Mechanism of Mammary Duct Stimulation 
by Tumor Transplants*

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SUMMARY

Subcutaneous epithelial Ehrlich ascites tumor (EAT) transplants stimulate adjacent mammary duct epithelium in C57BL mice. Present data describe a similar effect for Sarcoma 180, which is of connective tissue origin. Increased mitotic activity, with peak value on the 2d day of tumor growth, occurred in the epithelial cells of mammary ducts located in a 1-mm. area around the tumor nodule. Inward proliferation and enlargement of mammary duct cells resulted in increased height of epithelial lining and obliteration of duct lumen. The role of individual tumor components, namely, the ascitic fluid, the tumor cells, the necrotic and connective tissue, was determined. Ascitic fluid, devoid of tumor cells, had no effect on mammary duct epithelium. Nor was ascitic fluid required for growth of EAT cells, since saline-washed cells grew as usual and stimulated adjacent mammary duct epithelium. In vitro irradiation of tumor cells, which inhibited cell multiplication but not cell metabolism, decreased but did not eliminate the capacity of tumor cells to stimulate mammary duct cells. Implants of necrotic tissue did not affect adjacent mammary ducts significantly; nor did the connective tissue capsule, which surrounded transplants of boiled tumor cells, have any significant effect on adjacent mammary duct epithelium. Severe connective tissue proliferation and damage, as induced by paraffin pellets, did, however, produce a high mitotic activity in mammary duct cells, but only in those ducts which were in direct contact with the area of damage. Failure of EAT cells, growing in the vaginal wall of ovariectomized mice, to induce cornification of the overlying vaginal epithelium suggested that mammary duct stimulation by tumor transplants was not due to a local secretion of estrogen by the tumor cells.

These results suggested that living, but not necessarily dividing, tumor cells were required for mammary duct stimulation.

The presence of Ehrlich ascites tumor cells near the mammary gland of C57BL mice resulted in increased mitotic activity and enlargement of mammary duct cells located in a 1-mm. zone around the tumor nodule. This stimulation led to an increased height of the mammary duct lining with diminution of duct lumen (1, 2). The present investigation concerned the mechanism of this mammary duct stimulation by tumor transplants.

The first experiment in the series tested whether the stimulatory effect was specific for the Ehrlich ascites tumor or whether other tumors affected adjacent mammary tissue. For this purpose, we have used Sarcoma 180 which is of connective tissue origin, in contrast to the presumably epithelial nature of the Ehrlich ascites tumor.

Secondly, the role of the ascitic fluid, necrosis, and induced connective tissue in mammary duct stimulation was investigated. This was done because the Ehrlich ascites tumor, which stimulated mitotic activity in adjacent mammary duct epithelium, was injected underneath the skin in the ascitic phase and grew as a solid nodule, composed of a central area of necrosis, a peripheral rim of dividing cells, and a surrounding layer of connective tissue. Any of these tumor components, or the tumor-induced connective tissue, could therefore be responsible for the mammary duct stimulation. In addition, we tested whether the inhibition

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of cell division of the tumor cells by x-radiation, would obviate their capacity to stimulate adjacent mammary tissue.

Finally, since the mammary gland is sensitive to estrogen, we determined whether the mammary duct stimulation by tumor transplants could be due to the secretion of small, local quantities of estrogen by the tumor cells. This possibility was tested by growing the Ehrlich ascites tumor cells in the vaginal wall of adult ovariectomized mice and determining their capacity to induce cornification of the overlying vaginal epithelium.

MATERIALS AND METHODS

Three-month-old C57BL mice, bred in our own laboratory, were used. Conditions for maintenance of the mouse colony and diet have been described previously (2). The Ehrlich ascites tumor (EAT) was similar to the one used in our earlier studies (2). The Sarcoma 180 (S-180), in the ascitic form, was obtained from Dr. Sugura (Sloan Kettering Institute) in December, 1958, and has since been maintained by weekly intraperitoneal transfer in C57BL mice.

All experimental mice were ovariectomized, by dorsal approach, 10 days before the start of the experiment. One-tenth ml. of S-180 containing 15–30 × 10⁶ cells was injected, under aseptic conditions, underneath the second left nipple. The contralateral mammary gland was injected with 0.1 ml. of sterile saline and used as controls.

Ascitic fluid, devoid of tumor cells, was obtained by centrifuging the EAT for 5 minutes. A drop of fluid was checked microscopically to ascertain the absence of tumor cells. One-tenth ml. of this fluid was injected underneath the second nipple of 10-day ovariectomized mice. An equivalent amount of sterile saline was injected into the contralateral control mammary gland, and the animals were sacrificed 2 days later. The centrifuged cell portion was washed 3 times with sterile saline and used as controls.

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Irradiation of Ehrlich ascites tumor cells was performed in vitro. The tumor material was placed in a small test tube containing a few drops of a 20 per cent solution of potassium oxalate and the tube placed horizontally in a petri dish with crushed ice. A dose of 15,000 r in air was delivered by a General Electric Maximar x-ray machine, operated at 235 kv, 15 ma., with a 1.0 mm. Al and a 0.25 mm. Cu filter (HVL = 1.4 mm. Cu). Under these conditions, the dose rate was 453 r per minute as measured with a Victoreen r-meter. Immediately after irradiation, the cells were inoculated in the mammary gland region as described above. Animals were sacrificed 2 days later.

The presence of necrotic tissues near the mammary gland of ovariectomized mice was achieved by the fortuitous failure of some of the EAT transplants and of small tissue transplants from 18-day-old mouse embryos and adult kidney or lung to grow subcutaneously. The presence of these necrotic implants were sacrificed 2 days later.

To determine the effect of induced connective tissue on adjacent mammary duct epithelium, the EAT in the ascitic form was placed in a boiling water bath for 20 minutes, and the congealed pieces of tumor material, size 4 × 6 mm., were placed underneath the second left nipple of 10-day ovariectomized mice with a 13-gauge sterile trocar. An empty trocar of equivalent dimension was inserted in the contralateral gland. In another series, small, smooth paraffin pellets, dipped in alcohol, were inserted in the mammary gland region with a 13-gauge sterile trocar. The same operation was performed on the opposite side of the animal without insertion of a pellet.

All mice were treated with a dorsal subcutaneous injection of 0.1 mg. of colchicine, freshly dissolved in 0.25 ml. of saline, 6 hours prior to the time of sacrifice. Contralateral glands served as controls. Tumors were measured at autopsy. Biopsy specimens were embedded in paraffin, cut at 6μ, and stained with toluidine blue at pH 3.5. Microscopic measurements were made on mammary ducts located within a 1-mm. zone around the implant. Mitotic activity was determined as the number of mitotic figures per total number of mammary ducts observed and expressed as percentage values. Measurements of mammary duct diameter, height of mammary duct lining, and distance from the tumor were taken with an ocular micrometer.

To test the secretion of estrogen by the tumor cells, 0.1 ml. of EAT was injected into the vaginal wall of adult ovariectomized mice. The presence of normal estrous cycles before ovariectomy and their absence after the operation were ascertained before the tumor was inoculated. After the tumor was established the mice were smeared daily and the stage of the estrous cycle recorded. After 19–30 days animals were sacrificed and biopsy specimens taken to insure, by histological examination, a close proximity between tumor and vaginal epithelium.
RESULTS

Effect of Sarcoma 180 on mammary duct epithelium in castrated C57BL mice.—Chart 1 illustrates the stimulatory effect of S-180 on adjacent mammary gland tissue of ovariectomized C57BL mice. A sharp rise in mitotic activity of mammary duct cells occurred on the 1st day after tumor inoculation. This increased mitotic activity persisted for 1 more day (Chart 1), after which it declined until a level of 19 per cent was reached on the 24th day of tumor residence. Mitotic activity in these studies was expressed as the number of mitotic figures per total number of mammary ducts observed, stimulated as well as unstimulated. The number of mice and ducts used for determining this mitotic activity are indicated in Chart 2.

The shift to the right in this chart, with increased length of tumor residence, suggests that during the later stages of tumor growth there was an increase in the degree, as well as in the percentage of mammary duct stimulation. The range of epithelial heights, which was from 2 to 9 μ in control ducts, reached an upper limit of 34 μ on the 24th day of tumor growth. Nevertheless, normal-appearing ducts in and around the mammary duct stimulatory phenomenon, were in the ascitic form, the role of ascitic fluid per se was investigated. The cell-free fraction of EAT was injected underneath the nipple of six ovariectomized mice. Two days later no mammary duct stimulation could be observed. Control sections of the contralateral glands, given injections of an equivalent amount of saline, were indistinguishable from the mammary tissue injected with the ascitic fluid.

Although ascitic fluid by itself did not seem to affect the mammary duct cells, the possibility existed that it was required for the mammary duct stimulation by tumor cells. Our results, however, showed that EAT cells, washed thrice with saline and injected underneath the left nipple, grew as usual and stimulated adjacent mammary duct epithelium in the same fashion as did untreated EAT cells (2) (Fig. 3). Table 1 records the increased number of cell layers (one to four) lining the mammary ducts, the increased height of the mammary duct lining (up to 28.8 μ), and the mitotic activity (295 per cent on the 2d day). Figure 4 represents such a stimulated mammary duct, whereas Figure 5 shows a cross-section of a normal duct in the contralateral control region.

To test whether dividing cells were required for the mammary duct stimulation, the tumor transplants were present at all stages of tumor growth (Chart 2). As with the EAT (2), mammary duct stimulation was confined to an approximate 1-mm. zone around the tumor nodule. Figure 1 illustrates the high mitotic activity in a mammary duct stimulated by a 2-day-old S-180 transplant, whereas Figure 2 shows how this high mitotic activity could result in an increased height of the mammary duct lining with partial obliteration of duct lumen. This picture should be compared with Figure 5, which is a cross-section through a control mammary duct in the contralateral gland of a tumor-bearing mouse.

Effect of ascitic fluid, x-radiated tumor cells, necrosis, and connective tissue on adjacent mammary duct epithelium.—Since both tumors, active in...
cells were irradiated in vitro with 15,000 r. This dose had been shown to inhibit cell multiplication but allow cell metabolism to continue for 6 or 7 days (7). Two days after the subcutaneous inoculation of x-radiated tumor cells a nodule formed consisting of a central necrotic area and a rim of enlarged nondividing tumor cells (Fig. 6). The transplants were often encapsulated by a heavy layer of metachromatic connective tissue. These x-radiated tumor cells were capable of stimulating adjacent mammary duct epithelium as shown in Table 2 and Figure 7. However, the mitotic activity was not as high as that in mammary duct epithelium adjacent to actively growing tumor cells.

Since both the nonirradiated and the irradiated tumor transplants contained areas of necrosis and induced connective tissue formation, the role of these components in mammary duct stimulation was investigated. Fortunately, in some animals the tumor had failed to grow and was completely necrotic on the 2d day after inoculation. Table 2 indicates that these necrotic tumor cells did not produce any significant stimulation (3 per cent) in the adjacent mammary duct epithelium. These results were substantiated by additional data, represented in Table 2, which indicated that necrotic tissue of nonmalignant origin did not have a significant effect on adjacent mammary tissue either. The slight increased mitotic activity (25 per cent) was confined to ducts adjacent to the area of necrosis and was probably due to damage, as will become clear in the succeeding sections.

To induce connective tissue proliferation and test its role in mammary duct stimulation, we implanted tumor transplants, inactivated by boiling, in the mammary gland area. Table 3 indicates that these boiled tumor transplants, residing in the mammary gland region for 2 or 4 days, did not affect adjacent mammary ducts, even though the transplants became surrounded by a thin capsule of metachromatic connective tissue. The slight increase in mitotic activity (11 per cent) occurred only in ducts located inside or next to the area of connective tissue proliferation. Since the boiled tumor transplants had no significant effect on adjacent mammary duct epithelium, even though there was connective tissue proliferation, it was decided to further increase the amount of connective tissue proliferation by placing paraffin pellets in the mammary gland region. As Table 3 indicates, the presence of foreign pellets could stimulate mitotic activity in adjacent mammary duct epithelium (225 per cent). On analysis of these data it became apparent that mammary duct stimulation occurred only in ducts located within or next to the area of pellet-induced damage, whereas no mitotic activity occurred at a distance from the area of connective tissue proliferation. One could also correlate the mitotic activity in mammary ducts with the degree of damage inflicted by the pellet. Figure 8 represents a nontreated mammary duct close to an area of mild connective tissue proliferation, whereas Figure 9 shows an exceedingly high mitotic activity in a mammary duct located 200 μ away from a severely damaged area (Fig. 10).

Effect of EAT on the overlying vaginal epithelium of ovariectomized mice.—To determine whether local estrogen secretion by the tumor cells could be responsible for the mammary gland stimulation, the tumor cells were grown in the vaginal wall of six adult ovariectomized mice. These tumor cells were unable to induce cornification of the overlying vaginal epithelium. All these animals had normal estrous cycles before ovariectomy. Of a total of 90 vaginal smears, only two contained any cornified cells. Figure 11 demonstrates the close proximity of tumor and vaginal epithelium and the absence of cornified cells.

DISCUSSION

The purpose of the above experiments was to investigate the mechanism of mammary duct stimulation by subcutaneous tumor transplants. The above data first of all indicated that the stimulation was not specific for the EAT, since the S-180 exerted a similar mitogenic effect on mammary duct cells. The S-180, in contrast to the presumably epithelial nature of the EAT, was of connective tissue origin. Contrary to the EAT, which did not affect adjacent mammary duct cells until the 2d day after tumor implantation (2), the S-180 exerted its effect on adjacent mammary duct epithelium on the 1st day of tumor residence. The increased mitotic activity was still present on the 2d day of tumor growth, after which it declined. The difference in the pattern of stimulation between EAT and S-180 cannot be explained at the moment but might be owing to a difference in tumor growth rate or pattern.

Ascitic fluid, devoid of tumor cells, did not stimulate mammary duct epithelium. Nor was it required for the growth of EAT cells, because saline-washed tumor cells grew at a similar rate as untreated EAT cells and stimulated adjacent mammary duct epithelium as usual. This mammary duct stimulation, like that induced by untreated EAT, occurred up to a distance of 1 mm away from the tumor nodule, but not all ducts in this zone were affected.

We next tested whether dividing tumor cells
### TABLE 1

**Effect of Saline-washed Ehrlich Ascites Tumor Cells on Adjacent Mammary Duct Epithelium of 10-Day Ovariectomized Mice**

<table>
<thead>
<tr>
<th>Days of Tumor Growth</th>
<th>No. mice</th>
<th>Tumor size (mm.)</th>
<th>Mammary ducts</th>
<th>Lining</th>
<th>Mitotic activity (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>No. layers</td>
<td>Height (µm)</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>5×7</td>
<td>400</td>
<td>1-3</td>
<td>2.4-21.6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6×8</td>
<td>253</td>
<td>1-3</td>
<td>2.4-24</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>12×19</td>
<td>246</td>
<td>1-4</td>
<td>2.4-28.8</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td></td>
<td>225</td>
<td>1-2</td>
<td>2.4-12</td>
</tr>
</tbody>
</table>

### TABLE 2

**Effects of 2-Day-Old X-radiated Ehrlich Ascites Tumor Nodules and Necrotic Implants on Adjacent Mammary Duct Epithelium of Ovariectomized C57BL Mice**

<table>
<thead>
<tr>
<th>Type of Implant</th>
<th>No. mice</th>
<th>Size nodule (mm.)</th>
<th>Mammary ducts</th>
<th>Lining</th>
<th>Mitotic activity (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>No. layers</td>
<td>Height (µm)</td>
</tr>
<tr>
<td>X-rad. EAT</td>
<td>10</td>
<td>5×7</td>
<td>357</td>
<td>1-3</td>
<td>2.4-24</td>
</tr>
<tr>
<td>Necrotic tissue: EAT</td>
<td>5</td>
<td>5×7</td>
<td>90</td>
<td>1-2</td>
<td>4.8-14.4</td>
</tr>
<tr>
<td></td>
<td>Adult Embryonic</td>
<td>8</td>
<td>5×7</td>
<td>188</td>
<td>1-3</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
<td></td>
<td>402</td>
<td>1-2</td>
<td>2.4-14.4</td>
</tr>
</tbody>
</table>

### TABLE 3

**Effect of Boiled Tumor Transplants (B.T.) and Paraffin Pellets (P.P.) on Adjacent Mammary Duct Epithelium of 10-Day Ovariectomized Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. mice</th>
<th>Mammary ducts</th>
<th>Lining</th>
<th>Mitotic activity (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>No. layers</td>
<td>Height (µm)</td>
</tr>
<tr>
<td>B.T.—2 days</td>
<td>7</td>
<td>214</td>
<td>1-2</td>
<td>2.4-14.4</td>
</tr>
<tr>
<td>4 days</td>
<td>7</td>
<td>314</td>
<td>1-2</td>
<td>2.4-14.4</td>
</tr>
<tr>
<td>P.P.—2 days</td>
<td>8</td>
<td>291</td>
<td>1-3</td>
<td>2.4-21.6</td>
</tr>
<tr>
<td>4 days</td>
<td>6</td>
<td>149</td>
<td>1-3</td>
<td>2.4-21.6</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>223</td>
<td>1-2</td>
<td>2.4-14.4</td>
</tr>
</tbody>
</table>
were required for mammary duct stimulation. Multiplication of the EAT cells was inhibited by in vitro x-radiation with 15,000 r, a dose which was shown to inhibit cell division but allow cell metabolism to continue for 6 or 7 days (7). These irradiated tumor cells were capable of stimulating adjacent mammary duct epithelium, although to a lesser extent than nonirradiated tumor cells. These results suggested that the metabolizing tumor cells produced some factor(s) to which the mammary duct cells responded. A possible analogous phenomenon was described by Puck (8), who showed that irradiated cells served as a “feeder” layer for cell clones and by Révész (9), who found that irradiated tumor cells stimulated the growth of nonirradiated tumor cells. On the other hand, the irradiated tumor transplants, like the untreated nodules, contained areas of necrosis and connective tissue. Before ascribing the stimulatory effects of tumor transplants to the tumor cells per se, it was important to investigate the role of necrosis and of connective tissue in this phenomenon.

Implantation of necrotic tissues, of malignant or normal origin, did not significantly stimulate adjacent mammary duct epithelium. Nor did the connective tissue capsule, which formed around implanted boiled tumor transplants, affect the neighboring mammary ducts to any significant extent. The slight increase in mitotic activity that did occur was confined to those ducts located within or right next to the area of necrosis and/or connective tissue proliferation, and we believe that this mitotic activity was due to direct damage of the mammary ducts. Necrosis and connective tissue by themselves therefore did not seem to produce any substance which could affect the mammary duct cells at a distance. This does not mean that the necrotic areas and the tumor-induced connective tissue played no role in the stimulatory phenomenon, because both necrosis (6) and connective tissue (12) may be important for the growth of tumor cells. It is, therefore, possible that necrosis and/or connective tissue did affect the growth of tumor cells, which in turn stimulated the adjacent mammary ducts.

If one increased the amount of connective tissue proliferation and damage by implanting foreign pellets in the mammary gland region, one could “mimic” the mammary duct stimulation by tumor transplants. This raised the question whether stimulation of mammary duct cells by pellets and tumor transplants might have a common mechanism. Bullough (5) reported recently that epidermal damage resulted in increased mitotic activity in the surrounding epithelial cells up to a distance of 1 mm. Tumor cells do release catheptic enzymes in the surrounding tissues (10) and thus may cause destruction. Therefore, the above observed mitotic activity in the mammary duct cells may have been due to the damage caused by the growing tumor nodule.

On the other hand, recent evidence from studies on the stimulation of skin by the EAT (4) suggested that the stimulation of mammary ducts by the tumor transplants was not due to damage. These studies showed that implantation of the EAT beneath the dorsal skin of C57BL mice increased the mitotic activity in the overlying epidermis but did not stimulate the growth of resting hair follicles. Direct damage, on the other hand, would cause epidermal stimulation as well as the growth of adjacent resting hair follicles (3). These results suggested that the tumor elaborated some principle which stimulated the mitotic activity in the epidermis but which was different from the mitogenic factor produced by wounds.

The absence of keratinization of vaginal epithelium, overlying the growing EAT, seemed to indicate that the tumor cells did not produce estrogen, at least not in sufficient quantities to induce vaginal cornification. It appeared probable, therefore, that the mammary duct stimulation by tumor transplants was not due to a local secretion of small quantities of estrogen. This observation was already suggested by earlier findings1 that the mammary gland of a mammary

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Fig. 1.—High mitotic activity in a mammary duct located at a distance of 400 micra from a 2-day-old S-180 transplant. The black dots represent colchicine-arrested mitotic figures. x320.

Fig. 2.—Increased height of epithelial lining and intra- luminal projection in a mammary duct located 800 µ away from a 24-day-old S-180 transplant. X720.

Fig. 3.—High mitotic activity in a mammary duct located 250 µ away from a 8-day-old EAT transplant. The tumor cells were washed with saline before inoculation. Some of the colchicine-arrested mitoses are indicated by arrows. X420.

Fig. 4.—Stimulated mammary duct, located right next to a 24-day-old EAT transplant. As above, the cells were washed free of ascitic fluid prior to their inoculation. Note the increased number of cell layers in the mammary duct lining, the cell enlargement, and the diminution of duct lumen. X860.

Fig. 5.—Control mammary duct in the contralateral gland of an EAT-bearing mouse. At same magnification as the above stimulated duct. X860.

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1 Submitted for publication to the J. Nat. Cancer Inst.
FIG. 6.—Histological appearance of a 2-day-old nodule of x-radiated EAT cells. Note the central necrosis (bottom right), the peripheral rim of large, nondividing tumor cells, and the surrounding connective tissue (upper left). X150.

FIG. 7.—High mitotic activity in a mammary duct located 200 µ away from a 2-day-old irradiated EAT nodule. X390.

FIG. 8.—Mammary duct, 100 µ away from an area of mild connective tissue proliferation (upper right), induced by the 48-hour residence of a paraffin pellet. The mammary duct was not affected. X160.

FIG. 9.—High mitotic activity in the lining of a mammary duct located 200 µ away from an area of severe damage, inflicted by a 2-day-old paraffin pellet. The duct was surrounded by granulation tissue. This picture is a magnification of the insert in Fig. 10. X200.

FIG. 10.—Intense connective tissue proliferation and damage induced by a 2-day-old paraffin pellet. X40.

FIG. 11.—Ehrlich ascites tumor cells, growing in the vaginal wall of a 34-day ovariectomized, 5-month-old C57BL mouse. Note the absence of keratinization in the overlying vaginal epithelium. X270.
cancer-susceptible strain of mice did not respond as much to the tumor stimulus as did mammary tissue of a mammary cancer-resistant line of mice. Since the mammary glands of mammary cancer-susceptible mice were reported to be more sensitive to estrogen stimulation than those of mammary cancer-resistant lines (11), one would have expected a greater response to the tumor stimulus in the mammary cancer-susceptible mice if estrogen had been the underlying mechanism.

At the moment we cannot be conclusive about the exact nature of mammary duct stimulation by tumor transplants. We can suggest that living, but not necessarily dividing, tumor cells were required and that estrogen production, necrosis, or connective tissue proliferation by themselves were not responsible. Whether the tumor caused mammary duct stimulation by way of damage is a question which remains to be answered.

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REFERENCES
Mechanism of Mammary Duct Stimulation by Tumor Transplants

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