Table 1. Comparison of P^{32} distribution in fractions obtained by centrifugation (C) and by filtration (F) (three separate runs). Values are given as percentage of total activity in the TCA-insoluble fraction.

Method	Alcohol-soluble	RNA	DNA-residual	DNA
C	17.8	69.0	13.6	11.8
F	14.3	71.5	14.6	12.6*
C	17.2	68.0	14.5	12.4
F	14.7	72.5	12.8	10.8*
C	16.2	69.0	14.8	13.4
F	13.8	71.1	15.1	13.1*

* Defined in text.

residual fraction minus 2 percent of the total activity in the TCA-insoluble fraction. The validity of this step was further checked by carrying out the procedure on cultures of *E. coli* 15_{T^-} in thymine-deficient media (*9*). In confirmation of Cohen's findings, there was negligible incorporation of P^{32} into the DNA fraction, defined as above, in the absence of thymine. Incorporation into the RNA fraction, however, was linear with time under this condition of no DNA synthesis. The separation procedure has been found useful in further studies of "unbalanced growth" in *E. coli*, induced by the action of ultraviolet light (*10*).

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Leukemogenic Activity of Filtrates from Radiation-Induced Lymphoid Tumors of Mice

Abstract. Cell-free filtrates of x-ray-induced lymphoid tumors of strain C57BL/Ka mice have elicited, on injection into newborn isologous hosts, a lymphoma incidence of 15 to 19 percent. In control mice of the same subline, the incidence of spontaneous lymphoma is about 1 percent. No leukemogenic activity could be detected in filtrates from thymi harvested at 2 to 32 days following completion of x-ray treatment. Activity was evident at 64 days and was perhaps somewhat greater at 128 days. Serial cell-free passage of filtrates in newborn F_1 hybrid mice resulted in a marked increase in lymphoma incidence (69 percent), coupled with a shortening of the median latency. Supplementary x-irradiation failed to enhance the activity of filtrates after neonatal injection.

In most strains of mice the thymus is essential for the development of lymphatic leukemia and lymphosarcoma, either arising spontaneously or induced by ionizing radiation, hydrocarbons, or estrogen (*1*). In the case of the radiation-induced disease, however, it has been demonstrated that such tumors may develop from nonirradiated cells of thymic grafts implanted into irradiated hosts (*2*). This phenomenon of indirect induction may be interpreted in terms of the existence of a latent subcellular agent, analogous to the filtrable agent of spontaneous mouse leukemia demonstrated by Gross (*3*), which, having been "activated" by radiation, initiates malignant transformation in susceptible lymphoid cells. For the past 5 years, a series of experiments designed to test this possibility has been in progress in our laboratory (*4*).

Lymphoid tumors were induced in C57BL/Ka mice of both sexes by fractionated whole-body x-irradiation (four weekly doses of 168 r each) started at age 33 ± 3 days (*5*). The incidence of disseminated lymphomas resulting from this treatment is 80 to 90 percent, with an average latency of about 200 days (*6*). The animals were sacrificed and autopsied when unquestionable symptoms of the disease became apparent.

Cell-free extracts of the involved tissues were prepared by a modification of Gross' technique (*3*). Thymus, spleen, liver, and lymph nodes were removed and weighed, and iced Locke's solution was added to make a 20-percent suspension. This was homogenized and centrifuged at 7000*g* for 15 minutes. The supernatant was recentrifuged, and the cycle was repeated for a total of four centrifugations. The entire procedure was carried out at 4°C. The final supernatant was passed through a UF fritted glass filter. Impermeability of the filter to cells was ascertained by retention of *Escherichia coli*.

Filtrates were injected in 0.1-ml amounts either subcutaneously or intraperitoneally into C57BL/Ka or reciprocal F_1 (C57BL/Ka × BALB/c) hybrid mice of both sexes, aged 16 hours or less. The animals were maintained under standard laboratory conditions and were sacrificed when moribund, or when older than 600 days. Sections of the involved tissues were stained with hematoxylin and eosin. Only typical lymphocytic lymphosarcomas, identical to those arising after irradiation of strain C57BL (*7*), were tallied as "lymphomas."

Filtrates from isologous, x-ray-induced lymphoid tumors yielded ten (17 percent) disseminated lymphomas among 59 long-term survivors of neonatal injection (Table 1). In a second experiment, filtrates from tumor C43, a radiation-induced thymic lymphosarcoma of C57BL mice which has been carried by serial isologous transplantation for the past 7 years, gave a similar tumor incidence, indicating preservation of activity through some hundred successive transplant generations.

The only other neoplasms observed were reticuloendothelial tumors, ovarian tumors, and hepatomas, all of which occurred with about the expected frequency for aged, untreated mice of this strain.

In our subline of the C57BL strain there has been only one spontaneous lymphoma (1.3 percent) in a total of 74 untreated or saline-injected mice of both sexes maintained for 600 days or longer. A group of 25 C57BL/Ka mice injected when newborn with filtrates from isologous, spontaneous reticuloendothelial tumors and hemangiomas, and another group of 24 which had received filtrates from strain AK lymphoid tumors all remained free of lymphoma for over 600 days. Filtrates from normal tissues of C57BL/Ka mice injected into newborn isologous hosts have elicited no lymphomas to date (9 months) (*7a*).

The possibility that supplementary irradiation might be required to bring out the full potential of the latent leukemogenic agent was tested in another experiment. Newborn C57BL/Ka mice received lymphoma filtrate (the controls received saline) at birth, and a single, whole-body x-ray exposure of 200 r given concurrently or at 2, 6, 14, or 30 days of age. The incidence of lymphoma after the combined treatment was reduced to the level resulting from x-ray treatment alone (0 to 8 percent), except in the group irradiated 30 days after filtrate injection (17 percent).

Pooled thymus glands (from strain C57BL mice) obtained at serial intervals (2, 8, 32, 64, or 128 days) after fractionated, systemic irradiation were used for the preparation of filtrates and assayed to determine the time of first ap-

Table 1. Lymphomas in mice inoculated when newborn with filtrates from radiation-induced lymphoid tumors or from irradiated thymus glands.

Source of filtrate	Net No. of mice	Lymphomas		Latency (day)	
		No.	Percent	Median	Range
Experimental animals					
X-ray-induced lymphoma	59	10	17	582	253–688
Lymphoma C43	26	5	19	449	340–551
Thymus glands obtained:					
2 days postirradiation	27	0	0		
8 days postirradiation	21	0	0		
32 days postirradiation	21	0	0		
64 days postirradiation	39	3	8		370, 405,* 618
128 days postirradiation	20	3	15		333, 473, 580
Filtrate-induced lymphoma† (first passage)	6	2	33		83, 176‡
Filtrate-induced lymphoma† (second passage)	13	9	69	202	91–336
Controls					
Hemangioma	15	0	0		
Reticuloendothelial tumor	10	0	0		
Strain AK lymphoma	24	0	0		
Saline or no treatment	74	1	1§	565	

* Lymphoid tumor used for first passage.
† Assayed in newborn reciprocal F_1 (C57BL/Ka × BALB/c) hybrid mice.
‡ Lymphoid tumor used for second passage.
§ Statistical analysis: filtrates from x-ray-induced lymphoma and lymphoma C43 versus controls: χ^2 (corrected) = 17.760; $P < .001$.

pearance of leukemogenic activity. Microscopically identifiable lymphomas *in situ* first appear at about 30 to 50 days after irradiation (*8*). During the first 32 days after x-irradiation the filtrates were devoid of leukemogenic activity. Activity was evident in thymi harvested at 64 days and was perhaps somewhat greater by 128 days. The latency for lymphomas arising in mice injected with the 64- and 128-day filtrates was of the same order as that following injection of filtrates from frankly disseminated tumors.

Serial cell-free passage of a filtrate-induced lymphoma in newborn F_1 hybrid mice yielded lymphomas in two of six (33 percent) and in nine of 13 (69 percent) in the first two passages, with latency of 3 to 11 months. On transplantation, these tumors behaved as though they were of hybrid origin; this excludes the possibility that they were produced by contamination of the filtrate with intact tumor cells. Preliminary results of a subsequent cell-free passage indicate persistent activity of the agent.

It appears that cell-free filtrates from x-ray induced lymphoid tumors of C57BL mice can elicit the disease in compatible hosts. Similar results have recently been reported by Gross (*9*) for strain C3H. The active principle has been shown to have the following properties: (i) It is latent in untreated C57BL mice. (ii) Appropriate x-irradiation of the host "activates" it, and it can be recovered from at least some of the resulting lymphoid tumors. (iii) It passes bacterial filters. (iv) It exhibits specificity in that it causes only lymphoid tumors on injection into nonirradiated newborn isologous and compatible F_1 hybrid hosts. (v) Its potency is increased on serial passage, as was also noted by Gross (*9*). These characteristics appear to exclude all agents other than viruses and subcellular genetic determinants.

In many of its properties, the x-ray-activated leukemogenic agent bears, at first glance, a striking similarity to the temperate phage-lysogenic bacterial system (*10*). It does not, however, destroy its host cells on activation; instead, it causes them to proliferate more rapidly than normal. Moreover, the activity of "infectious" preparations is not enhanced (indeed, it was diminished) by x-irradiation of the host. Despite such differences, bacterial lysogenesis remains a useful model on which to base further investigations.

The demonstration of a leukemogenic filtrable agent in the cells of radiation-induced lymphoid tumors provides the first evidence directly linking the external carcinogens to viruses or virus-like agents. It thus lends new emphasis to the long-held view that all neoplasms result from such agents. Nonetheless, this thesis will require step-by-step experimental documentation for a variety of neoplasms and external carcinogens.

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5. Physical factors of irradiation were as follows: 120 kv (peak); 7.5 ma; 0.25 mm Cu + 1.0 mm Al added filter; 30 cm target-mouse distance; output, 25.5 r/min; HVL, 0.36 mm Cu.
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Biophysical Approach toward Tumor Regression in Mice

Abstract. An external electrical source of low magnitude was used in a series of experiments to alter inherent tumor potentials in mice. While no significant increase of tumor growth was noted in the acceleration group, total tumor regressions were obtained in the inhibition group. Preliminary studies with leukemia did not yield significant results.

It has been shown in the human being that a growing fetus or a growing uterine tumor will cause the uterus to be electronegative with respect to the outer abdominal surface (*1*). In the guinea pig and mouse, the tumor is also electronegative (*2*). This supports the many findings that a growing region is electronegative with respect to a slower growing or nongrowing region in the same organism, whether plant or animal (*3, 4*).

In plants, Lund has demonstrated growth acceleration and deceleration by an external potential source (*4*). The studies described here were based on the hypothesis that the growth rate of malignant tumor tissue would respond similarly to an external electrical source (*5*).

A rapidly growing type of tumor was chosen for initial experiments, and various control measures recommended by