NATURE AND CAUSES OF HOOKWORM ANEMIA

PREFACE

A number of studies have shown the prevalence of anemia in tropical areas—in great majority of the iron deficiency type—to be staggering; and it is probable that lack of iron is even more widespread than figures for circulating hemoglobin would indicate, if the rate of intestinal absorption for this substance can be taken as an early indication of its want.

Yet the reasons for the wide distribution of iron deficiency are not clearly known: in affected areas, often iron ingestion is relatively high, hookworm infections may be of low intensity, and there are no other obvious avenues of iron loss.

More information of a quantitative nature on the subject of iron metabolism in the tropics and its relation with anemia and hookworm was clearly needed, and, in 1955, we began measuring, in Venezuelan rural populations, chiefly by means of radioactive isotopes, various factors which influence the utilization and loss of iron. The present work is an attempt to bring together the studies done thus far.

We have centered the review around hookworm, because, at least in the Venezuelan context, it has emerged by far as the most important single cause of iron loss, and we suspect that this may be so in many other tropical areas of the world.

The conclusions reached on such a "geographic" disease as hookworm infection are naturally colored by the authors' personal experience with the particular conditions of nutrition, climate and customs prevailing in the particular areas under study. We have attempted, however, to correlate our experience with that of other workers in the field, by reviewing critically what we judged to be the pertinent literature.

Without anticipating too much of the substance of the monograph, two factors may be said to lie at the heart of the problem: a chronic drain of blood produced by the hookworm—whose order of magnitude we now know—and the relative unavailability for absorption of what would often seem an adequate supply of food iron—hunger in the midst of plenty. That in some cases the robbing of the host's blood by the worm may be compensated for by food iron is no reason for belittling the role of the parasite, as some are wont to do. This point of view is not unlike that of a bank president who, rather than firing a dishonest teller who every day pockets some of the organization's money, chooses to replace the daily loss from a special fund.

The present data, which might help shape up practical policy from a public health standpoint, give us, in addition, certain vistas of an interesting aspect of the disease: host-parasite relationship, or the manner in which the parasite influences its host (of which we now know a good deal, because of our naturally anthropocentric interest), or the host its parasite (of which we know but little).

It lies not within the scope of this work to speak of solutions, but the liberty afforded by the style of a preface permits some lines for a pressing appeal. Some of the remedies—vermifuges, the wearing of shoes—have been known for many years, and yet hookworm remains rampant, producing, we believe, enormous misery and suffering. If Norman Stoll's estimate is still valid, more than 600 million people on earth are infected by hookworm. Proper education and housing, and a general rise in the standard of living would help. But to this end are needed social changes, which do not depend directly upon those who, like ourselves, describe in detail some of the ills. If the work discussed here can provide even a small bit of the leaven required for such changes, we shall rest happy.

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I. RELATIONSHIP BETWEEN HOOKWORM AND ANEMIA

Cases of anemia may be observed in tropical areas without hookworm infection and, conversely, cases with infection without anemia; hence, the answer to the question whether there is such a thing as "hookworm anemia" should be perforce statistical; and the question might better be formulated thus: has it been shown, with convincing statistical significance, that hookworm infection in a community can lead to a higher incidence of anemia, and hence that hookworm infection is associated with such anemia as a cause to its effect?

True, most clinicians working in areas where heavy infection is understood an infection potentially leading to anemia in a group with a given nutritional level. In the Venezuelan rural context, for practical purposes, we draw the dividing line at infections represented by 2000 eggs/g of feces and consider cases above that value as "heavily" infected.

Studies Showing Correlation Between Hemoglobin and Worm Burden

The association between worm burden and "chlorosis" was mentioned early (Griesinger, 1854; Perroncito, 1880), but the problem does not seem to have been the object of thorough study until much later, when Darling et al. (1920) emphasized the direct relationship which they believed existed between burden and anemia. These workers felt, on the basis of the experience of the Uncinariasis Commission to the Orient, that such relationship was a rather close one. Part of their material from the Fiji islands was later submitted to statistical analysis by Stoll and Tseng (1925) who found the coefficient of correlation between worm burden (by direct parasite count) and hemoglobin, in 131 cases, to be $r = -0.634 \pm 0.035$, a "highly significant one."

Smillie (1922) studied 566 rural Brazilians of various ages, determining hemoglobin and performing direct parasite counts after worming. Although he made no statistical analysis of his results, his figures suggested a real negative relationship between burden and hemoglobin. There was no reduction in average hemoglobin with less than 25 worms, but, as the burden increased, the decrease in hemoglobin paralleled roughly the increase in number of worms, with individual variations, sometimes extreme.

The standardization of a method to count hookworm eggs in the feces (Stoll, 1923) made the determination of the burden-hemoglobin relationship easier. Stoll and Tseng (1925) studied this relationship in 337 Chinese from Southern Kiangsu Province. In the 273 positive cases, a correlation coefficient of "undoubted significance" of $r = -0.496 \pm 0.0030$ was found, but, although the general rule held, the great individual variations were again emphasized.

Carr (1926) studied the problem in 555 unselected individuals "probably free from malaria" from small rural Mexican communities. The correlation coefficient between hemoglobin and fecal egg-counts was $r = -0.551 \pm 0.020$, with a high degree of negative correlation. In this material, very light cases (50–500 eggs/g of feces) had
an average hemoglobin (73.9 ± 1.095%) which did not differ significantly from that of the negative cases (77.7 ± 2.011%). The difference in hemoglobin between the negative group and the group with 550–999 eggs/g (Hb = 66.0 ± 1.387%) was, however, significant. This suggested that a certain minimum load was required before hemoglobin began to be significantly affected.

In drawing a curve relating hemoglobin and egg counts, Hill and Andrews (1942) showed that the early part of the curve, which included the low egg-counts, was flat, indicating again that a certain minimal intensity of infection was needed before average hemoglobin began to drop. In these authors' material, the level at which this occurred was of the order of 5000 eggs/g of feces.

Although they did not submit their data to statistical evaluation, Keller et al. (1935) reached similar conclusions, and more recently Beaver (1951) has clearly and statistically established the point in studies on school-children in Georgia.

In three different groups of Chinese from the Szechwan Province of West China, Chang and co-workers (1949) consistently observed a decrease in hemoglobin with increasing burdens, which was highly significant in several of the groups studied. There was no significant correlation in other groups where only light infections prevailed.

Cort et al. (1929) studied two different groups in Panama. In the first, from Cocle Province, they found no statistical correlation between hemoglobin averages and egg counts, even though there were a number of heavily infected cases; in the second, correlation between the two parameters was statistically significant (r = -0.3414 ± 0.0646 in 95 children under 10 years, and r = -0.2637 ± 0.0412 in 241 adults and children over 10). They formulated the hypothesis that the apparent resistance to the anemia-producing effects of hookworm in the first group was due to a greater admixture of Negro blood, but this idea seems to have received no further support.

The studies by Kendrick (1927) are of particular interest, because they were carried out on a large group, mostly with light infections (both Necator and Ancylostoma), carefully analyzed by statistical methods. In 13,303 persons in the Mandapam camp, in India, Kendrick showed that there was "uniformly a progressive decrease in egg-counts with increase in haemoglobin content." This occurred even though many of the infections were light ones (three-fourths of the group had egg-counts of less than about 1000/g, and more than three-fourths probably harbored less than about 40 worms per person).

In a smaller group of 275 persons in a Madras jail, the correlation coefficient between hemoglobin and worm burden (based on egg-counts and total worm counts) was −0.11678 ± 0.040123. "For all practical purposes, the table may be regarded as normal, and the coefficient of correlation then indicates a negative association which is just significant when compared with its probable error." It is noteworthy that Kendrick reports as the "heaviest infected" cases in his large group of 13,303 persons, 20 individuals with a minimum of 103, a maximum of 374, and an average of 196 worms per patient, all of which are relatively small burdens.

Layrisse and Roche (1964a) have studied a group of 1142 rural Venezuelans from six hookworm infested communities. A general and significant relationship between circulating hemoglobin levels and hookworm load was found (Fig. 1). If a comparison is made between hemoglobin in the lightly infected or the noninfected group and that in groups with progressively increasing hookworm loads, the differences are found to be significant with worm loads represented by 2000 eggs/g of feces for women and children, and 5000 eggs/g of feces for "adult" men (over age 14) (Table 1). A significant correlation between burden and anemia has also been found, in given communities, by Smillie and Augustine (1926), Bassi (1936), White et al. (1957), and by Farid and Miale (1962). Such correlation will not usually appear, however, unless certain precautions are taken and certain conditions prevail.

Conditions for a Satisfactory Study

1) A sufficient number of cases must be studied, since individual variations may be extreme, and subjects may be found with infection and no anemia, and vice versa. Warnings in this respect have been given in the early literature. Thus Smillie (1922) stated: "There was such remarkable variations in individuals that we despaired at first of being able to arrive at any conclusions in regard to the effect of hookworm infection upon the hemoglobin index, but after a large series of examinations was made, certain definite tendencies became evident;" and Darling (1922)
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Figure 1. Relationship between hemoglobin levels and severity of hookworm infection in various Venezuelan rural communities. The number of cases in each group is shown in parentheses. Vertical lines indicate one standard deviation. The differences between the negative group and both groups with less than 2000 eggs/g of feces are not statistically significant. In all groups with more than 2000 eggs/g of feces, the difference with the negative group is significant (P < 0.01) (from Layrisse and Roche, 1964).

Also noted: “when sufficiently large numbers are taken and averaged, it may be seen that the amount of anemia is proportional to the number of worms.” What the actual number of patients studied must be cannot be stated a priori. This would depend on the number of severe cases in the series and on the “vulnerability” of the group studied. Thus, it would seem that in menstruating...
women and in growing children, a given burden may more readily produce anemia than in adult men, and the relationship between burden and anemia may be more readily demonstrated. (See studies by Chernin, 1954, and by Napier and Das Gupta, 1937.)

2) The series studied must include a sizable number of both light and fairly severe infections. A significant relationship between burden and anemia should not be expected to appear in groups which are fairly homogeneous with respect to intensity of infection, especially when dealing with light infections, which affect hemoglobin levels little, or at times not at all.

3) A statistical analysis should preferably be carried out. This implies that the variables involved—usually blood circulating hemoglobin and number of worms—are measured in a quantitative way. It is advisable to measure circulating hemoglobin by means of an accurate method, preferably photocolorimetric; however, even rough methods such as Talquist's, used in the earlier surveys, may yield satisfactory results, from a statistical point of view. To measure the worm burden, direct count after thorough worming is the most accurate method, provided one can insure complete stool collections; but this is not practical in large series, and the fecal egg-count by suitable methods (Stoll, 1923; Caldwell and Caldwell, 1926; Beaver, 1951) has proved to be satisfactory.

4) Other anemia-producing factors, such as certain dietary deficiencies, should not be paramount. It is conceivable that, if hemoglobin is already lowered markedly by, for example, folic deficiency, hookworm might not reduce it further in a significant manner, especially since equal loss of blood leads to relatively lower iron losses in the presence of lower circulating hemoglobin values. However, such situations of extreme dietary deficiencies which might occur, for example, in India, are infrequent in tropical America.

**Studies Showing No Correlation**

It is probably because the group they studied did not include some, or all, of the above desiderata, that a number of authors have found no

### TABLE 1

**Relationship between anemia and hookworm load in various age groups in rural areas of Venezuela**

*(From Layrisse and Roche, 1964)*

<table>
<thead>
<tr>
<th>Age (years) and sex</th>
<th>Control†</th>
<th>Egg count per gram of feces</th>
<th>100-900</th>
<th>1,000-1,900</th>
<th>2,000-2,900</th>
<th>3,000-3,900</th>
<th>4,000-4,900</th>
<th>5,000 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-6 M and F</td>
<td>110 (38)</td>
<td>P &gt; 0.20</td>
<td>106</td>
<td>12</td>
<td>6</td>
<td>&gt; 3,000</td>
<td>10</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &gt; 0.30</td>
<td>(50)</td>
<td>(100)</td>
<td></td>
<td></td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>7-14 M and F</td>
<td>119 (34)</td>
<td>P &gt; 0.20</td>
<td>259</td>
<td>90</td>
<td>30</td>
<td>&gt; 3,000</td>
<td>40</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &gt; 0.01</td>
<td>(41)</td>
<td>(51)</td>
<td>(67)</td>
<td></td>
<td>(78)</td>
<td></td>
</tr>
<tr>
<td>15-45 Females</td>
<td>50 (20)</td>
<td>P &gt; 0.10</td>
<td>115</td>
<td>33</td>
<td>5</td>
<td>&gt; 3,000</td>
<td>13</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &gt; 0.02</td>
<td>(31)</td>
<td>(39)</td>
<td>(80)</td>
<td></td>
<td>(77)</td>
<td></td>
</tr>
<tr>
<td>15-45 Males</td>
<td>21 (24)</td>
<td>P &gt; 0.50</td>
<td>66</td>
<td>18</td>
<td>14</td>
<td>8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &gt; 0.90</td>
<td>(20)</td>
<td>(22)</td>
<td>(43)</td>
<td>(50)</td>
<td>(50)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

* See Section II for definition of anemia.
† Control represents individuals with negative stools or with less than 100 eggs per gram of feces.
‡ The data in parentheses indicate percent of above number of individuals having anemia.
relation between burden and anemia. Beginning in 1930, a number of authors threw doubt on the relationship between burden and anemia. In nearly all studies quoted as supporting these doubts, the evidence presented is insufficient.

In some of the articles, the size of the series studied is clearly too small (Gordon, 1925; McCarthy, 1930; Rhoads et al., 1934; Napier and Das Gupta, 1937; Dick and McCarthy, 1946; Sautet, 1950; Yamasaki and Saruta, 1954; Kennedy, 1956; Foy and Kondi, 1960; Stott, 1960; Borrero et al., 1961); or the burden was not measured, or the methods used were inaccurate (Napier and Das Gupta, 1937 first series; Wilhelm, 1946; Sautet, 1950; Kennedy, 1956; Oldmeadow, 1956; Foy and Kondi, 1957; Friis-Hansen and McCullough, 1961; Foy and Kondi, 1961); or, finally, there were no heavy infections in the series (Dick and McCarthy, 1946; Chernin, 1954; Yamasaki and Saruta, 1954; Kennedy, 1956; Stott, 1960).

In the case of the often quoted, and apparently critical, article of Gordon (1925), and that of Fülleborn et al. (1928), some further comments are in order. The first author studied a group of 82 West African male natives. The point was made that there was no difference in burden, as measured by Stoll egg-counts, between a group of 57 subjects with hemoglobin levels ranging from 81 to 90 % and a group of 25 subjects with levels of 71 to 80 %. Since hemoglobin was measured by the Talquist method, whose intrinsic error is large, hardly any conclusion could be drawn from these data, especially in small, relatively homogeneous groups. In the ten heaviest infections studied (Stoll count from 11,200 to 23,100 eggs/g), on inspection of the data there does not appear to be a correlation between egg-counts and hemoglobin values, and roughly two-thirds of these cases fell into the “higher hemoglobin group” (81 to 90 % circulating hemoglobin). Gordon's study illustrates the fact that a subject can be heavily infected without the hemoglobin level being markedly reduced, at least for a time. But the number of cases in his series is clearly too small—especially the heavily infected group, composed of ten subjects—and he failed to demonstrate that hookworm infection will not affect hemoglobin levels in a community, because of the small number of patients and the relative homogeneity of the group.

Fülleborn et al. (1928) are also at times quoted as providing evidence that there is no relation between worm burden and anemia. Indeed, among the 308 individuals studied by them, from the Province of Corrientes, in the Argentine Republic, even though infections were not negligible, averaging around 5,000 eggs/g of feces, the average hemoglobin in adult males was 82.3 % and in adult females 74.4 %, as compared with individuals from Hamburg studied by the same group, who had 81.7 % and 74.6 %, respectively. This observation was attributed to the large quantity of meat consumed in the region (which may well have been the right explanation) or, tentatively, to the admixture of Indian blood. However, in the groups below age 20, there was indeed a progressive fall in hemoglobin, as the number of eggs in the stools increased, beginning with more than 1,000 eggs/g, even though animal protein ingestion was equally high in this age group.

Rhoads et al. (1934), in a study devoted chiefly to the etiological role of iron deficiency in hookworm anemia, were not impressed by the evidence in the literature regarding the relationship between burden and anemia, and wrote in

### TABLE 2

<table>
<thead>
<tr>
<th>Zone</th>
<th>Climate</th>
<th>State of nutrition</th>
<th>Hookworm</th>
<th>Prevalence of anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Source: Layrisse and Roche, 1964</td>
<td></td>
</tr>
<tr>
<td>Endemic</td>
<td>Hot and humid</td>
<td>Fair; little animal protein intake</td>
<td>Abundant No</td>
<td>46 30</td>
</tr>
<tr>
<td>Non-endemic</td>
<td>Hot and humid</td>
<td>Fair; good animal protein intake</td>
<td>No</td>
<td>16</td>
</tr>
</tbody>
</table>

* See Section II for definition of anemia.
their classical article, “this feature of the analysis was accordingly not exhaustively pursued.” They studied the correlation between those parameters in 17 subjects, performing direct worm counts in the stools. In the graph constructed by them, there was no “obvious correlation” but the cases are too few to permit a definite conclusion.

Before 1960, Henri Foy (Foy and Kondi, 1956, 1957; Foy, Kondi and Austin, 1958) felt that hookworms “may play a part” but “it may not be a paramount one” (Foy and Kondi, 1956). Since 1960, these authors have reversed their stand on the question and seem to have gone to the no less extreme view that all worm-free anemic cases must have been wormed and all heavily infected cases without anemia must have received iron (Foy and Kondi, 1960; Foy and Nelson, 1963).

Some of the studies which fail to meet all the criteria set forth above nevertheless provide valuable suggestive evidence in favor of a causal relationship between hookworm load and anemia. For example, in Chernin’s publication (1954) all the patients reported but one had less than 3000 eggs/g of feces and most (73.1%) had less than 1000 eggs/g. As could be expected, there was no consistent decrease in average hemoglobin value in this homogeneous, lightly infected group. In spite of this, mean hemoglobin in all hookworm positive females (10.34 g/100 ml) was significantly lower than in females free from infection (11.35 g). This would indicate that even small hookworm burdens may be sufficient to lower hemoglobin in a “vulnerable” group, in which the iron losses or requirements are higher, and the reserves presumably are lower. This point was also brought out in Layrisse and Roche’s study (1964a) where a significant lowering of average hemoglobin values appeared with smaller numbers of eggs in the feces, in menstruating women and growing children (Table 1).

Again Stott (1960), in a study designed to investigate average hemoglobin values as related to prevalence of infection in a given area, studied in detail a group of 92 infected subjects on the island of Mauritius, finding no relationship between hookworm burden and anemia ($P < 0.40$). The number of subjects was relatively small, however, and the average burden (84 worms per patient) was light. In spite of this, Stott gave suggestive evidence that there was indeed a relationship between hookworm infection and anemia, by showing in large groups that a higher prevalence of anemia in the island was found in areas where rainfall was high and hookworm infection frequent. A similar demonstration, by similar methods, was made on the island of Mahé by Foy and Kondi (1961).

There is only one study besides that of Cort et al. (1929), to our knowledge, which includes large groups of people, with heavy infections, and in which apparently no correlation whatsoever between burden and hemoglobin appeared to exist. This is the study quoted by Patwardhan (1961) and done in the State of Mysore in India. A total of 28,718 persons from this State were subjected to hemoglobin determinations and 7034 to fecal egg-counts, presumably by the Stoll method. Hemoglobin values in the various subgroups from different districts were unusually homogeneous (11.2 to 11.96 g/100 ml, no standard deviation or range given) although the prevalence of infection extended from 35.1% to 79.7% with the average Stoll count ranging from 600 to 11,000 eggs/g. In our experience, it is unique to observe average hemoglobin levels of, for example, 11.96 g/ml, such as is said to occur in the district of Shinaga, in the face of an average Stoll count of 11,302 eggs/g of feces, which implies very large burdens in a great proportion of the community. The Indian study must surely receive further confirmation.

We feel that, in most cases in which the material has been properly studied and the general rules set forth above followed, a definite and significant relationship has appeared. We cannot but repeat the early statement of Darling (1922) and make it our own: “The anemia and severity of the symptoms in general correspond with the number of hookworms harbored. This may not be apparent in the individual case, because individual resistance may cloak the malignant influence of the worms which are nonetheless causing losses of blood and drains on the individual’s powers.”

The Effect of Light Infections

Whereas it is clear that there is usually a gross but significant relationship between burden and anemia, it may be questioned whether light infections with egg-counts of the order of 2000 eggs/g or less may cause any anemia per se. In the early period of hookworm study, before 1923, the
tendency was to regard all infected patients as forming a homogeneous group. After a quantitative method of estimating hookworm loads had been devised (Stoll, 1923) and large groups could be studied, it became evident that light infections were frequently symptomless. Lately, the tendency has been to consider light infections as meaningless for the individual patient, outside of their public health significance as carriers.

According to some early observers, however, (Dock and Bass, 1910) for example, light cases are seen repeatedly to “improve in weight, feeling, appearance and hemoglobin after getting rid of what could be considered an insignificant infection.” We know of no well controlled study on this point, but some of the data in the literature would suggest that light infection may have indeed some significance in the lowering of average hemoglobin. For example, in Chernin’s (1954) female hookworm positive group, even though the burden was low, the drop in hemoglobin was significant by comparison with the uninfected females. Kendrick’s large, lightly infected, group showed significant negative correlation between hemoglobin values and burden. Kendrick (1927) stated (p. 229) however that *Necator* infections, averaging up to 25–30 worms or those producing counts of 700 to 900 eggs/g, would lead to no measurable reduction in the hemoglobin. In Stott’s series, on the island of Mauritius, even though infections were all obviously light, there was a strikingly higher prevalence of anemia in areas where hookworm infections were frequent (1960).

Similar findings were reported by Foy and Kondi (1961) on the island of Mahé, in the Seychelles. In Carr’s 1926 series, the group with as little as 550–999 eggs/g showed a significant hemoglobin decrease below that of the control group. In Layrisse and Roche’s 1964a series, although significant lowering of circulating hemoglobin did not occur before the number of eggs/g had reached 5000 for adult men and 2000 for women and children, perusal of Table 1 shows that, in every one of the groups considered, the percentage of subjects with anemia increased parallel to worm burdens, and continued to do so in a progressive fashion, well before the difference with the control group became significant. It is not unlikely that light infections might have shown significant hemoglobin lowering had a larger series been observed. Of course, infections which are light at the time of examination may have been much heavier previously, and this may account for some of the results observed.

It seems to us quite clear that, if proper precautions are observed, and provided there are included in the series a sufficient number of fairly severe infections, in a given community, hemoglobin in infected subjects is usually reduced significantly below the level of non-infected groups. This clearly means that there is such a thing as a “hookworm anemia,” a fact, as already stated, which few practical physicians would be inclined to doubt. Again, this does not imply that individual cases may not have anemia without infection and vice versa, but at least one author (Foy and Nelson, 1963) tends nowadays, after earlier doubts, to believe that very few, if any, iron deficiency anemias in infected zones are not due to hookworm as a principal cause. This is probably too extreme a view.

Layrisse and Roche (1964a) have attempted to obtain a general estimate of the contribution of hookworm as an anemia-producing factor in given rural communities. They studied two communities, geographically close to each other, in western Venezuela, one with hookworm and the other without. The results are shown in Table 2.

It is presumed that the difference in anemia prevalence in the non-infected groups of both zones is due to an alimentary factor, probably the quantity of available dietary iron. Similarly, the difference between prevalence of anemia in the infected and non-infected groups of the endemic zone (46% less 30% — 16%) would indicate the percentage of individuals in the endemic zone with anemia due to hookworm as a chief factor. Granted that the method used is uncertain, and the controls not entirely comparable, the results suggest that, in the given endemic zone, roughly one-third of all anemias could be attributed to hookworm infection as a major contributing cause.

It appears to us to have been abundantly shown, in a number of communities, that groups with varying degrees of hookworm infection have an average level of circulating hemoglobin significantly below that of uninfected groups living under similar conditions in the same community. Such groups could properly be said to exhibit *hookworm anemia*. In individual subjects, hookworm anemia would be presumably the lowering of circulating hemoglobin in an infected subject at least two standard deviations below the
average value in the uninfected subjects in the same area. But, since it is not possible at present to determine in individual cases to what extent previous infections might have caused iron drainage, and since factors other than hookworm may be at work to an undetermined extent, it will not be possible to determine with absolute certainty the causal diagnosis, and simple clinical judgment will have to be relied upon, aided if possible by egg-counts which furnish some information on the degree of blood loss.

**Summary**

Among 88 articles dealing directly with the relationship between worm burden and anemia in hookworm infection, 18 find a correlation, 10 do not find any, and 3 do not commit themselves. In only 14 of the articles was there a statistical analysis of the material, and in all of these there was a significant correlation between hemoglobin and worm burden.

It is clear, however, that in all series studied there were cases with heavy infections without anemia, as well as cases with anemia without infection, signifying that host-parasite relationship allows many slow progressive stages of adaptation of the host’s circulating hemoglobin to the parasite action. Several conditions should therefore be fulfilled when investigating the possible correlation between anemia and worm burden: (1) The series studied must be large enough; (2) it should contain a spectrum of cases, extending from lightly to heavily infected ones; (3) other anemia-producing factors, which might mask the effect of hookworm, should not be of undue importance; and (4) a statistical analysis of the data preferably should be carried out.

When such conditions are fulfilled, a significant negative correlation between worm burden and hemoglobin will appear. It is clear that hookworm can, and often does affect a population in such a way that the prevalence of anemia in it will be higher than it is in another, otherwise similar, population not so infected. It is in that sense that one can speak properly, from an epidemiological standpoint, of “hookworm anemia.”

In the individual subject, a decision as to the diagnosis of hookworm anemia will depend on clinical experience and judgment, based on a consideration of worm burden, possible length of infection, nutritional status of the individual, and the absence of other causes of anemia.

**II. CHARACTERISTICS OF HOOKWORM ANEMIA**

A number of changes have been reported to occur in various body fluids and tissues in association with hookworm anemia. Some of these, such as circulating erythrocytes, bone marrow morphology, or plasma and tissue iron, which have a direct bearing on the definition, and indeed on the question of the genesis of hookworm anemia, will be discussed here. (Since the literature on these points is abundant and repetitive, no attempt will be made to carry out an exhaustive review.)

**Circulating Hemoglobin** and Red Blood Cells

By definition, of course, circulating hemoglobin is reduced in anemia. A reduction in iron stores may exist, however, without hemoglobin values being influenced. That this may be frequent in a region where hookworm is endemic is shown by the presence, in non-anemic individuals, of markedly increased intestinal iron absorption (Section V).

The decision as to what constitutes lower normal values for hemoglobin is always somewhat arbitrary since, as has been pointed out by Bothwell and Finch (1962), any survey of so-called normal groups is likely to include a number of subjects with pathologically low hemoglobin, and this would tend to lower the average value. The establishment of normal values is further made difficult in countries where dietary deficiencies are rampant. We have therefore as a basis the values established by the World Health Organization (1959), with one exception which will be mentioned later. In our studies we have considered the minimum hemoglobin dividing value, below which anemia can be considered to exist, as follows: 11.5 grams per 100 ml in children from 2 to 6 years; 12.0 grams per 100 ml in children 7 to 14 years and nonpregnant women from 15 to 45 years; and 13.0 grams in men from 15 to 45 years. The latter value was taken, instead of the 14 grams per 100 ml given by the World Health Organization (1959) since most of the surveys on normal adult men report values in the vicinity of 16 grams per ml, with a standard deviation from 1.5 to 2; so that 13.0 grams per 100 ml seems to be a more appropriate value as a

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4 Hemoglobin is given throughout, unless otherwise specified, in the units utilized in the original reports.

4 Also administered cupric sulfate and fresh liver.
minimum for men. In studies other than our own, we have utilized the word anemia in the meaning given by the original authors.

There is usually a reduction in circulating red blood cells as well. As a rule, the decrease in hemoglobin is relatively the greater, so that the mean corpuscular hemoglobin concentration (MCHC) is diminished. Mean corpuscular volume (MCV) is also decreased. Microscopically, microcytic poikilocytic erythrocytes are observed as seen in hypochromic anemias from other causes. Such observations have been described in practically every report dealing with hookworm anemia, and it would be superfluous to review them here.

Occasionally, patients with heavy hookworm infection are found with either normal or high MCV. For example, in the series of Rhoads et al. (1934), 7 of the 83 subjects had normal MCV associated with a markedly reduced hemoglobin. Puerto Rico, where the study was conducted, is an area where the sprue syndrome is endemic and, as the authors themselves point out, deficiency of other anti-anemic principles may have masked in some cases the effects of iron lack. A few cases of macrocytosis associated with hookworm infection have been reported (Dock and Bass, 1910; Buckman, 1938; Cotti, 1937; Layrisse and Henriquez, 1953; Lehmann, 1949). Buckman's three cases responded to vermifuge and the administration of liver extract; Layrisse and Henriquez's cases responded to iron therapy, cobalt, and the administration of an adequate diet.

After heavy acute infections with hookworm in the human there is a rapid and apparently parallel reduction of circulating erythrocytes and hemoglobin, even in well-fed individuals (Kendrick, 1934; Ashford et al. 1933).

**Reticulocytes**

Acute hookworm (*Ancylostoma caninum*) infection in dogs evokes a marked reticulocytic response, varying from 16% to 63%, similar in degree to that caused by repeated bleeding of control dogs (Landsberg, 1937). We find no data on this point in early stages of infection in human beings.

In human chronic infections, reticulocytes are often found in normal percentage (Rhoads et al. 1934; Cotti, 1937; Brown and Otto, 1941; Yanagisawa et al., 1953). At times, however, the percentage may be slightly or markedly increased.

For example, of the 83 patients studied by Rhoads et al. (1934) in Puerto Rico, 51 had reticulocyte counts between 0.6% and 2.0%, 18 between 2.1% and 3.0% and 14 over 3%, reaching as high as 9% in one case (with 29% hemoglobin, and 30,700 eggs/g of feces). In 80 cases, studied in Costa Rica by Peña Chavarria and Rotter (1935), the average reticulocyte count was 6.4%. Occasionally, nucleated red cells (Rhoads et al., 1934; Cotti, 1937) or punctate basophilia (Cotti, 1937) may be found.

If reticulocytes are calculated in absolute values rather than in percentage, a somewhat elevated value is almost always found. Thus Layrisse et al. (1961) reported in 10 cases an average of 92,000 per cmm, which is almost twice normal (51,000 per cmm), all subjects but two having distinctly high values. This increase in reticulocytes would indicate a moderate increase in erythropoiesis.

**Bone Marrow**

Calmette and Breton (1905), in describing the pathology of ankylostomiasis, mentioned that the marrow in the long bones was “red” with a thick jelly consistency. De Marchis (1908) is quoted by Larizza and Ventura (1950) as having found, in a patient who died of hookworm anemia, that the bone marrow from the femoral diaphysis and from the ribs presented a red color and was rather abundant. Ashford and King (1907) examined the femoral marrow in two cases of heavy hookworm infection coming to autopsy and found it to be grayish red and very soft. The two photomicrographs by Dr. Gray published in the article show cellular hyperplasia, with many normoblasts.

By 1929, Huart, reviewing the then scanty bibliographical material, stated that “not a single investigator reports that he has found a bone-marrow such as is described in cases of aplastic anemia.” Since that time, a number of observers have enlarged on such comment.

Fieschi (1932) described the marrow in two cases of hookworm infection and anemia; the first was obviously a case of pernicious anemia, which responded to liver treatment; and the second had only “a few” worms but presented hypochromic anemia, with erythroblastic marrow. The author commented that in hookworm anemia the sternal marrow was “habitually active.” He pointed out the importance of the myelogram and stated that
the myelo-erythroblast ratio in hookworm infected subjects tends to unity or less.

A good description of the marrow in hookworm anemia is the early one made by Cruz (1933a), who studied it in 24 fatal cases of hookworm anemia, and in one biopsy specimen. In most of the cases the patient had not received iron therapy. The age varied from 3 to 68 years. The femoral marrow showed uniformly a red color, except in one case in which there was marked myeloid hyperplasia, and the marrow looked yellowish. Microscopically, in most cases, there was "an intense regeneration of the cellular parenchyma, with marked decrease of the fat cells..." In the white series, there was "always an intense myeloid reaction (eosinophil myelocytes). Megakaryocytes were at times increased, at times decreased in number... In some of the cases, an intense eosinophilic reaction of the marrow, one day only after finding a normal percent of eosinophils in the peripheral blood" was noted. "As for the red cell series, a large number of foci with orthochromatic erythroblasts with nuclei in pyknosis or in rhexis are observed. The red color noted macroscopically in the marrow is due to the large number of these cells loaded with hemoglobin... The structure of these cells, their coloring characteristics, size and shape are identical to those observed in the normal marrow..." No megaloblasts were observed. "In four cases, the sternal marrow was observed in addition to the femoral marrow. In two of the cases this was not essentially different; in two others, there was such an intense myeloid reaction that the erythroblastic response was partially masked..."

Cruz' thorough description, illustrated with 35 photomicrographs, has been many times confirmed, chiefly at first by the Brazilian school (see bibliography in Povoa, 1937). In Italy, Tronchetti (1939) observed the marrow obtained by sternal puncture in 11 living infected anemic subjects, and he reported active erythropoiesis in the marrow in all. The mean E/M ratio was 1.15, ranging from 0.91 to 1.67. In Egypt, Azmy Pasha and Zanaty (1939) studied the marrow in 7 cases with hookworm anemia, and found normoblastic hyperplasia. Similar findings have been reported by MacGregor (1944) in 16 cases, Aversa (1946) in 4, Bonnin and Moretti (1950) in 3, Languillon and Mauzé (1950) in 10, Matilla et al. (1951) in 64, de Pasquale and Quattrocchi (1953) in 9. The latter authors demonstrated a return of the marrow to normal after iron treatment.

Peña Chavarría and Rotter (1935) studied at autopsy 96 cases of hookworm anemia. In the younger age group (age 14 and below), one half showed "total erythropoiesis" and in the others there were fatty changes in at least one-half of the marrow compartment. In older age groups, one-half to one-third of the cases had similar evidence of inadequate regeneration of the bone marrow. However, the authors did not give microscopic description of the marrow tissue, and their evidence cannot be taken as adequate.

Layrisse et al. (1959, 1961) observed in infected anemic subjects a moderate increase of the erythroblastic series, as is usually the case in this type of anemia. The mean E/M ratio was 2.8 and 2.2 times normal.

Although erythroblastic hyperplasia is the common finding in anemia associated with hookworm infection, megaloblastic changes have been also reported. Tronchetti (1939) observed, in a 71-year-old infected subject, megaloblastic changes in the marrow similar to those seen in pernicious anemia. Layrisse et al. (1964) found megaloblastic changes in the marrow of four of their cases. Veles et al. (1965) made similar observations. Such observations might be theoretically explained by exogenous B12 or folate deficiency; there is often in addition, associated with heavy hookworm infections, impairment in folate absorption (see p. 1061). On the other hand, it has recently been demonstrated that iron deficiency may result in defective folate metabolism.

Chanarin et al. (1962) observed elevated formiminoglutamic acid in simple iron deficiency anemia, and Chanarin et al. (1965) found the highest prevalence of megaloblastic anemia of pregnancy in the most markedly iron depleted pregnant group. Vitale et al. (1965) have demonstrated a defect in the activity of the formimino transferase in the liver of rats with iron deficiency anemia.

Megaloblastic proliferation and giant metamyelocytes have been observed in the marrow of several of our cases, in which the serum levels of folic acid and of vitamin B12 were normal or not markedly reduced. We have observed recently a heavily infected case (39,200 eggs/g of feces) with striking megaloblastic proliferation, normal serum levels of folic acid and B12, and high excretion of FIGLU. Iron treatment alone corrected the iron
deficiency and induced normal erythroblastosis.

Five subjects with hypoplastic marrow associated with hookworm infection have been observed by the authors; four recovered when treated with iron and cobalt orally (Layrisse, 1951) and the other recovered with iron treatment alone (Layrisse et al. 1959).

It may be mentioned that the direct intramedullary injection of a ferric salt does not produce any significant change in 48 hours (Trincão et al., 1952a); nor does a large intravenous load of iron produce any noticeable effect on the marrow cells cultivated later in vitro (Trincão et al., 1952b).

In sum, the bone marrow in hookworm infection associated with iron deficiency anemia shows a moderate erythroblastic hyperplasia, with an E/M ratio in the vicinity of one. Outside of an increase in eosinophils, there is no abnormality in the granulocytic series. The number of megakaryocytes is either normal or slightly increased. Iron-stained hemosiderin is absent. Megaloblastic proliferation is occasionally observed and hypoplastic marrow is a rare event.

**Erythroid and Myeloid Metaplasia**

Erythropoiesis as well as myelopoiesis may appear in other organs. In eight of ten hookworm anemias examined at autopsy, Cruz (1934a) showed the presence of advanced erythroblasts in the spleen, and many foci of myeloid metaplasia in the same organ. No island of metaplasia was observed in the liver.

**Serum and Tissue Iron**

Absence of bone marrow hemosiderin has been reported in all 23 subjects studied by Layrisse et al. (1959, 1961). Similar observations have been made by Gilles et al. (1964) in 20 subjects with heavy infections and anemia in which hemosiderin was looked for. Serum iron is invariably found to be low in heavy infections (Ventura, 1949; Jans et al., 1955; Larissa and Ventura, 1959; Roche and Pérez Giménez, 1959; Layrisse et al., 1961; Gilles et al., 1961, 1964; Srikanthia and Belavady, 1962).

As might be expected, unsaturated iron binding protein is high (Larissa and Ventura, 1959; Layrisse et al., 1961; Srikanthia and Belavady, 1962). All these findings constitute clear evidence of a depletion in iron stores, which is further substantiated by a markedly increased iron absorption (see Section V).

In normal subjects, plasma iron turnover, as given by various authors, varies between 20 and 42 mg per day, or about 0.60 mg per 100 ml of blood per day, the majority of patients falling within 25% of this mean value (Bothwell and Finch, 1962).

In two iron-deficient cases not associated with hookworm infection, the plasma iron turnover was 0.83 and 0.27 mg/100 ml of whole blood, respectively (Wasserman et al., 1952). In eight patients studied by Bothwell et al. (1956) the mean value was 1.3 times normal (range 0.7 to 1.8). Bothwell et al. (1957) later found a mean of 1.3 times normal, with a range of 0.38 to 1.34 mg. Finch (in: Bothwell and Finch, 1962) appears to be the only one to have found low values; he mentions five cases with values between 20% and 60%.

In four cases with iron deficiency anemia associated with hookworm infection, Ventura et al. (1957) report a mean $T_{1/2} Fe^{2+}$ clearance of 40 minutes, a plasma disappearance half time of $30 \pm 2.6$ mm (S.E.), as compared with a normal of 110, and a plasma turnover of $26.7 \pm 0.97$ (S.E.) mg/24 hrs (normal = 21 to 32).

Layrisse and co-workers (1961) obtained, in 11 subjects with heavy hookworm infection and severe anemia, an average iron turnover 1.4 times normal, with a range between 0.60 to 1.85 mg per 100 ml of whole blood. In spontaneous infections with A. caninum in dogs, Krupp (1961a) found a rapid disappearance of injected $Fe^{2+}$ and an increased iron turnover.

The utilization of iron by the marrow to form new erythrocytes was complete, or almost complete, in the cases (non-infected) studied by Bothwell et al. (1956) and in those (infected) studied by Ventura and co-workers (1957) and by Layrisse et al. (1961).

Outside of Finch's five observations, it seems clear that iron turnover in iron deficient subjects, whether they are hookworm infected or not, is normal or, more often, somewhat elevated. This appears at first sight paradoxical. Bothwell and Finch (1962) mention as a possible explanation that the determination of plasma iron at very low levels is inaccurate. Henry, Sobel, and Chiamori (1958) suggest that other pigments can be extracted from the plasma precipitated with trichloracetic acid, which might introduce small
differences in the estimation of plasma iron. On the other hand, observations by Pollycove (1963) indicate that there is in iron deficiency an excess of intramedullary red cell destruction. Layrisse et al. (1965) have shown that there is a reduction in red cell survival in iron deficiency anemia, whether associated with hookworm or not. Such an excessive hemolysis may well be the explanation for the increased iron turnover observed in such cases (see Section VI).

Summary

Hookworm anemia, in its fully developed stage, is characterized by hypochromic microcytic circulating red blood cells. The percentage of reticulocytes is usually normal, but may show elevated values, and there may appear nucleated red cells and punctate basophilia.

The marrow shows morphological evidence of increased function: it is, as a rule, markedly hyperplastic, with an increase in normoblasts. There may be erythroid and myeloid metaplasia of the spleen. Rarely, a hypoplastic bone marrow may be observed.

Serum iron is almost invariably low, and the disappearance half-time of injected radioactive iron from the plasma is rapid, of the order of 30 to 50 minutes. Iron turnover is moderately elevated or normal. Utilization of iron for production of red blood cells is efficient, more than 90% of the injected iron usually appearing in the circulating erythrocytes.

These findings mark hookworm anemia as being of the iron deficiency type.

III. THE RESPONSE TO VARIOUS FORMS OF TREATMENT

The aim of this chapter is not to describe practical aspects of treatment in hookworm anemia, but rather critically to examine the response to various forms of therapy, insofar as they might give light on the nature and causes of the anemia.

The Effect of Iron Salts

Iron salts have been used since the early days of hookworm therapy as an adjuvant to worming, and many of the old clinicians advocated them (Lutz, 1885; Sandwith, 1894). After the first decade of the twentieth century, however, and based on the authority of such experts as Ashford, who felt that iron had a "remarkable lack of effect in the anemia of unciniaiasis" (Ashford and Gutierrez, 1911, p. 303) the substance was largely abandoned and Chandler (1929) in his classical textbook, makes only a passing mention of it.

Several authors, during the first quarter of the twentieth century, gave indeed objective evidence of the value of iron salts given without administering vermifuge (Liemberger, 1905; Kobayashi, 1927, 1929; Keefer and Yang 1929), but the question was finally settled only after the simultaneous publications of Cruz (1932, 1934b, c, and d) and of Rhoads and Castle (1932) and Rhoads et al. (1934). These authors showed clearly that, without removing the worms, it was possible to reach nearly normal hemoglobin levels with iron therapy (and hospital diet) alone. The response to iron was shown to be extremely rapid and dramatic.

These results have been widely confirmed in different parts of the world. As examples, we might quote, in Venezuela, Gabaldón (1933),4 in Egypt, Biggam and Ghalioungui (1934), in Costa Rica, Peña Chavarria and Rotter (1935), in Brazil, Povoa (1937), in India, Napier and Das Gupta (1937), Hynes et al. (1946), Gtour et al. (1962), in Central Africa, Lehmann (1949). The iron salt used was in general ferrous sulfate, and the dose of elemental iron of the order of 100-300 mg/day.

On the whole, the statement made by Heilig and Viswewar (1942) can be fully endorsed: "The way the severest degree of anaemia in ankylostomiasis improves if inorganic iron is supplied in sufficient quantities, is one of the most impressive experiences in clinical medicine. . . .” (Fig. 2).

The therapeutic doses of iron were large in the above cited studies, and the time of administration short. Cruz and Fimenta de Mello (1945) have administered smaller doses of iron, both alone or mixed with staple foods. Ferrous sulfate, which could be readily mixed with manioc flour, and ferric ammonium citrate, added to bean gravy, proved particularly successful, and one case was maintained at normal levels of hemoglobin, with ferrous sulfate added to manioc, at doses of approximately 0.2 g/day of the salt during a period of two years, without removal of the worms.

4 Also administered liver extract.
White et al. (1957) have administered small iron supplements (from 15 to 50 mg iron as ferrous sulfate) to hookworm-infected, anemic schoolchildren, for 12 to 14 weeks, and obtained good responses in hemoglobin, particularly in those children with low initial levels.

Roche and Layrisse (unpublished) have administered 60 mg of elemental iron daily during three months in the form of ferrous sulfate to subjects in a hookworm infected area, and a placebo to a neighboring community with practically the same prevalence and degree of hookworm infection. Figure 3 shows in the population treated with iron a gradual increase of hemoglobin, inversely proportional to the initial hemoglobin values. There was no evident change in the control group. In the small group with hemoglobin below 10 g/100 ml, one of the three individuals showed a rise in hemoglobin of 8.5 g, probably from iron treatment unbeknown to the observers, whereas in the two other subjects, the level went down.

On the basis of such response to iron, Cruz (1932) was first to state clearly that the basic mechanism of hookworm anemia was iron lack. In fact both he (Cruz, 1934b, c, and d) and other authors (Rhoads et al. 1934) tended to relegate hookworm to a secondary plane as a cause of the anemia, and felt that iron lack in the diet was the primary cause.

Whatever the case may be, these results show that absorption of iron salts is excellent in hookworm anemia, when they are administered in fairly large quantities (see p. 1044). Such iron as is absorbed is used rapidly and efficiently for the production of new red blood cells, and reticulocytes show a marked rise 2 to 4 days after beginning administration (Kobayashi, 1927; Cruz, 1934c; Rhoads et al. 1934). All this, together with the morphological and functional characteristics,
Roche et al. (1957b) is due to bone marrow arrest caused by infection or intoxication (Heilig and Viswewar, 1942; Chatterjee and Roy, 1951; Roche et al., 1957b) is due to bone marrow arrest caused by infection or intoxication (Heilig and Viswewar, 1942) or to the presence of very heavy hookworm loads, with large amounts of

![Graph showing the comparison of the effect on circulating hemoglobin of the routine daily oral administration of 60 mg of elemental iron in the form of iron sulfate, and of a placebo, on two neighboring and similar rural populations. In the treated group, the rise is inversely related to the initial hemoglobin, while the untreated group remains essentially unchanged (Layrisse and Roche, unpublished).]
bleeding (Roche et al. 1957b). The latter observation suggests that heavy bleeding may balance the effect of absorbed iron.

This is also probably the explanation for the failure of many iron-treated cases to reach completely normal hemoglobin levels. Not all authors believe that this is the case and opinions range widely from that of Heilig (1952), who felt that iron can raise hemoglobin "to any desired level," regardless of remaining infection, to that of Lehmann (1949)—with which we are inclined to agree—"Iron alone will not fully cure patients... Full recovery will take place after the removal of hookworms."

The data by Rhoads et al. (1934) can be readily analyzed (Table 3). Half of Rhoads et al.'s patients still had less than 70% circulating hemoglobin values at the end of iron treatment, before vermifuge was given.

Cruz' data (1934c) on his patients cannot be analyzed in table form, because each case was submitted to different therapeutic maneuvers. It appears, however, that of all the cases treated, only four (cases 3, 4, 15 and 18) reached what can be considered normal hemoglobin values, although most of them were treated and observed for considerable periods of time.

The Response to Vermifuge

The dramatic response of hookworm anemia to iron has tended to obscure the fact that patients do respond to vermifuge alone, although only after a protracted period of time. An analysis of this fact is of some importance in the interpretation of the pathogenesis of hookworm anemia.

In the reports by early authors, the favorable clinical response to vermifuges is taken for granted. Ashford et al. (1904) felt that light cases recovered readily without iron. In a series of cases selected by them to represent various grades of the disease, no iron treatment was given, in order to observe the effect of removal of the worms alone. The results were: "cured," 31.8%; "practically cured," 9.1%; improved, 45.5%; unimproved, 13.6%. Unfortunately, the authors did not give their criteria for "cure", nor the length of time needed to reach such cure. Average hemoglobin in the series, however, rose from 44.6% to 64.7%. The comment was made that "these percentages compared favorably with the general results," by which the authors probably meant that they compared with the cases treated with iron preparations.

Dock and Bass (1910), although they recommended the administration of iron, at least in the moderate and the severe cases, did not feel that it was essential. They give in detail the changes in blood values in 12 cases, but unfortunately, in only one of them (chart 9, p. 234) is there the specific mention "No iron."

Dock and Bass (1910) noted that, after vermifuge alone, red blood cells responded rapidly, followed later by hemoglobin. This is of course characteristic of the anemia of chronic bleeding, after the cause of bleeding has been removed, and has been noted by others (Ashford et al. 1933; Chandler, 1929).

Beginning in 1927, the point was emphatically made that hookworm anemia responded poorly, or not at all, to vermifuge alone. Kobayashi (1927) observed several cases treated with vermifuge alone and followed for a maximum of two weeks, and noted that there was no response in hemoglobin values. Cruz (1934d) presented in detail eight hospitalized cases, followed for from 32 to 300 days after being given vermifuge, without iron, and receiving either their "usual" diet or an iron poor one. The results were interpreted by Cruz as indicating a lack of response to diet alone, in absence of iron therapy. In fact, the three cases followed two months or less responded little or not at all; all other five cases responded, with increases in circulating hemoglobin which we have calculated to represent the result of absorption and utilization of food iron of at least from 1.2 to 5.5 mg per day. We have had a similar experience, under field conditions (see Section V and Fig. 8).

Rhoads et al.'s observations (1934) agreed essentially with Cruz'. They treated six field patients with vermifuge alone, and observed only an average rise from 38 to 41 % in circulating hemoglobin in 40 days in all six, and to 54 in three of them, followed for 90 days. This sort of response was not substantially modified by the addition of 300 g of meat and 1500 cc of milk daily in five other patients. They also treated 12 patients under hospital conditions with the removal of worms. The hemoglobin and other red blood cell values were then observed for about 16 days, and up to 20 weeks in one case. Responses were nil in eight of the cases, two of them actually showing a drop in hemoglobin and
the other four cases very modest rises. The American authors were struck by the difference between their observations and those made earlier by Ashford and his group in practically the same area. The discrepancy was in large part due to the short period of observation utilized by Rhoads et al. (1934).

That this was so transpires from data later obtained in children by members of the same group. Payne and Payne (1940), again in Puerto Rico, studied during prolonged periods of time hemoglobin changes after vermifugation, without giving iron (Table 4). "After anthelmintic treatment alone, the gain in hemoglobin in those children examined from 1 to 12 months after treatment was of doubtful significance. The gain which occurred was relatively more pronounced in children who showed moderate anemia than in the severe cases. At the end of six months appreciable progress had been made in restoration of hemoglobin, but only in the examination made 18 to 24 months after the initial treatment was the hemoglobin level of the study groups found to approximate the level in children of their age-group and community."

The latter part of the statement may be interpreted to mean that, 18 to 24 months after vermifuge, without iron treatment, hemoglobin levels do return to approximately normal levels. The meaning of this observation in relation to the absorption and utilization of food iron will be discussed in the chapter on absorption.

**Other Forms of Treatment**

Mention may be made of other forms of treatment, which have been used without success, such as copper salts (Povoa, 1937) and crude liver extracts (Rhoads et al. 1934).

Cruz (1934c) administered, together with iron, a number of substances such as tryptophane, histidine, crude liver, yeast, copper lactate, copper sulfate, cobaltous chloride, lecithin, cholesterol and arsenious acid, without any modification of the effect of iron on the blood values.

Quicker and more efficient effect has sometimes been claimed for iron given parenterally (Morisani, 1952; Biggam and Ghalioungui, 1934) or

---

**TABLE 3**

Response to iron treatment
(From Rhoads, Castle, Payne and Lawson, 1934)

<table>
<thead>
<tr>
<th>No. cases</th>
<th>Average days of treatment</th>
<th>Final hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24*</td>
<td>43.2</td>
<td>80-100 (12.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70-79 (37.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60-69 (12.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Below 60 (37.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(87.5%)</td>
</tr>
</tbody>
</table>

* Three of the 27 cases reported were treated for only 10 days. They are not included here.

**TABLE 4**

Heavily infected children who received anthelmintic alone, showing hemoglobin gains at different time intervals after first anthelmintic
(From Payne and Payne, 1940, Table 4, p. 130)

<table>
<thead>
<tr>
<th>Original hemoglobin (g/100 ml)</th>
<th>1-2 months</th>
<th>5-6 months</th>
<th>12 months</th>
<th>18-24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rio Grande Cidra Comerio</td>
<td>Rio Grande Cidra Guajataca Quebradas</td>
<td>Orocovis Quebradillas Guajataca</td>
<td>Orocovis Quebradas Bayanay</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Hb mean change (g/100 ml)</td>
<td>No.</td>
<td>Hb mean change (g/100 ml)</td>
</tr>
<tr>
<td>&lt;6.9</td>
<td>4</td>
<td>0.3</td>
<td>7</td>
<td>2.4</td>
</tr>
<tr>
<td>6.0-6.9</td>
<td>6</td>
<td>1.1</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>7.0-7.9</td>
<td>14</td>
<td>1.3</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>8.0-8.9</td>
<td>10</td>
<td>1.4</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>9.0-9.9</td>
<td>17</td>
<td>1.0</td>
<td>26</td>
<td>1.8</td>
</tr>
<tr>
<td>10.0-10.9</td>
<td>26</td>
<td>1.0</td>
<td>38</td>
<td>1.6</td>
</tr>
</tbody>
</table>
for iron given together with hydrochloric acid (Peña Chavarria et al. 1945). There is in the latter article no statistical study of the differences between the groups to which HCl was given and those without; such differences appear small and well within the methodical error of red blood cell counting. Layrisse (1951) has administered cobalt together with iron and found a faster response in hemoglobin. In two cases with marked edema, who did not respond to iron and dieting, the administration of vitamin B<sub>1</sub> resulted in diuresis and clinical improvement (McKenzie, 1939). Such cases are unusual, since edema responds usually well to iron and diet, and they must have had a concomitant vitamin B<sub>1</sub> deficiency. Trincão and co-workers (1955) made similar observations in two edematous cases, who had responded poorly to the accepted therapeutic measures. They found a markedly reduced vitamin B<sub>1</sub> excretion, however, in all subjects studied from the same region (Portuguese Guinea), whether they had hookworm, filariasis, sleeping sickness, or whether they appeared entirely normal. They did not feel that administration of vitamin B<sub>1</sub> modified in any way the response of cases with hookworm anemia.

Two cases of polyneuritis associated with hookworm anemia, and possibly due to beriberi have been reported by Hoff and Shaby (1939). There is no reason to think that the association was anything but casual.

Summary

The anemia associated with hookworm infection responds rapidly and dramatically to the administration of iron salts by mouth, so that circulating hemoglobin levels approaching normal may be reached in a matter of weeks. This confirms the iron deficient nature of this anemia. It may respond also, however, to worming with no change of diet, but in a much longer time: after such treatment, normal levels are not reached in less than 15 to 20 months. This latter observation suggests that dietary iron may be sufficient, in the areas studied, to maintain normal, or nearly normal hemoglobin levels.

IV. DIETARY FACTORS

Since dietary deficiencies of various types are often present in regions where hookworm infection is prevalent, it may become difficult to separate the effect on blood values of dietary factors from those due to parasitic infection. We shall attempt to review some of the available evidence in this respect and draw whatever conclusions might seem to be justified at present.

Gross Nutritional Status

Although malnutrition in areas affected by hookworm may be extreme, as is the case in certain regions of India, in others, such as Corrientes in Argentina (Fülleborn et al. 1928), dietary intake, including animal protein, may be excellent. Even in those areas in which anemia is frequent, there is most often no gross malnutrition. This point was brought out by Griesinger (1854) who, in his early description of “Egyptian Chlorosis” wrote that the patients “are as a rule not at all underweight, often even somewhat fat” (footnote 1, page 38).

Similar observations have been made by a number of other experienced observers. Thus Ashford and Gutiérrez (1911) noted that in hookworm disease in Puerto Rico “the nutrition is generally good” and, further “Emaciation... is not an usual phenomenon in uncinariasis, and when it occurs, it is likely to be due to intercurrent diseases, or severe digestive disturbances.” Chandler (1929), on the basis of his wide experience, mostly in India, wrote that “the hookworm patient seldom becomes emaciated. There is, in fact, some tendency for accumulation of fat...,” and Cotti (1937) working in Italy: “Very rarely is this anemia accompanied by loss of weight; as a general rule, the nutritional status is good.”

Foy and Kondi (1960) noted that “none of the patients was malnourished, and all had a well developed layer of subcutaneous fat. The diet of the grossly anaemic did not appear to differ from that of the non-anaemic patients.”

In Sheehy et al.’s (1962) 14 hookworm infected patients “none showed pronounced malnutrition, but all had lost weight;” however, nine of the 14 patients had diarrhea, which may have accounted for such weight loss.

Layrisse and Roche (1964a) carried out a screening dietary survey on 1142 subjects from heavily infected communities. Only 6.5% of the children in the survey were found to have weight

1 “Sind in der Regel gar nicht abgemagert, oft ziemlich fett” (p. 556).
2 “Ben difficilmente questa anemia si accompagna ad un dimagrimento; di regola generale le condizioni di nutrizione sono buone” (p. 242).
deficiency and no gross clinical malnutrition was observed (Fig. 4).

The above observations suggest that in hookworm infection there does not, as a rule, occur such extreme malnutrition as might account alone for the anemia; they also suggest that hookworm infection, even when severe, does not interfere with food utilization to an extent sufficient to lead to severe malnutrition.

Iron Intake

Since the publications of Cruz (1932, 1934a and b) and of Rhoads et al. (1932, 1934), great, and at times exclusive, importance has been given to dietary iron deficiency as the cause of tropical anemias, whether associated with hookworm or not. As a matter of fact, total iron ingestion appears to be relatively high in many regions where hookworm anemia prevails, if it is compared with iron ingestion in European or North American areas, where "the amount of iron furnished in the average normal diet is 12 to 15 mg per day" (Wintrobe, 1961).

To quote extreme examples, iron intake in Erythrea, around the year 1950, was calculated as 481 mg per day (sic!) on the average (Ferro-Luzzi, 1949-50), in the Indian Northwest Frontier 66 mg (Hynes et al., 1945) and in Western Nigeria 21 to 57 mg (Gilles et al. 1964). These are all areas where both anemia and hookworm are prevalent.

In Venezuela, where our studies have been carried out, Bengoa (1950) gives 16 mg as the quantity of iron consumed per day per person, both in laborers and in the middle class. In later studies, he found averages of 22 mg ± 5.8 (S.E.) in a mountain area (Planchart et al. 1951) and 12.6 mg in a warm rural area (Bengoa et al. 1952). The results suggest that iron intake in Venezuela varies somewhat in different regions, but remains generally adequate, or on the high side.

More recent studies, carried out by the Interdepartmental Committee on Nutrition for National Defense of the United States of America (1964)* have given an average, for various Venezuelan regions, of 16 mg of iron per person per day by the questionnaire method, and 18 mg by the recipe method.

The data given are almost always calculated from values in uncooked food. As pointed out by Bothwell and Finch (1962) this is only of limited value, since the amount of iron present in a particular foodstuff is profoundly affected by a number of factors, in particular by the manner of cultivation, which is highly individual in many of the tropical countries, and by the methods used in preparation. Actual chemical analysis of food iron have been performed on duplicate cooked servings, by Layrisse et al. (unpublished) on a total of 93 individuals from 13 families in an agricultural area of Venezuela where hookworm and anemia are endemic (Pequin, Estado Anzoátegui). The mean individual daily iron intake was 23 mg in 15 adult men, 17 mg in 16 adult women, 18 mg in 45 children of both sexes 7 to 14 years of age, and 13 mg in 19 children from 2 to 6 years of age. Chemical analyses carried out in aliquots from actual servings by the Interdepartmental Committee on Nutrition for National Defense of the United States of America (1964) gave a range of daily intake in adults from 19 to 51 mg.

* These studies were carried out in collaboration with the Venezuelan Institute of Nutrition.
The above data for iron ingestion show therefore that, in some of the tropical areas where iron is prevalent, iron intake is on the high side. The conclusion is inescapable that either a large proportion of the dietary iron is unavailable for absorption, or that iron losses are abnormally high, or both. The origin of the dietary iron is frequently not specified, but most of it seems to be of vegetable origin. Thus, for example, in Peshawar, Northwest Province of India, the total iron intake is on the average 66 mg per person per day, but only 1 mg is of animal origin (Hynes et al. 1945).

In Venezuela, Bengoa (1950) in people from Caracas belonging to the labor and middle class, found by the questionnaire method, that more than 70% of the iron intake comes from bread, cereals, and vegetables, and less than 5% from animal source. Similar results were obtained by the Interdepartmental Committee on Nutrition for National Defense in Venezuela (1963) (Fig. 5).

**Ingestion of Other Nutrients**

Mention will be made only of those studies which seem to have a bearing on the question of hookworm anemia.

**Protein.** Hookworm may lead to fecal loss of protein, as has been demonstrated by Gilles et al. (1964) and by Blackman et al. (1965); in addition, in endemic areas, protein ingestion is often inadequate. It is not clear, however, whether protein deprivation contributes significantly to the anemia associated with hookworm. Response of the anemia to a high protein diet is at best modest (see Section III). The anemia of protein deprivation in the rat is characteristically of a non-regenerative nature, as shown by the depression of the reticulocyte count (Aschkenazy, 1961) and the lowered red blood cell radioiron incorporation (Béthard et al., 1958; Reissmann, 1964), quite the opposite from what has been shown usually to occur in hookworm anemia (see Section II).

**Vitamin B12.** Low levels of vitamin B12 in the serum of heavily infected subjects have been reported by Layrisse et al. (1959). Eight out of 21 subjects showed values below 100 μg/ml serum by the Lactobacillus leichmannii method. In another series (Layrisse et al. 1964), using this time Euglena gracilis, the results were significantly lower than in normal subjects, but only one patient showed a value below 150 μg/ml serum. Shilling tests performed in both series showed normal absorption in all cases tested. The results are probably due to low animal protein ingestion; or possibly to malabsorption of vitamin B12 undetectable by the Shilling method. The cases in both series, even those with very low B12 serum values, responded to iron therapy alone; so that, presumably, these low values were not directly related to the observed anemia.

Nogueira and Perez (1959) have demonstrated high vitamin B12 activities in *Ancylostoma caninum* obtained from dogs (range: 648 to 3240 μg/g). Whether this indicates that hookworm may act as does *Diphylobothrium latum*, by robbing its host of the vitamin, is unlikely because of the small size of the worm.

**Folic acid.** Since people with hookworm infection are usually agricultural workers, their diet contains large amounts of vegetables, which should prevent folic acid deficiency. In Venezuela they do not usually, however, consume fresh vegetables as such, but rather boil them at length to produce a soup, or grill them. In a study performed in six families, made up of 47 individuals, living in an agricultural rural area, the actual folate intake was less than 50 mg per person per day in four of the six families studied. The diet contained vegetables with
deficient diet were given a normal diet, a reduction in number of eggs in the feces was noted. This was interpreted to mean in part that oviposition was impaired and in part that worms were lost; in fact, a number of the latter were observed in the feces. This was not the only explanation, however, since only part of the worms were expelled, and since a marked increase in egg production was noted when the host was again placed on a deficient diet. Foster and Cort (1935) later confirmed, on 12 dogs, that egg counts in the feces increased markedly when the animals were placed on deficient diets.

Otto and Landsberg (1940) studied 17 dogs from two litters, on a vitamin- and protein deficient diet (with approximately 7.5 mg of iron and 23.5 g protein per day) giving four of them 3000 to 6000 mg/iron ammonium citrate per day and keeping one dog per litter as controls on a normal diet. The dogs were then infected: all animals on the experimental deficient diet died, whether given supplemental iron or not. Both controls survived: in one of them circulating erythrocytes went down about 50% from the initial level, with reticulocyte response up to a maximum of 42%. The other developed no anemia. These experiments would indicate that diet, and particularly proteins, are of some importance in the resistance against the production of anemia by experimental hookworm infections. Whether these studies apply in man is not clear. In addition to the different species involved, it is seldom that such extreme burdens of infection associated with such profoundly deficient diets are found in a human context.

**Summary**

Patients with hookworm anemia are seldom underweight, and show as a rule a fair state of nutrition, unless causes other than hookworm intervene.

In many tropical regions where hookworm infection and iron deficiency anemia are prevalent, total dietary intake of iron, as compared to that in colder areas with a low incidence of iron deficiency, is actually high. In the areas where the dietary origin of the iron is reported, this seems to be mostly of a vegetable type.

A supplemented high dietary iron regimen may give rise to modest increases in hemoglobin levels in subjects with light infections. It is usually ineffective in subjects with heavy infections.

Experimental studies in dogs suggest, on the
other hand, that markedly deficient dietary regimens may act by lowering the resistance of the host to the parasitic infection, and possibly may lead to an increased blood loss from existing infections. Whether a similar situation may actually occur in man is not clear.

Anemia may be produced by hookworm infection, provided it is severe enough, in the face of a diet which appears adequate in every respect, including iron and protein intakes. On the other hand, a poor diet cannot but aggravate the harm done by hookworm, and may lead per se to iron deficiency anemia, even in the absence of hookworm infection. So that unsupported statements about diet being or not being an important cause in hookworm anemia in given areas should be replaced by quantitative determinations of iron ingestion and absorption from foods (cultivated in situ and cooked by local means, whenever possible) and iron losses through various routes.

V. INTESTINAL MORPHOLOGY AND FUNCTION

A study of intestinal morphology and function in hookworm infection is of particular interest since the parasite, through the changes produced by its attachment to the mucosa, could conceivably lead to impairment in the absorption of foodstuffs.

Various types of digestive systems may be observed in hookworm infection. These are generally vague and non-specific, ranging from epigastric distress to dirt-eating, but they may at times simulate the symptoms of a duodenal ulcer (Roig and Caffera Abadie, 1927; Yanikomshian and Shehadi, 1943). Details of clinical findings lie outside the scope of this monograph.

Pathology

Studies of the gross pathological changes associated with hookworm infection have been scarce. The earlier studies (Lutz, 1885; Leichtenstern, 1887) give few details. They describe the presence of thick mucus overlying the mucosa, often of a “light blood” or an “orange” color (Lutz, 1885; Leichtenstern, 1887) probably due to the admixture of blood; a few echymosis were observed, as well as minute erosions or ulcers (2–3 mm in diameter) giving at times a speckled appearance to the mucosa, often with little change in the surrounding tissue. The opinions of later authors have varied from Ashford and Gutierrez’ impression (1911), who felt that “the feeding ground of Necator americanus can easily be passed over without being noted” (p. 189) to that of Rotter (1931) who described extensive lesions, with localized mucosal and submucosal hemorrhages, necrosis of the adjacent tissue, and thickening of the submucosa. Rotter’s often quoted report gave the impression that the lesions were widespread: he wrote, “The usual picture found in nearly all cases of severe ankylostomiasis, consists in a pronounced thickening and sclerosing of the submucosa, which extends to a large portion of the intestinal canal with rather uniform intensity.”

It is probable that Rotter may have been describing extreme cases, since they were accompanied by cachexia and symptoms of enteritis and food malabsorption.

From the more recent data, obtained by means of capsule biopsy, and from our experience with dog infections, we are inclined to feel that the lesions inflicted by hookworm are remarkably local, limited to the area immediately surrounding the worm, and that most of the intestinal mucosa is spared, or at any rate, regenerates rapidly after the worm has left its site of implantation. Beker et al. (1961) reported on 39 subjects, with varying pathological conditions and commented briefly on the findings on six of these who harbored hookworm infections, of undetermined severity. These presented localized areas of epithelial flattening, with “hyperchromatism” of the nuclei and at times infiltration of the villi by lymphoid cells. The changes shown in the two published microphotographs seem minimal. Sheehy et al. (1962) described, in 14 selected cases with “massive infestation,” an abnormal histological picture, characterized by flattening and fusion of the villi (the latter in three cases), clear-cut fibrosis of the lamina propria in some of the cases and chronic inflammation of the lamina propria in all cases. The picture described by Sheehy et al. (1962), may have been influenced by the presence of other disease conditions, such as sprue, which is common in Puerto Rico (Gardner, 1958). In Sheehy’s group “all complained of gastrointestinal distress and nine had frank diarrheas,” “the stools were

10 “Das gewöhnliche Bild, das sich in fast allen Fällen von schwerer Ankylostomiasis findet, besteht in einer starken Verdickung und Sklerosierung der Unterschleimhaut, die in ziemlich gleicher Stärke sich über grosse Teile der Darwmwand erstreckt.”

11 In only three of the cases was a fecal egg count performed.
soft or watery, . . . and although they were never foamy in appearance they floated in water.”

Layrisse et al. (1964) have performed Crosby capsule biopsies in 22 cases with heavy hookworm anemia, in Venezuela, where sprue is a rarity (Gil Yépez et al., 1953; Agüero and Layrisse, 1958); their results differed from those reported by Sheehy et al. (1962). The mean length of the villi varied from 500 to 800 μm; the columnar epithelial cells were of normal size, well oriented, their nuclei and brush border well preserved. The crypt glands appeared slightly or moderately elongated in some cases. There was an apparent decrease of intracellular mucus, which suggests an increase in cell turnover. Cellular population of the lamina propria was normal, being represented chiefly by mononuclear cells. There was, in general, no evidence of atrophy of the mucosa. In one of the cases, section of a hookworm was seen. Although it was not possible to locate the site of the parasite mouth capsule, the structure of the subjacent mucosa was practically normal (Fig. 6).

Banwell (1962) reported, in a brief note, that there was no evidence of villous atrophy in the intestinal biopsy of eight East Africans infected with hookworm. In a specimen taken under direct vision at operation, Gilles et al. (1964), found a normal mucosa.

In four of the 22 cases reported by Layrisse et al. (1964), changes were more marked, one of them showing flattening and fusion of the villi, another frank atrophy, and two marked infiltration, specially around the base of the crypts, which, in one of the two cases, extended down to the muscularis mucosa and submucosa. There did not appear to be a relationship between hookworm burden and the severity of histological changes.

The findings by Layrisse et al. (1964), by Gilles et al. (1964), and by Banwell (1962) seem to indicate that hookworm infection per se produces, as a rule, inconspicuous histological changes in the intestinal mucosa, or that these changes, localized as they are, rapidly return to normal after the worm changes site.

Roentgenological Changes

Changes in the duodenal appearance characteristic of ulcer have been reported in association with hookworm infection (Pellegrini and Facci-Tosatti, 1934; Monat and Cooper, 1947). In the absence of proper statistical evidence, it is not possible to decide whether such association is more than casual. The series studied by Krause and Crilly (1943), and by Hodes and Keefer (1945), leave little doubt, however, that other types of roentgenological changes are of frequent occurrence. Of 44 subjects with hookworm disease, Krause and Crilly (1943) described “severe” changes in 26 and “minimal” in 14. Of 11 subjects with moderate changes, none returned to normal when reexamined after worming, although eight of them improved.
FIGURE 6. Histological changes in the jejunal mucosa of hookworm-infected patients. A: case 18, essentially normal. This is the picture in most cases. B: case 16. Although a section of the worm may be seen on the surface of the mucosa, and its head is probably anchored nearby, the villi show no alteration. C: case 14, showing flattening and fusion of the villi and infiltration of the lamina propria. D: case 22, exhibiting infiltration of the mucosa and submucosa (from Layrisse et al., 1964).
Hodes and Keefer (1945) made routine gastrointestinal studies of 125 infected U.S. soldiers, and found 60% of them with small intestine abnormalities. These, however, never affected the proximal half of the duodenum, and began generally in the distal half of the organ, or in the proximal jejunum, where the most marked changes were always found. No ulcer was demonstrated, but none of the infections were chronic. The x-ray changes are described and illustrated profusely in the original article: they consisted chiefly of excessive peristalsis and segmental contractions, with distortion of the mucosal pattern. Not infrequently, the changes improved only slowly after therapy and they at times remained present for months after the patient was clinically well; the authors felt this might be due to damage to the intramural nervous system.

In contrast, Yanikomshian and Shehadi (1943) found that normal appearance of the intestinal wall was restored in 11 to 24 days after vermifuge. All 14 of Sheehy et al.'s (1962) cases of severe infections had abnormal roentgenographic findings; “little abnormality” was demonstrated in the duodenum, but, in the jejunum, “the mucosal folds were usually thickened and irregular rather than feathery. A moderate degree of flocculation as well as pudding and segmentation was observed.”

**Gastric Acidity**

Rhoads et al. (1934) reported histamine-resistant achlorhydria in 13 of 54 patients with hookworm anemia; 11 responded to histamine but not to alcohol, and 30 responded to alcohol. Peptic activity was observed in all but seven patients. Srikanthia and Belavady (1962) demonstrated normal excretion of uropepsin in 20 subjects with hookworm. Garin et al. (1930) in 57 cases, most of them with light infection, found none with alcohol-resistant achlorhydria, and an approximately equal number with normal responses and with hypochlorhydria, in both the anemic (21 cases) and the non-anemic cases. Bonnin and Moretti's three heavily infected cases (1950) had normal acid content after histamine. Chevallier and Brumpt (1939) observed, by gastroscopy, an entirely normal pattern in their two cases.

Peña Chavarría et al. (1945), on the other hand, found five of ten children with hookworm anemia with “true achlorhydria” after alcohol stimulation, three with hypochlorhydria, and two normal. They treated these children with iron and HCl by mouth, and compared the response with that in 30 anemic children treated with iron alone. They felt that HCl administration accelerated the rise in blood values. Their results are not statistically analyzed, however, and in their charts (page 389) it may be observed that, after 45 days of treatment, the circulating red blood cells of the group taking HCl were only about 0.5 million higher (well within the limits of error) than those with iron alone; hemoglobin levels were about 8% above the control.

Heilig (1952) believed that, in hookworm anemia, hypochlorhydria and achlorhydria are “very frequent,” and do not improve upon disappearance of the anemia.

Fayez and Ragheb (1959) noted absence of free acid in 15 of 20 cases (A. duodenale) and a marked reduction in the other five. Histamine was not employed. Gastroscopic examination showed mucous folds which were “less apparent” than normal, excess mucus, and a “friable” mucosa which “in general . . . was atrophic.”

The reports are conflicting but, although hypochlorhydria is frequent, and achlorhydria not rare, this is not unlike the situation with iron-deficiency anemias unassociated with hookworm. Fifty to 60% of the cases with ordinary iron deficiency anemias may fail to secrete HCl in response to histamine and up to 83% in response to the Ewald test meal (Wintrobe, 1961).

Hypochlorhydria or achlorhydria might conceivably interfere with the absorption of food iron, and thus further aggravate iron deficiency anemia. No study seems to have been done of gastric acidity and food iron absorption together, but the latter is as a rule similar in infected and non-infected subjects from the same region.

**Absorption of Ionized Iron**

The response of most cases of hookworm anemia to the administration of iron by mouth, even in heavy infections, with more than 5000 eggs/g of feces, proves satisfactory, although some workers (McFadzean and Wong, 1952) feel that impairment of iron absorption is not infrequent in hookworm anemia and that the utilization of intravenously administered iron may be more efficient than that given orally.

Larizza and Ventura (1950) have determined
NATURE AND CAUSES OF "HOOKWORM ANEMIA"

serum iron in 12 patients with hookworm infection, 2, 4, and 6 hours after the oral administration of 1 g of hydrogen-reduced iron by mouth, and compared the results with those obtained in normal persons. The curve was similar in both groups during the first two hours; but, in the infected anemic group, the rise was less after four hours, and serum iron decreased markedly at the sixth hour, whereas in the normal subjects it continued to increase, probably a reflection of decreased tissue saturation rather than the result of deficient absorption in anemia.

The same authors later studied iron absorption, in five infected subjects, by means of the oral administration of 10 mg of hydrogen-reduced Fe⁵⁹ Ferric chloride (Ventura et al., 1956, 1957), measuring both the quantity of unabsorbed fecal iron and the circulating radioactive iron. Two of five patients showed increased absorption, one was normal and two had a markedly diminished absorption.

Martinez et al. (unpublished) have studied the absorption of ionized iron (1 mg elemental iron in the form of ascorbate) in 69 subjects living in an agricultural area (Pequin, Estado Anzoátegui, Venezuela) (Table 5). They used the double isotope (Fe⁵⁹, Fe⁵⁹) method described by Saylor and Finch (1953). While in eight normal noninfected adult men living in Caracas mean iron absorption was 16.8%, ranging from 2.7 to 24.4, a much higher absorption was found in all groups in the agricultural area, even in the absence of anemia; there seemed, however, to be a tendency toward higher absorption as severity of infection increased. These findings indicate that iron deficiency was present in practically all subjects living in the region, irrespective of whether they had anemia or not.

**Reabsorption of Hemoglobin Iron**

Foster and Landsberg (1934) mentioned the possibility that some of the iron lost into the intestinal tract in hookworm infection may be reabsorbed and hence made available for red blood cell production. Several lines of evidence indicate that such reabsorption actually takes place. Roche et al. (1957c) tagged the circulating red blood cells of chronically infected hookworm patients, with both radioiron Fe⁵⁹ and radio chromium Cr⁵¹ and measured differentially radioactivity from either isotope in both blood and stool. From Cr⁵¹ radioactivity the amount of blood lost into the gastrointestinal tract, and hence the quantity of iron lost in the intestine may be calculated. Measuring radioactivity from Fe⁵⁹, on the other hand, and comparing it with blood radioactivity indicates the actual quantities of iron lost in the feces. Roche and Pérez Giménez (1959) studied 14 infected patients in such fashion (the four patients previously reported by Roche et al., 1957c, were included in this group). They found an average of 44.1% (range 13.1-76.4) of the hemoglobin iron lost in the gastrointestinal tract not accounted for in the feces, and presumably reabsorbed. In 11 patients studied later by Layrisse et al. (1961) by the same methods, reabsorption was, on the average, 36.3%, with range 18.1 to 52.2. The group in Venezuela has now studied in this fashion a total of 40 patients (Table 6).

Gilles et al. (1964) have administered by duodenal tube, over a period of about 18 hours, Fe⁵⁹-tagged hemoglobin to two anemic subjects with heavy hookworm infections. Both patients

### TABLE 5

**Intestinal absorption of 1 mg of ionized iron using double isotope (Fe⁵⁹, Fe⁵⁹) technique**  
*(From Martinez, Carstens, Layrisse and Roche, unpublished)*

<table>
<thead>
<tr>
<th>Type of individuals</th>
<th>Area</th>
<th>Hookworm infection</th>
<th>No. individuals</th>
<th>% absorption</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologically normal</td>
<td>Caracas (urban area)</td>
<td>0</td>
<td>8</td>
<td>16.8</td>
<td>2.7-24.4</td>
</tr>
<tr>
<td></td>
<td>Pequin (rural area)</td>
<td>a. less than 500 eggs</td>
<td>1. M*</td>
<td>65.9</td>
<td>36.7-88.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. 500-1900 eggs</td>
<td>2. F</td>
<td>58.6</td>
<td>19.0-106.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. 2000 eggs</td>
<td>15</td>
<td>69.0</td>
<td>17.5-97.5</td>
</tr>
<tr>
<td></td>
<td>Pequin</td>
<td>Infected</td>
<td>11</td>
<td>87.1</td>
<td>39.0-107.0</td>
</tr>
<tr>
<td>Anemic</td>
<td>Pequin</td>
<td></td>
<td>19</td>
<td>88.3</td>
<td>61.2-106.4</td>
</tr>
</tbody>
</table>

* M, male; F, female.
TABLE 6
Iron absorption from the hemoglobin lost into the intestine in 40 hookworm infected cases

<table>
<thead>
<tr>
<th>Hemoglobin-iron (mg/day)</th>
<th>Percentage of hemoglobin-iron reabsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost into the gut</td>
<td>Excreted in the feces</td>
</tr>
<tr>
<td>7.6</td>
<td>4.6</td>
</tr>
<tr>
<td>(2.3—21.3)</td>
<td>(1.1—16.2)</td>
</tr>
</tbody>
</table>

showed a high absorption (47.1% and 60.4% of the administered iron, respectively) which was not substantially modified when the study was repeated after worming. The quantity of hemoglobin iron administered was of the order of 14—24 mg.

The degree of iron reabsorption found by the three groups mentioned above is high when compared to that obtained after giving Fe^59-tagged lysed rabbit red blood cells, which is of the order of 10% in normal and from 16% to 22% in iron deficient subjects (Callender et al., 1957; Turnbull et al., 1962; Layrisse et al., unpublished). Turnbull et al. (1962) also made the interesting observation that the absorption of hemoglobin iron is affected to a much lesser degree by iron deficiency or by phytates and ascorbic acid than that of ionized iron; they suggested that it followed an absorption pathway different from that followed by ionized iron.

Studies in progress in our laboratory have shown that, when Fe^59- and Cr^51-tagged rabbit hemoglobin is administered to humans, the value for iron absorbed is much less when determined from the resulting circulating Fe^59 than when calculated from the fecal difference between Cr^51 and Fe^59 excretion. Whether this means that part of the absorbed Fe^59 does not appear as transferrin iron is not clear. Studies are in process to determine the fate of the absorbed Fe^59.

Indirect evidence that hemoglobin iron is reabsorbed and reutilized for red blood cell synthesis has been advanced by Aparcedo et al. (1962). Radioactivity was measured in the upper and lower fourth of Fe^59 tagged, packed red blood cells in four patients with heavy hookworm infections, and compared during 120 days with that of three normal subjects. Although the subjects were few, the results were uniform and the standard deviations small, particularly after the 15-day sample. In the normal (Fig. 7) the curves cross toward the 30th day, indicating a migration downward of the radioactivity, as the initially tagged erythrocytes grow older and their density increases. Toward the end of the study, the curves cross again, presumably as the effete tagged red cell population is being destroyed, and its Fe^59 released, which goes on to tag a new generation of red blood cells, tending to appear again at the top of the hematocrit. In the hookworm-infected subject, on the other hand, Fe^59 lost in the feces is being constantly fed back into the emerging young erythrocytes, and hence radioactivity in the upper, lighter
and younger layer, remains constantly higher, and, in the bottom layer, lower.

It is clear that infected anemic subjects can efficiently absorb iron from the gut, both in the ionized form or originating from their own hemoglobin.

Absorption of Iron from Food

It is known that absorption of iron from food may be lower than that of ionized iron given on an empty stomach (McCance et al., 1943; Husain and Patwardhan, 1959a; Foy and Kondi, 1959). Hence, it is important to determine the absorption from staples, under conditions approaching normal. Unfortunately, only relatively few studies of this type have been performed. Because of technical uncertainties associated with other methods, we shall review here only those studies performed by incorporating radio iron biologically into the food under study. A summary of the results obtained may be seen in Table 7. In hemato logically normal subjects, presumably with normal iron reserves, absorption of iron from various types of animal food ranges from 10% to 25%; it appears to be distinctly lower from eggs. Absorption of iron from vegetable food seems generally less than that from animal origin. In iron deficient subjects, absorption of iron from both animal and vegetable food is increased, but to an apparently much lesser degree from the latter. It would seem therefore that iron from a predominantly vegetable diet would be less available for absorption than from an animal diet, specially in presence of iron deficiency. It is reasonable to think that this fact contributes to the iron deficiency so frequent in tropical areas.

The studies referred to deal with the absorption of staples administered singly, on an empty stomach. It would be of interest to know the effect of combining various types of foods, such as occurs under actual field conditions. An idea of what the absorption of food iron may be under such conditions is obtained by determining the increments in hemoglobin in anemic subjects consuming their usual diet during prolonged periods of time after worming. This was done during a period of 565 days, in 13 infected anemic subjects (Table 8 and Fig. 8). The increase in hemoglobin iron ranged from 0.36 to 1.1 mg per day but, in addition, there was a fecal iron loss, due to residual infections or re-infections, in spite of repeated worming. From egg counts, this fecal iron loss was estimated to range from 0.6 to 2.4 mg/day, so that food iron utilization was probably at least of the order of 1.7 to 2.8 mg per day, values not unlike those which may be calculated from data by Cruz (1954d) and those given by Finch et al. (1950) in phlebotomised patients with polycythemia vera. The rise of the hemoglobin to or towards normal after worming, on local diet only, and in spite of residual infections, suggests that, in the absence of hookworm infection, in the area studied (Pequin, Venezuela) food iron may be capable of sustaining hemoglobin levels adequately.

Absorption of Other Foodstuff

1) Protein. Nitrogen absorption has been found to be unaffected by "light" infections, of around 100 worms, with maximum of 515 (Holmes and Darke, 1959). In nine subjects with heavy infections and anemia, absorption was significantly reduced, as compared with five subjects, also with anemia, but without infection. The values for absorption were respectively (in percentages) 62.5 ± 3.1 for the infected group and 73.3 ± 1.9 (S.E.) for the non-infected. The hypothesis was emitted that two factors were operative in this absorption defect: the deficiency in dietary protein, which in turn led to deficiency of digestive enzymes, and possibly the secretion of anti-enzymes by the worm. It may be, however, that the protein lost into the gastrointestinal tract through bleeding might have accounted for the difference between the two groups, so that the increased quantity of fecal nitrogen might in part be due to loss rather than to lack of absorption. Presuming that the species involved was N. americanus, and assuming that average blood loss per worm was 0.03 mg per day, the patients studied by Holmes and Darke could be losing from 12.5 (in a six-year-old child) to 85 ml of blood per day into their gastrointestinal tract. They may hardly be said to have been on the same protein "diet" as the non-infected group. Gifies et al. (1964) have shown that actually some loss of plasma albumin (representing about 0.75 mg of N/day in three patients) does occur. Blackman et al. (1965), by means of I125 tagged plasma albumin in 17 patients, have reported an intestinal albumin loss of roughly 0.1 g per 100 N. americanus.

2) Sugars. Ten of the 14 cases studied by
<table>
<thead>
<tr>
<th>Type of food</th>
<th>References</th>
<th>Year</th>
<th>Normal</th>
<th>Iron deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of</td>
<td>Amount of food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>subjects</td>
<td>iron adminis-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tered (mg)</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Animal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit hemoglobin</td>
<td>Callender et al.</td>
<td>1957</td>
<td>27</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit hemoglobin</td>
<td>Turnbull et al.</td>
<td>1962</td>
<td>13</td>
<td>4.0-6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit hemoglobin</td>
<td>Hussain et al.</td>
<td>1965</td>
<td>10</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit hemoglobin</td>
<td>Layrisse et al.</td>
<td>1965</td>
<td>2</td>
<td>3.0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>Moore and Dubach</td>
<td>1951</td>
<td>10</td>
<td>3.6</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>Chodos et al.</td>
<td>1957</td>
<td>14</td>
<td>4.0-8.6</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken liver</td>
<td>Moore and Dubach</td>
<td>1951</td>
<td>3</td>
<td>10.0</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Chicken muscle</td>
<td>Moore and Dubach</td>
<td>1951</td>
<td>2</td>
<td>4.7</td>
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<tr>
<td>Ferritin</td>
<td>Hussain et al.</td>
<td>1965</td>
<td>8</td>
<td>3.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>Layrisse et al.</td>
<td>Unpublished</td>
<td>12</td>
<td>1.1</td>
</tr>
<tr>
<td>Beef</td>
<td>Layrisse et al.</td>
<td>Unpublished</td>
<td>7</td>
<td>4.0</td>
</tr>
<tr>
<td>B. Vegetable</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>Moore and Dubach</td>
<td>1961</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Mustard Greens</td>
<td>Moore and Dubach</td>
<td>1961</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Swiss Chard</td>
<td>Chodos et al.</td>
<td>1961</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>Beet Greens</td>
<td>Chodos et al.</td>
<td>1967</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>Hussain et al.</td>
<td>1965</td>
<td>21</td>
<td>3.7</td>
</tr>
<tr>
<td>Black Beans</td>
<td>Layrisse et al.</td>
<td>1966</td>
<td>14</td>
<td>3-4</td>
</tr>
<tr>
<td>Corn</td>
<td>Layrisse et al.</td>
<td>1966</td>
<td>7</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
TABLE 8
Utilization of food iron measured from the increase of the total circulating hemoglobin and iron loss due to hookworm infection (period of 605 days)

<table>
<thead>
<tr>
<th>Anemic subjects</th>
<th>No. cases</th>
<th>Initial Hb</th>
<th>Final Hb</th>
<th>Increase hemoglobin iron (mg/day)</th>
<th>Iron loss hookworm infection (mg/day)</th>
<th>Total iron utilized (minimal estimation) (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g/100 ml</td>
<td>Total g</td>
<td>g/100 ml</td>
<td>Total g</td>
<td></td>
</tr>
<tr>
<td>Children (2-6 years).....</td>
<td>2</td>
<td>9.6 107</td>
<td>11.7 168</td>
<td>0.36</td>
<td>2.35</td>
<td>2.71</td>
</tr>
<tr>
<td>Children (7-14 years)....</td>
<td>9</td>
<td>10.1 158</td>
<td>12.1 248</td>
<td>0.53</td>
<td>1.14</td>
<td>1.67</td>
</tr>
<tr>
<td>Adult men.................</td>
<td>2</td>
<td>8.6 287</td>
<td>13.3 466</td>
<td>1.06</td>
<td>0.60</td>
<td>1.66</td>
</tr>
</tbody>
</table>

FIGURE 8. Response of circulating hemoglobin to the administration of vermifuge. The values are near normal after 3 years even though there is continued fecal blood loss. Iron utilized from food for hemoglobin synthesis was calculated to be 2.7 mg per day. (P. A., 15-year-old male from a Venezuelan rural area. The patient remained in the same locality throughout the study, and consumed his usual diet.)

Sheehy et al. (1962) had impaired absorption of xylose. In 18 infected subjects studied by Layrisse et al. (1964) more recently, however, the urinary excretion of d-xylose after an oral load of 25 g was 6.48 ± 1.47 g which was not significantly different from that observed by the same authors in normal individuals (6.94 ± 1.26).

The ten infected subjects of Abdalla et al. (1963) showed normal absorption of d-xylose.

5) Fats. All 14 Puerto Rican cases of Sheehy et al. (1962) had impaired fat absorption. On the other hand, six cases studied in Venezuela by means of I\(^{131}\)-tagged oleic acid and ten cases studied by chemical means showed practically normal values for fecal fat excretion (Layrisse et al., 1964). Saravia et al. (1962) found, in Guatemala, in three of 13 hookworm infected cases, a fecal I\(^{131}\) excretion of more than 6% of the administered dose; in others the test was normal. Eight of Sheehy's cases had, in addition, impaired vitamin A absorption. Ten subjects studied by Abdalla et al. (1963) had an average fecal fat excretion of 3.74% ± 0.92 as compared to 4.2 ± 9.54 in ten controls.

The reported differences between the data from heavily hookworm infected subjects, from Sheehy's and from Layrisse's group, obtained respectively in Puerto Rico and Venezuela, may be explained, as in the case of the pathological changes of the intestinal mucosa, by the frequent occurrence of sprue in Puerto Rico, and its rarity in Venezuela. The Venezuelan studies show, at any rate, that heavy hookworm infection, per se, may not lead to impairment in the absorption of sugars and fats.

4) Vitamin B\(_12\). Layrisse et al. (1959) determined the Co\(_6\)\(^{15}\) vitamin B\(_12\) absorption in ten subjects with heavy hookworm infection by the Schilling's technique. In all of them the absorption was normal. Similar results were reported later by Layrisse et al. (1964). Schilling tests performed in ten of the 14 patients of Sheehy et al. (1962) were also reported as normal.

5) Folic acid. A definite impairment of folic acid absorption has been reported in the majority of 34 patients studied (Layrisse et al., 1959, 1964) (Fig. 9).

Folic acid malabsorption was usually not corrected for some time after the worms had been removed. Of ten patients tested after worming,
Figure 9. Folic acid absorption and serum folate activity in patients heavily infected with hookworm. Black circles indicate the cases in which blood morphological changes suggestive of folic acid or vitamin B12 deficiency were found (from Layrisse et al., 1964). Folic acid absorption is significantly impaired.

Eight continued to show impairment of folic acid absorption (Layrisse et al., 1959) and five of seven in a second study (Layrisse et al., 1964); this, in spite of an excellent response to iron therapy in all of them, and a normal clinical response. We can only speculate as to the cause of this impaired absorption, but it is interesting to note in this respect that the abnormalities observed by x-ray often remain present many days after worming (see page 1056).

6) Others. The maximum blood quinine level and the time necessary to reach it after oral administration of 20 mg of the drug were not affected by experimental A. caninum infection in two dogs (Andrews and Webb, 1942), infected with 88 and 75 worms respectively.

Summary
Various types of digestive symptoms have been reported in hookworm infection, ranging from vague abdominal distress to characteristic ulcer pains. Roentgenological examination may show excessive peristalsis and segmental contractions, with distortion of the mucosal pattern, usually most marked in the proximal jejunum. At times, the duodenum may be affected; typical ulcer changes...
have been described, but it is not clear whether their occurrence with hookworm infection may not have been accidental. The changes may remain unaffected for several months after worming.

Gross pathological lesions in the intestine are usually inconspicuous, although some authors disagree on this point. Microscopically, the intestinal mucosa, as obtained by means of capsule biopsy, is most often normal in appearance, but at times flattening and fusion of the villi, frank atrophy and marked infiltration may be observed.

Gastric acidity may be high, low, absent, or normal, as is the case in iron deficiency anemia from other causes.

Absorption of reduced iron is usually high, of the order of 80 to 70% of the administered dose. It is not unusual to find, in hookworm infected areas, patients who are uninfected and even non-anemic, and yet show a similarly high iron absorption. Data on iron absorption from staple food are still insufficient to reach definite conclusions; in general, absorption appears to be higher from animal than from vegetable sources.

Hemoglobin iron is well absorbed in hookworm infected anemic subjects. On the average 40% of the iron from hemoglobin lost into the intestinal tract is reabsorbed and reutilized.

VI. HEMOLYTIC PROCESS IN HOOKWORM ANEMIA

Although it is now generally abandoned, the theory has been advanced in the past that hookworms produced anemia in their host through a hemolytic process due to a secreted toxin. Evidence in favor of such a mechanism was drawn from experiments demonstrating the presence of weak hemolysis in extracts from *Ancylostoma duodenale* (Alessandrini, 1904; Preti, 1908; Whipple, 1909; Schwartz, 1920), *Necator americanus* (Noc, 1908; Whipple, 1909) or *Ancylostoma caninum* (Whipple, 1909; Fülleborn and Kikuth, 1929; Crus, 1933b). On the other hand, in well controlled experiments, Loeb and Fleisher (1910) failed to produce hemolysis with extracts of the whole worm, although they obtained, from whole worm powder, a marked inhibition of coagulation in vitro; they felt that the behavior of such extracts resembled that of cobra venom rather than that of hirudin.

Hemolysis as even a partial explanation of hookworm anemia was practically abandoned after 1920, so that in Chandler's textbook (1929) we do not find it mentioned. The consistent and significant finding of an increase in fecal urobilinogen excretion in heavily infected cases (Layrisse and Roche, 1962) and of a reduced red blood cell survival (Layrisse et al., 1965) made it necessary to study the question again, in order to determine whether a hemolytic process (1) is indeed involved in the anemia associated with hookworm; (2) is specific to this infection, that is, due to the presence of the worm itself; and (3) plays a role in the genesis of the anemia.

Erythrocyte Survival

Trincao et al. (1952) have studied red blood cell survival in hookworm-infected subjects. They transfused erythrocytes from four infected individuals “without anemia” into four compatible infected subjects with anemia and vice versa, and measured survival by the Ashby technique. The authors commented that survival time did not differ in the two groups, but made no statement concerning total survival time. Perusal of their charts shows that all survival times but one were abnormally short (of the order of 60-80 days). It must be mentioned that the group “without anemia” had hemoglobin values of 8.8, 10.15, 10.4 and 11.4 g/100 ml respectively. It would appear that the Portuguese workers demonstrated the presence of a short erythrocyte survival time in hookworm infected anemic subjects, although they did not interpret their results in that sense.

Larissa and Ventura (1959) (chart 1, page 241) studied erythrocyte survival, by the Ashby technique, in an unstated number of cases. While survival of erythrocytes from infected subjects appeared definitely shortened, averages fell within normal limits; but, in the absence of data on number of subjects and range obtained, final judgment is not possible.

Saif (1959) studied four cases with “severe hookworm anemia” and determined their CrT½ apparent survival time. The results were given with few details. In three of the cases the range was 21 to 27 days, as compared with a range of 25 to 39 days in five normal individuals and 28 to 37 days in six “ancylostoma infected patients” presumably without anemia and with mild infections. In a fourth patient, CrT½ was only 7 days when the hematocrit was 14%; the value was raised to 26 days when the hematocrit
reached 29%. The initial value was attributed to methodological factors rather than to actual increased hemolysis.

Roche et al. (1960) found an average half survival time (Cr") of 17 days (range 8 to 33, all patients but one showing a value of less than 21 days) as compared to 26 days (range 21 to 30) in 12 non-infected individuals. In the infected subjects, loss of Cr" in the feces was measured, and on this basis a correction in the survival curve was introduced. After such correction, most of the calculated survival times in the infected subjects turned out to be normal, or longer than normal. At the time, the authors felt that, in these patients, there did not exist any significant hemolytic process, but the method of correcting the curves was avowedly rough. Gilles et al. (1964) have stated that, in infected patients, Cr" survival curves were normal when corrected for Cr" fecal loss, but they did not give details of their results, nor the methods used for correcting their curves.

In 31 cases studied more recently (Layrisse et al., 1965) no relationship was found between intestinal blood loss and red blood cell survival time, both measured by the Cr" method. A half survival time as low as 10 days, for example, could be observed in patients who lost 100 ml of blood per day, as well as in subjects losing 30 to 50 ml. In ten subjects with pure A. duodenale infections, studied by Farid et al. (1965), half life of Cr" red cells ranged from 8 to 26 days, and again there was no apparent relationship between blood loss and red cell survival. A Cr" half life of 16 days was found, for example, in a case losing 45 ml of blood per day, while in another case with a Cr" half life of 8 days, the blood loss was 41 ml/day. In a more recent report Farid et al. (1965) observed a reduction of Cr" half life to less than 20 days in infected subjects. In two of these, surface radioactivity counting showed excess of Cr" over the spleen area, but since both patients had splenomegaly from schistosomiasis, the finding could not be attributed definitely to iron deficiency alone.

Some of these observations suggest that there are other factors, probably of a hemolytic nature, besides physiological destruction and intestinal blood loss, responsible for red blood cell destruction in these patients.

To determine whether such factors are associated with the hookworm, red cell survival was studied in infected subjects, before and after worming, and after iron treatment, (Layrisse et al., 1965). The results are shown in Table 9. In two of the subjects, some rise in survival time is seen after worming, but it remains distinctly abnormal, reaching normal values only after iron treatment. In more recent experiments in our laboratory (Martínez et al., unpublished) the subjects were not wormed and red cell survival was measured before and after iron treatment. Cr" T½ was corrected to normal value in four out of five cases even though in two of them blood loss was more than 40 ml per day. These results suggest that, in a hookworm infected anemic subject, there is indeed increased hemolysis, but that such hemolysis is not due to the worms only, since survival time remains abnormal after their removal, and rises to normal only after iron is given. Farid et al. (1965) have reported similar findings.

The possibility remained that elution of Cr" from circulating erythrocytes be abnormally high in iron deficient states. Studies were accordingly carried out, in four patients, with circulating red blood cells tagged with Di-iso-propyl-fluorophos-
NATURE AND CAUSES OF "HOOKWORM ANEMIA"

TABLE 10
Red cell survival, measured with DFP\textsuperscript{(a)}, in hookworm infected subjects before worming, after worming, and after iron treatment
(From Layrisse, Linares and Roche, 1985)

<table>
<thead>
<tr>
<th>Cases (all males)</th>
<th>Red cell survival</th>
<th>Hb (g/100 ml blood)</th>
<th>Hookworm eggs per g of feces</th>
<th>Probable intestinal blood loss (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hookworm infected subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. R.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before worming</td>
<td>49</td>
<td>7.2</td>
<td>13,677</td>
<td>28.7</td>
</tr>
<tr>
<td>After worming in own subject</td>
<td>67</td>
<td>7.5</td>
<td>279</td>
<td>0.6</td>
</tr>
<tr>
<td>After worming in normal subject</td>
<td>60</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After iron treatment</td>
<td>100</td>
<td>15.6</td>
<td>361</td>
<td>0.8</td>
</tr>
<tr>
<td>L. C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before worming</td>
<td>49</td>
<td>5.5</td>
<td>35,203</td>
<td>73.9</td>
</tr>
<tr>
<td>After worming in own subject</td>
<td>57</td>
<td>6.6</td>
<td>1,499</td>
<td>3.1</td>
</tr>
<tr>
<td>After worming in normal subject</td>
<td>65</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After iron treatment</td>
<td>80</td>
<td>12.5</td>
<td>1,321</td>
<td>2.8</td>
</tr>
<tr>
<td>E. R.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before worming</td>
<td>41</td>
<td>5.0</td>
<td>42,042</td>
<td>88.3</td>
</tr>
<tr>
<td>After worming in own subject</td>
<td>60</td>
<td>6.7</td>
<td>3,989</td>
<td>8.3</td>
</tr>
<tr>
<td>After iron treatment</td>
<td>110</td>
<td>13.8</td>
<td>1,061</td>
<td>2.2</td>
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<td>A. S.</td>
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<td></td>
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</tr>
<tr>
<td>Before worming</td>
<td>59</td>
<td>6.0</td>
<td>13,208</td>
<td>27.7</td>
</tr>
<tr>
<td>After worming in own subject</td>
<td>63</td>
<td>5.5</td>
<td>3,115</td>
<td>6.5</td>
</tr>
<tr>
<td>After worming in normal subject</td>
<td>64</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>After iron treatment</td>
<td>85</td>
<td>12.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Control: Normal subjects

| M. R. (m)         | 108               | 16.6               | 0                           |                                       |
| M. L. (m)         | 105               | 16.0               | 0                           |                                       |
| A. T. (m)         | 119               | 15.2               | 0                           |                                       |
| D. P. (m)         | 110               | 16.0               | 0                           |                                       |

\* The intestinal blood loss was approximately estimated from the number of hookworm eggs per g of feces (2.14 ml of blood per 1000 eggs per g).

The results may be seen in Table 10. Survival time again may be seen to rise after worming, probably because of the interruption of blood loss due to the parasite, but it remains abnormally low until after iron is given.

Survival of erythrocytes from infected subjects was not substantially changed when they were transfused to normal recipients but became nearly normal in splenectomized recipients (Layrisse et al., 1965) (Fig. 10). Cr\textsuperscript{51} surface counting in infected subjects with severe iron deficiency anemia, both before and after worming, showed excess radioactivity in the spleen area, indicating an excess red blood cell destruction (Diez et al., unpublished), thus confirming the findings of Farid et al. (1965).

The spleen is usually not palpable in hookworm anemia, although it may appear at times somewhat enlarged to percussion. At autopsy, in ten cases studied by Cruz (1934a), although myeloid metaplasia was often observed, the structure was otherwise normal, with no sclerosis, congestion or abnormal pigmentation, except in three of the cases, where age (case 5) or intercurrent infection (cases 9 and 10) could explain respectively the sclerosis and acute splenitis. The weight of the spleen in Cruz cases varied from 110 to 250 g.

If there is indeed increased hemolysis in iron deficiency anemia associated with hookworm infection, the question may be asked whether this is due, somehow, to the parasite, or whether it is simply a characteristic of severe iron deficiency anemia. Brown et al. (1944) gave this type of anemia as an example of anemia with normal red cell life span. Their studies, however, in which they injected red cell suspensions from stored blood not more than six days old, only demonstrated that subjects with iron deficiency anemia do not have extrinsic hemolytic factors able to destroy normal red cells. Temperley and Sharp (1962) did find, by tagging the subjects' own erythrocytes with Cr\textsuperscript{51}, a normal red blood cell survival in uncomplicated iron deficiency anemia.

A number of workers, on the other hand, have reported reduction of red cell life span in iron deficiency anemia. Rash et al. (1958) observed a mean Cr\textsuperscript{51} T½ of 17 days in 14 iron deficient anemic infants. Verloop et al. (1960) described eight cases of severe iron deficiency anemia with reduced red cell life span; two of the cases showed
FIGURE 10. Survival of circulating red cells from an infected subject (J. M.) after worming, in his own circulation, in a normal recipient, and in a splenectomised recipient, measured by means of DFP$^{32}$ (above) and Cr$^{51}$ (below) (from Layrisse et al., 1965). Erythrocyte life span is abnormally short both in the subject and in the normal recipient, but it increases markedly in the splenectomised subject.

a rapid fall in hemoglobin during the time of observation, and they were probably not uncomplicated cases of iron deficiency. Keidelling, quoted by Bothwell and Finch (1962), also found a moderate life span shortening in some of his subjects. Layrisse et al. (1965) found a definite reduction in DFP$^{32}$-tagged red cell life span in three out of four non-infected iron deficient women and, more recently, in our laboratory Dies et al. (unpublished) made similar observations in four out of seven cases. The studies by Dies et al. were carried out with both Cr$^{51}$ and DFP$^{32}$: in two of the four cases with a reduced DFP$^{32}$ life span, Cr$^{51}$ life span was within normal range. Cline and Berlin (1963) have stated that the Cr$^{51}$ method is not entirely reliable, because of variations in the rate of Cr$^{51}$ elution, which may mask minor reductions in survival time; this may be one explanation for Temperley and Sharp's (1962) report of normal survival in iron deficiency anemias, or perhaps the reduced span can only be demonstrated in profound deficiencies.

It is our distinct impression that, at least in the
markedly anemic iron deficient cases, there is a decreased erythrocyte life span, whether the cases are associated or not with hookworm, but more studies are needed to determine the true picture. However, the increased red cell destruction contributes probably little to the anemia, since iron released from red cell breakdown is in all likelihood efficiently reutilized by the bone marrow (Section II).

Collateral evidence in favor of a hemolytic process is suggested by the results obtained from other methods, which will be discussed in the following sections.

**Bile Pigment Metabolism**

Plasma bilirubin has been reported to be normal, even in the presence of heavy infections (Kobayashi, 1929; De Langen, 1934).

On the other hand, an increase in urobilinogen excretion has been reported by several authors. Fieschi (1932) observed this, in spite of a lowering of circulating hemoglobin, and he commented, "Certainly the observed values cannot be taken to mean that there is normal hemolysis, specially if account is taken of the markedly reduced hemoglobin values." Cotti (1937), in carefully performed studies, reached similar conclusions in 13 subjects, by relating urobilinogen excretion to circulating hemoglobin and body weight, and expressing the results as a "hemolytic index" whose normal value should be unity. In all but four of the cases, this index was high, the average of the 13 cases being 2.4 (average fecal urobilinogen was 73.3 mg/24 hrs and urinary urobilinogen 2.4 mg/24 hrs). The author concluded that there was, in hookworm anemia, a relative increase in hemoglobin turnover, and pointed out that this was similar to what occurred in ordinary secondary hypochromic anemia. Layrisse and Ventura (1950) attributed the evidence for hemolysis in hookworm anemia to a defect in the circulating erythrocytes rather than to a circulating toxin.

Layrisse et al. (1965) have also found a significant increase in bile pigment in the feces, as measured by the fecal urobilinogen index (Dameshek). This index was 0.48 ± 0.32 (S.D.) in 14 patients before worming, more than twice the upper limit of normal. After worming, in the same subjects, it became 0.28 ± 0.20, and after iron treatment 0.15 ± 0.10 (mg/g Hb/day). Although the index decreased significantly after removing the worms, it remained nonetheless elevated.

There is a significant correlation between the quantity of blood loss and fecal urobilinogen excretion (Layrisse et al., 1965), which suggests that these two factors are somewhat related. Turnbull et al. (1962) have submitted the hypothesis that iron from the hemoglobin molecule may be absorbed as a porphyrin complex. Roche and Pérez-Giménez (1959) and Layrisse et al., (1961) have shown that a considerable amount of hemoglobin iron lost into the intestinal tract is reabsorbed (see Section V). Recent experiments have shown, in dogs, that Fe⁺⁺ from tagged hemoglobin administered intragastrically appeared in portal blood partly as porphyrin and partly bound to transferrin (Brown et al., 1966).

It is well to point out that fecal urobilinogen excretion is a poor index of hemoglobin breakdown, and that speculations based on observing small changes in such index are to be regarded with caution (Wintrobe, 1961). These small changes become meaningful, however, when taken together with other evidence of increased hemolysis.

**Hemoglobinemia**

Plasma hemoglobin in 15 infected subjects with severe hookworm anemia showed a range of 0.5 to 2.8 mg per 100 ml of plasma (Layrisse et al., 1965). These results may be taken as further evidence that there is no abnormal intravascular hemolysis in this condition.

**Haptoglobins and Hemoglobin Turnover**

The finding of haptoglobin Hp⁺ or Hp, or both, in normal quantities, in the presence of a hemoglobin turnover approximately one half normal, amounting to an average of 0.08 g/kg of body weight/day, with a range of 0.03 to 0.17 (Layrisse et al., 1965) is again presumptive evidence against intravascular hemolysis in hookworm anemia (Jayle, 1962; Smithies, 1955; Brus and Lewis, 1959).

**Hypotonic Fragility of the Erythrocytes**

Hypotonic fragility of the erythrocytes in hookworm anemia has been found increased (Kobayashi, 1929; Aversa, 1946), normal (Fülle-
Summary

The idea that hookworm anemia was due in whole or in part to a hemolytic process, suggested by the finding, by several groups of early workers, of a weak hemolysin in worm extracts, has been now in general abandoned.

It appears nonetheless that increased destruction of circulating red blood cells, other than from loss into the gastrointestinal tract, may be associated with hookworm anemia. A moderate but significant increase in urobilinogen excretion has been reported by several authors. Crude red blood cell survival time is usually decreased, and is corrected only partially after worming. Erythrocyte survival measured by means of DFPE has also been found to be markedly decreased, whether the erythrocytes remain in the donor, or are transfused into a compatible, normal recipient. Worming does not bring survival time to normal. Survival of erythrocytes, however, becomes normal if these are transfused into a splenectomized recipient. Hence, the major site of destruction of the red blood cells is probably the spleen. An increased activity over the spleen by external counting has been reported.

The findings suggest that the shortened survival is due to an intrinsic defect of the erythrocytes, and it is felt that this is a characteristic of severe iron deficiency anemia, no matter what the cause. It is not likely that this shortened survival contributes to hookworm anemia, since iron from the effete erythrocytes is efficiently reutilized.

Plasma hemoglobin values and the finding of haptoglobin Hp or Hp', or both, in normal quantities, in presence of a hemoglobin turnover approximately one half normal, are again presumptive evidences against abnormal intravascular hemolysis in hookworm anemia.

VII. ASSESSMENT OF BLOOD AND IRON LOSS

1. Non-isotopic Methods

In the early period of hookworm research, during the late 19th century, blood loss was felt to constitute one of the central factors, if not the most important one, in the genesis of anemia associated with hookworm. During the first quarter of the present century, however, the idea became generally discredited; it was felt that a "toxic theory" better fitted the facts, and that blood loss due to hookworm was negligible. In his classical text, Chandler (1929) wrote (page 250): "It seems fair to assume, therefore, that chronic loss of blood from the intestine is at most a minor factor in hookworm disease, and certainly cannot account for the severe anemias so frequently seen in heavy infestations," and also, "There seems to be little ground for doubting that the anemia is due primarily to a toxic effect on the blood-forming organs."

After the work of Wells (1931), blood loss as a cause of the anemia regained favor, but still many authors doubted its importance, and, following Cruz (1934b, c and d) and Rhoads, Castle, Payne and Lawson (1934) attributed a more central role to deficient iron intake, or to losses by routes other than the fecal.

The chief reason for such fluctuations and uncertainties appears to have been the lack of accurate quantitative measurement. It is our purpose in this section to review the various attempts at estimating or measuring blood and iron loss in hookworm anemia by methods utilized before isotopes became generally available.

The Problem of Overt or Occult Blood in the Feces

One of the objections which has been raised to the idea that hookworm is a source of constant blood loss from the host has been that overt or occult blood was rarely found in the feces of infected subjects (Mackey, 1953). Reports vary indeed in this respect. Ashford and Gutiérrez (1911) stated that "of over 22,000 specimens of feces brought us in 1904 for examination, only six contained blood, and five blood and mucus from a naked-eyed inspection." Boycott (1911) mentioned that sometimes large quantities of blood could be found in the feces, specially in massive experimental infections "but in the majority of the cases, blood is very seldom found." De Langen (1934) later stated that he found positive benzidine test in only 32% of his infected cases, as compared to 15% in the non-infected. And finally Mackey (1953) states that in Dar es Salaam only a small proportion of those with
hookworm infection and anemia have positive occult blood.

On the other hand, the presence of bloody or tarry feces and of occult blood has frequently been reported. From an examination of the available literature, the general situation may be summarized as follows:

1) In acute infections, grossly bloody or tarry stools may at times be observed. Thus, in the acute Ancylostoma duodenale infection reported by Ashford, et al. (1933), five of the seven subjects exhibited at one time or another bloody or tarry stools. The total number of worms harbored by these five subjects was respectively 1439, 614, 64, 125, and 59. The two others, with 21 and seven worms respectively, never had gross evidence of fecal blood, but occult blood was not tested for. Leichtenstern (1887) spoke of observing "bloody diarrhea, at times true enterorrhagia."

Bass (1928, page 101) stated that in heavily infected cases, "there is so much blood lost that the intestinal contents are frequently of a chocolate-brown color or even reddish. Lower down, they become tarry in appearance. Even small numbers of worms give rise to enough hemorrhage to produce positive occult-blood."

He did not give, however, the figures on which such statements may have been based.

In heavy, acute experimental infection of the dog, the presence of visible blood in the stools is the rule. Thus Landsberg (1937) reported the presence of grossly bloody stools in seven dogs, six of them with lethal hookworm infections. Huart (1929) invariably observed gross blood in the feces of his experimentally infected dogs.

2) In chronic cases, the presence of grossly bloody or tarry stools is a rarity. On the other hand, the finding of occult blood is frequent, and may be the rule. Kouwenaar (1929, quoted by Huart, 1929) using a spectroscopic method found a positive reaction for blood in 32 (86.5%) of 37 patients with hookworm, as compared with seven (22.6%) of 31 patients free from hookworm. He noted a decrease in the positive reaction after administration of oil of chenopodium. Biggam and Ghaliounghi (1934), in reporting "a group of more than a hundred" cases, mentioned that "occult blood was nearly always found in the stools." In Cruz' reported series of 26 patients (1934c) five of the cases (1, 11, 15, 17, 21) showed positive Weber tests consistently and two more (4 and 5) occasionally. In the other 18, the test was not mentioned. Darke's (1950) nine heavily infected cases "invariably gave strongly positive tests for occult blood" (page 278).

Of the four cases reported by Aversa (1946), three had positive occult blood reactions; in the fourth, no statement is made about the matter. Arora (1951) stated having found occult blood in 80% of 91 cases he studied at Lucknow. In the experience of Gtour et al. (1962) occult blood is positive "in nearly all the cases."

Most of the studies we have quoted were not well controlled, but it can hardly be said that occult blood does not occur in acute and in chronic hookworm cases. It would be of interest to carry out such tests together with isotopic measurement of blood loss, since the differences found by the various authors may be a question of threshold sensitivity of the method employed.

Indirect and Rough Estimates of Blood Loss

A number of attempts have been made at assessing, indirectly or by rough methods, blood loss caused by hookworm.

Lutz (1885), while expressing the desirability of measuring directly blood loss caused by the worm, estimated—it is not clear by what means or method—that 500 worms (A. duodenale presumably?) would lead to a daily blood loss of "20 grams" of blood. This would be equivalent to 0.036 mm\(^5\) per worm per day. He stated clearly that blood loss due to the action of the worms could be sufficient to produce anemia with time.\(^{14}\)

In 1929, Huart reported his experimental work on 11 dogs. On two of these infected dogs, he attempted to assess the quantity of blood with that found in the feces of infected animals. He noted the inaccuracy of the method employed, and deplored the absence of an accurate way of measuring blood loss. Estimated blood loss varied from 7.0 to 40.8 ml per dog per day and from 0.007 to 0.118 ml per worm per day. Considering the inaccuracy of the method employed, these

\(^{14}\) "Wo würde der tagliche Blutverlust durch 500 Würmer 20 Gramm Betragen, was bei dem hohen Gehalte des Darmblutes an Nahrungstoffen schon genügen dürfte, um für sich allein mit der Zeit eine ausgesprochene Anämie hervorzurufen."
results are quite remarkable, and give at least an order of value for blood lost in dog hookworm infection.

Kendrick (1927) obtained satisfactory data in nine of 18 volunteers, infected artificially with A. duodenale, passing 1200 to 9000 eggs per g of feces, and harboring 72 to 508 worms. The circulating hemoglobin became reduced in these subjects by an average of 5.1 g per 100 ml of blood (assuming 100% hemoglobin = 15 g/100 ml) during an average 236 days of study. Too many assumptions need be made to reach a fairly accurate figure for the quantity of blood and iron losses, but it is clear that these must have been large indeed. Fülleborn and Kikuth (1929), in one experimentally infected dog, with a total of 1100 hookworms, found gross blood in the intestinal tract, after sacrifice on the 16th day after infection. The dog's last feces had been passed three days before death. Chemical analysis of the intestinal contents revealed 5.97 mg of iron in the small intestine and 150 mg in the large intestine. This was said to be respectively equivalent to 1.79 and 44.7 g of hemoglobin and 11.2 and 279.0 ml of "normal dog blood." Since the dog was probably anemic, the actual loss of blood may have been higher.

Foster and Landsberg (1934) bled five dogs repeatedly and measured amounts of blood varying from 28 to 43 ml per day. They felt, from their experience with hookworm infection, that the anemia developed by such dogs was similar in magnitude to that observed in heavily infected dogs, if one assumed a daily blood loss per hookworm of about 0.1 ml per day. In further experiments, they infected two litter mates and bled a third as a control, keeping circulating hemoglobin values in the latter roughly equivalent to that of the infected mates. It was found necessary to bleed the control an average of 41 ml per day during 62 days. No account of the number of worms harbored was given, so that the amount of blood lost per worm could not be estimated.

De Langen (1934) by making up various dilutions of feces and comparing the intensity of the benzidine reaction to that obtained with feces to which known amounts of blood were added, felt that most cases lost no more than 2–3 cc of blood per day by this route: "Very occasionally as much as 10 cc may be found, but on other days the faces of the same patient will be negative" (page 137).

Lehmann (1949) determined both circulating hemoglobin and blood volume in an infected patient, who was later found to harbor 830 worms. The patient lost roughly 36 g of circulating hemoglobin during one week and, after being wormed, gained 44 g in another week. It was assumed by Lehmann that 36–44 = 80 g represented the quantity of hemoglobin lost in one week. This would mean about 270 ml of blood per day, or 0.33 ml per worm per day.

Finally, mention may be made of artificial infections, used as palliative treatment for polycythemia vera. The favorable results obtained, although they do not prove the blood letting action of the worm, serve nonetheless to illustrate it. Duvoir et al. (1940) were first to utilize this method, in a patient followed up for three years (Duvoir et al., 1942). They applied percutaneously some 300 larvae to the thigh skin of a 57-year-old woman, with, 7,300,000 erythrocytes per cu mm. Occult blood appeared in the feces ("Meyer reaction") after 23 days, and eggs after 40 days. Red blood cell values had gone down to 3,570,000 per cu mm two months after the first occult blood appeared in the stools. Insufficient data regarding hemoglobin values were given, but it is interesting that the patient developed clear-cut hypochromia under treatment. In 1948, Brumpt and Gujar were able to report on a total of 25 cases treated with hookworm therapy, with excellent results according to the authors, although few details were given by them. Three hundred to 600 larvae were used. It was mentioned that melena was never observed, and that occult blood in the stool was positive "only intermittently." Nagaty and Zanaty reported one case, with good results (1949), and similarly Myhre and Wallace (1956).

**Direct Measurements**

The imaginative work of Wells (1931) gave the first direct evidence after Grassi's (see Section IX) of the blood letting ability of hookworm, and was the first to attempt to quantitate blood loss by individual worms. His elegant procedure and his description were so striking that his figure for blood loss per day per worm—inaccurate and incredibly elevated though it was—has been quoted widely and uncritically, in many articles and textbooks.

It is worth describing in some detail Wells' method. For the purpose of quantitating the amount of blood sucked by a single worm, its caudal portion was drawn by gentle suction into a capillary pipette. When a drop of blood was
emitted spontaneously by the worm's anus, the pipette was withdrawn, the red blood cells counted after suitable dilution, and the number of erythrocytes in the ejected blood compared with that in the dog's circulating blood. The size of the droplets, as measured in this manner, was found to vary from 0.05 to 0.50 cu mm. It was assumed that the average droplet measured 0.25 cu mm. The interval between each ejection of blood was then measured for varying periods of time. Assuming that the worm ejects blood at a similar rate during 24 hours, it was calculated that blood consumption per worm per day would be 0.84 cc of blood. It is not clear in the original paper how many experiments of this type were performed, and only one value is given. Although the experiment was ingenious, the lack of details as to variability of the results, the assumptions on which these are based and the inherent inaccuracy of the red blood cell counting method make the figure given unreliable. It was obviously too high, since a dog with only 100 hookworms could be losing 84 ml of blood per day, an improbable figure. A few years after Wells' publication, similar experiments were performed by Nishi (1933). After measuring the quantity of blood ejected by Ancyclostoma caninum by means of Wells' method, Nishi calculated, in 38 worms obtained from five different dogs and observed during an average of 25.4 minutes each, an average loss, extrapolated to 24 hours, of 0.144 ml per worm, and Ryo (1937), by similar methods, in 30 worms, an average loss of 0.149 ml/day.

Summary

Blood loss from the hookworm host was clearly implied to be a major cause of the associated anemia at least since 1879, but this idea has had a fluctuating fortune, chiefly because of the lack of a clear-cut quantitative study, until the utilization of radioactive isotopes.

A number of general estimates of blood loss in hookworm infection have been made, such as the direct observation of blood stained intestinal contents in autopsy material, the measurement of hemoglobin and blood volume at various phases of the infection, and the study of artificially infected cases of polycythemia vera. These studies suggest that blood loss can be indeed large. The finding of occult blood in the stool has not been uniform, but some question must be raised on the techniques employed, and, in general, the studies reported were poorly controlled.

In a number of the series, occult blood was found in the majority of the infected cases.

Several workers, since 1931, have measured directly, in the dog, the blood expelled by single hookworms, by counting the red blood cells issuing from the anus of the worms, and comparing with the circulating blood count. Values as high as 0.8 ml per worm per day have been obtained by this ingenious method, but such values are clearly too high.

VIII. ASSESSMENT OF BLOOD AND IRON LOSS

2. Isotopic methods

The general studies, set forth in the preceding section, furnished fair evidence that there was indeed blood loss associated with hookworm infections. Most of them were not, however, of a quantitative nature. Several of the older authors (Huart, 1929; Wells, 1931) had expressed the desirability of a method which would permit quantitative measurement of blood and iron loss in the feces. Isotopic methods have fulfilled this need. Hahn and Offutt (1949) were first to use them, in two infected dogs who received Fe\(^{55}\) tagged erythrocytes from donor dogs and in whom the decline in circulating radioactivity during periods of constant hematocrit reading was subsequently determined. They calculated, assuming that the red cell mass remained constant, that the two dogs lost one liter of blood in 27 and 16 days respectively, or 65 and 37 ml per day. The amount of blood calculated to have been lost through the action of the worms was said to have been roughly the same as that which had to be removed before infection to keep the hematocrit at the same low level. The authors did not count the number of worms harbored by their experimental animals, but they suggested that their method could serve to evaluate the quantity of blood lost per day per recovered worm.

Gerritsen et al. (1954) were first to use an isotopic method to measure blood loss in hookworm-infected humans. This was done by means of Fe\(^{55}\) administered by vein bound to transferrin, after which fecal and blood radioactivity were measured and compared. Hookworms were recovered after vermifuge. This procedure was performed on three subjects, found to be losing an average of 9.5, 14.1, and 18.8 ml of blood per day; this, divided by the number of worms, gave a blood loss of 0.022, 0.22, and 0.053 ml/worm/day respectively. The worm species was unstated. Account was not taken of the possibility that iron
may have been partly reabsorbed after being lost into the intestine (see Section V). Incomplete worming might explain the high value for daily blood loss per worm obtained in the second case, since the authors only state that egg concentration methods were employed to check the efficiency of worming "where possible" and do not mention in which of the cases this precaution was, or was not, taken.

After Gray and Sterling (1950) had shown that radioactive chromium, Cr⁴⁺, could be used to tag circulating erythrocytes, Owen et al. (1954) employed such method for assessing the amount of gastrointestinal bleeding, and Roche et al. (1957a) showed that the equivalent of an average of 1.27 ml/24 hours of chromium-tagged blood was lost in the feces of uninfected humans. This was confirmed by Ebaugh et al. (1958). In uninfected dogs, Clark et al. (1961) found the "blank" value of chromium excretion to be 0.5 ml per day. Further, little of Cr⁴⁺ from tagged erythrocytes introduced into the human gastrointestinal tract is reabsorbed (Roche et al. 1957a; Nabekura, 1959; Tasker, 1961). Within such limits, therefore, Cr⁴⁺ found in the feces of infected subjects may be said to originate from blood cells lost through the action of parasites, provided of course other sources of bleeding are excluded.

In their first report, Roche et al. (1957a) studied 21 subjects, with varying grades of infection, 16 of which had pure Necator americanus and the five remaining mixed Necator americanus-Ancylostoma duodenale. Four of the five mixed infections, however, had a low percentage of Ancylostoma so that a fair estimate of blood lost from that species could only be obtained in one case. After tagging circulating erythrocytes in the usual manner, Roche et al. (1957a) determined blood loss for at least 12 days, both before and after worming and counting of worms, from which the amount of blood lost per worm could be calculated. In 21 subjects, total blood loss varied between around 2 to 3 ml for subjects with less than 100 worms (and no anemia), and 99.3 ml per day in a subject with 2983 worms and a hemoglobin of 3.7 g/100 ml. One other person, with 3534 worms, was only studied for a single six-day period because of his condition, and lost 251.5 ml/day, but this was probably due to an acute enterorrhagia. In 12 of the subjects with pure Necator infections, studied 14 times (two of them were studied before and after two courses of vermifuge), total blood loss per worm per day varied between 0.012 and 0.064 ml with an average of 0.031 ± 0.017 (S.D.). In a single case, with a high percent of A. duodenale (total worms: 258, Ancylostoma 182 (71%), Necator 76 (29%)), after correction for blood loss by Necator, assuming an average loss of 0.031 per worm belonging to that species, blood loss per A. duodenale was found to be 0.205 ml/day, well beyond the amount found in any of the Necator infections. This would suggest that the consumption of blood by Ancylostoma is indeed greater than by Necator, a fact which has been suspected many times from clinical observations. This was later confirmed by Farid et al. (1965a) who found an average of 0.26 ml blood loss/worm/day in 12 Egyptians with pure A. duodenale infections.

Roche et al. (1957b) have studied blood loss, during longer periods of time (Fig. 11) in some of the subjects already reported upon in the same year (1957a). There was some suggestion, in three of the five cases, that blood loss diminished as blood values rose, either through transfusion or through iron therapy, but the authors have not been able to document this phenomenon further and neither Tasker (1961), nor Gilles et al. (1964) could confirm it.

Since the first studies by Roche et al. (1957a), several studies on total blood loss by the same group have appeared (Roche and Pérez Giménez, 1959; Layrisse et al., 1961) which have extended the earlier findings.

The results obtained by the group in Venezuela have been essentially confirmed in other countries with endemic hookworm disease. Ventura et al. (1957) and Larizza and Ventura (1959) have measured blood loss by means of Fe⁴⁺ in three anemic and four non-anemic infected subjects, and compared it to the results found in two normal individuals. The values for average blood loss per day (ml) from three four-day periods, were as follows: infected anemic, 21.2, 3.3, 16.1; infected non-anemic, 11.5, 1.8, 2.0, 13.3; normal individuals, 0.34, 0.22. No idea was given of the magnitude of the burden, and individual worms were not recovered. Because of the reabsorption of hemoglobin Fe⁴⁺, the actual blood loss may have been higher, particularly in the anemic subjects.

Tasker (1961) reported studies on 20 subjects, done with methods practically similar to those employed by Roche et al. (1957a), including correction for residual blood losses after worming. Daily blood loss was found to range from about 2 ml, with a light infection of about 20 worms, to
about 90 ml with a heavy infection of greater than 1500 worms. All the worms recovered were identified as *N. americanus*. As for blood loss per worm per day, the data from Tasker suggest that it is inversely proportional to the log of total hookworm burden, and it varied between about 0.1 ml/worm/day in light infections, to about 0.03 ml/worm/day for heavy infections. Reviewing our own data, we found a correlation coefficient between blood loss and log of hookworm burden of 0.30 ± 0.21, which is not significant (Martinez Torres et al., 1966). There is a "background" of "normal" Cr reference loss which, if not corrected for, may increase considerably the low values, while the high values remain practically unaffected; and this might help explain the divergence between Tasker’s results and our own in this respect.

Foy et al. (1958) reported two cases and Foy and Kondi (1960) 15 more cases, whose blood and iron losses were studied by isotopic means. Unfortunately, except for three cases (1, 4, and 6 of the 1960 article) there is no mention as to which was studied by means of Fe²⁺- and which by means of Cr⁶⁺-tagged cells. This is of some importance, since a considerable percent of hemoglobin-Fe²⁺ is reabsorbed from the intestine (see Section V) and therefore Fe²⁺ studies offer a good measurement of actual fecal iron loss but are inaccurate as far as blood loss is concerned. The data by Foy’s group is however of particular interest because their series contains a number of subjects with mixed *Necator* and *Ancylostoma* infections. With the reservations in mind that some of the cases may have had higher blood losses than shown, because the Fe²⁺ method was employed, we have calculated blood loss per worm from their data, excluding cases 10 and 11 of Foy and Kondi (1960), who harbored only nine and seven worms respectively. It may be shown thus that blood loss per *Necator* is distinctly lower than found by our own group, and that blood loss per worm increases as the admixture of *Ancylostoma* rises. If we take the value of 0.013 as representing

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**Figure 11.** Intestinal blood loss measured by means of Cr⁶⁺ in an infected patient (J. P., age 14) (from Roche et al., 1957). Blood loss is quickly reduced after worming.
TABLE 11
Measurement of blood loss in hookworm infection
(data from various authors)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>No.</th>
<th>Method used</th>
<th>Total blood loss (ml/day)</th>
<th>Necator americanus</th>
<th>Anclyloasma duodenale</th>
<th>Anclyloasma caninum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutz</td>
<td>1885</td>
<td>?</td>
<td>rough estimates</td>
<td>large</td>
<td></td>
<td></td>
<td>0.036</td>
</tr>
<tr>
<td>Kouwenaar cited by</td>
<td>1924</td>
<td>?</td>
<td>spectroscopy of feces</td>
<td>large</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Huart</td>
<td>1929</td>
<td>2</td>
<td>id.</td>
<td>7–40.8</td>
<td></td>
<td></td>
<td>0.007–0.118</td>
</tr>
<tr>
<td>Foster &amp; Landsberg</td>
<td>1934</td>
<td>7</td>
<td>parallel bleeding</td>
<td>28–43</td>
<td></td>
<td></td>
<td>About 0.08</td>
</tr>
<tr>
<td><strong>Rough estimates</strong></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Direct in vitro measurements</strong></td>
<td></td>
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<tr>
<td>Wells</td>
<td>1931</td>
<td>?</td>
<td>Wells’</td>
<td>—</td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>Nishi</td>
<td>1933</td>
<td>38</td>
<td>Wells’</td>
<td>—</td>
<td></td>
<td></td>
<td>0.144†</td>
</tr>
<tr>
<td>Ryo</td>
<td>1937</td>
<td>30</td>
<td>Wells’</td>
<td>—</td>
<td></td>
<td></td>
<td>0.149‡</td>
</tr>
<tr>
<td>Roche et al.</td>
<td>1960</td>
<td>35</td>
<td>in vitro chamber</td>
<td>—</td>
<td></td>
<td></td>
<td>0.054 ± 0.006 (S.E.)</td>
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<tr>
<td><strong>In vitro isotopic studies</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Hahn et al.</td>
<td>1949</td>
<td>2</td>
<td>fecal Fe**</td>
<td>37–65</td>
<td></td>
<td></td>
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<tr>
<td>Gerritzen et al.</td>
<td>1954</td>
<td>3</td>
<td>circulating Fe**</td>
<td>9.5–14.1–18.8</td>
<td>0.022–0.22‡</td>
<td></td>
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<tr>
<td>Roche et al.</td>
<td>1957a</td>
<td>14</td>
<td>fecal Cr⁴¹</td>
<td>2–99.3</td>
<td>0.031 ± 0.017 (S.D.)</td>
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<tr>
<td>Roche et al.</td>
<td>1957a</td>
<td>3</td>
<td>fecal Cr⁴¹</td>
<td>—</td>
<td></td>
<td></td>
<td>0.21</td>
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<td>Ventura et al.</td>
<td>1957</td>
<td>3</td>
<td>fecal Fe**</td>
<td>3.3–21.2</td>
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<tr>
<td>Study</td>
<td>Year</td>
<td>No.</td>
<td>Method</td>
<td>Range</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Nabekura</td>
<td>1960</td>
<td>47</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>1.6-15.0</td>
<td>0.07</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Foy et al.</td>
<td>1968</td>
<td>11</td>
<td>Cr&lt;sup&gt;41&lt;/sup&gt; or Fe&lt;sup&gt;41&lt;/sup&gt;</td>
<td>4.3-56.3</td>
<td>0.013</td>
<td></td>
<td></td>
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<tr>
<td>Foy et al.</td>
<td>1960</td>
<td>4</td>
<td>id.</td>
<td>20-85</td>
<td></td>
<td>0.14</td>
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<tr>
<td>Roche et al.</td>
<td>1960</td>
<td>4</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.015-0.063</td>
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<td>Tasker</td>
<td>1961</td>
<td>20</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>2-90</td>
<td>0.03-0.10</td>
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<tr>
<td>Clark et al.</td>
<td>1961</td>
<td></td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>3.57-9.15</td>
<td>0.07</td>
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<tr>
<td>Aly et al.</td>
<td>1962</td>
<td>39</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>3.9-25.8</td>
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<tr>
<td>Layrisse et al.</td>
<td>1961</td>
<td>11</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>15.2-106.0</td>
<td></td>
<td></td>
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<tr>
<td>Mahmood &amp; Woodruff</td>
<td>1964</td>
<td>18</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td>0.032 ± 0.035</td>
<td></td>
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<tr>
<td>Mahmood &amp; Woodruff</td>
<td>1964</td>
<td>15</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.152 ± 0.124</td>
<td></td>
</tr>
<tr>
<td>Gilles et al.</td>
<td>1964</td>
<td>13</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>0.7-75.5</td>
<td>0.02-0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farid et al.</td>
<td>1965a</td>
<td>12</td>
<td>fecal Cr&lt;sup&gt;41&lt;/sup&gt;</td>
<td>1.6-15.0</td>
<td></td>
<td>0.26 ± 0.045 (S.D.)</td>
<td></td>
</tr>
<tr>
<td>Blackman et al.</td>
<td>1965</td>
<td>17</td>
<td>I&lt;sup&gt;131&lt;/sup&gt; tagged albumin</td>
<td>63 ± 19.2 (S.D.) (plasma)</td>
<td>0.03 (plasma)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* No. of experiments.
† = add 0.216 ml from direct mucosal bleeding.
‡ = add 0.235 ml from direct mucosal bleeding.
§ = species unstated, probably *Necator americanus*. 

Nature and causes of "hookworm anemia"
blood loss per *Necator*, and correct for this loss in mixed infection, in the fashion indicated by Roche et al. (1957a), the probable loss by *Ancylostoma* in the four cases is about ten times blood loss from *Necator*; which confirms essentially Roche et al. (1957a), as well as the impression held by many clinicians that *A. duodenale* does lead to distinctly higher losses.

Aly et al. (1962) have measured blood loss by the Cr\(^{41}\) method in 39 patients with "pure ancylostoma infection." It is not clear however whether this means that the patients did not harbor parasites other than hookworm, or whether it is implied that they had only *A. duodenale*. Average hemoglobin in these cases was 64.3%, range 32–98, and mean daily blood loss 12.38 ml range 3.9–25.6. It was stated that there was "no correlation between the degree of anemia and the amount of blood loss caused by the ancylostoma worm," but no details were given as to severity of infection. Mahmood and Woodruff (1964) found an average blood loss in 15 cases with pure *A. duodenale* of 0.152 ± 0.124 and Farid et al. (1965), in 12 similar cases, an average of 0.26, with standard deviation ±0.045 ml, thus fully confirming that this species leads to a larger blood loss than *N. americanus*.

In the dog, Clark et al. (1961) determined blood loss by means of Cr\(^{41}\). Average blood loss per worm per day was 0.07 ml. Roche et al. (1960) in four dogs have reported quantities of 0.048, 0.063, 0.015, 0.022 ml/day/worm.

Table 11 summarizes the data thus far on blood consumed per worm by different species and in different hosts. From a perusal of the table and from the previous discussion, it will become apparent that such loss could hardly be called negligible. Blood loss per worm by *Ancylostoma caninum*, determined by various authors, is of the order of 0.05 ml/day, except for the values obtained by Wells’ method, which are all higher, and probably erroneous, if the isotopic method can be taken as a standard. Blood consumption by *A. caninum* as measured in vivo by an isotopic method (Clark et al., 1961; Roche et al., 1960) and in vitro (Roche et al., 1960) do not differ markedly. This point will be discussed further in another chapter (Section IX).

**Relation of Blood Loss and Number of Eggs in the Feces**

Any estimation based on egg counting in the stool must be made with caution, since the variables involved, in addition to those related to the methods utilized, are many. The number of female is almost always higher than that of male worms, depending, in part, upon the length of infection (Roche and Patrzek, 1966), and there is no general agreement on the number of eggs laid per day by females of different species. According to our own observations, in Venezuelan infected subjects, a female *Necator* lays 3406 ± 1,286 (S.D.) eggs per day (Martinez Torres et al., 1966), which agrees with the figure given by Hill (1926). The value is obviously quite variable and much higher figures have been reported, usually associated with very light infections; for instance, Davis (1924) reported 28,000 eggs per day per female and Beaver (1955), in human experimental infections (*N. americanus*) in which no more than two females were present, found as high as 25 to 50 thousand eggs per day.

Such variations are not unexpected, in view of the many factors which may affect egg laying, such as age of infection (Herrick, 1928; Chandler, 1929), nutrition of the host (Foster and Cort, 1932; 1935), total number of worms (Hill, 1926) and, possibly, egg laying cycles (Soper, 1927; Yarima and Machida, 1958), all of which have been insufficiently studied.

In spite of these factors of variation, Martinez Torres et al. (1966) have found, in a relatively homogeneous group of patients from Venezuela with *N. americanus* infections, a significant correlation between the number of eggs per 1000 g of stool per day and blood loss, as measured by Cr\(^{41}\) (Fig. 12). In 50 subjects, they give an average of 2.14 ± 1.01 ml (S.E.)/day/1000 eggs per g. This would correspond to 0.7 mg of iron in subjects with a normal circulating hemoglobin, a value which would obviously become less with lower hemoglobin levels.

Farid et al. (1965a) have reported a mean daily blood loss per 1000 eggs per gram of feces of 4.47 ml ± 1.16 (S.D.) in 12 cases with pure *A. duodenale* infection.

This type of information permits a rough evaluation of blood and iron loss in a given community, based on simple "quantitative" egg counts.

**Life Span of Hookworm; Its Relation to Total Blood Loss**

It is evident that blood loss at a given moment in infected subjects is related to the number of worms harbored by the host. However, blood loss has been studied with accuracy only during rela-
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for 12 months only but eggs could still be found by the direct centrifugal method, five years and four months after the date of infection. Caldwell and Caldwell (1931) followed during four years an infection in a laboratory assistant, finding no decrease in egg counts during this time. Palmer (1955) studied a self-inflicted experimental infection with N. americanus, in which the possibility of reinfection could be practically ruled out. The total production of eggs in 24 hours increased to a maximum after approximately a year and remained constant for almost four years, after which it decreased, in a nearly straight line with a slope of approximately 850,000 eggs per ten years or 85,000 per year. The feces were finally free from eggs 15 years after the original infection. The author estimated that his original infection was of the order of 200 worms, so that approximately 20 worms were lost per year. It is well to mention, however, that the infection studied by Palmer must have been a particularly hardy one since it resisted all attempts at eradication in the early period.

The evidence so far reviewed seems to indicate that infection by hookworm in the human species may last many years, although a general progressive loss is observed either at an early stage, as in the cases of Kendrick, or after several years, as in the case of Palmer.

As for infection in animals, Sarles (1929) found, in five puppies experimentally infected with A. caninum, an average span of 62 ± 6 weeks. After a maximum production of eggs had been reached, decrease was rapid and exponential, with a half time of the order of 6 to 10 weeks. All dogs utilized by Sarles were thoroughly wormed, but they had been infected previously, and it cannot be known to what extent immunological mechanisms played a part in the second infection. The rapid decrease of infection in dog might explain why a heavy load in this species is rarely found.

B) Naturally induced infections. Artificial infections are easier to study but they are not strictly comparable to those acquired in a natural way, since the latter invade the host probably in successive waves whose loss might be possibly accelerated through the effect of immunity, but this factor is poorly known in humans. The matter has been studied in different ways.

1) Simultaneous measurement, during variable lengths of time, of the number of worms or eggs in large groups of persons not subject to reinfection. Mhaskar (1920) examined the feces, after
worming, in a total of 1878 jail inmates in the Trichinopolis jail, in India. The percentage of infection, in 318 prisoners who had recently entered jail, was 96.1% and, in 33 subjects in jail from four to 16 years, 84.3%, which would indicate that this infection has a long duration. According to the author, reinfections were most unlikely under the hygienic conditions prevailing in the jail. When worms were counted after vermil- fugue, however, there was on the average a general decrease related to the time in jail. Thus, in those who had been jailed for eight months, the average number of worms was only half that in people who had just entered jail. After this time, the decrease was much slower and gradual. Chandler (1925-1926), made a similar study, counting this time the eggs in feces instead of the worms after vermi- fugue.

The total time of infection can be very long, since one of the cases was still infected after 20 years in jail. Chandler found, as Mhaskar had, that the conditions in jail were wholly unfavorable to reinfection.

The studies by Mhaskar and by Chandler indicate that there is a rapid decrease in the severity of infection in the subjects who are not reinfected. However, the data given by these authors are not in accordance with those obtained by others, as will be seen herewith.

b) Observations of actual loss of worms. Payne and Payne (1931) sieved the feces of three infected Puerto Rican patients during periods of 22 to 32 days and counted all spontaneously lost worms. This number was only 2.7% of the theoretical loss which could have been expected in accordance with the data given by Chandler.

c) Observations of infected subjects during a prolonged length of time. Maplestone (1930-1931), determined egg counts every month during a year in a group of jail inmates, in the same jail utilized previously by Chandler (1925-1926). The initial count was 1272/g and it increased on the average to 2222 in six months and was later reduced to 723 after one year. This would seem to indicate that the subjects had acquired a new infection during their stay in jail or possibly this may have been a reflection of egg laying by the worms acquired immediately before admission to jail. On the other hand, the number of eggs is so low that the probable error is high; so that we do not believe that a solid conclusion can be reached for Maplestone’s data.

Cruise and Pimenta de Mello (1948), in the course of studies upon the effect of ferrous sulfate on hookworm anemia, observed a 22-year-old man hospitalized during 15 months. Egg counts were 15,000/g at the start and remained with practically no variation during 14 months. Five counts made during this time varied between limits of 12,900 and 15,000/g. After 15 months, 1,051 worms were obtained after vermil- fugue.

It may be interesting in this respect that four of the five cases studied by Liermberger (1905) who had returned from Brazil to Germany two years before, still harbored 500 to 1515 worms.

Caldwell and Caldwell (1931) studied Cherokee children living during the humid summer months, June to mid-September, at home under conditions favorable to infection and remained at school from September to June, at which time they wore shoes and used latrines. Egg counts were performed during the school period in 73 of these children. There was no decrease in eggs, contrary to what might have been expected according to the hypothesis of Chandler. Chandler (1925) later attempted to reconcile these facts with his hypothesis and the reader is referred to his discussion.

Recently, Jeffery and Harrison (1963) followed 61 inmates of an insane asylum during seven years; the prevalence of infection went down only from 70.5% to 65.6%. The total number of eggs decreased, however, but not, by far, to the extent which could have been expected according to the data of Chandler and of Mhaskar.

The subject of the life-span of hookworms is evidently far from clear, but some general conclusions can be reached. The total duration of single infections both by N. americanus and by A. duodenale can be very long, of the order of eight to 16 years, while the life-span of A. caninum appears distinctly shorter. As for turnover, the studies done in India, with mixed infections, would indicate that it is rapid, requiring approximately three to six months for a decrease of 50% from the original level, while all the studies done in the Western hemisphere, in infections predominantly or exclusively with Necator, would indicate a slow turnover, of the order of years. Most of the infections studied in India were light; under these conditions, errors and variations in egg counting would be expected to be large.

We have not carried out systematic studies on hookworm life-span, but we feel, on the basis of reports from West Indies, Brazil, and the United States, and on clinical impression, that turnover of a given natural Necator infection in our geo-
graphical area is rather long, of the order of several years. It is probable, in addition, that in an endemic area, where infection has been long established, and where hygienic conditions are stagnant, infections exist in a steady state, wherein worm loss is matched overall by reinfec-
tion; so that, statistically, the long range picture of prevalence and intensity of infections is not too different from what may be observed in a single large scale survey. To that extent is extrapolation of individual studies on blood loss valid (see Con-
cluding Remarks).

Iron Losses in the Feces

It is by now evident that patients with hook-
worm infection may lose considerable quantities of iron via the feces. What these quantities actually are is summarized in Table 12.

Of the two isotopes, Fe\textsuperscript{55} and Cr\textsuperscript{51}, utilized to measure blood and iron losses, Fe\textsuperscript{55} gives a more accurate and direct idea of actual losses while since iron is in part reabsorbed (see Section V) the figures given for iron loss calculated from Cr\textsuperscript{51} measurements are probably too high.

Whether fecal iron loss is sufficient to account for the anemia associated with hookworm infection depends probably on a balance between the available dietary iron and total iron losses, including routes other than the fecal. This question will be discussed further in the last section.

Iron Loss through Sweat

Although the question of iron loss through sweat does not directly concern the hookworm, it is germane to discuss it here as a possible cause of iron deficiency anemia in the tropics (see Table 13).

Some authors (Mitchell and Hamilton, 1949; Adams, Leslie and Levin, 1950; Foy and Kondi, 1957; Hussain and Patwardhan, 1959b; Apte and Venkatachalam, 1962) have found values indicating that sweat may be a significant avenue of iron loss; while others (Johnston et al., 1950; Dubach et al., 1955) have reported low values. Both chemical and radioactive methods have been used, but a direct comparison of the results of the two in the same material has not been accomplished.

A number of difficulties associated with the methods used might help explain some of the disagreements, (not counting contamination which we presume did not occur). In particular, the conversion of the results from concentration of iron per ml of sweat to loss of iron per day in-volves a number of variables which are not well known, such as: the relationship between the amount of sweat during the experimental period and that produced under the subject's ordinary working conditions, the possible variation in iron content in different parts of the skin (see for example Apte and Venkatachalam, 1962, who found differences in upper and lower extremities) and the contribution of “cell free” and “cell rich” portions to physiological sweat, which has not been clearly defined. The cell-rich portion has been invariably found to be higher in iron content.

Finch (1959) has tagged circulating erythrocytes by means of an iron isotope with a 62.94 year half life (Fe\textsuperscript{55}) and followed blood radioactiv-
ity during prolonged periods of time. He found by this means total iron losses of about 0.6 mg per
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Site</th>
<th>Subjects</th>
<th>Condition of sampling</th>
<th>Sweat iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell and Hamilton</td>
<td>1949</td>
<td>Urbana, Ill., U.S.A.</td>
<td>Normal males, high Fe intake (around 54 mg per day)</td>
<td>Whole sweat, bulb heat. High heat and humidity</td>
<td>Conc. mg/L: 2 mg, Possible loss (mg/24 hrs.): 31†</td>
</tr>
<tr>
<td>Adams et al.</td>
<td>1950</td>
<td>Los Angeles, U.S.A.</td>
<td>25 normal subjects</td>
<td>Sweat from forearm</td>
<td>CR = 0.78-19.5†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CF = negligible‡</td>
</tr>
<tr>
<td>Foy and Kondi</td>
<td>1957</td>
<td>India</td>
<td>Not stated</td>
<td>Not stated</td>
<td>CR = 0.3-6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CF = 0.1-0.2</td>
</tr>
<tr>
<td>Hussain and Fatwardhan</td>
<td>1959 b</td>
<td>Coonoor, India</td>
<td>6 healthy adults</td>
<td>Sweat from forearm</td>
<td>CR = 1.61</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>CF = 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17 anemic women</td>
<td>Pre-treatment</td>
<td>CR = 0.44</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>CF = 0</td>
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<td></td>
<td></td>
<td>Post-treatment (42 days on iron)</td>
<td>CR = 1.45</td>
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<td></td>
<td></td>
<td>CF = 0.41</td>
</tr>
<tr>
<td>Hussain et al.</td>
<td>1960</td>
<td>Hyderabad, India</td>
<td>35 healthy males</td>
<td>Sweat from forearm</td>
<td>CR = 1.15 ± 0.055</td>
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<td></td>
<td></td>
<td></td>
<td>CF = 0.34 ± 0.016</td>
</tr>
<tr>
<td>Apta and Venkatachalam</td>
<td>1962</td>
<td>idem</td>
<td>16 adult healthy men</td>
<td>Upper extremities</td>
<td>CR = 0.33 ± 0.036</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>CF = 0.190 ± 0.013</td>
</tr>
<tr>
<td>Johnston et al.</td>
<td>1950</td>
<td></td>
<td>4 healthy women</td>
<td>Total sweat collect. filtered, cell free</td>
<td>CR = 0.52 ± 0.056</td>
</tr>
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<td></td>
<td></td>
<td>CF = 0.25 ± 0.024</td>
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<tr>
<td>Dubach et al.</td>
<td>1955</td>
<td>St. Louis, U.S.A.</td>
<td>2 healthy men and 1 healthy woman</td>
<td>Forearm studies Fe**</td>
<td>CR = 0.07-1.56</td>
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<td></td>
<td>1 man with hypoplastic an.</td>
<td></td>
<td>CF = 0.21-4.66</td>
</tr>
<tr>
<td>Moore</td>
<td>1961</td>
<td>St. Louis, U.S.A.</td>
<td>1 healthy male</td>
<td>Forearm studies chemical</td>
<td>CR = 0.15-0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CF = 0.10-0.74</td>
</tr>
</tbody>
</table>

* Assuming possible loss of 3 L/day in temperate zones.
† Calculated loss per 24 hrs in mg, given by the authors.
‡ CR, cell-rich sweat; CF, cell-free sweat.
day in adult men and non-menstruating women. These studies were carried out in Seattle, where a temperate climate prevails; similar studies were repeated later in a hot, hookworm free tropical area (Araya, Venezuela); the iron loss found during a two year period turned out to be of the same order of magnitude as that found in Seattle (Finch et al., unpublished) (Fig. 13). These findings suggest that the total loss of iron in a hot area, where sweating is abundant, but in which there is no hookworm infection, is higher than in a temperate climate.

Recent studies with iron 55 in an endemically infected area (Layrisse, unpublished) have indicated an increased turnover in lightly infected or non-infected subjects (of the order of 24% per year in adult men, and 40% in adult menstruating women). These findings bring up the two possibilities: (1) that bleeding from small hookworm infections may be relatively larger than hitherto suspected, and (2) that other agents (such as Rhodnius prolixus, the insect vector for Chagas' disease) may be leading to significant blood loss, or both.

Regarding blood loss due to the vectors of Chagas' disease, a single 5th instar nymph R. prolixus can draw in a single meal from 180 mg (Cirano and Zeledón, 1964) to 265 mg (Gómez Núñez, in Roche and Layrisse, 1964). Mayer et al. (1947) have reported 0.25 ml of blood per meal of adult Rhodnius. The 5th instar of Triatoma infestans, on the other hand, consumes from 300 to 400 mg of blood per meal (Cirano and Zeledón, 1964).

From measurement of single meals in various stages of development of the insect, combined with an estimate of the feeding frequency and the house density, it has been estimated by Gómez Núñez (in Layrisse and Roche, 1964) that a dweller of a rural Venezuelan house could lose from this cause an average of 0.7 ml of blood per day, which would represent 0.4 mg of iron loss. This quantity could help explain part of the increased iron turnover.

Summary

By means of isotopic measurement in dog and in human, blood loss from hookworm has been found to vary, from 2 to 3 ml per day in lightly infected subjects, to around 100 ml in heavy infections. Blood loss per worm is of the order of 0.08 ml per day for Necator americanus, and 0.06 for Ancylostoma caninum, and 0.16 for Ancylostoma duodenale.

Some of the iron lost with the blood into the intestine is reabsorbed (see Section V), but total fecal iron losses are nonetheless high.

Loss of iron through sweat has been mentioned as a possible additional factor in the iron deficiency observed in hot tropical areas. The results are conflicting, chemical methods yielding generally high iron losses, while isotopic methods give low values. A study of the loss of long lived radioactive iron Fe 55 from the circulating erythrocytes, in a hookworm free warm area, suggests that loss of iron through the sweat may not be an important anemia-producing factor.

For practical purposes, a rough, but statistically significant correlation has been established between the number of eggs in the feces and the blood—and iron—loss. Such correlation in Venezuelan subjects with pure N. americanus infections is: 8.1 ml of blood per day/1000 eggs/g of feces, or 0.7 mg of iron per day/1000 eggs/g of feces in an individual with normal hemoglobin values. Obviously, this latter value will diminish in the presence of anemia. In Egyptians, with pure A. duodenale infection, the loss of blood has been reported as 4.47 ± 1.16 ml/1000 eggs/g of feces.

IX. MECHANISM AND SIGNIFICANCE OF SUCTION BY HOOKWORM

It is clear that hookworm infection leads to much blood loss. Whether this is caused chiefly
through blood sucking by the worms, or through other mechanisms, becomes an academic question, and yet is of considerable biological interest. In this chapter, the manner in which the worm attaches itself to the mucosa and the way it sucks and expels fluid will be described; and the evidence both for and against its being a habitual blood sucker examined.

Mechanism of Suction

Grassi and Parona (1879) were first to describe the blood sucking activity of hookworm in vitro, but Wells' direct observations (1931) provided the first direct in vivo evidence that the worm could indeed be a blood sucker. After slitting locally the small intestine of animals anesthetized with sodium barbital and placing the intestine in a special clamp, which permitted direct observation while the worms remained fixed to the mucosa under conditions approaching the physiological, Wells observed that the worms move about in various directions by means of snake-like motions until, after an interval which might vary from a few minutes to several hours, "assisted by rapid flexing movements of the cephalic portion of the body" they become attached. When such attachment is superficial, the vessels become congested and stand out as a bluish network (Wells, 1931). There then appears on the surface a red disc-shaped spot, with its center limited by the sides of the buccal capsule of the parasite.

The worm then "with vigorous boring movements works its head between the villi to a deeper point of attachment." The first material sucked may not be blood but rather "clear fluid, mucus and clumps of epithelial cells." Wells mentions also that "in worms attached to the mucosa, one can often see fragments of epithelial detritus shining through the intestinal wall in the form of white mass."

After the hookworm has obtained a satisfactory spot, which may take several hours under experimental conditions but which is perhaps more rapid under natural conditions because of the close apposition of the intestinal wall, its 'body becomes quiet and esophageal movements become very rapid' (Wells, 1931).

It is probable that the blood-letting function of hookworm precedes egg-laying. Thus, in a therapeutic infection in human, occult blood in the feces was found after 20 days and eggs after 40 days (Duvoir et al., 1942). Clark et al. (1961) tagged with Cr the circulating erythrocytes of dogs, before infecting them with Ancylostoma caninum, and found an increase in fecal radioactivity about eight days after infection, while eggs appeared only one week later. They described two peaks in the quantity of fecal blood (Fig. 14), the first around day 13 due, according to the authors, to blood-sucking of the fourth stage larvae, followed by a dip, possibly associated with the last moulting, and a second peak around the 19th day.

It is possible that the process of suction may be facilitated by proteolytic substances such as described by Thorson (1956a) in extracts of the esophagus of A. caninum. The time between the moment in which hookworm fixes itself to the mucosa and the ejection of the first drop of blood is on average 7' 0" according to Nishi (1933) and 7' 20" according to Ryo (1937), both of whom made observations by Wells' method. The flow of blood may possibly be facilitated by substances which inhibit coagulation (Loeb and Smith, 1905) or prolong prothrombin time (Thorson, 1956b).

Roche and Martinez-Torres (1960) have designed a simple apparatus, by means of which the movements of the esophagus can be readily observed and filmed under the microscope (Fig. 15). The apparatus consists essentially of a thin rubber membrane, kept taut between two concentric plastic rings, as the skin on a drum. By means of a needle, the worm can be so set on this membrane that it remains caught, with its cephalic extremity on one side and its caudal end on the other (Fig. 16). The ring, with the membrane and the worm, are then placed horizontally under the objective of the microscope so that the cephalic extremity lies toward the observer, slightly compressed between membrane and coverslip and surrounded by a few drops of blood; the movements of the esophagus can then be readily observed (Fig. 17). The esophageal cavity, starting from the resting position, with its walls in apposition, executes rapid diastolic and systolic movements. The lumen of the esophagus expands while the anterior funnel-shaped valve opens. Blood flows rapidly into the open cavity, and is pushed backward towards the intestinal tract when the esophagus returns to its

An ordinary rubber prophylactic is satisfactory.
systolic position, while the anterior valve closes
and the posterior opens. A record of the electrical
signals associated with contraction of the esophag-
us may be obtained by inserting electrodes in the
chambers of the apparatus and connecting them
to an appropriate recording system (Fig. 18)
(Roche et al., 1962). The complete cycle, which
lasts, according to the oscilloscopic record, ap-
proximately 200 milliseconds, is repeated 120 to
200 times per minute. Once in a while, the sucking
movements cease entirely, for seconds or minutes,
to be resumed later.

At times the worm will regurgitate blood in
stead of ingesting it, and this is usually associated
with rapid and transitory oscillations of the
electroesophagogram, which we have termed B
wave (Roche et al., 1962) (Fig. 17). These are due
probably to a failure of coordination of the esoph-
agal musculature, leading to fibrillatory-like
movements.

After reaching the worm’s intestine, the blood
flows much more slowly; however, in an actively
sucking worm, transit time may be relatively
rapid. It is possible to measure in vitro the transit
time by means of the apparatus described above.
The worm is first allowed to suck plasma from the
anterior chamber, until it becomes transparent.
Plasma is then replaced by blood which can be
readily watched as it travels through the worm’s
body, and the time measured until the first anal
ejection of the blood. The electroesophagogram
may be used simultaneously to verify whether
the worm is sucking; in active worms, transit
time from mouth to first ejection has been found to
vary from 39" to 2' 30" (Roche and Patrzek, un-
published). When the worms do not suck, or suck
only intermittently, transit time can be much
longer, up to half an hour or more.

Nishi (1933) noted, in a particularly active
worm, a small piece of mucosa which traveled
through the length of the parasite in 52 seconds.
Wells (1931) noted that, although some worms
expelled the blood which filled them a few minutes
after they are removed from the mucosa, others
may be kept alive up to various days in saline
solution without losing the blood which they con-
tain. This latter observation is somewhat arti-
ficial, since Roche and Martinez Torres (1960) have shown that, in saline solutions, the sucking movements of the esophagus soon become abnormal and probably inefficient. In Ringer's solution, the worms do not transport Cr³⁺ from the anterior to the posterior compartment as does occur when they are sucking serum or blood (Warren and Guevara, 1962).

After blood reaches the rectal ampulla, it stagnates there for a while and is then expelled by means of forceful discrete ejections (Fig. 19) which take place at intervals varying between fractions of a second and several minutes (Wells, 1931; Roche and Martínez Torres, 1960). Nishi (1933) described the blood ejected through the anus of _A. caninum_ "like cigarette smoke." Wells (1931), Yamada and Inouye (1934), Nishi (1933), Ryo (1935), and Roche and Martínez Torres (1960)
have noted that the red cells which came out of the worms' anus looked whole and intact (Fig. 19). Roche and Martínez Torres (1960) have presented evidence, however, that part of the red cells are destroyed in their transit through the worm. After tagging them with Cr¹⁹ erythrocytes before feeding them to the worm, they determined simultaneously radioactivity and red cell counts in the anterior and the posterior chambers, and calculated independently, from both methods, the amount of blood transported; they found consistently a deficit, of the order of 40%, by the red cell counting method.

Little is known of the stimulus required for efficient and continuous blood sucking. In vitro, vigorous sucking motions may be observed only when the parasites are sucking blood, serum or plasma (Roche and Martínez Torres, 1960). These movements become abnormal, or cease entirely when saline solutions are utilized (Roche and Martínez Torres, 1960). This observation suggests that plasma contains one or various substances necessary for continuous suction by the worm.

**Significance of Blood-Sucking by Hookworm**

There is no doubt that hookworm may ingest blood in vivo as well as in vitro. It has been argued, however, that this is by no means the chief mechanism for blood loss produced by the worm, and indeed that blood letting is only incidental to its feeding on intestinal mucosa.

Nishi (1933) and Ryo (1937) felt that the loss of blood around the site of implantation of the worm's cephalic extremity was higher than blood loss from direct sucking. Nishi attempted to assess this quantity by carefully collecting, with a fine brush, the blood which oozed around the worm, shaking the brush immediately thereafter in counting fluid. He performed this operation repeatedly with 10 parasites, during observation periods lasting an average of 27' 20". The red
cells were then counted and the total loss extrapolated to 24 hrs, this loss being on an average 0.216 ml as compared with 0.144 ml estimated for direct suction. Ryo's figure in similar experiments was 0.235 ml. Such oozing around the worm was not mentioned in Wells' (1931) careful observations, and it is our impression that it compares in no way with blood letting which can be produced by the worm's efficient pumping. The similarity between the figures for total blood loss per worm in vivo, obtained by isotopic methods, and those obtained in vitro, where there is no blood letting other than that due to the worm's sucking (Roche, Martínez Torres, 1960) (Table 11) suggests that sources of bleeding other than direct sucking are minimal, at least with A. caninum.

It has been suggested, however, that hookworm is not a habitual blood sucker because it is found frequently empty at autopsy and because red blood cells have seldom been demonstrated histologically in its intestinal tract. Looss (1905, page 100) stated that "even in cases where dissection took place comparatively soon (3—6 hrs) after death, and in which a comparatively large percentage of the parasites were found still fixed to the mucus membrane of the host, the proportion of those containing blood was exceedingly small, indeed often there were actually none with blood in them." Similar comments have been made by Ashford and Gutiérrez (1911); by Mackey (1933), by Beaver et al. (1964), and by Sheehy and Floch (1964). Beaver et al. (1964) believe, in fact, that "one can now doubt whether hookworm actually sucks blood to satisfy their physiological needs" (page 292). They argue that it is difficult to demonstrate red blood cells in the intestine of the parasites, while epithelial cells are not infrequently found, and they explain blood loss chiefly through the lacerations of the mucosa produced by the worms. They state that "in the freshly opened intestine of infected dogs, blood is often seen oozing from the lacerated mucosa."

In figures published to show histological attachment of hookworm to the intestinal mucosa (Ashby and Gutiérrez, 1911; Sheehy and Floch, 1965), the worm's buccal capsule is often seen filled with a large pediculated plug of mucosal tissue, sometimes extending part way down the esophagus (Looss, 1905), whereas very seldom can blood cells be demonstrated histologically in the worm's...
intestinal tract. Hoepli (1927) mentions that, in *Ancylostoma duodenale*, epithelial cells, alone or in groups, could be found frequently in the buccal capsule and in the proximal part of the intestine.

Now, while it has not been proved beyond doubt that hookworm is a habitual blood sucker, we believe blood (or plasma) to be its chief source of food. It is true that a large number, often the majority of worms are found empty at autopsy. Grassi and Parona (1879) had already commented on this fact, and mentioned that failure to find blood in the worms does not imply that these are
not blood suckers; they mentioned that, when a cat is autopsied 24 hours after its death, hookworms are found to be empty, while, when autopsy is made immediately after death, they are found full of blood. These authors commented, further, that pathologists working in Egypt frequently found blood in the human hookworm, while those working in Italy did not; they explained this from the fact that the former performed autopsies a short time after death, and the latter a long time afterwards. Blanchard made similar comments (1889, page 775). In his experimental apparatus, Roche (unpublished) has demonstrated that, when the worm is actively sucking, transit time of blood through its body is short, of the order of 2 to 3 minutes; since no less than 8 to 10 minutes are required between sacrifice of the dog and the opening of its intestine, even when the parasites are maintained at a temperature of 20 to 22 degrees, and much longer before all worms are examined, they may readily empty themselves before actual examination.

We have sacrificed a large number of dogs to obtain worms for experimental purposes and we have not been impressed by the oozing of blood from the mucosa mentioned by Beaver et al. (1964). If a fasted infected dog is sacrificed, the mucosa is in general found intact, with only a few pin-point reddish spots, with no blood around them, and wherefrom blood does not, in our experience, ooze or flow. Hookworms are seen, single or in groups, attached to the mucosa or free, and they are generally found to be empty, but are always surrounded by pools of fresh-looking blood (which they have likely just ejected) (Fig. 20). The blood contained by some of the worms can be readily demonstrated by nicking the cuticle with fine eye scissors, following which a balloon-like hernia sprouts out of the wound, containing most of the blood previously held by the worm’s body. Upon pricking this balloon, a liquid rushes out, which can be demonstrated, with the usual stains, to be morphologically normal blood (Roche, unpublished). Looss (1905, page 101) had already noted that, in some worms prepared histologically, the blood “retains its characteristic appearance very distinctly even in animals which were not fresh when preserved but were in the act of decomposing.” Hsu (1938) found in the gut of _A. caninum_ red and white cells in various stages of disintegration, as have Garin et al. (1932).

Failure to demonstrate histologically blood cells in the intestine of hookworms is probably due to their emptying before the stain is able to reach the intestine. As for the plugs of mucosal tissue demonstrated in the buccal cavity of the worms, they are probably seen only in those worms which are deeply attached to the mucosa; most of the hookworms found at autopsy are in fact very loosely attached, or free.

The sucking of the parasite, however, is not limited to blood, since it will ingest with avidity, at least for a short time, saline solutions of diverse kinds, and even toxic products, such as formalin (Roche, 1965, unpublished). Looss (1905) has demonstrated hookworm eggs in the intestine of several worms, which suggests that these parasites may indeed ingest intestinal contents of their host at the time they wander about while going from site to site.

For a final proof that blood (or plasma) is indeed the habitual food of hookworm, there is need to demonstrate that it, and it alone, contains one or several factors essential to the worm physiology. Fernando and Wong (1964) have shown, by means of C14 glucose, that plasma, or a macromolecular fraction of plasma, is needed for glucose to be efficiently incorporated into the parasite’s glycogen. Although they did state that added India ink travelled varying distances irrespective of the medium the worms were in, Fernando and Wong did not demonstrate beyond doubt that the plasma did not act on the sucking activity of the worm rather than on mechanisms directly involved with glycogenesis; it also remains to be shown whether other tissues, readily available to hookworm, such as intestinal epithelium, does not contain also factors which facilitate incorporation of glucose into glycogen.

It is probable that active transport of substances occurs through the intestinal mucosa of the worm. Brown and Chowdhury (1959) have found the granular zone of the intestinal tract of _A. caninum_ to be full of mitochondria and Murakami (1961) demonstrated strongly positive reactions for acid phosphatase as well as alkaline phosphatase in the striated layer.

The fact that the transport of blood through the worm is rapid and the quantity of blood consumed high suggests that the worm may utilize chiefly easily diffusible substances, obtained from the plasma or from the blood of the host. One of these substances could be oxygen. The finding of a low glycogen content in the body of the worm (Von Brand and Otto, 1938; Fernando and Wong,
1964) suggests that the parasite is an aerobic organism and it would be logical to assume that it obtains the greater part, if not the whole, of the necessary oxygen from the ingested blood; it would thus be, in practice, a tissue parasite. Wells (1931) has noted that, as blood passes through the body of the worm, it may change color from bright red at the buccal end to bluish at the caudal extremity. He also observed that, during rapid suction and emission of blood, there was no change of color.

Oxygen consumption of *A. caninum* is in fact relatively high. In Warburg flasks, Warren and Guevara (1962) have reported a QO2 of 1600 μl/g wet weight/hr in female and 2200 in male, which means about 1.6 and 2.2 approximately per hookworm, or 38.4 and 52.8 μl/g wet weight/24 hrs. Taking the average consumption of blood as 0.05 ml/24 hr per female worm (Roche and Martínez Torres, 1960) under the best conditions, this blood could only furnish about 10 μl of oxygen per day, of which the greater portion would be contained in the erythrocytes. We must therefore suppose that either the results obtained in the artificial conditions of the Warburg method are too high or that the hookworm actually derives great part of its oxygen through the cuticle.

The cuticle may be permeable to oxygen since, when worms are maintained in blood under a coverslip for some time, blood close to the parasite becomes gradually bluish. If oxygen is then blown over the mucosa of the intestine when the worms are fixed, the blood in their interior takes on a bright red color in a few seconds (Wells, 1931). Even though the mammalian intestinal lumen is practically free from oxygen, there is a consid-

**Figure 20.** Small intestine of a dog at autopsy, photographed shortly after sacrificing the dog. *Left:* empty worms surrounded by localized areas of fresh-looking blood. *Right:* After removing the worms and washing the blood with water, the intestinal mucosa looks grossly intact.
erable oxygen gradient near the mucosa (Rogers, 1950) which might allow its utilization by the worm.

The electrical activity of the electrosesophogram remains normal during many hours when the worm ingests plasma free from red blood cells (Roche and Martinez Torres, 1962) or normal blood whose hemoglobin has been blocked with carbon monoxide (Roche, 1963). Hence it is probable that the organism uses chiefly oxygen dissolved in the plasma.

*A. caninum* survives in *vitro* under sterile conditions for a maximum of 15 days in a solution of Krebs Ringer with glucose, and up to 82 days in a 50% mixture of Krebs Ringer and serum (Komiya et al., 1956). It would seem therefore that for the normal physiology of the worm certain factors are needed besides those found in simple saline solutions. Fernando and Wong (1964) have demonstrated the need for non-diffusible plasma factors for the incorporation of C14-glucose into the worm glycogen. Warren and Guevara (1962) have determined the concentration of glucose, lactate and pyruvate in the anterior and the posterior compartments of the Roche and Martinez Torres' chamber, in which *A. caninum* was feeding on glucose-enriched serum. They measured the quantity of Cr5+ transported by the worm from the anterior to the posterior chamber, and calculated from this value the quantity of fluid transported. No glucose could be detected in the posterior chamber, although its concentration would have been well within the limits of sensitivity of the method, had it not been utilized. It was deduced that the worm consumed all the glucose made available through suction. A slight excess of pyruvate was found in the tail chamber, but pyruvate production was practically negligible when the experiments were repeated in well aerated Erlenmeyer flasks and the authors felt that pyruvate production in the *in vitro* chamber was due to the relatively anaerobic conditions prevailing therein.

The respiratory quotient (R.Q.) of *A. caninum* was reported by Warren and Guevara (1962) to be 0.43 for female and 0.56 for male worms in Ringer phosphate medium. Fernando and Wong (1964) suggested that this low R. Q. was due to retention of CO2 by the phosphate Krebs Ringer medium, and reported R.Q. values of hookworm in Krebs Ringer and air at pH 7.4, corrected for CO2 retention, as 0.97 ± 0.01, which they felt was indicative of a carbohydrate metabolism.

**Summary**

Hookworm has been observed to suck blood in *vivo* and in *vitro*. It may however suck other substances, including mucosal tissue, intestinal juices, and various artificial saline solutions. It is probable that the worms alternate between periods of esophageal activity and rest. During active periods, sucking motions occur from 180 to 800 times per minute and each motion is associated with an electrical signal which can be recorded as an "electrosesophagogram" by suitable devices. In active worms, transit time of blood from mouth to anus is short, of the order of 1 to 2 minutes.

At autopsy, many worms are found empty, but they are often surrounded by pools of apparently fresh blood, which suggests that they have emptied themselves recently, possibly as a result of the agonal intestinal movements.

Serum, if not blood, seems to be necessary for proper functioning of the worms' esophagus. It appears to be also needed for the incorporation of glucose into the worms' glycogen. Glucose is efficiently utilized and there seems to be a limited production of pyruvate. The quantity of blood lost in *vivo* by the infected host is of the same order of magnitude as that sucked by the worm in *vitro*.

The evidence indicates that the worm is indeed an habitual blood sucker or at any rate that it needs serum for its normal physiology.

**Concluding Remarks**

That anemia can be significantly associated with the hookworm in a given infected group, there is no doubt: a number of studies have shown this to be the case. Such anemia is of the iron deficiency type.

What anemia results from this association is, however, closely related to the background of iron intake of the subjects in whom it occurs, other nutritional factors appearing to be of little importance. Whether anemia does develop depends on a balance between iron utilised by the body for hemoglobin production and that which is lost via the hookworm, other channels of loss probably being negligible.

The total quantity of iron ingested daily in regions where hookworm is endemic, is, by the usual standards, adequate and often high; it is mostly of vegetable origin, however, and poorly absorbed, even though an increased capacity for absorption of ionized iron is often present. Consequently people living in rural tropical areas
probably receive a relatively inadequate supply of available food iron. What iron is absorbed is readily utilized by the marrow for hemoglobin synthesis.

Hookworm infection is grafted on this background of inadequate supply of available iron. There is no question that this worm does lead to the loss of large quantities of blood and iron, even though the question whether it needs blood for its normal physiology is still controversial. Such blood loss, in subjects who are already iron depleted, is of great importance in the causation of anemia in the tropics, and deserves far more attention from the public health point of view than it has generally received.

Whether hookworm or an inadequate supply of available iron assumes more importance depends on the quality of the local food and of the average hookworm load. In the Venezuelan rural context where this has been studied (Pequin), the diet seems to be sufficient to bring hemoglobin levels back to normal after worming, albeit in a protracted fashion, and hence hookworm would appear to be paramount in the production of iron deficiency. In other communities, a different situation may prevail.

Although in large groups a significant negative relationship generally results between hookworm load and anemia, it is a fact that individual cases are seen with anemia and no hookworm and vice versa. There are several possible explanations for such finding; one is that iron treatment may have been received by the patient unbeknown to the observers: this would result in a nearly normal hemoglobin in the presence of infection, but this is certainly a rare occurrence in the isolated areas studied by us; another is that the limited iron supply is sufficient to lead to iron deficiency, resulting in a low hemoglobin without infection. We feel, however, that the lack of correlation in certain individual subjects between load and anemia has to be sought in the temporal divorce between the cause of iron loss and the resulting lowering of hemoglobin.

Unfortunately, we do not know for certain the duration of the cause of blood loss, namely the hookworm infection. Assuming, however, that it may remain steady for years, either because of the long life of the parasites, or because of repeated infections, we may visualize the natural history of the development of hookworm anemia, based on some of the data discussed in the book, somewhat as follows. Let us assume a hookworm-infected male, 60 kg adult campesino, who, hypothetically, would have initially 15 g circulating hemoglobin per 100 ml of blood (Fig. 21); the blood volume would be around 3570 ml and the total body iron 3000 mg, distributed as 1950 mg of hemoglobin iron, 900 mg of iron stores, and the rest as myoglobin, cytochrome, etc. Let us suppose, further, that this subject receives at one stroke and maintains an infection of 700 Necator americanus, leading to a daily blood loss of 21 ml and to an egg output of about 17,000 per g of feces, which would represent at the start 10.6 mg of iron lost into the intestinal tract, of which about 36% would be reabsorbed. The subject would absorb from his ordinary diet around 2.7 mg of iron, so that the net loss would be 4 mg of iron per day. During the initial stage (Fig. 21), the iron stores first become depleted at a constant rate, without change in the hemoglobin value. At the same time, less iron is lost for the same amount of blood loss. This second stage would last about 800 days, at the end of which the hemoglobin value would become stabilized, iron utilisation matching the losses. Strictly speaking, it is only at this point that correlation between load and anemia could be perfect. Before this, we would find all intermediary stages of hemoglobin values, with the same given infection.

Such considerations help understand why an adult male subject does not become anemic, as a rule, with less than 5000 eggs per g of feces. These subjects would be presumably losing less than 12.5 ml of blood per day, which would represent less than 4 mg of iron. This loss would probably be replaced, providing no other source of bleeding, such as menstruation, or increased demands, such as pregnancy and growth, are present.

The above story assumes that infections remain fairly constant in degree during periods of up to 600 days. At the other extreme, however, let us assume an infection which is short lasting, taking as example the extreme case Chandler mentions of an infection which is reduced to half in 90 days. Supposing this to be the case, our infection with 1285 Necator can be calculated to lead to a total blood loss of the order of 1000 ml (500 mg of iron)

18 In an example of the same nature given in a previous publication (Layrisse, Paz, Blumenfeld and Roche, 1961) the values chosen for our hypothetical patient were somewhat different, as we had not then the information we now possess, in particular on iron absorption from dietary food.
in 90 days and 1500 ml (750 mg) in 180 days. If such infection is single and occurs in a perfectly normal individual, it would probably lead to an increased intestinal iron absorption, without affecting hemoglobin values, which would still be detectable after the infection has actually vanished. In a patient which is already iron depleted, such as is the case in the majority of our rural subjects, a hemoglobin decrease would occur soon after infection, but would be maximal long after the infection has disappeared, and hemoglobin could remain abnormally low for about 200 days after eggs are no longer found in the feces (calculating an absorption of about 5 mg per day). We would have then a subject with low hemoglobin but without an active infection.

In view of the above considerations, it becomes almost surprising that a significant negative correlation is indeed so often found in hookworm infected communities, and the relationship becomes even more impressive as an indication of causal relationship.

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