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Carcinogenic Action of Refined Tumor Factor Isolated from Mouse Leukemia Tissue.* (24883)

ROBERT KASSEL, LAWRENCE BURTON, FRANK FRIEDMAN, ANTONIO ROTTINO
Hodgkin's Disease Research Lab., St. Vincent's Hospital, N. Y.

Induction of leukemia and solid neoplasms in susceptible mice by injection of crude, cell-free filtrates of leukemic tissues has been reviewed by Gross(1). These crude, cell-free filtrates are heterogeneous, and contain a small amount of labile carcinogenic material along with enormous quantity of impurities (2). We had previously postulated(3) that variability in activity, lability, lack of reproducibility, etc., of crude filtrates could be eliminated by refining them. First attempts to purify the tumor factor by standard viral purification technics such as cold methanol fractionation(4), protamine precipitation(5) and genitron homogenization(6) were not successful. This communication presents results obtained when a modification of the method developed by Burton *et al.*(7) for purification of tumor-inducing factor (TIF) of *Drosophila* was employed.

Methods. Strains of mice used were originally obtained from Dr. Ludwik Gross and inbred in our lab for 3½ years. Incidence of spontaneous leukemia in AK (donor) strain has been approximately 85%, whereas only one spontaneous leukemia was found in several thousands of our C3H(f) host strain. We have not observed other spontaneous neoplasms in our C3H(f) line. Since, unlike the AK-C3H(f) hybrids, this

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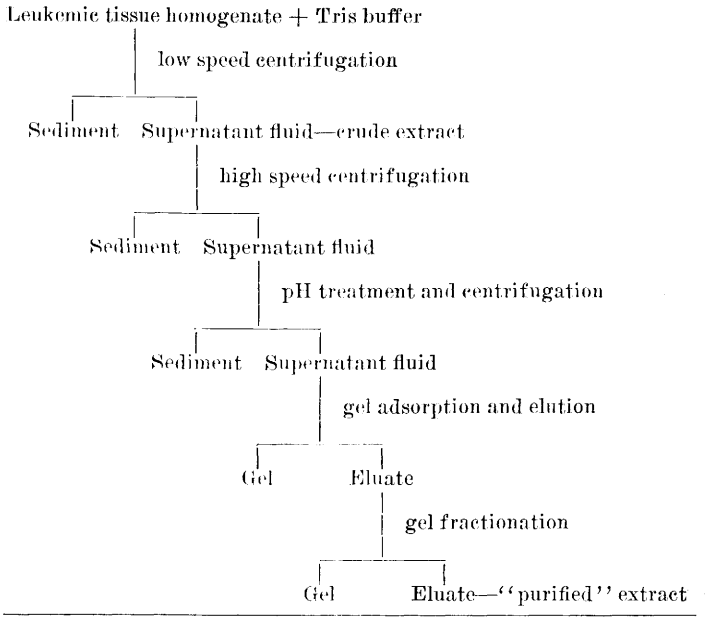
TABLE I. Modified Purification Process.
(After Burton *et al.*)



C3H(f) line apparently has a high resistance to spontaneous neoplasia, it was selected as source of host animals. Purification methods of Burton *et al.*(7) were modified where laboratory facilities and differences in tissue made it necessary. An outline of refinement process is presented in Table I. Heat treatment, ammonium sulfate precipitation and dialysis used by Burton *et al.*(7) in earlier work were omitted. Tissues (liver, spleen, lymph nodes, thymus, brain) from AK mice with spontaneous or transplanted leukemia and from C3H(f) mice with induced leukemia were homogenized in "tris" buffer, differentially centrifuged, pH treated, adsorbed on calcium phosphate gel, and eluted; these refined extracts were then ampulated and stored in dry ice. When 3 to 48 hours old, C3H(f) mice were inoculated either subcutaneously with 0.1 ml, or intravenously with 0.03 - 0.05 ml, of the extract. At weaning, surviving animals were toe clipped for identification and caged in groups of 8 or 10. Water and Purina chow were provided *ad lib.* and the diet supplemented with whole oats twice a week.

Results. The results clearly indicate high degree of tumor-inducing activity of extracts prepared by this technic (Table II: A, B, C and E). This activity was reproducible in 2 extracts prepared from different tissue sources at different times, and total tumor incidence appeared more dependent upon dosage than upon route of administration (Table II: Compare A with B and E). In each experiment where tumors were found, they were first palpable from 75 to 100 days post inoculation, but were suspected as early as 50 days in many animals. Induced fibrosarcomas and the parotid, adrenal and mammary carcinomas were histologically comparable to previous descriptions of such tumors(8,9,10).

The buffer was negative for tumor-inducing activity, confirmed by microscopic examination of histological sections of parotid glands from experimental animals, (Table II: F). Negative results from material stored at 0°C (Table II: G) showed lability of tumor factor preparations and the need for storage in dry ice, and necessity for adhering strictly to preparative procedure was shown by negative results when high speed centrifugation was omitted.

Discussion. A procedure has been developed for extracting and refining tumor factor (TF) contained in mouse leukemic tissue. The refined preparation repeatedly induced a

TABLE II. Tumor Induction with Purified Extract.

Exp.	Survived to weaning of No. inj.	Extract and No. days stored before use	Dose (ml) and route	No. tumors and tumor animals	Sacrificed at avg age of (days)	Tumor type and No.
A	9/26	#20, 3-5 days in dry ice	.1 SC	17/9	136	10 PT 1 FSa 4 MT 1 Myx 1 Adr
B	30/38	#20, 112 days in dry ice	.03–.05 IV	38/20	167	31 PT 3 MT 4 FSa
C	9/25	#20, 150 days in dry ice	.1 SC	20/9	147	16 PT 3 MT 1 OSa
D	"	#20, 30 days at 0°C	*Idem*	0	210	0
E	37/66	#1	.01–.05 SC	33/22	140	31 PT 1 MT 1 FSa
F	27/44	Tris 0.05 M pH 7.5 controls	.05 IV	0	240	0
	16/16		.1 SC	0	210 Still alive, no gross evidence of tumor	0
G	155/333	Prep. modified by omitting high speed centrifugation.	.05 IV .1 SC	0	210	0

PT, parotid gland carcinoma; MT, mammary carcinoma; FSa, fibrosarcoma; Myx, myxoma; Adr, adrenal ca.; OSa, osteogenic sarcoma.

Total 108 tumors
60 animals

variety of neoplasms in a high percentage of inoculated animals. Reproducibility of TF activity in repeated experiments involving large numbers of hosts from different litters, and predictable time of tumor appearance, testify to the enhanced potency achieved by using refined extracts of tumor tissue. Mammary tumors develop at an even earlier age than is reported when "milk factor" is injected. At 76 days it was suspected in one male animal, and when this animal was sacrificed at 104 days bilateral parotid tumors were found as well as mammary tumor. This accelerated tumor development gives to the method a decided advantage over use of crude-cell-free filtrates, and also the advantage of being subject to more precise control than tissue culture method.

That no leukemia developed in the experimental animals, but only a variety of solid tumors, is subject to several interpretations: as the action of a family of tumor viruses(1); as the action of one virus, *i.e.*, that called "polyoma" by Stewart and Eddy(10) and "pleuripotent" by Furth *et al.*(11); as the action of a single tumor factor in a preparation containing substances which modify the action of this factor. Different titers or ratios of these biologically active modifying substances, which may be concentrated or lost during the purification process, may govern the induction of specific tumors by TF.

Masking of tumor factor present in tissue extracts and the possibility that purification technics might overcome such masking has been discussed previously in a review of Hodgkin's disease(3). It was suggested then that the Drosophila technics of Burton and Friedman might be successfully applied to mammalian tissues.

That "tumor factor" could be isolated from sources so different as Drosophila and mice by use of similar technics has suggested the attempt to find this factor in Drosophila, fish lymphoma, chicken lymphoma, Lucké frog kidney tumor, etc. Should factors isolated

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)

DAVID M. DONALDSON AND JOHN R. MITCHELL
Dept. of Bacteriology, Brigham Young University and Dept. of Radiology, Utah Valley Hospital, Provo, Utah

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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