Pathogenesis of Anaemia in Hookworm Disease

III -- Hematic and organic modifications, induced by mere elimination of Anchylostoma and Necator, in individuals presenting intense anaemia.

by

W. O. CRUZ

In all cases of parasitism the animal organism is depredated either by a direct and constant action of the causal agent (and in such cases the cure of the disease always coincides with the disappearance of the parasite), or by lesions which, being conveyed by these agents, no longer decrease after the elimination of the parasite.

In the pathogenic interpretation of the anemic syndrome in anchylostomiasis one of the chief verifications, without any doubt, consists in knowing, whether the existing morbid state is induced by the direct action of the live agent or still persists after the disappearance of the parasite.

In anchylostomiasis no detailed investigation has been made in order to verify which are the modifications gone through by the organism liberated from the parasite; cure being obtained after the administration of various medicaments, at times of a very remarkable number of them, no verification has been performed in order to establish which of the substances employed are really active, for nobody doubted that the elimination of numberless worms was per force the only reason of the cure.

Notwithstanding this, many authors already insisted on the fact that after a mere elimination of the helminth, blood regeneration is not perceivable, or is very slight and slow. However, a definite documentation of this statement was not yet sought for, the investigators restricting themselves to informations about this fact in a superficial manner. Kobayashi (1) was the only one who carefully verified the persistence of the blood state after the cure with vermifuges; but as his observations were made only in 3 cases and were accompanied for 2 to 5 weeks (a very short period for a demonstrative documentation), we endeavoured to verify the same fact over a longer period and in a larger number of cases.

The importance of this study for the elucidation of the mechanism of the formation of anemia is so evident that it is a surprising thing that it has not already been investigated in detail and with the care required for fundamental observations.
OBSERVATIONS

We omit the description of the methods employed and of several other details in the cases studied, because we already have given informations of same in a previous paper (2).

INTERPRETATIONS

The characteristic features of anchylostomotic anemia have already been published by us (2). The verifications in the present cases, as seen from Table 1, agree with the results previously described. However, we noticed a slight increase of Vol. and Hb. indices, which leads us to consider the following data as the correct average of the hematic indices of this anemia:

Average of 35 cases

<table>
<thead>
<tr>
<th>Ht. mmc.</th>
<th>Hb. %</th>
<th>Hemat. %</th>
<th>Ind. Vol.</th>
<th>Ind. Hb.</th>
<th>Ind. Sat.</th>
<th>Ret. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>23</td>
<td>14</td>
<td>56</td>
<td>13</td>
<td>23</td>
<td>2 to 3</td>
</tr>
</tbody>
</table>

The distribution of the anemia per age and sex also agrees with the results already presented. We notice its predilection for ages under 15 years and for males. Reticulosis in these cases was slight, oscillating around 2%.

In Table 2 there are summarized the results obtained in 9 cases. The general average very fairly shows the essential hematic modification.
<table>
<thead>
<tr>
<th>Cases</th>
<th>Prior to Treatment</th>
<th>Indices</th>
<th>Days of Treatment</th>
<th>Treatment</th>
<th>Indices</th>
<th>Vermifuges</th>
<th>Daily Hb Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.B.C</td>
<td>cum.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.73</td>
<td>12.6</td>
<td>93</td>
<td>3.81</td>
<td>15.5</td>
<td>7</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>2.09</td>
<td>12.9</td>
<td>68</td>
<td>2.37</td>
<td>13.1</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.87</td>
<td>12.9</td>
<td>8</td>
<td>3.71</td>
<td>12.9</td>
<td>1</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>2.95</td>
<td>13.1</td>
<td>10</td>
<td>5.62</td>
<td>18.4</td>
<td>11</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>2.07</td>
<td>15.5</td>
<td>5</td>
<td>5.00</td>
<td>16.2</td>
<td>7</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>2.34</td>
<td>15.4</td>
<td>6</td>
<td>5.07</td>
<td>13.6</td>
<td>1</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>2.83</td>
<td>16.3</td>
<td>8</td>
<td>5.50</td>
<td>15.8</td>
<td>2</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>3.53</td>
<td>14.9</td>
<td>38</td>
<td>4.15</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.18</td>
<td>18.4</td>
<td>26</td>
<td>3.24</td>
<td>13.6</td>
<td>3</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.62</td>
<td>59</td>
<td>4.27</td>
<td>14.7</td>
<td>6</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

Average: 27.5 59 14.7 26
<table>
<thead>
<tr>
<th>Cases</th>
<th>Chenopodium mixture</th>
<th>Tetra-chloride</th>
<th>Chenopodium</th>
<th>Thymol</th>
<th>Nalfor beta</th>
<th>Number of days after the administration of vermifuges</th>
<th>Number of adverse reactions observed</th>
<th>Examination of the faeces for eggs of the worms</th>
<th>Increase of the number of R. B. C. per cm.</th>
<th>Modifications of the indices of volume and Hb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>530</td>
<td>600,000</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>500,000</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>more 2 m.</td>
<td>N</td>
<td>more 180,000</td>
<td>350,000</td>
<td>Decrease</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>more 2 d. 3</td>
<td>24 hours</td>
<td>N</td>
<td>more 150,000</td>
<td>400,000</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>30</td>
<td>more 2d.</td>
<td>N</td>
<td>Decrease of 400,000</td>
<td>500,000</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>40</td>
<td>1 M.</td>
<td>400,000</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>2 m. 20 d. 10 d.</td>
<td>N</td>
<td>more 1,100</td>
<td>600,000</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>11</td>
<td>N</td>
<td>500,000</td>
<td>500,000</td>
<td>Pronounced decrease</td>
</tr>
</tbody>
</table>
1 This total increase of 550,000 in 3 days, without the elimination of any worm, confirming the repeated negative examination of the faeces, remained constant for 1 month.

2 The Hb. Ind., after lowering initially, was increased until the Sat. Ind. became normal. The same took place in Case 5.
It is to be seen that after a long observation period (5 months), the hematic indices are not at all modified. The absence of reticulocytosis or of other phenomena indicating blood regeneration agree perfectly with the persistence of the degenerative features of the red blood cell. Nevertheless, in spite of the absence of a real regeneration, an increase of 2.45 M. of these cells per cmm. is verified. Hb. rate, by virtue of this increase of cells, has also risen but in an extremely slow manner, the average of 0.15% per diem. The daily increase of Hb. in peripheral blood, even during periods of rapid increase of red cells, is very far from attaining the figures induced by iron administration (1.7% per day, during the 1st regenerative period).

In order to understand the phenomenon in its whole extent, a detailed study in every particular case is required, which we pass on to show in Table 3. The interpretation of the data seen in Tables 2 and 3 does not differ from that published by us in a previous paper, in the cases in which vermifuges were administered, together with martial treatment, already during the period of regeneration, i.e. during that period when the blood aspect is stationary or very slowly modified. The general interpretation of this phenomenon may be summarized as follows:

1) The action of vermifuges upon blood is inconstant and may even fail.

2) When this action is noticeable, it proves to be independent of the observation period, of the number of eliminated worms or even of their presence (Case 3). It is not specific of a determined vermifuge, for this phenomenon was observed employing tetrachloride and thymol; with regard to chenopodium and beta naphtol, if any action exists, it is certainly much weaker than that of the aforesaid substances.

3) The blood modifications are essentially characterized by a numeric increase of red cells (see average result of Table 2). Vol. and Hb. indices initially undergo a decrease, at times pronounced, but always transient. Afterwards, an increase of Hb. Ind. is observed which leads the existing microcytes to saturation, if we prolong the period of observation sufficiently (Cases 5 and 6), for the Vol. Ind. remains stationary.

4) The numeric increase of red cells should not be interpreted as a true blood regeneration, for it is always accompanied by lack of reticulocytosis, by persistence of degenerated hematic indices and mainly by an increasing degeneration of the existing red cells, brought into evidence by the method of globular resistance (remarkable increase of the resistance).

5) The verified action seems to depend upon absorption of the medicament, so great is the rapidity which was observed in some cases, going even so far as to be verified 24 hours after administration of the vermicide (Case 3).
As quoted above, the increase of red cells and of Hb. rate must be considered as a degenerative phenomenon rather than a regenerative one. In Cases 5 and 6, the resistance of red cells against sodiochloric solutions showed a great increase of resistance after administration of vermilnuges. Thus, in Case 5, 35% of Hb. was diffused in a 3.6°/oo solution and 35% in a 2.8°/oo solution, the maximum resistance being met with in a 1.8°/oo solution. This result, which already demonstrates a great increase of resistance (vermilnuges having already been administered), was modified 2 months later, so as to denote a still greater resistance (48% were hemolyzed in a 2.8°/oo solution, the maximum resistance being met with in 1.2°/oo). In Case 6, 20% were hemolyzed in 3.6°/oo, 30% in 2.8°/oo, the maximum resistance being met with in 1.6°/oo. After another 3 months of observation it was verified that 50% were hemolyzed in 2.8°/oo, the maximum resistance being equal to 1°/oo.

The clinical aspect of these patients and the subjective disturbances complained of by them are facts interesting to be considered. It is verified that the increase of Hb. rate conveys an insignificant improvement in these individuals, who appear less dejected, maintaining however the citrine yellow colour commonly found in this disease. An important fact must be mentioned here: presenting the same Hb. rate, to exemplify say 50% of Hb., the aspect of the patients may be entirely different: thus, during iron treatment when the increasing blood figures pass this level (50% of Hb.), all main symptoms of the disease disappear or are very much attenuated, whereas on the contrary after administration of vermilnuges this Hb. rate merely presents an insignificant improvement, at times even totally absent, and the patient maintains the common aspect observed in advanced cases of anemia. Now, after the blood figures having reached 70 or 80% of Hb. thanks to martial treatment, if we then suspend this treatment and if patient is placed on a deficient diet, a blood regression is always observed, and then one verifies that on attaining 50% of Hb. rate the majority of the symptoms are already present to a rather pronounced degree. Thus, generally speaking, we may state that the intensity of the symptoms depends upon the Hb. rate (an anemia of 70%, whatever the condition of the patient may be, is always much less severe than an anemia of 30%), yet for an identical degree of anemia (according to the Hb. rate) we must take into consideration the state of martial regeneration, the pseudo-regeneration by virtue of vermilnuges and the degeneration induced by the helminth associated with the deficient diet. As already stated, the quality of the red cells is the main factor which determines the severity of the symptoms, the various facts described above being thus accounted for.

The morphologic aspect of the blood also demonstrates the absence
of real regenerative phenomena. Reticulosclerosis has never been observed, only 2 to 3% of these cells being seen, just as is verified in cases without treatment. Anisocytosis, poikilocytosis and intense oligochromemia persist during the whole period of cure by vermilicides. Moreover, there is observed absence of polychromatophilia, normoblasts and red cells with nuclear remainders.

**Blood regeneration induced by iron administration, after the previous application of vermicides:**

In Table 4 the results obtained in 7 cases are to be seen. In 2 (Cases 2 and 3), regeneration proceeded in a common manner, in the other cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>PRIOR TO TREATMENT</th>
<th>INDICES</th>
<th>Days of treatment</th>
<th>AFTER THE TREATMENT</th>
<th>INDICES</th>
<th>Maximum reticulosis o/o</th>
<th>Daily Hb. increase o/o</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>3.81</td>
<td>43</td>
<td>56</td>
<td>12.8</td>
<td>23</td>
<td>28</td>
<td>5.41</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>2.37</td>
<td>23</td>
<td>62</td>
<td>18.2</td>
<td>29</td>
<td>41</td>
<td>3.92</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>3.75</td>
<td>35</td>
<td>64</td>
<td>18.6</td>
<td>29</td>
<td>66</td>
<td>5.58</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5.45</td>
<td>72</td>
<td>62</td>
<td>18.2</td>
<td>29</td>
<td>61</td>
<td>4.95</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4.88</td>
<td>66</td>
<td>64</td>
<td>18.6</td>
<td>29</td>
<td>50</td>
<td>5.23</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>5.07</td>
<td>50</td>
<td>65</td>
<td>15.8</td>
<td>24</td>
<td>39</td>
<td>5.55</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>5.50</td>
<td>63</td>
<td>65</td>
<td>15.8</td>
<td>24</td>
<td>35</td>
<td>4.95</td>
</tr>
<tr>
<td>Average</td>
<td>4.40</td>
<td>50</td>
<td>60</td>
<td>15.4</td>
<td>26</td>
<td>41</td>
<td>5.15</td>
<td>85</td>
</tr>
</tbody>
</table>

The course of regeneration was identical with that observed in the cases reported by us in a previous paper and which, prior to treatment, presented, we obtained no complete normalization of these indices, by virtue of the long space of time required, and we terminated our observations when the Hb. Ind. reached 21 to 23 yy. As always observed in this regeneration type, reticulosclerosis and the daily increase of Hb. maintained in all cases an appreciably low level.

The graphic 2 shows clearly the aspect of the hematic curves during the period of vermicides administration and during the subsequent marcial treatment (the commencement of which is marked by a arrow).

In Case 7, we experimented the administration of a diet prepared with common salt mixed with ammoniacal ferrous sulphate (10%). After 24 days of treatment we verified an effect which by no means is to be compared to that induced by the same dose of iron administered orally, in ca-
psules. Nevertheless, considering the maximum reticulosis observed (17% on the 13th day of treatment), the increase of 14% of the Hb. rate as well as the slight improvement presented by the patient, we must interpret this result as encouraging new investigations.

In Case 3, we verified the minimum efficient dose of reduced iron for adults. The doses of 16 to 240 mgrs. per diem appeared to be inefficient, whilst the daily dose of 0.5 gr. induced an intense blood regeneration.

Though unnecessary by virtue of the constancy and intensity of blood regeneration induced by iron, we investigated (Case 2) a possible acceleration of the process by simultaneous administration of hydrochloric acid and iron. We verified no auxiliary action of this acid.

Anemiant process

In some cases, we endeavoured to verify what is the influence of various factors, mainly of dietary factors, upon the course of the anemia.

In Case 1, after administration of vermifuges, we kept the patient on a regime rich in iron for 1 month. The result observed (increase of Hb. Ind. and of the rate of this pigment) is most likely to be interpreted as a distant action of the vermifuges and not as an immediate action of the diet. The fact observed in this case was also observed in Cases 5 and 6, in which an increase of Hb. Ind. up to saturation of the red cells was seen, i.e. an increase of the Hb. Ind. along with the persistence of the small volume of the red cell.

In Case 2, after complete elimination of the helminthes, the aspect of a typical beriberi polyneuritis appeared. The administration of a diet rich in Vit. B improved and, afterwards, cured this disease. During 4 months no blood disturbance was observed, this case serving as a counter proof of those cases in which an anemiant process was verified due to the persistence of helminthic parasites.

In Case 3, immediately after the admission of the patient, we verified in 10 days a decrease of 10% in the Hb. rate. For a long time (4 months) the blood aspect remained stationary, despite the administration of small doses of reduced iron, which proved to be inefficient with regard to regeneration. After an efficient dose, Hb. reached 59%; afterwards, suspending martial medication and administering a diet poor in iron, blood regression proceeded very slowly, only returning to the figures prior to treatment after 8 months and 20 days. At the beginning, this regression did not take place, the persistence of the blood aspect being verified during 2 months and 10 days, after which the hematic figures were lowering progressively, along with a more intense degeneration of the Hb. Ind.
In Case 4, the administration of a diet poor in iron during 1 month did not modify the blood aspect, and a diet rich in Vit. B during 1 month and 10 days produced a slight alteration in the number of red cells.

In Case 7, on common diet, we verified that the blood aspect, after a slight regeneration induced by a brief iron treatment (Hb. rate from 22 to 36%), returned after 1 month to its former state.

We may summarize these results emphasizing their main points as follows: a) Some cases present a constancy of their blood aspect during a rather long period (2 to 3 months) independent of any treatment; b) An action of a diet rich in iron (Case 1) or in Vit. B (Case 4) is not proved in a positive manner; c) The blood regression is the more rapid the lower the Hb. rate, and at high rates the regression to the state prior to martial treatment may be extraordinarily slow (nearly 9 months in Case 3). The hematocrit index which was the most affected in the regressive process was, in these cases, the Hb. Ind.

Weber's blood test in the faeces gave negative results in Cases 5 and 6, in spite of the presence of predatory helminthes.

In Case 4, we verified the elimination of uric acid through urine during 25 days, without administering any medicament. The result was the daily average of 0.27 gr. The further observation of this patient was impossible, therefore we indicate only this separate result.

From the clinical viewpoint, the investigations here presented render conspicuous one of the symptoms of the disease — the coloration of the skin. As stated above, some symptoms decrease in intensity after the rise of Hb. rate induced by administration of vermifuges. Yet, the same does not occur with the coloration of the skin. During iron administration we observed a rapid recoloration of the tegument, when Hb. rate reached 40 to 50%; consequently, this symptom seems to depend upon the degenerated aspect of the circulating blood, and not upon the quantity of Hb., i.e. the yellow citrine coloration observed in severe cases of anemia seems to be due to hypochromia of the circulating red cells; by the way, we already mentioned above that this degeneration of red cells seems to determine most of the symptoms of the disease.

DISCUSSION

Some results of the present work are mere confirmations of facts already interpreted and discussed in a previous paper, in which we exposed the results of a study on the regenerative and degenerative phenomena of hookworm anemia (2). We rapidly recapitulate these confirmations as follows:
The characteristic features of the anemia in its extreme stages appeared the same as those previously described. The course of the anemia observed in some cases also presented no fact different from those previously indicated. Thus we verified: the decrease of the hematic figures occurs the more easily the lower the number of red cells; the fundamental alteration is verified in the decrease of the hematic indices, mainly of the Hb. Ind.; at advanced stages of the anemia a stationary blood aspect may be observed for a long period; diets rich in iron or Vit. B do not prevent the decrease of the hematic figures when regression begins at a level under normal. We moreover confirmed that the blood test in the faeces may be negative, despite the existence of numerous helminthes in intestine.

The hematic regeneration obtained by iron administration, after complete elimination of helminthic parasites, presented the same general features as the hematic regeneration induced by the same substance in the presence of numerous helminthes in human intestine. In the majority of the cases, the number of red cells prior to mural treatment was high; the regeneration curves then behaved as in the cases we observed previously and in which the number of red cells per cmm. was also high. Reticulosis was always small and the ascent of the hematic indices was, at times, rather slow.

In some cases, at the end of the treatment we observed the same number of red cells as at the beginning, in which case only a qualitative regeneration of red cells occurred. This fact also demonstrates the existence of a hematic destruction concomitant with a regenerative neo-formation. We already stated that the regeneration obtained by some authors by means of a preliminary elimination of the helminthes presents the same general aspect as that we obtained independent of this elimination. The results here presented may severe as a counter proof, as the regeneration appeared to be similar to those observed formerly, which goes to prove still in another way that the helminthic infestation plays no rôle in the phenomena of blood regeneration induced by iron administration.

With regard to the minimum dosage, we succeeded in obtaining good results with 0.5 gr. of reduced iron in an adult. The administration of a diet prepared with common salt mixed with ammoniacal ferrous sulphate induced a partial regeneration of blood. Although this trial does not show a definite result, the maximum reticulosis observed (17%) and the improvement of the patient induce us to believe in the possibility of the use of this method, when conveniently employed, in the prophylaxis of the disease.
Aspects verified in the blood image after administration of vermifuges.

As a matter of fact, the phenomenon we will begin our discussion with has nothing to do with the anemia under study or with the direct activity of helminthic parasites, as we verified that the blood modifications occurring after administration of vermifuges are not related to the helminthic disinfestation, for it is a mere result of the assimilation of the vermicidal substances and of their further action on the economy of the diseased organism. The simple increase of red cells has also been observed in cases in which iron had been administered prior to the vermifugal medication. Here we will connect certain facts with one another and also with the fact here referred to; and for this purpose, we must begin describing some verifications not related to anchylostomiasis.

Formerly, the only organs considered to be important in the metabolism of the hematic elements belonging to the red series were bone marrow and spleen. After Whipple’s studies, the interference and even the direct relationship of other organs, such as the kidneys and mainly the liver, are demonstrated in the process of blood formation and destruction. This latter organ not merely interferes with blood metabolism through the function of cells of the reticulo-endothelial system (Kupffer’s cells) in the formation of gall, but also through the elaboration and storing up of many of hemocyto-forming substances or factors. The importance of this organ for blood metabolism is brought into evidence by a number of experiments: the total removal of liver from frogs induce in them an anemia resembling human pernicious anemia; in pernicious anemia or in experimental anemiae induced by hemorrhages, the administration of liver suffices to induce a quick and complete blood normalization.

Some connexions have already been pointed out between hepatic changes and the metabolism of some substances which are essential for blood formation. Raposo (3), in a paper on the reticulo-endothelial system, states:

«With regard to liver, there is a very significant experiment by Eppinger: if the intravenous injection of iron is preceded by an injection of alcohol into the portal vein and if the hepatic epithelial cells are changed by virtue of this process or through a slight phosphorus poisoning, iron accumulates not only in Kupffer’s cells but equally in glandular cells. That is, these elements fix no large quantities of iron unless they be changed and after fixation of same by Kupffer’s cells».

With regard to the metabolism of bile pigment, the same author states:
From the Kupffer's cells bile pigment would pass to the hepatic cells proper which would eliminate it through the biliary canaliculi. With regard to bile pigment there would take place something similar to what we referred to in Eppinger's experiments: appearance of iron in Kupffer's cells first, and only subsequently in the glandular cells.

These facts and several others more thoroughly demonstrate the direct participation of the hepatic functions in hematic metabolism.

Here we only wish to focalize certain hepatic lesions induced by some substances and the modifications of the hematic aspect existing in these cases.

**Intoxication by chloroform:**

A great number of authors (Howland, Muskens, Wells (4), Whipple and Sperry (5), Stiles and Mc Donald, Ostertag, etc.) verified that chloroform, beyond a certain dose, is a violent poison, mainly for the hepatic cells. Both in man and in numerous other mammals (rabbit, guinea-pig, rat, cat and dog) or even in fowl (pigeon) this substance induces a hepatic lesion of more or less constant morphological features. A central hepatic necrosis is concerned, i.e. a necrosis of hepatic cells which are around the central venula of the lobule. Such necrosis, in some cases, comprises almost all lobular cells, for, with the exception of one or two layers of these cells around the spaces of the portal vein, all the others appear necrotic. Other changes, such as peritoneal ecchymoses and hemorrhages and fatty degeneration of the pancreas, kidneys and heart are met with in a moderate degree. Even in every slight anaesthesia these hepatic lesions are verified to a reduced extent, which lesions disappear some days afterwards by virtue of an active myotic regeneration of the noble cells of the parenchyma, which thus induces a complete recomposition of the organ.

**Intoxication by phosphorus:**

The chief morphological alteration is observed in the liver in which fatty degeneration is found on the periphery of the lobules. The general aspect of this organ resembles that verified in acute yellow atrophy, yet differing from same by more voluminous fat drops. Moderate fatty degeneration is also observed in pancreas, heart, intestinal glands and musculature, vessels and muscles of the trunk. In cases of acute human intoxication death occurs on the very first days; cases which resist up to the 10th or 14th day are extremely rare.

**Intoxication by carbon tetrachloride:**

Intoxications by this substance have been verified in man and other mammals. A detailed study was made in dogs by Gardner and collaborators (16) who verified hepatic changes even 12 hours after the administration
of the drug. These changes resemble those observed in chloroform intoxication, i.e. they consist of a central necrosis of lobular cells. These authors verified, moreover, that other changes, apart from the hepatic, may appear in an inconstant manner, and also that the hepatic regeneration beginning 3 or 4 days after the administration is not yet completed after 5 weeks time. Meyer and Pessôa (7) verified that the severity of the lesions varies in inverse proportion to the age of the animal, and that the acting mechanism of the substance is the same as that of chloroform.

The studies of Minot and Cutler (8) and, later on, those of Minot (9) came to elucidate the intimate mechanism of the intoxication. These authors verified that the hepatic change, owing to a hypofunction of this organ, would induce a disorder in another organ (presumably parathyroid) which controls the metabolism of guanidin. This disorder would occasion a lesser elimination of this substance, giving rise to hyperguanidinemia thus induced or an acute intoxication by guanidin is brought into evidence by a larger concentration in blood of lactic acid and subsequent exaggerated elimination through urine. This loss of lactic acid is a serious drainage of carbohydrates which induces acute hypoglycemia followed by death. These authors also verified that calcium administration, preventing accumulation of lactic acid in blood and quickening glycogenolysis in liver thus impeding hypoglycemia, prevents death of the intoxicated animals.

**Intoxication by thymol:**

The studies on intoxication by this substance are neither numerous nor detailed. Ostwald (10) gives the following summarized description of the pathologo-anatomical aspect:

«Innerlich in kleinen Gaben laengere Zeit gerecht, bewirkt es bei Kaninchen, gleich dem Benzol und Phenol, Leberverfettung und Nierenlæsonen» (Orally administered in small doses for some time, it occasions in rabbit, like benzol and phenol, fatty degeneration of liver and changes in kidneys).

Thus we see that the pathologo-anatomical aspect of this intoxication is also analogous to those above described concerning other poisons.

Apart from these substances focalized in particular, a number of others exerts an action, the mechanism of which is quite comparable to the above described. With regard to acute hepatic necrosis induced by chloroform and carbon tetrachloride Wells (11) states:

«In the acute degeneration of the liver caused by chloroform, carbon tetrachloride and other halogen derivatives of the aliphatic hydrocarbons
we find autolysis or solution of the structural elements of the liver, blood and urine. *These halogen poisons are characterized by their specific effect on the liver*, the other organs of the body being practically unaffected by them. Moreover, within the liver itself, only the hepatic cells proper are injured, the epithelium of the bile ducts and the stroma cells being little if at all affected. The action of chloroform, carbon tetrachloride and other halogens on the liver is produced by the hydrochloric acid formed within the liver cells. Such poisons are specially likely to attack fatty livers, presumably because the fat-soluble poisons accumulate in greater quantity in such livers, these poisons act by interfering with the oxidation enzymes of the liver cells, leaving the autolytic enzymes and the lipase free to digest the liver cells and to form fat. Any poison which causes severe injury of the liver cells without at the same time destroying the autolytic enzymes so that the liver cells die and undergo rapid autolysis, may produce a condition similar to acute yellow atrophy of the liver. Amongst such poisons may be mentioned phosphorus, arsenic (salvarsan), the nitrophenols (picric acid), trinitrotoluene, phenylcinechominic acid (atophan), dinitro-bezene and other aromatic compounds containing the benzene ring.

From this we infer that a group of numerous substances, when administered in determined doses, has the common property of inducing in man an acute specific change of the noble cells of the liver. This change morphologically may appear as a necrosis or an intense fatty degeneration, the essential feature of the change being not only the specific affinity to the hepatic cell but also its acuteness. Allied to this group of substances there exist morbid entities the pathologo-anatomical aspect of which resembles very much this one already described. We mean acute yellow atrophy of the liver, a disease of varying etiology, and yellow fever. The latter disease has often been confounded with cases of phosphorus intoxication, as may be learned from Cazanove and Robert's (12), and Strathairn's (13) papers. The hepatic change is one of the most salient changes in yellow fever, and a good deal of the symptomatology of this disease must be imputed merely to a hepatic hypofunction. The importance of this lesion is prominent in the pathologo-anatomical aspect of this disease; after Torres work (14) who described intranuclear inclusions in the hepatic cells even at the beginning of the disease, it was verified that one of the earliest changes apart from nephritis (clinically observed on the first days of the disease) is exactly acute hepatitis.

Thus far we focalized these various intoxications and diseases insisting on the specific nature of the change of the hepatic cell in order to connect this fact with a hematological verification of fairly well fixed features, also described in the various conditions referred to.
Even in former treatises on hematology there are references to hyperglobulia in intoxication by various substances. In Besançon and Labbé's treatise (15) of 1904, there is the following passage:

**Intoxication phosphorée.**—C'est sans doute en concentrant le sang par le moyen des vomissements abondants que l'intoxication phosphorée produit une hyperglobulie; en effet, l'hyperglobulie n'apparait que quand les vomissements se produisent. On a trouvé dans ces cas 6.800.000 (Badt), 5.150.000 (Grawitz) globules rouges, et même plus encore (Taussig, V. Jaksch, Limbeck) (Phosphorus intoxication.—There is no doubt that, by concentrating blood by means of abundant vomiting, phosphorus intoxication produces a hyperglobulia; indeed, hyperglobulia does not appear unless vomitings occur. In such cases 6.800.000 (Badt), 5.150.000 (Grawitz) red cells and even more (Taussig, V. Jaksch, Limbeck) have been found).

Rieu (16) refers to secondary hyperglobuliae connected with blood intoxication by carbonic oxide, phosphorus and benzol.

Already Ferrata (17) places hyperglobulia induced by phosphorus intoxication in the group of hyperglobuliae due to exaggerated blood neoformation, on the basis of Auberti's works who verified hyperplasia of marrow and myeloid transformation of spleen in rabbits intoxicated by this substance. He also quotes the hyperglobuliae induced by arsenic, carbonic oxide, hematic and medullary opotherapy.

In cases of prolonged chloroformic anaesthesia of dogs we also observed accentuated hyperglobulia, one or two days after the administration, or even a few hours after the beginning of the anaesthesia.

Studying the mechanism of hyperglobulia induced by cobalt and manganese, Underhill and collaborators (18) verified the following hematic readings:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmc.</td>
<td>grs.</td>
<td>%</td>
<td>uc.</td>
<td>yy</td>
<td>%</td>
</tr>
<tr>
<td>Normal rats</td>
<td>8.3</td>
<td>14.3</td>
<td>49</td>
<td>60</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>Rats (cobalt)</td>
<td>12</td>
<td>21.5</td>
<td>75</td>
<td>61</td>
<td>16.8</td>
<td>29</td>
</tr>
<tr>
<td>Rats (cobalt and manganese)</td>
<td>10</td>
<td>17</td>
<td>61</td>
<td>60</td>
<td>17</td>
<td>29</td>
</tr>
</tbody>
</table>

From this the conclusion may be drawn that the increase of red cells, just as we verified in anchylostomiasis after administration of tetra-chloride and thymol, is merely quantitative, there being no modification of the indices of volume, hemoglobin and saturation. Apart from this, these authors (19) verified that the hyperglobulia is not a result of blood concentration, but a true increase of red cells. In performing determinations con-
cerning the total volume of blood, they observed that the total quantity of plasma slightly varies, whilst the quantity of red cells increases very considerably. Consequently, not a permanency of the number of red cells along with a diminution of plasma quantity is verified but the contrary; i.e. a quantitative permanence of plasma along with a numeric increase of red cells.

According to Mascherpa (20), intoxication by cobalt produces severe renal changes. We have no knowledge of the existence of a concomitant hepatic lesion; nevertheless, a mere renal lesion is also liable to be interpreted by a relationship between kidney and blood metabolism, this so maintaining accordance with Whipple's classical experiments who verified a positive action of a diet constituted by kidney, besides the well known efficiency of liver diet.

In yellow fever, Sodré and Couto (21) verified a numeric increase of red cells, at times of great intensity. These authors interpreted the fact by a diminution of the physiological destruction of red cells accompanied by a habitual neo-formation. Lobo (22) confirms this fact giving it the same interpretation, and moreover verifying in one case that the increase of red cells may not appear on the first days of the disease, but supervene during the following days.

The said hyperglobulinae are always verified in cases of intoxication, whilst the numeric increase of red cells we observed after administration of carbon tetrachloride and thymol takes place after the administration of a medical dose, i.e. seemingly innocuous for the human organism. Nevertheless according to the aforesaid experiments on intoxication in dogs by this substance, even after the administration of a small dose, a moderate necrosis may appear followed soon afterwards by a prompt regeneration of the hepatic cells. Consequently, it is admissible to think that even the administration of medical doses of these vermifuges always induces a moderate and transient hepatic lesion, which however, just as occurs in cases of intoxication, has a repercussion on blood brought into evidence through a numeric increase of red cells.

All the facts here described evidently have many points of contact and even seem to constitute a peculiar phenomenon which we will summarize in general lines as follows:

Some substances (chloroform, carbon tetrachloride, thymol, benzol, arsenic, cobalt, phosphorus, etc.) when administered in large doses or even in medical doses, induce a numeric increase of red cells (without modifying their qualitative features) and changes mainly of the liver (of the kidney by cobalt), inducing one to think of a direct or indirect rela-
tionship of the hepatic cells (perhaps also of the renal cells) and blood control.

What can the mechanism of this controlling action of the liver be? Evidently, in order to elucidate this, it will be opportune to clear up at first what the mechanism of hyperglobulia is.

Three hypotheses have been ventured by several of the above mentioned authors:

a) Excessive blood regeneration;
b) Mere blood concentration due to plasma extravasation from the circulation outward;
c) Decrease in the physiological destruction of red cells and, in spite of same, persistence of the habitual neo-formation.

In the quotations above, we could see that these three hypotheses have been equally sustained by various authors. Our own verifications permit no definite conclusion; however, they indicate in a fairly clear manner the insufficiency of one of these hypotheses.

In our examinations, there never appeared the signs met with in true blood regeneration. Thus, we never verified either an appreciable increase of reticulocytes or such an aspect of our slides which would denote neo-formation (polychromatophilia, red cells with nuclear remainders, etc.). On the other hand, the hematic indices either remain unaltered for long months, or present a really insignificant increase. The clinical aspect of the patient is entirely different from that observed in true hematic regeneration induced by iron; the symptoms become simply attenuated, in an almost imperceptible manner, whilst in martial regeneration they disappear entirely after 1 or 2 months of treatment. Another additional fact which indicates a pseudo-regeneration in this hyperglobulia is the rapidity of this increase of red cells verified in some cases; thus, we observed 24 hours after tetrachloride administration a numeric increase of red cells of such intensity, not comparable even to the most violent martial regenerations in hypochromic anemiae, or to those verified after liver administration in pernicious anemia.

Also, the dependency of the increase of red cells upon the administration of verminfuges tells in disfavour of a true exaggerated neo-formation on the part of bone marrow, for we verified that after administration of a verminfuge the number of red cells rises to a determined level where it remains stationary; then another dose is necessary in order to observe a new rise, followed by a new stationing on this higher level. Now, this aspect has nothing to do with that observed in true hematic regenerations,
in which the regeneration, unless it be constantly supported by the administra-
tion of its inducing factor, remains stationary and, soon afterwards, quickly returns to the primary hematic state.

With regard to the hypothesis concerning the decrease of globular destruction, it is at variance with some phenomena which show a strict interrelationship between blood destruction and its formation, denoting that the latter depends upon the former; nevertheless, this does not mean that in this particular case of an acute hepatic lesion this interrelationship is undone. In order to elucidate the value of this idea or of the other which gives credence to a mere blood concentration, more detailed investigations are wanted, mainly such as will verify the total blood volume in animals intoxicated by these drugs.

Consequently, if even the hematic mechanism of this phenomenon is not known yet, much less will be the question concerning the liver action in blood control. We will recall that this action may be a direct property of the hepatic cells or an indirect one by means of a secretory function of these cells, acting in another organ. We already quoted above the remark made by Minot, recollecting the action of the parathyroids in the mechanism of guanidineemia, observed in the intoxication by carbon tetrachloride. We also wish to focalize the possible action of the hypophysis in this phenomenon, for Moelig and Bates (23), in cases of polycythemia, verified hypophysary changes which they interpreted as the causal factor of the phenomenon.

As one may see, the subject here dealt with is entirely unknown in its essence, and its elucidation, in all likelihood, contributes to render clearer the phenomena concerning blood metabolism.

From the therapeutical viewpoint, i. e. regarding the patient's improvement, the increase of red cells verified after administration of vermiluges is of secondary importance. We already stated above that the symptoms of the disease decrease in an almost imperceptible manner after the increase of degenerated red cells, the contrary occurring when the increase arises from the launch into circulation of normal red cells. In combination with iron treatment, or better, after a previous intense martial medication, the administration of tetrachloride is advisable, owing to the concomitant action of disinfestation and hyperglobulization. It must be borne in mind that this increase of red cells must be interpreted rather as a degeneration than as a regeneration of blood, and as it is a phenomenon which has been little studied, it is quite possible that its action on the organism conveys unapparent malign consequences to other organs of the economy.
The chief fact here observed is not important for the elucidation of the pathogenesis of the anemia, as it is a trivial case of a slight intoxication by poisonous substances. Nonetheless, a fundamental fact to be taken into consideration in these pathogenic theories is the verification of the absence of hematic modifications merely imputable to the elimination of helminthic parasites. It is, however, of no use again to insist upon it here, for we already referred amply in previous papers to its significance in the pathogenesis of the anemia.

**SUMMARY**

We studied, from the hematological viewpoint, 10 straightforward cases of anchylostomiasis for a long time. One of the patients was under observation for more than 2 years, this ample observation being justified by the reliability at which we aimed that the mere elimination of helminthes conveys no hematic modification, not even a remote one.

The hematological study consisted of 200 routine examinations (determination of the number of red cells, of Hb. rate, hematokrit, and in many a case of the rate of reticulocytosis and examination of slides), practised on all our patients, and of determinations of globular resistance and other hematological investigations practised in some of our cases.

The greater part of our study consisted in a careful observation of the blood and of the clinical symptoms of our cases, prior to and after the administration of vermifuges until complete elimination of the intestinal parasites. By means of various diets we also sought to verify the influence on the hematic aspect of the said elimination combined with the effect of different nutritive factors contained in these diets. We endeavoured to verify the regenerative phenomena after iron administration and we also had the opportunity of observing the course of the anemia and of confirming thus the results already published.

The results showed the importance of the absorption of the vermicidal medicaments employed and therefore we focalized several similar facts endeavouring to connect them with the fact here verified.

We then discussed the therapeutical method in the light of the results presented, and we referred to the importance of same for the correct elucidation of the pathogenesis of the disease.

**CONCLUSIONS**

1) On observing some hookworm patients during a long period, after administration of some vermifuges (carbon tetrachloride and thymol), we verified remarkable modifications in the blood
aspect, side by side with insignificant improvements of the symptoms of the disease.

2) These hematic modifications are essentially constituted by a mere numeric increase of red cells, i.e. an increase which is not accompanied by qualitative modifications of these cells. We observed a complete absence of signs of blood regeneration, such as increase of the rate of reticulocytes, intense polychromatophilia, red cells with nuclear remainders etc. We verified that this increase of red cells is accompanied by a remarkable increase of resistance of the red cells against hypotonic salt solutions, which indicates a larger qualitative blood degeneration, despite the globular numeric increase observed.

3) We verified that the elimination of helminthes has nothing to do with the blood modifications described above, but that such modifications are in direct relationship to the substances employed as vermifuges, after their assimilation by the intestine.

4) The action here verified after administration of carbon tetrachloride and thymol is analogous to that of some other substances (chloroform, phosphorus, benzol, etc.) which have in common the property of inducing an acute specific lesion of the hepatic cells. Considerations on this phenomenon induce the belief in a possible rôle of the liver in blood control, apart from the actions, already known, of this organ on blood metabolism.

5) Iron administration after complete elimination of helminthes induced a blood regeneration, the general aspect of which is comparable to that already verified by us independently from the said elimination.

6) The absence of blood modifications ascribable to elimination of the intestinal parasites and the resemblance of the blood regenerations induced by iron both in the presence of helminthes in the intestine and after previous elimination of these parasites are very important verifications for the elucidation of the pathogenesis of the disease, and tend to confirm the essential importance of an organic insufficiency (iron deficiency) in the determination of the anemic syndrome and of the disease.