15244

Antigenic Character of the Cancer Milk Agent in Mice.*

ROBERT G. GREEN, MARYE M. MOOSEY, AND JOHN J. BITTNER.

From the Department of Bacteriology and Immunology and the Division of Cancer Biology of the Department of Physiology, University of Minnesota Medical School, Minneapolis.

The influence of milk in the transmission of mammary cancer in mice described by Bittner[1] was determined by him to be filterable.[2,3] The colloidal dimension of the influence was ascertained by means of ultracentrifugation by Visscher, Green, Bittner, Ball and Siedentopf,[4] and these authors suggested that the agent might be classed as a filterable virus. Additional data showing that the agent is filterable have been presented by Andervont and Bryan[5] and by Bittner, Evans and Green.[6] We have pursued studies on the antigenic character of the milk agent to gain further information as to its general characterization and in particular as to its properties as a virus.

Studies on the antigenic nature of the mouse-tumor agent have been pursued by Andervont and Bryan,[5] who have recently reported on the neutralization of the milk influence by immune serums. Two immune serums were prepared by them, each from one rabbit. These serums apparently neutralized the mouse mammary-tumor milk agent, as only 1 of 36 mice injected with tumor material mixed with the antiserums developed a tumor, while 10 of 15 mice injected with tumor material mixed with normal rabbit serum developed tumors.

In our investigations of the antigenic character of the milk agent, we have utilized as controls the agent suspended in saline solution and the agent treated with normal serum and with serum from animals immunized with normal tissue. As the agent we have used high-speed centrifugates collected between a first run at 15,000 g and a second at 95,000 g. These centrifugates, which were previously demonstrated[4] to contain much of the milk agent, have been injected into rabbits and white rats to produce antiserums. Five doses of centrifugate, each consisting of a 4-g equivalent of spontaneous mouse tumor suspended in 3.5 cc of saline solution, were injected into rabbits at 5-day intervals. The rats were immunized similarly, except that each immunizing dose was 2 cc and contained the equivalent of 1 g of mammary-tumor tissue. The animals were bled for the production of antiserum from 7 to 10 days after the last injection. In the first experiment on the neutralization of the agent, 90 mice were injected in 3 groups of 30 mice each. Group 1 was injected with a centrifugate equivalent to 0.25 g of tumor tissue suspended in 0.5 cc saline solution. At the end of 11½ months, 25 of the 30 mice had developed mammary cancer. The 30 mice in Group 2 were injected with a centrifugate equivalent to 0.25 g of tumor tissue suspended in 0.5 cc of rabbit immune serum after the mixture had stood at room temperature 2 hours. The antiserum was a pooled mixture of the serums of 3 rabbits immunized as described above. At 11½ months, 2 of the 30 mice had developed tumors. The 30 mice in Group 3 were injected with similar amounts of centrifugate suspended in 0.5 cc of normal rabbit serum. Of these, 14 had developed breast cancer at 11½ months. All inoculated mice in these groups were of the

* This investigation has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Cancer Research Fund of the Graduate School of the University of Minnesota, and the Citizens Aid Society of Minneapolis.

1 Bittner, J. J., *Science*, 1936, **84**, 162.

2 Bittner, J. J., *Science*, 1942, **95**, 462.

3 Bittner, J. J., *Cancer Research*, 1942, **2**, 710.

4 Visscher, M. B., Green, R. G., Bittner, J. J., Ball, Z. B., and Siedentopf, H. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 94.

5 Andervont, H. B., and Bryan, W. R., *J. Nat. Cancer Inst.*, 1944, **5**, 143.

6 Bittner, J. J., Evans, C. A., and Green, R. G., *Science*, 1945, **101**, 95.

TABLE I.
Neutralization of Mouse Milk Agent with Rat Immune Serum.

No. mice	Tumor centrifugate mixed with	Tumors produced in mo.					
		7½	8	9	10	11½	13½
24	Spontaneous tumor antiserum	0	0	0	0	0	0
24	Transplant tumor antiserum	0	0	0	0	0	0
24	Normal tissue antiserum	1	3	7	10	12	21
24	Normal rat serum	2	4	8	9	12	13
24	0.9% saline sol.	6	10	15	15	17	18

Dosage: Centrifugate equivalent of 0.2 g tumor tissue mixed with 0.18 cc antiserum in 0.9% saline to make total volume of 0.23 cc. The mixtures were held at room temperature for 2 hours before injection.

Note: All injected mice subjected to forced breeding. Mice 1-mo-old ZBC stock without milk influence.

ZBC strain, mice which were susceptible to, but did not carry, the tumor agent. After inoculation, the mice were maintained as forced breeders in order to stimulate maximum cancer development. These results confirm the findings of Andervont and Bryan.[5]

A second experiment on the antigenic properties of the milk agent has yielded similar results 14 months after its inception. The antiserum used to neutralize the milk agent in the latter experiment was prepared from laboratory rats as described above. Three antiserums were prepared: one from spontaneous tumor tissue; one from transplanted tumor tissue; and one from normal mouse tissue consisting of heart, liver, spleen and kidney. Each antiserum, as finally prepared, was a pooled serum from 3 immunized rats. In addition, a pool of normal rat serum was prepared by combining the serums from 3 uninjected rats. ZBC mice not carrying the milk influence were injected at 1 month of age in groups of 24 with centrifugates of tumor tissue mixed and incubated for 2 hours with the various antiserums. One control group received infective centrifugate suspended in 0.9% saline solution. The appearance of tumors at different intervals and the number ultimately produced are shown in Table I.

Of the 2 groups of mice totaling 48 that received spontaneous tumor antiserum and transplanted tumor antiserum, none has developed cancer at 13½ months. Twenty-one mice of the 24 receiving normal tissue antiserum have developed cancer, and 13 of the 24 that received the normal rat serum have developed cancer. Of the 24 control mice, which received the infective centrifugate without the addition of serum, 18 have become cancerous. These additional data obtained with antiserums made from the closely related rat would seem to establish rather definitely that the infective agent in mouse mammary carcinoma is antigenic.

Demonstration that the milk influence of Bittner is an antigen becomes of greatest importance in its characterization. First termed an *influence* and later an *agent* or an *incitor*, its antigenic capacity, together with other attributes, would seem to put it in the class of filterable viruses. Although it is difficult to describe a virus accurately, its most definitive characters are: (1) reproduction only in association with living host cells, (2) small size, usually ultramicroscopic, and (3) antigenic capacity. All 3 basic criteria are now known to be met by the milk agent. Inasmuch as many tumors in animals are known to be virus infections, it would seem reasonable from the evidence at hand to consider the mammary cancer of mice an infectious process and its cause a filterable virus.

The antiserum produced in rats by the injection of normal mouse tissues failed to produce any adverse effect upon cancer-formation by the agent. Of the 3 groups of controls, those receiving the normal tissue antiserum have presented the largest number of cancerous mice. This indicates that the agent is antigenically different from mouse tissue and is not derived therefrom.

Normal rabbit serum and normal rat serum both seem to contain something that is antagonistic to the cancer agent. Both normal

serums effected a retarding of tumor development to a rate below that observed with the agent in saline solution, and ultimately a smaller number of tumors was produced.

Conclusions. The milk factor of mammary mouse carcinoma is highly antigenic and stimulates the formation of antibodies in both rabbits and rats. The mouse cancer antiserum neutralizes and renders inactive mouse cancer centrifugates. Equally effective antiserums are prepared from spontaneous and from transplant tumors. Normal rabbit and rat serums have a slight effect in inactivating the mouse cancer virus. The finding that antiserum against normal mouse tissue does not have any neutralizing or adverse effect on the milk agent indicates that the agent is a virus of exogenous origin.

15245

Cell-Blockade in Canine Distemper.*

ROBERT G. GREEN AND CYRIL S. STULBERG.

From the Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis.

The interference phenomenon of animal viruses was first observed by Hoskins[1] and by Magrassi[2] independently. Magrassi observed an interference between nonencephalitic and encephalitic herpes viruses in rabbits, and Hoskins reported an interference between neurotropic and pantropic yellow fever viruses in monkeys. Subsequent investigations by Findlay and MacCallum,[3] Dalldorf,[4] Jungeblut and Sanders,[5] Ziegler, Lavin and Horsfall,[6] the Henles,[7] and others have shown that the cell-blockade, or interference phenomenon, can occur in many animal viruses under experimental conditions.

We have conducted experimental studies of simultaneous infections of young foxes with virulent distemper virus and a distemper virus modified by ferret passage, and found that the phenomenon of interference, or cell-blockade, can be demonstrated for these viruses. Foxes inoculated *intranasally* with virulent distemper virus can be protected from fatal infection by subsequent inoculation of the ferret-passage distemper virus, which is of low virulence for foxes and dogs. In foxes injected *intramuscularly* with the virulent distemper virus, the course of the infection is also blocked by the introduction of the modified virus either 3 days before, or at the same time that, the virulent distemper virus is given. However, if the interval elapsing between the intramuscular injection of the virulent virus and the subsequent inoculation of distemperoid virus is as long as 3 days, there is very little interference with the course of the virulent infection.

Materials and Methods. The animals inoculated were red fox pups 10 to 16 weeks old. In any one experiment the foxes were very nearly the same age. The virulent distemper virus was of dog origin, and had been passed experimentally through foxes until it acquired an extremely high virulence for that animal. The ferret-passage virus was the distemperoid virus described by Green.[8]

* Aided by a grant from the Medical Research Fund of the Graduate School of the University of Minnesota and by experimental facilities and financial support furnished by Fromm Laboratories, Inc., Grafton, Wisc.

1 Hoskins, M., *Am. J. Trop. Med.*, 1935, **15**, 675.

2 Magrassi, F., *Z. f. Hyg. u. Infektionskr.*, 1935, **117**, 501, 573.

3 Findlay, G. M., and MacCallum, F. O., *J. Path. and Bact.*, 1937, **44**, 405.

4 Dalldorf, G., *J. Imm.*, 1939, **37**, 245.

5 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 127.

6 Ziegler, J. E., Jr., Lavin, G. I., and Horsfall, F. L., *J. Exp. Med.*, 1944, **79**, 379.

7 Henle, W., and Henle, G., *Am. J. Med. Sci.*, 1944, **207**, 705.

8 Green, R. G., *Am. J. Hyg.*, 1945, **41**, 7.