Summary

Rabbits treated with large doses of progesterone for up to 763 days developed numerous cysts of the endometrium, sometimes associated with atypical hyperplasia. Active mammary secretion also occurred. After large doses of testosterone for up to 763 days 1 animal developed 2 adenomatous polyps of the endometrium, but there were no other noteworthy endometrial changes. Neither hormone produced significant alterations in other tissues including ovary, adrenal, thyroid, or pituitary gland. No precancerous endometrial changes or cancers were found.

Introduction

Various uterine abnormalities have been produced experimentally in rabbits by excessive estrogen stimulation in a previous study (3). These included endometrial polyps, hyperplasias, and carcinomas. The estrogen used was stilbestrol in oil. It was not considered a direct carcinogen, since a host reaction that included pituitary and adrenal cortical hyperactivity was implicated in the animals that developed endometrial carcinoma (6).

The clinical use of both progestogens and androgens has increased and often involves relatively large doses. A study was therefore undertaken to determine particularly whether progesterone and testosterone might have any long-term effects upon the endometrium similar to those of estrogen. We also wished to study abnormalities that might result in other tissues and to compare them with the experimental effects of excessive estrogen.

Materials and Methods

Female rabbits of mixed breeds were employed. They were obtained commercially, ostensibly as young virgin adults, averaging slightly over 3 kg in weight. The experiment was started with 56 animals, 19 of which were treated with progesterone, 21 with testosterone and the remaining 16 served as controls. They were maintained and fed under identical conditions; they were housed singly. All animals were observed for 10 days before beginning injections of hormones.

The progesterone preparation was hydroxyprogesterone caproate (Delalutin) which was suspended in sesame oil in a concentration of 125 mg/ml. The i.m. injections of progesterone were given on alternate weeks in doses calculated by kg weight of animal to correspond with a maximal recommended dose for humans (250 mg). Since the rabbits averaged slightly over 3 kg in weight, the average dose was about 13 mg of the progesterone preparation.

The testosterone preparation used was testosterone ethanate (Delatestryl) suspended in sesame oil in a concentration of 200 mg/ml. The dose was calculated as with the progesterone on per kg body weight of the animal to correspond with a maximal dose (300 mg) to humans; the average dose was about 15 mg.

Complete autopsies were performed on all rabbits when the animals were found dead or were sacrificed in extremis. At the end of 763 days of hormonal treatment the experiment terminated and the surviving rabbits were killed and autopsied.

Weights of the animals at intervals during life and at autopsy were recorded. After dissections performed as soon as possible after death, important solid organs were weighed and microscopic sections of the heart, lungs, trachea, spleen, pancreas, stomach, intestines, liver, adrenals, kidneys, bladder, vagina, cervix, uterus, oviducts, ovaries, lymph nodes, thyroid, skin, breasts, bone, bone marrow, brain, and pituitary were examined, stained routinely with hematoxylin and eosin. Slides were examined and reviewed by both investigators independently.

Pituitary cell counts of 25 anterior lobes were made, using Pearse's PAS trichrome stain and counting about 3200 cells per case.

Results

The duration of treatment to time of death of the animals varied from 178 to 763 days in the progesterone-treated group and from 54 to 763 days for the testosterone-treated group (Table 1). The controls were followed from 47 to 636 days. In 1 control animal the tissues were too autolyzed to allow interpretation. Nine of the 21 testosterone-treated animals and 8 of the 19 progesterone-treated animals survived the complete experiment. Since a number of animals died before the termination, it was possible to study certain changes in various stages of development.

Endometrial Changes

CONTROL ANIMALS. Secretory droplets in some of the epithelial cells were found at all ages, although not in all animals. In 1 older rabbit there was slight adenomatous hyperplasia of the endometrium and in 3 older animals there were a few cysts less...
TABLE 1

| Observation Period (days) of Female Rabbits Treated i.m. with Progesterone or Testosterone |
|---------------------------------|---------------------------------|---------------------------------|
| Control (15 rabbits, untreated) | Progesterone, av. 20 mg/month (19 rabbits) | Testosterone, av. 30 mg/month (21 rabbits) |
| 47                              | 178                              | 54                              |
| 170                             | 207                              | 169                             |
| 200                             | 350                              | 173                             |
| 223                             | 392 (2 animals)                  | 231                             |
| 280                             | 421                              | 255                             |
| 294                             | 502                              | 438                             |
| 311                             | 511                              | 504                             |
| 369                             | 552                              | 552 (3 animals)                 |
| 375                             | 623                              | 636                             |
| 370                             | 690                              | 710                             |
| 636 (5 animals)                 | 703 (8 animals)                  | 763 (9 animals)                 |

than 0.3 mm in diameter. In 2 instances there were small non-neoplastic polyps with a fibrous core.

PROGESTERONE-TREATED ANIMALS. The endometrium showed striking changes in the great majority. Secretory droplets in the cells decreased progressively with the duration of treatment until 552 days, after which secretion in individual cells was no longer found. Fourteen of the 19 cases showed cyst formation. The endometrial cysts were found as early as the 267th day, at which time they were small and resembled those seen in the control animals. With increasing duration of treatment, the cysts became progressively larger and more numerous (Fig. 1).

Seven of the 8 animals surviving the entire length of the experiment had numerous large cysts that often measured several mm in diameter (Figs. 2, 3). These cysts were unilocular and lined with a single layer of cuboidal or columnar epithelium, often ciliated (Fig. 4). The cysts contained a watery fluid and occasionally a few desquamated cells. There were no polyps of any kind, except for the polypoid cysts.

In 7 of the animals there was an associated atypical hyperplasia of the epithelium in the endometrium between the cysts. This occurred mostly in older animals, but was present once after 392 days of therapy (Fig. 5). The hyperplasia included irregularly shaped glands of varying size, hyperchromatic nuclei, pleomorphism of cells and nuclei, and occasional mitoses. There was no secretory activity in these hyperplastic foci.

TESTOSTERONE-TREATED ANIMALS. The endometrium generally resembled that of the control animals except that it was less active and secretion was rarely present in individual cells. Four cases showed a mild adenomatous hyperplasia (Fig. 6), and in 2 there were minute cysts similar to those seen in the control animals. There were no fibrous polyps, but in 1 rabbit 2 neoplastic adenomatous polyps were found after 763 days (Fig. 7).

OTHER ORGANS AND TISSUES

There were no significant changes in body weight or in the weights of any of the individual organs in the treated as compared with the controls. The breast in the testosterone-treated animals was often indistinguishable from the control animals but tended to be less active. The progesterone-treated animals uniformly showed grossly and microscopically an active lobuloplastic pattern with secretion and often a moderate dilatation of mammary ducts (Figs. 8, 9). No significant or distinctive changes were found either grossly or microscopically in other tissues including the ovary, adrenal, thyroid, or pituitary gland in the treated as compared with the untreated animals.

In the testosterone-treated animals the endometrium usually resembled that of the controls, but in 1 case there were 2 adenomatous polyps. These polyps were similar to those previously found after estrogenic stimulation.

Except for the expected secretory activity of the breast in the progesterone-treated rabbits (2), there were no noteworthy changes in any organs other than the endometrium. This is in contrast to findings previously reported in rabbits given large doses of estrogen where there frequently were significant changes in the ovaries, adrenal and pituitary glands. (1, 6).

Neither progesterone nor testosterone injected in oil in relatively large amounts continuously over long periods had any demonstrable carcinogenic effect. The 2 instances of malignant lymphoma were regarded as coincidental, particularly since 1 occurred in a control animal.

ACKNOWLEDGMENTS

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REFERENCES


MARCH 1966
Multiple small cysts in endometrium of animal treated with progesterone for 690 days. The cysts were mostly small, but larger than the cysts occasionally found in the endometrium of controls and testosterone-treated rabbits. The endometrial glands between the cysts were locally hyperplastic and distorted. × 30.

Fig. 2. Larger cysts of the endometrium in animal treated with progesterone for 763 days. The cysts contained watery fluid often with a few desquamated cells. × 30.

Fig. 3. Very large cysts in endometrium of animal treated with progesterone for 763 days. × 30.

Fig. 4. A portion of cyst wall and adjacent stroma. The cysts were lined with a single layer of cuboidal or columnar epithelium. Cilia were frequently identified. × 400.

Fig. 5. Focus of "atypical" hyperplasia in animal treated with progesterone for 392 days. Such abnormal glands were found in about half of the cases where there were endometrial cysts. × 125.

Fig. 6. Mild focal adenomatous hyperplasia of the endometrium in testosterone-treated animal after 763 days. × 125.

Fig. 7. Adenomatous polyp of endometrium in animal treated with testosterone for 763 days. The adjacent endometrium is inactive and characteristic of that found in most testosterone-treated animals, as well as in control rabbits. × 30.

Fig. 8. Progesterone treatment stimulated secretion in the developed mammary lobules and ducts. × 125.

Fig. 9. Breast tissue of control rabbits of corresponding age was inactive. × 125.
Endometrial Changes after Hormones
Endometrial Changes after Prolonged Progesterone and Testosterone Administration to Rabbits

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