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Mammary Carcinoma in Mice Bearing a Transplantable Mammotropic Tumor, Carrying the Bittner Virus.* (28777)

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Induction of transplantable mammotropic pituitary tumors (MtT) in LAF_1 (C57L × A/He) female mice by radiation was reported in 1956(1). Later, the estrogen-induced pituitary tumors were also identified as mammotropic(2). Subsequent studies of numerous MtT induced by radiations and estrogens led to the conclusion that the hormones of MtT alone are not carcinogenic(3). In LAF_1 mice the mammary tumor virus of Bittner (MTV) alone failed to induce mammary tumors (MT) in adult LAF_1 female mice, but MtT rendered such mice susceptible to MTV(3). Similarly MtT renders adult female rats susceptible to induction of MT by subthreshold doses of 3-methylcholanthrene(4) and radiation(5).

Recently an MtT strain, used in co-carcinogenesis studies with MTV(3), appears to have been "contaminated" with MTV so that it now alone produces in adult LAF_1 female mice not only an extraordinary mammary gland hyperplasia with anaplasia but also numerous MT.

Materials and methods. Mice. Virgin female LAF_1 (C57L/J × A/HeJ) mice, about 6 to 8 weeks old, supplied by the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine were fed commercial pellets and tap water *ad libitum*. The incidence of mammary carcinoma in these mice was about 1% (3,6-8).

MtT. The functional MtT strain 38 used arose in a female LAF_1/J mouse that had been given 550 r X-rays over the head and neck and 0.05 mg pellets of diethylstilbestrol (DES) renewed at 3-month intervals(2). First passage tumors of this strain were used

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TABLE I. Incidence of Mammary Tumor in LAF_1 Mice Bearing MtT 38.

Passage	No. of mice		Incidence of MT (%)	Mean latency of MT* (days)	Mean age of mice with MT (days)	Mean latency of MtT (days)	Mean duration of MtT (days)	Mean age of mice at death (days)
	Total	With MT						
Original	4‡	0	0	—	—	150	90	290
I	6	0	0	—	—	160	40	240
II	7	0	0	—	—	130	65	235
III	22	1	4.5	120	300	140	80	280
IV	28	9	31.0	155	305	155	75	265
V	14	10	71.4	115	280	140	85	265
V adrex†	7	0	0	—	—	130	60	230
Controls	31§	0	0	—	—	—	—	365

* Period from MtT take to MT development.
† Recipients were adrenalectomized. MtT is the same as used in passage V.
‡ Bearing MtT.
§ Without MtT.

in the co-carcinogenesis studies with MTV (3). This strain causes a very marked, predominantly alveolar hyperplasia of the mammary gland with secretion and increase of body weight and weights of several organs. Lymphopenia, atrophy of the thymus and hypertrophy and hyperplasia of the zona fasciculata of the adrenal cortex are interpreted as indicating AtH (ACTH) activity. Virus-infected and non-infected mice were kept in different rooms. The main line of MtT 38 was maintained through passages in isologous mice, separated from those infected with virus. Routine transplantations were made by intramuscular injection of tumor mince in the right thigh of 6- to 8-week-old mice. In the original and second passages, 0.05 mg pellets of DES were implanted in some mice when the MtT grafts were made, in order to promote the growth of MtT. The pellets were renewed at 2-month intervals. In the present experiments DES was not used. In most passages, 5 to 10 untreated normal siblings served as controls.

Follow-up. The mice were inspected monthly or biweekly and killed when the grafted MtT became about 1 cm in average diameter, or when an MT became palpable. All mice were autopsied. MtT in mice whose mammary glands showed a high mammotropic activity was used to make successive passages. For microscopic study cervical and inguinal mammary pads were fixed in Bouin's fixative and stained with hematoxylin-eosin solution. Whenever MT was found at autopsy additional sections were taken from different mammary pads and MT.

MTV. In earlier experiments(3) the source of MTV was milk of pregnant DBA mice, received from Dr. D. H. Moore, Rockefeller Inst. of Medical Research, New York.

Results. Incidence of MT (Table I). No MT was observed in the first 3 successive passages of MtT. From the third passage on, the incidence of MT rapidly increased from 4.5% to 71.4% in the fifth (latest) passage. The mean latency of MtT was 146 days and the period from MtT-take to MT-development was about 4 to 5 months. In most mice, several MT were found on gross and more on microscopic examination.

Mammary tumors. Fig. 1 illustrates the mammary gland hyperplasia and tumors noted in all mammary pads of a mouse that had been examined. In general, the small MT were firm and the large ones soft. The latter had scattered necrotic and cystic areas. Fig. 2 shows microscopic tumors and anaplastic ("pretumorous") changes in hyperplastic mammary glands with milk secretion. Most MT were simple (Fig. 3, 4) or papillary (Fig. 5) adenocarcinomas and a few squamous cell carcinomas (Fig. 6). The anaplastic ("pretumorous") changes were similar to those seen in mice which received MtT and virus(3). *Electronmicrographs* of MtT (Fig. 7) and mammary gland (Fig. 8) of a mouse bearing a third passage MtT disclosed the presence of MTV-like virus particles. This mouse had a grafted MtT of about 8 mm in

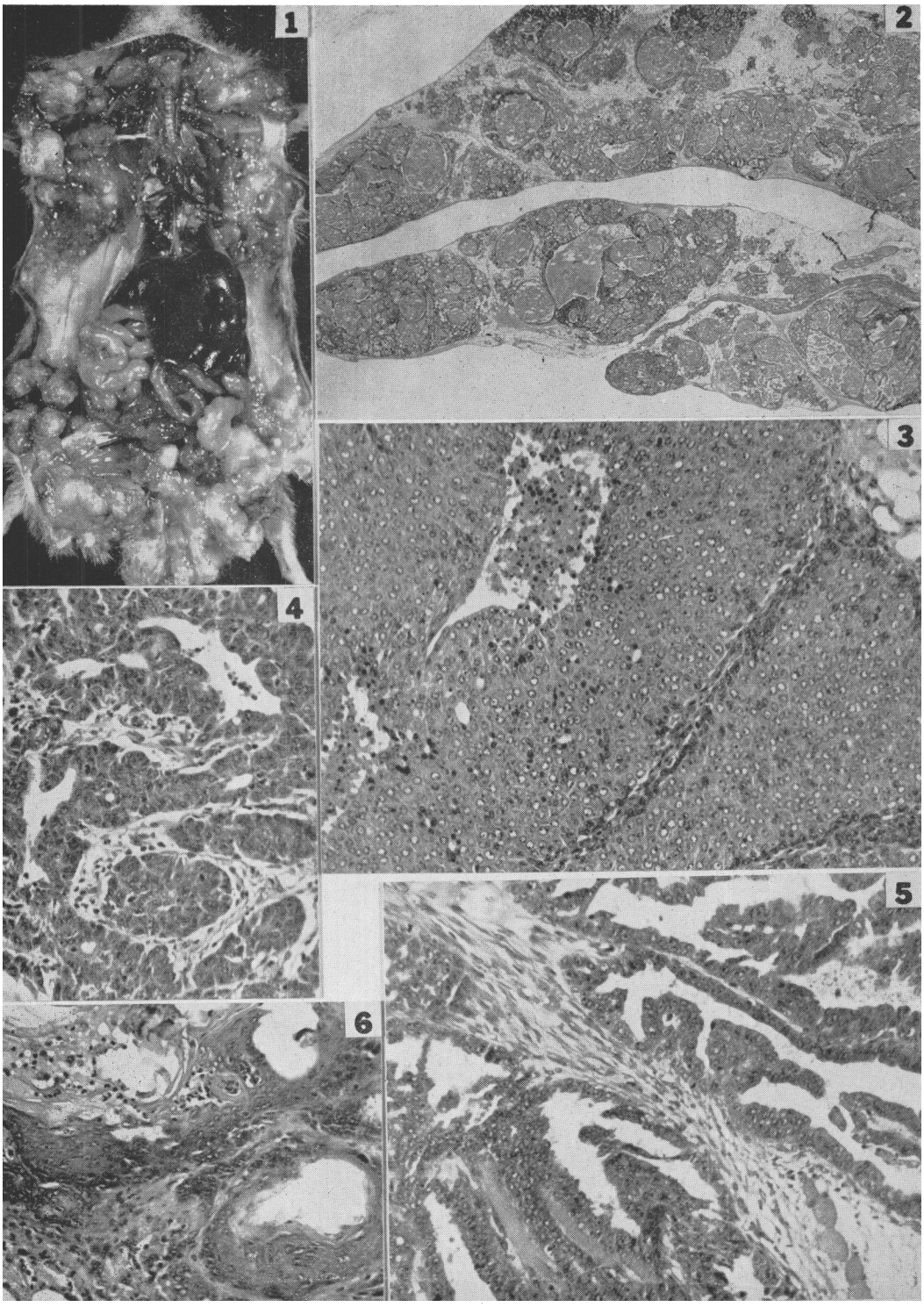

FIG. 1. All mammary pads of this mouse bearing MtT are hyperplastic and anaplastic and contain many small MT.
FIG. 2. Microscopic appearance of mammary pads. × 6.4.
FIG. 3. Medullary carcinoma. × 190.
FIGS. 4 and 5. Adenocarcinomas. × 190.
FIG. 6. Squamous carcinoma. × 190.

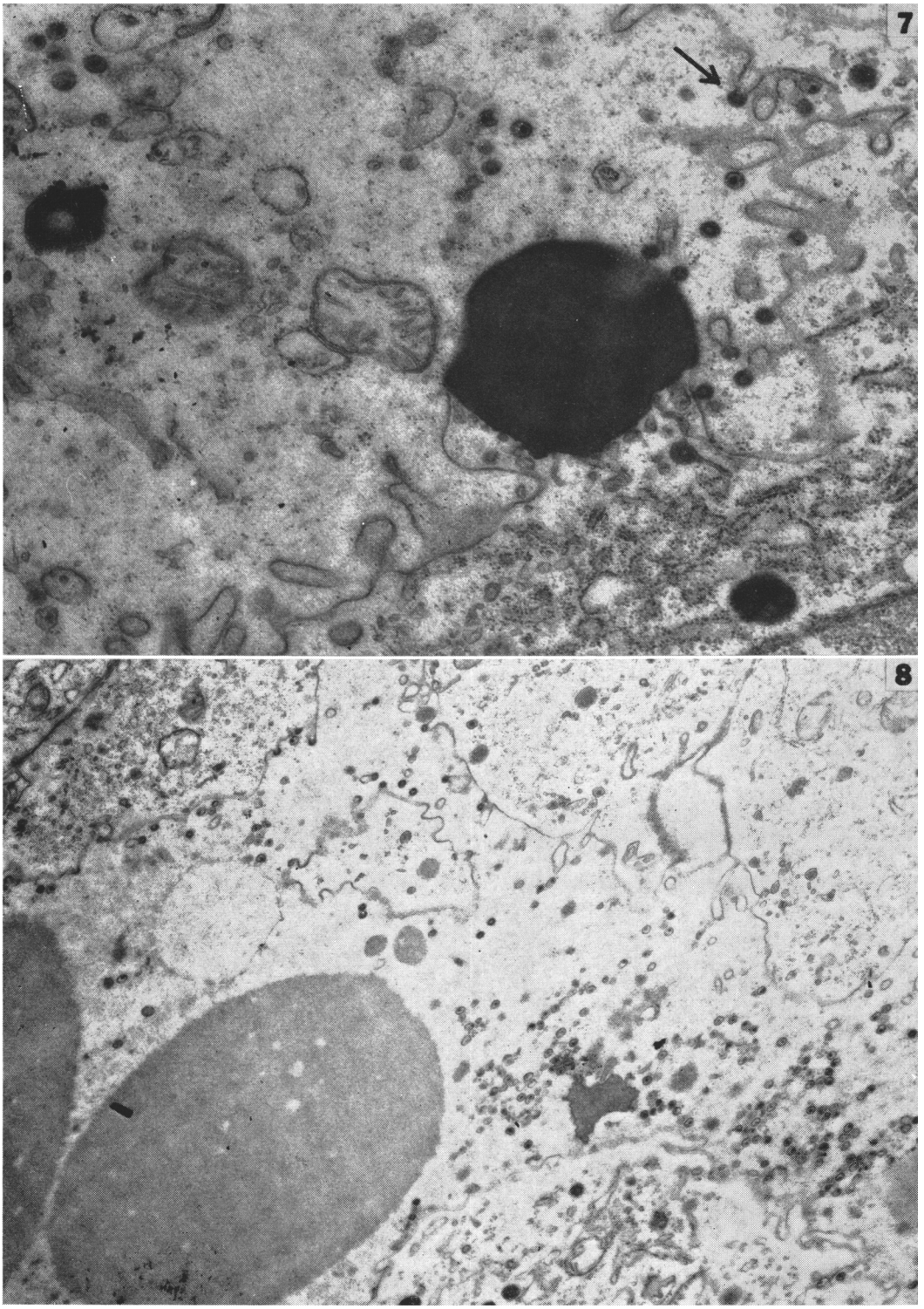

FIG. 7. Electron micrograph of MtT. Arrows point to virus. × 32,000.
FIG. 8. Electron micrograph of mammary gland of the same mouse as in Fig. 7. × 13,000.
(Magnifications given are approximate.)

diameter in the right thigh and well stimulated mammary glands with no macroscopic MT. (No sections of the mammary glands were taken from this mouse for light microscopy.) No virus particles were found in MtT grafts of the first passages, studied in the co-carcinogenesis series in collaboration with Dmochowsky(9). Further electronmicroscopic examinations and bioassays of MTV are in progress.

The effect of adrenalectomy on tumor development. MtT 38 has a moderate AtH (ACTH) activity(2,10). Adrenalectomy was found to enhance the growth of those MtT which have AtH activity, and their sommatotropin (StH) and mammotropin (MtH) activities(10). Growth of mammary gland is stimulated by MtT (MtH) even in adrenalectomized hosts but the gland becomes fibrous and devoid of milk secretion. Therefore it was of interest to find out whether MT induction was enhanced or inhibited by adrenalectomy. The latter was found to be true in this preliminary trial (Table I).

Discussion. In the genesis of MT in mice, 3 main factors have been established: genetic, hormonal, and viral(11). Several hormones are known to influence the development of mammary gland (cf. 12) and MT (cf. 13). In our experience hormones are not primary carcinogens but powerful stimulators (promoters) in carcinogenesis and play a determining role in initiation and maintenance of growth of MT(2-5,14).

Mammary tumor is rare in LAF_1/J mice (3,6-8) and this strain is resistant to induction of MT with estrogens(2). The present study suggests that exposure of mice to functional MtT carrying MTV creates a vicious circle: MtT stimulates growth of and milk secretion of the mammary gland, thereby promoting viral replication and increasing the concentration of MTV in the host. This virus is known to be present in the blood, so that MtT becomes carrier of the virus. The arrow in Fig. 7 suggests virus formation on a villus of the plasma membrane in MtT. The increasing incidence of MT through the last 3 successive passages can be best explained by assuming that the concentration of MTV increased in the course of these passages.

The way in which MtT 38 picked up a virus is merely conjectural. There are two likely possibilities: a) The virus is either that received from Dr. D. H. Moore, used in co-carcinogenesis studies(3) and "picked up" by MtT as a laboratory infection or laboratory error; or b) MtT activated a latent virus carried in LAF_1 mice. The A/He, parent of LAF_1, is known to carry MTV and it is possible that some LAF_1 mice carry MTV in latent form. It is noteworthy, however, that during some 6 years' study of several highly functional MtT, there was no indication that it alone produces MT, with the exception of an experiment reported by Haran-Ghera(8). She resected MtT repeatedly and thereby extended the life span of MtT bearing mice to 12 months and observed 8.8% MT in these mice. Further, it is now generally believed that most strains of mice carry MTV and that genetic factors can suppress viral activity. The high capacity of MtT 38 to produce MT was observed by us only after its use in co-carcinogenesis studies with MTV. To clarify the situation, several presumably MTV-free MtT strains, preserved in the frozen state, are now being resuscitated for studies of their capacity to activate a latent MTV or to establish that they do not carry MTV.

It is well known that milk is a rich source of MTV. Since adrenalectomy of MtT hosts is followed by better growth of MtT, and loss of milk secretion(10), it is possible that prevention of MT-development by adrenalectomy is due to loss of milk secretion. This in turn suggests that viral multiplication is enhanced by corticoids, and that procedures which stimulate milk secretion might actuate MTV carried in latent form. The classical demonstration of the beneficial effects of adrenalectomy on the growth of human MT by Huggins(15) is usually explained by removal of this accessory source of estrogens (16). The above explanation points to the possibility of controlling viral replication and thereby MT induction by adrenalectomy. Further, corticoids may have a direct effect on some MT. These possibilities are being analyzed.

Summary. 1. A subline of mammotropic

tumor strain 38, used in previous co-carcinogenesis studies with Bittner's virus (MTV), now appears to carry this virus and causes mammary tumors in adult mice of a strain (LAF_1) in which spontaneous mammary tumors are very rare. This strain has marked mammary gland stimulating, marked adrenotropic and moderate growth-promoting activities. 2. Numerous similar MtT strains studied earlier failed to induce mammary tumors. 3. Electronmicrographs showed MTV-like particles in both mammotropic tumor and mammary gland. 4. In a small experiment no mammary tumors developed in bilaterally adrenalectomized mice bearing this mammotropic tumor, even though adrenalectomy enhanced the growth of the mammotropic tumor. Prevention of induction of mammary tumor by adrenalectomy might be related to inhibition of milk secretion and reduction of viral concentration. 5. Cancerization by this mammotropic tumor is extraordinary so that almost every mammary pad examined had microscopic mammary carcinomas (both glandular and squamous) and anaplastic (presumably precancerous) changes.

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Enhancement of Metastatic Calcification Produced with Parathyroid Extract by Parathyroidectomy and Adrenalectomy. (28778)

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The present experiments were prompted by the following observations: During attempts to maintain parathyroidectomized (PTX) rats on calcium lactate and daily injections of 50 units of bovine parathyroid extract (PTE), it was noted that this treatment caused death associated with widespread calcific changes within several days. Similarly treated intact rats survived for many weeks and exhibited only mild changes. Adrenalectomized (ADX) rats were also seen to tolerate less PTE than their intact controls. Attempts were made to quantitate the calcific changes with the aid of radiocalcium.

Methods. Male Holtzman rats fed Purina Laboratory Chow and weighing from 125 to 150 g were used in all experiments. Parathyroidectomies, by electrocautery, and adrenalectomies were performed under ether anesthesia. All operated rats, as well as their controls, were placed on a 2% calcium lactate

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