HORMONE-INDUCED CHANGES IN GRAFTED RAT MAMMARY GLAND TUMORS (RMK-1)

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By now it is established that different results are obtained from treatment of grafted strains of animal tumors and clinical results, even when adequate doses of identical preparations are used for identical tumor strains. For example, the use of sarcolysin, which consistently causes the disappearance of certain grafted rat sarcomas [5] is ineffectual with respect to human tumors [1]. On the other hand, many methods of hormone therapy which have a negligible therapeutic effect on grafted mouse mammary gland tumors [6, 8, 9, 10, 11, 14, 15], are widely used clinically for the treatment of this kind of tumor [7, 12, 16-21].

It therefore follows that strains of animal tumors cannot be used as experimental models for the development of rational methods of a chemical or hormone therapy in man.

Perhaps one of the reasons for this difference is the gradual change in the properties of the experimental tumors through repeated transplantation from one animal to another. Indeed, in experimental oncology, it has long been known that attempts to graft spontaneous tumors are by no means always successful. However, if the tumor does take, then in subsequent generations, the percentage of successful grafts increases greatly, and at the same time there is an increase of malignancy.

It is natural to suppose that the change in the fundamental properties of the tumor during successive graftings should be associated with a change in the sensitivity of the tumor to the action of therapeutic agents. The object of the present investigation has been to test this hypothesis.

METHOD

As an experimental model we used a grafted rat mammary gland tumor (RMK-1) which we had found to be very sensitive to hormone action [2, 3]. With regard to localization, etiology, and reactivity of this model tumor corresponded completely to human mammary gland cancer.

As our previous experiments showed, the reactivity of the first generation of RMK-1 resembled that of human mammary gland cancer. Thus, by using the standard methods of hormone therapy [4] as applied clinically directed either to suppressing the function of the source of estrogens (ovariectomy and ovariectomy + adrenalectomy + cortisone) or to suppress the follicle-stimulating function of the hypophysis (hexestrol, or hexestrol + ovariectomy + cortisone). RMK-1 reacted by a marked reduction of growth. On the other hand, the first generation RMK-1 reacted with a marked increase of growth to the application of small doses of hexestrol, a result which was important clinically because to some extent it answered the question of what dose of hexestrol to give to counteract tumors.

In order to answer this problem, i.e., to determine any possible relationship between a change in the reactivity of a tumor and the increase of its malignancy brought about by repetitive transplantations, we used all the methods described in all successive generations of RMK-1 from the 7th to the 32nd.

Altogether experiments were carried out on 365 rats (from impure lines) which were born from females of the generations mentioned above into which RMK-1 tumors had been implanted. The conditions of the experiment corresponded to those carried out on the first generation. On account of the increased rate of growth of the tumor as the number of generations increased, after the 18th generation the treatment was begun on the 6th-8th day after

grafting, and was continued for not less than 3 weeks. The hormonal preparations were injected daily subcutaneously as a suspension in physiological saline. Hexestrol was given as 0.005, 0.2, and 2 mg per day; testosterone propionate was given as 20 mg per day, and cortisone as 1 mg per day. The operation was performed at the beginning of the hormone treatment.

RESULTS

The results of the investigations are shown in the table, and for comparison we give our results, published previously, of the hormonal treatment of the second generation [3].

Change of Tumor Growth (as percentage) in the Different Generations of RMK-1, and the Influence of Various Methods of Hormone Treatment

Method	Generation									
	2nd*		7th		10th		18th		32nd	
	Number of rats	Result	Number of rats	Result	Number of rats	Result	Number of rats	Result	Number of rats	Result
Control	15		10		—	—	6		21	
Ovariectomy	10	— 60	10	+ 24	—	—	11	+ 11	16	+ 43
Control	12		—	—	—	—	6		21	
Ovariectomy + adrenalectomy + 1 mg per day cortisons	10	— 65		—	—	—	8	—65	19	—48
Control	—		—		—	—	6		12	
2 mg per day hexestrol	—	—	—	—	—		10	—28	10	+ 23
Control	15		10		10		32		21	
0.2 mg per day hexestrol	8	— 91	11	—83	10	—13	34	—11	20	+ 12
Control	15		—		10		6		21	
0.2 mg per day hexestrol + ovariectomy + 1 mg per day cortisone	6	— 85	—	—	10	—29	8	—54	20	—50
Control	9		—		—		32		12	
0.005 mg per day hexestrol	6	+ 118	—	—	—	—	33	+ 37	10	— 9
Control	8		—		—		32		9	
20 mg per day testosterone propionate	8	+ 87	—	—	—	—	27	0	9	—27

Note. The minus sign indicates suppression of growth, the plus sign stimulation of growth.
* Results for the second generation were taken from our previous publication [3].

From the results obtained it emerges that even after the fourth generation, ovariectomy caused no suppression of growth of RMK-1, but on the other hand, from the 7th generation onwards it actually had a stimulating influence. This stimulating effect increased with the number of generations, and at the 32nd generation, tumor growth stimulation amounted to 43%. In addition, when ovariectomy was combined with adrenalectomy and cortisone, once more suppression of tumor growth was observed. Thus, by the 18th generation, growth suppression amounted to 65%, and in the 32nd generation it was 48%.

When 0.2 mg of hexestrol were given after the 10th generation, there was practically no tumor growth suppression. Thus, at the 10th generation the suppression was 13%, at the 18th 11%, and at the 32nd there was actually a 12% stimulation of growth. An increase of the dose of mexestrol to 2 mg in no way enhanced the therapeutic effect. However, 0.2 mg of hexestrol combined with ovariectomy and cortisone applied between the 18th and 32nd generations continued to exert a considerable suppression of tumor growth, although in comparison with the suppression in the first generations, the suppression was reduced somewhat, and at the 18th generation was 54%, and at the 32nd 50%.

All doses of hexestrol (0.005 mg per day) at the 18th generation stimulated tumor growth only by 37%, and at the 32nd generation had practically no stimulant effect.

The injection of 20 mg testosterone propionate at the 18th and 32nd generations differed from its effect on the first generations by causing a negligible stimulation of RMK-1 growth.

The results obtained therefore indicate that in successive transplantations, RMK-1 lost its sensitivity to the effect of ovariectomy and to hexestrol. At the same time there was some reduction of sensitivity to ovariectomy combined with adrenalectomy and cortisone, also to hexestrol combined with ovariectomy and cortisone, when, besides a suppression of the main sources of estrogens or follicle stimulating hypophyseal hormone, there was also some action on the compensatory sources of these same hormones.

A comparison of the action of identical methods of hormone therapy on different RMK-1 generations from the 2nd to the 32nd, it becomes obvious that successively transplanted mammary gland tumors are not suited to the development of hormone therapy methods, because if we began investigations in a manner accepted in this kind of work, by the time the RMK-1 strain had become established (i.e., by the 25-30th generation of tumor) we would not be able to determine the reactivity of this tumor to any of a number of hormonal influences.

Also, from the results obtained it follows that the development of hormone therapy methods can be carried out not only on spontaneous mammary gland tumors but also on the first generations of transplanted tumors, which is a result of considerable practical significance.

SUMMARY

A study was made of the reactivity to various hormones of the 2nd to 32nd generation of spontaneous rat mammary carcinoma (RMK-1) transplanted into randomly bred weanling rats. After the 10th generation, RMK-1 lost its reactivity to ovariectomy and hexestrol therapy, and became less sensitive to ovariectomy combined with adrenalectomy and cortisone treatment, as well as to ovariectomy combined with hexestrol and cortisone treatment. The loss of reactivity of RMK-1 to some hormones during repeated transplantations demonstrates that transplantable mammary cancers should not be used as an experimental model on which to base clinical methods of hormone therapy for this particular tumor.

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