testicles."[10] Their observations were confirmed by Casey[8] and by Besredka, Mazat, Besnard, Gross, Bardach, Laval,[11] Saphir, Appel, Strauss,[12] Cheever, and Janeway.[13] Besredka and Gross reported that this immunity could be produced only by the tumor cells and that it could not be induced by the intracutaneous inoculation of normal tissues.[11] In Fig. 1 it will be seen that the New Zealand white rabbit shows no evidence of developing at about 40 days an immunity or resistance following intratesticular inoculation as is true for this same breed following subcutaneous inoculation or for common hybrids by intratesticular inoculation as reported by Brown and Pearce[9,10] and by Malluche.[14]

*Summary and conclusions.* Standard values were compiled for the Brown-Pearce tumor in New Zealand white male rabbits, 42 being inoculated intratesticularly and 66 subcutaneously. The breed was highly resistant to subcutaneous inoculation, (mortality, 13.6%; metastatic foci, 3.2; metastatic tumor 13.1 cc per animal inoculated) and susceptible to intratesticular inoculation (mortality 62.7%, metastatic foci 11.4 and metastatic tumor 90.5 cc per animal inoculated). Between 40 and 60 days after intratesticular inoculation the mortality curve continued to rise at about the same rate as between 20 and 40 days. This was in contrast to the immune or resistance reaction which seemed to set in between 40 and 60 days following subcutaneous inoculation of this breed, or following intratesticular inoculation of most other breeds.

10 Pearce, Louise, and Brown, Wade H., *J. Exp. Med.*, 1923, **37**, 811.

11 Besredka, A., Mazat, L., and Besnard, P., *Comp. rend. Acad. d. sc.*, 1935, **201**, 170; Gross, L., *Am. J. Cancer*, 1937, **31**, 609; Besredka, A., and Bardack, M., *Comp. rend. Acad. d. sc.*, 1936, **203**, 2193; Besredka, A., *Cancer Bruxelles*, 1935, **12**, 115; Besredka, A., Mazat, L., Laval, P., and Besnard, P., *Ann. Inst. Pasteur*, 1936, **36**, 125; Besredka, A., and Gross, L., *Ann. Inst. Pasteur*, 1936, **57**, 342; 1938, **60**, 5, 465; 1939, **62**, 253.

12 Saphir, O., and Appel, M., *Am. J. Cancer*, 1940, **38**, 55; Saphir, O., Appel, M., and Strauss, A. A., *Cancer Research*, 1941, **1**, 545.

13 Cheever, F. S., and Janeway, C. A., *Cancer Research*, 1941, **1**, 23.

14 Malluche, H., *Beitr. Z. klin. Chir.*, 1938, **167**, 481.

## 16794

## Selective Blocking of Host Resistance to Malignant Neoplasm (Brown-Pearce Tumor in New Zealand White Rabbits).

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In the preceding paper[1] it was reported that the New Zealand White rabbit is highly susceptible following intratesticular and highly resistant following subcutaneous transplantation of the Brown-Pearce tumor. In the mortality curve an immune or resistance reaction could be detected at about 40-60 days following subcutaneous inoculation but little or none following intratesticular inoculation of the tumor. The present experiments were designed to test the extent to which the XYZ factor[2] might overcome this immune or resistance reaction of the New Zealand white to this tumor following subcutaneous inoculation.

* The work was aided by a grant from the Committee on Growth of the National Research Council, acting for the American Cancer Society.

1 Casey, Albert E., Meyers, L., and Drysdale, George R., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 576.

2 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816; *Am. J. Cancer*, 1934, **21**, 760; 1936, **26**, 276; 1939, **35**, 354.

TABLE I.
Preparation of and Injection Interval for XYZ Factor from Brown-Pearce Tumor (134 New Zealand Whites).

| Exp. | Preparation: Temp. | Preparation: Storage, days | Preparation: Covering | Injection XYZ: Site | Injection XYZ: Dosage, g | Injection XYZ: Interval, days | Animals inoculated Brown-Pearce tumor: Exp. | Animals inoculated Brown-Pearce tumor: Controls |
|---|---|---|---|---|---|---|---|---|
| 1. a. | 24°F | 15 | Paraffin | S.C. | 0.0001 | 19 | 4 S.C. | 4 S.C. |
| b. | 24 " | 15 | " | " | 0.01 | 19 | 5 " | 5 " |
| c. | 24 " | 15 | " | " | 1.0 | 19 | 7 " | 7 " |
| 2. a. | 34 " | 25 | Glycerin | " | 0.0001 | 16 | 2 " | 2 " |
| b. | 34 " | 25 | " | " | 0.01 | 16 | 3 " | 3 " |
| c. | 34 " | 25 | " | " | 1.0 | 16 | 3 " | 5 " |
| 3. | —22°C | *30?* | None | " | 0.02 | 15 | 9 " | 8 " |
| 4. | —22 " | *30?* | " | " | 0.02 | 16 | 5 " | 8 " |
| 5. a. | 0°F | 244 | Glycerin | I.T. | 0.1 | 11 | 3 " | 4 " |
| b. | 0 " | 244 | " | " | $(0.1)^2$ | 11 | 4 " | 4 " |
| 6. a. | 0 " | 296 | " | " | 0.1 | 11 | 4 " | 4 " |
| b | 0 " | 296 | " | " | $(0.1)^2$ | 11 | 4 " | 4 " |
| 7. | 0 " | 51 | None | S.C. | 0.1 | 21 | 1 " | 2 " |
| | | | | Subtotal (Subcutaneous) | | | 54 | 52 |
| 1. | 18 " | 14 | Paraffin | I.T. | 0.1 | 14 | 5 I.T. | 8 I.T. |
| 2. | 18 " | 10 | " | " | 0.1 | 10 | 6 " | 9 " |
| | | | | Subtotal (Intratesticular) | | | 11 | 17 |
| | | | | Total subcutaneous and intratesticular | | | 65 | 69 |

*Material and Methods.* There were 13 experiments involving 106 New Zealand white rabbits inoculated subcutaneously and several experiments involving 28 New Zealand white rabbits inoculated intratesticularly. In the former 52 controls and 54 experimental animals were inoculated subcutaneously with 0.2 to 0.3 cc of an emulsion of tumor tissue, and in the latter 17 controls (including 6 stock animals) and 11 experimental animals inoculated intratesticularly with a similar dosage.

The XYZ material consisted of aseptically removed Brown-Pearce tumor (from 7 different animals) covered with paraffin, or layered with 50% glycerine in normal saline, or even left in a sterile container uncovered. The tumor tissue was refrigerated at 24°-34° F for 15-25 days (always covered with paraffin or glycerin at these temperatures), or at 0° F or lower for periods up to 300 days (Table I). Immediately after removal from the ice chamber amounts of the refrigerated tumor tissue (in the equivalent of 0.0001 to 1.0 g) were emulsified in normal saline and given as a single injection (see exceptions below) subcutaneously or intratesticularly 11-21 days prior to subcutaneous or intratesticular transplantation of the Brown-Pearce tumor (Table I). One group (in Experiments 5 and 6) was injected twice with XYZ material after a week's interval. The variations in the dosage of the frozen material seemed not to be significant.[3] About half of the animals were obtained directly from the breeder and it was often possible to divide them so that one litter mate would be in the experimental and one in the control group. In any event the controls and experimental animals were matched according to breed and weight before beginning the experiments with equal numbers in each. About 10% of both experimental and control animals died from intercurrent disease, and were eliminated, thus accounting for the unequal groups (Table I). The ex-

[3] Casey, Albert E., *Cancer Research*, 1941, **1**, 134.

TABLE II.
Brown-Pearce Tumor in New Zealand White Rabbits as Influenced by the Prior Injection of the Brown-Pearce XYZ Factor (Subcutaneous Transplantation of Tumor).

| | Non-regressed tumor, necropsy Animals | % | Metastases Animals | % | Died from metastases Animals | % | Total animals |
|---|---|---|---|---|---|---|---|
| XYZ | 39 | (72) | 35 | (65) | 25 | (46) | 54 |
| Controls | 24 | (46) | 17 | (33) | 4 | ( 8) | 52 |
| Chi square | 9.7 | | 10.9 | | 19.9 | | |
| Prob. | 0.01 sig. | | 0.01 sig. | | 0.01 sig. | | |

| | Metastatic Foci (or Sites) per Animal. Inoculated Mean | Variance | With tumor Mean | Variance | With metastases Mean | Variance |
|---|---|---|---|---|---|---|
| XYZ | 8.0 | 1.6 | 11.1 | 2.3 | 12.4 | 2.4 |
| Controls | 2.6 | 0.6 | 5.6 | 2.4 | 7.9 | 3.7 |
| Diff. | 5.4 ± 1.5 | | 5.5 ± 2.2 | | 4.5 ± 2.5 | |
| Prob. | 0.01 sig. | | 0.01 sig. | | 0.07 | |

| | Volume of the Metastases per Animal. Inoculated Mean, cc | Variance | With tumor Mean, cc | Variance | With metastases Mean, cc | Variance |
|---|---|---|---|---|---|---|
| XYZ | 38.7 | 55.9 | 53.6 | 88.4 | 59.8 | 99.5 |
| Controls | 11.2 | 25.1 | 24.3 | 106.8 | 34.4 | 195.2 |
| Diff. | 27.5 ± 9.0 cc | | 29.3 ± 13.9 cc | | 25.4 ± 17.1 cc | |
| Prob. | 0.01 sig. | | 0.03 prob. sig. | | 0.15 | |

| | Volume of Primary and Metastatic Tumor per Animal. Inoculated Mean, cc | Variance | With tumor Mean, cc | Variance |
|---|---|---|---|---|
| XYZ | 60.4 | 135.9 | 83.6 | 214.4 |
| Controls | 17.8 | 53.7 | 38.5 | 223.3 |
| Diff. | 42.6 ± 13.8 cc | | 45.1 ± 20.9 cc | |
| Prob. | 0.01 sig. | | 0.03 prob. sig. | |

TABLE III.
Brown-Pearce Tumor in New Zealand White Rabbits as Influenced by the Prior Injection of the Brown-Pearce XYZ Factor (Intratesticular Transplantation of Tumor).

| | Non-regressed tumor, necropsy XYZ | Controls | Incidence of metastases XYZ | Controls | Died from metastases XYZ | Controls | Total inoculated XYZ | Controls |
|---|---|---|---|---|---|---|---|---|
| Animals | 11 | 16 | 11 | 16 | 11 | 11 | 11 | 17 |
| Percent | (100) | (94) | (100) | (94) | (100) | (65) | (100) | (100) |

| | Metastatic foci (sites) | | Volume of metastases, cc | | Total tumor primary and metastatic, cc | | | |
|---|---|---|---|---|---|---|---|---|
| Per animal inoculated | 23.5 | 13.7 | 170.3 | 53.8 | 189.4 | 73.3 | 11 | 17 |
| | diff. 9.8 ± 3.0 | | diff. 116.5 ± 22.1 | | diff. 116.1 ± 19.5 | | | |
| | t = 3.2 | | t = 5 | | t = 6.0 | | | |
| | P = 0.01—sig. | | P = 0.01—sig. | | P = 0.01—sig. | | | |
| Per animal with tumor | 23.5 | 14.6 | 170.3 | 57.3 | 189.4 | 77.9 | 11 | 16 |
| | diff. 8.9 ± 2.3 | | diff. 113.0 ± 23.6 | | diff. 111.5 ± 25.1 | | | |
| | t = 3.87 | | t = 4.8 | | t = 4.4 | | | |
| | P = 0.01—sig. | | P = 0.01—sig. | | P = 0.01—sig. | | | |

perimental and control animals of two groups were only 3 months of age at the time of inoculation; the others were 4-12 months of age. All except one were males (the exception was among the 54 XYZ animals inoculated subcutaneously).

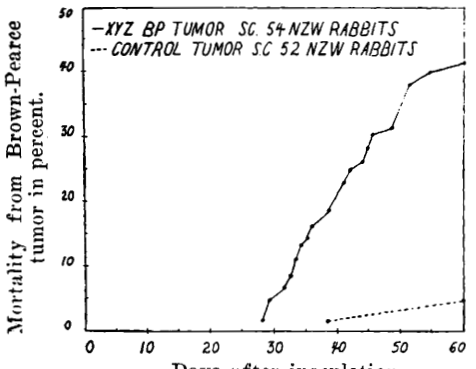
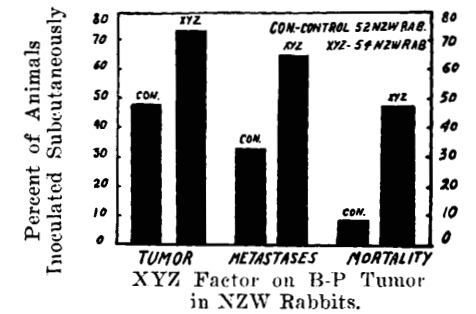
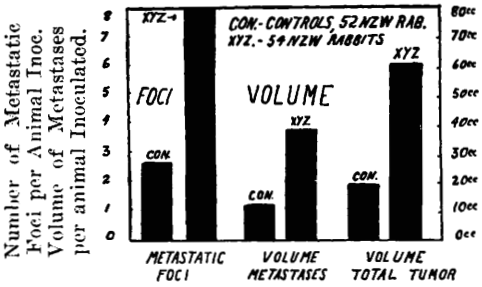



FIG. 1.




XYZ Factor on B-P Tumor in NZW Rabbits.




XYZ Factor on B-P Tumor in NZW Rabbits.

FIG. 2.

Both the control and experimental animals in each group were transplanted with the same emulsion and dosage of Brown-Pearce tumor. The animals were observed at weekly intervals and surviving animals sacrificed at 50 to 90 days. Careful necropsies according to a plan described in the preceding paper, were checked in each instance by the preparation of slides for microscopic examination. The size of the primary and metastatic tumor was measured at necropsy by water displacement.

*Results.* Among the 54 XYZ animals inoculated subcutaneously and the 11 XYZ animals inoculated intratesticularly with the Brown-Pearce tumor there was a greater incidence of and larger primary tumors, a greater incidence and volume of, and more numerous metastases and a greater mortality from the tumor in a shorter interval after inoculation than among their respective controls (52 controls were inoculated subcutaneously and 17 intratesticularly. Tables II and III, Fig. 1, 2, 3 and 4). Each of the differences was statistically significant for the groups inoculated subcutaneously and for those inoculated intratesticularly with two exceptions. The exceptions relate to the intratesticular inoculation in that 16 of the 17 controls (94%) had non-regressed primary tumor and metastases at necropsy, as compared with 11 (100%) among the XYZ injected animals. This afforded no opportunity to show a statistically significant XYZ effect.

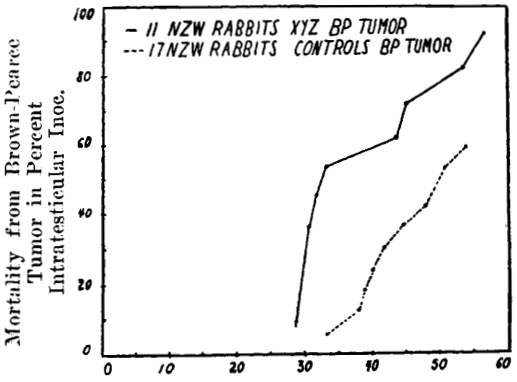



FIG. 3.

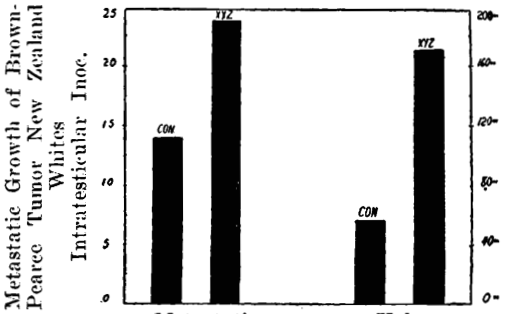



FIG. 4.

*Discussion.* The production of a state of

hypersusceptibility to tumor transplantation has been, during the past fifty years, fraught with great difficulties. The only recorded instance where published observations could be confirmed by other workers was the demonstration by Haaland,[4] by Leitch,[5] and by Casey[6] that such a state could be produced in the mouse using Bashford carcinoma 63. Sterile Bashford carcinoma 63 tissue was frozen and preserved anaerobically in the frozen state for 10-21 days. An emulsion of the frozen tumor tissue, no longer capable of growth, was injected subcutaneously into mice 10-21 days before subcutaneous transplantation of the same tumor. The animals so injected grew larger primary tumors than their controls not so injected. Some 200 control and 200 experimental animals were employed by the three authors. No metastases were observed and no further observations have been recorded.

Casey[2] was the first to demonstrate that similarly frozen and anaerobically preserved Brown-Pearce tumor tissue, no longer capable of growth, would when injected (1.0 to 0.0001 g) prior to tumor transplantation result not only in a greater incidence of and larger primary tumors but also in a greater incidence. number and volume of the metastases and in a greater mortality from the tumor in a shorter interval after transplantation than in control animals not so injected. The factor responsible was thermolabile[7] probably specific for the Brown-Pearce tumor (homologous),[6,8] filtrable through a Berkefeld "V" filter, differed from the Duran-Reynals spreading factor and effective even when injected into animals carrying the tumor. Because the phenomenon had no known counterpart in biology and the nature of the factor was unknown, the name

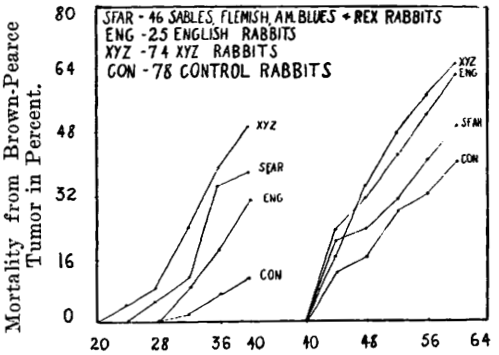


FIG. 5.

"XYZ" was given. Our total experience to date with the unaltered Brown-Pearce XYZ factor (including the present data) consists of 14 experiments involving 74 XYZ and 78 control animals in which the material was injected 10-21 days prior to intratesticular inoculation of this tumor, and 11 experiments involving 84 XYZ and 79 control animals inoculated subcutaneously. In each one of the 25 experiments during a 17 year period was the XYZ effect observed. The mortality curves for the groups inoculated intratesticularly were calculated first for the period up to 40 days and secondly for the period of 40-61 days after tumor transplantation and are presented (Fig. 5). The separation of mortality into the two periods was affected because the host factors influencing the first 40 days seem to differ from the host factors influencing the course of the tumor 40 to 60 days.[10,12] The animal breeds used varied widely but were always matched between controls and experimental animals; the consistency of the results bears this out.

Animals of average resistance to this neo-

4 Haaland, M., *Proc. Roy. Soc. London*, 1910, **82**, 293; *Lancet*, 1910, **1**, 787.

5 Letich, A., *Lancet*, 1910, **1**, 991.

6 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 674.

7 Casey, Albert E., and Moragues-Gonzales, Vincent, *Am. J. Cancer*, 1940, **38**, 59.

8 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1025; 1934, **31**, 663; 1939, **42**, 731.

9 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 111.

10 Malluche, H., *Beitr. Z. klin. Chir.*, 1938, **167**, 481.

11 Kidd, John G., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 292; *J. Exp. Med.*, 1946, **83**, 227.

12 Casey, Albert E., and Drysdale, George R., *Cancer Research*, 1947, **7**, 728.

13 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 666.

plasm (controls) became somewhat more susceptible than the animals of the most susceptible breeds (English, Flemish, Sable, American Blue and Rex) (Fig. 5). The factor has not been obtained (using identical techniques) from other neoplasms of the mouse or rabbit, nor from the rabbit spleen, skeletal muscle, or rabbit testis. The effect of the material has not in our hands resembled anaphylaxis or sensitization of the usual sort. Necroses do not appear at the site of repeated injections and animals injected weekly for almost a year were still highly susceptible and none died because of the injections. Histologically there seems less necrosis in the tumor tissue of XYZ animals and more cells seem alive. The prolonged storage in the frozen state seems to inactivate or mask such inhibitory factors as may be present in the fresh tumor tissue or the surrounding intercellular fluids.[12]

Kidd,[11] using our technique, froze Brown-Pearce tumor tissue and kept it frozen for 1-2 months at -22° C until ready for use. He then injected a saline emulsion of the frozen and non-viable tumor tissue, in the manner of our experiments, 7-20 days prior to intramuscular transplantation of the tumor. Among the 16 "blue cross" control rabbits was one animal at 30-35 days with progressively enlarging primary tumor (6%), none had metastases (0%) and none died from the tumor (0%). Among the 53 "blue cross" XYZ animals (called "immunized animals" by Kidd) there were 18 at 30-35 days with progressively enlarging primary tumors (34%), at least 10 had metastates (19%) and 10 died from metastases (19%). Evaluation of his work is difficult since his experiments were terminated at 30-35 instead of at the usual 60 days, control animals were few and autopsies were incomplete in that no enumerations of the incidence, number and volume of the metastases was recorded. Nevertheless his "immunized" (XYZ injected) animals had significantly more enlarging tumors ($X^2=4.7$, $P=0.035$), and among those with tumors a greater mortality from metastases ($X^2=5.6$, $n=1$, $P=0.02$) than their respective controls. The term, "immunized," used by Kidd with respect to the XYZ injected animals is, we believe, ill considered.

Several years ago Dr. Kidd kindly sent us 2 samples of frozen Brown-Pearce tumor tissue tested by him for antigen. This nonviable tumor tissue was injected by us into New Zealand white rabbits 15-16 days prior to subcutaneous transplantation of the tumor. The animals comprise Groups 3 and 4 in the present experiments (Table I). Among the 16 controls 3 had non-regressed tumors at necropsy (19%), 2 had metastases (13%), and none had died (0%); the metastatic foci averaged 1.3 and the total tumor 3.6 cc per animal inoculated. Among the 14 XYZ animals 8 had non-regressed tumor at necropsy (57%), 5 had metastases (36%), 4 died from metastases (29%); the metastatic foci averaged 5.8 and the total tumor 26.4 cc per animal inoculated. Combining Dr. Kidd's animals with enlarging primary tumors at 30-35 days with our animals having non-regressed primary tumor at 60 days there were 26 such animals among the 67 "immunized" (XYZ injected), as compared with 4 among the 32 controls ($X^2=7.1$, $n=1$, $P=0.01$– significant). Similarly the deaths from tumor were 14 among the 67 "immunized" (XYZ) and none among the 32 controls ($X^2=8.8$, $n=1$, $P=0.01$– significant).

Kidd like ourselves encountered the XYZ phenomenon while trying to immunize with frozen tumor tissue. An antibody response to the frozen tumor tissue was obtained by him in 22 of the 53 "blue cross" rabbits in the form of complement fixing antibodies with titers of 1-2 to 1-64; and each of the 13 rabbits which failed to grow a primary tumor was in this group. Such an antibody response was absent or insignificant among rabbits of other breeds and mixtures when tested by Cheever,[14] by Jacobs and Houghton[15] and by Kidd.[11] The antibody response seems largely limited to the "blue cross" rabbit, which is a Lilac cross developed by Dr. Wade H. Brown for stock use at the Rocke-

14 Cheever, F. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 517.

15 Jacobs, J. J., and Houghton, J. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 88.

feller Institute. The Lilac and its close relative, the Havana, have the highest natural resistance of any breeds tested against the Brown-Pearce tumor. No Havana rabbit has ever died from the tumor. Dmochowski[16] and Jacobs and Houghton[15] comment that it is "necessary to exclude the obvious likelihood that the antigenic substance is part of the cellular elements of the tumor" which result "in the formation of antibodies" when injected into a genetically different host. The antibodies described by Kidd seem to bear no relation to the growth of the Brown-Pearce tumor in animals successfully transplanted (in direct contrast to the XYZ effect). Among the 9 animals with antibodies and successfully transplanted 5 had enlarging primary tumors at the termination of the experiments (55%) and there were 2 deaths from metastases (22%), as compared with 13 enlarging primary tumors (42%) and 8 deaths from metastases (26%) among the 31 animals without antibodies and successfully transplanted. The differences were not significant.

Known mammalian viruses[17] are thermolabile (55-60°C) yet Kidd has applied the term "virus" to the thermostable (65°C) antigen in the cells of the Brown-Pearce tumor causing antibody response in the Lilac cross or Blue cross rabbit. He disregards the specific filtrable thermolabile (56°C) XYZ factor, uniformly present in the frozen Brown-Pearce tumor tissue, and capable of abrogating or completely blocking host resistance to this neoplasm. Its effect is not limited to certain strains of rabbits. The only biologically comparable filtrable thermolabile factor in mammalian tumors is the milk factor, discovered more recently by Bittner.[18] This agent is also specific, thermolabile (56°C), filtrable, and also does not immunize but renders even resistant strains of mice highly susceptible to the development of a specific neoplasm. It also causes no necrosis, or obvious reaction in the host.

*Summary and Conclusions.* 1. Of 134 New Zealand White rabbits in 15 experiments 65 were given an injection of 1.0 to 0.0001 g of Brown-Pearce tumor tissue (no longer viable) which had been kept frozen anaerobically for 10-296 days. Viable Brown-Pearce tumor tussue was transplanted 10-21 days later into the testes of 54 and beneath the skin in 11 of the 65 animals; also inoculated at the same time were the 69 controls, 52 subcutaneously and 17 intratesticularly.

2. The experimental animals had a significantly greater incidence of and larger primary tumors, a greater incidence and volume of, and more numerous metastases and a greater mortality from the tumor in a shorter interval after inoculation than their respective controls, by both the intratesticular and subcutaneous routes.

3. This effect (XYZ factor) was not confined to the New Zealand White breed as common rabbit hybrids of average resistance to the Brown-Pearce tumor could be rendered more susceptible than even the most susceptible breeds such as the English, Sable, Flemish and Rex. Even the relatively resistant blue-cross or Lilac cross rabbit became more susceptible upon injection of the frozen tumor tissue.

4. The mechanism of the XYZ phenomenon seems to be influenced by the inactivation or masking by prolonged freezing of inhibitory factors present in the fresh tumor. The frozen material seems to act by blocking host resistance to a specific tumor.

16 Domochowski, L., *Compt. rendue de la Soc. de Biol.*, 1938, 349.

17 Seiffert, Gustav, Virus Diseases in Man, Animal and Plant, Philosophical Libr., New York, 1944, 49.

18 Bittner, J. J., *Science*, 1936, **84**, 162; Andervont, H. B., Mammary Tumors in Mice, A.A.A.S., Washington, 1945, 123.