LYMPHOMATOSIS, MYELOMATOSIS, AND ENDOTHELIOMA OF CHICKENS CAUSED BY A FILTERABLE AGENT*

I. TRANSMISSION EXPERIMENTS

BY J. FURTH, M.D.

WITH THE ASSISTANCE OF CHARLES BREDEIS

(From the Department of Pathology, Cornell University Medical College, New York)

PLATES 14 AND 15

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The filterable agent that will be described here produces in chickens leukosis of types that hitherto have not been successfully transmitted, namely lymphomatosis and myelomatosis, sometimes with leukemia and sometimes with tumor formation. In many chickens inoculated with this strain leukosis was associated with the formation of tumors that histologically resembled endothelioma. Infiltration of nerves often occurred among chickens inoculated with this strain, and in a few birds paresis or paralysis of legs or wings was noted. Thus this transmissible strain possesses characteristics ascribed to three different types of filterable agents; namely, (a) the agent of Ellermann and Bang (1) that causes leukosis of chickens; (b) the agents of Rous that stimulate connective tissues of chickens to neoplastic growth and may cause endothelioma (Begg (2)); and (c) the agent of Pappenheimer, Dunn, and Cone (3) that produces fowl paralysis.

In this article evidence will be presented to show that lymphomatosis, myelomatosis, endothelioma, and fowl paralysis occurring among the passages of this strain are caused by filterable agents. In the paper that follows, the relationship of these diseases and the anatomical lesions associated with them will be discussed. The available evidence is in favor of the assumption that a single agent can produce lymphomatosis, with or without paralysis, myelomatosis, and endothelioma.

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Nomenclature

Leukosis will be used as a collective term to designate diseases characterized by autonomous growth of the cells of the blood-forming organs of chickens. Leukosis is subdivided into lymphoid, myeloid, and erythroleukosis, according to the three types of blood-forming systems which may become affected. The adjectives leukemic, subleukemic, and aleukemic are used to indicate the degree of blood involvement.

In erythroleukosis the primitive erythroblasts multiply rapidly in the bone marrow (4), and, without maturing, enter the circulation in large numbers and accumulate in the pulp of the spleen and the capillaries of organs such as lungs and liver.

Myeloid leukosis occurs in two distinct forms: myeloblastomatosis and myelocytomatosis. Myeloblastomatosis is characterized by the appearance of enormous numbers of large mononuclear cells, presumably myeloblasts (Ellermann), in the blood. This type of leukosis is not associated with tumors and is caused by the same agent that causes erythroleukosis (5). In myelocytomatosis, myelocytes are numerous in the blood, whereas myeloblasts are few or absent. This form is usually associated with tumor formation. The differentiation between myeloblastomatosis and myelocytomatosis becomes unavoidable in the study of transmissible leukosis because the agent of the common type of transmissible leukosis (5, 6) produces erythroleukosis and myeloblastomatosis almost exclusively, while the strain to be described here produces lymphomatosis and myelocytomatosis. Mathews (7) has noted the difference between myeloid leukemia of Ellermann and myelocytomatosis, and described the latter disease under the name of "leukochloroma."

Lymphomatosis (lymphoid leukosis) of chickens is identical with the similar disease of mammals. It has been described recently by Mathews and Walkey (8).

Endothelioma presents great variation in location and in the histological characteristics of its lesions. It may occur as a solid tumor composed of large round cells, of which several are binucleated, or as an angiomatous growth with extensive hemorrhages, or, most commonly, as a tumor consisting of pleomorphic cells with numerous large necrotic areas surrounded by multinucleated giant cells. The nuclei of all these cells are similar: they are large and spherical, contain little chromatin (indicated by their pale color in hematoxylin-eosin-stained preparations), and have a large nucleolus.

Transmissible Strains.—The common type of transmissible strain described in previous reports (5) will be referred to as Strain 1, the strain described here as Strain 2.

LITERATURE

1. Chicken Leukosis.—Ellermann and Bang have stated that all types of avian leukosis are caused by one filterable virus, but the evidence presented by them is only sufficient to show that two types of leukosis, namely erythro- and myeloid
leukosis, are transmissible and are caused by the same filterable agent (5, 6). Andersen and Bang (9) failed, in numerous attempts, to transmit lymphatic leukosis, and Bang (10) expressed recently the view that lymphatic leukosis had so far not been successfully transmitted. Erythroleukosis and myeloid leukosis, on the other hand, are readily transmissible. Furth (5), Engelbreth-Holm (6), Stubbs and Furth (11), and Olson (12) have described transmissible strains that produced erythroleukosis and myeloid leukosis. Jármai (13) described a strain that produced only erythroleukosis. Battaglia and Leinati (14) observed a strain that produced erythroleukosis, myeloid leukemia, and “hemocytoblastic myelosis” (identical with Ellermann’s lymphoid leukosis).

2. Fowl Paralysis.—Van der Walle and Winkler-Junius (15) were the first to suggest, though on insufficient evidence, that fowl paralysis is caused by a filterable virus. The extensive investigations of Pappenheimer, Dunn, and Cone (3) have shown that fowl paralysis is transmissible. About 25 per cent of the chickens inoculated by them developed paralysis, whereas in uninoculated control chickens the incidence of this disease was about 7 per cent. The anatomical lesion leading to paralysis is, according to these investigators, in some instances an inflammatory process (edema, accumulation of lymphocytes, plasma cells, and monocytes) and in other instances, a neoplastic process (lymphoma or lymphosarcoma). They observed lymphomatous infiltrations of viscera in about 10 per cent of the paralyzed chickens and presented suggestive evidence that transmission of fowl paralysis is due to a filterable virus. Further evidence for the causation of fowl paralysis by a filterable agent was presented by Seifried (16), while the data presented by Biely, Palmer, and Asmundson (17) strengthen the evidence that the lymphoid infiltration in the nervous system and in the viscera may occur in response to a common agent.

3. Rous Sarcoma.—Begg (2) describes a strain of chicken sarcoma in which the predominating type of cell resembles endothelium. The filterable agent of this tumor causes accumulation of lymphocytes and macrophages, followed by a tumor-like growth of endothelium of blood vessels with metastases in distant organs. The agent of Begg’s endothelioma does not affect the blood-forming system and is immunologically related to other types of Rous sarcoma (Andrewes (18)). Roffo’s round cell sarcoma (19) is possibly another link between chicken leukosis and Rous sarcoma.

Notes on the Presentation of Experimental Data; Difficulties in Their Interpretation

The figures shown in the tables refer to inoculations made before Dec. 31, 1932. An occasional observation made since will be mentioned in the text. Birds dying of intercurrent diseases from 1 to 2 months after inoculation are listed in separate columns, those dying two months after inoculation without showing any evidence of leukemia are given among the unsuccessful inoculations.

The disturbances in which the leukocyte count exceeded 100,000 and immature
leukocytes formed the majority of the cells are called "leukemic." Those in which the leukocyte count was below 100,000 and sufficient immature leukocytes were present in the blood to make the type of leukosis recognizable are called "sub-leukemic," and the rest are called "aleukemic."

Instances in which the disturbance of one system was distinctly predominant are classified as myelomatosis, lymphomatosis, or erythroleukosis, those in which the involvement of two or more systems was conspicuous are given as "atypical" leukemia.

The incubation period of leukemia is variable; most of the chickens developed leukemia from 2 weeks to 2 months after inoculations, but in rare instances the incubation period was as long as 4 months. Thus our destruction of the animals after approximately 5 months is not an ideal procedure, but so far we have been able on account of limited space to keep only a few of the inoculated animals alive for longer periods.

Whereas in some instances it was extremely difficult to determine from post-mortem appearances the success of inoculations and the type of leukemia, diagnosis could easily be made from blood smears. Basophile lymphocytes (Fig. 4), often containing minute vacuoles (Fig. 5), were present in the blood smears of most of the chickens successfully inoculated with this strain, even in cases in which the hyperplasia of the myeloid and erythroblastic tissues was predominant. Since such cells were absent in the blood smears of all control chickens, including those injected with Strain 1, their presence in the blood was characteristic of Strain 2 and distinguished the erythroleukosis and myeloid leukemia of Strain 2 from those of Strain 1.

Origin of the Transmissible Strain 2

This strain originated in a Barred Rock chicken (No. 2255) of about 8 months of age. The chicken was received November 19, 1931, weighing 670 gm., and was kept as an uninjected control among chickens inoculated with the transmissible Strain 1. This was done to find out whether leukemia is transmitted by natural means from diseased to healthy animals. There was no evidence that healthy animals acquire leukemia by contact with leukemic chickens and the type of lymphomatosis which developed in this chicken was different from that caused by any known transmissible strain. Therefore it must be regarded as a spontaneous disease.

Blood smears of this chicken taken from Nov. 20, 1931, to Feb. 27, 1932, were normal. The blood smear taken on Mar. 28 showed a few immature red cells. By Apr. 13, the abdomen of this chicken was distended by fluid, and the blood smear showed many polychrome erythrocytes and a few polychrome erythroblasts. On Apr. 23, immature lymphocytes were in the circulation in large num-
bers, the white cell count was 17,500, and 7 days later it was 130,000, most of the cells being large lymphocytes (Figs. 3, 4), many in mitosis. Post mortem the carcass weighed 1050 gm., the liver 100 gm., the spleen 7 gm. The liver was thickly studded with partly confluent tumor nodes (Figs. 2, 6) and similar tumor nodes were found in the spleen, heart, and kidneys (Figs. 1, 9). The size of these tumors varied from those just visible to those measuring 1 cm. across. They were composed of large round cells like lymphocytes, and other larger, paler stained cells which appeared to form a syncytial mass (Fig. 8). The bone marrow was reddish grey and contained minute, well defined, greyish, tumor-like areas.

Transmission Experiments

The results of the inoculations with blood and emulsion of tumor cells of this chicken are shown in Table I, and some of the salient data on the successfully inoculated chickens are shown in Table II.

Thus all birds successfully inoculated developed lymphomatosis, two aleukemic, three subleukemic, and one leukemic. One chicken (No. 2572) died 53 days after inoculation with severe anemia associated with osteosclerosis (osteodystrophia).

Text-fig. 1 shows the sequence of passages attempted subsequently from 66 chickens and more details of the first 37 passages are given in Table III.

Of the attempts to transfer leukosis from 53 chickens only eight were unsuccessful (Text-fig. 1).

Table III shows that about one-third of all inoculated chickens developed leukosis. More than one-half (72) of the successfully inoculated chickens developed lymphomatosis, and in a smaller number of chickens (38) several blood-forming systems (including the lymphatic system) were markedly affected. In 19 birds myelomatosis was predominant, and other blood-forming systems were affected slightly, if at all. In eight birds the alterations histologically resembled erythroleukosis. Endothelioma with giant cell formation was found in 11 birds. Instances of all these diseases will be described in the paper that follows. Myelomatosis appeared in the second passage, endothelioma with giant cell formation in the third passage, and both reappeared in all subsequent passages.

In the following section the results of the transmission experiments will be analyzed, and attempted transmissions with material contain-
TABLE I
The Results of Injections with Blood and Tumor Cells of Chicken 2255 (Spontaneous Leukemic Lymphomatosis)

| Injection Method with Blood | Chicks Weighing | Chickens Weighing | Successful Transmissions | Intercurrent Disease |
|-----------------------------|----------------|------------------|--------------------------|---------------------|
| Intravenous                 | 150 to 350 gm  | 600 to 1000 gm   | 20                       | 1                   |
| Intramuscular and subcutaneous | 230 to 290 gm  | 650 to 690 gm    | 6                        | 1                   |
|                             | 250 to 300 gm  | 590 to 690 gm    | 3                        | 0                   |

TABLE II
Data on Chickens Successfully Inoculated with Material from Chicken 2255 (Spontaneous Leukemic Lymphomatosis)

| Chicken No. | Weight of Bird | Material Injected | Route of Injection | Amount Injected | Incubation Period | Duration of Illness | Length of Life after Inoculation | Type of Leukemia |
|-------------|----------------|-------------------|--------------------|-----------------|------------------|---------------------|-----------------------------------|------------------|
| 2573        | 1000           | Blood             | iv.                | 21              | 16               | 9                   | K* 25                             | sL               |
| 2643        | 650            | "                 | "                  | 21              | 14              | 14                  | D* 143                           | sL               |
| 2644        | 625            | "                 | "                  | 1              | 1               | D 84                | sL                                |                  |
| 2645        | 595            | "                 | "                  | 7              | 20              | D 84                | sL                                |                  |
| 2618        | 250            | Tumor             | im.                | 7              | 1               | K 8                 | aL                                |                  |
| 2619        | 290            | "                 | "                  | 1              | 1               | D 80                | aL                                |                  |

* D = died, K = killed.

Abbreviations Used in Text-Fig. 1 and in All Tables

Types of Disease.—L = lymphoid leukosis; Mb = myeloblastomatosis; Mc = myelocytomatosis; Er = erythroleukosis; En = endothelioma; Eng = endothelioma with giant cell formation; Sc = sarcoma formed by cells other than round cells; a (used only in Text-fig. 1) = atypical (mixed) leukosis; an = anemia; p = paralysis (neurolymphomatosis) without L of viscera.

Degree of Blood Involvement.—i = leukemic; s = subleukemic; a (used only in the tables) = aleukemic.

Route of Injection.—iv. = intravenous; im. = intramuscular; sc. = subcutaneous; ip. = intraperitoneal.

O = unsuccessful transmission; x = transmission attempted recently.
| Donor | No. of passages | Type of leukemia | Material injected | Route of injection | No. of transmissions | No. died of intercurrent disease | Type of leukemia |
|-------|----------------|------------------|-------------------|-------------------|---------------------|-----------------------------|-----------------|
|       |                |                  |                   |                   | Successful | Uncertain | Unsuccessful | Within 1 mo. | From 1 to 2 | Lymphomas | Myeloblastomas | Myeloblastoses | Erythroleukosis | Atypical | Unint. |
| 2255  |                | IL               | Spontaneous       | Blood             | sc. and im. | 1         | 0             | 3           | 4         | 1         | 1         | 1         | 1         | 1         | 1     |
| 2572  |                | Anemia           | Blood             | Blood             | sc. and im. | 1         | 0             | 0           | 3         | 1         | 1         | 1         | 1         | 1         | 1     |
| 2573  |                | sL               | “                 | Tumor             | im. and ip. | 0         | 0             | 0           | 4         | 2         | 1         | 1         | 1         | 1         | 1     |
| 2618  |                | aL               | Blood             | “                 | sc. and im. | 0         | 0             | 0           | 3         | 1         | 1         | 1         | 1         | 1         | 1     |
| 2619  |                | aL               | Blood             | “                 | sc. and im. | 1         | 0             | 0           | 7         | 1         | 1         | 1         | 1         | 1         | 1     |
| 2643  |                | IL               | Blood             | Dried blood       | sc. and im. | 21        | 0             | 0           | 10        | 1         | 11        | 2         | 1         | 4         | 3     |
| 2645  |                | sL               | Blood             | “                 | sc. and im. | 0         | 0             | 0           | 4         | 1         | 2         | 1         | 1         | 1         | 1     |
| 2768  |                | Atypical         | Blood             | “                 | sc. and im. | 13        | 1             | 0           | 4         | 2         | 1         | 2         | 3         | 1         | 1     |
| #   | Type | ID | Stage | Tumor | Treatment | iv | 1 | 2 | 1 | 1 | 1 | 2 |
|-----|------|----|-------|-------|-----------|----|---|---|---|---|---|---|
| 2778| aL   | II | Blood | iv.   | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| 2775| Atypical | II | Blood | iv.   | 4 | 0 | 7 | 1 | 1 | 2 | 1 | 1 |
| 2779| sL   | II | Blood | iv.   | 3 | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| 2801| Atypical | II | "    | "    | 3 | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| 2805| "    | II | Blood | "    | 2 | 0 | 3 | 1 | 1 | 2†| 2†| 2†|
| 2860| Anemia | II | "    | "    | 0 | 0 | 6 | 1 | 1 | 1 | 1 | 1 |
| 2698| aL   | II | Tumor | "    | 0 | 0 | 4 | 1 | 1 | 1 | 1 | 1 |
| 2748| iMc  | II | Blood | "    | 0 | 0 | 5 | 1 | 1 | 1 | 1 | 1 |
| 2870| iMb  | II | "    | "    | 4 | 0 | 2 | 1 | 3†| 1 | 1 | 1 |
| 2833| Atypical | III | Blood | "    | 0 | 0 | 1 | 4 | 1 | 1 | 1 | 1 |
| 2851| "    | III | "    | "    | 2 | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| 2923| sL   | III | "    | "    | 2 | 1 | 1 | 2†| 2†| 2†| 2†| 2†|
| 2925| Atypical | III | "    | "    | 5 | 0 | 0 | 3 | 2 | 2 | 2 | 2 |

* Non-leukemic tumor.
† Endothelioma, with giant cell formation (one sign for each instance).
### TABLE III—Concluded

| No. | Type of leukemia | No. of passages | Material injected | Route of injection | No. of transmissions | No. died of intercurrent disease | Type of leukemia |
|-----|------------------|----------------|-------------------|--------------------|----------------------|---------------------------------|-----------------|
| 2926 | LL               | III            | Blood             | iv.                | 0 0 2                | 2                              | Lymphomatosis    |
|      |                  |                | Plasma            | "                  | 1 0 3                |                                |                 |
| 2970 | sL               | III            | Blood             | "                  | 3 0 1                | 2 1                            | Monocytic leukemia |
|      |                  |                | Plasma            | "                  | 1 1 2                | 1                              | Myeloblastic     |
| 2836 | sL               | III            | Blood             | "                  | 0 0 6                |                                |                |
| 2843 | Atypical         | III            | "                 | "                  | 4 0 1                | 1 2 1                          |                 |
| 2968 | "                | III            | "                 | "                  | 4 0 8                | 1 2                            | Monoblastic       |
|      |                  |                | Plasma            | "                  | 2 1 4                | 1                              |                |
| 3045 | LL               | III            | Blood             | "                  | 2 0 2                | 1                              |                 |
| 3143 | sL               | III            | "                 | "                  | 1 0 2*               | 1 1                            |                |
| 2930 | aL               | III            | Tumor             | "                  | 1 0 4                | 1†                             |                 |
| 2934 | sMc              | III            | Blood             | "                  | 1 0 3                | 1                              |                |
| 2976 | sL               | IV             | "                 | "                  | 4 0 0                | 2*                             | 1 1              |
|   | Atypical, Sa |   | Tumor | im | 0 | 0 | 4 |   |   |   |   |   |   |
|---|-------------|---|-------|----|---|---|---|----|---|---|---|---|---|---|
| 2997 | sL | IV | Blood | iv. | 2 | 1 | 5 | 1 | 2 | 1 | 1 |   |   |   |
|     |     |     | Filtrate | “ | 3 | 0 | 3 | 3 |   |   |   |   |   |   |
| 3035 | sL | IV | Blood | “ | 5 | 0 | 0 | 2 |   |   |   | 3 |   |   |
|     |     |     | Filtrate | “ | 2 | 0 | 6 |   |   |   |   | 2 |   |   |
| 3125 | Atypical | IV | Blood | “ | 3 | 0 | 1 |   | 1 |   |   | 2 |   |   |
|     |     |     | Filtrate | “ | 0 | 0 | 6 |   |   |   |   |   |   |   |
| 3035 | IMc | IV | Blood | “ | 3 | 0 | 1 |   | 1 |   |   | 2 |   |   |
| 3202 | Atypical | V | Filtrate | “ | 1 | 0 | 4 | 1 |   |   |   |   | 1 |   |
| 3087 | sL | V | Blood | “ | 2 | 0 | 2 | 1 | 2 |   |   |   |   |   |   |
| 3240 | Atypical | V | “ | 5 | 0 | 0 | 2 |   |   |   |   | 3 |   |   |
| 3227 | aMc Eng | V | “ | 3 | 0 | 2 |   | 2 |   |   | 1 |   |   |   |
| 3229 | Atypical | V | “ | 3 | 0 | 2 | 1 |   |   | 2 |   |   |   |   |
| Total |   |   |   |   | 150 | 6 | 212 | 38 | 36 | 72 | 17 | 2 | 8 | 38 | 13 |
ing immature, viable lymphocytes will be considered separately from attempted transmissions made with material free from viable cells.

All birds used in this study (including the controls) were Barred Rock chickens raised for us on one farm. For technique, see our previous communications (5).

**Transmission of Strain 2 by Material Containing Viable Lymphocytes**

Transmission of this strain was more often successful with blood than with an emulsion of tumor cells (Table IV). Inoculations with blood were successful both in chicks weighing from 100 to 300 gm. and in chickens weighing from 600 to 1200 gm. In the latter about

| Material injected          | Route of injection | No. of transmissions | No. of intercurrent disease | Type of leukemia |
|----------------------------|--------------------|-----------------------|----------------------------|------------------|
| Blood injected into chicks | iv.                | 15                    | 16                         | 6                |
| Blood injected into young chickens | “”               | 107                   | 93                         | 16               |
| Tumor tissue               | “”                | 8                     | 0                          | 28               |
| Tumor tissue               | im. and sc.       | 1                     | 0                          | 1                |

| Total                      | 131                | 163                   | 27                         | 34               |

TABLE IV

Inoculations with Material Containing Viable Lymphocytes

one-half of all injected birds developed lymphomatosis, and the death rate due to intercurrent diseases was much lower than among baby chicks (Table IV). Transmission by an emulsion of tumor cells was much more successful by intravenous than by subcutaneous or intramuscular injections. In the seven experiments recorded in Table IV, only one of the 19 chickens injected intramuscularly died of leukemia.

In more recent experiments not described fully in this report, subcutaneous and intramuscular inoculations were more often successful, but with one exception there was no tumor formation at the site of injection. On the other hand, subcutaneous transmissions of lympho-
matosis of mice (20) were never successful unless a tumor developed at the site of inoculation and tumors could be produced with lymphocytes obtained from the blood. The significance of this observation will be discussed later.

TABLE V

Data on the Relationship between the Success of Inoculation and Amount of Blood Injected

| Donor   | No. of chicken injected | Amount of blood injected | Result of inoculation |
|---------|-------------------------|--------------------------|-----------------------|
| 2255, IL | 2642, 1                 | Killed after 152 days*   | Negative              |
| 2255, IL | 2645, 1                 | Died after 84 days       | sL                    |
| 2255, IL | 2643, 4                 | Killed after 44 days     | IL                    |
| 2255, IL | 2644, 10                | Died after 132 days      | Negative              |
| 2255, IL | 2644, 14                | ' ' ' 147 ' '           | IL                    |
| 2968, atypical | 3160, 0.5            | Died after 40 days       | sL                    |
| 2968, atypical | 3161, 0.5            | ' ' ' 62 ' '            | sL                    |
| 2968, atypical | 3162, 0.5            | Alive and healthy        |                       |
| 2968, atypical | 3163, 10              | ' ' ' '                |                       |
| 2968, atypical | 3164, 20              | Died after 32 days       | E                     |
| 2643, IL | 5 chicks                | Died of leukemia after 67, 75, and 123 days |                       |
| 2643, IL | 5 ' ' 1                 | 3 ' ' ' ' ' ' 34, 75, ' ' 116 ' ' |                       |
| 2643, IL | 4 chickens              | Died of leukemia after 38, 49, 55, and 97 days |                       |
| 2643, IL | 2 ' ' 2                 | 1 ' ' ' ' ' ' 41 days    |                       |
| 2643, IL | 4 chickens              | Died with leukemia after 40, 54, and 88 days |                       |
| 2643, IL | 3 ' ' 1                 | 2 ' ' ' ' ' ' 51 and 86 days |                       |

* After injection.

The relationship between the success of inoculation and the amount of blood injected has thus far not been studied systematically. The observations shown in Table V suggest that the concentration of the agent in the blood is great and that the success of inoculation depends rather on the individual susceptibility of the chickens than on the inoculating dose.
Table VI shows that all types of disease produced by material containing viable blood cells are also produced by material free from viable cells with the exception of myeloblastomatosis.

**Transmission by Filtered and Unfiltered Plasma**

Inoculations with unfiltered plasma were successful in about 36 per cent of the injected chickens, but after filtration through a Berkefeld N filter this percentage decreased to about 21.

The plasma was obtained by spinning heparinized blood at 1000 R.P.M. for 5 minutes, and then spinning the plasma twice at 3000 R.P.M., 10 minutes each time.

### TABLE VI

**Intravenous Inoculations with Material Free from Viable Leukocytes**

| Material injected                  | No. of transmissions | No. died of intercurrent disease | Type of leukaemia |
|-----------------------------------|----------------------|---------------------------------|-------------------|
|                                   | Successful | Unsuccessful | Within 1 mo. | From 1 to 2 mo. | Lymphosarcoma | Myeloblastoma | Myeloblastoma | Erythroblastoma | Atypical | Untyped |
| Plasma, unfiltered                 | 11         | 2            | 18          | 3              | 4            | 3            | 1            | 2            | 1        |
| Plasma, passed through Berkefeld filter | 6         | 0            | 23          | 1              | 3            | 3            | 2            | 1            | 2        |
| Dried blood                         | 4          | 0            | 5           | 1              | 2            | 4            |              |              |          |
| Glycerinated blood                 | 4          | 0            | 3           | 1              | 2            | 4            |              |              |          |
| Blood frozen at -70°C. for 30 min. | 2          | 0            | 1           | 1              | 2            | 4            |              |              |          |
| Total                              | 23         | 2            | 50          | 2              | 9            | 11           | 3            | 2            | 5        | 2        |

After each centrifugation, the plasma was pipetted off with great care to avoid contamination by blood cells. From 0.2 to 3 cc. of plasma was injected intravenously into each bird.

In filtration tests the plasma was first passed through a coarse Berkefeld filter, approximately 1 3/8 x 5/8 inches in size, and then refiltered through a medium or fine filter of the same size at a pressure of 40 cm. Hg. The filters were tested and washed with Locke’s solution before filtration. Their quality is indicated by the following figures.
From 0.2 to 5 cc. of filtrate was injected intravenously into each bird. For the results of the individual tests see Table III.

It is highly probable that the disease produced by unfiltered leukemic plasma was due to the cell-free agent that it contained and not to contaminating blood cells and that the smaller number of successful inoculations with filtered plasma was due to a partial retention of the agent by the filters.

In three more recent experiments plasma filtered through a medium filter caused leukosis in about two-thirds of the 22 inoculated chickens. These experiments will not be fully described because several of the chickens injected in this series are still alive.

**Transmission Experiments with Dried Blood**

The following experiment indicates that drying does not destroy the transmitting agent of Strain 2.

**Procedure.**—Loosely packed blood cells were frozen with the aid of solid carbon dioxide and dried in high vacuum over phosphorus pentoxide. In the desiccator the material to be dried rested on a metal box into which solid carbon dioxide was inserted through an outlet on the top of the desiccator. With this arrangement drying took place while the material was in the frozen state. The dried material was then kept in the ice box in sealed test-tubes.

**Experiment 1.**—40 cc. of blood was drawn from a chicken with subleukemic lymphomatosis (No. 3039) and the plasma, filtered through a Berkefeld N filter, was injected into eight chickens in amounts of 0.2 to 2 cc. Loosely packed cells from the same chicken were injected in amounts of 0.01 cc. into five chickens; cells 87 days after drying were injected in amounts corresponding to 1.2 cc. of loosely packed cells into another five; all injections were intravenous. Filtered plasma caused leukosis in only two of the eight injected chickens; one recovered and the other was killed 26 days after injection. Blood cells caused leukosis in all of the five injected chickens and they died 39, 45, 45, 109, and 128 days after in-
jection. Four of the five chickens injected with dried blood developed leukemia; two died 27 and 76 days after injection, two were killed 35 and 76 days after injection.

It is evident from this experiment that the dried blood, kept in a sealed tube for 87 days in the ice box, contained much of the active agent, whereas fresh plasma of the same bird, passed through a Berkefeld filter, contained little.

Experiment 2.—Four chickens were inoculated with dried whole blood, taken from a chicken with leukemic lymphomatosis (No. 2643), and kept for 103 days in the ice box. From 12 to 83 mg. of dried blood taken up in Locke solution was injected into each bird (1 mg. of dried blood was equal to 0.01 cc. of fresh whole blood). All chickens appeared healthy when killed 157 days after injection. The fresh blood of the same chicken, injected in amounts of 0.1 cc. into chickens of the same size, caused leukemia in all four chickens injected.

Thus in this experiment drying of blood resulted in inactivation of the transmitting agent. Quantitative relations were ignored in both experiments, but are being considered in the experiments now under way.

Miscellaneous Experiments with Material Free from Viable Cells

Glycerination.—In one experiment five chickens were injected with from 0.07 to 0.7 cc. of whole blood, taken from a chicken with atypical leukemia (No. 2768), and kept for 65 days in approximately 80 per cent glycerin. Three of these chickens died of intercurrent diseases, 34, 44, and 86 days after injection, without showing any lesions suggestive of leukemia and two remained healthy and were killed 158 days after injection. Fresh blood from the same chicken caused leukemia in 10 of 12 chickens injected with from 1 to 4 cc.

In another experiment, loosely packed cells of a chicken with atypical leukemia (No. 2976) were kept for 113 days in glycerin (3 cc. cells plus 10 cc. concentrated glycerin c.p.) and were injected into five chickens in amounts corresponding to 0.5 cc. of cells. All of these birds remained healthy. All of five chickens injected with 0.25 to 0.5 cc. of fresh blood from the same chicken died with leukemia 43, 74, 82, and 83 days after injection.

Thus, attempts to preserve the transmitting agent in approximately 80 per cent glycerin were unsuccessful in two experiments.

Freezing.—Freezing at approximately −70°C. for 1/2 hour caused little if any deterioration of the transmitting agent. Three chickens were injected with 0.005 cc. of loosely packed fresh blood cells and
three chickens with the same amount of cells after they had been kept at $-70^\circ$C. for 1/2 hour. Two chickens of each group developed leukosis, those injected with fresh blood after 15 to 25 days, those injected with frozen blood after 29 days.

**Technique.**—0.5 cc. of the cells was sealed in a test-tube which was immersed in ether and kept for 1/2 hour in a thermos bottle containing one-half of its volume of solid carbon dioxide.

Experiments with mouse leukemia indicate that the death point of leukemic lymphocytes is, at temperatures below the freezing point, between $-10^\circ$ and $-20^\circ$C. There is little evidence in favor of the view of Cramer and Foulds (21) that mammalian tumors are caused by invisible agents that share with the filterable agents of avian tumors the property of resisting freezing and thawing but do not share filterability and resistance to drying. Our experiments show that freezing at temperatures below $-30^\circ$C. injures little if at all the filterable agents of leukosis of chickens, but destroys blood cells and destroys the ability of malignant lymphocytes of mice to transmit lymphomatosis (20). It is a matter of dispute whether there is a difference in resistance to low temperature between cells of vertebrates on the one hand and microorganisms and viruses on the other hand (cf. Rivers (22)); there are not enough experimental data to settle this question, for most, if not all, of the experiments on resistance of organisms to freezing were concerned with the process of freezing and thawing and did not record the actual temperature to which all cells were exposed. Our experiments suggest that freezing and thawing at temperatures below $-30^\circ$C. for 30 minutes is a very good procedure to destroy living cells without inactivation of agents that transmit leukosis of chickens. It would be highly desirable to search with this procedure for the presence of agents in chicken tumors not transmissible by filtrates, for demonstration of filterability often fails because of technical difficulties such as association of the virus with larger particles retained by the filter.

**Control Material**

For comparison with the types of diseases caused by and characteristic of Strain 2, the incidence of tumors, leukemia, and related condi-
tions occurring among all of the chickens kept in our animal rooms throughout these transmission experiments was analyzed (Table VII). This table includes 298 chickens that were inoculated with transmissible leukosis, Strain 1, 20 chickens used for miscellaneous transmission experiments, 14 chickens injected with various chemicals, and 16 uninjected chickens, all kept in our animal rooms during this period. No immature lymphocytes characteristic of Strain 2 have been seen in the blood smears of these chickens.

**TABLE VII**

Survey of All Chickens Kept in Our Animal Rooms during the Period of Experimentation with Strain 2

| Chicken                  | No. of chickens in group | Free from Leukosis and Transplantation | Type of Leukosis | Myelocytomatosis | Uncertain for Leukosis | No. died of Intercurrent Disease |
|--------------------------|--------------------------|---------------------------------------|------------------|------------------|------------------------|----------------------------------|
| Uninjected               | 16                       | 11                                    | 0                | 1                | 0                      | 1                                |
| Injected with various chemicals | 14                       | 12                                    | 0                | 0                | 0                      | 2                                |
| Transmission experiments |                          |                                       |                  |                  |                        |                                  |
| Strain 1                 | 298                      | 120                                   | 137              | 0                | 0                      | 1                                |
| Strain 2                 | 431                      | 209                                   | 10               | 17               | 72                     | 38†                              |
| Miscellaneous            | 20                       | 16                                    | 0                | 0                | 1                      | 0                                |

* In 13 instances the type of leukosis was not determined.
† 2 instances of sarcoma occurred in association with leukosis.

One chicken, injected with tumor cells deriving from a chicken with spontaneous aleukemic lymphomatosis (given in the group of miscellaneous transmissions), died of aleukemic lymphomatosis. Attempted subpassages made from this bird into six chickens (given also among miscellaneous transmissions) were unsuccessful.

In none of the chickens injected with Strain 1 was myelocytomatosis found, but it was found in one of the uninjected controls. This spontaneous instance of myelocytomatosis was associated with endothelioma, the only presumably spontaneous tumor of this type I have ever seen. It is noteworthy that during the period of these investigations
(April to December, 1932) the birds were kept side by side in meshed wire cages, and in several instances birds injected with different material were kept in the same cage. Yet there is no evidence that Strain 1 became contaminated by Strain 2, or Strain 2 by Strain 1. Since December, 1932, the cages holding the birds injected with different strains of leukosis have been kept in separate groups but in the same room. Uninjected chickens have been kept as controls, some separate from and some among the inoculated chickens. No transmissible leukosis has so far (May, 1933) been observed among approximately 30 uninjected control chickens thus treated.

DISCUSSION

Are all types of leukosis observed among the passages of Strain 2 caused by a single agent?

The data presented indicate that lymphomatosis, myelocytomatosis, and endothelioma of fowls are transmissible diseases. Of 294 chickens injected with cell-containing material (Table IV), 131 developed leukosis, 60 of the latter had lymphomatosis, 14 myelocytomatosis, and 10 instances of leukosis were associated with endothelioma. Among 73 chickens inoculated with material free from viable cells (Table VI) 23 instances of leukosis were observed; 11 chickens developed lymphomatosis, 3 myelocytomatosis, and 1 instance of leukosis was associated with endothelioma. Since myelocytoma and endothelioma are rare as spontaneous diseases, it is probable that they too are caused by a filterable agent.

Indeed the experiments suggest that lymphomatosis, myelocytomatosis, and endothelioma are caused by the same agent. Whether transmissions are made from apparently pure instances of lymphomatosis or of myelocytomatosis, there occur instances of both lymphomatosis and myelocytomatosis among the successfully inoculated birds. Lymphomatosis usually occurs more often than myelocytomatosis. Endothelioma free from lymphomatosis and myelocytomatosis was not observed. Of 13 instances of endothelioma with giant cell formation, nine occurred among passages made from a leukotic fowl apparently free from this disease. Our examination of small pieces of tissue does not exclude the possibility that minute lesions of endothelioma escaped discovery at the postmortem examina-
tion. But one would expect a higher incidence of endothelioma from transfers made from apparently pure cases of that disease. Similarly, were different viruses responsible for lymphomatosis, on the one hand, and myelomatosis, on the other, one would expect among the successfully inoculated chickens a great incidence of the type from which the transfer is made, but this is not the case. Nevertheless, the evidence presented here does not exclude the possibility that we are dealing with an intimate mixture of viruses, and, for this reason, further efforts toward separation are being made. Transmission experiments are being conducted with washed cells of tumors composed of one type of cell, and cross-immunity tests, including tests with Rous sarcoma, are being made. The experiments thus far performed indicate that sarcoma can be grafted upon chickens that are resistant to repeated inoculations of leukemia Strains 1 and 2.

Another inference drawn from these observations is that transmission is accomplished mainly by the filterable agent and not by multiplication of the introduced cells.

Intramuscular or subcutaneous inoculations with this strain are, with rare exceptions, not followed by tumor formation at the site of injection, whereas tumors and leukemia of mammals (20) and Rous sarcoma of chickens, including endothelioma of Begg, produce tumors at the site of injection. After subcutaneous injection of tumor tissue derived from two spontaneous instances of Rous sarcoma (one occurred in an un.injected control chicken, one in a chicken inoculated with leukemia Strain 2) tumors developed readily at the site of injection. The agents of Rous sarcoma are capable of producing tumors in the subcutaneous tissue because the cells that they stimulate are present there, whereas the agents of leukemia have to reach blood-forming organs to find cells that can be stimulated by them. Lack of tumor formation after subcutaneous and intramuscular injection of leukemic lymphocytes or myelocytes supports the opinion that transmission is accomplished mainly by the filterable agent.

Morphologically, there is a similarity between endothelioma of Begg and endothelioma occurring among the passages of Strain 2. The former is not associated with leukemia and stimulates endothelium of young capillaries, at the site of injection, to neoplastic growth. Strain
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2 does not stimulate endothelium at the site of injection, and endothelioma caused by it is always associated with leukosis.

The data presented by us show that there are at least two different agents that cause leukosis. Since the visceral alterations caused by the virus of neurolymphomatosis of Pappenheimer, Dunn, and Cone are leukotic in nature, we may regard their virus as a third virus that stimulates cells of the hemopoietic system to apparently unrestricted multiplication. The agent of Jármai that has affinity for the erythropoietic tissue only may represent a fourth type of transmissible leukosis.

The relation of neurolymphomatosis to this strain of lymphomatosis, which is also capable of infiltrating nerves and causing paresis or paralysis, will be discussed more fully in the report that follows. Suffice it to say here that the agent of neurolymphomatosis does not produce blood changes, whereas immature lymphocytes appear in the blood of most of the chickens successfully inoculated with our strain. Myelomatosis and endothelioma are not caused by the strain of Pappenheimer and coworkers, and lymphomatosis of viscera occurs only in 10 per cent of their paralyzed chickens. On the contrary, lymphomatosis of the viscera is the primary change produced by our Strain 2, and it is doubtful whether the two instances of paralysis without infiltration of viscera, occurring among the passages of our strain, are caused by the agent of Strain 2, for attempted transmission of one of these cases was unsuccessful. Infectious paralysis of Marek (cf. 16) and Seifried (16) is a primary inflammatory degenerative disturbance of the nervous system, and is unassociated with lymphomatosis of the viscera. Strain 2 is a primary neoplastic disturbance of hemopoietic tissues with secondary invasion of the nerves, and neurolymphomatosis of Pappenheimer and coworkers is sometimes lymphomatosis of both viscera and nerves, and sometimes paralysis caused by inflammation of nerves unassociated with lymphomatosis of viscera.

Claude and Murphy (23) have compared the filterable agents of avian tumors with the substance described by Griffith and by Alloway that transforms one type of pneumococcus into another. Applying this analogy to Strain 2 one would expect that the filterable agent derived from myelocytomatosis would produce myelocytomatosis only, and that derived from endothelioma would produce endothelioma only,
but the evidence presented shows that the type of leukosis produced is independent of that from which the agent is obtained.

The filterable agents of avian tumors and leukosis are growth stimuli; the disturbance caused by them depends on the type of cell attacked, on the character of the stimulus (agent), and on some additional unknown factors. Strain 2 is an example of a single agent that produces several types of diseases; whatever the type of the disease, the identity of the agent remains unchanged.

**SUMMARY**

A new transmissible strain of leukosis of chickens is described that causes (a) lymphomatosis with or without tumor formation, and with or without leukemia, (b) myelocytomatosis with or without leukemia, and (c) endothelioma. All these diseases are transmissible by material free from viable cells, and the available evidence indicates that they are caused by a single filterable agent.

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EXPLANATION OF PLATES

The blood smears were stained with a combination of Wright and Giemsa's solutions, the sections with hematoxylin-eosin. The magnifications given are approximate.

**PLATE 14**

**Fig. 1.** Photograph of the ovary and kidneys of Chicken 2255 (spontaneous leukemic lymphomatosis). There are numerous tumor nodules in the kidneys; the ovary seems normal.

**Fig. 2.** Photograph of the liver of the same chicken. The liver is thickly studded with partly confluent tumor nodes.

**Fig. 3.** Blood smear of Chicken 2255 showing large numbers of basophilic lymphocytes. × 450.

**Fig. 4.** Same with higher magnification (× 1500).

**Fig. 5.** Lymphocytes characteristic for Strain 2 in the blood of a chicken with subleukemic lymphomatosis. They are basophilic and contain small vacuoles and azure granules. × 1300.

**PLATE 15**

**Fig. 6.** The liver of Chicken 2255 showing extensive lymphomatosis infiltrations. × 90.

**Fig. 7.** The spleen of Chicken 2255 showing the character of infiltrations. × 270.

**Fig. 8.** Syncytial mass of tumor cells infiltrating the liver. × 700.

**Fig. 9.** The kidney of Chicken 2255 showing extensive lymphomatosis infiltrations. × 90.
(Furth: Virus causing lymphomatosis of chickens.  I)
(Furth: Virus causing lymphomatosis of chickens. 1)