

THE CARCINOGENIC ACTIVITY OF THE MILK FACTOR AFTER STORAGE FOR 6 YEARS IN THE DRY STATE

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(Received November 15, 1957. Presented by Active Member of the AMN SSSR L. A. Zil'ber)

In previous papers on this problem [3, 4] it has been shown that the virus isolated from macerated tissue of a mammary gland tumor of mice together with the so-called nucleoprotein fraction (in short, the milk factor), when contained in an ampule sealed in vacuo at -70°C , does not lose its carcinogenic properties for 2 years if it is kept at $+4^{\circ}\text{C}$. A method was also described for isolating the virus from the nucleoprotein components and of the further preparation required for its long storage.

In the present paper we give the results of two more experiments, in which two samples containing the nucleoprotein virus were tested after a far longer storage period in the dry state.

EXPERIMENTAL METHOD

One of the two samples of virus tested was opened on December 13, 1955, i.e., 5 years and 10 months from its date of preparation. The contents of the ampule were added to physiological saline and injected into 27 young female mice soon after birth — aged from 1-10 days (see table). The contents of another similar ampule were injected into 30 newborn female mice on February 13, 1956, i.e., 6 years from the date of preparation. Each ampule contained 286 mg of nucleoproteins, corresponding to 1075 mg of the original tumor tissue [3]; consequently, in the first experiment each mouse received 10.6 mg of nucleoproteins from 40 mg of original tumor tissue and in the second experiment each mouse received 9.5 mg of nucleoproteins from 35.8 mg of original tumor tissue. Just as in the two previous experiments, the material was injected as a single dose subcutaneously in the right groin directly into the next to the last mammary gland.

In both experiments the recipient mice belonged to the CC-57 white strain of the 30th inbred generation. Females of this strain do not develop carcinoma of the mammary glands [1, 2, 5, 6] but are susceptible to milk factor, and in experiments to test the carcinogenic activity of this factor, carried out according to a standard method with extract from recent tumors, on the average 50-55% of them developed carcinoma of the mammary glands [6].

On reaching the adult state the injected mice were removed from their mothers and marked according to a definite system; males were placed with them and remained with the females for not less than one year. Throughout the whole experiment the mice were examined once a month and any dying from any cause were autopsied, and their internal organs examined in detail. Data on the duration of survival of the experimental females and of the tumors arising in them are presented in the third and fourth lines of the table (IIIa, IIIb). Since the results of the tests of both samples are essentially the same, in the fifth line of the table they are given jointly for the two experiments.

In the first and second lines of the table (I, II) for the sake of convenience the results of previously published experiments (I and II) carried out with specimens of virus stored for 1 and 2 years respectively are given. In the

Experiment No.	Length of storage of virus	No. of mice injected	Dose per mouse (in mg)	No. of injections	Age of recipient mice																			Tumors of the mammary glands produced	
					at expt. start	months																		No.	%
						days																			
						7	8	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24			
I	1 year (3)	20	53	14.3	1	5-9	17	17	16	16	11	11	10	8	5	4	3	2	2	2	9	8	7	10	62.5
II	2 years (4)	27	40	10.6	1	5-17	26	26	25	24	20	17	13	12	11	7	6	4	2	2	9	9	8	4	15.4
IIIa	5 years 10 months	27	40	10.6	1	3-10	15	15	15	15	15	14	14	14	14	13	12	10	9	9	7	7	6	1	—
IIIb	6 years	30	35.8	9.5	1	1-9	17	17	17	17	17	17	16	15	14	10	10	10	8	8	7	7	6	1	—
	Total in expts.						32	32	32	32	32	31	30	29	28	23	22	20	17	17	16	15	11	1	3.1
	IIIa and IIIb	57	—	—	—	—	17	7	10	13	14	13	8	1	7	24	23	4	2	1	3	—	—	—	—
	Control (6)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

sixth line are given the control results showing the absence of carcinoma of the mammary glands from mice of the CC-57 white strain between 1953 and 1957 [6].

The males are not included in the results of all these experiments since in these experimental conditions it would serve no useful purpose.

EXPERIMENTAL RESULTS

32 mice attained the "cancer" age for tumors of the mammary glands, i.e., 8 months. Later on the number of mice gradually fell. 22 females survived 18 months and a few lived for 2 years and more (see table).

Among this number of mice a mammary gland tumor developed in only one of the females in experiment IIIb, into which the test sample of virus was injected after storage for 6 years in the dry state. The tumor grew very slowly and at the end of the 3rd month of observation it was no bigger than a pea. Although testing of this tumor for the presence of milk factor is not yet completed, there are nevertheless grounds for believing that it developed under the influence of milk factor which still retained feeble activity in the sample of virus investigated. This is also shown indirectly by the fact that this particular tumor was one of a small number developing in CC-57 white mice in our other experiments devoted to testing the carcinogenic activity of tissue extracts from tumors of mice and man and they were completely absent from CC-57 white mice both in 1953-1957 and in the course of all the previous years [1, 2, 5, 6].

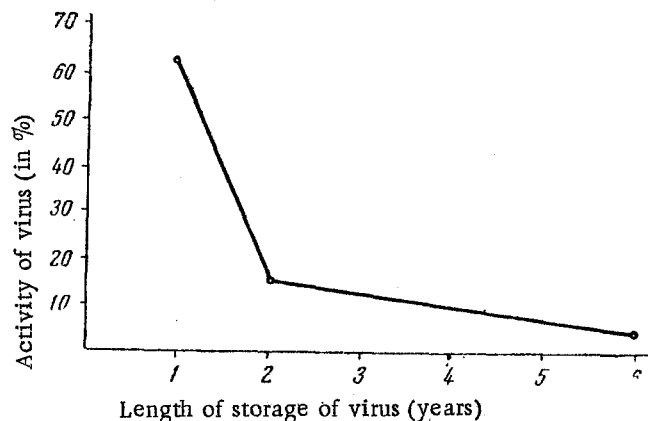
It should be mentioned that with increasing age of the tested virus and with a reduction in its biological activity tumors arose in older mice. This in experiment I (virus stored 1 year) the "cancer" age of the experimental mice varied from 9 to 19 months; in experiment II (virus stored 2 years) it varied from 12 to 16 months, and in experiment IIIb the only tumor of the mammary gland developed in a mouse 20 months after the injection of the virus.

In experiments I, II and III, besides carcinoma of the mammary gland, other tumors developed in other situations, in particular tumors of the lungs and of a few other organs. However the types and incidence of these tumors did not exceed the limits of their spontaneous development in CC-57 white mice.

The results of all three experiments thus show that the virus of carcinoma of the mammary glands under

The results of all three experiments thus show that the virus of carcinoma of the mammary glands under

these experimental conditions retains its carcinogenic activity for a comparatively long time. If the number of tumors in experiments I, II and III is related to the number of 8-month-old mice, i.e., at the "cancer" age for tumors in this situation, the percentage of mice with tumors in these experiments is 62.5, 15.4 and 3.1 respectively. The relation between the biological activity of the milk factor and the length of its storage period is shown graphically (see figure).



Graph of the relation between the carcinogenic activity of the virus (ordinate axis) and the length of its storage (abscissa) in the dry state.

The above values of the incidence of tumor formation are probably less than half their true value since it is known that tumors of the mammary glands arise on the average in only 50-55% of females of the CC-57 white strain of mice, even when the milk factor for the experiment is isolated from primary, i.e., spontaneous tumors or from recently excised transplanted tumors [6].

SUMMARY

The results of the conclusive experiment in the series concerning the blastomatogenic activity of the milk factor after a 6-year storage in the dry state are given. The experiments showed that the activity of the milk factor did not materially decrease after a one-year storage; however, it disappeared almost entirely when kept for six years the dry state.

LITERATURE CITED

- [1] V. I. Gel'shtein, F. D. Mashbits, *Biull. Eksptl. Biol. i Med.* 29, 5 (1950).
- [2] N. N. Medvedev, *Biull. Eksptl. Biol. i Med.* 24, 1 (1947).
- [3] N. N. Medvedev, *Med. Zhur. Ukrain.* 24, 3 (1954).
- [4] N. N. Medvedev, *Doklady Akad. Nauk SSSR*, 100, 5 (1955).
- [5] N. N. Medvedev, *Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel. Biol.* 62, 4 (1957).
- [6] N. N. Medvedev, *Doklady Akad. Nauk SSSR* 119, 2 (1958).*

* See English translation.