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Received November 4, 1959. P.S.E.B.M., 1960, v103.

Serologic Comparison of Mammary Tumor Viruses from 3 Strains of Mice.* (25454)

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The antigenic nature of the mouse mammary tumor virus is recognized. Sera of rabbits, rats, and guinea pigs(1-3) previously injected with extracts of virus-containing tissues are capable of neutralizing the virus in tumor extracts, both *in vivo* and *in vitro*. Normal sera of these animals, on the other hand, have little or no effect on the virus. The present experiment was designed to investigate possible antigenic differences between mammary tumor viruses possessed by different strains of mice. To eliminate any possible effect arising from tissue antigen differences, one strain (BALB/cCrgl) served as donor for all viruses. A comparison was made between the antigenicity of the viruses carried by 2 different strains of mice (C3H/Crgl and A/Crgl) and also between the viruses carried in a strain (A/Crgl) and a subline of it (A/Crgl/3, formerly designated A/vi), which are known to differ in effect upon tumor development(4). In addition, possible tissue differences were investigated by a comparison of one virus in 2 genetically different hosts (A/Crgl virus in A/Crgl and in BALB/cCrgl mice). The effectiveness of rabbit antisera in neutralizing the activity of the viruses was measured by tumor incidence in susceptible virus-free test mice injected with virus and antiserum.

Materials and methods. Antigen preparation: Mammary tissue was taken from the following groups of mice (Table I): BALB/c females (virus-free C); BALB/c females fostered on C3H (C3H virus in C); BALB/c females fostered on A/3 (A/3 virus in C); BALB/c females fostered on A (A virus in C); and A females (A virus in A). The donor females were permitted to breed, and were sacrificed during the third week of their second lactation. Mammary gland pairs #2, 3, and 4 were dissected out. The mammary glands from 2 females in a group were homogenized in a Waring blendor with 24 ml sterile saline for a total of 2 min. The mixture was spun in an International Clinical Centrifuge for 30 min at 1540 g. The pellets were discarded and the supernatants were then spun for 90 min in the multispeed attachment of an International Model U Centrifuge (maximum 30,000 g). The supernatants were discarded, and the pellets were resuspended in saline (.5 ml/g of original tissue weight for injection into rabbits, 2 ml/g of original tissue weight for injection into mice). The solu-

* Conducted during tenure of N.S.F. predoctoral fellowships. Aided by Am. Cancer Soc. grant and by Univ. of California cancer research funds. The helpful advice of Professor K. B. DeOme is gratefully acknowledged.

TABLE I. Mammary Tumor Development in BALB/c Females Given Various Injections of Mammary Tumor Virus and Rabbit Antiserum.

Antigen source for antiserum	Virus	No. of mice	No. with tumors	% tumors	Mean tumor age (mo)
No serum	A virus in C	24	10	42	12.6
	A/3 *idem*	23	14	61	8.2
	C3H "	24	13	54	10.7
	A virus in A	22	7	32	11.6
Normal rabbit serum	A virus in C	23	12	52	11.9
	A/3 *idem*	25	13	52	8.5
	C3H "	16	6	38	11.3
	A virus in A	20	9	45	12.3
Virus-free C	A virus in C	19	11	58	11.0
	A/3 *idem*	20	16	80	8.3
	C3H "	19	8	42	10.4
	A virus in A	16	7	44	13.6
A virus in C	A virus in C	30	1	3	16.5
	A/3 *idem*	16	0		
	C3H "	26	0		
	A virus in A	28	0		
A/3 virus in C	A virus in C	25	0		
	A/3 *idem*	15	0		
C3H virus in C	A virus in C	27	1	4	8.5
	C3H *idem*	27	0		
A virus in A	A virus in C	27	1	4	14.0
	A virus in A	26	0		

tion was cleared by spinning in the International Clinical Centrifuge for 15 min and was placed in the refrigerator overnight. The material was kept under refrigeration during the preparation procedure. *Antiserum preparation*: Six-month-old male albino rabbits were injected intramuscularly once weekly for 4 weeks with the antigen preparation derived from 1 g of lactating mammary tissue. Four days after last injection, blood was withdrawn from heart, allowed to clot overnight, and centrifuged to separate serum from the clot. At the same time, blood was collected from 2 rabbits which had not received any injections. *Neutralization*: Equal amounts of rabbit serum and saline were combined in test tubes with the virus preparation from 1 g of lactating mammary tissue in each 20 ml of final solution. They stood at room temperature for 2 hours. Each mouse received .2 ml liquid intraperitoneally. Therefore, the total amount of virus preparation given to each test mouse was that derived from .01 g of lactating mammary tissue. The test animals were 17 to 24-day-old BALB/c females, weaned at 5 weeks and bred for 2 litters. The offspring were discarded within 24 hours after birth. All tumorous females were sacrificed. Histologic sections were made of tumors taken from approximately ¾ of tumor-bearing animals. The experiment was terminated when tumor-free animals were 18 mos. old.

Results are summarized in Table I. Test mice which received virus alone, or virus combined with normal rabbit serum, or virus combined with serum from rabbits previously injected with virus-free BALB/c tissue, developed mammary tumors. Thus the injected dose of virus was sufficient to cause tumor development, and the viruses are not inhibited by normal rabbit serum nor by antisera against virus-free tissue.

However, in experiments where the virus preparation was mixed with rabbit antiserum against a mammary tumor virus, tumor development did not occur (with 3 isolated exceptions). Neutralization occurred regardless of whether the antiserum had been prepared against the same or another virus, either a virus from a different strain of mice, or a virus from the same strain (different subline) but with a different effect upon tumor development.

Bittner and his co-workers(3,5,6) using A, C3H, and (A x C3H) F_1 mice have reported that the tissues of the donor mouse may have

a reversible effect upon ability of antisera to neutralize the virus. In the present experiment the source of the virus had no effect upon efficiency of the neutralization: antisera against A virus neutralized the A virus from both BALB/c mice and A mice, regardless of whether the virus preparations used in antisera production had been derived from A mice or BALB/c mice.

In control groups a significantly earlier age of tumor development was found in the group of mice given A/3 virus, than in groups given A (in BALB/c or in A tissue) or C3H virus. This difference with regard to the A/3 and the A viruses will be discussed elsewhere.

Summary. No antigenic differences were found when the mouse mammary tumor viruses from 3 different strains or sublines (one in 2 different host strains) were compared, using the technic of *in vitro* virus neutralization with rabbit antisera followed by injection into susceptible virus-free test mice. Normal rabbit serum and antiserum against virus-free tissue did not neutralize the virus. Antiserum against any of the viruses neutralized that virus and all others tested. Neutralization of the virus was not affected by the tissue source of the virus.

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Received November 9, 1959. P.S.E.B.M., 1960, v103.

Experimental Preparation Showing Effect of Reserpine on Tissue Resistance.* (25455)

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Reserpine has been shown to be a potent agent in abetting ulcer diathesis both clinically and in the laboratory(1,2,3). The mechanism of acute ulcer production by reserpine is not clearly understood, although it has been shown that intravenous injection of reserpine causes a marked increase in HCl secretion in human patients(2) and also augments Heidenhain pouch secretion in the dog(3). Whether reserpine has any effect on tissue resistance, however, has not been clearly assessed. Susceptibility of cat's esophagus to the erosive action of human gastric juice has been well documented in this laboratory(4). Using a modification of this technic, the peripheral effect of reserpine has been observed and is here reported.

* Supported by U.S.P.H.S. Research Fund, Donald J. Cowling Fund for Surgical Research, Malignant Disease Research Fund, and Jay and Rose Phillips Fund for Surgical Research.

Method. Adult mongrel cats were used. The animals were divided into 2 groups. In both groups, the animals were anesthetized with pentobarbital, 30 mg/kg, and positive pressure respiration was maintained automatically through a cervical tracheostomy. In Group I the cervical esophagus and gastric cardia were cannulated for outflow tracts. A left thoracotomy through 7th interspace was achieved and the esophagus mobilized. A No. 26 T-tube with ¾ cm "limbs" was inserted through longitudinal incision in mid esophagus, each limb being secured in place by an umbilical tape. By alternate occlusion of the limbs of the T-tube, the upper or lower segments of the esophagus could be perfused separately. In Group II animals the entire esophagus was utilized for perfusion, with the perfusate being introduced through a cannula in the cervical esophagus and drained at 20 cm outflow pressure at the lower end of the