EFFECT OF SEX OF THE HOST ON SEX CHROMATIN OF TUMORS

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After subcutaneous implantation of rat sarcoma SSK into rats (males and females) the percentage of cells containing sex chromatin was found to be 2-3 times lower in males than in females. The percentage of these cells was lower in male mice with a transplanted spontaneous adenocarcinoma of the mammary gland than in the female from which the tumor was obtained.

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Information on the effect of hormones on the number of Barr's particles in cell nuclei is conflicting. No final answer to the problem has yet been obtained, although its urgent clinical importance is obvious. The effectiveness of hormonal treatment of breast carcinoma in man is known to be directly dependent on the content of sex chromatin in the tumor cells [2]. However, it is not known how hormones influence the sex chromatin of tumor cells. It is interesting to note that, according to some data [16], the behavior of sex chromatin in the cells of hormonally dependent and independent tumors is identical, while according to other information [10, 14], cases of noncoincidence of the sex of the host and the presence or absence of sex chromatin in the tumor are more frequently observed when the tumors develop in organs under endocrine control (breast, prostate).

So far as normal cells are concerned, here also opinions differ. Some investigators [3, 4, 9] describe resistance of the sex chromatin to hormones and independence of its behavior of hormonal action. Attention has been drawn to the fact that Barr's particles appear in mammalian cells before the laying down of the primitive gonads. Onuma and Nishikawa [13] observed no changes whatever in the localization and morphology of Barr's particles after gonadectomy and injection of sex hormones into castrated male and female animals (bulls and cows, goats, etc.). In the light of Lyon's hypothesis [11, 12] concerning the formation of sex chromatin in the early stages of embryonic development from the X-chromosome, which has entered into a heteropycnotic state, the experiments of Onuma and Nishikawa, which were carried out on males, cannot be accepted as demonstrative: in adult animals, with no sex chromatin (males), no sex chromatin can be formed in the somatic cells.

However, there is information in the literature [8, 15] to show that the number of Barr's particles in the buccal cells of newborn girls and their mothers is dependent on hormones: in the first two days after birth the number of cells with Barr's particles was at a minimum, and after the 3rd day it increased up to the normal level. It is also known that fluctuations in the content of sex chromatin are also dependent on the menstrual cycle, on drug therapy, and so on [15]. It has been found that shock doses of corticosteroids lower the content of sex chromatin in the buccal cells of patients with dermatoses [1, 5].

We have studied the effect of the host's sex on the number of Barr's particles in cells of a transplantable rat sarcoma SSK and adenocarcinoma of the mammary gland.

EXPERIMENTAL METHOD

In the first experiments 29 (15 males and 14 females) noninbred albino rats weighing 100-130 g were used. A sarcoma SSK was implanted subcutaneously into all the animals at the same time. The animals were sacrificed 15 days after implantation of the tumor. Pieces of the tumors were fixed in 10% neutral formalin solution and embedded in paraffin wax. Sections were stained by Feulgen's method. Sex chromatin was counted in 200 cells of each section.

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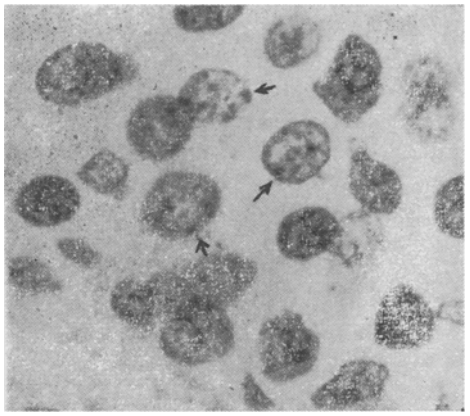

Fig. 1. Sarcoma SSK inoculated into a female rat. Nuclei of tumor cells containing granules of sex chromatin (marked by arrows) can be seen. Feulgen, 1028 ×.

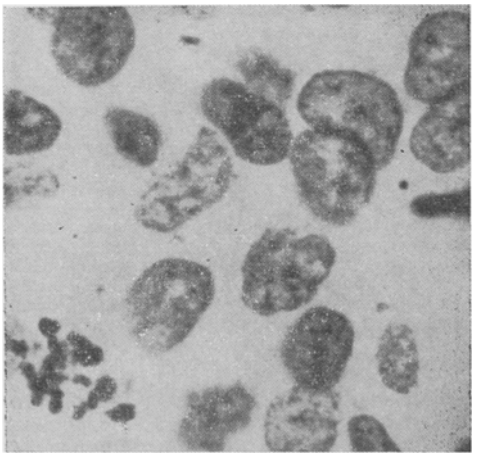

Fig. 2. Sarcoma SSK inoculated into a male rat. Nuclei of tumor cells are without granules of sex chromatin. Dark granules of autosomal chromocenters can be seen. Mitotic figure below on the left. Feulgen, 1028 ×.

When selecting the tumor strain the following circumstances were taken into account. First, transplantable sarcoma SSK was derived from a hormonally dependent tumor arising spontaneously in the mammary gland tissue of a female rat in 1960 [7]. Second, as is known from the literature [6] and as we ourselves have established, a well marked relationship exists between growth of sarcoma SSK and the host's sex; the tumor in females grows more rapidly and has a more malignant course, metastasizing earlier and more often, causing earlier death of the host, and giving a higher percentage of successful takes than in males. In males cases of absorption of the tumor are frequently observed, and its latent period of growth is much longer than in females (9-15 and 5-9 days respectively).

The second experiment was carried out on mice of line C3H. A tumor was extirpated from a female mouse with a spontaneously developing adenocarcinoma of the mammary gland, minced and mixed with sterile physiological saline, and used to inoculate two male mice of the same line. On the 20th day of growth the male mice were killed. Tissue of the tumor taken from the males and the female was fixed in 10% neutral formalin and embedded in paraffin wax. Sections were stained by Feulgen's method. The number of Barr's particles was counted in 200 cells.

EXPERIMENTAL RESULTS

The percentage of cells containing sex chromatin was much lower in cells of the sarcoma SSK implanted into males than in cells of the sarcoma implanted into females (9 ± 1 and 26.4 ± 1.5 respectively). The difference between the indices was statistically significant ($T = 9.7$, $P < 0.001$). In the female mouse 32% of tumor cells contained Barr's particles, compared with 11 and 16% in the two males.

The results point unambiguously to the presence of a close relationship between the incidence of Barr's particles in tumor cells and the hormonal background of the host organism; the number of cells containing sex chromatin in the transplantable rat sarcoma SSK and spontaneous adenocarcinoma of the mammary gland of C3H mice was 2 or 3 times lower when the tumors were implanted into males. Without a parallel karyologic analysis it is impossible to say what lies at the basis of this phenomenon: uncoiling of the X-chromosome forming sex chromatin, or selection of cells without this X-chromosome in the case of implantation of the tumor into males. Since sarcoma SSK belongs to the category of transplantable tumors, capable of being transmitted for many years among animals of both sexes, the process regulating the formation of granules of sex chromatin in the tumor cell nuclei is evidently reversible in character. The direction of the process at any particular time depends on hormonal influences: if the tumor is inoculated into females the number of cells with Barr's particles increases, while if implanted into males, on the other hand, it decreases (Figs. 1 and 2).

It may be asked why masculinization (loss of sex chromatin) of breast carcinoma cells in man is accompanied by an increase in the malignancy of the tumor [2], whereas essentially the same phenomenon (selection of cells without sex chromatin) in transplantable sarcoma SSK takes place against the background of marked inhibition of growth of the tumor when inoculated into males. It may be objected that the hormonal background is totally different in the female organism (influence of estrogens) in which the mammary gland tumor develops. However, this objection is invalid, because it is well known that the masculinized mammary

gland tumor in women treated with androgens acquires an even more malignant course and the prognosis of the disease under these circumstances is considerably worsened*.

It is not known to what degree the absence of correlation between masculinization of the tumor and its malignancy can be ascribed to the fact that carcinoma of the breast in man is a primary tumor while sarcoma SSK in rats is a transplanted tumor. A paradoxical situation thus arises: the tumor at the earlier stage of progression (spontaneous carcinoma of the breast in man), if its cells become "masculinized," responds to exogenous androgens by an increase in the intensity of its growth and metastasization, while the transplanted tumor, the long-developing sarcoma SSK, responds to endogenous androgens in the body of the male recipient by slowing of growth and metastasization and by a decrease in the rate of successful takes. A further study of this problem is required.

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*No comparison was made of the rate of growth and degree of malignancy of the spontaneous mammary gland adenocarcinoma in C3H mice when inoculated into males and females.