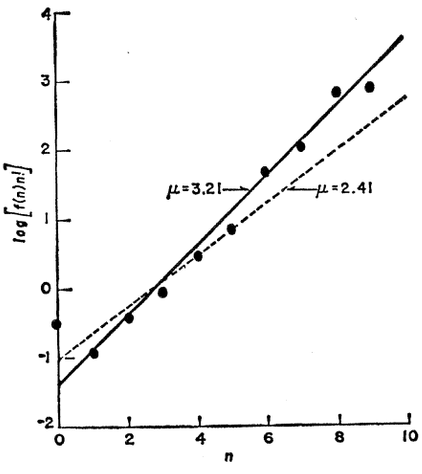

Fig. 2. Graph of observed grain-count frequencies from the data in Table 1. See text for method of plotting and interpretation.

tion. The zero class, represented by the point on the ordinate line, is very much too large. A population average calculated from all of the counts in Table 1 has the value 2.41. The line corresponding to a Poisson distribution having this mean value is also drawn on the graph. Even here, the observed zero class is too large, and in addition, this function does not fit the other data points.

It could be argued that a large zero class might indicate only the presence of some nuclei in which RNA synthesis was slower than in the general population. In such a case, the nuclei with zero-grain nucleoli should in general have only a small grain count over the second nucleolus. This was not observed. On the contrary, slightly more than half of the nuclei (52 percent) had one unlabeled nucleolus, and these nuclei accounted for 40 percent of all the silver grains. Since one of the two nucleoli in each had no grains, it is clear that zero grains over a nucleolus were not correlated with a low grain count over the partner. This is in itself evidence of asynchrony.

Thus, the nucleoli scored in this experiment are members of at least two classes; about 75 percent were synthesizing RNA during the pulse period and 25 percent were not. All the nucleoli engaged in synthesis were doing so, on the whole, at about the same rate, since their grain-counts are distributed as expected for a homogeneous population of radioactive units.

It is important to stress that the large zero class represents intracellular rather than cellular asynchrony, since all nonradioactive nucleoli included in the scoring were paired with normally labeled radioactive ones.

Root meristem is an asynchronous population of cells undergoing both mitosis and differentiation. Several important studies have been reported on variations in cell size, mitotic index, intermitotic time, and patterns of macromolecule synthesis within different zones (*8*). Asynchrony of RNA synthesis in the nucleoli of some, but not all cells of the meristem may be an early signal of cellular differentiation within the tissue. Our observation favors the idea that nucleoli have individual specificity of function within a single nucleus, since their rates of RNA synthesis may be very different at some point in the cell cycle.

Note added in proof. Since this manuscript was submitted for publication, it has been reported that the nucleoli of pea seedlings are centers for the methylation of transfer RNA (*9*). Nucleolar RNA from the same source can be hybridized with denatured homologous DNA (*10*), and the hybridizable stretches of DNA are complementary to ribosomal RNA. Further evidence is presented that some ribosomal RNA is manufactured on non-nucleolar chromatin and then transferred to the nucleolus. The authors cite evidence from their own and other laboratories which suggests that the ribosomal RNA's may be structurally diverse.

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Radiation-Induced Mouse Leukemia: Consistent Occurrence of an Extra and a Marker Chromosome

Abstract. *Granulocytic leukemia, induced in the primary mouse by x-irradiation, was serially transmitted to RF/Up mice. An extra chromosome, as well as a morphologicaly unusual chromosome, was found in the bone marrow cells of all the leukemic mice that had been injected previously either with leukemic spleen cells or with cell-free ultracentrifugates. This suggests that the changes in the chromosomes are caused by a virus.*

Granulocytic leukemia, induced in a mouse of the RF/Up strain by x-irradiation, was serially transmitted more than 25 times by inoculation of leukemic spleen cells, cell-free spleen ultracentrifugate, or plasma, to RF/Up recipient mice (*1*). The transmissability of the leukemia by cell-free filtrates containing virus particles was demonstrated by Parsons *et al.* (*2*), by means of electron microscopy. The similarity of the clinical and pathologic features of this disease (*2*) to those of chronic granulocytic leukemia of humans, and the regular occurrence of the Ph[1] chromosome in patients with the latter disease (*3*) prompted a search for a similarly characteristic abnormality of the chromosomes in RF/Up mice with this leukemia. In the marrow of all such mice examined to date we have found cells which show a consistent karyotypic abnormality.

The first mouse studied was a female who received an inoculum of plasma ultracentrifugate at birth. All the other recipient mice were 10-week-old males. The inoculum of leukemic spleen cells was prepared from tissue obtained from freshly killed leukemic donors. The spleens were minced with surgical scissors in chilled, sterile Tyrode's solution to give a suspension of 1.0 to 1.5 $\times 10^8$ nucleated cells per milliliter. The re-

sulting suspension was injected into a tail vein, 0.5 ml per recipient. The ultracentrifugates were prepared by centrifuging suspensions of spleen cells at 1800*g* for 20 minutes, and centrifuging the resulting supernatant fraction at 30,000*g* for 1 hour. The supernatant fluid resulting from the second centrifugation was injected into a tail vein, 0.5 to 1.0 ml per recipient. The "plasma" was prepared in a similar way with pooled, fresh, whole blood from leukemic donors; that is, the blood was centrifuged at 1800*g* for 20 minutes and the supernatant fraction centrifuged at 30,000*g* for 1 hour. The resulting supernatant fluid was injected into a facial vein of the neonatal recipient, in a dose of 0.05 ml. All materials were kept at 3°C while being prepared. After receiving the injections at Oak Ridge, the mice were shipped by air to Pittsburgh, where they were observed for signs of leukemia: pallor, listlessness, splenomegaly. The diagnosis was then confirmed by examination of the peripheral blood for the presence of leukocytosis and immature myeloid cells, and by subsequent necropsy.

The marrow of mice found to have the disease was prepared for cytogenetic examination as follows. From a living mouse, anesthetized with ether, marrow was aspirated from the femur by a technique reported previously (*4*). The marrow was suspended in 0.85 percent NaCl containing 1 μg/ml of diacetylmethylcolchicine (*5*) and maintained for 2 hours at room temperature. After hypotonic treatment with 0.25 percent buffered saline, and fixation in Carnoy's fixative, the material was dropped on slides and dried in air. After staining with Giemsa stain and mounting, the slides were scanned and photomicrographs made of suitable mitotic cells in metaphase. Chromosome counts and morphological observations were made and, in some instances, karyotypic analyses were performed, both photographic material and direct microscopic observation being used for verification.

Cytogenetic examination of all leukemic mice revealed a significant proportion of marrow cells with 41 chromosomes instead of the normal modal number of 40, and of marrow cells with an apparently identical, morphologically abnormal chromosome (Table 1). This "marker" chromosome, shown in Fig. 1 (including inserts *A*, *B*, and *C*) was consistently of medium size, and was not one of the largest or smallest pairs, nor one of the pairs identifiable by virtue of secondary constrictions adjacent to the centromere (*6*). It was seen in the female as well as in the males and does not resemble the Y chromosome. Karyotypic analysis showed that it was in the size range of the 13th to 16th pairs. The morphological abnormality consisted of a region of secondary constriction with achromatic staining properties on each chromatid, and was situated about two-thirds of the distance from the centromere to the distal end. The two chromatids appeared adherent to each other in this region in 94 percent of the 85 marker chromosomes studied. The marker was present in greatest frequency in cells having 41 chromosomes. Comparable abnormalities were not observed in the controls that were not inoculated, nor in the inoculated animals which failed to develop leukemia during the period of observation.

The consistent occurrence of an extra chromosome and of a marker chromosome in the abnormal cells of the leukemic marrow is noteworthy. Since not all the bone marrow cells sampled were necessarily leukemic, the absence of the marker and the presence of the normal 40 chromosomes in some cells is not unexpected. Discordance between the presence of the marker and an abnormal chromosome count may be the result of technical imperfections, or of biological variability of the processes which result in the aberrations, such as the postulated "stickiness" phenomenon described here.

The fact that these changes in the chromosomes are induced by inoculums presumed to be cell-free is of particular interest. The transmissibility of the leukemia by cell-free filtrates containing virus particles has already been demonstrated (*1*, *2*). Although the cell-free induction of the chromosomal aberration remains to be verified by experiment (such tests are currently in progress, inoculums of cell-free filtrate being used), it is doubtful that the inoculated spleen or plasma ultracentrifugate contained intact cells after centrifugation for 1 hour at 30,000*g*. Careful search of such fluids with the microscope invariably failed to disclose cells. It is tentatively assumed, therefore, that the leukemia and coexisting chromosomal abnormalities were virus-induced. Additional support for this assumption is provided by the preliminary studies of another granulocytic leukemia, induced independently by neutron irradiation of a female RF/Up mouse from the same colony, and serially transmitted by cells and by cell-free ultracentrifugate inoculums to mice of the same strain. Similar clinical, pathological, and chromosomal changes appear to characterize this

Table 1. Chromosome counts and marker distribution in the bone marrow cells of RF/Up mice inoculated with leukemic spleen cells or cell-free ultracentrifugates. The numbers in parentheses indicate the number of cells containing the marker chromosome. All mice had granulocytic leukemia except those instances noted in the table.

No. of mice	Time after injection (days)	Chromosome counts						
		<	38	39	40	41	42	>
		Plasma ultracentrifugate after 21st successive passage						
1	35	1	1	1	5	17 (6)	1	1
		Spleen ultracentrifugate after 22nd successive passage						
1	44	0	2	4	7	18 (2)	8 (2)	0
		Spleen cells after the 23rd to 27th successive passage						
1	9	1 (1)	1	1	10	6 (4)	1	0
1	13	0	1 (1)	0	7 (7)	12 (9)	0	0
1*	13	1	2	0	6	9	1	0
1	13	0	0	0	1	9 (5)	0	0
1	31	0	1	1 (1)	7 (3)	11 (9)	0	0
4†	133‡	3	0	6	44	1	0	0
		Spleen ultracentrifugate after 23rd to 27th successive passage						
1	36	0	0	2 (1)	4 (2)	13 (11)	1 (1)	0
1	47	0	0	1 (1)	2 (1)	14 (9)	2	1 (1)
1§	48	—	—	—	—	—	—	—
1	57	0	3 (2)	0	0	17 (12)	0	0
14†	133‡	1	4	3	66	0	0	0
		Noninjected controls						
24		4	4	15	239	10	0	1

* Preparation unsatisfactory for detailed morphological study. † Mice injected, but showing no evidence of leukemia. ‡ Approximately 133 to 140 days. § Died and decomposed before cytogenetic studies could be made.

leukemia as well (Fig. 1, inserts *D* and *E*). This suggests that the same virus was present in the primary animal of this leukemia line and that it therefore produces the same changes on inoculation into the recipient animals (*7*).

The mechanisms by which the extra chromosome and the structural abnormality are produced are not known, although viruses have been reported to cause aberrations in chromosomes in vitro (*8*) and in vivo (*9*). The morphological similarity of the marker chromosome found in this study to some of the aberrant chromosomes described by Moorhead and Saksela (*10*), is noteworthy. In human cells transformed in vitro by the virus SV_{40}, they reported abnormally stretched and exaggerated secondary constrictions, as well as breakage, at sites where such constrictions normally occur. They interpreted this as preliminary evidence that the SV_{40} virus affects specific chromosome regions preferentially because of structural weaknesses in these particular regions. However, this is not certain because, as they pointed out, some of the most prominent, normally occurring sites of secondary constriction remained unaffected, while the material in some sites which are rarely seen under normal conditions, did appear exaggerated. Furthermore, the points of breakage and "induced" constriction seen in the cultured leukocytes of patients with measles and chickenpox (*9*) were scattered at random over the various chromosomes. Finally, no normally occurring site of secondary constriction has been reported, or seen by us, at the location of the involved region in mouse chromosomes of the size of our marker.

The constancy and specificity of the observed changes indicate that they differ from the varied aneuploidy and structural abnormalities noted during the growth of many other types of neoplastic cells. There are two abnormalities, however, to which they may bear some resemblance. One is the consistently occurring marker, the diminutive G group chromosome called the Ph^1, which is regularly present in chronic granulocytic leukemia of humans (*3*). The other is the shift to 41 chromosomes in 15 or 16 Swiss stock mice that developed thymic lymphosarcoma after one or two injections of 7,12-dimethylbenz[*a*]anthracene (*11*). In the latter, a "single small chromosome resembling the Y chromosome in shape and size" was seen in "a number of lymphosarcomas." The mechanisms by which these changes are produced has not been demonstrated.

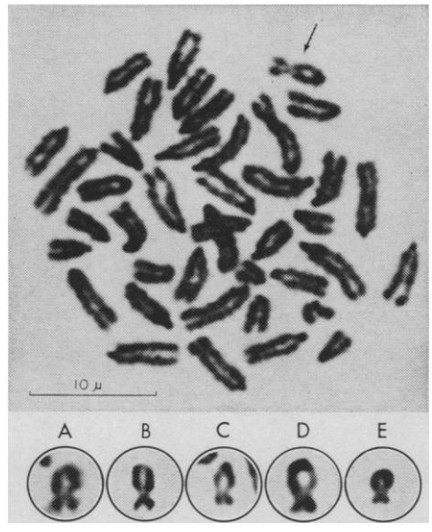

Fig. 1. Bone marrow cell in metaphase from a mouse inoculated with a cell-free ultracentrifugate of leukemia spleen cells after the 23rd to 27th successive passage from the primary x-irradiated donor. Arrow indicates the morphologically abnormal marker chromosome. Giemsa stain, light microscope. Inserts are typical marker chromosomes from five other mice with leukemia, which received the following inoculums: (*A*) plasma ultracentrifugate, after 21st successive passage from primary x-irradiated donor; (*B*) spleen cells, after 23rd to 27th successive passage from the same primary donor; (*C*) same as (*B*); (*D*) spleen cells, after 7th successive passage from a primary donor in which the leukemia was induced by neutron irradiation; (*E*) same as (*D*).

A simple hypothesis which may account for our findings is as follows. (i) A virus present in the primary irradiated animal becomes active after exposure to radiation and produces granulocytic leukemia in the primary animal and, on cell-free passage, in other nonirradiated mice. (ii) The virus affects a specific region on one of the two chromatids of a particular chromosome (possibly containing leukocyte control genes) for which it has an affinity, producing morphological changes, as well as an accompanying period of abnormal stickiness. (iii) The stickiness in turn results in adherence of an unaffected chromatid, probably the sister one, in the next cell division, so that the chromatids fail to separate. (iv) This results in a daughter cell with an extra, but morphologically normal, chromosome, possibly a duplicate of the affected one, as well as the one with the morphological abnormality. (v) Since the chromosome, including its newly altered area, is duplicated during the synthetic phase, the next metaphase reveals a chromosome in which both chromatids show the same abnormality, as well as an unaltered extra chromosome. (vi) The initial stickiness is subsequently diminished, possibly paralleling a change in the viral material to an integrated form (*12*), so that the chromatids no longer fail to separate in subsequent cell divisions despite the frequent apparent adhesion of the two altered sister chromatids. (vii) A stable 41 chromosome line with a marker results. Experiments are in progress to test the validity of this hypothesis.

The marked increase in incidence of granulocytic leukemia in the RF/Up mouse, which is readily produced by exposure to radiation (*13*), and the consistent presence of an extra and a marker chromosome in the disease, bring to mind five findings in man, which possibly are related. These are: (i) the presence of persistent chromosome aberrations in the blood cells of individuals irradiated by accident or by therapeutic intent (*14*); (ii) the increased incidence of leukemia, and of the granulocytic variety in particular, in the irradiated human populations studied (*14*); (iii) the consistent presence of a deletion in a small, 21-22 or G group, acrocentric chromosome, the Ph^1 in chronic granulocytic leukemia of humans (*3*); (iv) the significantly increased frequency of occurrence of aberrations in the chromosomes of certain groups (13-15 or D; 21-22 or G; and probably 6-12, X or C groups) in acute leukemia of humans (*15*); and (v) the markedly increased incidence of all forms of leukemia at all ages in the mongoloid (Down's syndrome) population (*16*), in which an extra small acrocentric chromosome in the 21-22 or G group is present in all body cells. Whether any of these alterations in the chromosomes are involved in producing leukemia remains to be determined. The importance of chromosomal aberrations in the pathogenesis of other viral neoplasms has been postulated (*12*). However, the work of Nowell *et al.* (*17*) indicates that granulocytic leukemias can occur in rodents without their chromosomes showing any obvious consistent abnormality.

Some of the significance of our findings lies in their possible service as evidence that the viral and somatic mutation concepts of leukemogenesis are very closely related, at least in this

in vivo test system. Thus, exposure to radiation may alter an indigenous quiescent virus, or its environment, giving it the ability to alter the chromosomes of blood cells which, may in turn give rise to the functionally malignant cells of leukemia.

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Lymphocyte Interaction: A Potential Histocompatibility Test in vitro

Abstract. *Lymphocytes from two unrelated individuals, cultured together in the same tube, undergo morphological transformation to large cells and divide. Both of these parameters may be estimated quantitatively. There is a correlation between the degree of this response and the degree of cross-reactivity of grafts from the two individuals placed on a third unrelated recipient.*

In order to evaluate potential donors for tissue transplants, it would be desirable to be able to test the compatibility of donor and recipient in vitro. Such a test might also prove useful in tissue typing. This report describes an approach to such a system in which peripheral blood lymphocytes are utilized. Genetic similarity between the donor and recipient of a transplanted tissue appears to be the major factor responsible for the success of a transplant. In skin transplantation, if the donor and recipient are genetically identical, the graft will "take" (*1*). In humans, the chance for success with kidney transplantation increases if the donor and the recipient are blood relatives. With identical twins, there is uniform immunological success.

Much work has been devoted in recent years to the problem of selecting suitable donors for tissue transplantation. In human subjects, Rapaport *et al.* (*2*, *3*) and Wilson *et al.* (*4*) have tested for histocompatibility by placing successive skin grafts from prospective donor-recipient pairs on a third unrelated individual. If A and B are the two members of the donor-recipient pair, and C is a third unrelated individual, a skin graft from A is placed on C. At a given time after the rejection of this graft, when C is sensitized to A, a skin graft from B is placed on C. If C responds to B's graft with a second-set reaction, this suggests that A and B may share transplantation (histocompatibility) antigens. Rapaport *et al.* (*2*) have been careful to indicate an alternate interpretation, relating to the contribution of hypersensitization or antigen competition to the results observed.

Table 1. The percentage of large cells (LC) and mitoses (M) in mixtures of lymphocytes cultured for 4 to 7 days.

Source of lymphocytes	After 4 days		After 7 days	
	LC	M	LC	M
X and Y	36.2	0.4	74.3	8.1
X and Z	53.1	1.4	75.7	7.6
Y and Z	32.1	0.3	52.0	3.9

This possibility is supported by the observation that the incidence of individuals exhibiting cross-reactivity appears to increase with increasing dosage of antigen, when leukocyte preparations instead of skin are used to induce sensitivity (*5*).

Brent and Medawar, using guinea pigs (*6*), and Gray and Russell, using human beings (*7*), are testing differences in immunological constitution between donor and recipient. Lymphocytes from the recipient are injected intradermally into a series of potential donors. The intensity of the delayed type-response to the lymphocytes is correlated with the rapidity of breakdown of subsequent grafts from the donors to the recipient. There is an inverse relationship between the reaction of the donor to the recipient's lymphocytes and the survival time of the donor's graft.

The immune capabilities of human peripheral blood lymphocytes in vitro have been demonstrated (*8*). These cells produce γ-globulin (*9*), presumably in the form of specific antibodies (*10*), when stimulated by antigens to which the donor of the lymphocytes is sensitized. Bain *et al.* (*11*) have shown that when human peripheral blood leukocytes from two individuals are mixed, the incorporation of thymidine into DNA is stimulated. We have also shown that in such a mixture of cells, lymphocytes are stimulated to enlarge and divide (*8*). The percentage of large cells and mitoses can be estimated quantitatively.

The lymphocytes are prepared from human peripheral blood as follows. Venous blood is mixed wtih heparin and allowed to sediment at 37°C (1 to 2 hours), and then supernatant plasma is withdrawn. This is mixed with one volume of Eagle's "minimum essential medium" (*12*) modified for suspension culture and placed in a flint-glass pre-