They do emphasize the need to determine the relation between the electrophysiological abnormalities of the dystrophic muscle fiber membrane and the biochemical abnormalities of dystrophic muscles.

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8. The chickens were bred and maintained by the Department of Avian Sciences at the University of California, Davis.
9. The DPH (Dilantin, Parke-Davis) was administered in a diluent of 40 percent propylene glycol and 10 percent alcohol in water adjusted to *p*H 12. The dose of DPH, high by comparison to the typical human dose [see (*7*)], was chosen on the basis of preliminary trials with 50 chicks in which a single injection of 20 mg of DPH per kilogram produced rapid improvement in righting ability with no behavioral signs of involvement of the central nervous system.
10. This time period was chosen because exhaustion scores on days 1 to 12 after hatching were not sufficiently different to distinguish among the four groups (see Fig. 1).
11. The activity of LDH was measured at 340 nm, with 6 m*M* pyruvate being used as substrate [see G. H. Cardinet, R. A. Freedland, W. S. Tyler, L. M. Julian, *Am. J. Vet. Res.* **33**, 1671 (1972)].
12. The AChE activity was measured at 412 nm, with 75 m*M* acetylthiocholine iodide and 0.1 mmole of the selective inhibitor tetraisopropylpyrophosphoramide [see G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **1**, 88 (1961)].
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3 August 1976; revised 18 October 1976

Effect of Delta-9-Tetrahydrocannabinol on Uterine and Vaginal Cytology of Ovariectomized Rats

Abstract. *The estrogen-like activity of delta-9-tetrahydrocannabinol (Δ^9-THC), an active component of marihuana, as measured by uterine weight gain and vaginal smear techniques in ovariectomized rats, is reflected in histological examination of uterine and vaginal tissues. Doses of 1, 2.5, and 10 milligrams of Δ^9-THC per kilogram elicit hypertrophy and hyperplasia of the uterus; the dose of 2.5 milligrams per kilogram is most effective. There is an increase in stratification of vaginal epithelium with doses of 2.5 and 10 milligrams per kilogram; cornifying cells are seen with 2.5 milligrams per kilogram.*

A recent report (*1*) demonstrates that administration of delta-9-tetrahydrocannabinol (Δ^9-THC), one of the major components of marihuana, in doses within ranges used in human male studies (*2*), has significant estrogenic activity in ovariectomized rats as measured by uterine weight gain and vaginal smear bioassay techniques. We now report the results of histological examination of tissues obtained in the earlier study (*1*) from ovariectomized rats treated with Δ^9-THC. Cytologic studies of these tissues show that Δ^9-THC induces hyperplasia and hypertrophy of the uterus. Doses of 2.5 and 10 mg of Δ^9-THC per kilogram elicit an increase in the number of cell layers in the vaginal epithelium; cornifying cells are seen at the 2.5 mg/kg dose.

The effects of estradiol benzoate (EB) or Δ^9-THC, or both, were studied in ovariectomized rats by using the following parameters: the uterine weight gain and vaginal smear analyses for estrogens (*3, 4*) and histologic examination of uterus and vagina (*5*). Estrogen dosage was based on requirements for estrogen in spayed rats (*3, 4*). The doses of Δ^9-THC were based on our preliminary studies.

At 75 days of age, 55 female rats of the Charles River (CD) strain were bilaterally ovariectomized by removing the coiled fallopian tubes and ovarian capsule with the surrounding fat to assure complete removal of ovarian tissue. The animals were grouped so that the average body weight of each group was between 184 and 189 g, and treated as follows: group 1, sham ovariectomized rats treated intraperitoneally with saline to serve as controls for ovariectomy. Groups 2 to 5, ovariectomized rats treated with either sesame oil intraperitoneally or subcutaneously, or with saline intraperitoneally or subcutaneously to serve as controls for injection trauma, vehicle, and route of vehicle administration. The experimental groups were: group 6, EB, 2 μg/kg per day; groups 7 to 9, Δ^9-THC, 1, 2.5, or 10 mg/kg per day, respectively; group 10, EB (2 μg/kg per day) plus Δ^9-THC (10 mg/kg per day). Treatment with 0.2 ml of the appropriate compound was begun on the day of operation and continued daily for 14 days. Estradiol benzoate and Δ^9-THC were administered in sesame oil according to common usage: estrogens administered subcutaneously (*3, 4, 6*), Δ^9-THC administered intraperitoneally (*7*). Daily vaginal smears were obtained according to the method described by Zarrow *et al.* (*3*), and the animals were weighed twice weekly.

At the termination of the experiment, on the 14th day, the animals were killed by guillotine and examined for complete removal of both ovaries. Reproductive tissue, from the vagina to the tip of the uterine horn, was excised. Uteri were removed, stripped of adhering fat and connective tissue, and weighed. The tissues were fixed in Carnoy's solution (*8*). Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin (*8*). Step sections from comparable levels of mid-horn and vagina of each group were examined. Vaginal smears were coded, and read independently by two individuals.

Histologic examination of mid-horn sections obtained from control rats treated intraperitoneally with sesame oil (Fig. 1) show the effects of ovariectomy in the rat: the uterus is small and poorly

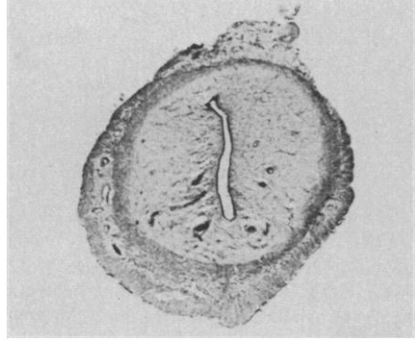


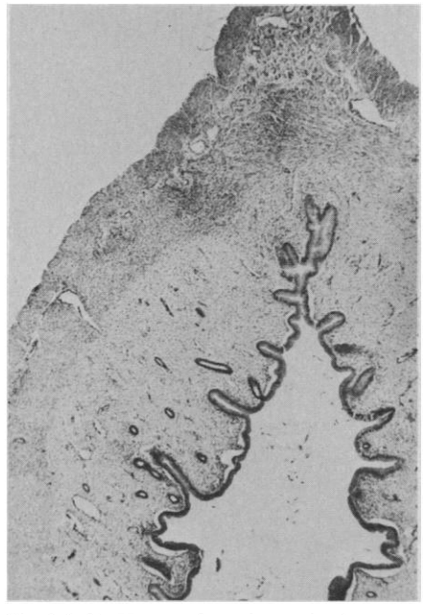
Fig. 1. Uterus of control ovariectomized rat. The organ is small and poorly vascularized. The lumen is slit-like, and the surface epithelium, the endometrium, and the myometrium are atrophied. Endometrial glands are collapsed (×22).

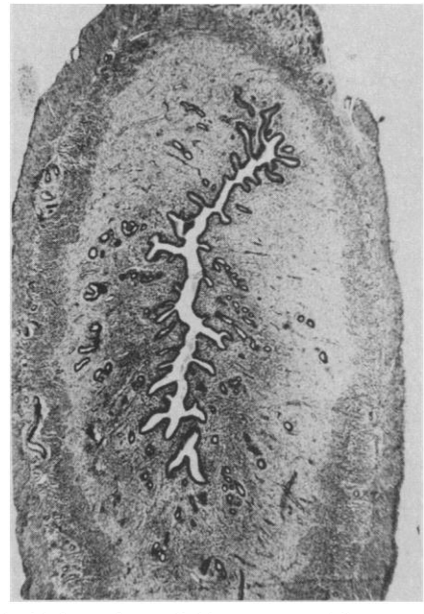
vascularized; the lumen is slit-like; the surface epithelium, endometrium, and myometrium are atrophied; and the endometrial glands are collapsed and involuted. As seen in Fig. 2, EB (2 μg/kg) induces recovery from the castrate condition. The enlargement of lumen, and hypertrophy and hyperplasia of the muscular layer and endometrial stroma allow only a portion of the uterine circumference to appear in the field; stimulation of the surface epithelium and endometrial glands is also apparent. Administration of 2.5 mg of Δ^9-THC per kilogram elicits distention of the lumen and growth of surface epithelium, endometrial stroma, and myometrium; proliferation of endometrial glands is evident (Fig. 3).

When compared to ovariectomized controls, mid-horn sections obtained from rats treated with 1 or 10 mg of Δ^9-THC per kilogram (groups 7 and 9) show histologic evidence of hyperplasia and hypertrophy. The most striking result seen in groups 7 and 9 (1 and 10 mg of Δ^9-THC per kilogram) is stimulation of the endometrial glands. A comparison of mid-horn sections obtained from rats treated with Δ^9-THC shows that 2.5 mg of Δ^9-THC per kilogram induces enhanced growth of surface epithelium, endometrial stroma, and myometrium, and increased proliferation of endometrial glands over that obtained with 1 and 10 mg of Δ^9-THC per kilogram.

In rats treated with both 2 μg of EB and 10 mg of Δ^9-THC per kilogram (group 10), the mid-horn section is somewhat smaller in diameter than in animals treated with EB alone (group 6), but there is little difference in the number and height of the epithelial columnar cells between the two groups. However, the endometrial glands appear somewhat increased in number and lumenal size after treatment with EB plus Δ^9-THC, when compared with animals treated with EB alone.

In sum: the uteri of ovariectomized rats treated with either 2 μg of EB per





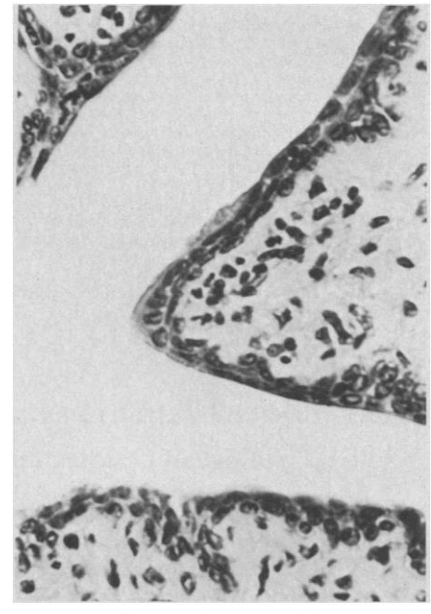
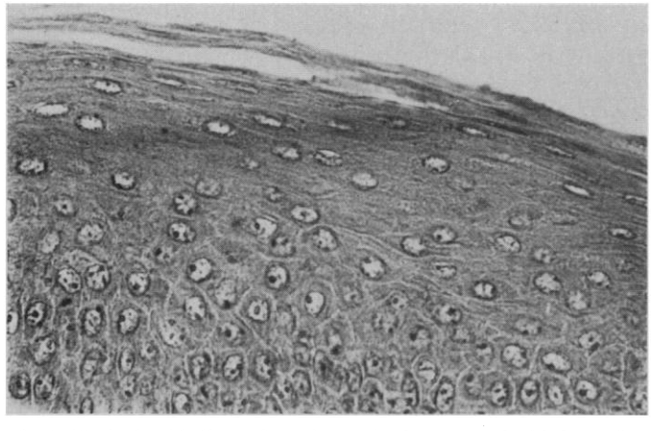


Fig. 2 (left). Uterus of ovariectomized rat treated with 2 μg of estradiol benzoate per kilogram of body weight. Compared to control, hypertrophy and hyperplasia of the endometrium and myometrium, distention of lumen, and stimulation of surface epithelium and endometrial glands are evident (×22). Fig. 3 (center). Uterus of ovariectomized rat treated with 2.5 mg of Δ^9-THC per kilogram. Compared to control, enlargement of lumen, increase in cellular size, and mitotic activity of surface epithelium, endometrial stroma, and myometrium are apparent. Glandular proliferation is also evident (×22). Fig. 4 (right). Vaginal epithelium of control ovariectomized rat. The stratified squamous epithelium is thin (two to three layers in thickness) (×400).



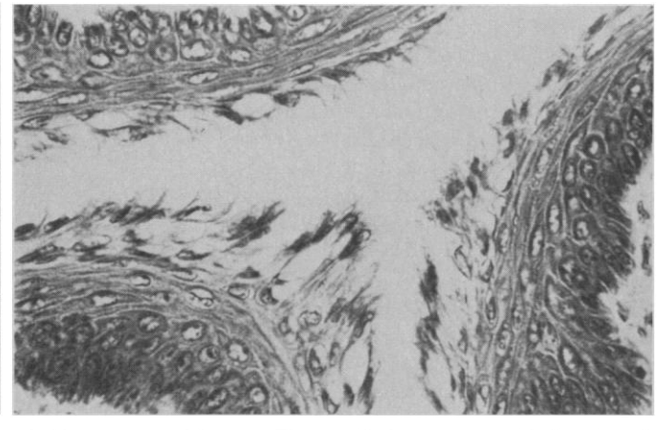


Fig. 5. Vaginal epithelium of ovariectomized rat treated with 2 μg of estradiol benzoate per kilogram. The stratified squamous epithelium is well developed. The superficial layer is cornified (×400). Fig. 6. Vaginal epithelium of ovariectomized rat treated with 2.5 mg of Δ^9-THC per kilogram. The stratified squamous epithelium shows cells undergoing cornification. In many areas, mucified cells form the superficial layer (×400).

kilogram alone (group 6, Fig. 2) or with 2.5 mg of Δ^9-THC per kilogram alone (group 8, Fig. 3) show extensive growth of the epithelium, endometrial glands and stroma, and muscular layer. Neither 1 mg nor 10 mg of Δ^9-THC per kilogram (groups 7 and 9) was as effective as 2.5 mg of Δ^9-THC per kilogram in stimulation of uterine growth. When animals are treated with EB plus Δ^9-THC (group 10), total horn diameter is diminished, and the endometrial glands are more prominent when compared to treatment with EB alone (group 6).

Sections obtained from the vaginas show the following results. In ovariectomized controls, the epithelium is thin, 2 to 3 cell layers thick (Fig. 4). With administration of EB, the vaginal wall is well stratified, and many layers of cells are found under the superficial cornified epithelium (Fig. 5). When rats are treated with 2.5 mg of Δ^9-THC per kilogram, there is an increase in vaginal stratification which may vary from 5 to 12 cell layers. The most characteristic picture seen is a superficial layer of mucoid cells which overlies cells undergoing cornification (Fig. 6).

Ovariectomized rats treated with 1 mg of Δ^9-THC per kilogram show little evidence of stimulation; the epithelium is rarely more than 2 to 4 cell layers in thickness, and there is little difference in appearance between this group and controls. When ovariectomized rats are treated with 10 mg of Δ^9-THC per kilogram, the vaginal epithelium is uneven: in some areas there are 2 to 3 cell layers, and in other areas the epithelium is 12 to 14 cell layers thick. In this group, some stratified layers show mucification. Incipient cornification is less clearly defined with 10 mg of Δ^9-THC per kilogram than with 2.5 mg/kg. Treatment of spayed rats with 10 mg of Δ^9-THC per kilogram plus 2 μg of EB per kilogram results in proliferation and cornification to a degree similar to that obtained with EB alone.

Examination of vaginal smears shows that in control rats there is no stimulation, and the smears show the animals to be in diestrus. In animals treated with Δ^9-THC alone, leukocyte counts are depressed, and the epithelial cells, some of which are cornified, are increased over the controls. In rats treated with EB alone, or with EB plus Δ^9-THC, the vaginas are cornified, but not mucified, and vaginal smears show vaginal estrus.

In the earlier study (*1*), EB had the expected uterotrophic effect as measured by increased uterine weights (*3, 4, 9, 10*); all three doses of Δ^9-THC had significant ($P < .01$; $P < .001$) but less pronounced uterotrophic activities than EB. In that report (*1*), the mean uterine weights plus or minus the standard errors of the means, expressed as milligrams of organ weight per 100 g of body weight, were: groups 2 to 5 (controls), 76.7 ± 2.8; group 6 (EB), 277.8 ± 16.7; group 7 (1 mg of Δ^9-THC), 98.9 ± 7.7; group 8 (2.5 mg of Δ^9-THC), 174.8 ± 20.9; group 9 (10 mg of Δ^9-THC), 118.6 ± 8.4; and group 10 (EB plus Δ^9-THC), 248.8 ± 24.4.

Histologic examination of uterine and vaginal tissues indicates that in the ovariectomized rat, Δ^9-THC is less effective than EB, but does have estrogenic activity as measured by hypertrophy and hyperplasia of uterine and vaginal tissue. These histologic changes are consistent with the changes in vaginal smear cytology and uterotrophic effect of Δ^9-THC observed in these animals (*1*).

Different doses of Δ^9-THC induce changes in the weights and cytology of the uterus, vagina, and vaginal smears. A low dose (1 mg/kg) results in a significant gain in uterine weight, which appears to be largely a reflection of stimulation of the growth of the uterine epithelium and the endometrial glands. At this dose, Δ^9-THC produces little change in the cytology of the vaginal epithelium, but vaginal smears show fewer leukocytes than ovariectomized controls.

At a dose of 2.5 mg of Δ^9-THC per kilogram, there is a more striking increase in uterine weight gain. Cytologically, the uterus shows hyperplasia and hypertrophy of the surface epithelium, endometrial stroma and glands, and myometrium. Again, metrial glandular development seems striking with Δ^9-THC administration. At this dose, Δ^9-THC produces vaginal stratification; here, cells which appear mucified overlie cells undergoing cornification. Depending on the dosage, this pattern of a superficial layer of mucus cells which overlies a cornifying vaginal epithelium can be seen in ovariectomized rats and mice with the administration of estrogen alone (*11–16*), or with administration of estrogen and progesterone (*11–13, 16–18*). When compared to controls, vaginal smears from rats treated with 2.5 mg of Δ^9-THC per kilogram show fewer leukocytes and more epithelial cells, some of which are cornified. Depending on the dosage, vaginal smears obtained from ovariectomized rats and mice treated with estrogen alone or progesterone and estrogen show leukocytes and epithelial cells, and a few cornified cells (*3*, p. 71; *14, 17*).

At 10 mg of Δ^9-THC per kilogram, uterine weight is significantly increased; stimulation of the epithelium and development of endometrial glands resembles that seen with the 1 mg/kg dose. However, vaginal histology shows an uneven response to the 10 mg/kg dose, and the smears show fewer leukocytes, and an increase in epithelial cells when compared to controls.

At this point, it is too early to determine whether the effect of Δ^9-THC on uterine and vaginal tissue is direct, indirect, or both. The possibility exists that Δ^9-THC may increase the secretion of adrenal steroids which, in turn, may influence the response of the castrate vagina and uterus to Δ^9-THC. It has been shown that Δ^9-THC stimulates adrenal activity (*19*) and elevates plasma corticosterone levels (*20*). It is also known that adrenal cortical hyperactivity has an inhibitory influence on the responsiveness of the castrate uterus to administered estrogen (*21*). It will be of interest to examine the effects of Δ^9-THC on adrenalectomized-ovariectomized rats.

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