Experimental Studies on Reticulosarcoma

By Akira Sakamoto, M.D.

In a previous paper on experimental leukemia the author has demonstrated that various kinds of human leukemias could be transmitted serially to fowls by cell-free filtrates. Liver emulsions derived from 7 cases of leukemia (acute and chronic myeloid or lymphatic leukemia, chloroleukemia, aleukemic lymphatic leukemia and myelosis erythraemica) were administered to each series of white leghorns intravenously, occasionally intracutaneously or intramuscularly. Over 1,000 fowls were tested in the course of experiments extending over a period of four to six years and the following major results were obtained:

1. Lesions produced in fowls were found to be either (a) myelosarcoma, myelosis and myeloid metaplasia or (b) reticulosarcoma, reticulosis and related conditions.

2. These changes presented themselves without regard to the nature of the materials used for inoculation. Hyperplasia of the immature myeloid tissue or that of the reticulo-endothelial cells can arise in one generation of the same strain, and in the next generation either the former or the latter may occur.

3. Often greyish white tumors were noted, ranging in size from those as large as a sparrow’s egg in myelosarcoma to a hen’s egg in reticulosarcoma.

4. The liver showed lesions most frequently, followed by the spleen, bone marrow, kidney and rarely the heart and skin. Pathologic changes in the liver, spleen and bone marrow were not always found simultaneously; often one or two of these organs were totally free, contrary to cases of the spontaneous chicken leukosis.

5. Marked anemia was usually absent and the blood picture was characterized by leukopenia without immature cells. Throughout our experiments no evidence of erythroleukosis was encountered. This is in striking contrast to the experience of others with chicken leukosis.

6. In each series hemorrhagic diathesis manifested itself more or less, resulting sometimes in death due to hemorrhage.

7. The causative agent passed through Chamberland L1, L2, L3 filters.

8. The agent increased in pathogenicity when cultured on the chorio-allantoic membrane of chick embryo.

On these grounds I believe that there is no possibility of confusion with infective agents of chicken leukosis, and that human leukemia is an infectious neoplastic disease caused by a specific virus.

In addition, we have noted the peculiar phenomenon in each series of experiments that different forms of the lesions (hyperplasia of myeloid tissue and of...
reticulo-endothelial cells) occur regularly within the same strain. This convinced me that reticulosarcoma and leukemia might be caused by the same virus, the differences being attributable to differences in tissue reactions stimulated by the same agent. I proceeded, accordingly, to prove this concept by carrying out experiments with human reticulosarcomas similar to our previous work on leukemia.

**Experimental Methods**

The material used for inoculation was derived from two typical cases of lymphatic reticulosarcomas in adults, which originated in the nasopharyngeal cavity, and three typical cases of myeloid reticulosarcomatosis affecting the flat bones in young children. The latter consisted of extensive hyperplasia of polymorphocellular types of reticulum cells with giant cells present in many flat bones and lymph nodes, and was characterized by an osteolytic x-ray picture, especially of the pelvis. The author hopes to publish the particulars in detail in English later. In each case the blood picture proved to be aleukemic. White leghorns, regardless of sex and age were employed.

The tumors or enlarged lymph nodes were ground, saline was added to make a 1 per cent. emulsion, centrifugated at 3,000 r.p.m. for ten minutes. The supernatant fluid was injected intravenously; at times intramuscularly; usually in amounts of 1 cc. into each of 2 or 3 fowls. In the first generation unfiltered emulsions were used for inoculation, but in subsequent generations Chamberland L2 filtrates of liver emulsions of birds dying from the inoculation were used. A single injection was given to each fowl, and the blood was examined at least once a month.

The experiments came to an end March 12, 1945, when our laboratories were completely destroyed by the war.

1. **Experiments on Lymphatic Reticulosarcomas**

The material from a patient (K.) was given to fowls through 5 generations totalling 27 birds and they were investigated in the course of three years and three months. Material from another patient (T.), was given to 14 animals representing 3 generations over a period of two years and seven months.

The pathologic findings showed (table 1) myelosis or myeloid metaplasia on one hand, reticulosarcoma, reticulosis or hyperplasia of reticulum cells on the other. It is believed that these are all to be regarded as findings typical of the author’s experimental leukemia. In the first generation fowl inoculated with human materials marked lesions were produced, and there was no evidence of lymphatic tissue changes throughout the experiment. The blood picture was characterized by a slight anemia without immature erythrocytes and by marked leukopenia, sometimes by a leukocytosis, in either case polymorphonuclear leukocytes increasing at the expense of lymphocytes. All of the cases have been found to be aleukemic, showing, if any, only a few immature leukocytes just before death.

To get a general view, brief reference will be made to one selected series of experiments (table 1). In this and subsequent tables birds listed without mention of cause of death or the significant findings, did not show any evidence of possibly specific pathologic-histologic findings so far as it concerns the survey of each liver, spleen and bone marrow. Their cause of death was, therefore, often
Table 1—A Series of Feud Passages from One of the First Generation (No. 958) Derived from Patient K.

<table>
<thead>
<tr>
<th>Passage</th>
<th>Event</th>
<th>Days</th>
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<tbody>
<tr>
<td>958♀</td>
<td>Heart enlargement</td>
<td>101</td>
</tr>
<tr>
<td>958♀</td>
<td>Reticulosarcoma (heart)</td>
<td>101</td>
</tr>
<tr>
<td>957♀</td>
<td>Reticulosarcoma (L, S, M)</td>
<td>101</td>
</tr>
<tr>
<td>956♀</td>
<td>Dead (231 day)</td>
<td>231</td>
</tr>
<tr>
<td>961♂</td>
<td>Ret. hyperplasia (L, S)</td>
<td>264</td>
</tr>
<tr>
<td>962♀</td>
<td>M. metaplasia (L, S)</td>
<td>1029</td>
</tr>
<tr>
<td>994♀</td>
<td>Dead (765 day)</td>
<td>765</td>
</tr>
<tr>
<td>16♀</td>
<td>M. metaplasia</td>
<td>1042</td>
</tr>
<tr>
<td>45♀</td>
<td>M. metaplasia (L)</td>
<td>181</td>
</tr>
<tr>
<td>47♀</td>
<td>Dead (140 day)</td>
<td>140</td>
</tr>
<tr>
<td>49♀</td>
<td>Dead (66 day)</td>
<td>66</td>
</tr>
<tr>
<td>53♀</td>
<td>M. metaplasia (L)</td>
<td>23</td>
</tr>
<tr>
<td>54♀</td>
<td>Killed (370 day)</td>
<td>370</td>
</tr>
<tr>
<td>46♀</td>
<td>Dead (75 day)</td>
<td>75</td>
</tr>
<tr>
<td>48♀</td>
<td>M. metaplasia (L)</td>
<td>425</td>
</tr>
</tbody>
</table>

L₆ means a filtration through Chamberlin L₆; ret hyperplasia = hyperplasia of reticulum cells; m. metaplasia = myeloid metaplasia; L = liver; S = spleen; K = kidney; M = bone marrow.
unknown, sometimes thought to be due to coccidiosis or to disorders of the respiratory tract.

**Fowl No. 958:** Liver; 66.5 Gm. Smears stained with Giemsa presented variously sized plasma cell-like and lymphoblast-like cells, averaging 5-7 for each field of vision (magnification of 900 is used throughout). Histologically there were rather diffuse accumulations of reticulum cells, occasionally of eosinophile myelocytes. The endothelial cells of capillaries in trabeculae were swollen and continuations between the neighboring cells were manifest, revealing a slight increase in lattice fibers by silver method. Spleen: 4.7 Gm. In Giemsa smears, large rhombic reticulum cells predominated with many vacuoles, cytoplasm of which were stained deeply basophilic, while the normal splenic cells were sparse. On section the latter were replaced by an immense number of cells resembling large plasma cells. There was reduction in lattice fibers. Of special interest is the swelling of the heart (37 Gm.), where a grey medullary tumor was noted. Microscopically polymorphous reticulum cells resembling lymphoblasts apparently at very primitive stages formed a tumor, infiltrating between neighboring muscle fibers just as in the heart of human lymphatic leukemia. All of the newly formed cells were characterized by their abundant cytoplasm and larger nucleus which is double the size of that of a lymphocyte, and by the multiplicity of cell forms. There was no increase in lattice fibers. Of special interest is the swelling of the heart (37 Gm.), where a grey medullary tumor was noted. Microscopically polymorphous reticulum cells resembling lymphoblasts apparently at very primitive stages formed a tumor, infiltrating between neighboring muscle fibers just as in the heart of human lymphatic leukemia. All of the newly formed cells were characterized by their abundant cytoplasm and larger nucleus which is double the size of that of a lymphocyte, and by the multiplicity of cell forms. There was no increase in lattice fibers.

**Fowl No. 961:** 2230 Gm.; was injected intravenously with 1 cc. of a supernatant fluid prepared by the 200 times diluted liver emulsion. During this experiment, 2 cc. of a 5 per cent indiar suspension was intravenously injected twice in two days. The fowl died on the one hundred and fifteenth day following the indiar inoculation, weighing 1370 Gm. Liver: 23.5 Gm. Smears stained by Giemsa's method revealed some histiocytes and an average of 2 pseudoeosinophil leukocytes for each field of vision. On histologic examination the interstitium showed many foci of accumulations of cells loaded with indiar ink. This is interpreted to be typical of reticular hyperplasia. Spleen: 0.5 Gm. Sections revealed disappearance of follicles and cells containing many granules of indiar ink were scattered all over the organ. In marrow smears normal erythrocytes were predominant.

**Fowl No. 997:** 1440 Gm. The amount of 0.5 cc. of a 1:100 liver emulsion filtrate was inoculated intracutaneously in the neck, which resulted in death with body weight 1100 Gm. Liver: 79.0 Gm. (the heaviest in this paper). Brownish and reddish in color with numerous grey spots on the surface. Giemsa smears showed myeloblasts and myelocytes with nuclei composed of fine chromatin and with distinct nucleoli, averaging from one to two per visual field. Microscopically it revealed large and medium-sized perportal cell accumulations without mitoses, which mainly consisted of large, irregular in shape, non-granular myelocytes and myeloblasts with abundant cytoplasm and undivided round nuclei. Individual cells were placed close to each other, but not interconnected. Scattered endothelium cells and normoblast-like cells were found in the accumulations. The capillaries contained a few erythrocytes. Spleen: 9.0 Gm. Liver-like in color. On the cut surface diffuse distribution of grey fine spots was noted. Giemsa smears revealed many myeloblasts and myelocytes, there being 10 or more in each field of vision. On section the follicle design in this organ was not completely seen. Extensive diffuse proliferations of primitive myeloid cells of the same kind as those in the liver infiltrations were detected instead of the normal pulp cells, associated with fairly pronounced hyaline degeneration in the walls of blood vessels and with scattered small foci of hemorrhages and necrosis. In the infiltrations pyknotic cells as seen in figure 5 were met with in large numbers. There was also moderate fibrosis. Bone marrow: smears showed predominance of myeloblasts and myelocytes as seen in the liver.
EXPERIMENTAL STUDIES ON RETICULOSARCOMA

On the whole, these findings in all the organs were closely analogous to those of leukemia in man.

The successive blood examinations were done on 12 occasions after injection (table 2). The white cells fluctuated between 1,500 and 39,000 in number without immature elements. The red cell count was 2,170,000 before death, approximately the same as before injection, i. e. 2,300,000.

Fowl No. 48: dead with extreme marasmus. Liver: 22 Gm. Smears showed an average of 3-4 cells resembling monocytes or myeloblasts in each field of vision. Histologically the Kupffer cells were swollen with brown pigments. There were considerable periportal groups.

Table 2.—Fowl No. 997—Myelosis (Liver, Spleen, Kidney, Marrow) Absence of Severe Anemia or Immature Leukocytes, Repeated Occasional Occurrence of Leukopenia Are to be Noted

<table>
<thead>
<tr>
<th>Date</th>
<th>Body Weight (Gm.)</th>
<th>Erythrocytes 1,000</th>
<th>Hb. %</th>
<th>Leukocytes 1,000</th>
<th>Platelets 1,000</th>
<th>Lymphocytes</th>
<th>Monocytes %</th>
<th>Pseudo. %</th>
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<tbody>
<tr>
<td>12/14/42</td>
<td>2030</td>
<td>2,300</td>
<td>60</td>
<td>21.0</td>
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<td>0</td>
<td>80</td>
<td>0</td>
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<tr>
<td>12/18/42</td>
<td>0.5 cc. of the filtrate of a 1 per cent liver emulsion was inoculated intracutaneously</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4/43</td>
<td>1945</td>
<td>2,400</td>
<td>58</td>
<td>26.0</td>
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<td>5</td>
<td>71</td>
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<td>2/12/43</td>
<td>1780</td>
<td>2,450</td>
<td>62</td>
<td>2.5</td>
<td>37</td>
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</tr>
<tr>
<td>2/27/43</td>
<td>1970</td>
<td>2,350</td>
<td>63</td>
<td>21.0</td>
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<td>0</td>
<td>84</td>
<td>6</td>
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<td>3/7/43</td>
<td>1800</td>
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<td>6</td>
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<td>4</td>
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<td>10</td>
<td>76</td>
<td>2</td>
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<td>5/14/43</td>
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<td>1,790</td>
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<td>27.0</td>
<td>18</td>
<td>11</td>
<td>49</td>
<td>4</td>
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<td>6/25/43</td>
<td>1610</td>
<td>1,360</td>
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<td>6</td>
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<td>1</td>
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<tr>
<td>7/10/43</td>
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<td>1,400</td>
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<td>7/24/43</td>
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<td>0</td>
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<td>22</td>
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<td>8/23/43*</td>
<td>1590</td>
<td>2,350</td>
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<td>22.0</td>
<td>26</td>
<td>4</td>
<td>54</td>
<td>4</td>
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<tr>
<td>11/13/43*</td>
<td>1510</td>
<td>740</td>
<td>20</td>
<td>1.5</td>
<td>34</td>
<td>4</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>11/29/43*</td>
<td>1135</td>
<td>2,170</td>
<td>51</td>
<td>8.7</td>
<td>13</td>
<td>87(?</td>
<td>0</td>
<td>6</td>
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<tr>
<td>12/4/43</td>
<td>1100</td>
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<td></td>
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</table>

* Erythrocytes present numerous polychromasie erythroblasts and those with hemoglobin content and anisocytosis.

It is to be noted as in our experimental leukemia, that some organs in the same fowl were characterized by hyperplasia of myeloid tissue, while others were characterized by hyperplasia of reticulo-endothelial cells. Both of these pathologic changes sometimes coexist prominently in the same organ, e.g., Fowl No. 42. Liver: 56 Gm. Myeloid infiltrations with a little proliferation of reticulum cells were present, forming in some areas small rounded nodules of myeloid metaplasia. Spleen: 2.0 Gm. Follicles were atrophic. Slight fibrosis. A typical hyperplasia of reticulum cells and scattered foci of myeloid metaplasia coexisted. The bone marrow presented a reduction in leukopoiesis.
In brief, from patient K.: in the course of a period of three years and three months, 27 fowls in 5 generations were investigated. The pronounced lesions were: reticulosarcoma and reticulosis in 1, hyperplasia of the reticulum cells in 3, myelosis in 1, myeloid metaplasia in 6 and reticulo-endothelial hyperplasia associated with myeloid metaplasia in 1 case. These then gave manifest infection in at least 44.4 per cent of cases.

From patient T.: over a period of 2 years and 7 months, a total of 14 birds (apart from 7 cases, of which 4 were burned to death by bombing and 3 were lost); through 3 generations were used, in which the following changes were produced: 4 cases with myeloid metaplasia and 1 with myeloid metaplasia and hyperplasia of reticulum cells, giving the infection rate of 71.4 per cent.

2. Experiments with Human Myeloid Reticulosarcomatosis

On the basis of 3 cases similar experiments to those of the previous lymphatic ones were carried out. The fowl liver emulsions, however, were inoculated in a more concentrated form, i.e., in a dilution of 1:50–200 in series of experiments in the second and third patient, and in a dilution of 1:1,000–5,000 in the first patient, usually in doses of 1 cc. of the supernatant fluid following centrifugation.

A. The first case (I. K.).

(1) The first administration was made using the patient’s blood, and in subsequent passages serially by means of infected fowl livers. The results are summarized in table 3. From this table some important cases will be selected and illustrated as follows.

The first intravenous administration was performed with 2 cc. of a mixture, 2 cc. of the patient’s blood and 6 cc. of distilled water.

Fowl No. 573: 1350 Gm. After five months following the inoculation, the animal was given again 2 cc. of the blood of No. 632, after which the fowl was killed by venesection on the twenty-first day. The weight of the bird was 1850 Gm. Fowl No. 632 is of the third generation in another series of experiments in which the enlarged lymph node of the same patient was used as inoculation material. This reinjection is an exceptional procedure. Liver: 31.5 Gm. Histologically localized perivascular infiltrations with myelocytes were met with in Glisson’s capsules, revealing at times follicle-like foci of myeloid metaplasia. There was also an increase in reticulum cells and connective tissue, especially marked swelling of vascular adventitia. Spleen: 1.7 Gm. Sections showed fibrosis and perivascular swelling of adventitia and accumulations of myelocytes. In some areas eosinophilia was noted. In brief, both organs exhibited a clear evidence of allergic changes associated with myeloid metaplasia.

Fowl No. 866: an intramuscular injection of 1 cc. of a 1:5000 liver emulsion from the second fowl passage was given. The blood picture was examined on 21 occasions up to about three months before death, showing practically no change throughout. Liver: 39 Gm. Spleen: 1 Gm. A large amount of bile-like fluid was present in the abdominal cavity. On the intestinal loop hundreds of small round tumors were found scattered on the mesentery from the sand-stomach and below along its entire length (fig. 1). Most of the tumors were of pinhead size and grey in color. Nowhere was there to be found any primary tumor in other organs.

Smears stained with Giemsa: in the blood smears leukocytes appeared to be increased. Specifically young myeloblast-like cells were prominent. Furthermore, it was noticed that there were a number of reticulum-cell—like cells resembling plasma cells, which were usually long ovoid cells with abundant cytoplasm, having at times vacuoles and large, round nuclei with fine reticular chromatin. In the marrow films larger myeloblast-like cells with clearly
Table 3.—Essential Results in Serial Fowl Passages with the Blood of the First Patient

<table>
<thead>
<tr>
<th>Generation</th>
<th>Event</th>
<th>Description</th>
</tr>
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<tr>
<td>First</td>
<td>killed (184 day)</td>
<td>573♂ m. metaplasia (L, S)</td>
</tr>
<tr>
<td></td>
<td>dead (19 day)</td>
<td>572♂ m. metaplasia (S)</td>
</tr>
<tr>
<td></td>
<td>killed (753 day)</td>
<td>575♂ m. metaplasia (L) end, hyperplasia (S) swelling of Kupffer's cells</td>
</tr>
<tr>
<td></td>
<td>dead (23 day)</td>
<td>601♂ m. metaplasia (L)</td>
</tr>
<tr>
<td></td>
<td>dead (35 day)</td>
<td>631♂ ret. hyperplasia (S)</td>
</tr>
<tr>
<td></td>
<td>dead (28 day)</td>
<td>690♂ m. metaplasia (L)</td>
</tr>
<tr>
<td></td>
<td>dead (14 day)</td>
<td>712♂ m. metaplasia (S)</td>
</tr>
<tr>
<td></td>
<td>dead (14 day)</td>
<td>729♂ m. metaplasia (S)</td>
</tr>
<tr>
<td></td>
<td>dead (30 day)</td>
<td>788♂ m. metaplasia (S)</td>
</tr>
<tr>
<td></td>
<td>dead (72 day)</td>
<td>789♂ m. metaplasia (S)</td>
</tr>
<tr>
<td></td>
<td>killed (1057 day)</td>
<td>830♂ ret. hyperplasia (L)</td>
</tr>
<tr>
<td></td>
<td>a large hematoma of S.</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>dead (294 day)</td>
<td>688♂ ret. endotheliosis (L, S, M)</td>
</tr>
<tr>
<td></td>
<td>dead (443 day)</td>
<td>866♂ myelosarcomaosis (mesentery, L, S, K, M)</td>
</tr>
<tr>
<td></td>
<td>dead (448 day)</td>
<td>507♂ m. metaplasia (S)</td>
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<td></td>
<td>dead (155 day)</td>
<td>845♂ m. metaplasia (L)</td>
</tr>
<tr>
<td></td>
<td>killed (294 day)</td>
<td>944♂ killed (774 day)</td>
</tr>
<tr>
<td></td>
<td>ret. hyperplasia (L, S)</td>
<td></td>
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</table>
visible nuclei made up about two-thirds of all cells. The liver smears contained a number of those cells with large nuclei or large nucleoli resembling human myeloma cells. Also in the spleen smears myeloblast-like cells with similar nucleoli were noted. Moreover, the smears made from the mesenteric tumors presented numerous myeloblast-like features in the trabeculae (fig. 2), especially in Glisson's capsules, eosinophil myelocytes accumulating in places. The Kupffer cells were swollen and reticulum fibers showed no increase. The spleen had a homogeneous structure with disappearance of follicles and infiltration of myeloblasts and eosinophil myelocytes just as seen in the liver. The mesentery showed similarly immature cells in the liver. These form the tumor with light circular areas, in which the cells were loosely present. These areas appeared to be made of tissues resembling myxomatous or gelatinous fibers, while argyrophilic fibers were present in a limited amount in the tumors.

FIG. 1.—Fowl No. 866. Numerous nodular formations (myelosarcoma) on the mesentery.

The pancreas adjacent to the tumors showed moderate infiltrations of the tumor cells which extended into its parenchyma. A similar infiltration was also detected in a lesser degree in the kidneys.

From the above findings it was concluded that the fowl showed a state of diffuse myelosarcomatosis.

Fowl No. 688: 1140 Gm. was inoculated intramuscularly with 1 cc. of a 0.1 per cent emulsion prepared from the liver and a piece of the tumor from No. 866. It weighed 1685 Gm. when it died. There was no anemia. In the blood films oval or spindle-shaped cells resembling endothelium cells were met. Liver: 67.5 Gm. The smears revealed small monocyte-like cells with intensive, deeply stained basophil cytoplasm. Spleen: 2.5 Gm. On examining the stained films from the bone marrow, a large number of cells about the size of small lympho-
cytes resembling human normoblasts were found instead of ordinary myelocytes. Microscopically in the liver, perivascular collections of reticulum cells with slight increase in argynophilic fibers were encountered. The splenic pulp is infiltrated with reticulum cells, small, round or irregular-shaped endothelium cells predominating with little or no lymphocytes. There was also fibrosis without follicles. Moderate amounts of reticulum fibers were demonstrable. This picture was consistent with reticulo-endotheliosis.

_Feed No. 845_: 1630 Gm. was given 1 cc. of a filtrate of a 0.5 per cent liver emulsion intravenously. Thereafter, repeated blood examinations were performed over a period of four months, until marked anemia suddenly occurred. The red cell count fell to 1,000,000; hemoglobin 20 per cent Sahli. Two weeks later the animal died, at which time the hemoglobin was 10 per cent or less. The comb appeared intensely anemic. Liver: 30.5 Gm. It was yellowish in

**Fig. 2.** - Myeloblasts in higher magnification in the liver of figure 1. X720.

color. On section it revealed from a moderate to severe degree of perivascular myelocytic accumulation, in places with some foci of eosinophil myelocytes. Spleen: 1.6 Gm. Rosy in color, histologically atrophic. Leukocytes were found scattered throughout the organ.

In summary, in the course of a five year and one month period, in 15 among 28 fowls through 11 generations, the following alterations have been produced: myelosarcomatosis in 1 case, myeloid metaplasia in 10, hyperplasia of the reticulum cells in 5, reticulo-endotheliosis in 1 and hematoma of the spleen in 1, some birds being afflicted with more than one pathologic change.

(2) Another series of experiments was run, in which a lymph node of the patient was used as experimental material, extending over a period of three years and seven months through 7 generations, comprising 21 birds. The positive re-
sults obtained in 11 fowls were as follows: myeloid metaplasia in 9 cases, hyperplasia of the reticulum cells in 4, hyperplasia of the reticulo-endothelial cells in 2. Also, in this series of experiments, moderate hemorrhagic diathesis manifested itself. Linear hemorrhage usually in the muscles was noted in 6 fowls, some of which showed many hemorrhagic areas. A few typical instances of them will be briefly illustrated below.

_Fowl No. 776_: was of the sixth generation, 4 generations of which had been infected with filtrates passed through Chamberland L₂. The bird died on the six hundred and twenty-ninth day. Three days previous to death the red cell count was 2.42 M, and hemoglobin 59 per cent Sahli. The blood smear presented a little increase in pseudoeosinophils.

At autopsy hemorrhages in the breast muscles were found. Liver: 34 Gm. On section it showed numerous primitive myelocytes including pseudoeosinophils in perivascular tissue and in the Glisson capsules in nodular shape. Spleen: 1 Gm. The tissue was homogeneous in appearance and the pulp was hyperplastic, showing in many places gland-like structures. Moderate hyperplasia of the reticulo-endothelium was met. Films from the bone marrow disclosed a moderate increase in myelocytes.

B. Experiments using 12 fowls were conducted with materials obtained from a cervical lymph node of the second patient (Ka.) extending for two years and eight months. The final results may be summarized as follows: 1 fowl lost and 3 destroyed by a bomb. Of the remaining 8, myeloid metaplasia in 2 cases, both reticular hyperplasia and hypertrophy of Kupffer cells loaded with blood pigments in a case.

C. The third patient (Ha.). The corresponding series of experiments is summarized in table 4.

_Fowl No. 27_: 1235 Gm. was inoculated with 2.5 cc. of a hemorrhagic puncture fluid from the patient’s chest cavity. Liver: 44.8 Gm. It showed some yellowish pinhead-sized spots on the surface. Microscopically, perivascular and periportal accumulations of myelocytes (often containing eosinophil granules) were found forming numerous nodules. Frequently large tumor cells such as those found in the spleen were met. Blood pigments suggestive of old hemorrhage were also seen. On the whole, the picture reminds one of leukemic findings in man. Spleen, 3.0 Gm. revealed in Giemsa staining preparations many lymphocyte-like cells with round nuclei and deep basophilic cytoplasm. Histologically the tissue was diffuse in structure with no follicles remaining. The pulp tissue was found diffusely hypertrophic with an special increase in characteristic, large, primitive cells with distinct nuclei, which were highly suggestive of tumor cells found in the fowl of the next generation, No. 58. The marrow was firm and its stained preparation contained mainly myelocytes.

_Fowl No. 58_: 1320 Gm. was inoculated intravenously with 1 cc. of a 1:100 diluted liver emulsion prepared from the fowl, No. 27, causing death with weight of 950 Gm. No anemia was evident. Liver, 37 Gm. had one bean-sized, greyish tumor and many small spots on it. On section this mass showed numerous tumor cells, with sharply defined margin (figs. 3 and 4.). There were also many accumulations of similar newly formed cells producing variously-sized nodules, corresponding to the minute spots. These tumor cells were irregular in shape, rich in nuclear chromatin with relatively ample cytoplasm and connected by pseudopod-like processes of the cytoplasm. Here and there these reticulum tumor cells were seen in hepatic cords and in places myeloblasts (some eosinophil granules) formed nodules around small vessels, which were interpreted to be the foci of myeloid metaplasia. It is worthy of note that the reticulum cells of the vascular endothelium formed nodular elevation. Spleen, 3.5 Gm., on gross examination presented 4 tumors varying in size from a pinhead to a small bean. In Giemsa stained smears lymphoblast-like cells were increased. On section the fol-
EXPERIMENTAL STUDIES ON RETICULOSARCOMA

licles appeared indistinct and hyaline substance was often met with. The normal cells were
replaced in most parts by tumor cells, which often formed masses consisting of the same reti-
culum cells as found in the liver. Heart, 5.7 Gm. contained a bean-sized grey tumor in its
wall. Microscopically it showed a typical picture of reticulosarcoma, similar to that in the
liver and spleen, while the same newly formed tissue formed small nodules here and there in
between muscle fascicles. Intestine: on the blind gut some ten oval greyish thick masses
each about the size of a horse-bean were found, corresponding to intestinal follicles. Histol-
ogically a newly formed reticulum tissue was demonstrated with features of malignant tu-
mor, invading profusely into the neighboring muscles. Necrosis was evident on the surface
of the tumor. Kidneys: enlarged with greyish spots. On section infiltrations with reticulum
cells were also well marked. Bone marrow appeared to be normal. Blood smears showed a
subnormal number of leukocytes with no pathologic elements. Lungs were unchanged.

Summarizing the above findings the lesions in the fowl are to be considered as those of
reticulosarcomatosis with systemic involvement.

Fowl No. 59: Spleen, 1.0 Gm. presented histologically a disappearance of follicles and the
following characteristic cells predominated in every part. Medium-sized, irregular round
cells with abundant cytoplasm, which stained light blue with hematoxylin-eosin were seen.
Their nuclei were small, round, and eccentric and stained dark blue. Some of these cells were
characterized by small, quite rounded form with scarce cytoplasm and highly suggestive of
normoblasts in man (fig. 5). In the Giemsa smears these cells resembled plasma cells with
depth basophilic cytoplasm or monocytes, but no cells which are to be regarded as immature
erythrocytes were found. It may remind one of pyknotic cells as often seen in tissues which
have undergone post mortem decomposition, although this finding has nothing to do with
such decomposition in the author's preparations. These cells were frequently met with in
fowls from other patients too. These changes are regarded here for the time being as those of
reticulo-endothelia character. Liver: 34.5 Gm. On section, in addition to a large necrotic
area, it showed a number of small foci, consisting mainly of reticulo-endothelial cells such as
found in the spleen.

Using the liver of this patient, another series of experiments was carried out.

<table>
<thead>
<tr>
<th>First generation</th>
<th>Second generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 ♀ myelosis (L, S, M)</td>
<td>57 ♀ m. metaplasia (L)</td>
</tr>
<tr>
<td>(dead 152 day)</td>
<td>(dead 178 day)</td>
</tr>
<tr>
<td>59 ♀ hyperplasia of reti. end. cells (L, S)</td>
<td>59 ♀ hyperplasia of reti. end. cells (L, S)</td>
</tr>
<tr>
<td>(dead 64 day)</td>
<td>(dead 212 day)</td>
</tr>
<tr>
<td>58 ♀ reticulosarcomatosis (Heart, L, S, K, Intestine)</td>
<td>58 ♀ reticulosarcomatosis (Heart, L, S, K, Intestine)</td>
</tr>
</tbody>
</table>

The entire results of these two series may be summarized in the following:
Throughout the entire series, covering a period of one year and six months, 11
fowls were tested, of which 2 were lost, and 3 were killed in the war fire. Of the
remaining 6 birds, 5 developed marked lesions, namely, reticulosarcomatosis in
1 case, hyperplasia of reticulo-endothelial cells in another, myelosis in the third,
and myeloid metaplasia in 2.
(3) Control experiments.
Using the blood from 5 patients with various kinds of purpura, 5 series of

![Fig. 3.](image)
Fig. 3.—Fowl No. 58. Reticular sarcoma in the liver. X720.

![Fig. 4.](image)
Fig. 4.—Same as figure 3 but with tumor cells in higher magnification. X720.

experiments were made. A detailed account of the following 140 control fowls observed under comparable conditions follows.
A first series of experiments includes a total of 27 fowls which have been studied over a period of five and one-half years through 8 generations; a second series of 60 fowls over a three year and four month period through 13 generations; a third group of 20 birds over a year and a month; a fourth group of 14 birds in the course of three years and ten months; and a fifth series of experiments consisted of 19 animals, having been observed for four years. Every case of all 140 fowls has been subjected to studies of Giemsa smears taken from the liver, spleen, marrow and blood, and sections have been made from about one-third of all of the birds tested, whenever it was regarded necessary and advisable. This experiment was at first intended to transmit purpura to fowls, but none of the animals showed any evidence of specific lesions; nor any focus of myeloid metaplasia or of reticulum cells. On the contrary, most of the birds revealed perivascular cell accumulations in livers, while there were found regularly, foci of lymphoid cells in normal fowls. A number of livers tested showed foci of various sizes of necrosis more or less. Spleens tend to give an atrophic picture, frequently with phagocytes containing brown pigments.

In other series of experiments with tuberculous lymphoma in man (3 fowls of the first generation survived one thousand and ninety-five days) and with the blood of a case with Jaksch-Hayem anemia (8 birds in two years and five
months) all failed to demonstrate any significant lesions on gross and microscopic examination.

COMMENT

A review and comparison of all the results in the experiments above described with those of our previous experimental leukemia may be summarized as follows:

1. From the very first transmission to fowls marked pathologic changes have been produced in each series of experiments on reticulosarcoma. This depended upon the concentration of the injected emulsion and the frequency of administration. In general the manifest infection occurred in about one-half of tested fowls prepared by doses of higher concentration (a few hundred times) in this experiment, while in our previous work on leukemia the final result was found to be successful in about 11 per cent, using small doses of a few thousand times emulsion. Furthermore, the transmission rate might have been larger, if the administration were carried out repeatedly into the same birds instead of single administrations in our study.

2. The induced lesions proved to be entirely similar qualitatively to those of our experimental leukemia. Microscopically tumors manifested themselves in the birds too. Proliferations of primitive cells were found as a rule perivascularly, often with formation of nodules, and less often intravascularly as compared with those of experimental leukemia.

3. In general, filtration of the test materials caused regularly a fair reduction in the pathogenicity of the agent just as in the experiments on Rous sarcoma.

4. The enlargement of the liver and spleen were noted to a lesser degree than in those of our experimental leukemia.

5. In the fowls myeloid lesions were always produced, irrespective of the nature of the original materials used.

6. Changes in the bone marrow were less pronounced in this experiment, the parenchyma almost always presenting fat cells.

It is worthy of note that throughout our study no evidence of lymphatic cell proliferation has been proved in respect to its staining with Giemsa and to the histologic features of each cell. In addition, overall pictures of the spleen tested are characterized by their follicle atrophy, especially with no germinal centers, which is contrary to any lymphatic productive change, particularly to that found in spontaneous chicken lymphoid leukosis.

Since Ellermann, much work has been done on chicken leukosis manifesting different types of leukosis in several countries as cited in the author’s preceding paper. A reticular type is, however, rarely encountered (Furth? and Breedis9), while in my experiments, this type regularly developed. Further, there was not a single case of erythroleukosis in either preceding or present experiments of the author, which is in sharp contrast to spontaneous leukoses, that is, the relationship of hyperplasia of the reticulum cell to the author’s experimental human leukemia and reticulosarcomas has always been that of the erythroleukosis to the natural chicken myelosis. In general, natural chicken leukosis, especially myelosis, seems to be of great rarity in Japan. Furthermore, Giemsa smears
were consistently studied from the liver, spleen, marrow and blood of each bird
tested in our study. And, it has been proved to be very helpful and valuable
for prompt and accurate diagnosis of pathologic changes in them, especially
regarding whether cells of lesions concern a lymphatic or myeloid type, endothe-
lium or reticular. According to my long term experience, such Giemsa smears
have never failed to detect reticular or myeloid changes in advance, whenever
their histologic study revealed such lesions.

Transmission of the human leukemia has also been studied by several workers.
Minot and Isaacs,8 and Gramen9 tried in vain to produce leukemia by means of
transfusion of leukemic patient’s blood. On the other hand, a number of experi-
ments on production of the human leukemia in various kinds of animals have
been reported, of which some noteworthy results may be mentioned. Sellards
and Baetjer10 maintained that they had succeeded in producing abortive leukemic
changes in mice tested by materials of man. So far, however, none has claimed
the success of transmission of human leukemia with cell-free materials.

Now as to leukemia associated with sarcoma, such experiments have been
reported in chicken leukemia by Engelbrecht-Holm,11 Pikovski12 and others. Re-
garding human reticulosarcoma, the author, however, could find no compatible
experimental references available here.

From the experimental data presented, it may be said that leukemic reticulo-
endotheliosis (Downey),14 a type of the human leukemia, or a mixed form of
reticulosarcoma with myelosis (Lubbers),15 is present.

SUMMARY

1. Five human lymphatic and myeloid reticulosarcomas were transmitted to
fowls in series.
2. The pathologic changes produced in fowls consisted in proliferation of
reticulo-endothelial cells (reticulosarcoma and allied conditions) or those of im-
mature myeloid cells (myelosarcoma, myelosis or myeloid metaplasia). These
different forms of leukosis may occur alternately in subsequent generations or
simultaneously in the same bird. Neither erythroleukosis nor lymphatic leukosis
was encountered. These observations conform with our earlier experience gained
in experimental studies of human leukemia.
3. The blood picture of infected fowls was consistently aleukemic either with
marked leukopenia or slight leukocytosis. There was usually an increase in ma-
ture granulocytes at the expense of lymphocytes. Anemia was usually slight.
4. The causative agent can pass through a Chamberland L2 filter.
5. The infective agent can be demonstrated in the blood of patients, in fluid
from the thoracic cavity, in the enlarged lymph nodes as well as in the human
tumors.

REFERENCES

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Experimental Studies on Reticulosarcoma

AKIRA S. SAKAMOTO