Electron Microscopic Observations on Primary and Serially Passaged Radiation-induced Myeloid Leukemias of the RF Mouse

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SUMMARY

RF mice with primary and passaged radiation-induced myeloid leukemia were examined with the electron microscope for the presence of virus particles. The spleen, liver, and bone marrow of nearly all such mice examined contained small numbers of these particles. In one of two animals in which other tissues (thymus, pancreas, salivary glands, lymph nodes, omentum, lung, and brain) were examined, the thymus and pancreas also showed small numbers of particles. Much larger numbers of particles of the same morphology were seen in tissue cultures derived from the serially passaged leukemias. One control tissue culture, but none of the control tissues, contained similar particles. The particles resembled those associated with AKR mouse lymphoid leukemia (Type C in Bernhard’s morphological classification of tumor viruses). Although cell-free filtrates of the particle-containing tissues have shown lymphoid and myeloid leukemogenic activity, the possible etiologic significance of the particles remains to be established.

Although spontaneous myeloid leukemia occurs infrequently in most strains of mice (6), its incidence approaches 3–5 per cent in mice of the RF strain (1, 29). A marked increase in the incidence of the disease occurs in RF mice following whole-body ionizing irradiation (9, 29), along with an appreciable incidence of thymic lymphomas (31). Other agents apart from x-rays, such as indole (8) or 3-hydroxy-anthranilic acid (7), also markedly increase the incidence of myeloid leukemia in this strain. In view of the importance of viruses as etiological agents in mouse leukemia (15) and the well known depression of the immunological defense mechanism by x-rays, it is natural to suppose that in some radiation-induced leukemias a latent tumor virus is responsible. Cell-free passage of lymphoid leukemias induced by radiation has been successful in the low-leukemia C3H (16) and C57BL/Ka (19) mice, and particles resembling tumor viruses have been observed in the electron microscope in these leukemias (5, 18). Preliminary data encouraged the working hypothesis that radiation-induced leukemias in the RF strain were also transmissible by cell-free agents (29).

A filtrate-induced myeloid leukemia of the mouse has been extensively investigated by Graffi (11, 13), but the disease was produced by inoculation of cell-free extracts of various sarcomas and carcinomas. This leukemia is transferable by cell-free filtrates into Agnes-Bluhm mice as well as into Wistar rats. In the original transfer of S-37 tumor filtrates to mice, a marked increase in the incidence of myeloid leukemia was obtained if the recipient was given whole-body irradiation with x-rays just before, or just after, the inoculation (14). More recently, the Gross virus has been found to give myeloid leukemia if the recipient mice are thymectomized after inoculation (17).

In this report, a preliminary summary of our attempts to establish the role of tumor viruses in the etiology of radiation-induced myeloid leukemias of...
The pathology of the disease is described, as observed by light and electron microscopy in primary leukemias (directly induced by x-rays) and in leukemias developing after a number of serial passages of the tumor cells. The significance of virus particles observed in vivo and in vitro with the electron microscope is discussed.

MATERIALS AND METHODS

Mice and irradiation conditions.—The mice with primary radiation-induced leukemia were randomly bred males of the RF/Up strain, 4–12 months old, previously exposed to 150–450 r of whole-body x-radiation at 8–10 weeks of age. The radiation factors, conditions of treatment and diagnosis, and clinicopathologic characteristics of the leukemia were identical to those described previously (31), unless otherwise indicated.

Mice with transplanted leukemia were randomly bred and inbred RF/Up males and females given inoculations at less than 1 day of age (“newborn”) or at 65–75 days of age (“adult”). Some groups of adult recipients were exposed to 200–300 r whole-body 300 kvP x-radiation within 24 hours before or after inoculation (Table 1). After injection, the mice were inspected periodically for signs of leukemia or other effects. Males and females were housed separately, eight to ten per cage, with Purina Laboratory Chow and drinking water freely accessible at all times.

**Cellular transfer technique.—** Mice showing enlargement of the spleen, pallor of ears and tail, and

**TABLE 1**

INCIDENCE OF MYELOID LEUKEMIA IN RELATION TO TYPE OF PASSAGE MATERIAL INOCULATED

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>Passage Material:</th>
<th>Whole Cells</th>
<th>Supernate</th>
<th>Filtrate</th>
</tr>
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<tr>
<td></td>
<td>Recipient:</td>
<td>Irrad. adult</td>
<td>Newborn</td>
<td>Irrad. adult</td>
</tr>
<tr>
<td>1</td>
<td>No. pos./no. injected:</td>
<td>5/20</td>
<td>142</td>
<td>48–272</td>
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<tr>
<td></td>
<td>Latency (days) / mode</td>
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<td>30</td>
<td>29–217</td>
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<td></td>
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<td>39</td>
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<td>33/69</td>
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* 200–300 r within 24 hours before injection.
† 300 r within 24 hours before or after injection.
increased numbers of promyelocytes and myelocytes in the peripheral blood were killed, and their spleens removed aseptically. Part of the spleen (and also liver, kidney, bone marrow, and lymph nodes) was removed for light and electron microscopy. The remainder of the spleen was minced with scissors in 8–10 ml. of cold, sterile Tyrode’s solution to give a suspension of 100–130 × 10⁶ nucleated cells/ml. Such a suspension was inoculated intravenously, 0.1–0.5 ml. into each adult recipient by tail vein, and 0.05 ml. into each newborn recipient by facial vein. Similar suspensions prepared from normal spleens were correspondingly inoculated into controls.

**Supernate passages.**—In some passages (Table 1), aliquots of the spleen cell suspension were centrifuged in the cold (0–5° C.) for 20 minutes at 1500 × g. The resulting supernate was pipetted from the centrifuge tube and injected intravenously into recipients in the same volume as was used with the original cell suspension. Although the supernate invariably appeared grossly free of cellular contaminants, on microscopic examination it was found to contain up to three cells per cu. mm. Such a suspension was inoculated in adult and newborn recipients in the amount used to give a suspension of 100–130 × 10⁶ nucleated cells/ml. In no instance was growth obtained in the centrifugation procedure, which was carried out in the earliest experiments. In no instance was growth obtained in the filtrate, although the organism was recovered from the fluid before filtration. On completion of the filtration procedure, which was carried out in the cold, filtrate was inoculated intravenously into adult and newborn recipients in the amount used with the other passage materials.

**Cell-free filtrate passages.**—In all later passages (Table 1), aliquots of the aforementioned supernates were subjected to filtration before inoculation. This was accomplished by passing the fluid through a Selas 03 filter, under a negative pressure of 9 mm. Hg. To check the integrity of the filter, *Escherichia coli* were added, and the fluid was cultured in nutrient broth before and after filtration (this procedure was omitted in the earliest experiments). In no instance was growth obtained in the filtrate, although the organism was recovered from the fluid before filtration. On completion of the filtration procedure, which was carried out in the cold, filtrate was inoculated intravenously into adult and newborn recipients in the amount used with the other passage materials.

**Tissue culture technic.**—The most successful method tried for tissue culture of the normal and leukemic spleen cells was that of Manaker (21). The only variant of the technic in the present work was the occasional substitution of medium 199 for the Yeastolate medium (this, however, did not appear to have any particular advantage). Aureomycin (25 mg/ml), penicillin (120 units/ml), and streptomycin (0.12 mg/ml) were added to the medium at all stages of the culture. Two or more tubes were taken from each culture for electron microscopy at 7, 10, 14, 17, 21, and 24 days, provided cell growth on the walls of the tube was evident under the light microscope.

**Electron microscope technics.**—In most experiments, the spleen, liver, kidney, and bone marrow were examined. In other cases, lymph nodes, salivary glands, thymus, pancreas, lung, brain, and white blood cells were also taken. Small pieces (1-mm. cubes) were removed from all parts of the organ examined. Tissue was fixed for 90 minutes in Palade’s osmic acid (24) and dehydrated in a graded series of alcohols or acetones. In most cases, the tissue was embedded in methacrylate and polymerized by ultraviolet light (27). All methacrylate sections were stained for 7 minutes with 50 per cent saturated lead subacetate (4) in an atmosphere of nitrogen (26). In some cases, the tissue block was stained with an acetone solution of potassium permanganate (25) following dehydration in acetone. The stained tissue was then embedded in Epon 812 Epoxy resin (20). Sections of unstained tissue, embedded in Epon, were stained for 1 minute with lead subacetate.

Special handling was required for the small quantities of cells present in the tissue cultures. With aseptic technic, the cells were scraped from the wall of the tube with a scraper fashioned from a silicone-rubber stopper. The cells were sedimented by centrifugation, and the medium was completely removed, quickly frozen, and stored at −65° C. Two ml. of Palade’s osmic acid were added to the cells, and the resulting cell suspension was transferred to small, pointed tubes and centrifuged. The osmic acid was then decanted, and the cells were washed twice more in osmic acid. It was found that admixture of the medium with osmic acid or delay in adding the osmic acid to the scraped cells gave poor fixation. The cells were dehydrated and embedded as described above.

Sections were cut on a Porter-Blum microtome with a 1.8-mm., 50° angle, diamond knife (Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela) and examined either in an RCA EMU-3e electron microscope or in a Siemens Elmiskop I. In searching for virus particles two to four blocks of each tissue were examined, and 1 grid of sections was taken from each of two levels of every block, under a magnification of 9,000×.

**RESULTS**

**Clinico-pathological features of the leukemia.**—Whether spontaneous, radiation-induced, or produced by serial passage of leukemic spleen cells, centrifuged supernates, or filtrates, the myeloid leukemias all showed essentially the same gross features. The first signs of leukemia were pallor, weight loss, and ruffling of the fur. Mice showing these signs were examined by abdominal palpation for splenomegaly, a constant feature of the disease (Fig. 1). In addition to enlargement of the spleen, the liver was usually enlarged. The lymph nodes
and thymus were generally of normal size or atrophic.

The peripheral blood invariably contained primitive myeloid cells and usually showed signs of leukocytosis. In the primary disease, the shift to the left was not extreme; mature granulocytes were present in normal or increased numbers, and cells as primitive as the promyelocyte were rare (<1 per cent). With successive passages, however, the shift to the left became more pronounced, promyelocytes and myeloblasts occurred with increasing frequency (~10 per cent) in the blood, and mature polymorphonuclear cells decreased in number. In almost all cases, occasional normoblasts were noted along with polychromasia of the red blood cells.

Histologically, the bone marrow and spleen showed leukemic infiltration in all cases. The next most commonly involved organ was the liver (Figs. 2–5). Infiltration of other tissues was variable and seldom pronounced. In both primary and passaged leukemias, the diffusely infiltrated femoral marrow was frequently necrotic, necrosis in some cases extending throughout the entire marrow cavity. Marrow necrosis was noted in more than half of the animals examined, without relation to age or previous irradiation. The cellular composition of the leukemic infiltrate varied, depending on the number of previous passages. In the primary disease, mature granulocytes were conspicuous (Figs. 2 and 3), whereas after several passages maturation was less advanced (Figs. 4 and 5). Even after 22 passages, however, promyelocytes and myelocytes continued to be more numerous than myeloblasts, and metamyelocytes remained common. Aside from the tendency toward dedifferentiation, which occurred on serial transplantation, the disease conformed morphologically to earlier descriptions (1, 6, 30). The cases resulting from inoculation of supernates and filtrates showed essentially the same dedifferentiation as those induced by cell suspensions. Preliminary diagnosis was made by means of the blood film (Fig. 7) and spleen imprint (Fig. 6). Final diagnosis was based on the histologic examination of spleen, bone marrow, and other organs.

An electron micrograph of promyelocytes from the spleen of a mouse which developed leukemia following inoculation of a filtrate is shown in Figure 8. The primitive character of the leukemic cells is indicated by the even dispersion of the chromatin. The mitochondria are more ovoid and less cylindrical than in more mature granulocytes. A late-stage metamyelocyte is shown in Figure 9. The nucleus is ring-shaped but appears separated into lobes, because the plane of sectioning has passed through indentations into the nucleus. The chromatin shows marked unevenness of distribution, especially at the nuclear membranes. The mitochondria are cylindrical. The endoplasmic reticulum is present as flattened sacs.

The granulocytes from most leukemic animals appeared identical with those seen in normal mouse bone marrow. However, in the leukemic mice receiving serially passaged spleen cells and in which peripheral blood showed myeloblasts and promyelocytes, abnormal cells were seen (Fig. 10). The cytoplasm of such dedifferentiated cells was unusually empty of granules and other structures. The nucleus was round, with evenly dispersed chromatin. The general appearance was similar to that of the lymphoblast except that sometimes granules were present in the cytoplasm.

Results of the transfer of leukemic spleen cells, centrifuged supernatants, and filtrates.—The pedigree of serial passages shown in Chart 1 indicates the relation among those materials utilized in the electron microscope work reported herein. Some fluctuation in incidence occurred from one passage to another, but, in general, the incidence of takes increased (Table 1) between the first and ninth passages of cells (since mice are still developing leukemia in the ninth and tenth passages, the apparent fall in incidence in these passages is presumably due to incompleteness of the data). It can also be seen that transfer of leukemic cells into irradiated 8- to 10-week-old mice and into nonirradiated newborn mice is about equally effective. The inoculation of supernates from low-speed centrifugation of leukemic spleen-cell suspensions was surprisingly effective. Although only four passages were carried out with such material, it was not significantly less effective than a much larger number of cells—i.e., on inoculating up to 0.5 ml. of supernates, a maximum of 1500 cells was given, whereas 12 X 10^6 to 60 X 10^6 cells were inoculated in the transfer of uncentrifuged cell suspensions. Insufficient data are available to permit comparison of the effectiveness of supernates in young adult as opposed to newborn recipients. A significant number of positive results was obtained with filtrates, but only in one of the cases tabulated was the integrity of the filter verified simultaneously with E. coli broth; the same filters, however, have not shown any leakage of bacterial cells to date, and 22 of 507 unirradiated mice since inoculated with E. coli-controlled filtrates have developed leukemia within 25 weeks after injection, whereas none of 1751 noninjected controls have become positive during the same age interval.

Also noteworthy was the occurrence of neoplasms other than myeloid leukemia in unirradiated filtrate-inoculated newborn recipients—i.e.,

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ten out of 156 such animals developed thymic lymphomas within 71–180 days after inoculation, an incidence many times higher than that in un.injected RF controls (31).

The modal value of the latency in the various passages decreased with successive cell transfers. Tissue culture fluids from cultures showing virus particles in and around the cells were inoculated into newborn RF and C3H recipients and into irradiated adult RF recipients. To date, however, the preliminary results of such experiments are negative for myeloid leukemia, although two thymic lymphomas and one generalized leukemia have developed among 94 newborn RF recipients. One other recipient developed tumors of both ovary and mammary gland, and another developed tumors of the liver and adrenal. No tumors have developed thus far in 40 newborn RF and eighteen newborn C3H mice inoculated with unused tissue culture medium.

Electron microscopy of tissues from leukemic and control mice.—The results of electron microscope examinations for virus particles are shown in Table 2. No particles were seen in the tissues (spleen, liver, and bone marrow) of six control mice (young adults 8–16 weeks old); in three of the six controls, normal isologous spleen cells had been inoculated at the age of 8 weeks, and the animals were killed 6–8 weeks later.

The tissues (in most cases, spleen, liver, bone marrow, and kidney and, in three cases, the peripheral white blood cells and ultracentrifuged [45,000 X g for 40 minutes] plasma pellets) of eighteen primary radiation-induced myeloid leukemias were examined in the electron microscope for virus particles; in nearly all cases long searching showed a few particles in the spleen. Most often these particles (80–110 mμ diameter with a 45–55 mμ dense center) were present between myeloblasts and promyelocytes in an area of leukemic infiltration,
but in a few cases single particles were seen in vacuoles in the cytoplasm of these cells. In five cases, particles were seen more frequently, and these animals were recorded as being positive. No structures suggestive of inclusion bodies were seen. Of the eighteen primary leukemias examined, nine were possibly complicated by earlier inoculation of materials derived from lymphomatous AKR or RF tissues. In most cases, however, the material (a vaccine prepared against AKR leukemia cells for another experiment) would not be expected to contain live AKR virus (15). Furthermore, none of the mice showed any evidence of lymphoid leukemia. Spontaneous myeloid leukemias have not developed leukemia after inoculation of filtrates all showed a slightly increased frequency of particles. In one of these animals, particles were fairly numerous (Fig. 11).

In two mice into which the passage of leukemia cells was made directly after birth, other tissues (salivary glands, lymph nodes, thymus, pancreas, omentum, lung, and brain) were examined to determine whether any reservoirs of particles might be present at such sites. In one of the animals, a small number of particles was seen in the thymus and pancreas.

**Electron microscopy of cultured leukemic and control tissues.—** Attempts to selectively grow out cells

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<th>DIAGNOSIS ON MATERIAL EXAMINED</th>
<th>TYPE OF SPLEEN CELLS TRANSFERRED</th>
<th>X-RAY DOSE TO RECIPIENT (R)</th>
<th>AGE OF RECIPIENT AT TRANSFER MORTON</th>
<th>LATENCY (MONTHS)</th>
<th>NO. POSITIVE FOR PARTICLES/NO. EXAMINED</th>
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<td>Nonleukemic, control</td>
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* Spleen, liver, and bone marrow (see text).

been available thus far for electron microscope examination.

In the spleen (Fig. 10) of a mouse with early leukemia developing 1 month after the second successive passage of leukemic spleen cells, many particles were seen. The particles had an outer diameter of 90–120 mJ and an inner dense portion with a diameter of 40–55 mJ; they appeared similar to those seen in primary leukemias. The tissues of eight other irradiated mice with passaged leukemic spleen cells showed no appreciable increase in frequency of particles over that found in primary leukemias. The frequency of particles was slightly greater in the tissues of three newborn recipients in which leukemia developed 3–4 weeks following administration of leukemic spleen cells. In all instances, particles were seen most frequently in the spleen. The tissues of four animals de-
recorded as positive for virus particles when recognizable particles of about 100 mμ diameter having a dense center were seen in at least two (nonserial) sections or in different blocks of such sedimented cells (Figs. 12-18). When particles were seen at all, they were present most frequently at 7-14 days from the start of the culture. Hence, cultures were called negative if well preserved cells were seen in at least one of the specimens taken at the 7th, 10th, or 14th days, and virus particles were lacking.

Of five satisfactory tissue culture preparations of spleen cells from mice with primary myeloid leukemia, only one (Figs. 12-14) was positive for particles. In contrast, eleven of nineteen satisfactory cultures prepared from adult mice given inoculations in the 1st to 6th passage of leukemic spleen cells, showed particles 7-14 days following the start of the culture (Figs. 16-18). Of nine cultures prepared from newborn recipients developing myeloid leukemia, only two were definitely positive. Altogether, therefore, about one-half of the cultures were positive in the third and subsequent passages (Chart 1).

One of the control tissue cultures prepared from six normal 10-week-old RF mice showed similar virus particles. Cultures from three other nonleukemic mice (the absence of neoplasm was confirmed histologically) killed at 12 months of age were negative for particles. No particles were, likewise, seen in the remaining group of six controls, into which one passage of normal spleen cells had been made at 8 weeks of age followed the next day by 300 r whole-body x-radiation. These mice were killed (without signs of leukemia) after 7 weeks, which represented the latent period for development of leukemia when leukemic cells were passed into similar animals. Altogether, therefore, only one out of fifteen control mouse tissue cultures was positive for particles.

DISCUSSION

The present work has shown that particles of tumor-virus morphology are a relatively constant feature (although often present only in small numbers) of the radiation-induced myeloid leukemia of RF mice. Although it is not established that these particles represent a latent tumor virus responsible for the increased incidence of myeloid leukemia after irradiation, they are associated with filtrates having leukemogenic activity. The relatively low leukemogenic potency of such filtrates is not altogether surprising, since more active filtrates were obtained from the AKR mouse lymphoid leukemia only after several successive cell-free passages through newborn mice (15).

The initially low activity of our filtrates and those from the AKR leukemia, which has a spontaneous incidence as high as 80-100 per cent, and the narrow range of mouse strains receptive to the AKR virus contrast with the situation in certain other mouse leukemias. For example, highly active filtrates with much less strain specificity have been reported by Moloney (23) for another lymphoid leukemia of the mouse. The mouse myeloid leukemia of Graffi (11-13) could also be readily passed by cell-free filtrates. It may be significant, however, that the viruses responsible for the leukemias of both Moloney and Graffi were being carried in certain unrelated mouse sarcomas and carcinomas. The presence of leukemia viruses in such tumors may have been due to frequent exposure of the tumors to virus infection during repeated passages in one or more strains of mice. Thus it is conceivable that carcinoma- or sarcoma-adapted viruses whose main site of action is the hematopoietic system should not be very host-cell-specific. If this is the case, it is not surprising that the viruses are leukemogenic in a variety of mouse strains. No such opportunities for selection of leukemia viruses have occurred in the inbred mouse strains which spontaneously develop a high incidence of leukemia—e.g., in the AKR strain, which was developed by Furth (10) by inbreeding of leukemic sublines of mice. Since the spontaneous myeloid leukemia of RF mice appears similar to the type induced by radiation in the same strain, any virus causing the disease might also be expected to show a narrow range of host susceptibility. For this reason, it was considered desirable to use isologous (RF) recipients in these experiments. Furthermore, the spontaneous incidence of myeloid leukemia is low enough (8 per cent) to allow demonstration of the leukemogenic activity of filtrates in unirradiated RF recipients. On the other hand, it is conceivable that a virus present in this strain and occasionally giving rise to spontaneous leukemia might also have immunized a significant proportion of the animals in the colony, thus interfering with the induction of the disease in all recipients.

The virus particles in tissues and spleen cell cultures of leukemic (and one control) mice show three main features. First, the particles appear morphologically similar in all specimens examined. Second, the particles have the general morphology of the larger-sized tumor viruses. Third, the particles were seen much more frequently and in larger numbers in tissue cultures from mice developing leukemia after serial passages of leukemic cells. The particles described in the present report resemble particularly the virus of AK lymphoid leukemia, Type C in Bernhard's classification (9).
Similar, but not identical, particles were also seen in the leukemias of Moloney and Friend (3). In a few cases, Type A particles (3) of doughnut shape without a dense center were seen in our tissue cultures. Type B particles with an eccentric nucleoid (3) were not seen. The similarity of the Type C particles in the spleens of our primary and serially passaged leukemic mice, in tissue cultures of leukemic spleen cells and in one control spleen-cell culture, is striking. The particles contain a single outer membrane (Fig. 18) 100 m/¿ in diameter and 35 A in thickness. Their inner dense center has a diameter of 45–55 m/¿ and does not appear to have a limiting membrane. The dense center contains fibrils of a width of 40 A similar to those found in many chromosomes of nuclei with disperse chromatin (28).

The morphological similarity of the described particles with those seen in AK lymphatic leukemia, raises the question of whether the same agent is present in the RF strain in which it produces predominantly myeloid leukemia, although thymic lymphomas also occur both spontaneously and after irradiation in such mice (29). In a few cases, moreover, our transfer of leukemic filtrates into RF mice resulted in thymic lymphomas at a greater-than-expected frequency, a result seemingly at variance with that noted earlier with unpasaged AKR lymphomatous brain filtrates (29). Consistent with this hypothesis are the observations of Gross (17) that myeloid leukemia occurs in thymectomized CSH mice following inoculation of AK lymphatic leukemia virus and of J. Furth (personal communication) that the AK lymphoid leukemia virus of Gross increases the incidence of myeloid leukemia in irradiated RF/Jax mice.

Particles were found most frequently in tissue cultures derived from animals developing leukemia after injection of serially passaged leukemic cells. This result could be interpreted as an increase in activity of a virus (either by modification of the virus or by increase in numbers of virus particles, or both) with serial passage. The incubation period was also dramatically shortened, from 1½ months to 10 days, by serial passages, and the leukemic cells appearing in the peripheral blood became progressively more primitive. Although these changes in latent period and malignant cell type might be due to selection or modification of the injected cells rather than of any injected virus, the result might be equally well interpreted as the effect of increased activity of a latent tumor virus. There was no suggestion of the increasing occurrence of another disease apart from myeloid leukemia as the serial passages progressed. Since the particles retained the same morphology but increased in number, it seems probable that serial passage merely increased the concentration of the particles seen in the primary leukemia.

REFERENCES


**FIG. 1.**—Mouse with myeloid leukemia, showing enlargement of spleen and liver and atrophy of thymus and lymph nodes. ×3/4.

**FIG. 2.**—Liver from mouse with primary myeloid leukemia, showing diffuse leukemic infiltration. H. & E., ×150.

**FIG. 3.**—Detail from Fig. 2, showing moderate maturation of myeloid cells in leukemic infiltration around central vein. H. & E., ×600.

**FIG. 4.**—Liver from mouse from transplanted myeloid leukemia, showing predominantly less mature cells infiltrating around central vein than those in the primary leukemia illustrated in Fig. 3. H. & E., ×600.

**FIG. 5.**—Detail from Fig. 4, further illustrating the immature character of the leukemic cells. H. & E., ×1500.

**FIG. 6.**—Spleen imprint from mouse with transplanted myeloid leukemia, showing predominance of promyelocytes and myeloblasts. Wright-Giemsa, ×1500.

**FIG. 7.**—Blood smear from mouse with transplanted myeloid leukemia, showing presence of promyelocytes and myeloblasts. Wright-Giemsa, ×1500.
Fig. 8.—Closely packed promyelocytes in the spleen of an unirradiated adult recipient (RF 7795) in the 9th-passage generation, which developed filtrate-induced myeloid leukemia after a latent period of 3 months. The cells in the field are classified as early promyelocytes, since the nuclei (N) are only slightly flattened, the chromatin is evenly distributed and moderate numbers of granules (G) are present in the cytoplasm. The promyelocytes illustrated closely resemble normal promyelocytes, but such cells were sometimes seen with empty-looking cytoplasm and a more irregular nucleus. Embedded in Epon and the section stained for 1 minute in lead subacetate. (G = Golgi zone, M = mitochondria.) X9,400.

Fig. 9.—A metamyelocyte from the spleen of the same animal shown in Fig. 5. The typical ring-shaped nucleus (N) appears to be separated into four parts, because the plane of sectioning passes through indentations in the main nuclear mass. Characteristically, the chromatin is more aggregated, especially adjacent to the nuclear membranes. Granules (G) of varying density are shown at the center of the cell. The mitochondria (M) are more cylindrical than in earlier stages of the granulocyte. Embedded in Epon and the section stained for 1 minute with lead subacetate. X10,200.

Fig. 10.—Portion of the spleen of an irradiated adult recipient in the 2d-passage generation (RF 7401), which developed leukemia 1 month after injection of leukemic spleen cells. The spleen showed large numbers of virus particles (V) at the plasma membranes of cells. The cells (L) show a particularly empty cytoplasm, together with a large nucleus containing evenly distributed chromatin. The cells may be lymphoblasts inside a lymphoid follicle or atypical leukemia cells. It may be significant that this was the earliest leukemia diagnosed. The virus particles (V) have an outer membrane of 90–140 mµ diameter and an inner nucleoid of 40–55 mµ diameter. This and all of the following electron microscope specimens were embedded in methacrylate and the sections stained 7 minutes with lead subacetate (P), unless otherwise stated. X23,500.

Fig. 11.—Portion of the spleen of a newborn recipient in the 8th passage generation (RF 7361) which had received filtrate 4 weeks earlier. In the spaces between the cells (L), among some cell debris, there are virus particles of 85–100 mµ diameter similar to those shown in Fig. 10. X26,000.
Fig. 1:—Cell from 10-day tissue culture of spleen of a mouse (RF 15075) which developed myeloid leukemia (primary) 8 months after 300 r of whole-body x-radiation at the age of 10 weeks. An inclusion body (I) of virus particles (V) appears to be present in the cytoplasm. Larger particles (100 mμ, V₁) with a dense center appear to be mixed with smaller particles (50-70 mμ, V₂) of nearly uniform density. Similar particles (V) are placed outside the cell at the convoluted plasma membrane (P). (N = nucleus; ER = endoplasmic reticulum.) X31,500.

Fig. 13:—Portion of another cell from the same culture as shown in Fig. 12. Virus particles (V), of 100-110 mμ, are seen in the cytoplasm of a large monocyte-like cell. One particle (V₁) appears to be attached by a neck to a membrane. Another particle (V₂) is present inside a granular body. (F = fat vacuole; ER = endoplasmic reticulum.) X47,000.

Fig. 14:—Portion of another cell from the same culture as Figs. 12 and 13. Large numbers of virus particles (V) are situated in smooth-walled vacuoles, presumably formed by pinocytosis. (N = nucleus; M = mitochondria; GB = granular body; ER = endoplasmic reticulum.) X45,000.

Fig. 15:—Myeloblast from a 14-day tissue culture of spleen cells of the same mouse (RF 7401) as shown in Fig. 10. However, the predominant cells in the tissue cultures were monocytes and macrophages. The chromatin of the nucleus (N) is fairly even. Round and elongated mitochondria (M) are distributed in the cytoplasm. The endoplasmic reticulum (ER) is present as small flattened sacs. Small, dense mitochondria or possible early granules (GR) at present. A few virus-like particles (V), similar to those seen in the original spleen specimens (Fig. 10), are shown outside of the cell. X11,000.
FIG. 16.—Portion of a large mononuclear cell in a 14-day spleen culture from an irradiated adult recipient in the 2nd-passage generation (RF 5402), which developed leukemia 5 weeks after the transfer of leukemic spleen cells. Large numbers of virus particles (V) are present at the outside of the cell. An increased number of granular bodies (GB) are present in the cytoplasm and one particle (V1) is inside a granular body. (P = plasma membrane; F = fat bodies; ER = endoplasmic reticulum.) X38,000.

FIG. 17.—A portion of the same cell as shown in Fig. 13 photographed at higher magnification to show the ultrastructure of the virus particles (V). The particles inside the granular body (GB), (V1) appear to have the same kind of structure as the particles at the plasma membrane (P). The granular body (GB) has a trace of a double outer wall and double inner membranes (indicated by the arrows). The particles appear to have a single outer wall of diameter 80–100 μm. In most particles (V2), there is a denser center of 45–50 μm diameter but in others (V3) the periphery of the particles is denser than the center. Cytoplasmic inclusion of particles or evidence of budding from the plasma membrane was seen only rarely. X133,000.
Fig. 18.—Part of another large group of particles found in the same tissue culture as shown in Figs. 16 and 17. The outer membrane is 35 Å thick and appears single when sectioned normally (M₁). In other particles it appears double (M₂), but this may be due to oblique sectioning and to the section’s being slightly thick. The dense center (N) appears to have no definite membrane, but inside appears to contain filaments (F) of 40 Å diameter. There is also a suggestion of fibrils of diameter 20 Å (indicated by arrows) being present. X300,000.
Electron Microscopic Observations on Primary and Serially Passaged Radiation-induced Myeloid Leukemias of the RF Mouse

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