14519

Viability of a Transmissible Fowl Tumor (Olson) upon Storage at Low Temperatures.

B. R. BURMESTER, C. A. BRANDLY, AND C. O. PRICKETT. (Introduced by M. C. Winternitz.)

From the Regional Poultry Research Laboratory, Agricultural Research Administration, United States Department of Agriculture, East Lansing, Mich.

A fowl tumor under study at this Laboratory during the past year and one-half was obtained through the courtesy of Dr. Carl Olson, Jr. It was described[1] as a transmissible lymphoid tumor having cells with "relatively large vesicular nuclei and distinct nucleoli." That the tumor appears to be highly virulent is indicated by the high tumor incident when transplants to the pectoral muscle are made and by the rapid rate at which it can be serially transferred.[1,2] Its malignancy is indicated by the occurrence of early and extensive metastases.[1,2] Transmission by a cell-free inoculum has thus far not been demonstrated.

It is well known[3,4] that the agents producing neoplasms in the fowl will survive low temperatures for a considerable period, and that most[5,6,7] but not all mammalian neoplasms will survive freezing under certain conditions. The viability of this tumor[1] after storage has thus far not been reported. If inocula can be frozen and stored under certain conditions, the work of maintaining a constant and known supply for experimental use can be materially reduced. To examine this possibility we have tested several samples of inoculum from different transfers for the ability of the minced tumor to survive low temperatures.

Procedure. Tumors grown in the pectoral muscle of chickens maintained in serial transfer (7-day interval) were excised aseptically, minced with scissors or a tissue mincer and placed in pyrex glass tubes and Lusteroid* tubes. The former were sealed by means of a gas-oxygen flame, the latter with screw cap and adhesive tape. The samples were frozen by immersing the tubes in 95% ethyl alcohol and lowering the temperature between 0°C and −20°C at the rate of 1° per minute by the addition of small pieces of solid CO_2. The temperature was then reduced more rapidly to −60°C by the addition of larger pieces of solid CO_2. This method is similar to that described by Breedis[8] for the slow freezing of leukemic cells. After freezing, the tubes were transferred to a CO_2 icebox having a temperature range of −65°C at the top and −76°C at the bottom of the box. Tumor samples were thawed just prior to inoculation by placing the tubes under running tap water.

Tumor samples were tested by diluting minced[9] tumor with equal parts of Tyrode's solution, saline, or heavy mineral oil, after which 1 cc of the suspension was injected into the pectoral muscle.

Results and Discussion. Results of viability tests on tumors from 9 different donors are given in Table I. All of the tumor samples tested before freezing produced takes in all birds inoculated with the exception of Nos. IV and VI (25% and 80% takes respectively). These are typical of results of other inoculations of this tumor (unfrozen) at the time these samples were obtained. The tumor samples tested after freezing by the method outlined and inoculated immediately (without storage) gave no indication of a decrease in activity.

1 Olson, Carl, Jr., *Cancer Res.*, 1941, **1**, 384.

2 Unpublished data obtained at this Laboratory.

3 Rous, P., and Murphy, J. B., *J. Exp. Med.*, 1914, **19**, 52.

4 Furth, J., *J. Exp. Med.*, 1932, **55**, 495.

5 Snell, G. D., and Cloudman, A. M., *Cancer Res.*, 1943, **3**, 396.

6 Breedis, C., and Furth, J., *Science*, **88**, 531.

7 Mider, C. B., and Morton, J. J., *Am. J. Cancer*, 1939, **35**, 502.

* Obtained from Lusteroid Container Co., South Orange, N.J.

8 Breedis, C., *J. Exp. Med.*, 1942, **76**, 221.

9 Olson, Carl, Jr., *Am. J. Vet. Res.*, 1941, **2**, 295.

TABLE I.
Viability of Minced Tumor after Freezing and Storage at —65° to —76°C.

Donor	Transfer No.	Tumor incidence T/I*			Storage period (days)
		Fresh inoculum not frozen	Inoculum frozen only	Inoculum frozen and stored	
I	1	12/12	—	2/6	28
II	3	—	—	3/3	10
III	4	12/12	—	7/7	182
				7/7	391
IV	4	1/4	2/4	8/8	384
V	6	3/3	—	2/7	369
VI	10	4/5	—	7/7	328
VII	12	4/4	3/3	8/8	102
				2/7	307
VIII	18	3/3	—	7/8	245
IX	20	4/4	—	8/8	225

*T—No. of birds that developed a tumor at site of inoculation.
I—No. of birds inoculated.

Samples tested after freezing and storing for 10 to 391 days in a CO_2 icebox produced 100% takes in 7 of 11 tests. Two inoculations showed an apparent increase in activity; whereas, 4 tests showed a decrease after storage. One of these, sample VII, produced tumors in all of the 8 birds inoculated after 102 days of storage but only 2 of 7 birds developed tumors after 307 days of storage. However, there appeared to be no definite relation between the length of period stored and loss in activity. The minced tumors that showed a decrease in activity were among those stored in Lusteroid tubes, while those stored in pyrex glass produced takes in all birds inoculated with tumor stored up to 391 days. It may be possible that inadequate seals were obtained with screw cap and tape and thus sufficient CO_2 was allowed to enter some of the Lusteroid tubes and partially inactivate the minced tumor.

Tumors obtained from recipients of 4 of the storage tests described were transferred in one case in the fresh unfrozen state, and in 3 other cases after the tumors had been frozen and stored for 22 to 229 days.

Typical tumors developed in all of 34 birds inoculated. Thus, the storage of minced tumor tissue at low temperatures has no apparent effect upon its ability to produce typical tumors after one passage in birds or upon its viability during low temperature storage after one transfer in birds.

Conclusion. When the transmissible fowl tumor (Olson) was frozen slowly and stored at −65°C to −76°C for 10 to 391 days, no significant reduction or alteration in its capacity to produce typical tumors in the pectoral muscle was detected by methods employed.

14520

"Folic Acid" a Tumor Growth Inhibitor.

C. LEUCHTENBERGER, R. LEWISOHN, D. LASZLO, AND R. LEUCHTENBERGER.
From the Laboratories of Mount Sinai Hospital, New York City.

Studies on tumor growth inhibitors, carried out in this laboratory, suggested the possible importance of "folic acid" for malignant growth. Evidence has recently been presented that inositol, another member of the vitamin B-complex, inhibited tumor growth, whereas 8 other crystalline B-vitamins were ineffective.[1]

In the following we report experiments in which the action of a "folic acid concentrate"*