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Influence of Vaccine Virus on a Transmissible Leukemia of Mice.*

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The relation of extraneous viruses to neoplasms has been the subject of comparatively few investigations, scattered over the past 25 years. Levaditi and his associates[1,2] found that certain pox viruses proliferate in epitheliomas of rodents and sometimes cause necrosis and impairment of growth of the tumors. Rivers and Pearce[3,4] studied the influence of Virus III on a rabbit carcinoma, but could find no appreciable alteration of the degree of malignancy.

More recently, it has been demonstrated that vaccine virus will proliferate in mouse sarcoma 180, and furthermore, that transplants of fragments of infected tumor grow much more slowly and less successfully than controls.[5] The explanation was offered that this effect was produced by a direct action of the virus on the neoplastic cells, perhaps by a kind of competitive biochemical antagonism. The study has now been extended to leukemias of mice. These neoplasms provide an exceptionally satisfactory test material, both in their close resemblance to the corresponding human disease and in their readily predictable and high grade of malignancy.

Mice of a closely inbred line of the Ak strain and a leukemia designated 9417 have been employed. This tumor is lymphoid in type and at post-mortem the dominant lesions are usually found to be in liver, spleen, and mesenteric nodes, all of which may be greatly enlarged.

The strain of vaccine virus used was adapted to mouse brain more than a year ago, and has been carried along since by frequent intracerebral passage.[5] It is lethal when inoculated directly into the brain, but moderate doses may be given subcutaneously or intravenously without the development of any gross lesions or impairment of health. The virus was given as a suspension of infected mouse brain in 5 parts of plain broth containing 1,000 units per cc of both penicillin and streptomycin and 0.5 mgm heparin.

Leukemia 9417 is readily transmitted within this strain of mice, becoming established in, and killing approximately 100% of the hosts within a few weeks.[6] For transmission, the enlarged spleen of an animal dying of the disease was macerated in 10 cc of normal saline, and 0.1 cc was injected intraperitoneally.

In a first experiment leukemic cells were implanted in 50 young mice. Of these, 23 were kept as controls and received no further treatment. The remaining 27 were given vaccinia virus parenterally after periods of time sufficiently long to allow no reasonable doubt that the leukemia had meanwhile become well-established. One group of 17 received on the seventh day after the leukemic transplant 0.2 cc of vaccine virus suspension intravenously. A second group of 10 mice were given, on the fifteenth day, 0.2 cc of virus suspension intravenously and at the same time 0.5 cc intraperitoneally. The rates at which the control and the treated

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1 Levaditi, C., and Nicolau, S., *Compt. rend. Soc. de biol.*, 1922, **87**, 498.

2 Levaditi, C., and Nicolau, S., *Compt. rend Acad. de Sc.*, 1922, **174**, 1649.

3 Pearce, L., and Rivers, T. M., *J. Exp. Med.*, 1927, **46**, 81.

4 Rivers, T. M., and Pearce, L., *J. Exp. Med.*, 1925, **42**, 523.

5 Turner, J. C., and Mulliken, B., *Cancer Research*, 1947, **7**, 774.

6 Burchenal, J. H., Lester, R. A., Riley, J. B., and Rhoads, C. P., *Cancer*, 1948, **1**, 399.

TABLE I.
Number of Mice Surviving After Inoculation of Leukemic Cells.

Day:	13	16	19	22	25	54
Controls	23/23	3/23	0	—	—	—
Treated with virus on Day 7	17/17	7/17	6/17	4/17	2/17	2/17
Treated with virus on Day 15	10/10	7/10	6/10	4/10	2/10	2/10

TABLE II.
Number of Mice Surviving After Inoculation of Leukemic Cells.

Day:	13	16	19	22	25	28	35
Controls	19/20	16/20	9/20	4/20	2/20	1/20	1/20
Treated with virus on Day 7	13/15	7/15	5/15	4/15	4/15	4/15	4/15
Treated with virus on Day 12	15/15	12/15	7/15	4/15	2/15	2/15	2/15

animals died may be seen in Table I.

It may be noted that all of the control mice were dead on the 19th day after inoculation of leukemic cells. In contrast, deaths occurred less rapidly in both groups receiving virus, and no less than 4 animals (15%) were still alive at least until the 54th day after transplantation of leukemia. Two have survived 194 days.

The experiment was repeated with the modification that the group of animals treated late in the disease received the injection of virus on the 12th day instead of on the 15th day. There were 20 control mice and 15 mice in each of the groups receiving virus. Table II records the findings.

It is apparent that the leukemia is now somewhat less acute than before, yet among the controls the mortality at the end of the 4th week is nearly 100%. A difference between the groups is first evident at that time when, with all but one of the control animals dead there are still 6 (20%) of the 30 treated mice alive. Furthermore, 4 of these have now survived at least until the 170th day after transplantation of the leukemia.

All of the mice that died, both treated and untreated, showed gross evidence of leukemia, with enlargement of liver, spleen, or lymph nodes. Portions of the organs removed from animals that had received virus were examined for vaccinia by intradermal injection of rabbits and by inoculation of chorioallantoic membranes. Virus was demonstrable in about 25%. Whether this represents a fair estimate of the presence of virus is not clear, since it may well be that antibody was circulating in sufficient concentration to interfere with the tests.

It would appear that the introduction of vaccinia virus by parenteral injection in the course of mouse leukemia may result in the prolongation of life of a significant number of hosts. Whether the animals still alive will ultimately die of leukemia remains to be seen. It could be supposed that they will, and that the delay in the time of their death is merely a consequence of undernutrition brought on by systemic vaccinial infection; but no evidence of appreciable complicating general injury to the hosts has yet been uncovered. The weights of all groups of animals were taken several times a week, and none failed to gain.

It is hardly possible to compare the prolongation of life of leukemic mice brought about by orthodox chemotherapeutic or physical agents with the effect observed as a result of the injection of virus. In one case it is customary to employ repeated doses of the material on trial and to begin very soon after the leukemia has been transplanted into the subject. Here, on the other hand, a dose of virus has been given only on a single day and that only relatively late in the course of the disease.

The mechanism of the observed effect is not clear. It is reasonable to suppose that a direct action of virus on neoplastic cells takes place, but the reaction may then be modified by the operation of immune bodies.

Summary. In 2 experiments, 100 mice of the inbred Ak strain were inoculated with leukemia 9417. One week or two weeks later

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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1 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816.

2 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 760.

3 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 776.

4 Casey, Albert E., *Cancer Research*, 1941, **1**, 134.

5 Casey, Albert E., *Arch. Path.*, 1937, **23**, 741.

6 Casey, Albert E., *Am. J. Cancer*, 1939, **35**, 354.

7 Coman, Dale R., McCutcheon, Morton, and Ziedman, Irving, *Cancer Research*, 1947, **7**, 383.

8 McCutcheon, Morton, and Coman, Dale R., *Cancer Research*, 1947, **7**, 379.

9 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 731.

10 Casey, Albert E., unpublished reports.

11 Haaland, M., *The Lancet*, 1910, **88**, 787.

12 Leitch, Archibald, *The Lancet*, 1910, **88**, 991.

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

14 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1025.

15 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 663.

16 Stern, K., and Willheim, R., *The Biochemistry of Malignant Tumors*, Reference Press, Brooklyn, N. Y., 1943, 714.