

Development of Leiomyomas in Female Rats with an Endocrine Imbalance*

CARROLL A. PFEIFFER, PH.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut)

Smooth muscle tumors of the uterus are rare in rats. In our colony such tumors have never been found. It is true, however, that only a fraction of the colony has been allowed to reach extreme old age before autopsy. Bullock and Curtis (1) have data on over 2450 rats with cysticercus tumors and 489 rats with independent or primary spontaneous tumors. In the latter group there were 48 uterine tumors of which 2 were myosarcomas and 4 were leiomyomas. Therefore, the presence of leiomyomas in 4 of 16 endocrine imbalance female rats that had survived more than 3 years seems well worth reporting.

MATERIAL AND METHODS

One hundred and eighty-five newborn female rats received testis grafts in the neck region by the technique of Pfeiffer (6). The method of administering the cold anesthesia in this technique has been gradually modified so that the animals are no longer chilled in the refrigerator but instead are placed on the ice in the freezing trays which are set out at room temperature. As soon as the animals become unresponsive to tactile stimuli, which occurs in a very few minutes, they are removed and quickly operated. If they begin to respond to pain stimuli before the operation is completed they are placed back on the ice and the operation is finished there. If this is not convenient they are allowed to cool down again and removed to finish the operation. With a little practice the animals can be placed on the ice a few at a time so that deep anesthesia is attained at almost the exact time that the animal is needed for operation. The short exposure to cold and the rapid return to normal temperature greatly reduces the mortality.

The donor was always a littermate of the host. Usually 2 testes were transplanted into each female (142 cases), but due to the variations in the sex ratio in the litters, the number of testes grafted ranged from 1 to 4. It has been shown that 1 testis graft, if it takes well, is sufficient to produce the endocrine imbalance (6) but 2 or more increase the chance of a good take. Subsequent to weaning, these animals were fed a commercial dog chow diet which varied somewhat throughout the ex-

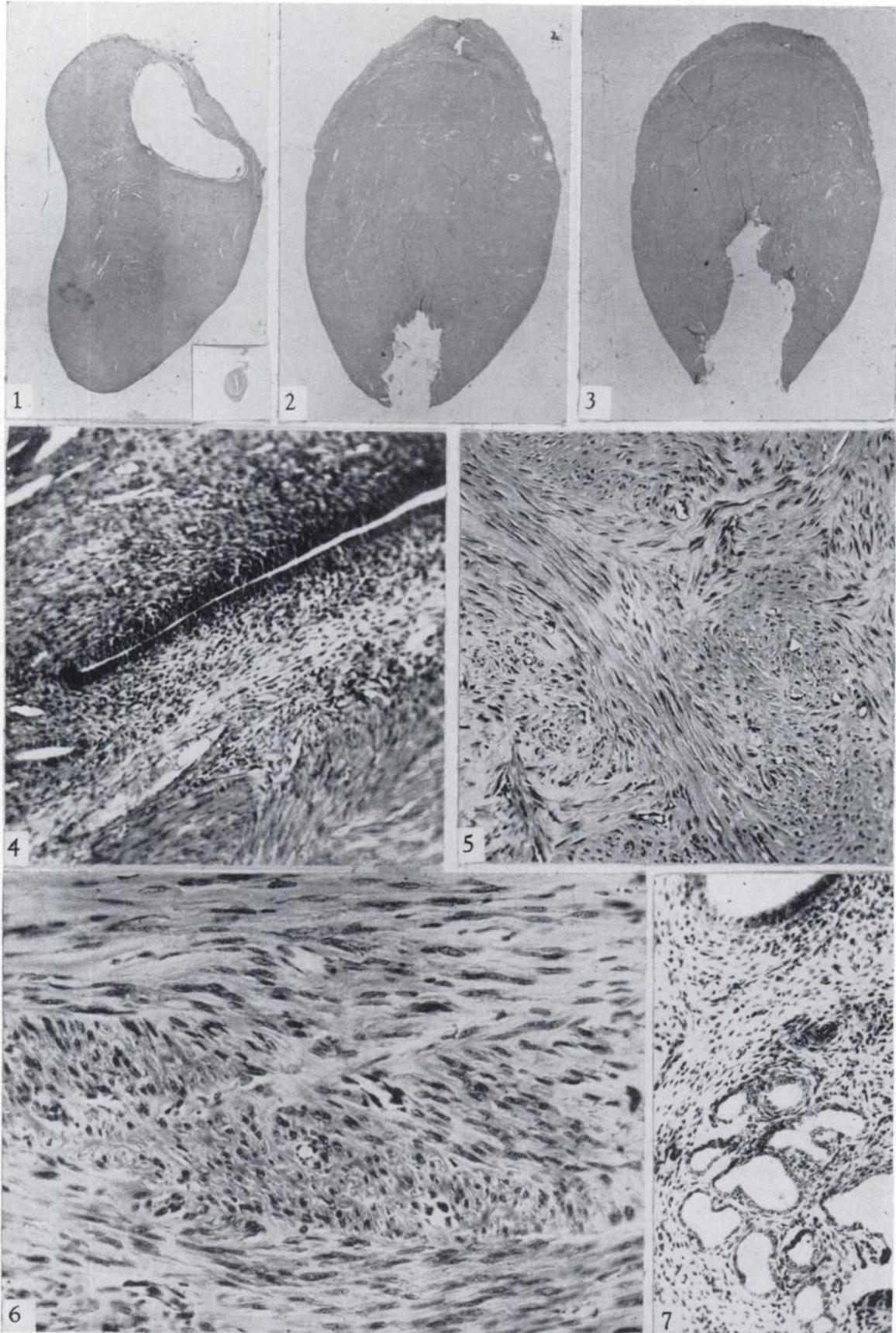
periment due to wartime conditions and animal food shortages. However, they were kept under the same conditions as the rats in the breeding colony which served as controls. All tissues were preserved in Bouin's fluid, imbedded in paraffin, sectioned at $8\ \mu$ and stained with Erlich's hematoxylin and triosin or mordanted with Zenker's fluid and then stained with either Mallory's or Masson's connective tissue stain.

OBSERVATIONS

An endocrine imbalance is usually established in the female rats with testis transplants by the time they reach sexual maturity. This fact is expressed by the presence of constant estrus as shown by Pfeiffer (6). Vaginal smears were not followed continuously during the present study because the constant estrus condition has been well demonstrated by the hundreds of animals of this type described previously (6, 7, 8), and used in unpublished investigations. Pyometra is usually observed in these animals beginning after 6 months of age and becoming progressively more common as the period of constant estrus lengthens. The condition becomes increasingly severe until the major part, or even the whole, of the genital tract is involved. As the pyometra becomes more extreme, the uterus ulcerates into the body cavity, forms adhesions and often ulcerates through the body wall to the exterior or into the digestive tract, resulting in the death of the animal. Very few animals survive the pyometra, but there is some evidence, although proof is lacking, that all the animals that do live until senility have at some time had pyometra.

Of the 185 animals under observation only 16 survived for more than 3 years. Four of these 16 animals had uterine tumors which on histological examination proved to be leiomyomas. One of the animals had 3 tumors; one (25×15 mm.) was located in the lower third of the right cornu, another (10×8 mm.) was situated in about the middle of this horn, and the third was located near the cervical end of the left horn of the uterus. The other 3 animals each had a single tumor; in 1 it

* This investigation has been supported by grants from The Anna Fuller Fund and the National Cancer Institute (U.S.P.H.S.).



Figs. 1-7

was in the right horn and in the other 2 it was in the left horn. In all of these animals there was evidence of pyometra.

All of the tumors except the largest one were eccentric in respect to the rest of the uterus, a condition which indicates that the tumor started as a localized area in one side of the uterus. In the animals which did not have any tumors the uterine wall was somewhat thickened. This was especially evident in comparison with the uteri of old normal female rats. The ovaries of the animals with tumors had much the same gross appearance as the ovaries of senile animals. There was no luteal tissue that could be detected macroscopically. There was, however, some evidence that luteal function was not completely suppressed in some of the animals which had not developed tumors. Occasionally a remnant of a testis graft was found at the time of autopsy.

With hematoxylin and triosin stain all the tumors appeared to be composed entirely of smooth muscle cells. However, with connective tissue stains (Mallory's and Masson's) it was seen that varying amounts of connective tissue were present. The smaller tumors were composed entirely of smooth muscle (Fig. 6), but as the tumors became larger, fine collagenous fibers became interspersed among some of the muscle fibers. There was a gradual increase in the fibrous tissue until in the largest tumor it greatly predominated, with large areas of connective tissue separating the smooth muscle portions of the tumor. There were also large areas of mixed muscle and connective tissue (Fig. 5). The tumors had the appearance that as they increased in size the proliferation of connec-

tive tissue greatly exceeded that of smooth muscle.

On the mesometrial side of the largest tumor (Fig. 1) there was a fairly large necrotic area which was presumably the former lumen of the uterus and is interpreted as having resulted from pyometra. The thin wall on the mesometrial side had the same appearance as the subserosa of any uterus in which there is marked pyometra. On the opposite side the necrosis had extended to the tumor tissue, leaving no indication of an intervening endometrium. The tumor was well vascularized, and there was no evidence of necrosis in the tumor proper. It had apparently grown slowly, allowing complete organization of muscle bundles and vascular channels, both of which became separated by large amounts of connective tissue. The tumor was completely encapsulated, and there was no evidence of invasion or metastasis. From the histological appearance of the tumor it is unlikely that it would ever have become malignant.

The four smaller tumors showed very clearly that each arose as a single hyperplastic muscle growth which, as it increased in size, displaced the remainder of the uterus in such a way that the latter formed an eccentric cap over one side of the tumor (Figs. 2 and 3). Except when the pyometra was extreme, all of the layers of the uterus were complete in this cap, including the endometrial glands (Fig. 4). The muscle layers on the tumor side of the lumen were lost in, and continuous with, the muscle fibers of the tumor. The serosa and the subserosal layers of connective tissue were stretched to form the capsule of the tumor. It was impossible to determine whether the tumor

FIGS. 1-7.—All of the tissues were sectioned at 8μ and stained with Erlich's hematoxylin and triosin.

FIG. 1.—An extremely low power view of a cross section of the largest leiomyoma found in an endocrine imbalance female rat, showing the general characteristics of the tumor. The cavity is where pyometra had involved and distended the original lumen of the uterus. The thin wall is similar to that found around the large necrotic mass produced by pyometra. The other side is composed of tumor tissue. For comparison, a cross section of the uninvolved horn of the uterus at the same magnification as the tumor is shown in the small insert. $\times 2.5$.

FIGS. 2 and 3.—Cross sections of medium sized tumors illustrating how the smooth muscle tumor forms in a localized area in the muscle layer. As the tumor grows, the serosa remains intact and the remainder of the uterus becomes stretched over the tumor as a relatively thin eccentric cap, shown at the extreme top of each photograph. The tumor tissue is slightly darker than the rest of the uterus so that the junction between them can be seen even at this magnification. The small cavity in the uterine tissue at the very top of each section is the uterine lumen. The ragged outline at the lower edge is due to the fact that these are not quite complete cross sections. $\times 8$.

FIG. 4.—Photomicrograph of the stretched normal uterine tissue which forms a cap over the tumor. The uterine lumen, which may be seen extending diagonally across the photograph, has been elongated by the stretching of the uterus over the tumor. Note the relatively low columnar epithelial lining of the lumen, the normal uterine glands at the left of the lumen and the normal tunica propria. The muscle tissue of the tumor may be seen in the lower right hand corner of the photograph. $\times 110$.

FIG. 5.—An area of a leiomyoma showing both smooth muscle and connective tissue in a mixed portion of the tumor. $\times 110$.

FIG. 6.—A high power view of the smooth muscle which comprises the entire tumor when it is small. $\times 220$.

FIG. 7.—A portion of the tunica propria of an uninvolved segment of the uterus, illustrating the chronic low estrogen effect. The uterine epithelium at the top of the picture is only slightly stimulated. The glands are well developed without hypertrophy of the epithelial cell lining. $\times 110$.

started in the circular or in the longitudinal muscle layers, but it certainly must have involved both at an earlier stage than we have been able to observe.

The portions of the uterine horns not involved in the tumors showed some hypertrophy. If the mucosa was eroded at the tumor level it was usually almost completely absent throughout the remainder of the uterine horn. Conversely, if the endometrial epithelium was intact in the tumor region it was complete at other levels, but the tunica propria was thickened, and the uterine glands were often distended (Fig. 7). In all cases the epithelium was low columnar (Fig. 7) indicating that the estrogen level was relatively low. The tunica propria was thickened by increased amounts of collagenous tissue. Both circular and longitudinal muscle layers were hypertrophied, and the entire uterus was heavily vascularized.

DISCUSSION

The tumors in the rats' uteri described here are similar in all respects to the fibromyomas which occur so frequently in the human. Approximately 20 per cent of all women have fibromyomas. The incidence is much higher in unmarried women beyond middle life, but as the gynecologists point out, these figures are misleading since a great many of these tumors give no symptoms. Curtis (2) states that "the origin of myomatous tumors remains unexplained, although evidence is at hand which indicates that disturbed balance of the ovarian hormones, notably hyperestrinism, is an important etiologic factor." Earlier, Witherpoon (10) had suggested that there is a correlation between hyperestrinism and the occurrence of fibromyomas. Credence is given to this suggestion by the fact that not only have fibroids been produced in guinea pigs by prolonged treatment with estrogen (5) but they have been produced with ease in this animal with many kinds of estrogenic compounds (3, 4). This hypothesis is, however, far from being generally accepted.

Leiomyomas are extremely rare in rats, although Bullock and Curtis (1) have shown that they do occasionally occur. Therefore, it is felt that the fact that 4 of 16 constant estrus female rats which survived over 3 years developed leiomyomas would indicate that the endocrine imbalance imposed on these animals had increased the chance of the formation of such tumors. These animals could not, of course, become pregnant, which fact places them in a different category from the females usually found in a colony and more nearly likens them to the unmarried group in

the human. It may be that the conditions of pregnancy, which alter the relatively short cyclic changes in the uterus, have in themselves an inhibitory effect on the production of leiomyomas. Multiple leiomyomas have not previously been described in the rat, a fact commented on by Bullock and Curtis (1). It has been shown in the present investigation, however, that multiple myomas of the uterus occur in rats just as they do in humans.

The endocrine imbalance in these rats keeps the uterus under the influence of a chronic low level of estrogen which allows pyometra to develop in almost all of the animals. This is undoubtedly due to the continuous exposure of the uterus to the bacterial flora of the vagina (9). It is felt that when the animals are able to survive the pyometra until old age, the chronic stimulation of the smooth muscle by the estrogen greatly enhances the chances of tumor formation. The fact that the pyometra prevents prolonged survival, together with the fact that leiomyomas are rare in the rat, makes it extremely difficult to analyze the factors responsible for the tumors described here. It is doubted that the administration of exogenous hormones would be as successful in stimulating the formation of tumors while avoiding the lethal effects of pyometra as were the endogenous hormones produced under this endocrine imbalance.

SUMMARY

Of 185 endocrine imbalance female rats, 16 survived over 3 years. Leiomyomas were present in 4 of these 16 animals. One animal had multiple tumors; 2 were located in the middle and lower third of the right cornu, and a third was near the cervical end of the left cornu. The other 3 animals had single tumors. In one of these the tumor was located in the right cornu. In the other 2 it was in the left cornu. It is believed that the chronic estrogen stimulation resulting from the endocrine imbalance increases the chance of smooth muscle tumor formation.

REFERENCES

1. BULLOCK, F. D., and CURTIS, M. R. Spontaneous Tumors of the Rat. *J. Cancer Research*, 14:1-115, 1930.
2. CURTIS, A. H. *A Text Book of Gynecology*. 5th ed. Philadelphia: W. B. Saunders Co., 1946.
3. LIPSCHÜTZ, A., and VARGAS, L., JR. Tumorigenic Powers of Stilboestrol and Follicular Hormones. *Lancet*, 1:541-543, 1940.
4. LIPSCHÜTZ, A., VARGAS, L., JR., EGANA, E., and BRUZZONE, S. Conjunctive Tumorigenesis Elicited by Different Artificial Estrogens. *Proc. Soc. Exper. Biol. and Med.*, 48:467-471, 1941.
5. NELSON, W. O. Endometrial and Myometrial Changes,

- Including Fibromyomatous Nodules, Induced in the Uterus of the Guinea Pig by the Prolonged Administration of Oestrogenic Hormone. *Anat. Rec.*, **68**:99-102, 1937.
6. PFEIFFER, C. A. Sexual Differences of the Hypophyses and Their Determination by the Gonads. *Am. J. Anat.*, **58**:195-225, 1936.
 7. PFEIFFER, C. A. Hypophyseal Gonadotropic Hormones and the Luteinization Phenomenon in the Rat. *Anat. Rec.*, **67**:159-175, 1937.
 8. PFEIFFER, C. A. Interstitial Cell Stimulation and Luteinization under the Influence of Male and Female Hypophyses. *Yale J. Biol. and Med.* **14**:619-630, 1942.
 9. WEINSTEIN, L., GARDNER, W. U., and ALLEN E. Bacteriology of the Uterus with Special Reference to Estrogenic Hormones and the Problem of Pyometra. *Proc. Soc. Exper. Biol. and Med.*, **37**:391-393, 1938.
 10. WITHERSPOON, J. T. A Possible Cause of Uterine Fibroids. *Endocrinology*, **17**:703-708, 1933.