The Etiology of Leukemia: The Status of the Virus as Causative Agent—A Review

By S. O. Schwartz and H. M. Schoolman

A REVIVAL OF INTEREST in the virus theory of causation of leukemia, since Engelbreth-Holm and Frederiksen concluded that their experiments had been in error because of the unreproducibility of their results, was spurred by the observations by Gross in 1951 and subsequently, and confirmed in 1956 by Wooley and Small, namely, that cell-free filtrates of leukemic AK tumors could induce leukemia in a low leukemic strain of mice when inoculated into newborn animals. Since then, additional studies have been gradually supporting evidence for the virus theory of the etiology of leukemia. Graffi produced leukemia in newborn mice by the use of extracts of malignant mouse tumors. Latarjet accelerated the development of leukemia in AK mice by inoculating them with leukemic tissue extracts when newly born. Kaliss demonstrated the transmissibility of mouse neoplasia with lyophilized tumor tissue; whereas, Magrassi and Mas y Magro were able to produce diffuse disease in guinea pigs with tissues and tissue extracts derived from patients having leukemia. Bergol's reported in 1957 the induction of leukemia in newborn and adult mice by extracts of tissues of patients dying of leukemia. Achievements in the field were the induction of leukemia in adult Swiss mice by cell-free tumor extracts reported by Charlotte Friend; the production of leukemia and multiple tumors by the use of tissue culture extracts reported by Sarah Stewart; and the extensive studies of the nature and immunologic characteristics of the viruses of the fowl leukoses by Beard and his colleagues.

Because of the smallness of viruses, their obligate intracellular existence, the difficulty of culturing and the lack of understanding relating to their multiplication, a great deal of the evidence to date has been based on indirect experiments; however, in 1957 Dmochowski and his associates reported the demonstration by means of electron microscopy of virus-like particles in the cervical lymph node of a patient having acute lymphatic leukemia.

Our interest in the etiology of leukemia was stimulated years ago by the observation that striking similarities existed between infectious diseases and the onset and course of many leukemias. To demonstrate that leukemia had an infectious origin, we reasoned that the approach would have to be by way of immunologic methods. Bacterial or fungal infections were not instrumental agents according to ample available evidence. The search logically led to viruses.
Agglutinability and Precipitability

Kidd had demonstrated that normal guinea pig serum contained a factor which in vivo protected C3H mice against the 6C3HED Gardner lymphosarcoma. Following this lead, we tested normal guinea pig serum against tissues of the AKR mouse. The leukemic cells gave strong agglutinin and precipitin reactions. Neither blood nor tissues of animals which did not show clinical or hematologic evidence of leukemia showed positive reactions.

About this time we observed that there was a widening of the cervical spinal canal in AKR animals in which tumor cell suspensions had been transplanted. Histologic examination failed to reveal recognizable leukemic infiltration in the cervical spinal canal, but material from that area produced a disseminated lymphosarcoma when transplanted into AKR mice. When material from that area, and subsequently from the brain, was tested against guinea pig serum, positive precipitin or agglutinin reactions were obtained (Table 1). These reactions were in titers of the same order of magnitude as those obtained with leukemic cells, even though significant numbers of leukemic cells were not demonstrable. Apparently, the positive precipitin reaction when brain and spinal cord material was used could not be attributed to either contamination by leukemic cells or infiltration by those cells; moreover, the results did not suggest contamination by serum, for thoroughly washed tissues gave higher titers than those given by serum alone. The possibility that the antigen was absorbed from the serum by cells of the brain and spinal cord was unsubstantiated by in vitro studies, because normal brain incubated with leukemic mouse serum failed to give a positive precipitin reaction.

On the basis of those observations, we suggested in our original communication that the mesenchymal cells of the brain underwent a transformation detectable by immunologic methods before morphologic changes could take place. We concluded: "It follows from this interpretation that so-called infiltration of the brain and other organs may actually be the morphologic transformation of the mesenchymal cells already present. This would represent a structural counterpart of the metabolic process which could be dem-

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* C3H mice represent an inbred line with a very low incidence of spontaneous leukemia.
† 6C3HED Gardner lymphosarcoma is a lymphoma transplantable in C3H mice.
¶ AKR mice are a highly inbred strain with a high incidence of spontaneous leukemia.
THE VIRUS AS CAUSATIVE AGENT IN LEUKEMIA

281

...onstrated earlier by immunologic methods. Morphologic transformation, subsequent proliferation, and perhaps mobilization of existing mesenchymal cells may be the means by which organs that are not strictly reticulo-endothelial organs become ‘infiltrated’ with AKR leukemia.”

Our interpretation, therefore, was that leukemia was a systemic disease representing, not a spontaneous, lawless multiplication of a single cell or group of cells, but rather the response of the host to an offensive agent. This would account for the morphologic identity of leukemic cells with cells that may be present in the normal marrow and lymphoid organs. This also explains why biochemical and immunologic changes may take place without any recognizable morphologic alteration. This thesis implies that the agent is probably active even in areas where demonstrable morphologic change is not evident. We proved this when cell-free filtrates of the brain and spinal cord accelerated the development of leukemia in AKR mice.

Specificity: Demonstrable Reaction with Human Leukemic Tissues

Having demonstrated these interesting phenomena regarding both the agglutinability and precipitability of mouse leukemic tissues, it was obvious to inquire as to specificity, and also as to whether a similar reaction could be demonstrated with human leukemic tissues. Subsequent studies revealed that human leukemic white cells, but not nonleukemic white cells, were also agglutinated by normal guinea pig serum. At about that time Gardner and his co-workers reported the detection of antigenic modification of red blood cells induced by the virus of Newcastle disease. This suggested to us the further possibility that if leukemia were a virus infection, a similar modification of the red cells might be detected in patients having leukemia, as well as in leukemic animals.

When specific anti-sera are prepared for red cells obtained from patients having chronic lymphocytic leukemia, or from mice having spontaneous AKR leukemia, antigenic modification of the red cell is demonstrable. Furthermore, a cross-agglutination was demonstrated among various human leukemias as well as between human and mouse leukemia. Inasmuch as a cross agglutination had also been demonstrated in previous studies with white cells, evidence was furthered that not only are viruses instrumental, but that human and mouse leukemia might be etiologically similar. Meanwhile, experiments were being expanded regarding cell-free filtrates of brain. Minor modifications have been made in the technic of preparing the extracts, although the fundamental method has remained unchanged through the years. The method now being used is as follows:

1. One-half of the mouse brain is removed immediately after death and placed in sterile physiologic saline solution in a 10 mm. by 75 mm. test tube. If the brain is not to be processed immediately, the tube is stoppered with a cotton plug and stored at -15 C.

2. Mortar and pestle for grinding, a 100 cc. graduated cylinder and a flask are kept in the freezer so that they will be cold.

*Half a brain is used because the other half serves as histologic control.
3. An ice bath, consisting of a pan filled with ice cubes and ice water, is kept in the refrigerator at 4 C., along with a flask of buffered saline solution pH 7.4.

4. The cold mortar is placed in the ice bath. The brain and a small amount of saline solution is poured into the mortar along with some sand. The brain is ground for about 10 minutes until only a fine suspension remains. This is poured into the graduate and the mortar is rinsed with the buffered saline solution. The same saline solution is used to dilute the brain to 100 cc.

5. After it has been mixed, this preparation is transferred to a flask and is allowed to stand in the refrigerator for 24 hours. (This step does not seem to be crucial, some preparations having been processed immediately.)

6. If the supernatant fluid is relatively clear, it is removed and Seitz-filtered. If it is cloudy, it is centrifuged at 3000 rpm for 15 minutes in a cold centrifuge at 4 C. and then Seitz-filtered.

7. A culture of *E. coli* is used to contaminate the supernatant.*

8. The supernatant is filtered through a Seitz sterilizing pad.

9. A sample specimen is taken for bacterial culture before and after filtration.

10. The filtrate either is used immediately for inoculation or is stored until needed in sterile cotton-plugged tubes in the freezer at -15 C.!

11. The mice are inoculated with either 0.5 cc. of the filtrate intraperitoneally or 0.075 to 0.1 cc. intracerebrally.

12. From 4 to 12-week-old mice are usually used as recipients.

*Acceleration of Development of Lymphoblastoma in AKR Mice Following Inoculation with Cell-free Brain Filtrates of Leukemic AKR Mice*45

In the first series of experiments attempts were made to determine whether the filtrate could produce acceleration in the development of leukemia in a leukemia-susceptible strain of mice. For that purpose AKR mice were used. In this strain, leukemia occurs spontaneously with a frequency of approximately 75 to 80 per cent by the end of a year. Recognizable leukemia is first seen at about 24 weeks and thenceforth the incidence gradually increases. In our control group of 460 animals, 377 died of leukemia by the 52nd week.

If such animals are inoculated with filtrates prepared from the brains of leukemic AKR mice some time between the 4th and 12th weeks of life, leukemia develops in about 50 per cent (181/373) by the age of 22 weeks,

*The *E. coli* is used only for the purpose of testing the integrity of the filter pad. All brain filtrates, whether from leukemic or control sources, are identically treated throughout the processing of the material.

| The material has been kept frozen successfully at approximately -15 C. for a number of months.
| The AKR mice used in these experiments either were bred by us from a breeding stock originally obtained from Drs. J. Furth and A. C. Upton or were obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine. The Swiss mice were obtained from the Harlan Small Animal Industries, Cumberland, Indiana. The C3H/He mice were bred from a pedigreed breeding stock obtained from the Jackson Memorial Laboratories; the dba mice were bred from a stock originally obtained from Dr. Albert Tannenbaum, Chicago. |
the disease developing in from 2 to 16 weeks, with an average of about 4 weeks (fig. 1). Since spontaneous leukemia begins to appear at 24 weeks, we considered negative all animals that failed to show frank gross or histologic evidence of leukemia by the age of 22 weeks. Thus, leukemia develops in about 50 per cent of the injected animals at an age when spontaneous leukemia is unknown in this strain.

In its gross and microscopic appearances, the experimentally-induced disease is in every way identical with that occurring spontaneously (fig. 2). Further, there is no significant difference whether one uses the intracerebral or the intraperitoneal route, whether male or female animals are used or whether the source of the filtrate is from mice whose disease developed spontaneously or was induced by cell-free filtrates or tissue transplants.

Filtrates prepared in an identical manner from nonleukemic AKR brains or from the AKR tumors failed to accelerate the development of the lymphoblastoma. Heat (65° for 45 minutes) inactivated the leukemic brain filtrates. Because the filtrate prepared from the tumor failed to accelerate the development of the lymphoblastoma, whereas the brain filtrates exerted such a remarkable effect, the explanation seemed likely that the difference lay in an adjuvant action of brain. To test this possibility, filtrates were prepared from the brain and tumor of the same leukemic AKR mouse. In addition, a third preparation was made from equal parts of this tumor and nonleukemic Swiss mouse brain. The various filtrates were diluted with saline solu-
tion to make their concentrations similar. When the filtrates were injected into AKR mice, the brain filtrate accelerated the development of the lymphoblastoma in 12 of 20 animals; whereas neither the tumor filtrate nor the tumor added to the brain filtrate had any influence. Thus, under the conditions of the experiments, the activity of the brain filtrates could not be ascribed to an adjuvant action of brain, but only to the active agent contained in the leukemic brains.
THE VIRUS AS CAUSATIVE AGENT IN LEUKEMIA

Table 2.—Results of Control Filtrates

<table>
<thead>
<tr>
<th>Material Injected</th>
<th>No. of Experiments</th>
<th>No. of Swiss Mice Having Leukemia: Number Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free filtrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemic Swiss Mouse Brain</td>
<td>14</td>
<td>158/278</td>
</tr>
<tr>
<td>Cell-free filtrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemic DBA Brain</td>
<td>2</td>
<td>45/52</td>
</tr>
<tr>
<td>Leukemic Swiss Mouse Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat Inactivated</td>
<td>3</td>
<td>2/78</td>
</tr>
<tr>
<td>Leukemic DBA Brain</td>
<td>1</td>
<td>0/25</td>
</tr>
<tr>
<td>Heat Inactivated</td>
<td>6</td>
<td>0/274</td>
</tr>
<tr>
<td>Nonleukemic Swiss Mouse Brain</td>
<td>2</td>
<td>0/57</td>
</tr>
<tr>
<td>Tumor of Swiss Mouse Leukemia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Tumor Cell Suspensions | | |
|------------------------| | |
| Swiss                  | 22 | 100/293                                          |
| DBA                    | 2  | 7/18                                             |

**Induction of Leukemia in Swiss Mice by Means of Filtrates of Leukemic Mouse Brain**

Because of the high incidence of spontaneous leukemia in the AKR strain, we attempted to reproduce the results with mice of a strain in which spontaneous leukemia seldom occurs. This became possible when a 33-week-old Swiss female mouse that had been caged with AKR males for the purpose of hybrid-breeding exhibited a spontaneous lymphoblastoma characterized grossly by generalized lymphadenopathy, a mesenteric tumor, enlarged thymus and pronounced hepatosplenomegaly. The gross and microscopic characteristics of this mouse, unlike the ones in subsequent passages, were indistinguishable from the spontaneous lymphoblastoma of AKR mice. Tumor-cell suspensions obtained from that mouse grew in both Swiss and dba mice but failed to grow in AKR mice. Subsequent experiments in this series were performed with cell-free filtrates derived from the brains and tumors of dba and Swiss mice whose lymphoblastoma was originally derived from that one animal. Cell-free brain filtrates induced a lymphoblastoma in 158 of 278 of the Swiss mice inoculated. Filtrates prepared from the brains of leukemic dba mice which had been injected with Swiss brain filtrates or tumor-cell suspensions produced a lymphoblastoma in 45 of 52 Swiss mice injected. Control filtrates, consisting of material derived from nonleukemic animal brains and heat-inactivated filtrates, caused the development of the lymphoblastoma in only 2 of 434 mice. Both of the animals in which lymphoblastoma developed had been inoculated with heat-inactivated leukemic brain filtrates. The results are summarized in table 2.

Figure 3 illustrates the rate of development of the lymphoblastoma in Swiss mice following inoculation with cell-free filtrates of leukemic mouse brain. The lymphoblastoma may be manifest as early as the 5th day follow-

*The incidence of spontaneous leukemia in our dba and Swiss mice is less than 2 per cent at 12 months of age.
Inoculation of cell-free filtrates of leukemic brain.

Gross manifestations of lymphoblastoma differ significantly in the Swiss and AKR mice. In the Swiss mice a distinct peripheral lymphadenopathy is rare. Slight enlargement of the thymus is frequent; but only in an occasional animal does the thymic tumor approach the proportions commonly seen in the AKR lymphoblastoma. Splenomegaly is significant in about half the animals, but moderate to severe hepatomegaly is present in 75 per cent. The outstanding finding is a massive diffuse infiltration of the mesentery and frequent invasion of the urogenital organs (fig 4). This is true whether the filtrate is inoculated intraperitoneally or intracerebrally. Ascites is present in about 25 per cent of animals injected intraperitoneally and in about 10 per cent of those injected intracerebrally. Primitive lymphoid cells, from 2 to 80 per cent, were in the blood in 10 of the 26 cases in which the blood was studied; whereas replacement of from 15 to 95 per cent of the marrow cells by lymphoblasts took place in 8 of 31 animals. The histologic observations were similar to those seen in the AKR mouse; they showed an intense extensive diffuse infiltration of all organs by a primitive lymphoid cell.

Acceleration of Leukemia in AKR Mice by Means of Cell-free Brain Filtrates of Humans Who Died of Leukemia

Attempts were made to transmit leukemia to AKR mice or to accelerate the development of leukemia in AKR mice by the use of filtrates prepared from the brains of patients who died of leukemia. Those filtrates were prepared in a manner similar to that used for the preparation of mouse extracts.
Fig. 4.—Swiss mouse two weeks after the intracerebral inoculation of 0.075 cc. of cell-free filtrate of leukemic Swiss mouse brain. Note extensive infiltration of mesentery and genital organs.

About 250 mg. of brain per 100 cc. of saline solution were used. The development of the AKR lymphoblastoma was accelerated in about 40 percent of animals injected (fig. 5). The rate of development as well as the gross and microscopic appearance of the lymphoblastoma were similar to those obtained with filtrates prepared from the brains of AKR mice.

Filtrates that gave positive results are summarized in table 3. Seven of the ten filtrates were from patients with acute myeloblastic leukemia; but the resulting leukemia of the mouse was lymphoblastic, uninfluenced by the type of leukemia that provided the source of the filtrate.

Table 4 summarizes the results of filtrates that failed to accelerate the development of AKR lymphoblastoma. Four of those filtrates were prepared from the brains of patients with acute lymphoblastic leukemia; three were prepared from the brains of patients with acute myeloblastic leukemia. Any number of reasons may be found to explain the negative results in those experiments. It is conceivable that no virus was present; but it is far more likely that the negative results are related to the quantity of the virus or its alteration by treatment or postmortem changes. However, within the lim-
INOCULATION OF CELL FREE FILTRATES OF LEUKEMIC BRAIN

Fig. 5.—Acceleration of the development of leukemia in leukemia-susceptible (AKR) mice following inoculation with cell-free brain filtrates of patients dying of acute leukemia. 
Inset: Rate of development of leukemia following inoculation with cell-free filtrates regardless of age of animals at time of injection.

Table 3.—Filtrates Giving Positive Results

<table>
<thead>
<tr>
<th>Source of Filtrate</th>
<th>No. of Experiments</th>
<th>No. of Mice Having Leukemia Before 22 Weeks: Mice Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Acute Myeloblastic</td>
<td>3</td>
<td>24/40</td>
</tr>
<tr>
<td>B. Acute Myeloblastic</td>
<td>3</td>
<td>24/51</td>
</tr>
<tr>
<td>H. Acute Myeloblastic</td>
<td>4</td>
<td>3/63</td>
</tr>
<tr>
<td>W.S. Acute Myeloblastic</td>
<td>1</td>
<td>19/25</td>
</tr>
<tr>
<td>E.P. Acute Myeloblastic</td>
<td>1</td>
<td>14/26</td>
</tr>
<tr>
<td>J.K. Acute Myeloblastic</td>
<td>1</td>
<td>31/50</td>
</tr>
<tr>
<td>F.T. Acute Myeloblastic</td>
<td>1</td>
<td>21/25</td>
</tr>
<tr>
<td>J.C. Acute Lymphoblastic</td>
<td>1</td>
<td>20/25</td>
</tr>
<tr>
<td>H.G. Acute Monoblastic</td>
<td>1</td>
<td>19/25</td>
</tr>
<tr>
<td>M.G. Stem Cell</td>
<td>1</td>
<td>30/50</td>
</tr>
<tr>
<td>Pooled Leukemias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, B, H, P, M</td>
<td>3</td>
<td>20/61</td>
</tr>
<tr>
<td>Pooled Leukemias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G. and J.K.</td>
<td>1</td>
<td>23/50</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>248/491</td>
</tr>
</tbody>
</table>

For purposes of control, 550 mice were injected with filtrates prepared from nonleukemic human brains and with heat-inactivated filtrates of human leukemic brain. In two of the animals in the control group, lymphoblastoma
THE VIRUS AS CAUSATIVE AGENT IN LEUKEMIA

Table 4.—Filtrates Failing to Accelerate Development of AKR Lymphoblastoma

<table>
<thead>
<tr>
<th>Source of Filtrate</th>
<th>No. of Experiments</th>
<th>No. of Mice Having Leukemia Before 22 Weeks: Mice Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.F. Acute Myeloblastic</td>
<td>1</td>
<td>0/12</td>
</tr>
<tr>
<td>J.P. Acute Myeloblastic</td>
<td>1</td>
<td>0/12</td>
</tr>
<tr>
<td>E.K. Acute Myeloblastic</td>
<td>1</td>
<td>0/25</td>
</tr>
<tr>
<td>E.S. Acute Monoblastic</td>
<td>1</td>
<td>0/25</td>
</tr>
<tr>
<td>R. Acute Monoblastic</td>
<td>2</td>
<td>0/25</td>
</tr>
<tr>
<td>P. Acute Lymphoblastic</td>
<td>2</td>
<td>0/36</td>
</tr>
<tr>
<td>M. Acute Lymphoblastic</td>
<td>2</td>
<td>0/50</td>
</tr>
<tr>
<td>P.L. Acute Lymphoblastic</td>
<td>1</td>
<td>0/10</td>
</tr>
<tr>
<td>M.H. Acute Lymphoblastic</td>
<td>1</td>
<td>0/42</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0/237</td>
</tr>
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</table>

Table 5.—Effect of Human Brain Filtrates on Acceleration of AKR Lymphoma

<table>
<thead>
<tr>
<th>Source of Filtrate</th>
<th>Controls No. of Experiments</th>
<th>No. of Mice Having Leukemia: Mice Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free Filtrates of Leukemic Humans Brains, Heat-inactivated</td>
<td>15</td>
<td>1/184</td>
</tr>
<tr>
<td>Heat-inactivated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemic (Pooled)</td>
<td>3</td>
<td>1/51</td>
</tr>
<tr>
<td>Nonleukemic</td>
<td>8</td>
<td>0/253</td>
</tr>
<tr>
<td>Nonleukemic (Pooled)</td>
<td>4</td>
<td>0/62</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2/530</td>
</tr>
</tbody>
</table>

developed. Both animals had been inoculated with heat-inactivated leukemic brain filtrates. The experiments are summarized in table 5.

Serial Passage of Cell-free Brain Filtrates Through Heterologous Strains

In order to rule out the possibility of cellular or cell fragment contamination, and to elucidate the behavior of the filterable agent, another series of experiments was devised:

Filtrates were prepared from the brains of leukemic AKR and Swiss mice and from patients dying of leukemia. Those filtrates were serially passed through Swiss mice in the case of the AKR mouse and human brains, and through C3HeB mice in the case of Swiss mice. Parallel experiments were performed with the use of intraperitoneal and intracerebral routes of injection. The experiment was carried out as follows: The cell-free filtrate was inoculated (intracerebrally or intraperitoneally) into a nonsusceptible mouse. At the end of 72 hours the mouse was killed and a new filtrate prepared from its brain. That filtrate was reinoculated into similar nonsusceptible mice, and the procedure was repeated. After five such passages the filtrate was injected into groups of mice of the original strain in the case of AKR and Swiss mice, and into AKR mice when the original source of the filtrate was human brain. Of 160 animals injected with filtrates whose original source was leukemic brain, leukemia developed in 115 (table 6).

Heat-inactivated filtrates and filtrates prepared from nonleukemic mouse
and human brain were used for controls and were similarly passed. Leukemia did not develop in any of the 180 animals injected with these filtrates.

In two additional control experiments, tumor-cell suspensions of Swiss mice having leukemia were injected intraperitoneally into C3Heb mice at the start of the serial passage. At the same time, filtrates prepared from the brains of the same mice which served as the source of the tumor were passed through other C3Heb mice. After five passages leukemia developed in only one of the 40 Swiss mice of the tumor cell line; whereas of the 40 animals injected with the brains line, leukemia developed in 17 (table 7).

If one assumes 100 per cent recovery of the active agent in each passage, the effect of five passages is to dilute the original material to approximately $10^{13}$. When the original cell-free brain filtrate is diluted beyond $10^4$, no activity can be demonstrated.
DISCUSSION

The two basic postulates on which our experiments were based were that leukemia is virus-induced and that leukemia is a systemic disease whose morphologic manifestations represent a host reaction which is primarily defensive. Those postulates are reinforced by the reported experiments, although the evidence remains circumstantial. Nonetheless, certain facts have been established:

1. In a high leukemic strain of mice the development of a malignant lymphoma can be accelerated by a cell-free filtrate obtained from the brain of a leukemic mouse of a homologous strain.

2. In a high leukemic strain of mice, the development of a malignant lymphoma can be accelerated by a cell-free filtrate from the brain of a leukemic human being.

3. In a low leukemic strain of mice the development of a malignant lymphoma can be induced by a cell-free filtrate obtained from the brain of a leukemic mouse of a homologous strain.

4. The so-called malignancy of the lymphoma is evidenced by its progressive growth and its fatal outcome; by its transplantability; by its invasiveness; and by its histologic appearance.

5. The filtrates that transmit the disease are cell-free and the passage does not depend on cell fragments or on cell particles.

6. The agent passes through both Seitz and Berkefeld bacterial filters.

7. Heat (65° for 45 minutes) inactivates the filtrate.

8. The activity of the filtrate is retained after prolonged freezing.

9. The filtrate still shows activity after exposure to 50,000 r delivered by Cobalt60.

10. Activity of the filtrate can be demonstrated after it has been passed serially through several animals of a heterologous strain, which would in theory dilute the original material to less than 10⁻¹⁰, whereas no activity can be demonstrated when the original material is diluted to 10⁻⁵. Only the assumption of self-perpetuation and multiplication can satisfactorily explain this phenomenon.

The reduced infectivity of the agent derived from tumor explains why so many investigators had been unsuccessful in inducing leukemia with tumor extracts, notwithstanding the demonstration now of an active agent in tumor. This reduced infectivity may be the result of the virus being in a noninfective state or it may be the result of the host response which binds, neutralizes, or in part destroys the virus. Zil’ber and his associates demonstrated a neutralizing effect of tumor tissue, which supports our view. Whatever the reason, the infectivity of tumor extract is quantitatively reduced when compared with brain extract. This has been confirmed by Krischke and his associates. Quantitative diminution of infectivity may explain why it was not until newborn animals were injected that leukemia was consistently induced with cell-free extracts of tumor.

Are we dealing with one virus or several different viruses? The evidence is sketchy but favors the interpretation that several different viruses are in-
volved. Eckert's demonstration of the enzymatic specificity of the viruses of the fowl leukemia lends support. We do not believe that the different responses of the host necessarily imply different viruses, nor that the same host response necessarily implies the same virus. We prefer to postulate that the host response is the determinant in the morphologic manifestation of the disease. This is the reason that the AKR lymphoblastoma is the same regardless of whether it arises spontaneously, is induced by filtrates prepared from AKR leukemic brain, or is induced by filtrates prepared from the brains of patients dying of acute leukemia of all cell types.

Drawing an analogy for the human being, this interpretation could explain why different types of leukemias occur in different age groups. In children, in whom the host response is usually lymphoid in character, the characteristic leukemia is acute lymphoblastic. In the aged, in whom the host response is also lymphoid in character, we again see lymphatic leukemia, but in the more indolent form. Between these age extremes of life, leukemia is myelogenous in type, and characteristically the response to other types of infections is also primarily granulocytic.

Does the virus simply accelerate the development of leukemia or does it actually induce it? The high incidence of leukemia in a strain in which its spontaneous occurrence is rare and the rapidity with which it develops after a single inoculation would certainly belie the possibility that the virus is only ancillary or augmenting in influence. This question cannot yet be unequivocally answered. Many other significant factors are contributory. Indeed, any of the factors that change the host environment or condition the "soil" will have profound effects on the responsiveness to the virus. Thus roentgen rays, endocrine alterations, thymectomies, chemicals and other agents act either to increase or retard the susceptibility of the host. The influences of age and heredity are certain.

Growing evidence heavily favors the virus theory of the etiology of the leukemias. It must be recognized, however, that proof of this in itself would not resolve the problem. The most crucial of the unanswered questions is how all factors act and interact to cause leukemia. What is the mechanism? Do all factors simply prepare a chemical milieu which acts as an irritant to which the host responds; or do they diminish the resistance of the host sufficiently so as to make him unable to cast off the invading organism; or is the organism present in the cells from birth and does it only become active under such conditions that in some way alter the cell?

Perhaps the tentative acceptance of the virus theory of etiology will help channel the energies of investigators. Acceptance and substantiation of a virus causation should encourage the development of immunologic or chemical agents that would confer active or passive immunity in the host.

Summario in Interlingua

Es presentate un revista summari del historia de experimentos e investigaciones relative al theorias que le agente causative in le leucemias es un factor viral.

Es describite in detalio un serie de experimentos in muses, per que le
autores visava a testar le duo postulatos (1) que leucemia es inducite per un virus e (2) que leucemia es un morbo systemic, characterisate per manifestationes morphologic que representa un reaction del organismo-hospite de natura primarimente defensive.

Le autores conclude que lor experimentos servi a reinfotiar le supra-citate postulatos, ben que le provas remane indirecte. In omne caso, certe factos pote esser reguardate como establite. Illos es:

1. In muses de un racia a alte susceptibilitate leucemic le disveloppamento de un lymphoma maligne pote esser accelerate per acellular filtratos cerebral ab muses leucemic de racia homologe.
2. In muses de un racia a alte susceptibilitate leucemic le disveloppamento de un lymphoma maligne pote esser accelerate per acellular filtratos cerebral ab humanos leucemic.
3. In muses de un racia a basse susceptibilitate leucemic le disveloppamento de un lymphoma maligne pote esser inducite per acellular filtratos cerebral ab muses leucemic de racia homologe.
4. Le describite effectos es supprimite per calefaction del filtratos a 65 C durante 45 minutas.
5. Le describite effectos del filtratos es intacte post prolongate periodos de congelation.
6. Le activitate del filtratos non dispare post exposition a 50.000 r ab cobalt$^{60}$.
7. Le activitate del filtrato pote esser demonstrate post su passage serial a transverso plure animales de racia heterologe, resultante—theoricamente—in un dilution del material original per un factor de $10^{13}$, durante que nulle activitate pote esser demonstrate quando le material original es diluite directemente per un factor de $10^{-5}$. Iste phenomeno se explica satisfactorimente solo per le supposition de auto-perpetuation e multiplication.

REFERENCES
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30. Unpublished data.


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