57 animals received parenteral injections of living vaccinia virus. The 43 controls received no treatment.

Of the controls all but one (98%) were dead within 4 weeks after the leukemic transplant. The lives of at least 10 (18%) of the mice receiving virus seemed to be significantly prolonged, and 6 of these have survived more than 5 months.

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Search for the Brown-Pearce Tumor XYZ Factor in Rabbit Spleen.

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Some years ago it was reported that frozen and preserved Brown-Pearce tumor tissue contained a factor called, for lack of a better term, the XYZ factor, which, when injected 2 weeks before the Brown-Pearce tumor transplantation resulted in increased incidence of, and larger metastases, and a shorter survival period after transplantation.[1-4] The factor was different from the Duran-Reynals spreading factor (hyaluronidase),[5-8] nor was the factor present in normal rabbit testicle,[5,6] in the tissue of an adenocarcinoma of the rabbit uterus (Greene),[9] in Bashford Carcinoma 63,[10] nor in Sarcoma 180 of the mouse.[10] A review of the literature revealed that Haaland[11] and Leitch[12] had described a similar factor for Bashford Carcinoma 63 and it was possible to confirm their work.[13] The Bashford Carcinoma 63 XYZ factor seemed to be specific in that the growth of this tumor was not affected by similarly prepared material from mouse Carcinoma 48, mouse Sarcoma 37, nor by the XYZ factor from the Brown-Pearce tumor.[14,15] Much has been written concerning the presence of inhibiting and enhancing materials said to be present in the spleen.[16] In resuming work on the Brown-Pearce XYZ factor after a lapse of some 10 years, preliminary experiments were performed in an attempt to recover the factor from the spleens of normal rabbits and of rabbits 2 weeks after the injection of the factor.

Materials and methods. In the 2 experiments 52 rabbits were employed. Each rabbit was about 3 months of age, except for

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TABLE I.
Course of Brown-Pearce Tumor in Rabbits Transplanted Subcutaneously.

	Rabbits, No.	Tumor takes	Tumor per animal inoculated: Met. foci, No.	Vol. met., cc	Total tumor, cc	Per animal with tumor: Met. foci, No.
Spleen (XYZ)	12	3 (25%)	0.3	2.1	11.7	1.3
Spleen (normal)	12	4 (33%)	1.8	10.7	18.5	5.5
Controls	13	3 (23%)	1.7	19.3	33.4	7.3
XYZ (BP tumor)	3	2 (67%)	10.7	45.0	112.7	16.0
8 Prior Experiments with the Subcutaneous Inoculation of BP Tumor.						
Controls	52	16 (31%)	2.7	12.8	21.6	4.4
XYZ (BP tumor)	58	44 (76%)	7.7	31.1	46.7	10.2

7 adult males used in obtaining the transfer material and in the preparation of the spleens. The young animals were divided into matched groups of the same breed and all were obtained from the same local breeder.

The spleens were prepared as follows: Two normal rabbits were killed by air injection and the spleen removed aseptically, placed in a sterile tube and stored in the deep freeze chamber at 0°F. The preserved spleen after 14 days in the first and 68 days in the second experiment, was removed from the deep freeze, emulsified in normal saline (1-3 dilution) and 0.3 cc of the emulsion injected subcutaneously into 5 rabbits in the first experiment and into 8 rabbits in the second experiment.

For XYZ material a rabbit bearing the Brown-Pearce tumor was killed by air injection 13 days after intratesticular transplantation. The large testicular tumor was removed aseptically, 1.5 cc used immediately for transfer (A) and 6 cc placed into sterile tubes without glycerin or other preservative and stored at 0°F (B). The transfer tissue (A) was emulsified in 4 cc of normal saline and 0.2 cc of the emulsion injected subcutaneously or intratesticularly into 15 rabbits. The transplantation was successful indicating that the tumor used was viable. One portion of the frozen tumor tissue (B) was removed 7 days later, 3 cc emulsified in 10 cc of normal saline and 0.33 cc of the emulsion was injected subcutaneously into the back of 2 rabbits which were killed by air injection 16 days later, the spleens removed aseptically, placed in sterile tubes, and stored in the deep freeze chamber at 0°F (C). The spleens thus obtained (C) were removed from the deep freeze after 14 days in the first and 68 days in the second experiment, emulsified in normal saline (1-3 dilution) and 0.3 cc of the emulsion injected subcutaneously into 5 rabbits in the first and into 8 rabbits in the second experiment. In the first experiment a second batch of frozen Brown-Pearce tumor tissue (XYZ material, marked B) was removed after 37 days in the deep freeze, emulsified in normal saline (1-3 dilution) and 0.3 cc injected subcutaneously into 5 rabbits. The material used was sterile when cultured in thioglycollate medium, and microscopic sections of biopsies of the frozen and fresh spleen and tumor were made and examined at each stage of the experiment.

In the first experiment, 8 days after injection of the spleen emulsions and the Brown-Pearce XYZ material, each animal in the 3 groups (with spleen from XYZ animal, with spleen from normal animal, with Brown-Pearce XYZ material; one animal had died meanwhile of intercurrent disease) together with the previously matched but uninoculated controls were injected subcutaneously into the back in numerical order with an emulsion of Brown-Pearce tumor tissue in normal saline.

In the second experiment there were 3 groups of 8 animals in each. The first group was injected with an emulsion of spleen preserved 68 days at 0°F from the animal which had received Brown-Pearce XYZ (as described above); the second group was injected with an emulsion of spleen taken from a

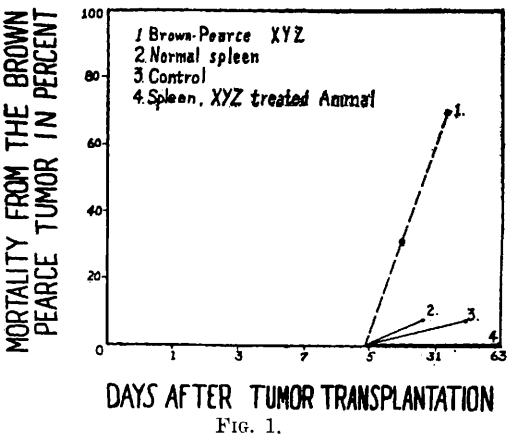

FIG. 1.

normal rabbit and preserved for 68 days at 0°F; the third group were control animals. Twenty days after the injection of the splenic emulsions into the 2 experimental groups the animals of these 2 and those of the control group (24 animals in all) were injected subcutaneously with 0.2 cc of an emulsion (1-3 dilution) of Brown-Pearce tumor tissue in numerical order. In addition a stock animal (NZW) was injected intratesticularly with 0.2 cc of the emulsion. Biopsy showed that this tumor was nearly all living, the animal having been inoculated intratesticularly.

Results. The results are summarized in Fig. 1 and in Table I. One animal from the controls and one from the spleen XYZ series, another from the Brown-Pearce XYZ group and one from the normal spleen group died of intercurrent disease between 2 and 17 days after tumor transplantation. These were eliminated from the results because mortality from the tumor does not occur until 20 days or longer after inoculation.

There was no statistically significant difference in the success of transplantation, in the mortality, in the number of metastatic foci, nor in their volume, nor in the total tumor primary and metastatic per animal inoculated between the 3 groups. There was no evidence that the emulsions of the splenic tissue had in any way significantly affected the course of the Brown-Pearce tumor.

The Brown-Pearce XYZ group containing only 3 animals could not be analyzed statistically but it was consistent with previous experience on the intracutaneous inoculation of the Brown-Pearce tumor (Table I). In 8 prior experiments at the Rockefeller Institute,[3] at the Louisiana State University[10] and at the Baptist Hospitals[10] there were 52 controls and 58 B.P. XYZ animals inoculated with Brown-Pearce tumor subcutaneously into young rabbits. The incidence of tumor proven by autopsy was among all animals inoculated, 31% in the controls and 76% among the XYZ treated animals, metastatic foci 2.7 and 7.7, volumes of metastatic tumor 12.8 and 31.1 cc, total tumor 21.6 and 46.7 cc; metastatic foci among animals with tumor was 4.4 in the controls and 10.2 in the XYZ animals. The results in the XYZ group in the present experiments were in accord with this prior data.

Discussion. Stern and Willheim published a review of the voluminous literature on the effect of spleen extracts on transplanted tumors.[16] Much of the work reviewed was done without controls or without an adequate number of animals for statistical analysis. Woglom[17] found that fresh splenic implants increased resistance to tumor transplantation but that crushed, frozen or thawed mouse spleen had no effect upon resistance to Bashford carcinoma 63 of the mouse. Fardon, Brotzge and Loeffler[18] obtained resistance to mouse mammary carcinoma 15091 A by means of extracts of fresh (unfrozen) mouse spleen and not with heterologous spleen. Since our work was done with frozen and not with fresh spleen it is in accord with previously published work and there is no reason to suspect that the Brown-Pearce XYZ factor may be found in the spleen of normal or of XYZ injected animals.

Summary. The Brown-Pearce tumor XYZ factor could not be prepared from the spleen of normal rabbits nor from the spleens of rabbits previously injected with the factor. There were two experiments comprising 52 rabbits and further controlled by prior experiments involving 110 young rabbits likewise injected intra- or subcutaneously with the Brown-Pearce tumor.

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