COMPARISON OF THE ESTROUS CYCLES IN MICE OF HIGH AND LOW CANCER LINES

S. S. Laguchev

From the Laboratory of Histophysiology (Head — Candidate Biol. Sci. V. N. Dobrokhotov) of the Institute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received November 11, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Many experimental investigations and clinical observations have now shown that the development of the different forms of pathological proliferation of the epithelium of the mammary glands, the so-called mastopathies and carcinoma of the mammary glands into which they often are transformed, are associated with disturbance of the balance of the estrogenic hormones and the hormones of the adenohypophysis in the animal body. Since the anterior lobe of the hypophysis secretes several hormones which act on both the ovaries and the mammary glands, and the ovary, in turn, acts on the hypophysis with its own hormones, the hormonal interralationships between these organs are highly complex.

Oncologists and endrocrinologists have carried out numerous experimental investigations dealing with the hormonal balance both in mice often affected by carcinoma of the mammary glands and in mice resistant to carcinoma. Attempts to discover the nature of the hormonal disturbances in the so-called high cancer lines of mice, by comparison with the low cancer lines, have been far from successful.

One of the experimental methods enabling the hormonal function of the ovaries and the hypophysis to be assessed in the method of study of the estrous cycle. The character of the estrous cycle in individual animals reflects the inidividual balance between the ovarian and hypophyseal hormones and the rhythm of secretion of these hormones.

The duration of the various stages of the estrous cycle in mice of high and low cancer lines has been studied by many workers, but their findings are extremely contradictory.

Some workers [6, 7] assert that in mice of high cancer lines estrus is much more prolonged and diestrus much shorter than the corresponding stages of the estrous cycle in mice of low cancer lines. According to the findings of other workers [1, 3, 5], no differences can be found in the total duration of the estrous cycle and in the duration of its various stages in mice of low and high cancer lines.

The aim of our investigation was to compare the characteristics of the estrous cycles in mice of high and low cancer lines, and also to study the influence of the time of year on the course of the estrous cycles.

EXPERIMENTAL METHOD

Experiments were carried out on 40 female mice of known lines. As a high cancer line we selected line "A", and as low cancer line "C_{57}" (black). Vaginal smears were taken twice a day from the mice for a period of 3 weeks, stained by the Romanowsky — Giemsa method and examined under the microscope. Mice in which no cyclic changes were found in the vagina were excluded from the experiment. In order to detect seasonal changes in the estrous cycles, in a first series of experiments vaginal smears were investigated in February, and in a second series, in May.

Estrous Cycles in Mice of Lines "A" and "C_{57}"

Time of year	Line	Number of animals	Duration of experiment, in days	Mean duration in days, of				
				whole cycle	estrus	metestrus	diestrus	proestrus
February	«A»	10	21	5,19	2,28	0,80	1,68	0,43
February	«C_{57}»	11	22	5,38	1,48	0,54	2,95	0,42
May	«A»	9	22	5,80	2,09	0,76	2,36	0,60
May	«C_{57}»	10	22	5,80	1,52	0,71	2,97	0,58

EXPERIMENTAL RESULTS

The investigations showed that in healthy mice, kept a normal room temperature during the winter months, the estrous cycles did not cease. In each of the mice investigated, the estrous cycle was individual in its character. Furthermore, in the majority of mice, the duration of the individual stages of the estrous cycle changed in the course of the experiment. It was impossible to detect any relationship whatever between the character of the estrous cycle and the age of the mice.

The character of the winter and spring cycles in the mice is illustrated in the table.

A characteristic feature of some animals of line "A" was the sudden onset of an extremely prolonged estrus, which was followed by a relatively correct alternation of the stages in their usual length. The duration of estrus in these cases reached 4-8 days, but diestrus, on the other hand, was considerably shortened. In the majority of line "A" animals the length of diestrus in the individual cycles was shortened to 1-1½ days. In the animals of the low cancer line, in no case did estrus last longer than 2½ days, and in occasional cycles it was shortened to 24 hours. In many of the females of this line diestrus was extremely prolonged, lasting for 5-13 days. When the mean values were calculated (see table), the extremely individual differences which were characteristic of each line were to a large extent concealed.

Comparison of the estrous cycles of lines "A" and "C_{57}" in February shows that the mean duration of estrus was much longer in mice of line "A" than in those of line "C_{57}". So far as diestrus was concerned, here the opposite relationship was observed, i.e., in the mice of line "A" the duration of diestrus was much less than in the mice of line "C_{57}". Statistical analysis of the mean values of the duration of estrus and diestrus in the mice of these lines showed that the differences described above were significant (P=0.0001). The mean duration of the estrous cycles in these two lines was almost the same. It also follows from the figures in the table that metestrus in mice of line "C_{57}" in winter was much shorter than in mice of line "A".

From a comparison of the winter and spring cycles it can be seen that the duration of estrus and diestrus in the mice of line "C_{57}" hardly changed in the course of the year. The total length of the cycle increased slightly in May, and there was a corresponding increase in the duration of proestrus and especially of metestrus. The physiological meaning of these phenomena is probably an increase in the possibility of fertilization, since fertilization in mice occurs at the end of estrus and during metestrus [4].

In contrast to the "C_{57}" line, in the females of line "A" the spring pattern of the estrous cycle was changed (see table). In the first place attention was drawn to the considerable lengthening of diestrus, which did not, however, reach the mean length of diestrus in the mice of the low cancer line. Conversely there was a slight decrease in the duration of estrus. The whole cycle was lengthened, just as in the case of the mice of line "C_{57}". The duration of metestrus and proestrus was essentially unchanged.

Comparison of the cycles in the mice of lines "A" and "C_{57}" in May showed that the relationship which had been observed when these cycles were compared in February was maintained, although it was more feebly expressed. As before, estrus was more prolonged in the mice of line "A", and diestrus was shortened by comparison with the duration of these phases in the line "C_{57}" mice. Statistical analysis showed that the difference between these values was marginally significant (P = 0.03). Thus in spring, in the period of high sexual activity and intensive reproduction, the character of the sexual cycle of the mice of the high cancer line approximated to the character of the sexual cycle of normal mice.

Our findings were in agreement with the experimental results of Lacassagne [7] and Harde [6], and in disagreement with the conclusions of Bonser [5], L. P. Grigoliya [1] and N. I. Nuzhdin [3].

The cause of this discrepancy between the results is very difficult to account for. Estrous cycles in different high cancer lines and sublines of mice may possibly have essential differences and, although Lacassagne's, Harde's and our own results were obtained in mice of different lines, it is impossible to transfer them to all high cancer lines of mice. In this way it is easy to explain the difference in Bonser's results. It is far more difficult to find the reason for the discrpancy between our findings and the experimental results of N. I. Nuzhdin and his co-workers [3], since we obtained our families of high cancer line mice from the same nursery. Possibly in this case the character of the cycles was affected by differences in the conditions under which the mice were kept. In the Institute of Genetics of the AN SSSR the mice were kept in an artificially illuminated basement, and in our institute in a room in sunlight. Other differences were present in their conditions of keeping.

The method of conduct of the experiments differed considerably. We took smears from the mice not once but twice a day, and our experiments were much shorter in duration. It seems to us that our method was superior for when smears are taken only once a day it is easy to miss the short stages of the estrous cycle (proestrus and metestrus) and erroneously to combine two cycles into one, and in any case it is impossible to determine accurately the lengths of the individual stages of the cycle. The prolonged taking of vaginal smears often leads to distortion of the estrous cycle and even to the development of a state of pseudopregnacy.

We received the impression that the estrous cycles were extremely labile and easily altered their character under the influence of many exogenous and endogenous causes. In spite of the negative results obtained by several authors, the question of the peculiarities of the estrous cycles in mice of high cancer lines cannot, therefore, be regarded as answered. The conclusive solution of this problem is of great interest to experimental oncology. If the estrous cycle in high cancer lines of mice really presents special features, it will be possible to use these features to define the estrous cycle or, in other words, the hormonal type, characteristic of animals developing carcinoma of the mammary gland soonest. By using the type of estrous cycle as a prognostic sign, it will be possible not only to select animals for an experiment, but also to maintain high cancer lines of animals without having to cross only brothers and sisters, which give grossly weakened generations.

High cancer mice are known to be less fertile than ordinary mice of no pure line. It is very probable that this is determined by peculiarities of their hormonal balance. It is all the more strange that, in breeding high cancer lines of mice in many institutions, the ordinary method of selection is used: selection for breeding of the most fertile females. In this case, however, high fertility is a sign of a normal hormonal balance. Is this not the reason why many high cancer lines of mice gradually lose their specific characteristics, and why the incidence of development of tumors among them falls considerably [2].

SUMMARY

In February and May the author studied the duration of the estrous cycle stages in female mice of high "A" and low "C_{57}" cancer lines. It was established that the average duration of the estrus is much greater and of diestrus much shorter in the "A" than that of the corresponding stages in the "C_{57}" line.

The estrous cycles of the "C_{57}" line females do not undergo any significant seasonal changes while in the "A" line they approach the character of the normal mice spring cycles.

Desviations from the normal duration of the estrous cycle stages in the high cancer line mice denote a disturbed hypophyseal - ovarian hormonal balance.

LITERATURE CITED

[1] L. P. Grigoliya, Byull. Éksptl. Biol. i Med. 30, 11, 369-372 (1950).

[2] L. L. Malyugina and O. G. Prokof'eva, Voprosy Onkol. 2, 197-203 (1957).

[3] N. I. Nuzhdin, N. I. Shapiro and O. N. Petrova, Collected Papers on Radiobiology, 113-149 (Moscow, 1955) [In Russian].

[4] S. Bloch, Verhandl. der naturforsch. Ges. in Basel. Bd. 67, 2, 269-297 (1956).

[5] G. M. Bonser, J. Path. a. Bact. 41, 33-42 (1935).

[6] E. Harde, Compt. rend. Soc. biol. 116, 999-1001 (1934).

[7] A. Lacassagne, Compt. rend. Soc. biol. 115, 937-939 (1934).