now in progress to determine whether other agents will serve as satisfactory adjuvants with Japanese encephalitis vaccine.

At the present time no information is available regarding the capacity of this vaccine either alone or with adjuvants to immunize man.

Summary. 1. Highly infectious suspensions of embryo tissue have been prepared from embryonated chick eggs inoculated with the Japanese B virus. Infective titers of $10^{-7.5}$ to $10^{-8.5}$ were regularly encountered under suitable conditions.

2. Formalinized vaccines made from 20% suspensions of infected chick embryo tissue are capable of immunizing mice against intraperitoneal infection with the virus of Japanese B encephalitis. Such vaccines are comparable in potency to the Japanese B encephalitis vaccines prepared from infected mouse brain and used by the U. S. Army.

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Cytotoxic Property of Mouse Cancer Antiserum.*

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In our investigations of the milk factor,[1] or the Bittner virus, associated with mammary carcinoma of mice, an antiserum prepared from rabbits has been tested for its toxicity for cancer cells. In his studies, Andervont[2] found that an antiserum prepared in rabbits by injecting as antigens the centrifugates of mouse tumor, neutralized the milk factor, while normal serums failed to do so. Green, Moosey and Bittner[3] likewise have shown that antiserums prepared from immunized rabbits, as well as from immunized rats, neutralized the agent. We have prepared additional control serums by immunizing rats and rabbits against normal mouse tissue centrifugates, and these have failed to neutralize the virus. This finding demonstrates that the milk factor is antigenically unlike mouse tissue and might more properly be considered a foreign virus. In the experiments here reported a rabbit antiserum prepared against the Bittner virus has been tested for its toxic properties for mammary cancer cells when mixed with them *in vitro*.

Three rabbits were immunized by 5 weekly injections of cancer centrifugate given intraperitoneally. A suspension of mouse cancer tissue was homogenized and subjected to fractional centrifugation. The first sediment, obtained by centrifugation for 1½ hours at 14,000 X gravity, was discarded. The supernatant suspension was again centrifuged 1 hour at 100,000 X gravity. This second sediment was collected, suspended in a 0.9% saline solution and injected in a dosage corresponding to 2 g of original tumor tissue. The rabbits were bled for serum 14 days after the fifth injection of centrifugate. An antiserum against normal lactating mouse mammary tissue was prepared in an identical manner by subjecting lactating breast tissue to the same processes. The normal breast tissue was obtained from mice susceptible to, but not carrying, the Bittner virus. An additional control serum was made from normal rabbit blood. Each of the 3 serums, as finally prepared, represented a pooled serum from 3 rabbits. The 3 serums were test-

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

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

3 Green, R. G., Moosey, M. M., and Bittner, J. J., *Cancer Research*, 1945, **5**, 588.

TABLE I.
Cytotoxic Property of Mouse Cancer Antiserum.
Rabbit antiserum—C3H tumor—ZBC mice without milk factor.

No. mice	Tumor cell suspension injected with	No. tumors in 10	15	40	60 days
10	Cancer antiserum	0	0	0	0
8*	Normal mammary antiserum	1	3	8	8
10	Normal rabbit serum	1	1	5	7
10	Saline sol.	10	10	10	10

* Two died from intercurrent infection.

ed for their comparative cytotoxicity.

To test the serums, a cancer-cell suspension for the production of transplant tumors was obtained as follows. A mass of cancer tissue was forced through a tissue press. The macerated cancer tissue was then suspended in saline solution and allowed to settle for 30 minutes. A heavy sediment of tissue masses settled out and the supernatant suspension of cancer cells and small cellular masses was removed for injection into susceptible mice. The concentrated cancer-cell suspension was then diluted 10 times. To 4 portions of the cell suspension were added, respectively, 5 volumes of cancer antiserum, normal tissue antiserum, normal rabbit serum, and 0.9% saline solution. All mixtures were treated in the same manner. They were held at room temperature 3 hours, and then in a refrigerator at 7° C for another 3-hour period. Each preparation was then injected in a dosage of 0.6 cc, intraperitoneally, into a separate group of 10 mice. The dose given each mouse represented 0.1 cc of a 1 to 10 suspension of cancer cells and 0.5 cc serum or 0.9% saline solution.

The formation of tumors at various intervals is shown in Table I.

It is evident from the data that the cancer antiserum completely inhibited growth of cancer cells. Both the normal rabbit serum and the normal mammary tissue antiserum effected a delay in the development of tumors. The normal rabbit serum appears to have inhibited slightly the growth of tumor cells, since only 7 mice of the 10 injected displayed tumors at the end of 60 days.

Tumors developed in all mice injected with tumor cells in saline solution much more rapidly than they developed in mice receiving cancer cells in normal rabbit serum or in normal mammary tissue antiserum. There appears, therefore, to be something in normal rabbit serum which interferes with the rapid growth of cancer cells. This effect of normal serum is not increased, but possibly decreased, by immunization with normal tissue centrifugates.

These results seem to demonstrate that an antiserum produced from mouse cancer-cell centrifugates is capable not only of neutralizing the virus but also of inhibiting the growth of cancer cells produced by the virus.

Conclusions. An immune serum prepared in rabbits against the mouse mammary carcinoma milk factor, or Bittner virus, when mixed with cancer cells for a period of 6 hours completely inhibits their growth so that transplant tumors do not develop. Control tumors developed after similar treatment of tumor cells with serum from normal rabbits and with serum from rabbits immunized with normal lactating mouse breast tissue.