frozen state, should facilitate attempts to isolate this agent from human sources, and to define further its role as a respiratory pathogen.

Summary. Coe virus dissociates from infected cells, but 25% to 50% of virus is still intracellular at time of maximum cytopathic effect. The virus was relatively stable when exposed to repeated freezing and thawing, and to storage at −70°C, 4°C, and room temperature. At 37°C, 90% of the virus was inactivated within 3 days, and less than 1% of particles were infectious after 20 days. Coe virus is rapidly inactivated at 50°C, but a few particles survive 30 minutes at this temperature. Coe virus is ether resistant and resistant to inactivation at pH 6 to 9.

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1. Lennette, E. H., Fox, V. L., Schmidt, N. J., Culver, J. O., *Am. J. Hyg.,* 1958, v68, 272.

2. Jordan, W. S., Jr., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 872.

3. Ginsberg, H. S., Gold, E., Jordan, W. S., Jr., *ibid.,* 1955, v89, 66.

4. Ginsberg, H. S., *ibid.,* 1956, v93, 48.

5. Pereira, M. S., Pereira, H. G., *Lancet,* 1959, v2, 539.

6. Lehmann-Grube, F., Syverton, J. T., *Am. J. Hyg.,* 1959, v69, 161.

7. Ginsberg, H. S., *J. Exp. Med.,* 1958, v107, 133.

8. Jawetz, E., Kimura, S. J., Hanna, L., Coleman, V. R., Thygeson, P., Nicholas, A., *Am. J. Ophth.,* 1955, v40, 200.

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Development of Myeloid (Chloro-) Leukemia in Thymectomized C3H Mice Following Inoculation of Lymphatic Leukemia Virus.* (25576)

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Results of recent preliminary experiments suggested(1) that thymectomy renders mice of an otherwise susceptible strain (C3H) resistant to leukemogenic action of a highly potent lymphatic leukemia virus (passage A). This report deals with a) further observations of injected, and thymectomized mice for delayed development of leukemia; b) an attempt to restore susceptibility by subsequent implantation of normal mouse thymus, and c) the unexpected development of myeloid (chloro-) leukemia in some of the thymectomized mice inoculated with the lymphatic leukemia virus, and in one additional non-thymectomized mouse inoculated with the same virus and implanted with normal mouse thymus.

Methods. Passage A strain of leukemic virus initially derived from spontaneous Ak leukemia(2) and then passed serially by cell-free inoculations through newborn mice(3,4) was used. Now in its 21st serial passage, this virus induces consistently lymphatic leukemia in 85 to 100% of suckling C3H mice (Bittner subline) inoculated at less than 10 days of age. The average latency is about 2½ to 3½ months. Adult mice are also susceptible; up to 65% develop leukemia following intraperitoneal inoculation of the virus, but the latency is prolonged, varying usually from 4 to 6 months. Leukemic filtrates (Selas 02) of 20% concentration were prepared(3,4) from pooled leukemic organs from several C3H donors with primary, passage A virus-induced leukemia. All mice were of the C3H, or foster-nursed C3H(f) lines, both of Bittner substrain, bred in our laboratory. Spontaneous lymphatic leukemia in untreated mice of our C3H and C3H(f) colonies does not exceed 0.5%(4). We have never seen myeloid leukemia developing spontaneously in these mice.

Results. Resistance of thymectomized mice to inoculation of virus. It appeared previously that thymectomy either a) following, or b) preceding inoculation of passage A virus ren-

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TABLE I. Inoculation of Leukemic Filtrates into Suckling C3H Mice, Followed by Thymectomy.

Thymectomized*						Controls					
No. of mice inoc.†	No. dev. leuk.‡	Leuk. inc., %	Avg age leuk. dev., mo	No. dev. s.c. Sa	Avg age Sa dev., mo	No. of mice inoc.†	No. dev. leuk.‡	Leuk. inc., %	Avg age leuk. dev., mo	No. dev. par. tum.	Avg age tum. dev., mo
60	26§	43	10	5	9	85	79	93	3.6	2	4.8

* Avg age at thymectomy 32 days.
† 0.10 to 0.15 ml of 20% passage A fil. s.c. or i.p. Avg age at inoc., 3 days.
‡ Avg age thymectomized mice died, or survived, with no signs of leukemia, 14.5 mo.
§ 8 mice among these developed myelogenous leukemia at 12 to 14 mo of age.

dered mice resistant to development of leukemia. The reservation was made, however, that leukemia might still develop later in some thymectomized mice since the average age of surviving animals in group a) was only 4½ months(1). This indeed was the case. A considerable number of mice injected when newborn with virus and subsequently thymectomized, eventually developed leukemia. The incidence among controls was nevertheless substantially higher and leukemia developed earlier (Table I).

Of 60† mice inoculated with passage A leukemia virus at average age of 3 days, and thymectomized about a month later, *i.e.*, at an average age of 32 days, 26 (43%) developed leukemia at average age of 10 months. Of these, 8 developed myelogenous leukemia (chloroleukemia), in 4 the leukemias were unidentified, and in 14 lymphatic disease occurred. The remaining mice survived with no signs of leukemia to average age of 14.5 months. Of 85 litter-mate controls, inoculated simultaneously with the same filtrates, but not thymectomized, 79, or 93% developed lymphatic leukemia, after average latency of only 3.6 months. Thus, thymectomy performed after inoculation of the virus, inhibited development of the disease in some animals, or delayed it considerably in others. A different form of leukemia, hitherto not observed in our mice, that had been inoculated with the same virus, appeared in some of the thymectomized group. A few mice also developed subcutaneous fibro-myxo-sarcomas which were probably unrelated to inoculation of the leukemia virus. Passage A filtrates contain the parotid tumor virus(5), capable of inducing, among other tumors, also subcutaneous sarcomas. Thymectomy rendered most of these mice refractory to development of leukemia and prolonged their lives. Subcutaneous sarcomas developed, usually late, in some of these mice.

On the other hand when thymectomy was performed prior to inoculation of leukemia virus, no leukemia resulted even after prolonged observation. Thirty-nine† young adult C3H mice were thymectomized when 4 to 6 weeks old, and inoculated a few days later with passage A filtrate. None had developed leukemia, although all were observed until an average age of 15 months. Of 41 non-thymectomized control mice, inoculated simultaneously, when approximately 2 months old, with the same leukemic filtrate, 26 (63%) developed lymphatic leukemia at an average age of 8.7 months (Table II).

TABLE II. Effect of Thymectomy, Preceding Inoculation of Leukemic Filtrates, on the Incidence of Virus-Induced Leukemia.

Thymectomized				Controls				
Age at thym., days	No. of mice inoc.*	No. dev. leuk.	Avg surv. age, mo	No. of mice inoc.*	No. dev. leuk.	Leuk. inc., %	Avg age leuk. dev., mo	Avg surv. age, mo
55	39	0	15	41	26	63	8.7	14

* 0.5 ml of 20% passage A filtrate i.p. each. Avg age at inoc., 59 days.

† Several mice were added to this series since the last report was published(1).

TABLE III. Effect of Subcutaneous Implantation of Normal Thymuses on Susceptibility of Thymectomized C3H Mice to Leukemogenic Action of Passage A Filtrates.

	Filtrate only	Filtrate then thymectomy† followed by implantation of normal thymus‡	Filtrate then thymectomy‖
No. of mice inoc.*	36	20	5
No. dev. leuk.	35	18§	2
Leuk. inc., %	97	90	40
Avg age leuk. dev., mo	3.6	8.3	8.5

* Avg age at inoculation, 6 days.
† Interval between inoculation of filtrate and thymectomy, 1 mo.
‡ Interval between thymectomy and implantation of thymus, 7 to 30 days, avg 15 days.
§ 2 of these leukemias were myeloid (chloroleukemia).
‖ The No. of mice in this column is small, but is supported by similar data in Table I.

Attempt to restore susceptibility of thymectomized mice by implantation of normal thymus. Suckling C3H mice less than 8 days old were inoculated with passage A filtrate. After approximately 3 weeks these mice were divided into 2 groups. One group served as control. Mice of the other group were thymectomized. Seven to 30 days (average 15 days) after thymectomy, 20 thymectomized mice received implants of normal thymuses immediately after aseptic removal from young, approximately 3 weeks old, healthy, untreated C3H mice. Implants of whole thymuses from 2 donors were inserted through a small incision in skin of the back of each thymectomized mouse, and gently pushed into the subcutaneous tissue toward the chest. The wound was closed by one or 2 interrupted sutures. Of 36 control mice inoculated with the filtrate, 35 (97%) developed leukemia after average latency of 3.6 months. Of 20 mice inoculated with the filtrate, thymectomized later, and subsequently implanted with thymuses, 18 (90%) developed leukemia at an average age of 8 months (Table III). At least 2 of these leukemias were myeloid (chloroleukemia). In some of these mice a large tumor developed at site of subcutaneous implantation of normal thymuses; these mice also showed, however, evidence of generalized lymphatic leukemia, such as large spleens and livers, infiltrated with lymphoid cells, and large peripheral lymph nodes. In a small additional control group, 5 mice had been inoculated with the leukemic filtrate and were later thymectomized, but did not receive subsequent thymus implants. Only 2 of these developed leukemia after a latency of 8.5 months. These experiments suggest that susceptibility of thymectomized C3H mice to leukemogenic action of passage A virus could be restored by implanting into such mice normal thymuses from young, healthy C3H mice.

Development of myelogenous leukemia in mice that had been inoculated with passage A filtrates. Some C3H mice inoculated when newborn with passage A filtrates, and subsequently thymectomized, remained healthy for a time, but when approximately 12 months old, developed palpable spleens and enlarged peripheral lymph nodes. Autopsy of one of these leukemic mice revealed a green color of the peripheral lymph nodes and a more detailed examination, including microscopic studies of blood and other organs revealed myeloid leukemia. Within a few weeks 7 other mice with a similar form of leukemia were found among surviving filtrate-injected and subsequently thymectomized C3H mice. Myeloid leukemia also developed in 3 additional mice that had been inoculated with passage A virus; only 2 of these 3 mice had been thymectomized, but all 3 received subcutaneous normal C3H thymus implants.

C3H mice not inoculated with passage A filtrate, though thymectomized, did not develop myelogenous leukemia. In an unrelated experiment, 22 normal C3H mice not inoculated with leukemic filtrate were thymectomized when 3 to 4 weeks old. They were then observed without further treatment until they were 14 to 15 months old. None developed leukemia.

Morphology. All 11 mice with primary myeloid leukemia presented similar pathology. The moderately enlarged superficial and internal lymph nodes were in most instances of a characteristic light green color suggesting the diagnosis of chloroleukemia. The green color was particularly pronounced in 7 out of the

11 mice examined. Spleens were large, but livers were only slightly enlarged and of brown color. The number of peripheral white blood cells was increased (to 149,000/cu mm). Blood smears revealed in all 11 mice the presence of myeloblasts, myelocytes, metamyelocytes and unidentified large mononuclear cells; there were numerous myeloid cells and polymorphonuclear leukocytes in all phases of maturity, but relatively few lymphocytes. Nucleated red blood cells were also present; in one instance the number of nucleated red blood cells was considerable (103 nucleated RBC per 100 WBC). There was rapidly progressing anemia with a marked anisocytosis and polychromasia of erythrocytes. One leukemia could be identified as erythroblastic.

Transplantation of myeloid leukemia. Cell suspensions(2-4) from C3H donors with myeloid leukemia were inoculated intraperitoneally into young adult, 4 week old C3H mice. Leukemia developed in most of them after a latency of 2 to 6 weeks. The latency was shortened in subsequent transplantations. The characteristic green color of the superficial and internal lymph nodes was, however, considerably less pronounced in those mice that developed leukemia following transplantation of leukemic cells, than in initial donors with primary, virus-induced, leukemia. Some mice with transplanted leukemia revealed a varying amount of ascitic fluid and presence of multiple leukemic tumors in the peritoneal cavity resulting probably from the intraperitoneally implanted leukemic cells. Some mice had peripheral blood changes characteristic for myelogenous leukemia; in some of the C3H mice with transplanted myeloid leukemia, the number of white blood cells reached 500,000 and even 800,000/cu mm, with a large proportion of myeloblasts and immature myeloid cells. Frequently, however, lymphoid leukemia resulted, with a high proportion of lymphoblasts.

Discussion. The fact that removal of thymus conferred resistance to C3H mice to passage A leukemogenic virus was observed in our laboratory(1) and independently by Levinthal and her associates(6). Removal of the thymus prior to inoculation of the virus was most effective, since such thymectomized animals did not develop leukemia even after long interval of time. On the other hand, when thymectomy followed inoculation of the virus, some mice eventually developed leukemia, either lymphatic, or myelogenous. There was, however, a substantial difference between these 2 groups in age at which inoculation of virus was performed. Animals thymectomized first received the virus when approximately 4 or 5 weeks old. Mice of the other group received the virus shortly after birth, *i.e.*, at time of higher susceptibility. This could explain why a substantial number of mice in this group later developed leukemia. Levinthal and her associates did not observe development of leukemia in mice that had been inoculated with the virus and then thymectomized, but their experiments were terminated when the mice were only 7 to 9 months old(6). It is conceivable that at least some of their thymectomized mice could have developed leukemia later. It is rather difficult to remove completely both thymic lobes in an operative procedure requiring skill and speed to avoid hemorrhage and respiratory difficulties. We assume therefore, that in at least some of our mice that developed lymphatic leukemia in spite of thymectomy, parts of their thymic lobes might have been left after operation.

Implantation of a normal thymus into thymectomized animals restored their susceptibility to leukemogenic virus. In previous similar experiments by Law(7), Kaplan(8), Carnes(9), and their associates, implantation of normal thymus into thymectomized mice also restored their susceptibility to carcinogen or radiation-induced leukemia, respectively. More recently Miller(10) also observed that susceptibility of thymectomized C3H mice to inoculation of passage A leukemic virus could be restored even several months after thymectomy, by implantation of a normal, homologous thymus. These experiments suggest that thymus is not the only organ where the leukemic virus is stored. Animals that had been injected with the virus and were later thymectomized, apparently still carried the virus.

The most startling finding was the recent observation of development of myeloid leuke-

mia in some of the thymectomized mice inoculated with passage A leukemia virus. That form of leukemia has not been hitherto observed in normal C3H mice receiving only passage A virus without additional treatment. In previous experiments several hundred newborn and suckling C3H mice had been inoculated with passage A filtrates. Up to 95% of the inoculated mice developed lymphatic leukemia. Over 70 blood counts were recently made on these mice. In some mice (not exceeding 20%) the disease was aleukemic. Peripheral blood examination revealed in the majority increased numbers of white blood cells (average 53,300/cu mm) predominantly of the lymphocytic series (average 65% of lymphocytes) with presence of lymphoblasts (average 7.1%) consistent with the diagnosis of lymphatic leukemia. Quite frequently, however, the blood picture became leukemic only in terminal phases of the disease.

Although there were not always significant peripheral blood changes, the plasma of these mice contained the virus. Filtrates of plasma from C3H mice with passage-A-virus-induced leukemia were almost as leukemogenic on inoculation tests as the highest concentrations of "organ filtrates"(3,4) of spleens, livers, thymic tumors, and mesenteric and peripheral lymph nodes from the same donors. This observation (unpublished) suggested that the leukemic virus is present in relatively high concentration in blood plasma of mice with passage-A-virus-induced leukemia.

Development of myelogenous leukemia in some C3H mice inoculated with passage A (lymphatic) leukemia virus is of considerable interest. Most of these mice were thymectomized after inoculation with the agent. One is tempted to speculate that removal of thymus had a significant relation to development of myelogenous leukemia in these mice. Another mouse also received passage A virus, but instead of having the thymus removed, received a subcutaneous implantation of an additional thymus from a homologous host. Here again there may be some obscure, perhaps indirect, relation between the implanted thymus and development of myelogenous leukemia.

It could be assumed that development of myelogenous leukemia was "spontaneous," and had no relation to injection of the virus. It would be difficult nevertheless to accept such an explanation. We have never seen spontaneous myelogenous leukemia among untreated mice of our C3H or C3H(f) colonies observed during the past 15 years. One could assume, on the other hand, that myelogenous leukemia developed in these mice as a result of inoculation of passage A leukemia virus. If so, a) the same virus might cause either lymphatic or myelogenous forms of leukemia, b) we may be dealing with a mixture of distinct leukemogenic viruses.

A similar phenomenon was observed in mice of the Ak strain, which carry, and transmit naturally from one generation to another, a leukemic agent(2) presumably identical with that contained in passage A filtrates(3). If left undisturbed, up to 90% of Ak mice develop leukemia spontaneously, as a rule lymphoid, when about 7 to 12 months old. Development of myelogenous leukemia in untreated Ak mice has been reported, but is exceedingly rare(11,12). We have not seen myelogenous leukemia among many hundreds of spontaneous Ak leukemias studied during the past 15 years.

Incidence of spontaneous leukemia in mice of the Ak strain could be considerably reduced by thymectomy(13). Furth observed, however, that among thymectomized Ak mice whose life was prolonged, several later developed myelogenous leukemia(12). Chloroleukemia was also observed in a mouse of the Ak strain(14), but that particular animal had been irradiated.

The first transmission of chloroleukemia in mice by cell transplantation was reported by Hall and Knocke(15). More recently, Graffi observed frequent development of chloroleukemia in mice inoculated with filtrates prepared from several transplantable mouse tumors. Only 10% of the myelogenous (chloro) leukemias observed by Graffi(16) could be transmitted by cell transplantation, but at least some of them could be transmitted by filtrates. The myelogenous leukemias we observed could be readily transplanted by cell graft, retaining their myelogenous blood morphology, or changing to lymphatic form. Attempts to transmit these leukemias to new-

born and suckling C3H mice by filtrates are now in progress in our laboratory.‡

Burmester *et al.*(17) observed recently that when cell-free extracts prepared from a presumably pure strain of avian myeloblastosis A were inoculated into susceptible chicks, either myeloblastosis, visceral lymphoblastosis (lymphatic leukosis), renal adenocarcinomas, or osteopetrosis resulted. All combinations were observed except a concurrent development of myeloblastosis and lymphatic leukosis. In another series Burmester *et al.* observed that filtrates prepared from chicken erythroblastosis strain R induced either erythroblastosis, or visceral lymphomatosis. The fundamental question remains to be answered whether a single virus is capable of inducing different forms of chicken leukosis, or whether we are dealing with a mixture of distinct, although possibly related, leukemogenic agents. The problem is similar in the mouse.

Summary. 1. Thirty-nine C3H mice were thymectomized at 4 to 6 weeks of age and inoculated a few days later with passage A leukemic filtrates. None developed leukemia, although observed until they were 15 months old. Of 41 non-thymectomized adult control mice injected simultaneously with the same filtrates, 63% developed leukemia at 8.7 months average age. 2. Of 60 C3H mice inoculated with passage A leukemic virus at average age of 3 days and thymectomized a month later, 26 (43%) developed leukemia at 10 months average age. Among leukemic mice in this group, 8 developed myelogenous (chloro) leukemia. Of 85 litter-mate controls injected simultaneously with the same filtrates, but not thymectomized, 93% developed lymphatic leukemia at 3.6 months average age. 3. Myelogenous leukemia developing in some virus-injected and subsequently thymectomized mice could be readily transplanted to mice of the same strain, retaining its myelogenous form or changing to lymphoid. 4. Susceptibility of thymectomized mice to the leukemogenic action of passage A virus could be restored by subcutaneous implantation of thymuses from young normal C3H mice. Of 20 C3H mice injected with the leukemic virus, then thymectomized, and which subsequently received subcutaneous implants of normal C3H thymuses, 18 developed leukemia (2 of these were myelogenous), as compared with 35 of 36 non-thymectomized controls that had received the same filtrates.

‡ As this manuscript goes to press, among the 117 C3H mice inoculated at an average age of 2 days with filtrates (Selas 02) prepared from myelogenous leukemias, 21 mice (18%) have thus far developed either thymic lymphosarcomas, or generalized lymphatic leukemia, at an average age of 3 months; a high proportion of lymphoblasts in peripheral blood was noticed in some of them.

1. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 325.
2. ———, *ibid.*, 1951, v76, 27.
3. ———, *ibid.*, 1957, v94, 767.
4. ———, *ibid.*, 1958, v97, 300.
5. ———, *ibid.*, 1953, v83, 414.
6. Levinthal, J. D., Buffett, R. F., Furth, J., *ibid.*, 1959, v100, 610.
7. Law, L. W., Miller, J. H., *J. Nat. Cancer Inst.*, 1950, v11, 425.
8. Kaplan, H. S., Carnes, W. H., Brown, M. B., Hirsch, B. B., *Cancer Research*, 1956, v16, 422.
9. Carnes, W. H., Kaplan, H. S., Brown, M. B., Hirsch, B. B., *ibid.*, 1956, v16, 429.
10. Miller, J. F. A. P., *Nature*, 1959, v184, 1809.
11. Barnes, W. A., Sisman, J. E., *Am. J. Cancer*, 1939, v37, 1.
12. Furth, J., *J. Geront.*, 1946, v1, 46.
13. McEndy, D. P., Boon, M. C., Furth, J., *Cancer Research*, 1944, v4, 377.
14. Flory, C. M., Furth, J., Saxton, J. A., Reiner, L., *ibid.*, 1943, v3, 729.
15. Hall, J. W., Knocke, F. J., *Am. J. Path.*, 1938, v14, 217.
16. Graffi, A., Bielka, H., Fey, F., *Acta haemat.*, 1956, v15, 145.
17. Burmester, B. R., Walter, W. G., Gross, M. A., Fontes, A. K., *J. Nat. Cancer Inst.*, 1959, v23, 277.

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