ONCOLOGY

THE SIGNIFICANCE OF HIGHER NERVOUS ACTIVITY IN THE APPEARANCE AND DEVELOPMENT OF TUMORS OF THE BREAST IN MICE

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There are several reports in the literature of studies of the relationship between growth of tumors in various animals and aspects of higher nervous activity.

These studies may be divided into 2 groups: the first group consists of investigations of the appearance of tumors in animals after trauma to the nervous system [3, 5, 6, 10]; the second group is made up of investigations in which tumor growth was studied in animals with normal higher nervous activity [4, 8].

In earlier investigations on mice of the C_{57}-black strain, injected subcutaneously with a carcinogenic compound, we showed that in animals with mobile nervous processes, induced tumors appeared less often than in animals with inert nervous processes. On the basis of these findings we suggested that there is an essential relationship between the degree of mobility of its nervous processes and the resistance of an animal to carcinogenic agents.

The present investigation, a continuation of these earlier studies, was carried out on female mice belonging to the high-cancer strain C_3HA, a considerable proportion of which develop "spontaneous" tumors of the breast [7], i. e., tumors due to estrogenic stimulation and to the so-called milk factor with a definite hereditary susceptibility.

EXPERIMENTAL METHOD

In the experiment 112 females of the C_3HA strain were used, in 50 of which (47.7%), aged between 7 and 12 months, tumors of the breast had developed, while 62 mice were without tumors. Of these 112 mice, 96 (aged from 7 to 12 months) were investigated by the alimentary conditioned reflex method [1, 2, 9]. Before investigation all the animals received a diet in excess of normal requirements. During the investigation (2-4 months) they were put on a normal diet (25 calories per mouse per day with protein accounting for 18% of the calorific value), while at the end of the investigation they were formed into groups of 4-6 animals and again put on the ordinary diet.

The physiological investigation of the mice consisted of production of 2 conditioned reflexes (positive – to a bell, and inhibitory – to a light) and the bilateral modification of these reflexes. The mobility of the nervous processes was measured by the rate of modification of the reflexes in these mice, the criteria for the assessment of this property of the nervous system being: 1) the onset of modification, i. e., that state of conditioned reflex activity of the animals in which for the first time the reflex in response to a reinforced stimulus was greater in magnitude than the reflex to the stimulus when not reinforced by food, 2) the end of modification, characterized

by prolonged (not less than 5 successive experiments) dominance of the positive over the inhibitory reflex, and 3) total modification, in which the magnitude of the positive and inhibitory conditioned reflexes regained its initial value as it was before modification.

After 5 months all the experimental animals were subjected to regular inspection – observations were made of the time of appearance of tumors of the breast, the situation and number of tumor nodules. After death, all the animals were examined and the tumors and the lungs, containing metastases, examined microscopically. Of the 50 mice which developed tumors, multiple nodules were observed in 23 (46%) and metastases of the tumor in the lungs were found in 14 mice (28%).

The majority of the tumors of the breast were adenocarcinomas and small-cell intraacinous carcinomas, or tumors of mixed structure. Solid carcinomas, cystadenocarcinomas and adeno-acanthomas were observed in isolated cases.

Some of the mice (numbering 49) from the age of one month and before the beginning of the investigation (7-12 months) were kept in individual cages measuring 10 × 10 × 20 cm, and the remaining 63 mice in groups of 8-26 mice in large cages. These two groups differed sharply in the degree of mobility of their nervous processes: in the animals kept separately the mobility of the nervous processes was found to be on the average twice as high as that of the mice living in groups (12 experiments were required to modify the reflexes in the "individual" mice and 20 experiments for the "group" mice). As regards the proportion which formed tumors, these groups showed no difference from each other: in the first group tumors appeared in 40.5% of cases and in the second group in 40%.

EXPERIMENTAL RESULTS

The difference in the keeping of the animals, and in particular the difference in the average degree of mobility of the nervous processes, makes it essential to make a separate analysis of the results obtained from the 2 groups of mice mentioned.

TABLE 1

Statistical Treatment of the Results of Mobility of Nervous Processes in Healthy and Tumor-Bearing Mice

Modification of conditioned reflexes	Mice without tumors			Mice with tumors		
	no. of specimens	$M_1 \pm m_1$	coeff. of variability, %	no. of specimens	$M_2 \pm m_2$	coeff. of variability, %
		Mice kept separately				
Onset of modification	25	4.56±0.42	45	17	5.00±0.6	49
End of modification	24	11.04±1.00	44	17	13.00±1.8	66
Total modification	24	14.45±1.40	47	17	17.82±3.0	70
		Mice kept together				
Onset of modification	32	4.19±0.47	63	22	6.1±0.9	70
End of modification	31	17.39±1.7	55	21	23.7±4.31	84
Total modification	31	22.7±2.2	54	21	28.5±4.5	68

In Table 1 are shown the results, treated statistically, of experiments on mice kept separately. It is clear from these results that by all criteria the mobility of the nervous processes among unaffected animals is higher than in the group of animals with tumors. This difference is slight, however, not statistically significant, and may only be detected as a tendency towards higher mobility of nervous processes in unaffected mice. Here also are indicated the results of experiments on mice kept together. As can be seen, in the mice with tumors the mobility of the nervous processes is considerably less than in the group of animals without tumors. These differences are more marked than in the animals kept separately, nevertheless they are not statistically significant.

On comparing these results the following problems naturally arise. Why are the differences in the degree of mobility of nervous processes between unaffected and affected mice more pronounced in the animals kept together in groups? Why, in spite of the quite large variations in the degree of mobility of nervous processes between affected and unaffected animals kept in groups, are these differences not statistically significant?

In regard to the first question it must be stated that in mice kept separately the mobility of nervous processes was found to be considerably higher than in animals kept together; this could to some extent equalize the differences in this particular property of the nervous system between affected and unaffected animals. The answer to the second question may be found in the degree of variability of the results obtained. While in the group of unaffected animals the coefficient of variability is 66%, in the group of mice which developed tumors it is 84% (for cases of total modification of reflexes). It may easily be calculated that if the mobility of nervous processes in the group of affected animals varied to the same extent as in the unaffected group, the differences in the degree of mobility between these two groups of mice would be statistically significant.

We shall consider this last question in somewhat greater detail.

In Table 1 results are shown which also illustrate the degree of variability of nervous processes in affected and unaffected animals. It can be seen from these figures that in every case the variability of this particular property is considerably higher in mice with tumors than in animals without. This fact can be accounted for in two ways. In the first place there is greater homogeneity in the properties of the nervous system, especially in the strength of the nervous processes, in mice with a higher mobility of the nervous system, i. e., in the mice unaffected by tumors, while among animals with tumors and with a more inert nervous system the higher nervous activity is of a type which shows extremes of strength. In the second place it has to be pointed out that the mice were studied at a period when some of them were beginning to develop malignant neoplasms. It might be suggested that the presence of tumors in certain animals was a factor which increased the variability of our findings, especially in view of similar results in the work of N. M. Turkevich. However, if all the animals with tumors are divided into 2 groups (those examined before tumors were discovered and those with tumors), and comparing their degree of variability of mobility of nervous processes, we observed significant differences, although in the opposite direction: in investigation of mice with tumors the coeffident of variability was 81%, and in investigation of mice before tumors were found – 91%. No difference was found in the degree of mobility of nervous processes between these groups of animals: in the first group the end of modification of the reflexes occurred on the average after 20.2± 3.9 experiments, and in the second group – after 18.2± 3.6 experiments. Thus the presence or absence of tumors at the time of the investigation of higher nervous activity is not the main cause of the lowered mobility and the increased variability of the results in the affected mice.

TABLE 2

Mobility of Nervous Processes in Various Groups of Mice Kept Separately and in Groups

Group of mice	No. of mice	Average duration of life in days	Mobility of nervous processes in mice kept separately	Mobility of nervous processes in mice kept together
With solitary tumor nodules without metastases	13	48	4.15-12.92-16.38	4.7-18.4-28.5
With multiple tumor nodules without metastases	12	44		
With solitary tumor nodules and metastases in the lungs	6	103	5.75-15.75-22.5	8.16-23.5-33.16
With multiple tumor nodules and metastases in the lungs	7	94		

From the results shown in Table 2 it can be seen that metastases from the breast tumors in the lungs were observed in those mice which survived long enough. Analysis of the degree of mobility of nervous processes in animals dying with and without metastases showed that the mobility of nervous processes was lower in the first than in the second, i. e., among mice with metastases there was a predominance of animals with more inert processes.

TABLE 3

Time of Appearance of Tumors and Length of Life of Affected Mice

Characteristics of the higher nervous activity of the animals	No. of specimens	Extreme variants	$M_1 + m_1$
Times of appearance of the primary tumor (in days)			
With mobile nervous processes	25	245-600	386.6 ± 15.0
With inert nervous processes	13	239-540	403 ± 30.7
Duration of life of animals with tumors (in days)			
With mobile nervous processes	25	0-123	47.0 ± 7.8
With inert nervous processes	13	0-164	57.0 ± 13.5

The facts which we obtained may be analyzed from the point of view of the time of onset of the primary tumors and the duration of life of the affected animals in relation to the degree of mobility of nervous processes. For this purpose all the affected mice were divided into 2 groups differing significantly in the degree of mobility of their nervous processes. The relevant data is shown in Table 3, from which it can be seen that tumors appeared somewhat sooner in mice with mobile nervous processes, and that these animals died after a shorter time than mice with an inert nervous system.

From a summing up of the material presented and a comparison with results obtained from earlier experiments with carcinogenic compounds of mice of the C_{57} strain the following conclusions can be drawn.

There is undoubtedly a relationship, which can be revealed by keeping and studying animals under certain conditions, between the degree of mobility of nervous processes as determined by the modification of two conditioned reflexes, and the resistance of the animals to factors causing malignant neoplasms. The same relationship also exists between metastasis formation and the degree of mobility of nervous processes, i. e., among mice with metastases from a tumor of the breast there is a predominance of mice with more inert nervous processes.

In spite of the fact that a smaller percentage of mice with higher mobility of nervous processes develop tumors, the tumors appear sooner, and the affected animals die rather more quickly than mice with a less mobile nervous system.

SUMMARY

The appearance and development of tumors of mammary glands in female mice of the highly-cancerous line C_3HA was studied. The peculiarities of the higher nervous activity were investigated with the aid of the motor food method (E. A. Ganike – 1935, 1939 and 1941 and V. K. Fedorov – 1951). The following was established.

1. There is a direct relationship between the degree of mobility of the nervous processes and the resistance of the organism to the "milk factor." This alone was detected only in definite conditions of keeping and investigation of animals.

2. The same relationship exists between the process of metastasis and the degree of mobility of the nervous processes, i. e., mice with inert nervous processes prevailed among the animals with metastases of the tumors.

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THE LEUKEMIA-PRODUCING ACTIVITY OF CELL-FREE FILTRATES OF HUMAN LEUKEMIC TISSUE

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The ability to pass through a semipermeable membrane is a characteristic property of the so-called filtrable viruses. By the use of special filters it is possible to isolate from a tissue extract a virus component, leaving undamaged cells and bacterial microorganisms on the filter [2]. If injection of the filtrate into an animal causes the appearance of a disease, this implies that the filtrate contains a virus.

The possibility of transmission of a tumor from an affected animal to a healthy one by means of a filtrate is the main factor in support of the virus etiology of these tumors. Tumors of this sort include carcinoma of the breast and leukemia in mice, papilloma and fibroma in rabbits, fibroma in deer, sarcoma and leukemia in fowl and so on.

There are no indications in the literature of the possibility of transmission of leukemia in man by means of cell-free filtrates. We have produced leukemia in mice by injecting them with centrifuged extracts of human leukemic tissue in which the presence of whole cells was practically impossible. However, the objection that isolated undamaged cells may still be left in the extract persists.

For this reason it was essential to attempt to produce leukemia in mice by means of cell-free filtrates of human leukemic tissue. In the present paper we give the results of experiments undertaken in this direction.

EXPERIMENTAL METHOD

We prepared cell-free filtrates from tissue from lymphatic glands, blood, brain and tumor-like leukemia infiltrations from 4 human patients suffering or dying from acute leukemias (hemocytoblastoses). In control experiments we used extracts of donated blood and of brain tissue from a patient dying from vascular disease. We had studied previously the biological activity of extracts of "normal" lymphatic glands: the extracts possessed no leukemia-producing activity [1]. In the same report the findings are given of the leukemia-producing activity of extracts of human sarcoma (as a control of tumor-like leukemia infiltration).

* In Russian.