The Mammary Tumor Milk Agent Given to Adult Female Mice Following Splenectomy and Vital Staining*

PAULO R. F. BORGES

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

Studies on the mammary tumor milk agent of the mouse (14) would be facilitated by the development of a rapid test for its detection. The usual method of testing for the presence of this agent requires that test animals be observed for a long period of time before an estimate can be made of the percentage of mammary tumors obtained, as the latent period of the milk agent runs from 6 to 24 months (3).

By the use of complement-fixation tests Bennison (8) obtained results showing that spleen extracts from mice possessing the mammary tumor milk agent fix more complement than spleen extracts from agent-free mice. Recently Imagawa, Green, and Halvorson (20) reported a precipitin test for mouse tissues containing the milk agent. Results from the use of such time-saving tests in routine work are eagerly awaited.

Experiments have shown that mice of susceptible strains become more resistant to the milk agent with increasing age (4, 5). Adult mice given the milk agent develop few mammary tumors (2, 10, 13). However, Dmochowski (17) succeeded in inducing a high incidence of breast tumors by injecting repeated doses of dried breast tumor tissue into 4 months old hybrid female mice which were genetically susceptible to mammary tumors, but which lacked the milk agent.

Considering that the role played by the reticuloendothelial system in the resistance to the growth of tumors remains unsettled (21), the following experiments were undertaken to ascertain whether it would be possible to hasten the action of the milk agent in old mice submitted to splenectomy and vital staining, hoping to develop a more rapid bioassay technique for the milk agent.[1]

MATERIALS AND METHODS

Hybrid BDF_1 (C57 Black ♀ × dba ♂) and BAF_1 (C57 Black ♀ × A ♂) mice were used as test animals. The age of the mice at the beginning of the experiments ranged from 10 to 15 months. All were breeding females, born and raised in the Roscoe B. Jackson Memorial Laboratory. The animals were kept on a diet of Purina Fox Chow pellets with an unlimited supply of water.

BDF_1 and BAF_1 hybrids are genetically susceptible to mammary tumors (9, 12, 24), but as they do not obtain the milk agent while being nursed by their low-cancer strain C57 Black mothers, they show a low incidence of mammary tumors under normal conditions. However, BDF_1 and BAF_1 are suitable as test animals as they show an increased percentage of breast tumors when supplied with the milk agent (9, 11, 17, 23).

Splenectomy was performed under nembutal anesthesia.

Sterile 0.5 per cent aqueous solution of trypan blue or 1 per cent aqueous solution of congo red was used in each of two groups of experiments. The injections of the dyes were given intraperitoneally, and started the following day after splenectomy was performed. Mice of the trypan blue group received injections for 2 months. In the beginning 0.2 cc. to 0.5 cc. of the dye was given at 3 to 7 day intervals until a definite blue color of the skin was shown. Later, doses of 0.2 cc. given at 4-day intervals were maintained until the end of the experiment. Mice of the congo red group were injected daily with 0.1 cc. to 0.3 cc. of the dye during the 4 days following splenectomy. From then on 0.3 cc. of congo red was injected every other day, occasionally at 3-day intervals, for 57 days.

Freshly excised spontaneous mammary tumors from C3H female mice were used as a source of the milk agent. Minced mammary tumors were suspended in 0.9 per cent sodium chloride solution in the proportion of 1 gm. of tumor tissue in 30 cc. of sodium chloride solution. The suspensions were cleared by centrifugation at 2000 r.p.m. for 10 minutes. The supernatant fluid was first passed through a Seitz filter, and then through a porcelain bacteria-tight "Selas" candle No. 02. Each mouse was injected intraperitoneally with 0.5 cc. of mam-

* This investigation was aided by a Finney-Howell Research Foundation Fellowship.

[1] These experiments were interrupted and partially ruined by the Bar Harbor fire in October, 1947.

mary tumor filtrate at 4 to 13 day intervals for the trypan blue group, and weekly for the congo red group. The injections of the filtrate were given on days not coinciding with the administration of either trypan blue or congo red.

Mice subjected to splenectomy and injected with trypan blue or congo red (but no milk agent), and non-splenectomized mice given the milk agent alone (single or repeated doses) were run as controls.

The animals were kept under observation for 8 to 26 weeks until the experiments were accidentally interrupted. The results of these experiments, which were negative, are shown in Table 1.

TABLE 1

ATTEMPTS TO INDUCE MAMMARY TUMORS IN BREEDING FEMALE MICE BY INTRAPERITONEAL ADMINISTRATION OF THE MAMMARY TUMOR MILK AGENT FOLLOWING SPLENECTOMY AND VITAL STAINING

Group	Stock	Age at the beginning of the experiments *Months*	Number of mice	Treatment*	Number of injections: Trypan blue†	Congo red‡	Milk agent§	Time under observation *Weeks*	Average age of mice at death *Months*	Results
Experimental	BDF_1	13–15	7	Sp.-TB-MA	15		6	18	17.5	Negative
"	BAF_1	10–14	7	"	15		5	18	15.1	"
Control	"	10–14	9	Sp.-TB	15			17	15.3	"
"	"	10–14	9	MA			5	17	15.4	"
"	"	11	9	"			1	26	17.0	"
Experimental	"	11–14	8	Sp.-CR-MA		27	8	8	15.3	"
Control	"	11–14	6	Sp.-CR		27		8	15.5	"

* Sp., Splenectomy; TB, Trypan blue; MA, Milk agent; CR, Congo red.

† 0.5 per cent aqueous solution; 0.2 cc. to 0.5 cc. intraperitoneally.

‡ 1 per cent aqueous solution; 0.1 cc. to 0.3 cc. intraperitoneally.

§ Cell-free filtrate of spontaneous mammary tumor from C3H mice (1 gm. of tumor tissue in 30 cc. of 0.9 per cent sodium chloride solution); 0.5 cc. of filtrate intraperitoneally.

DISCUSSION

The purpose of the experiments reported here was to investigate whether it would be possible to hasten the appearance of mammary tumors in genetically susceptible hybrid mice, and thus work out a more rapid method of testing for the presence of the mammary tumor milk agent.

By injecting 4 months old BAF_1 breeding females with 1.5 gm. of dried breast tumor tissue, divided in 12 doses, Dmochowski (17) succeeded in obtaining a high percentage of mammary tumors after 13 to 26 months. The relative resistance of adult mice to the milk agent is thus believed to have been overcome by large doses of the agent.

The opinions about the role played by the reticulo-endothelial system in the resistance to tumor growth are discordant (7, 15, 16, 18, 19, 22, 25, 26). However, a great number of experiments on this subject seems to indicate that a decrease in the number of active reticulo-endothelial elements lowers the resistance to the growth of tumors. The contradictory results thus far obtained can be explained by the difference of materials and methods used by individual investigators, and by the great capacity of the reticulo-endothelial system to regenerate and to perform vicarious functions. This capacity is so pronounced that it cannot be decided to what extent procedures intended to produce a blockade will in fact determine a stimulation of the reticulo-endothelial system. Aschoff (6) himself recognized that it is difficult, and even impossible, to suppress altogether and permanently the functions of the reticulo-endothelial system. Yet it has been demonstrated that vital staining lowers or suppresses the resistance of animals to transplanted tumors (1, 27).

There are some possible factors to account for the negative results obtained in the present experiments: 1) The mice might have attained an age when the milk agent is no longer effective; 2) The dose of milk agent administered might not have been large enough to overcome their resistance; 3) The time during which the animals were kept under observation might not have been long enough for mammary tumors to develop; 4) The presence of trypan blue or congo red in the mouse might have interfered with the specific activity of the milk agent.

The very purpose of the present experiments explains why these factors were not obviated, and why mice which had been observed for 8 weeks were also included in the table.

SUMMARY

Attempts were made to develop a more rapid method of testing for the presence of the mouse mammary tumor milk agent.

Repeated doses of filtrates of freshly excised spontaneous C3H mammary tumors failed to induce mammary tumors in 10 to 15 months old BDF_1 and BAF_1 breeding females. The mice which previously had been submitted to splenectomy and

vital staining with trypan blue or congo red were kept under observation for 8 to 18 weeks, until the experiments were accidentally interrupted.

ACKNOWLEDGMENTS

The author is indebted to Dr. L. W. Law for suggesting these experiments, and to Mr. Sidney Chason for his assistance.

REFERENCES

1. Andervont, H. B. Studies on Immunity Induced by Mouse Sarcoma 180. Pub. Health Rep., **47**:1859–1877, 1932.
2. Andervont, H. B. Susceptibility of Young and of Adult Mice to the Mammary-tumor Agent. J. Nat. Cancer Inst., **5**:397–401, 1945.
3. Andervont, H. B. The Milk Influence in the Genesis of Mammary Tumors. A.A.A.S. Publ. No. 22 on Mammary Tumors in Mice, pp. 123–139, 1945.
4. Andervont, H. B., with assist. of McEleney, W. J. Effect of Ingestion of Strain C3H Milk in the Production of Mammary Tumors in Strain C3H Mice of Different Ages. J. Nat. Cancer Inst., **2**:13–16, 1941.
5. Andervont, H. B., Shimkin, M. B., and Bryan, W. R. Technique Suitable for Quantitative Studies on the Mammary Tumor Inciter of Mice. J. Nat. Cancer Inst., **3**:309–318, 1942.
6. Aschoff, L. Das reticulo-endotheliale System. Ergebn. d. inn. Med. u. Kinderh., **26**:1–118, 1924.
7. Baroni, V., and Comsia, O. Blocage du système réticulo-endothélial par l'encre de Chine et immunité envers le sarcome de Jensen. Compt. rend. Soc. de biol., **111**:1108–1111, 1932.
8. Bennison, B. E. Serologic Studies on Mouse Mammary Tumors. Program abstracts, Fourth Intern. Cancer Research Congress, St. Louis, Mo., U.S.A., 1947.
9. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155–168, 1940.
10. Bittner, J. J. Inciting Influences in the Etiology of Mammary Cancer in Mice. A.A.A.S. Research Conference on Cancer, pp. 63–95, 1945.
11. Bittner, J. J. Observations on the Inherited Susceptibility to Spontaneous Mammary Cancer in Mice. Cancer Research, **4**:159–167, 1944.
12. Bittner, J. J. Possible Method of Transmission of Susceptibility to Breast Cancer in Mice. Am. J. Cancer, **39**:104–113, 1940.
13. Bittner, J. J. Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice. Cancer Research, **2**:710–721, 1942.
14. Bittner, J. J. The Mammary Tumor Milk Agent. Ann. New York Acad. Sc., **49**:69–73, 1947.
15. Brüda, B. E. Über erfolgreiche Züchtung von Mäusetumoren auf entmilzten Ratten. Klin. Wchnschr., **7**:27, 1928.
16. Büngeler, W. Geschwulsttransplantation am gespeicherten Tier. Frankf. Zeitschr. f. Path., **35**:67–82, 1927.
17. Dmochowski, L. Age and Dosage in the Induction of Breast Cancer in Mice by the Mouse Mammary Tumour Agent. Brit. J. Exper. Path., **26**:192–197, 1945.
18. Dobrovolskaïa-Zavadskaïa, N., and Samssonow, N. L'influence de la splénectomie sur les greffes du cancer expérimental. Compt. rend. Soc. de Biol., **92**:1222–1224, 1925.
19. Erdmann, R. Erzeugung des Flexner-Jobling-Tumors durch Filtrate. Ztschr. f. Krebsforsch., **27**:69–82, 1928.
20. Imagawa, D. T., Green, R. G. (Deceased), and Halvorson, H. O. A Precipitin Test for Antigens Present in Mouse Tissues Containing the Milk Agent. Proc. Soc. Exper. Biol. and Med., **68**:162–166, 1948.
21. Jaffé, R. H. The Reticulo-endothelial System in Immunity. Physiol. Rev., **11**:227–327, 1931.
22. Munck, W. Über den Einfluss von Vital-Trypanblauspeicherung auf das Wachstum von verimpften Mäusecarcinom und -sarkom. (Verläufige Mitteilung). Ztschr. f. Krebsforsch., **26**:377–385, 1928.
23. Murray, W. S. Studies on the Effect of Foster Nursing and Its Relation to the Development of Mammary Carcinoma in the Mouse. Cancer Research, **1**:790–792, 1941.
24. Murray, W. S., and Little, C. C. Chromosomal and Extrachromosomal Influence in Relation to the Incidence of Mammary Tumors in Mice. Am. J. Cancer, **37**:536–552, 1939.
25. Oser, E. G., and Pribram, E. E. Ueber die Bedeutung der Milz in dem an malignem Tumor erkrankten Organismus und die Beeinflussung von Tumoren durch Milzbrei. Ztschr. f. exper. Path. u. Therap., **12**:295–302, 1913.
26. Psaromitas, D. Zur Frage des Wachstums artfremder Tumoren bei gespeicherten Tieren. Ztschr. f. Krebsforsch., **30**:77–105, 1930.
27. Saphir, O., and Appel, M. Attempts to Abrogate Immunity to the Brown-Pearce Carcinoma. Cancer Research, **3**:767–769, 1943.