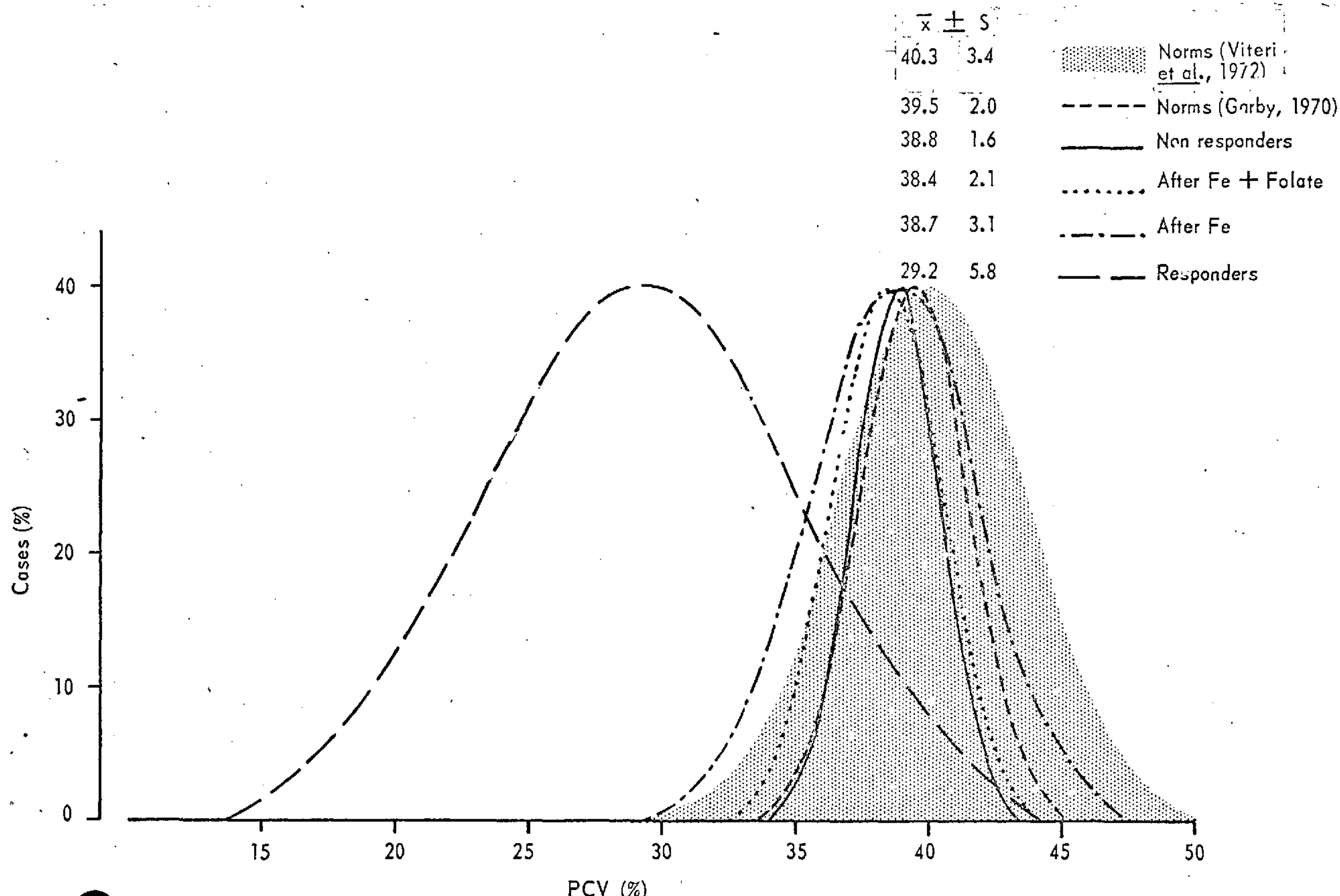

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FIGURE 24

ESTIMATED RISK OF BELONGING TO A POPULATION WITH SUBNORMAL PCV, ASSOCIATED WITH VARIOUS PCV VALUES IN WOMEN 21-49 YEARS OF AGE IN THE LOWLANDS OF GUATEMALA

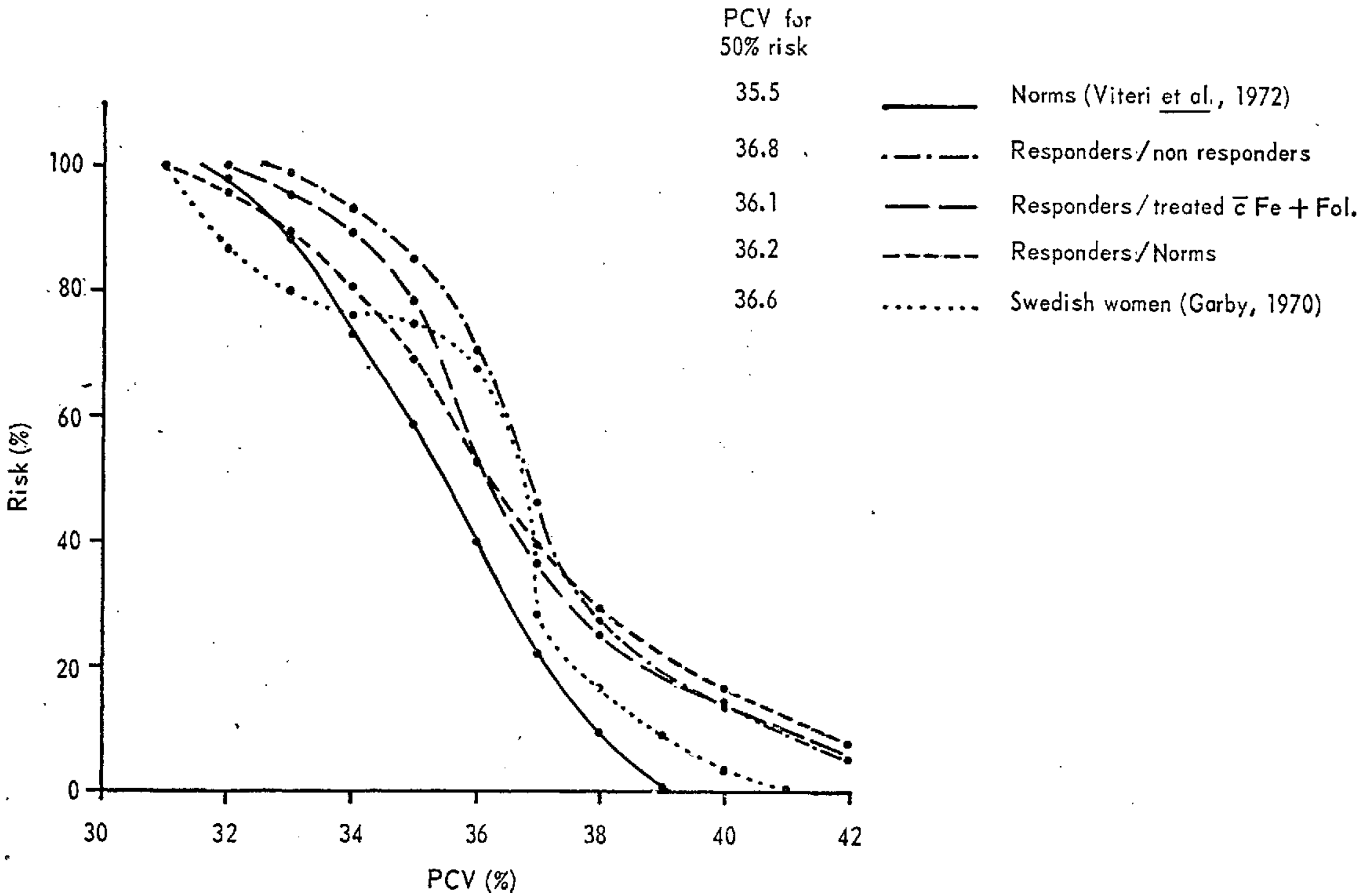

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FIGURE 25

PACKED CELL VOLUME IN TWO GROUPS OF LOWLAND GUATEMALAN AGRICULTURAL LABOURERS, BEFORE (T_0) AND AFTER 4 MONTHS (T_4) OF TREATMENT WITH PLACEBO OR ORAL IRON

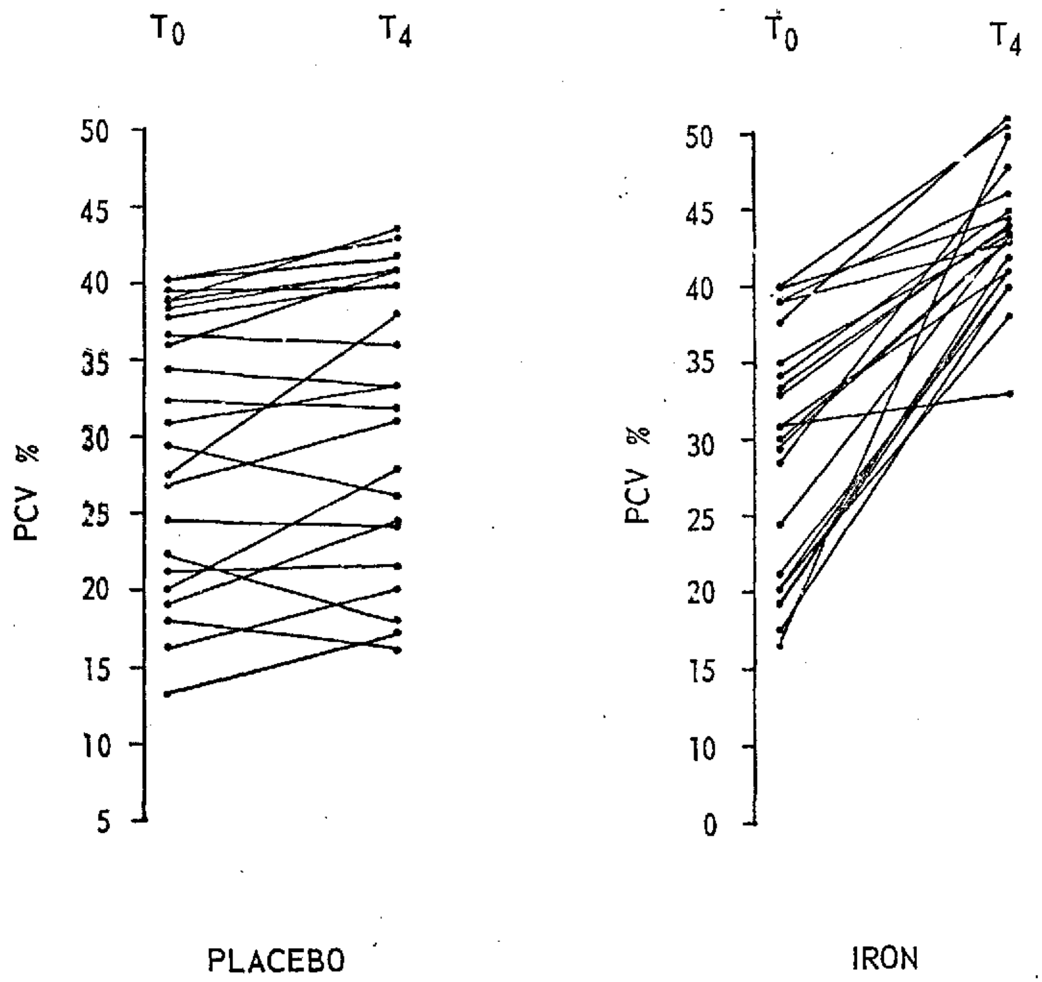

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FIGURE 26

HARVARD STEP TEST SCORE IN TWO GROUPS OF LOWLAND GUATEMALAN AGRICULTURAL LABOURERS, BEFORE (To) AND AFTER 4 MONTHS (T_4) OF TREATMENT WITH PLACEBO OR ORAL IRON

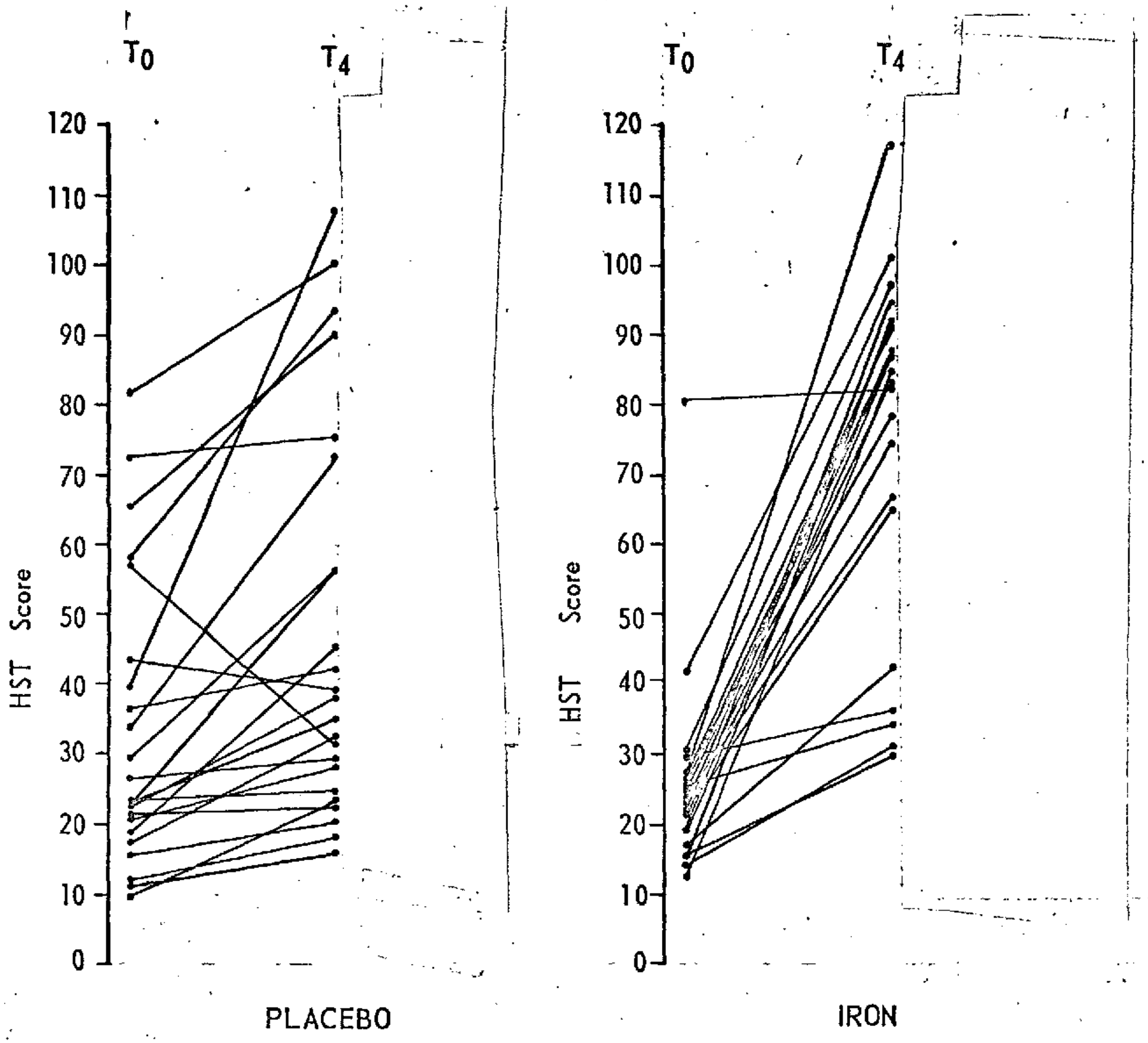

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FIGURE 27

RESULTS OF HARVARD STEP TEST SCORE WHEN ALL THE SUBJECTS, REGARDLESS OF THERAPY, WERE GROUPED BY PACKED CELL VOLUME

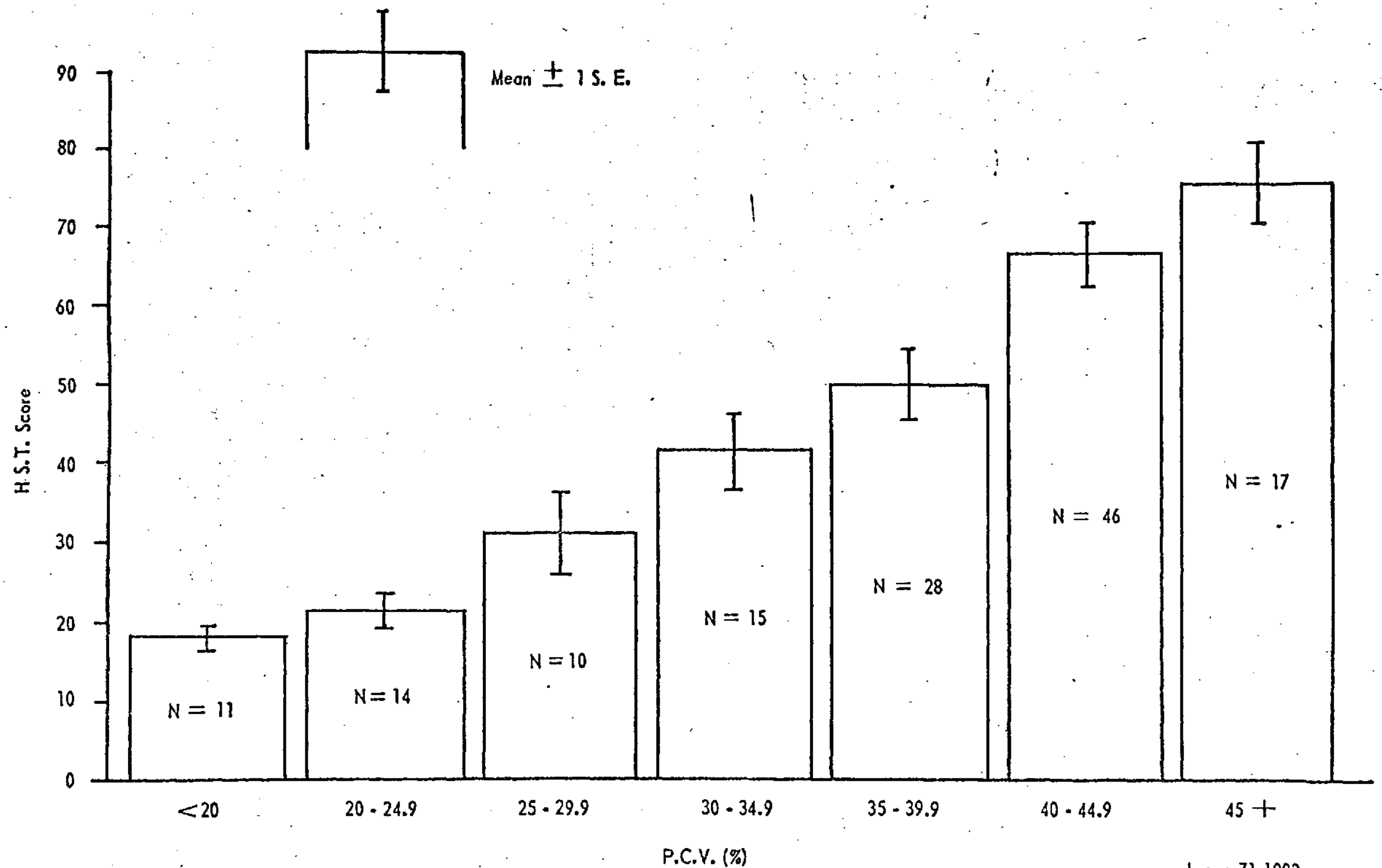

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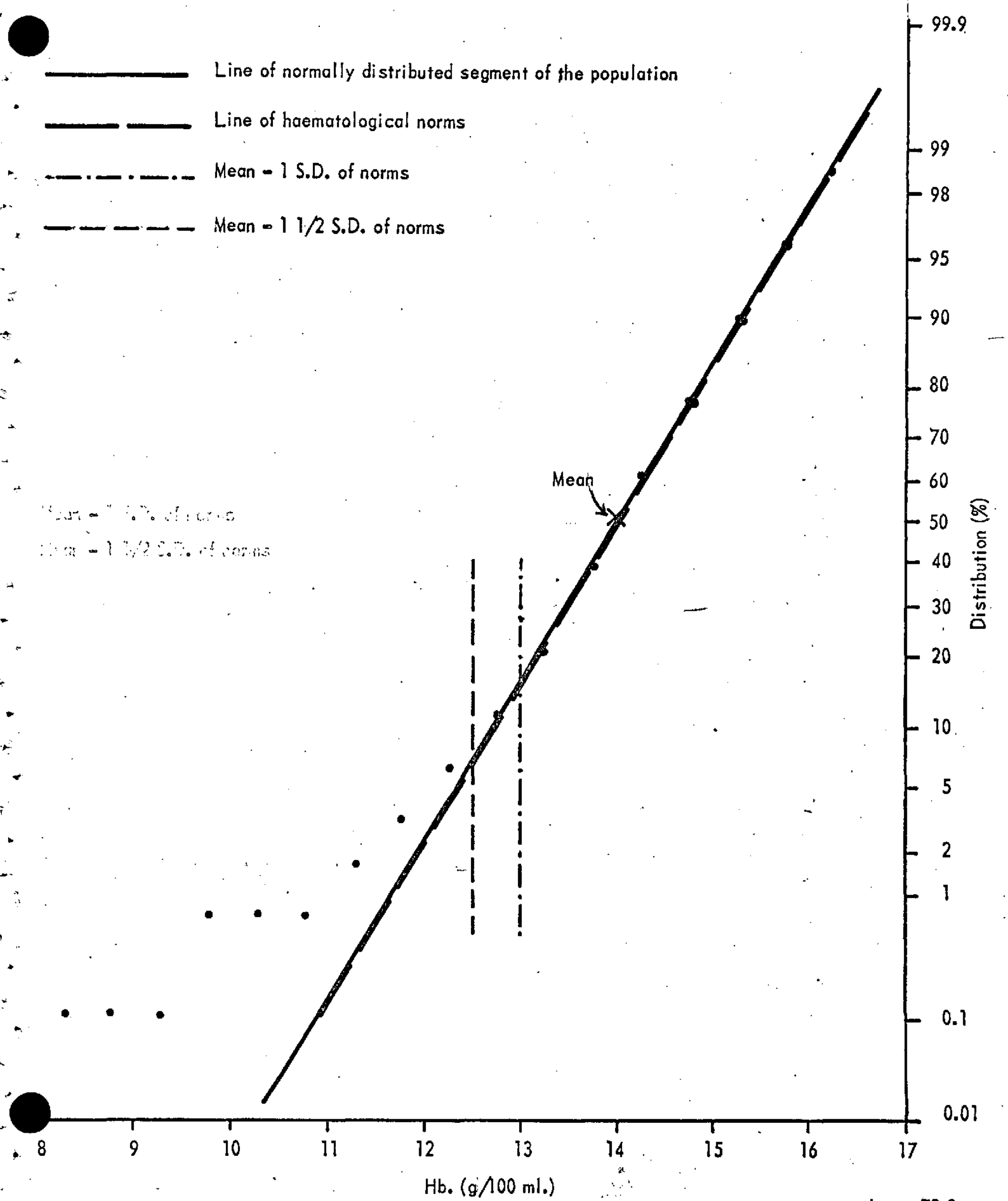
Fig. . 28

DISTRIBUTION OF CASES (%) BY HAEMOGLOBIN LEVELS. CHILDREN 9-12 YEARS OLD. ALTITUDE 750-1499 m.



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