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THE MODE OF ACTION OF OVARIAN HORMONES IN THE INDUCTION OF MAMMARY CANCER IN MICE*

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Abstract—Prolactin seems to be the predominant agent in hormonal mammary gland carcinogenesis in mice. Oestrogens act mainly by promoting prolactin production. Progesterone is effective only in the presence of prolactin, acting synergistically with it.

MANY years ago it was demonstrated that the ovaries play an important part in the development of mammary cancer in mice.[1] After the natural oestrogens had been isolated, Lacassagne[2] reported the induction of mammary carcinoma in male mice by long-term treatment with one of these preparations. Later investigations confirmed the carcinogenic activity of estrogenic hormones on the mammary gland in various mouse strains.

All the earlier experiments in this field have been performed on animals infected with the so-called mammary tumour agent. The application of oestrogenic hormones was found to be as effective in ovariectomized females as in orchidectomized males It could be shown that the effect was dose-dependent: the higher the dose, the higher is the tumour incidence and the earlier do neoplasms appear. This was true at least for physiological or near physiological dosage schedules; at higher doses toxicity may affect the end-result. Another condition for the induction of mammary cancer by oestrogens was found to be the continued application of the drugs over a long time. Discontinuous treatment with oestrone in drinking-water at a 5-day-interval greatly reduced the tumour incidence as compared with continuous treatment at half the dose level, the total dose given to each group thus being the same.[3–5]

The strains of mice used in the study of mammary gland carcinogenesis can be divided into two groups. To the first group belong those strains in which the mammary tumour agent is normally present and carried over from generation to generation by the mother's milk; to mention a few: strains C_3H, DBA, WLL and A. These strains can be made permanently agent-free by the conventional method of foster-nursing immediately after birth on C57BL females or by ova transfer into pseudopregnant C57BL animals. After this procedure these sublines show a much lower tumour incidence at a much later age as compared with the strains from which they are derived.

To the second group of mice belong those strains which are naturally free of the mammary tumour agent and cannot propagate the agent because they either do not transmit it to the offspring or do so for a few generations only (strains such as 0_{20}, CBA and C57BL).

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The results of experiments with oestrogen treatment of castrated male mice of the various agent-free strains and their F_1 hybrids indicated that no tumours could be induced, with the exception of the C_3H_f subline and its hybrids. Generally speaking, mammary tumour induction by oestrogens in mice is possible only in the presence of the mammary tumour agent.[6] The mechanism of action of the synergism or combined activity of the virus and the steroids has yet to be elucidated.

By far the best method to induce mammary cancer in intact female mice purely by hormonal stimulation is the implantation of additional hypophyses to sites remote from the hypothalamus. This technique has been studied in our laboratory for a number of years.[6–9] Functional activity of a hypophyseal graft is of course possible only when isografts are used or grafts of tissue of either of the parent strains to a F_1 hybrid. The mechanism of action of the hypophyseal isografts seems clear-cut. The production of all hormones of the adenohypophysis, except prolactin, is dependent on a close contact with the hypothalamus, which acts by means of the stimulatory releasing factors. Prolactin function, however, is regulated by the hypothalamus by an inhibitory mechanism, also neurohumoral, the so-called prolactin inhibiting factor. Therefore, disconnected hypophyses or hypophyseal isografts produce prolactin continuously and nothing but prolactin.

By grafting additional hypophyses, preferably to the kidney, it has been possible to induce mammary tumours in a large number of mouse strains and their F_1 hybrids, all free of the mammary tumour agent. These tumours are in no way different from those occurring spontaneously in mice. In some strains this technique induced only a small incidence of tumours. It is therefore obvious that a genetic factor is also of importance in the genesis of mammary carcinoma. In a recent investigation it was shown that this difference must be brought back to a genetically determined degree of susceptibility of the mammary gland tissue in the various strains studied. It could be proved that transplanted mammary glands of the strains with a low incidence are much more resistant to the development of tumours than those of other strains transplanted under equal environmental circumstances in the common F_1 hybrids.[10]

In mice with the mammary tumour agent, implantation of additional hypophyses also leads to an earlier appearance of mammary tumours. In otherwise intact female animals the prolactin released by the hypophyseal isografts has a direct effect on the mammary gland, and also an indirect action by stimulating ovarian progesterone production. Ultimately the carcinogenic process occurs through the combined action of prolactin, oestrogens and progesterone. A quantitative analysis of the hormonal situation in the graft-bearing female animals is complicated by the fact that frequently the grafts show progressive growth in the course of time, with a concomitant increase in prolactin secretion. In intact and castrated male animals the hypophyseal isograft technique generally is ineffective, not only because of absence of the ovarian hormones, but also because the grafts remain extremely small, producing only minimal amounts of prolactin. The discovery that graft growth, and thus also prolactin production, can be regulated by exogenous oestrogen treatment[8], has opened the way to a more detailed analysis of hormonal mammary gland carcinogenesis. Table 1 shows the results of a representative group of experiments.

Castrated male mice of the agent-free (C57BL $\times$ CBA)F_1 hybrid were given a single hypophyseal isograft in the left kidney at an age of 6 weeks. In the first group no further treatment was given, the isografts remained small and no mammary

TABLE 1. MAMMARY TUMOURS IN CASTRATED (C57BL × CBA)F_1 MALE MICE WITH HYPOPHYSEAL ISOGRAFTS

Treatment	No. of animals	With tumour		Without tumour	Weight isograft in mg		Weight hypophysis *in situ* in mg at 450 days
		%	Av. age (days)	Av. age at death (days)	At 300 days	At 450 days	
1.	36	0	—	587	2	3	2
2. High dose* oestrogen continuous	37	0	—	439	—	70	70
3. High dose† oestrogen discontinuous	31	65	488	504	—	200	3
4. Low dose‡ oestrogen continuous	35	40	466	477	30	300	5
5. Progesterone§	12	25	577	553	2	3	2
6. Low dose oestrogen continuous + progesterone	42	100	271	—	25	—	—

* 2 mg oestrone/l. drinking-water.
† 2 mg oestrone/l. drinking-water at 5-day-intervals.
‡ 0·25 mg oestrone/l. drinking-water.
§ 3 × 2 mg progesterone pellets s.c. weekly.

tumours were found. In the second group, receiving high-dose oestrogen treatment, both hypophysis *in situ* and hypophyseal isograft grew at the same rate, but still no mammary tumours did occur. In the third and fourth groups the oestrogen dosage was selected in such a manner that maximal isograft growth occurred, leaving the hypophysis *in situ* relatively unimpaired. In both groups considerable mammary tumour incidences were observed. It can be concluded that in these experiments the oestrogen treatment acts mainly by increasing prolactin production of the isografts, and only secondarily by stimulating the mammary gland: this points to prolactin as the main carcinogenic agent in mammary tumour virus-free animals. In agent-carrying animals without isografts the carcinogenic activity of oestrogen then would depend mainly on an increase of the prolactin production of the hypophysis *in situ*, for which high-dose treatment is optimal.

The last two groups of Table 1 illustrate the role of progesterone. This hormone does not seem to affect the growth and function of either the hypophysis *in situ* or the hypophyseal isograft. However, even in the presence of only moderate prolactin levels, progesterone seems to act synergistically, inducing up to 25 per cent mammary tumours. Combined with suitable oestrogen treatment, which stimulates the prolactin production of the isografts, progesterone is very effective in promoting mammary gland carcinogenesis, resulting in no less than 100 per cent tumours at a very early age. Progesterone treatment in animals without hypophyseal isografts has as yet not produced a single mammary tumour.

REFERENCES

1. A. E. C. LATHROP and L. LOEB, *J. Cancer Res.* **1**, 1 (1916).
2. A. LACASSAGNE, *C. r. hebd. Séanc. Acad. Sci.* **195**, 630 (1932).
3. O. MÜHLBOCK and L. M. BOOT, in *Ciba Foundation Symposium on Carcinogenesis* (Eds. G. E. W. WOLSTENHOLME and M. O'CONNOR), pp. 83–90. Churchill, London (1959).
4. O. MÜHLBOCK in *Biological Activities of Steroids in Relation to Cancer* (Eds. G. PINCUS and E. P. VOLLMER), pp. 331–342. Academic Press, New York (1960).
5. O. MÜHLBOCK and L. M. BOOT, in *Hormonal Steroids, Biochemistry, Pharmacology and Therapeutics*, Proceedings of the First International Congress on Hormonal Steroids, Vol. II (Eds. L. MARTINI and A. PECILE), pp. 397–401. Academic Press, New York (1965).
6. O. MÜHLBOCK and L. M. BOOT, *Cancer Res.* **19**, 402 (1959).
7. L. M. BOOT, O. MÜHLBOCK, G. RÖPCKE and W. VAN EBBENHORST TENGBERGEN, *Cancer Res.* **22**, 713 (1962).
8. L. M. BOOT, G. RÖPCKE and O. MÜHLBOCK, in *Proceedings of the Second International Congress of Endocrinology*, pp. 1058–1062. Exc. Med. Congr. Ser. No. 83 (1965).
9. L. M. BOOT and G. RÖPCKE, *Cancer Res.* **26**, 1492 (1966).
10. A. DUX and O. MÜHLBOCK, *Int. J. Cancer* **1**, 5 (1966).