from such diverse sources give rise to the same variety of tumors in the C3H(f) mouse as have been found in the experiment just completed, a tumor-inducing mechanism common to Metazoan life will have been demonstrated for the first time.

The successful application of purification technics to the isolation of tumor factor from Hodgkin's disease tissue and other neoplastic tissues from human beings will be presented later.

Summary. 1) A procedure has been developed for extracting and refining tumor factor contained in mouse leukemic tissue. The refined preparation repeatedly induced a variety of neoplasms in high percentage of inoculated animals, but no leukemia was induced. 2) Results were reproducible and time of tumor appearance predictable. Tumors developed at earlier age than when crude cell-free filtrates are used. 3) This work suggests that an attempt be made to isolate tumor factor from Drosophila, fish lymphoma, chicken lymphoma, Lucké frog kidney tumor, etc., so that a tumor-inducing mechanism common to Metazoan life may be demonstrable. 4) By purification technic discussed, tumor factor has been isolated from Hodgkin's disease tissue and other neoplastic tissues from human beings to be reported.

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Immunization Against Ehrlich's Ascites Carcinoma with X-Irradiated Tumor Cells.* (24884)

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In 1910 Contamin(1) produced immunity to transplanted tumors in mice by pre-treatment with tumor cells that had been x-irradiated *in vitro*. Since that time varying degrees of immunity have been obtained against mouse sarcoma-180(2) and mouse Ehrlich ascites tumor(3) following treatment with x-irradiated cells. In the present study, immunization procedures with irradiated cells were carried out both before and after challenge with Ehrlich's ascites carcinoma.

Methods. Swiss mice of mixed sexes were used. They weighed from 17 to 20 g when experiments were initiated. The material used for immunization and challenge injections consisted of pooled ascites exudate collected from 2 or more mice that had received intraperitoneal (ip) transplants one to 3 weeks previously. Five units of heparin were added to each ml of exudate when ascites fluid was collected to prevent clotting. Tumor cells employed for challenge transplants were withdrawn from the peritoneal cavity, counted with a hemacytometer, diluted with physiological saline solution (PSS) and injected into recipient mice within one hour from time of collection. The number of tumor cells used in the challenge injection varied in different experiments. Tumor cells used for immuniza-

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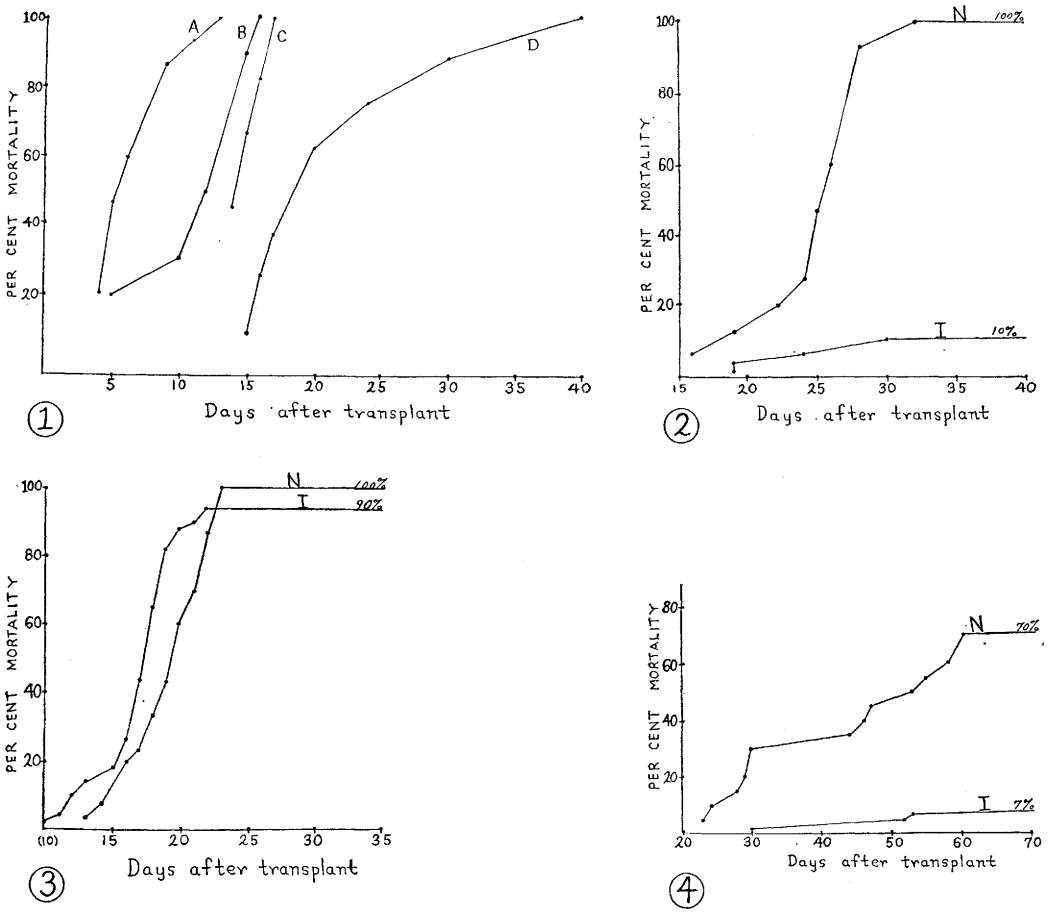

FIG. 1. Effect of different irradiation doses on capacity to induce tumors. (A) non-irradiated cells, (B) cells exposed to 500 r, (C) 1000 r, (D) 2500 r.

FIG. 2. Immunization with single inj. of irradiated tumor cells prior to ip challenge. (I) Immunized mice, (N) non-immunized controls.

FIG. 3. Immunization with irradiated cells after ip challenge. (I) Immunized mice, (N) non-immunized controls.

FIG. 4. Immunization with irradiated cells after sc challenge. (I) Immunized mice, (N) non-immunized controls.

tion were prepared as follows: 20 ml of heparinized ascites fluid were placed in a plastic petri dish, exposed to x-irradiation in air, and diluted wth PSS solution to give the desired cellular concentration. The x-ray factors used were: 250 K. V., 15 ma, hvl 0.77 mm cu, t.o.d. 40 cm, output 280 r/min. In all cases immunizing injections were administered within an hour after x-ray exposure. Irradiation dose and number of cells in the vaccine varied. When mice received multiple injections, fresh vaccines were prepared each day that immunizing injections were administered.

Results. To determine what dose of x-irradiation was necessary to interfere with successful tumor transplant, the ascites fluid was exposed to varying doses (500 to 100,000 r) of x-irradiation *in vitro*. Mice were divided into groups of 10 and given single ip injections containing 5,000,000 cells that had received different irradiation exposures. Twenty mice served as controls and received non-irradiated cells. Daily mortalities following these injections are recorded in Fig. 1. In no case did tumors develop if the irradiation dose was 4,000 r or greater. Relationships between mortality curves in Fig. 1 are

TABLE I. Effective Immunization against Ehrlich's Ascites Carcinoma with Cells Exposed to X-irradiation *In Vitro*.

			Immunization				Tumor transplant		Mortality		
Exp.	Group	No. mice	Start*	Inj.†	X-ray	Cells/inj. × 1000	Route	No. cells × 1000	Ratio	%	P value‡
1	Immunized	50	20 days before transplant	1	4,000 to 13,000 r	5,000	IP	2	5/50	10	<.001
	Control	15		0		Non-immunized	"	"	15/15	100	
2	Immunized	50	1 day after transplant	5§	10,000 r	20	"	16.5	45/50	90	>.1
	Control	30		0		Non-immunized	"	"	30/30	100	
3	Immunized	55	1 day after transplant	5§	10,000 r	20	SC	"	4/55	7	<.001
	Control	20		0		Non-immunized	"	"	14/20	70	

* Time that single or first inj. of irradiated cells was given in relation to inj. of viable cells.
† All immunizing inj. were given by intraper. route.
‡ P values calculated by Chi square.
§ The 5 immunizing inj. were administered over a 10-day period.

similar to those seen when mice are challenged with different numbers of cells. Consequently, increase in survival time that follows an increase in irradiation dose might be due to a decrease in number of viable cells.

In the first immunization experiment, irradiated cells were administered prior to challenge with viable non-irradiated cells. The 50 mice in the immunized group received an ip injection of 5,000,000 cells that had been irradiated *in vitro* with doses from 4,000 to 13,000 r. Twenty days after the single injection of irradiated cells the immunized mice and a control group of normal mice received ip transplants containing 2,000 non-irradiated cells/mouse. Mortality curves (Fig. 2), demonstrate the protective effect of immunization on subsequent challenge. Other pre-immunization experiments have been carried out and essentially the same results obtained.

An attempt was made to protect mice against the tumor by initiating immunization procedures after tumor transplantation. The mice were challenged by either ip or subcutaneous route with 16,500 nonirradiated cells. Both routes of challenge were employed to see if different results would be seen between the rapidly growing tumor in the peritoneal cavity and the slower growing tumor in subcutaneous tissues. One day after challenge the first immunization injection was given. The immunization schedule consisted of 5 ip injections over a 10-day period. Each immunizing injection contained 20,000 cells that had received a 10,000 r x-ray exposure. Daily mortalities following the ip challenge and post-immunization are recorded in Fig. 3. Even though mice in the immunized group seemed to die faster, 10% survived; whereas, all control animals died. The difference in mortality between immunized and normal mice is more pronounced following the sc challenge (Fig. 4). A 10-fold reduction in mortality was obtained when immunization was started following sc transplantations. These results have been confirmed in recent experiments. Results of immunization experiments are summarized in Table I.

In addition to the challenge experiments in which mortality was measured, attempts were made to determine if Ehrlich's ascites cells could be isolated from immunized mice that had received ip transplants but did not develop tumors. Thirty-three to 40 days after ip challenge, the survivors were sacrificed and their peritoneal cavities washed with 5 ml of PSS. Two and one-half ml of this PSS containing the peritoneal cells was then injected into normal mice. Of 60 mice injected with these exudates, only one developed an Ehrlich's ascites tumor. Since we have been able to induce tumors with as few as 2 normal tu-

mor cells, it is speculated that immunization has a lethal rather than a static effect on transplanted tumors.

Discussion. McKee *et al.*(3) were unable to induce immunity by treatment with Ehrlich's ascites cells that had been killed by desiccation, freezing and thawing, mechanical grinding, and supersonic waves. The possibility exists that the physical-chemical changes of tumor antigens induced by *in vitro* x-irradiation are extensive enough to make the antigens less homologous and more antigenic for the mouse without destroying antigenic specificity. Indirect evidence for this is manifested by recent observations made in this laboratory. The agglutinins against normal Ehrlich's tumor cells are more readily produced and demonstrated in sera of animals that have been exposed to higher irradiation doses.

Summary. Pre-immunization with irradiated Ehrlich's ascites tumor cells protected mice against subsequent ip transplants of viable cells. Immunization procedures started one day after sc challenge with the tumor, significantly reduced mortality. Such post-immunization did not inhibit the rapid multiplication that follows ip transplantation of the tumor. The evidence indicated that the immunization procedures employed resulted in a lethal rather than a static effect on transplanted tumor cells.

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Passage of Bacteriophages from Mother to Foetus in the Rat.* (24885)

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During a study of tolerance to homografts produced in rats by direct injection of particulate antigens into foetuses, the question arose whether similar antigens can cross the intact placental barrier under natural conditions. Passage of antibodies and similar large molecules from mother to foetus has been shown by studies of Brambell and his associates(1). Whether larger particles can cross the placental barrier has not been answered satisfactorily. Bacteriophages offer special advantages in such a study. They are larger than protein molecules, they multiply only in specific host bacteria and their presence can easily be detected by testing for plaque forming activity, produced only by intact viable phage. Since phage is not able to penetrate and multiply in cells other than susceptible bacteria, it is free from the objection that may be raised against animal viruses, namely, that the latter may traverse the placenta by direct extension of an infectious process. The passage of coliphage and staphylococcal phage across the placental barrier was investigated by Grasset(2), who obtained no evidence for such passage in rabbits and guinea pigs; Blair and Reeves(3), using high titer phage suspensions, reported passage in the guinea pig. However, Nattan-Larrier *et al.*(4) were unable to repeat the latter results. Burckhardt(5) reported passage of coliphage from mother to foetal blood in guinea pigs. Recently Keller and Engley(6) and Keller(7) published quantitative studies on passage of *Bacillus megatherium phage* through gastrointestinal and renal barriers and through intact skin of mice.

Materials and methods. Four bacterio-

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