THE RELATION OF LEUKOSIS TO SARCOMA OF CHICKENS*

I. SARCOMA AND ERYTHEROLEUKOSIS (STRAIN 13)

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PLATES 24 TO 28

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All known causative agents of leukosis and sarcoma of chickens stimulate either a single type of cell or several closely related types of cells to unrestricted multiplication (1, 2). The association of sarcoma with leukosis was noted by us in 1930 among chickens inoculated with our leukosis Strain 1 and also as a spontaneous disease (3); but the etiological relationship of these two diseases was not determined. There was no evidence that the agent of leukosis Strain 1 is capable of producing both sarcoma and leukosis. McIntosh (4) has shown recently that treatment with tar is followed by the development of neoplasms that are transmissible by filterable viruses. This finding suggests that viruses that produce neoplasms are widespread among chickens.

Oberling and Guérin (5) described an agent that in their opinion is capable of producing both leukosis and sarcoma, and probably also carcinoma. They suggest that the common agent of leukosis may “mutate” into an agent with affinity for both mesodermal and ectodermal tissues. Their experiments have been repeated with the same results by Troisier (6) and partially confirmed by Rothe Meyer and Engelbreth-Holm (7), the latter workers having described a strain that produces sarcoma and leukosis but not carcinoma. There are no reports on the incidence of neoplasms among the uninjected control chickens by any of these workers. The experiments described here were undertaken to determine whether a transmissible disease of

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fowls, known as Strain 13 is caused by one or several viruses. This strain is characterized by the formation of endothelial neoplasms in the blood-forming organs often associated with erythroblastic proliferation.

One of us (May, 1933) made intravenous passages with our leukosis Strain 1, from a chicken (No. 442) that had erythroleukosis, into four healthy chickens.

![Text-fig. 1. Origin of Strain 13.](image)

**Notes to the Text-Figures**

Abbreviations: S = sarcoma, E = erythroleukosis, M = myeloid leukemia, 0 = inoculation unsuccessful.

Route of injections: | = intramuscular, : = intravenous, ! = intramuscular and intravenous.

*Microscopic examination was omitted.

In addition to erythroleukosis, two of the inoculated chickens developed sarcoma at the site of intravenous injection. One developed erythroleukosis in association with sarcomatosis of the blood-forming organs, and the fourth was apparently unaffected (Text-fig. 1). Since that time more than thirty-five passages have been made with either blood or tumor tissue and the inoculated birds developed sarcoma or sarcoma in combination with erythroleukosis. The diseases transmissible with material deriving from Fowl 442 will be named Strain 13.

Fowl 442, from which Strain 13 originated, was injected intravenously with dried blood of No. 2256 on Apr. 4, 1933. Jan. 18, 1932, three chickens were
injected with fresh blood of No. 2256, and one of them developed erythroleukosis.
From Jan. 20, 1932, to Aug. 9, 1934, twenty-nine chickens were injected with
dried blood of No. 2256, of which twelve developed erythroleukosis or myeloid
leukemia as indicated by the examination of the blood and by the gross findings
at necropsy. The blood-forming organs from six of these chickens were studied
microscopically and showed leukemia unassociated with sarcoma (Text-fig. 1). No.
442 was not examined microscopically. Although the blood smear and gross
postmortem findings of this chicken were characteristic of erythroleukosis, its
blood produced sarcoma about the injected wing vein in two of the three chickens
that were inoculated (Text-fig. 1). These data indicate that Strain 13 arose in
No. 442. The agent of Strain 1 has either assumed the ability to produce sarcoma
or a sarcoma virus present in No. 442 has contaminated it.

We have assumed that in Strain 13 we were dealing with a mixture
of two agents and have attempted to separate them. The problem
proved to be intricate. The agent of this sarcoma strain had an
affinity for connective tissue cells as well as for endothelial cells of the
blood-forming organs (bone marrow, spleen, and liver) and affected
most chickens, whether injected intramuscularly or intravenously.
In most instances of erythroleukosis that were studied microscopically,
the small sample of marrow examined showed diffuse sarcomatosis or
endothelial proliferation with a profound derangement of the vascular
bed of the marrow; but sarcoma of the injected breast muscle was
often found unassociated with erythroleukosis. It seemed doubtful
whether the erythropoietic changes produced by Strain 13 were
identical with the classical erythroleukosis described by Ellermann,
for they may have been secondary to the endothelial lesions caused by
this virus. In most instances they could be readily distinguished on
gross postmortem examination from primary erythroleukosis. Eryth-
roleukosis of Ellermann is characterized by progressive, seemingly
autonomous proliferation of erythroblasts that invade the blood and
accumulate in the pulp of the spleen and in the capillaries of many
organs; e.g., liver, lung. Retarded or arrested maturation and leuko-
stasis distinguish erythroleukosis from secondary erythroblastic
hyperplasia, the latter being associated with normal maturation of
erthroblasts and with no leukostasis. Most instances of erythro-
leukosis produced by Strain 13 are modified by coexistent diffuse
endothelial neoplasms, but pure instances of erythroleukosis of the
bone marrow occur among the passages of this strain and histological
Anatomical Characteristics

Tumors produced with intramuscular inoculations of this strain (Fig. 1) are firm and exude large amounts of mucinous material. They often contain cysts filled with such material (Fig. 7), and hemorrhages. They grow rapidly, and 2 to 3 weeks after injection into young chickens may occupy most of the breast muscle. Softening and hemorrhages into the surrounding tissues frequently occur. Microscopically, the tumors are composed of cells that resemble closely those of the common spindle cell sarcomata (Fig. 8) and occasionally they contain multinuclear giant cells (Fig. 9). Yet it is doubtful whether these cells are of connective tissue origin, because the malignant cells of the tumor caused by the virus of Begg and Murray in the breast muscle are similar to those produced by Strain 13 but have been traced to endothelium by these observers.

On gross examination internal sarcomatosis was usually suggested by hematomata, with no gross tumors, or occasionally gray tumor nodules or diffuse infiltrations. The liver, spleen (Fig. 3), and bone marrow (Fig. 4), and the gonads were the most frequent sites of neoplasms whose endothelial character was indicated by continuity with endothelium, and presence of blood in channels formed by tumor cells. Microscopically, proliferations of endothelial cells associated with derangement of the vascular system were usually but not always found at the site of the hematomata. Diffuse sarcomatosis of the spleen with replacement of the lymphoid tissue and dilatation of the sinuses is shown in Fig. 11. Neoplastic proliferation of endothelial cells in the lumen of a blood vessel of the spleen is shown in Fig. 12.

Erythroblastic proliferation caused by Strain 13 is, in most instances, readily distinguishable from that produced by the common leukosis strains (e.g. our Strain 1) by gross examination of either the bone marrow or the spleen. In the usual type of erythroleukosis of Ellermann, the bone marrow is firm, red, and can be removed as a solid cylindrical mass, but in Sarcoma 13 the marrow is usually soft, "watery," and hemorrhagic (Fig. 4). The spleen in the more common erythroleukosis (Fig. 2) is greatly enlarged and is uniformly red, whereas in Strain 13 it is only slightly or moderately enlarged, and is gray because of replacement with tumor tissue; or it contains one or several hematomata (Fig. 3).

Intravenous inoculation in very young chickens is frequently followed by conspicuous weakness of the legs. This is apparently due to extensive hemorrhage in the bone marrow (Fig. 4) that can often be seen through the intact cortex of femur and tibia, and occasionally separates the proximal epiphysis from the diaphysis. A tumor that measured about 2 x 1 x 0.5 cm., and had invaded the cortex of the tibia, was found in a young chicken (Fig. 4).

Sections of the bone marrow from 52 chickens that were successfully inoculated with Strain 13 were examined.
(a) 17 showed neither erythroleukosis nor sarcoma of the bone marrow; (b) 21 showed both; (c) 6 showed evidence of sarcomatosis of the bone marrow; (d) 7 showed evidence of erythroblastic proliferation resembling erythroleukosis of Ellermann, and sarcoma in some organ other than bone marrow; (e) 1 showed erythroleukosis unassociated with sarcomatosis. Thus only one (No. 73) of the chickens studied microscopically had pure erythroleukosis indistinguishable from that produced by Strain 1. In addition, however, seven chickens (group d) showed erythroblastic proliferation of the bone marrow unassociated with maturation of erythroblasts and sarcoma of the muscles, spleen, or some other organ. In four of these birds erythroleukosis was advanced, in three it was slight. Several birds had hematoma in organs other than the bone marrow but the erythroblastic hyperplasia of the marrow, unlike that which follows loss of blood, was unassociated with maturation and was indistinguishable from that which characterizes incipient erythroleukosis (Fig. 2 (8)). These findings strangely support the assumption that the virus of Strain 13 may stimulate independently erythroblasts as well as endothelial cells. It is possible that parts of the unexamined marrow of some of the eight chickens included in groups d and e had sarcomatous changes, but it does not seem probable that this is true for all members of these groups. Moreover, the occurrence of hemorrhage or sarcoma in one part of the bone marrow does not explain proliferation of erythroblasts with arrested maturation in other parts of the marrow.

Sarcomata were found in the ovary, testis (Fig. 10), and less frequently in the lung, kidney, skin (Figs. 15-17), and heart. Hemorrhage from spleen or liver was often the immediate cause of death.

The histogenesis of the changes produced by this strain needs further investigation. The tumors produced in the breast muscle resemble those of connective tissue origin (Figs. 7-9), while most neoplasms found in the skin and the internal organs appear to arise in situ from endothelium. Examples of endothelial neoplasms are shown in Fig. 5 (bone marrow), Figs. 15-17 (skin), Figs. 13 and 14 (ovary). Endothelial hyperplasia, such as shown in Fig. 18, was often found in the liver of chickens injected with Strain 13. Side by side with distinctly endothelial neoplasms there were solid masses of sarcoma cells whose origin was not determined. The changes in the spleen, characteristic for Strain 13 and illustrated in Figs. 11 and 12, have the morphological characteristics of a neoplasm that has arisen through malignant transformation of endothelial cells of the spleen. Similar alterations were found in the bone marrow, ovary (Figs. 13, 14), and testis.

The formation of blood cells from endothelial cells was not seen in any of these preparations. The channels lined with neoplastic endothelium either contained normal blood cells (Figs. 5, 18) or, in the presence of coexistent erythroleukosis, they contained basophile erythroblasts (Figs. 10, 13, 14).
Transmission Experiments

A survey of the passages made with Strain 13 is shown in Text-fig. 2. In this figure only those chickens are recorded (fifth to the eighteenth subpassages) from which further subpassages were made and details of significant experiments will be given in the text.

Text-fig. 2 resembles the similar figure of Rothe Meyer and Engelbreth-Holm which shows the passages of their strain that produces both sarcoma and leukosis. These authors found that intravenous transmission produces erythroleukosis almost exclusively while intramuscular transmission produces sarcoma occasionally associated with erythroleukosis. The results of our first five passages appeared to confirm fully the findings of Rothe Meyer and Engelbreth-Holm, until it was discovered that the chicken inoculated intravenously and believed to have erythroleukosis only, had also diffuse endothelial sarcomatosis of several organs. Diffuse endothelial neoplasm of the blood-forming organs has never before been observed in the chicken, so far as we are aware, and distinguishes the avian sarcomata thus far described from our Sarcoma 13.

Intramuscular Inoculations.—Oberling and Guérin believe that the type of disease produced by the common agent of leukosis and sarcoma depends mainly on the route of introduction, and that we have failed to note the ability of our leukosis Strain 1 to produce sarcoma because our inoculations were intravenous. This, however, is not the case, for our leukosis Strains 1, 2, and 5 do not produce spindle celled or fibroblastic tumors resembling the Rous sarcomata after intramuscular inoculations. The intravenous route of transmission of Strain 1 was chosen by us after preliminary experiments had made evident that leukosis Strain 1 is best transmitted by intravenous inoculations.

The causative agent of leukosis Strain 1 after intramuscular inoculations produces systemic disease (erythroleukosis or, less often, myeloblastic leukemia) with no tumors at the site of injection; but most adult chickens inoculated in this manner remain healthy. Sarcomata such as those studied by Rous, on the contrary, are often readily transmitted by intramuscular injections, and it is possible that if this route of introduction is practiced with a mixed virus of leukosis and a sarcoma, it may occasionally lead to the isolation of the sarcoma virus. This, however, was not observed. Successive intramuscular inocula-
Text-Fig. 2. Passages of Strain 13.
tions, examples of which are shown in Text-figs. 3 and 4, produced either seemingly pure sarcoma or sarcoma in combination with erythro-leukosis; but among the passages made from chickens with apparently pure sarcoma, there occurred again sarcoma and leukosis. Most chickens with metastatic sarcoma of the bone marrow showed the blood picture of either anemia or erythroleukosis.

Sarcoma 13 was usually associated with extensive hemorrhages, and the microscopic differentiation of erythroleukosis from secondary anemia was difficult. It is possible that the small sample of marrow taken for microscopic examination was normal, while foci of sarcoma or leukosis were present in parts of the unexamined marrow. It is also possible that a chicken with no evidence of erythroleukosis after inoculation still carried the introduced virus. However, with few exceptions, when the blood smear indicated anemia or erythroleukosis, microscopic examination of the marrow showed diffuse sarcomatosis with or without erythroblastic hyperplasia. The reverse was also true; when the circulating blood appeared normal, the marrow showed only slight or no evidence of sarcomatosis.

Successive intravenous subpassages (see Text-fig. 2), with blood of chickens that showed anemia or erythroleukosis, have likewise failed to isolate the agent of erythroleukosis; but all chickens successfully inoculated by the intravenous route and examined microscopically, showed, with two exceptions, sarcomatosis of the blood-forming organs.

Chickens inoculated intramuscularly and intravenously with minute amounts of blood (0.00001 cc.) from a chicken that had both sarcoma and erythroleukosis, developed seemingly pure erythroleukosis. A similar subpassage with minute amounts of blood (0.00001 cc.) from one of these chickens, yielded again seemingly pure erythroleukosis. Inoculation with a large amount of blood (1 cc.) from the same chicken produced, however, sarcoma in the injected breast muscle. These puzzling results were fully explained by the microscopic examination which showed that the chicken injected with 0.00001 cc. of blood and believed to have pure erythroleukosis, also had an extensive diffuse sarcomatosis of the blood-forming organs. These experiments are more fully described below. Successive intravenous subpassages failed to yield a virus that would produce erythroleukosis only. It
cannot be expected to do so because intravenous inoculation produces diffuse sarcomatosis of the internal organs as regularly as intramuscular injection produces sarcoma of the injected muscle.

**Attempts to Separate the Hypothetical Sarcoma Agent from the Hypothetical Leukosis Agent by Injecting Diminishing Amounts of Blood or Tumor Tissue**

If Strain 13 is composed of a mixture of two agents and if there is a difference in the effective concentration of these agents in a given inoculum, then by injecting diminishing amounts of it one should reach a dose that produces only sarcoma or leukosis. Since the concentration of the transmitting agent of leukosis in the blood is variable and may be as high as one millionth of a cubic centimeter (9) and each dose has to be tested on several chickens, the experiments reported here cannot be regarded as complete.

**Intramuscular Titration.**—The blood of a chicken that had sarcoma and leukosis was inoculated in diminishing amounts into the muscles of the left wing and left breast of six chickens, with the following results (Passage X i).

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Amount injected</th>
<th>Period of observation</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>275</td>
<td>0.1 cc.</td>
<td>D 16</td>
<td>Sarcoma and erythroleukosis</td>
</tr>
<tr>
<td>274</td>
<td>0.1 cc.</td>
<td>D 30</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>273</td>
<td>0.001 cc.</td>
<td>D 26</td>
<td>Sarcoma and erythroleukosis</td>
</tr>
<tr>
<td>272</td>
<td>0.001 cc.</td>
<td>D 24</td>
<td>Sarcoma and erythroleukosis</td>
</tr>
<tr>
<td>271</td>
<td>0.00001 cc.</td>
<td>D 21</td>
<td>Erythroleukosis</td>
</tr>
<tr>
<td>270</td>
<td>0.00001 cc.</td>
<td>D 30</td>
<td>Erythroleukosis</td>
</tr>
</tbody>
</table>

**Abbreviations Used in the Tabulations**

D = died, K = killed. The figures that follow the letters D or K show the period of observation, in days, after inoculation; e. g. "D 28" denotes that the chicken died 28 days after inoculation.

Route of injection: i.m. = intramuscular, i.v. = intravenous.

Most of the chickens used were young Barred Rocks, or White Leghorns; their age varied from a few to about 100 days.

Intramuscular injection of 0.001 cc. of blood produced both erythroleukosis and sarcoma, but 0.00001 cc. produced erythroleukosis only, with no tumor at the site of injection. No tissues were taken for microscopic examination from the
chickens with seemingly pure erythroleukosis. Subpassage was made from one of these chickens (No. 271) by injecting 0.00001 cc. of its blood into the wing vein and pectoral muscle of three chickens (Passage XI h). These chickens also died of erythroleukosis in from 36 to 50 days after the inoculation with no grossly detectable tumors in the injected breast muscle. The microscopic examination of one of these chickens (No. 293) showed extensive diffuse sarcomatosis of the blood-forming organs. At the time these experiments were made the significance of internal sarcomatosis was not appreciated and, with the exception stated, the diagnosis was based solely on blood smears and on gross postmortem examination. It now appears highly probable that all chickens of this series had sarcomatosis of the bone marrow because of the multiple hematomata noted in the gross in these organs. When large amounts of blood (1 cc.) from No. 293 were injected intramuscularly into each of two chickens, sarcoma developed in both at the site of injection (Passage XII g). Microscopic study revealed that No. 293 had internal sarcomatosis as well as erythroleukosis.

These experiments indicate that the blood of chickens with internal sarcomatosis transmits the disease in amounts as small as 0.00001 cc. The results now to be described suggest that the intravenous route of injection may be superior to the intramuscular route for the demonstration of small amounts of virus.

In an earlier experiment (Passage IX d) diminishing amounts of dried blood from a chicken (No. 3116) that had sarcoma and showed no gross or microscopic evidence of leukosis, were injected intramuscularly and intravenously into fifteen chickens with the following results.

<table>
<thead>
<tr>
<th>Amount injected</th>
<th>Route of injection</th>
<th>Period of observation</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>i.m.</td>
<td>D 39. Sarcoma and erythroleukosis. K 124. Sarcoma*</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>i.v.</td>
<td>D 45. Sarcoma and erythroleukosis</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>i.m.</td>
<td>D 84. Sarcoma. * K 84. Negative</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>i.v.</td>
<td>D 26. Erythroleukosis. * D 30. Erythroleukosis*</td>
<td></td>
</tr>
<tr>
<td>0.002</td>
<td>i.m.</td>
<td>K 84. Negative. K 84. Negative</td>
<td></td>
</tr>
<tr>
<td>0.002</td>
<td>i.v.</td>
<td>D 51. Erythroleukosis. * K 90. Negative</td>
<td></td>
</tr>
<tr>
<td>0.0002</td>
<td>i.m.</td>
<td>D 56. Negative. K 84. Negative</td>
<td></td>
</tr>
<tr>
<td>0.0002</td>
<td>i.v.</td>
<td>K 90. Negative. K 90. Negative</td>
<td></td>
</tr>
</tbody>
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Negative means that there was no evidence of leukosis and sarcoma.
* These chickens have not been examined microscopically.

The results of this experiment were essentially the same as those already described. Small amounts (0.01 and 0.002 cc.) of dried blood
were more active after intravenous than after intramuscular injection. Small amounts of blood appeared to produce erythroleukosis only; larger amounts of blood produced both leukemia and sarcoma. We now believe that all of these chickens with “pure” erythroleukosis had, in addition, an internal sarcomatosis. In the previous experiment, fresh blood of leukotic chickens was used for inoculations; in the present experiment, dried blood was used from a chicken that during life showed no evidence of leukemia, and on postmortem examination appeared to be free of leukemia.

A similar experiment (Passage VIII f, g), made with sarcoma obtained from a chicken (No. 3972) that showed no gross or microscopic evidence of leukemia, was less successful. Three chickens injected intravenously with 0.5 cc. of blood remained healthy; but one of two chickens that were injected intravenously with 0.5 cc. of a tumor extract died of erythroleukosis. Also, one of two chickens injected intravenously with 0.01 cc. of the same tumor extract died of erythro-leukosis.

These experiments show that small amounts of blood or tumor tissue inoculated intravenously produce erythroleukosis, and larger amounts inoculated intramuscularly produce sarcoma at the site of injection, even though the chicken whose tissues were used for inoculation had sarcoma unassociated with erythroleukosis. These results strongly support the view that the same agent that produced sarcoma was responsible for the development of erythroblastic proliferation.
Inoculation of Chickens Immune to Leukosis

Chickens that are resistant to one inoculation with tissues containing leukotic cells are, with rare exceptions, resistant to reinjections with similar material (10) and can therefore be regarded as immune to leukosis. Most chickens immune to leukosis Strain 1 were resistant to inoculations with leukosis Strain 2 (10).

In the next experiments, chickens that were resistant to repeated injections with leukotic tissues were injected with Strain 13 and developed sarcoma unassociated with leukosis. This procedure appeared to be promising in the separation of the assumed agents of sarcoma and of leukosis; but in the subpassages made from such chickens leukosis invariably made its appearance.

Experiment I.—(Passage VIII b.) Five chickens that resisted repeated inoculations with tissues of leukotic chickens were injected intramuscularly with tumor tissue of Strain 13. All five chickens developed sarcoma unassociated with leukosis. Sarcoma also developed in all of the four injected control chickens, and in one chicken it was associated with erythroleukosis combined with myeloid leukemia. Three control chickens that had sarcoma only, died or were killed within 25 days after injection, whereas the fourth, that also had leukosis, lived for 45 days. It is probable that the first three chickens would have developed blood changes if the rapidly growing tumors had not caused their death soon after the injection. The leukosis-immune chickens were killed 20, 48, 56, 239, and 278 days, respectively, after injection.

The following is a brief history of these immune chickens.

No. 2960 was inoculated June 27, 1932, with blood, leukosis Strain 1; Dec. 29, with plasma, leukosis Strain 1; June 26, 1933, with blood, leukosis Strain 2; Oct. 5, with tumor tissue, sarcoma Strain 13. It was killed Dec. 25 and had two tumor nodules, each measuring about 3 cm. in longest diameter at the sites of injection. There was no evidence of metastases or leukosis.

No. 3116 was inoculated Dec. 3, 1932, with blood, leukosis Strain 2; June 8, with blood, leukosis Strain 2; Oct. 5, with tumor tissue, sarcoma Strain 13. It was killed Nov. 13, when the tumor in the injected breast muscle measured 7 x 3 x 2 cm. There was a somewhat smaller tumor in the injected leg muscles. There was no evidence of metastases or leukosis.

No. 3410 was inoculated Jan. 24, 1933, with dried blood, leukosis Strain 2; June 26, with fresh blood, leukosis Strain 2; Oct. 5, with tumor tissue, sarcoma Strain 13. It was killed Dec. 1, and the postmortem findings were similar to those of No. 3116.

No. 3422 was inoculated Jan. 24, 1933, with blood, leukosis Strain 1; June 26, with blood, leukosis Strain 2; Oct. 5, with tumor tissue, sarcoma Strain 13. A
tumor nodule that was about 1.5 cm. across, developed in the injected muscles Nov. 21, but slowly regressed.

No. 3530 was inoculated June 16, 1933, with blood, leukosis Strain 2, and Nov. 5, with tumor tissue, sarcoma Strain 13. The tumors that developed at the sites of injections measured about 2.5 x 1 x 1 cm., Nov. 24, in both breast and leg muscles, but slowly regressed. The regressing tumor was successfully implanted Jan. 19 in three of four chickens injected. No. 3530 was reinjected intramuscularly with sarcoma Strain 13, but remained healthy.

Experiment 2.—Eight immune and two control chickens were injected with tissue of sarcoma Strain 13. The two control and four of the immune chickens developed sarcoma; the controls also had severe anemia, whereas the blood of the immune chickens appeared normal. One of the immune chickens (No. 3528) died with adenocarcinoma of the oviduct and extensive carcinomatosis of the peritoneum. Three chickens were inoculated with tissues of this tumor but all remained healthy. This was the only carcinoma found among the chickens injected with this strain. There appears to be no etiological relationship between carcinoma and Strain 13.

The following is a brief history of the immune chickens that were successfully reinjected with sarcoma Strain 13.

No. 2277 was inoculated Dec. 9, 1931, and Apr. 18, 1932, with blood, leukemia Strain 1; Dec. 3, 1932, with blood, leukemia Strain 2; May 3, 1933, with tumor tissue, leukemia Strain 2; July 26 and Nov. 1, with tumor tissue, sarcoma Strain 12. The first injection with sarcoma Strain 13, on Jan. 8, 1934, was unsuccessful, but the second injection of tumor tissue of this strain, made Feb. 26, was followed by the development of a tumor in the injected leg muscles. Three of the chickens injected in July with this tumor developed sarcoma as well as erythroleukosis.

No. 3108 was inoculated in a similar manner, twice with blood of leukemia, Strain 2; twice with tumor, sarcoma Strain 12; twice with sarcoma Strain 13. Only the second injection with Sarcoma 13 was successful. The first injection with Strain 13 was made with material of low virulence.

No. 3152. The history of this chicken is similar to No. 3108, but in this immune chicken there were extensive metastatic sarcomata in the liver and kidneys. The spleen and bone marrow appeared normal and there was no evidence of leukemia.

No. 3521 received two intravenous inoculations with leukemia Strain 2 followed by intramuscular inoculations with sarcoma Strains 11 and 12, and finally by intravenous and intramuscular inoculations with Strain 13. Sarcomata were found in the liver and kidney but there was no tumor at the site of intramuscular injection and it is doubtful whether the sarcomata were actually caused by Strain 13.

The history of the four chickens immune to leukemia in which subsequent inoculation with sarcoma Strain 13 was unsuccessful was similar to the histories given above.
The experiments show that resistance to leukemia is not associated with resistance to Sarcoma 13. Chickens immune to leukemia, when inoculated with Strain 13, appeared to develop pure sarcoma, but erythroleukosis invariably occurred in the subpassages made with sarcoma tissue from these chickens. It is probable that some of these leukemia-immune chickens were carriers of the leukemia virus, but we expected that some of them would be free of the virus of leukemia as well as immune to it. In none of these chickens, however, was Strain 13 deprived of its ability to produce leukemia.

The experiments can be interpreted in several ways. It can be assumed that the virus of sarcoma Strain 13 is unrelated to the virus of leukemia Strain 1. The chickens immune to leukemia developed pure sarcoma after intramuscular injection with sarcoma Strain 13, and did not develop erythroleukosis because the sarcoma did not metastasize to the bone marrow. Localization of the disease to the site of injection can be partially explained by old age of the immune chickens, and also by the fact that most of them were destroyed soon after the sarcoma made its appearance. It is possible that successful intravenous inoculation of sarcoma Strain 13 into chickens presumably immune to leukemia would produce erythroleukosis.

Since the presence of live cells prevents the inactivation of the associated virus by immune sera (11), it seemed possible that if chickens immune to leukemia are inoculated with cell-free extract of Sarcoma 13, they might destroy the hypothetical leukemia virus but not the sarcoma virus.

Experiment 3.—In Passage X b, four chickens were injected intramuscularly with desiccated tumor tissue of Sarcoma 13 after two unsuccessful inoculations with leukemia Strain 1. All remained healthy, whereas four of five young control birds injected in a similar manner developed tumors at the site of inoculation. 78 days later the same four chickens, immune to leukemia Strain 1, were reinjected for the second time with sarcoma tissue exposed to –30°C. during 30 minutes (Passage X j), this time with success. Three of the four chickens developed sarcoma; it regressed in two. Tumor tissue from the third chicken was injected intramuscularly into three chickens and produced only sarcoma (Passage XI g), but in the later subpassages, made by intravenous and intramuscular inoculation, erythroleukosis again appeared.

Experiment 4.—Each of six immune and four control chickens received intramuscular injections of dried blood and dried spleen and intravenous injection of
dried blood (Strain 13). All remained healthy and four controls developed sarcoma with anemia or erythroleukosis.

Each dose was approximately 10 mg. Of the six immune chickens injected, three were resistant to leukemia Strain 1, sarcoma Strains 11 and 12; two were resistant to leukemia Strain 2, sarcoma Strains 11 and 12; and one was resistant to sarcoma Strains 11 and 12. Incidentally, this experiment shows that the transmitting agent of Strain 13 present in the blood and spleen, is readily preserved by drying.

These experiments suggest that most chickens immune to leukemia are resistant to the cell-free virus of Sarcoma 13 and that those that are susceptible are unable to deprive the virus of Strain 13 of its ability to produce erythroleukosis. The studies of Andrewes (12) indicate that immunity to the viruses of chicken tumors is not strictly specific. Further investigations are needed to determine whether resistance to a cell-free virus develops in birds with advancing age and whether it can be produced by inoculations with a heterologous virus.

Neutralization Experiments

In some animals that recover from sarcoma, or leukemia, or that have the disease in a chronic form, antibodies that neutralize the free oncogenic agent appear in the blood. The antibodies directed against the agents of sarcomata have been thoroughly investigated by Andrewes (12), who found that they possess some specificity in addition to a group effect. Antibodies active against the agents of leukemia, according to the few observations made (10, 13), also exert group and specific effects. It seemed possible that sera of chickens resistant to repeated inoculations with leukotic tissues would neutralize the hypothetical leukemia component of Strain 13.

The difficulties are numerous in conducting such neutralization experiments with leukemia agents on a scale large enough to be conclusive. In similar experiments with sarcoma a single chicken may serve as a test object for several serum-virus mixtures; in leukemia several chickens are required for each mixture. Some normal sera inhibit the action of the agent (12). The concentration of neutralizing substances in the serum is apparently small, and the determination of the optimal amount of serum and virus required to demonstrate
specific neutralization would require extensive preliminary experiments.

In one experiment (Passage 4304) none of six chickens developed tumors following injection with tumor filtrate, incubated at 37°C during 1/2 hour with varying amounts of a pooled serum of six leukosis-immune chickens.

In another experiment (Passage X j) five chickens were injected intramuscularly with a mixture of pooled sera obtained from chickens immune to leukosis and presumably cell-free tumor extract, kept at 37°C during 1 hour. They all developed sarcoma and none showed leukotic blood changes; only one (No. 266) was examined microscopically. The first two subpassages made from this chicken produced sarcoma only, but in the third subpassage erythroleukosis appeared again.

The immune sera were heated to 56°C during 40 minutes. The proportions of serum and extract varied, and the chicken from which further subpassages were made (No. 266) received a mixture containing 0.5 cc. of serum, 0.5 cc. of tumor extract, and 1 drop of fresh normal chicken serum. The remaining chickens received mixtures of serum and tumor extract in various proportions, the extremes of which were 0.1 cc. virus plus 0.9 cc. serum and 0.9 cc. virus plus 0.1 cc. serum.

In a third experiment (Passage XI b) an extract of desiccated tumor tissue was mixed with an equal volume of pooled, inactivated immune sera and incubated during 2 hours at 37°C. This mixture was injected intramuscularly into five chickens without ill effect, whereas all of the five control chickens injected with tumor extract alone developed sarcoma or erythroleukosis. The four immune chickens whose blood was used in this experiment were reinjected with the same tumor extract, and remained healthy.

These experiments suggest that the sera of chickens immune to leukosis have an inhibiting or neutralizing effect on the virus of Strain 13 as concerns its ability to produce tumors as well as leukosis. Neutralization is not always complete, however.

Transmission of Sarcoma 13 with Material Free from Living Cells

Desiccation.—Most, but not all, viruses of sarcoma and of leukosis are resistant to desiccation (1). It seemed possible that Strain 13 is caused by two viruses that differ in resistance to desiccation and that this procedure might separate them. But it was found that dried blood and tumor tissue of Sarcoma 13 produced both sarcoma and
erythroleukosis. Five of six dried samples tested produced tumors in all injected chickens; one sample was inactive.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Dried material</th>
<th>No. injected</th>
<th>No. of successful injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>3971</td>
<td>Tumor</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3116</td>
<td>Tumor</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4078</td>
<td>Tumor</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4304</td>
<td>Blood</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3014</td>
<td>Blood and spleen</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>3116</td>
<td>Blood</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

The desiccation in these experiments was done in the frozen state, as previously described (2). In one experiment tumor tissue was also dried *in vacuo* at room temperature. There was no significant difference between the tissues dried *in vacuo* in the frozen state and those dried at room temperature.

<table>
<thead>
<tr>
<th></th>
<th>Weight of fresh material</th>
<th>Loss of weight</th>
<th>Result of inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried in the frozen state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 min. in desiccator</td>
<td>0.946</td>
<td>28.5</td>
<td>Successful</td>
</tr>
<tr>
<td>1½ hrs. in desiccator</td>
<td>1.001</td>
<td>60.0</td>
<td>Unsuccessful</td>
</tr>
<tr>
<td>19 hrs. in desiccator</td>
<td>1.072</td>
<td>85.8</td>
<td>Successful</td>
</tr>
<tr>
<td>7 days in desiccator</td>
<td>—</td>
<td>85.8</td>
<td>Successful</td>
</tr>
<tr>
<td>Dried in the unfrozen state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 min. in desiccator</td>
<td>1.048</td>
<td>67.8</td>
<td>Successful</td>
</tr>
<tr>
<td>19 hrs. in desiccator</td>
<td>0.866</td>
<td>85.8</td>
<td>Successful</td>
</tr>
</tbody>
</table>

The foregoing experiment was undertaken for two reasons: (a) There are no data available that compare the results of drying oncogenic viruses in the frozen and unfrozen state. (b) It was thought that if the virus is living, there might be an optimum degree of dehydration beyond which it might perish. Dehydration, however, was complete under the conditions of the experiment within 19 hours *in vacuo*; when phosphorous pentoxide was renewed after this time and the material was kept in high vacuum for an additional 6 days, there was
no further loss of weight or of activity. From the chemical standpoint the materials thus dried are probably not water-free.

Freezing of tissue from Sarcoma 13 was done by submerging the minced tumor in alcohol chilled to $-30^\circ$C. during 30 minutes in a sealed test tube. Frozen and unfrozen material was tested on the same three chickens and there was no significant difference in the rate of development of tumor from the fresh and from the frozen material.

DISCUSSION

The evidence here presented supports the view that a single oncogenic virus may stimulate two different types of cells to seemingly neoplastic growth. This possibility has been suggested by several recent investigators (5, 7, 2) but abundant contradictory data have accumulated in the 2 decades since Rous and his associates first described viruses causing chicken sarcomata of various types. Several viruses with well defined individual characteristics have been described and none of them produced leukemia (1).

The strain here described produces sarcoma (endothelioma) and erythroleukosis. It originated in a chicken injected with leukemia Strain 1, which produces erythroleukosis and, less often, myeloblastic leukemia. Did Strain 13 arise from leukemia Strain 1 as a result of a “mutation” as suggested by Oberling and Guérin (5), or did Strain 1 become contaminated with a virus of a sarcoma that affects endothelial cells of the blood-forming organs? The characteristics of Strain 1 remained unchanged during 5 years of animal passages with the possible exceptional modification described here. Strain 13 also has remained constant during 1½ years of studies in which approximately 300 chickens have been used.

The experiments of McIntosh already quoted (4) may be taken to indicate that tumor-producing viruses are common among chickens. We found them among the experimental birds as well as among the uninjected controls. The possibility that Strain 13 consists of a mixture of two viruses seems, therefore, very likely, but the tests thus far applied have failed to separate them. However, a sarcoma agent that stimulates endothelial cells of the blood-forming organs of almost every young chicken inoculated, and discharges a virus into the blood in high concentration, is obviously not easily separable from a leukemia
agent that stimulates primitive blood cells but otherwise behaves like
the sarcoma agent.

Several factors are necessary for a virus to produce manifest disease. The number of disease-producing units in the new host must exceed a certain minimum. This is the probable explanation of the incubation period of leukosis, during which anatomical changes are not detected. It may be supposed that after intramuscular inoculation of Strain 13, the hypothetical sarcoma virus undergoes rapid multiplication and leads to the death of the animal before the leukosis virus reaches a concentration necessary to produce manifest disease. In the sub-passages of sarcoma both viruses are carried over into the new host but become manifest only under conditions that allow them to multiply and reach the concentration that would produce disease. This assumption is contradicted by the experiment that showed that a minute amount of the sarcoma tissue (0.00001 cc.) of an apparently leukosis-free chicken produced erythroleukosis only, whereas larger amounts produced sarcoma. It evidently requires a larger dose or a longer period of time to produce sarcoma in the breast muscle than it does to cause diffuse sarcomatosis and erythroleukosis of internal organs, such as spleen and bone marrow.

The observations suggest the possibility that the virus of Strain 13 produces sarcoma primarily and that erythroleukosis is the result of profuse secondary sarcomatosis of the bone marrow. Against this view are the histological characteristics of erythroleukosis that are indicative of a primary proliferative process. There was no difference in this regard between Strain 1 and Strain 13. Attempts to induce erythroleukosis by bleeding or chemicals that injure red cells have not been wholly successful, but pyrodin produced erythroblastic hyperplasia of the marrow resembling closely that produced by the leukosis viruses (3). Erythroleukosis unassociated with sarcoma was seen in very few chickens and in these cases only a small piece of marrow was examined. Sarcoma of the breast muscles may exist over long periods of time without causing blood changes. Histological examinations showed that the virus of Strain 13 caused a profound derangement of the vascular system with extensive hematomata, often with only scant evidence of malignant proliferation. It may be that this derangement is often the first step in the neoplastic transformation of
endothelium. The relation of this virus to endothelial cells and to primitive erythroblasts, as well as the very nature of erythroleukosis, needs further investigation. Erythroleukosis does not appear to be an etiological entity.

It is evident that the adenocarcinoma of the oviduct which was encountered in a leukemia-immune chicken killed 4 months after intravenous and intramuscular inoculations with Strain 13 was not produced by it since the three chickens into which the tumor tissue was implanted remained healthy. There is no evidence to support the opinion of Oberling and Guérin that viruses which produce neoplasms possess a high degree of adaptability, for the characteristics of both Strains 1 and 13 have remained constant during the past 18 months.

Tissue culture studies offer a less expensive and more promising procedure to determine whether a single virus is capable of producing both sarcoma and leukemia. Such studies have shown (14) that sarcoma cells of Strain 13 incubated at 38°C. retain the ability to produce sarcoma with erythroleukosis during a period of 67 days.

The experiments here described do not warrant definite conclusions regarding the origin and nature of Strain 13. The possibilities considered may be summarized as follows:

1. A single virus known as Strain 13 produces sarcoma (endothelioma) and erythroleukosis. (a) It developed as a variant of the virus of leukemia Strain 1. (b) It occurred spontaneously in a chicken inoculated with Strain 1, but has no etiological relationship to this strain.

2. Strain 13 consists of two viruses, one producing sarcoma (endothelioma), the other (the virus of Strain 1) erythroleukosis. The sarcoma virus occurred independently in a chicken inoculated with the leukemia strain.

SUMMARY

A transmissible strain of sarcoma (Strain 13) is described that took its origin in a chicken inoculated with leukemia of Strain 1. The virus of Strain 13 produces sarcoma in the breast muscle injected with it and after intravenous inoculation it produces diffuse sarcomatosis of the spleen, bone marrow, and several other organs.
The available evidence suggests that Strain 13 is caused by a single virus with ability to produce diffuse endothelial growth in the blood-forming organs associated with extensive hematomata, and erythroblastic proliferation.

The strain can be readily transmitted by material free from live cells and preserved by drying. As small amounts of blood as 0.00001 cc. from chickens with sarcomatosis of the internal organs transmit Strain 13 by the intravenous route.

By implantation sarcoma of Strain 13 can be transferred to chickens that are resistant to repeated inoculations with leukotic viruses.

BIBLIOGRAPHY


EXPLANATION OF PLATES

The photographs of the gross material were made from specimens preserved in Kaiserling solution, and the photomicrographs from sections stained with hematoxylin and azure II-eosin solutions according to the technic of Maximow. The magnifications shown are only approximate.

PLATE 24

Fig. 1. Sarcoma produced in the breast muscle by intramuscular injection of tumor tissue of Strain 13.
LEUKOSIS, AND SARCOMA OF CHICKENS. I

Fig. 2. Diffuse sarcomatosis of the spleen (S) contrasted with erythroleukosis (E) of that organ. Sarcomatosis causes slight enlargement of the spleen, increased consistency, and grey-white discoloration. Erythroleukosis causes moderate or great enlargement of the spleen and grey-red discoloration.

Fig. 3. Hematomata in the spleen and liver produced by proliferation of endothelial cells, with disruption of blood vessels. The immediate cause of death was hemorrhage from the liver.

Plate 25

Fig. 4. Sarcomatosis (S) Strain 13 of the bone marrow contrasted with erythroleukosis (E). In the marrow of chickens with sarcomatosis there are solid tumor masses (t) filling the cavity of the marrow and infiltrating the cortex of the bone; cysts (c), and hemorrhages (h). In erythroleukosis the marrow is uniformly grey-red.

Fig. 5. Bone marrow in Sarcoma 13 showing cavities lined with endothelial cells and filled with serum and nucleated erythrocytes. × 75.

Fig. 6. Bone marrow in Sarcoma 13 showing dense masses of spindle-shaped tumor cells. × 350.

Plate 26

Fig. 7. Mucinous degeneration with formation of cavities in sarcoma of the breast muscle. Azure II stains this mucinous substance blue. × 75.

Fig. 8. Shows the predominant type of cell of Sarcoma 13 produced by intramuscular inoculation. There are mitotic figures. × 650.

Fig. 9. Multinuclear giant cells in sarcoma of the breast muscle, produced by Strain 13. × 650.

Fig. 10. Section from a testis showing cavities lined by endothelial cells and filled with erythroblasts and erythrocytes. × 400.

Plate 27

Fig. 11. Diffuse sarcomatosis of the spleen with replacement of lymphoid tissue. The dilated sinuses are filled with erythroblasts and erythrocytes. × 350. The gross appearance of such a spleen is shown in Fig. 2.

Fig. 12. Malignant proliferation of endothelial cells narrowing the lumen of a blood vessel in the spleen. The vessel is filled with erythroblasts and erythrocytes and there is normal lymphoid tissue about the vessel. × 350.

Figs. 13 and 14. Diffuse sarcomatosis of the ovary. The neoplastic endothelial cells form sinusoidal channels filled with erythroblasts. Magnifications: Fig. 13 × 130; Fig. 14 × 400.

Plate 28

Fig. 15. Cavernous endothelioma of the skin containing erythrocytes in variable numbers. × 65.
FIG. 16. Shows another microscopic field of the same endothelioma. The cavities are smaller and there is solid tumor tissue in the lower part of the field. × 65.

FIG. 17. Higher magnification of the same endothelioma of the skin, showing the endovascular formation of tortuous blood channels arising seemingly from the endothelial cells lining the cavities. × 250.

FIG. 18. Diffuse hypertrophy and hyperplasia of endothelium of the liver, probably neoplastic. The capillaries are greatly distended with blood cells, and the liver cells are atrophied. The arrows point to remnants of liver cells. × 300.
(Stubbs and Furth: Leukosis, and sarcoma of chickens. I)
(Stubbs and Furth: Leukosis, and sarcoma of chickens. I)