Tumors Produced by Estradiol Benzoate in the Guinea Pig

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(Received for publication April 21, 1941)

Nelson (4) injected 32 guinea pigs with various estrogens for periods ranging from 2 to 10 months, and in 6 of these animals succeeded in producing in the cornua or fundus of the uterus one or more growths which appeared to be definitely fibromyomatous. Lipschütz (2) and collaborators, in an extended series of experiments, produced similar tumors not only on the uterus but on many of the abdominal organs, including the stomach, spleen, and omentum. He noted that ovariectomized guinea pigs almost invariably developed growths after the administration twice weekly for 2 to 3 months of 80 mgm. of estradiol benzoate. The tumors that resulted were not observed to undergo malignant change.

A second group of 3 male guinea pigs received estradiol benzoate pellets in the same manner, without preliminary operative procedure. Two pellets weighed 25 mgm., the third 50 mgm. One of the animals with a 25 mgm. pellet was allowed to live 90 days, the other two were kept for 148 days and then killed.

RESULTS

Each animal of the first group had well over a hundred small, firm, white, translucent nodules of varying size; the majority were sessile, a few pedunculated (Table I). Each nodule was roughly spherical and measured from less than 0.5 mm. to 2 cm. in diameter. The tumors were scattered irregularly over the peritoneal surface (Fig. 1). The sites of predilection seemed to be the surface of the spleen, stomach, and adjacent diaphragm, the terminal ileum, and omentum. Careful examination revealed no similar changes either on the other serous surfaces, including the pleura and pericardium, or in the parenchymatous tissues.

The vaginal cuff in four instances was the site of a very much larger nodule than was found elsewhere. These were directly associated with the suture used in closing the cuff. On section, three of them were found to have small central abscess pockets which drained into the vagina.

The pellets, found to be almost their original size,
were encapsulated by a thin layer of connective tissue. Beyond this no gross sign of local reaction was seen. The musculature beneath the pellets showed no reaction, and the underlying peritoneum showed no greater tendency toward tumor formation.

Some tumors were typical fibromyomas, the fibrocytes and muscle cells differentiating clearly with Masson’s stain. Muscle fibrils could also be demonstrated with phosphotungstic acid hematoxylin. The great majority of small nodules, however, presented a somewhat different picture. There were no elements within them which stained specifically for muscle. The nuclei of the cells were narrow and ribbon-like, and the abundant intercellular tissue stained green with Masson’s stain, indicating its collagenous nature. These would have to be classified as fibromas.

Many of these nodules had a third element within them which was somewhat confusing. This consisted of large cells with vesicular nuclei; and with cytoplasm which was colored more or less pink with Masson’s stain. Some of these cells were occasionally caught in division. These cells occurred in clusters in some areas, while in others they appeared to line ducts and crevices within the tumor. When the ducts were traced to the surface it could be seen that these cells covered the tumor. They were often arranged several layers deep, and at times were markedly hypertrophied. Projecting from the surface as columnar-like epithelium, their free ends were destined to give the whole a tear-drop appearance (Fig. 2). This was particularly marked at the edge of the tumor where the cells were not spread out and a fairly steep gradation to the usual flat serosal cell type occurred in a very short distance from the tumor. The relation of these cells to the serosal cell has not been established, but it is clear that they continued into the above-described crevices, and appeared as acinar-like masses within the connective tissue stroma of the nodules.1 Both in the crevices and in the acinar-like arrangement, these cells at times formed several layers, and again showed evidence of growth on the surface (Fig. 3). Efforts to demonstrate muscle fibrils within them met with failure.

Around the central portion of three growths on the vaginal cuff there was a fibrous wall infiltrated by a small number of polymorphonuclear leucocytes.

TABLE II: MALE GUINEA PIGS RECEIVING ESTRADIOL PELLET SUBCUTANEOUSLY

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>Estradiol benzoate mgm.</th>
<th>Period in days</th>
<th>Site of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>25</td>
<td>148</td>
<td>Testes</td>
</tr>
<tr>
<td>22</td>
<td>25</td>
<td>90</td>
<td>Stomach</td>
</tr>
<tr>
<td>29</td>
<td>50</td>
<td>148</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diaphragm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parietal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peritoneum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mesentery</td>
</tr>
</tbody>
</table>
| + = presence of tumor; - = no tumor present.

Among the second group, (Table II), nothing remarkable was found in one guinea pig exposed to a 25 mgm. pellet for 90 days. The second animal, 148 days after the pellet had been implanted, showed only a few small nodules on the lateral abdominal wall. However, the third, which had harbored a 50 mgm. pellet for 148 days, had several hundred small nodules entirely similar to those of the first group. Here, in addition to the usual sites, many small nodules were present on the serosal surface of the testes.

It should be emphasized that similar nodules have not been found in any of the many guinea pigs autopsied during the course of various experiments in the laboratory.

Histologically, all of the tumors appeared to originate from the serosal or subserosal layer of the peritoneum. They projected into the cavity, atrophy being the only indication of change in the underlying tissues. Among the second group, (Table II), nothing remarkable was found in one guinea pig exposed to a 25 mgm. pellet for 90 days. The second animal, 148 days after the pellet had been implanted, showed only a few small nodules on the lateral abdominal wall. However, the third, which had harbored a 50 mgm. pellet for 148 days, had several hundred small nodules entirely similar to those of the first group. Here, in addition to the usual sites, many small nodules were present on the serosal surface of the testes.

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1 Since this work was completed, Lipschütz and Vargas (3) have noted an intimate relation between the cells of the serosa and certain cells of the tumor. This is in agreement with the present findings.
This was surrounded by a much larger mass of quite young granulation tissue which closely resembled the majority of the tumors in structure.

In sections, the positions of the pellets of estradiol benzoate were indicated by a nuclear free matrix, from which the estrogen had been dissolved by the dehydrating agents. This was surrounded by a thin layer of connective tissue. Within this tissue were many vacuolated giant cells, particularly near the periphery, while strands of capillaries and granulation tissue ramified further into the matrix.

In all except one female, the vaginal mucosa showed the large foamy cells typical of estrogenic stimulation. In this animal no pellet could be found at autopsy. It was therefore assumed that the pellet had sloughed out. Small nodules remained on the peritoneum, however, which had been produced while the pellet was in place.

**DISCUSSION**

The high incidence of these tumors, 100 per cent in the first group of 9 guinea pigs, together with their multiplicity, sites of origin, and the constancy of involvement of these sites, is significant and substantiates the previous work of Lipschütz.

The absence of uterine tissue seemed to have exerted no effect on the production, frequency, or distribution of tumors resulting from estradiol benzoate.

Why these tumors should have such a widespread distribution throughout the abdominal cavity, but be absent from all other parts of the body, is not clear.

Fig. 2 (Left).—Showing the marked hypertrophy and hyperplasia of the serosal covering cells of a tumor. Mag. X 150.

Fig. 3 (Right).—Duct-like extensions or inclusions composed of hyperplastic serosal cells. Mag. X 300.

The tumors consisted of proliferating fibrous tissue and occasionally muscle tissue. Structures interpreted as the participation of serosal cells in the tumor are a prominent and highly peculiar feature. The relation of this hyperplastic cell to the formation of the tumor requires further investigation.

Three of the four large tumors found on the cuff of the vagina appeared to have been formed about an abscess pocket. Since these three were composed particularly of granulation tissue, the question is raised whether they may represent estrogenic stimulation of granulation tissue. The abdominal scars showed no tendency toward keloid formation, but this is hardly
to be expected since the wounds were well healed before the pellet was introduced.

SUMMARY AND CONCLUSIONS

1. Ovariectomized and hysterectomized female guinea pigs and a male guinea pig which received a 50 mgm. pellet of estradiol benzoate subcutaneously developed tumors in the peritoneal cavity in 100 per cent of the cases.

2. Muscle fibers were found in only a small number of these tumors; the majority of tumors appeared to be fibromas.

3. The absence of uterine tissue had no effect either on the frequency or distribution of these tumors.

4. The serosal cells seemed to participate in the formation of the tumor. They occurred as small aggregations of actively growing cells where the tumor and serosa joined, and also lined crevices and extended into the tumor as pseudo-acinar structures.

5. An extensive overgrowth of granulation tissue, histologically similar in many respects to the tumors, occurred in relation to three abscess pockets.

REFERENCES


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