FURTHER STUDIES ON THE BLOOD AND THE HEMATOPOIETIC
TISSUES IN MALIGNANT PANLEUCOPENIA OF CATS

BY WILLIAM D. HAMMON,* M.D., AND JOHN F. ENDERS, PH.D.

(From the Department of Bacteriology and Immunology, The Harvard Medical School, Boston)

PLATES 41 AND 42

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In a recent report (1) we described a highly fatal epizootic disease of cats which, we demonstrated, is caused by a filterable agent. The usual clinical course is characterized by a fulminating leucopenia associated with 1 or 2 days of manifest illness during which vomiting, moderate elevation of temperature and progressive anorexia and weakness are almost constant features. A large series of autopsies with tissue sections showed the constant, outstanding pathological changes restricted to the bone marrow, lymphoid tissues and intestines. In these affected organs eosinophilic intranuclear inclusion bodies were found to be a characteristic feature. We have more recently, through the observation and autopsy of approximately 50 more kittens suffering from this infection, noted several pathological changes, not previously described, related to the blood and the hematopoietic tissues. The description of these which forms the matter of this report is of value, we believe, in the interpretation of the causes underlying the observed quantitative abnormalities of the cellular elements of the blood.

Effects on the Leucocytes and Leucopoietic Tissues

Prior to any manifest symptoms of illness and frequently before a rise in temperature, a fall in the leucocyte count occurs which, as previously described, may represent a decrease of the lymphocytes, the polymorphonuclear cells or both. After studying this larger series of animals it has become more obvious that during the 2nd to the 4th day after inoculation there is in most cases a more conspicuous decrease in the number of lympho-

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cytes than in the number of the polymorphonuclear cells. This is, however, usually of only short duration and may easily go unnoticed if daily counts are not made. Chart 1 shows such a course in one kitten which died and in another which recovered.

Studies made by us on the blood of normal kittens (2) show that under ordinary circumstances the number of lymphocytes in the circulation remains relatively constant, despite wide physiological fluctuations in the total leucocyte count, which are due almost entirely to changes in the number of circulating polymorphonuclear cells. Such marked reduction of the lymphocytes as occurs during the incubation period of this disease becomes more significant when considered in respect to this otherwise normal constancy.

Confirmation of this observation regarding an early effect on the lymphocytic system was obtained in the study of tissue sections from 3 kittens killed during the incubation period, prior to the onset of any symptoms.

Cat 1-80 killed when the total leucocyte count had fallen to 9550, showed no significant changes in the bone marrow or in any other of the tissues which we examine as routine, except the lymphoid tissue. The large ileoceleal lymph node (Fig. 2) revealed an early lesion with signs of both stimulation and degeneration of the germinal centers, and in many follicles only a narrow, peripheral ring of small lymphocytes presented a normal appearance (cf. Fig. 1, a normal follicle). An unusually large number of immature mononuclear germinal cells containing typical intranuclear inclusion bodies were found in these affected follicles (Fig. 5A). In cats 1-84 and 1-85 killed at a slightly more advanced stage with leucocyte counts of 5200 and 7600 respectively,
but without symptoms or temperature elevation, the lymph nodes revealed more advanced changes, the follicles being now hypoplastic, edematous and necrotic. The changes noted were almost as extensive as those seen during the terminal stage of the disease (Figs. 3 and 4). In addition the bone marrow presented a most unusual picture. Instead of the marked aplasia of all the blood-forming cells with the exception usually of the megakaryocytes, hyperemia, and the presence of many degenerating cells seen characteristically later in the disease, these marrows under low power appeared quite normal (Fig. 8; cf. Fig. 7, normal bone marrow). However, on examination under higher magnification (Figs. 5B, 6A and 6B) it was noted that a number of the primitive and immature cells of both the granulocytic and erythrocytic series contained well defined eosinophilic intranuclear inclusion bodies. A few granulocytes, however, which had differentiated to the extent of possessing simple lobulated nuclei, both in the neutrophilic and eosinophilic series, contained inclusion bodies but most of the inclusion bodies were seen in the more primitive cells.

From these observations relative to the blood and the hematopoietic tissues it would appear that we are justified in calling this disease a panleucopenia or,—as far as the hematopoietic tissues are concerned,—a pancytopenia.

Although in several other virus diseases a mild leucopenia may occur during the acute phase, this is the only instance of such which has come to our attention in which there is found direct evidence, in the nature of clearly defined inclusion bodies, of an attack by a virus on the blood-forming elements.

Broadhurst, MacLean and Saurino (3) and Broadhurst, Cameron, MacLean and Saurino (4) reported finding in stained blood smears from patients with measles and scarlet fever respectively, the presence of various globular bodies and granules in and being extruded from the lymphocytes. The numerous photographs offered in support of this show lymphocytes which appear to us entirely similar to certain cells frequently encountered in smears of normal blood rendered abnormal by physical changes taking place while being spread and dried. They find similar “inclusion bodies” in both diseases and in each case offer these as evidence in favor of a virus etiology.

Findlay (5) reports the presence of cytoplasmic inclusion bodies in polymorphonuclear leucocytes, eosinophiles and large mononuclear cells of the blood of sheep infected with tick-borne fever. These may be resolved into particles resembling elementary bodies. This disease was first described by Gordon, MacLeod and associates (6-8) while working with louping ill, but up to the present the etiological agent has not been demonstrated to be a filterable virus. We have been unable to find any reference regarding the presence or absence of quantitative blood cell changes during the course of the infection.

Mack, in 1911 (9), described in connection with equine infectious anemia small “coccus-like bodies” in the erythrocytes. These she stated were present in all cases of this disease in which she had had the opportunity to study properly fixed and stained blood smears, and were not found in the blood of normal horses or those suffering from other infections. We can find no confirmation of these observations.
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Since Gamma (10) and Favre (11) described cytoplasmic inclusion bodies (Gamma-Favre bodies) in the lymph nodes of patients suffering with lymphogranuloma inguinale, their presence has been confirmed by many observers. Findlay (12), however, considers these to be, in all likelihood, nuclear extrusions and similar to the bodies occurring in normal lymph nodes noted by Flemming in 1885. The former, however, does attach some significance to smaller cytoplasmic bodies found in lymphoid cells, which consist of metachromatic staining granules of about 0.2 to 0.3μ in diameter. These were previously described by Gay Prieto (13), Miyagawa and his associates (14) and others. Miyagawa and his associates noted these granules in neutrophilic leucocytes and histiocytes in tissue sections from both man and experimental hosts. They believe them to represent actual virus particles. According to Todd (15), a mild degree of leucocytosis is generally present during the early stages of the infection in man. Findlay (12) classifies the virus as one which produces proliferative rather than degenerative cellular processes. He regards the essential pathological change in any organ affected, both in man and experimental animals, as a proliferation of the cells lining the lymph channels. Except for migrating polymorphonuclear cells, the reaction is restricted to the mononuclear series of cells. This reaction, though predominantly proliferative in character, appears to resemble in certain respects that encountered in panleucopenia of cats.

The injurious effects noted on the tissues of the marrow and lymph nodes in panleucopenia are limited to the immature and stem cells. No cells containing inclusion bodies have ever been seen in smears of the peripheral blood. The adult leucocytes of the marrow and peripheral blood, though greatly reduced in number, show no nuclear abnormalities. Not infrequently, however, the polymorphonuclears of the circulating blood appear "toxic" with vacuolated cytoplasm.

The reduced number of mature leucocytes, we feel, may be accounted for by failure of the marrow and lymph nodes to replace those which have been discarded by the normal physiological process, of which the mechanism is at present poorly understood.

Effects on the Erythrocytes

In our previous communication we pointed out that there was occasionally a slight fall in the number of circulating erythrocytes during the course of the disease and that this was more apparent in animals which were recovering, due possibly to rehydration. It was also noted that in two animals examined at death the serum icterus was definitely increased. At that time no explanation of the mechanism of these changes suggested itself. After observing a larger number of animals it became apparent that in many instances during the acute course of the infection no anemia could be demonstrated, although at autopsy the marrow was almost or completely aplastic (Fig. 9). These apparently paradoxical phenomena may possibly be explained as follows: The life of mature erythrocytes is without much
doubt many times longer than that of the leucocytes, and, since there is evidence in the hematopoietic tissues of damage only to the primitive blood-forming cells, the erythrocyte count would not be expected to fall conspicuously during the course of an illness associated with dehydration (vomiting and refusal of liquids) of 3 or 4 days' duration. The normal mechanism for the destruction of old erythrocytes, without replacement of these by new cells, would satisfactorily account for a slight anemia in certain individuals. With this consideration in mind the blood was examined for evidence of a higher percentage of old cells (increased erythrocytic fragility) and a decrease in the percentage of the reticulocytes.

We found in making reticulocyte counts on 3 normal kittens (2) that the normal percentage of reticulocytes was so low (in one instance none was found after counting 3000 erythrocytes) that no very significant decrease would be noted in case all erythropoiesis should be suddenly terminated. Accordingly, we do not attach great significance to the fact that we found no reticulocytes in blood smears made during the course of the illness, although Krumbhaar (16) reports 0.2 per cent as the average reticulocyte count in cats and gives the normal range as 0 to 0.4 per cent.

In normal kittens we found hemolysis of erythrocytes to begin at 0.58 to 0.60 per cent NaCl and to be complete at from 0.46 to 0.48 per cent (2). The fragility range was tested on 3 experimentally infected animals at different times during the course of the disease and in one case during early recovery (see Table 1). In these, hemolysis began at from 0.64 to 0.74 per cent and was complete at from 0.50 to 0.58 per cent, indicating that hemolysis began and was complete in more nearly the isotonic range of saline concentration. In 2 of the animals the fragility of the cells was tested before inoculation and found to be within the normal range as given above.

As further evidence of increased fragility, the cells of one of the above kittens, No. 2-11 (see Table 1), were seen repeatedly to hemolyze in the counting chamber, when diluted with Hayem's solution which had become slightly hypertonic through evaporation, but the same solution produced no such effect on the cells of normal cats or on those of this animal previous to infection. To follow up this observation the fragility test was made, with the blood from 2 normal animals and human blood as controls. The tests were repeated the following day with the same results.

Since our series of observations on changes in erythrocytic fragility is small, and since such wide variation in this respect between normal animals has been found by other workers (17, 18), it is impossible to regard these results as affording a conclusive demonstration of increased erythrocytic
fragility. They do, however, suggest that this has occurred. We do not desire to stress this point, but since our results are consistent and appear to fit in with the whole expected picture, we record them to be interpreted as possibly significant.

**The Changes Associated with Recovery**

Study of sections of bone marrow made on animals killed within a few days of the onset of recovery, as demonstrated by a rapid rise in the leucocyte count, showed a similar picture to that reflected in the smears of the

### TABLE I

<table>
<thead>
<tr>
<th>Kitten</th>
<th>Date</th>
<th>Clinical condition</th>
<th>Beginning hemolysis</th>
<th>Complete hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Per cent NaCl</td>
<td>Per cent NaCl</td>
</tr>
<tr>
<td>2-11</td>
<td>Jan. 25</td>
<td>Inoculated</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Feb. 10</td>
<td>Normal leucocyte count with mild symptoms</td>
<td>0.74</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>&quot; 11</td>
<td>Same*</td>
<td>0.74</td>
<td>0.58</td>
</tr>
<tr>
<td>2-14</td>
<td>Mar. 9</td>
<td>Inoculated</td>
<td>0.58</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>&quot; 17</td>
<td>Ill, leucopenia</td>
<td>0.60</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>&quot; 22</td>
<td>Improving</td>
<td>0.66</td>
<td>0.50</td>
</tr>
<tr>
<td>2-15</td>
<td>&quot; 9</td>
<td>Exposed</td>
<td>0.58</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>&quot; 17</td>
<td>Dying, W.B.C. 450</td>
<td>0.64</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*This animal ran a prolonged subacute course. When sacrificed shortly after this determination, the marrow showed complete regeneration.*

peripheral blood. For the first few days the marrow and smear both present the appearance of a malignant change, so rapid is the proliferation of immature cells, but this is restricted to the leucocyte series. No regeneration in the red cell precursors appears till about the 4th or 5th day. This delay may possibly be attributed to the early mechanical crowding of the marrow by the more rapidly growing myeloblasts and myelocytes (Fig. 10). During the later phase numerous polychromatophilic and nucleated red cells may appear in the circulating blood. A slight rise in the reticulocyte count was noted in one animal in which the reticulocyte count on the 4th to the 7th day after the beginning of recovery was 0.4, 0.45, 0.1 and 0.5 per cent respectively (based on a count of 2000 erythrocytes).

**SUMMARY**

The most conspicuous clinical finding during the course of this virus disease is a fulminating panleucopenia. The earliest significant decrease
noted is usually in the number of the lymphocytes. From the study of lymph nodes and bone marrow during the incubation period and throughout the illness, it appears that failure of leucopoiesis is the cause of leucopenia. Inclusion bodies in the primitive blood cells of the marrow suggest a direct action of the virus on these cells. When recovery occurs a marked myelogenous leucemoid response is noted.

Available data indicate the presence of a mild anemia due to a failure in erythropoiesis, less marked than the leucopenia probably because of the longer life of the adult circulating erythrocytes. The erythrocytes appear to have an increased fragility and the serum has a slight increase in icterus, suggesting an increased mean erythrocyte age. During recovery erythropoiesis does not begin until after the myeloid marrow response has begun to subside, due possibly to previous mechanical crowding of the marrow by the more rapidly growing myeloblasts and myelocytes.

BIBLIOGRAPHY

EXPLANATION OF PLATES

All photomicrographs in the following plates are of tissue sections stained with eosin-methylene blue. All animals were killed with ether and portions of tissue fixed immediately in Zenker's solution. The bone marrows were not decalcified.

PLATE 41

Fig. 1. Typical follicle from the mesenteric lymph node of a normal kitten. × 260.

Fig. 2. Follicle from a mesenteric lymph node of kitten 1-80, typical of the earliest pathological changes (increased size of germinal center with signs of stimulation and degeneration combined). This animal was sacrificed during the incubation period at onset of leucopenia (9550 cells). Fig. 5A is an enlargement from this same node. Such changes were not found in the marrow of this kitten. × 260.

Fig. 3. Follicle from mesenteric lymph node of kitten killed in the terminal stage of the disease (150 leucocytes). This follicle was probably originally comparable to those of Figs. 1 and 2. There is marked aplasia, necrosis, edema, widening of sinusoids and increase of reticulum cells. × 260.

Fig. 4. Another area of the same lymph node shown in Fig. 3 showing the widened sinusoids which contain large numbers of macrophages. × 260.

Fig. 5A. Area from lymph node of kitten 1-80 (Fig. 2 low power) with a central cell containing an eosinophilic intranuclear inclusion body, which is divided by a strand of chromatin material joining the two portions of the nucleolus. × 1350.

Figs. 5B, 6A and 6B. Three areas from the femoral marrow of kitten 1-85 (Fig. 8 low power of same marrow) each with a central cell containing one or two eosinophilic intranuclear inclusion bodies. × 1350.
(Hammon and Enders: Further studies on panleucopenia of cats)
PLATE 42

Fig. 7. Bone marrow from the proximal end of the femur of a normal kitten. × 320.

Fig. 8. Femoral marrow from kitten 1-85 sacrificed during the incubation period soon after onset of leucopenia (7600 leucocytes). No aplasia noted, but many cells are undergoing degenerative processes. × 260.

Fig. 9. Femoral marrow at terminal stage of the disease (same animal as that of lymph node, Figs. 3 and 4). This marrow is almost completely aplastic. The remaining cells are for the most part plasma cells and mature erythrocytes. The whole of the marrow presents about the same appearance as the part photographed. × 260.

Fig. 10. Femoral marrow of kitten 1-56, early recovery phase. The marrow is crowded with myeloblasts and myelocytes. × 260.