Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 99 OCTOBER, 1958 No. 1

Induction of Tumors by a Virus-Like Agent(s)* Released by Tissue Culture. (24227)

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Borrel first held the concept that a virus might be a causative agent in cancer. However, viruses resemble bacteria, protozoa and fungi in causing lesions which range from suppurative or necrotizing lesions to granulomatous or proliferative ones depending on severity and type of injury. Neoplasia may represent an extreme example of the proliferative type of reaction or develop as a result of hyperplasia. Rous(1) reported transmission of a sarcoma in a Plymouth Rock hen by cell-free filtrates. Our knowledge of cell-free filtrates and cell particulates as they effect neoplastic processes has increased enormously since this period(2-26 and Table I). Gross has reported that filtered cell-free leukemia extracts of AK mice when inoculated into newborn C_3H mice can induce leukemia, parotid tumors and subcutaneous sarcomas (2,3,4). Recently Gross(5) reported the high susceptibility of 1- to 14-day-old C_3H mice to "passage A" leukemia filtrates inducing leukemia but not parotid tumors or sarcomas. Stewart(6) produced neoplasms of the parotid gland and adrenal gland in mice inoculated with cell-free extracts or filtrates of leukemic mouse tissues. Drs. Stewart *et al.* and Eddy *et al.*(7,8) reported a tissue culture method to increase the tumor-producing capacity of leukemic extracts or parotid gland tumors by incubating them in tissue cultures of cells from monkey kidney or from mouse embryos. The increase in tumor-producing capacity was shown by production of a wider variety of tumors and a pronounced decrease in the tumor latent period. The tumor-producing effect from these fluids of tissue culture preparations was *neither strain nor species specific*(7,8,9).

This report confirms and extends, by using inbred strains of mice, rats, and hamsters, the recent observations of Stewart and Eddy relative to induction of multiple types of cancers in hamsters(9), mice(7,8), and rats(10) with a virus-like agent derived from a mouse tumor propagated in mouse embryo tissue culture.

Material and method. Tissue cultures were prepared as reported by Eddy *et al.*(9). Swiss embryos of the ICR-Ha strain approximately in the 12th day of gestation were used for our mouse embryo tissue culture preparations.

Sources of tumor-producing material. Tissue culture fluids containing the virus-like agent were obtained from Dr. Stewart in November, 1957. These were unfiltered fluid #3919, 3791, and D-3919 (lyophilized). Our own tissue culture fluids (M-1-1 and M-1-2)

* Supported in part by the Dorothy H. and Lewis Rosenstiel Foundation and the Jane Coffin Childs Memorial Fund.

TABLE I. Induction of Tumors by Cell-Free Extracts in Mammals.

Investigator	Source of material	Responsive recipient	Resulting lesions
Gross (1951-1958)	AKR and C_{58} tissues	Newborn and young C_3H/Bi, C_{57} Bl	Lymphatic leukemia, few parotid tumors and associated neoplasms
Stewart and Eddy (1955-1958)	AK leukemia, Swiss salivary tumors and other tissues	Newborn mice C_3Hf x AKR F_1 Swiss Newborn and young hamsters Newborn rats " rabbits	Multiple types of neoplasms and/or hyperplasia
Schwartz *et al.* (1957-1958)	Swiss leukemia C_3H " AKR " Human "	Adult Swiss " C_3H " AKR " AKR	Only brain filtrate is effective in producing lymphatic leukemia
Friend (1957-1958)	Ehrlich neoplasm	Adult Swiss, DBA/2	Leukemic-like disease
Graffi *et al.* (1956-1958)	Ehrlich neoplasm	Young Agnes-Bluhm mice	Acute and chronic chloroleukemia
Mirand *et al.* (1958)	Swiss salivary tumors	Newborn DBA/1, AKR/Sn 57 Black/Ha, Swiss/ICR-Ha AKR x Swiss F_1 Newborn and young hamsters Newborn rats	Multiple types of neoplasms and/or hyperplasia

containing the tumor inducing agent(s) were obtained by inoculating Swiss embryo (ICR-Ha) tissue cultures with minces of a parotid tumor from a Swiss mouse (NIH). Culture fluids to which *E. coli* suspensions were added were filtered through HA grade millipore filters and fluids were tested before and after filtering for *E. coli*. Newborn mice were inoculated with filtered and unfiltered tissue fluids from these tissue cultures.

Control tissue cultures. Control tissue culture fluids were also obtained from Dr. Stewart. Our own control tissue culture fluids were obtained from Swiss embryo (ICR-Ha) tissue cultures inoculated with normal Swiss parotid tissue.

Hamsters. Hamsters (*Cricetus anuratus*) used in this study were obtained from the Nat. Inst. of Health and Blue Spruce Farms. Cannibalism of newborn and young hamsters by their mothers was a serious problem. The hamsters, at time of injection, varied in age from newborn to 2 weeks. The dose of tissue culture fluid administered to each animal was 0.1 ml by subcutaneous injection.

Mice. Mice used in this study were bred at the Institute. These were C_{57} Black/Ha, DBA/1, Swiss/ICR-Ha, AKR/Snell and AKR x Swiss F_1 hybrids. Some Swiss mice of the NIH strain obtained from Dr. Stewart were used. Newborn mice, less than 24 hours old, were injected subcutaneously with 0.1 ml of tissue culture fluid.

Rats. Rats of the Sprague-Dawley strain were obtained from Holtzman Co., Madison, Wisc. Newborn rats less than 24 hours old were injected subcutaneously with 0.1 ml of tissue culture fluid.

Results. Table II presents a summary of types and sites of tumors induced in animals by different tissue culture fluids of Dr. Stewart and of our own (M-1-1 and M-1-2).

Table III presents induction of tumors in animals injected subcutaneously when less than 24 hours old with 0.1 ml of unfiltered tissue culture fluid received from Dr. Stewart. The average tumor latent periods for hamsters, mice and rats were 29, 75, and 180 days, respectively. Three out of the 6 AKR/Sn mice receiving tissue culture fluid 3791 developed both leukemias and parotid tumors in 2½ months, while in the others only parotid tumors were observed. It is significant that C_{57} Bl/Ha mice, a "low tumor" strain, developed parotid tumors with tissue culture fluid, #3919 and D-3919. DBA/1 mice, high mammary tumor strain, developed salivary gland tumors and osteogenic sarcomas.

TABLE II. Induction of Tumors in Animals by Different Tissue Culture Fluids.

Animal	Type	Site	Avg latent period (days)
Hamster	Sarcoma	Heart, kidney, blood vessel, stomach, mediastinum, lung, subcut.	29
	Vascular*	Lung, liver	
Mouse	Leukemia	Thymus, lymph node, liver	75
	Osteogenic sarcoma	Rib, vertebra, shoulder	
	Sarcoma	Lung, kidney, subcut., heart	
	Mixed–carcinoma (pleomorphic)	Salivary glands (parotid, submaxillary, sublingual)	
Rat	Squamous cell carcinoma	Lip	180
	Sarcoma	Caecum, kidney,† lung	

* Duran-Reynals also observed vascular lesions in chickens(26).
† Kidney tumor in its F_3 transplantable generation; 100% takes.

In Table IV are data from our own tissue culture fluids prepared by the technic of Eddy and Stewart(9). It can be observed that tissue culture fluids of the first week from first passage (M-1-1) displayed tumor-inducing ability in hamsters injected shortly after birth. Moreover, tissue culture fluids of the first and second week from second passage (M-1-2) displayed tumor inducing ability in newborn mice and newborn hamsters. The average latent periods were similar to those in Table II.

Recently 2 DBA/1 mice, one Swiss/ICR-Ha mouse, and one hamster injected when newborn with control tissue culture fluid (line 1) developed parotid tumors and in the hamster multiple tumors (heart, kidney, lung, and liver). Although rigid precautions in technic were observed, the possibility should be considered that the virus-like agent(s) contaminated our control tissue culture fluid or that the normal Swiss mouse embryo harbors in low concentration a virus-like agent(s) and that embryo cells *in vitro* increase its concentration. The fact that only one batch of control material (line 1) was oncogenic adds credence particularly to the first possibility.

Attempts to transplant some of these induced tumors occurring in inbred mouse strains, particularly parotid, into the strain of origin have been successful, when newborn and pre-conditioned irradiated hosts were used. This result should be considered in interpreting neoplastic nature of the growths observed.

Discussion. Stewart and Eddy have re-

TABLE III. Induction of Tumors in Newborn Animals by a Virus-Like Agent(s) in Tissue Culture Fluids Obtained from Dr. S. Stewart.

Tissue culture strain	Animal	Injected	Effective survival	No. animals died with tumors	Surviving without tumors
3919	Hamsters	115	5	5	0
	Swiss/ICR-Ha	31	20	8	10 (2)
	C_{57} Black/Ha	8	3	1	2
	DBA/1	5	3	3	0
	Rat	91	32	8	12 (12)
3791	Swiss/ICR-Ha	50	24	2	17 (5)
	C_{57} Black/Ha	10	8	1	6 (1)
	DBA/1	10	4	0	3 (1)
	AKR/Sn	7	6	6	0
	AKR x Swiss F_1	9	9	2	7
D-3919	DBA/1	8	0	0	0
	C_{57} Black/Ha	6	4	2	1 (1)
	Rat	29	11	1	8 (2)

Control fluids inj. into control animals showed to date no evidence of tumor development.
( ) Animals died without tumors.

TABLE IV. Induction of Tumors in Newborn Animals with Virus-Like Agents in Tissue Culture Fluids.*

Tissue culture fluid (Line M-1)	Animal	Injected	Effective survival	No. animals died with tumors	Surviving without tumors
(A) First passage					
M-1-1 2nd wk (unfiltered)	Hamster	11	5	4	0 (1)
	Swiss/ICR-Ha	13	10	0	7 (3)
Idem (filtered)	Hamster	10	3	2	0 (1)
	Swiss/ICR-Ha	7	0	0	0
(B) Second passage					
M-1-2 1st wk (unfiltered)	Swiss/ICR-Ha	11	8	0	6 (2)
	C_{57} Black/Ha	4	2	1	1
Idem (filtered)	Swiss/ICR-Ha	16	12	6	4 (2)
	C_{57} Black/Ha	7	5	0	5
M-1-2 2nd wk (unfiltered)	DBA/1	6	2	2†	0
	Swiss/ICR-Ha	11	8	4	4
Idem (filtered)	Hamsters‡	10	3	3	0
	Swiss/ICR-Ha	15	0	0	0
	DBA/1	3	2	1	0 (1)

Control tissue culture fluids of first and second wk did not show to date evidence of tumor development.

( ) Animals died without tumors.

* Mouse embryo tissue culture inoculated with minces from Swiss mouse parotid tumor.

† Intranuclear inclusion bodies in kidney cells.

‡ 14-day-old hamsters.

ferred to the tumor inducing agent(s) as the SE-polyoma virus. Virological data reported by Eddy(10) and Stewart(11) suggest the existence of one virus rather than a family of viruses. Further study of this facet is indicated. It is surprising, indeed, to observe that this virus-like agent(s) is not strain or species specific. The variety of tumors that one sees in the injected animals appears to be determined by host susceptibility to the virus-like agent(s). The same response is frequently seen with carcinogens. Although the hamster appears to be very susceptible to the agent as judged by a very short latent period, the mouse comes down with a greater variety of tumors. Whether these tumors that arise are multiple primaries or metastatic tumors is still not definitely established. However, it appears that they are not metastatic in nature.

Among the responsive recipients, newborn mice and rats, as well as newborn and 2-week-old hamsters are susceptible to inoculation of the SE-polyoma virus or virus-like agent(s). In studies on chicken leukosis(15,16,17,18) and on Rous Sarcoma(19) and other related studies (Table I) very young and/or young recipients are also more sensitive to cell-free extracts. Whether there is an optimum age factor involved in the susceptibility of recipients to the SE-polyoma virus remains to be more fully examined. The data from the laboratory of Stewart and Eddy seem to support the tentative conclusion that there is an optimum age factor. Generally there seems to be decreased susceptibility and increased latent period with advancing age. This, of course, agrees quite well with the findings of others who have induced tumors with cell-free filtrates. Immunological immaturity of the newborn, genetic features of the host, metabolic state of the host cells and presence of other viruses may all play a role in vulnerability of the host tissues to the SE-polyoma virus.

It has been reported(11) that when breeding units of mice are maintained in the same room with mice that have been injected with tissue culture fluids the newborn mice from such a colony remain resistant to the virus-like agent(s) in the tissue culture fluid. This is suggestive of immunity after exposure. It is possible that passive transfer of antibodies from the mothers may have conferred some

degree of immunity to the newborns. This problem is under investigation in our laboratory.

The intracellular mechanisms underlying viral oncogenesis remain to be elucidated. Whether tumor formation results from alteration of cells directly by viral parasitism of specific substances, such as RNA, or is mediated more indirectly through interference with homeostatic growth regulation in the host, or both, is still unanswered.

Stasney *et al.*(24) and Paschkis *et al.*(25) induced lymphosarcomas and hepatomas in rats with chromatin and to a much lesser extent with mitochondria fractions from both tumors. Even though tissue culture fluids still possessed tumor inducing properties upon being passed through filters as small as a pore size of 77 mμ(10), the possibility remains that the tumors might be related to cellular particulates rather than a virus. However, it remains to be shown whether the hypothetical activity of such cell particulates and infectious agents is experimentally separable.

Summary. (1) The observations reported by Stewart and Eddy have been confirmed and extended by using inbred strains of mice, rats, and hamsters treated with tissue culture fluids prepared by Stewart and Eddy and at this laboratory. (2) Injection of such tissue culture fluids into newborn and young animals produced multiple types of tumors in mice of DBA/1, Swiss/ICR-Ha, C_{57} Black/Ha, AKR x Swiss F_1, and AKR/Sn strains as well as in hamsters and rats. (3) Although most control tissue culture fluids possessed no tumor inducing ability, in one instance a control tissue culture fluid (line 1) did produce parotid tumors in 2 DBA/mice, 1 Swiss/ICR-Ha mouse, and multiple tumors in 1 hamster. (4) Transplantation of some of these induced tumors occurring in inbred mouse strains has been successful into the strain of origin if newborn and preconditioned irradiated hosts are used.

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Received June 30, 1958. P.S.E.B.M., 1958, v99.