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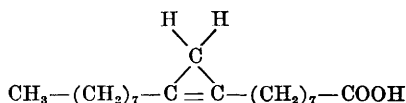
Histomorphological Changes in Reproductive Organs of Rats Fed Cyclopropenoid Fatty Acids.*† (31074)

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(Introduced by A. R. Kemmerer)

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Physiological disturbances involving sexual development and reproduction in the chicken and the rat have been coupled to the ingestion of dietary lipids containing cyclopropenoid fatty acids(1). The effects of cottonseed oil or cottonseed products containing cottonseed oil on sexual development and egg production in the avian species have been extensively reported(1,2). Studies with rats have utilized *Sterculia foetida* oil as a rich source of cyclopropenoid fatty acid(3).

The biologically active fraction of *S. foetida* oil contains sterculic and malvalic acids in a 10 to 1 ratio(2). Sterculic is a C₁₉ cyclopropenoid fatty acid whose structure is as follows (4):



Malvalic acid has one less C between the cyclopropene ring and the carboxyl end(5).

Schneider(6) reported that daily dietary supplements of 200 mg *S. foetida* oil (30-50% cyclopropenoid fatty acids) fed by capsule to sexually immature pullets starting at 16 weeks of age, impaired ovary-oviduct-uterus and comb development and prevented egg production. When these hens were sacrificed at one year of age, no mature ova were observed in the ovaries. In fertilized eggs from hens fed 50 mg *S. foetida* oil per day, embryonic de-

velopment ceased after 5 days of incubation (7).

Sexual development of female rats maintained on a 1% safflower-3% *S. foetida* oil diet from weaning was significantly retarded, estrous cycles were prolonged and irregular, and the reproductive organs (ovaries-oviducts-uteri) were significantly smaller than those of controls(3).

To date, only the gross effects of the cyclopropenoid fatty acids have been reported. This paper reports some of the accompanying histomorphological changes in reproductive tissues as a result of cyclopropenoid fatty acid ingestion.

Methods. Sprague-Dawley female and male rats were maintained from weaning to 6 or 12 months of age on complete purified diets containing either 4% safflower oil, hereafter designated as control, 2% safflower-2% *S. foetida* oil, or 1% safflower-3% *S. foetida* oil, hereafter designated as 2% or 3% *S. foetida* oil diets. Drinking water was provided *ad libitum*. Estrous cycles were characterized by daily vaginal smears taken at approximately the same time each morning by the method of Long and Evans(8). Rats found in proestrus were killed by concussion the next day and the reproductive organs anterior to the cervix were removed, weighed, and fixed in Bouin's fluid. Ovaries and the attached oviducts and mid-portions of uteri were paraffin embedded, sectioned serially at 10 μ and stained with hematoxylin and eosin. Testes and epididymides were excised from *S. foetida* oil and control oil fed rats and were processed in the same manner.

In breeding studies the females and males were maintained in individual cages during

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the day where they were fed their respective diets. In the late afternoon, remaining food was removed from the cages and females were placed in males' cages, 2 females and one male per cage, until 8:00 a.m. the following morning when the females were returned to their respective cages. Weight gains, pregnancies, and litter sizes and weights were recorded. Control, 2% and 3% *S. foetida* oil fed females were mated with control males. 2% and 3% *S. foetida* oil fed males were also crossed with each of the 3 groups of females(9).

Results. Histological examination and comparison of the ovaries from rats fed *S. foetida* or control oil showed no apparent differences in the production of ova, although follicular development appeared to be depressed in those fed *S. foetida* oil. There was a higher incidence of degeneration of primary follicles in *S. foetida* oil fed rats in which atrophic changes in the ova, nuclear karyolysis and cytoplasmolysis, were seen. In addition, there was a higher incidence of atresia in small- and medium-sized vesicular follicles (Fig. 1B). The histomorphological criteria used for determining atresia of follicles were nuclear karyolysis, cytoplasmolysis, and division and fragmentation of the ovum, nuclear pyknosis and fragmentation and lack of mitoses in the granulosa cells, dissociation of the granulosa into the liquor folliculi, and the invasion and replacement of the granulosa by other cells. Many of the atretic small vesicular follicles had been or were in the process of being turned into lobules of interstitial tissue with central cavities formed by the zonae pellucidae of completely or partially degenerated ova (Fig. 2). The granulosa of the atretic medium-sized follicles was invaded by cells proliferated by the theca interna and appeared to be undergoing luteinization. Of the small number of apparently viable mature follicles present in the *S. foetida* oil fed rats, very few were comparable in size or physical appearance to those of control rats (Fig. 1A). Several large follicles were seen in which the granulosa had continued to proliferate until the entire follicle was filled with an almost solid mass of granulosa cells (Fig. 1C). The granulosa had been invaded by thecal cells and vascular tissue and was undergoing degeneration about

the small central cavity. No ova were found within these follicles.

The sizes and stages of development and regression of corpora lutea of *S. foetida* oil fed rats were as variable as those of control rats. However, the overall amount of luteal tissue appears to be less in *S. foetida* oil fed rats and medium and large corpora lutea have a characteristic expansion of the sinusoids and fatty infiltration not observed in corpora lutea of comparable size from control rats (Fig. 1D, E). Regression of several corpora lutea of *S. foetida* oil fed rats involved extensive fatty infiltration (Fig. 1F) not seen in the involution of corpora lutea of control rats.

No histomorphological changes in the oviducts resulted from feeding *S. foetida* oil. The uteri of *S. foetida* oil fed rats, however, were much smaller than those of control rats as a result of a lack of development of the endometria and myometria. The muscle fiber bundles of the myometria, especially those of the longitudinal muscle layer, were loosely arranged and lacked the compact appearance of those of the controls. The underdeveloped endometria contained fewer uterine glands than in controls.

The testes and epididymides of *S. foetida* oil fed males showed no apparent difference when compared with controls. Sperm production in the seminiferous tubules and storage of sperm in the epididymides appeared normal in *S. foetida* oil fed rats.

Results of the initial breeding experiment as shown in Table I indicate complete reproductive failure at the 3% *S. foetida* oil level and a low reproductive rate with stillborn litters at the 2% *S. foetida* oil level. In these

TABLE I. Comparison of Reproductive Performance of Rats Fed Diets With and Without Cyclopropenoid Fatty Acids.

Group	No. of rats mated and type of diet fed	No. of litters born	Avg No. of young per litter
I	(9) C* × (3) C	9	8.4
II	(9) 2% <i>S.f.</i> † × (3) C	2§	6.0§
III	(9) 3% <i>S.f.</i> ‡ × (3) C	0	—

() = No. of rats.

* 4% safflower oil control diet.

† 2% safflower oil—2% *S. foetida* oil diet.

‡ 1% safflower oil—3% *S. foetida* oil diet.

§ Stillborn.

studies, 2 and 3% *S. foetida* oil fed males were successfully bred to control females.

Discussion. Histomorphological changes in

follicular development of *S. foetida* oil fed rats could be correlated with their irregular and prolonged estrous cycles. In vaginal smears

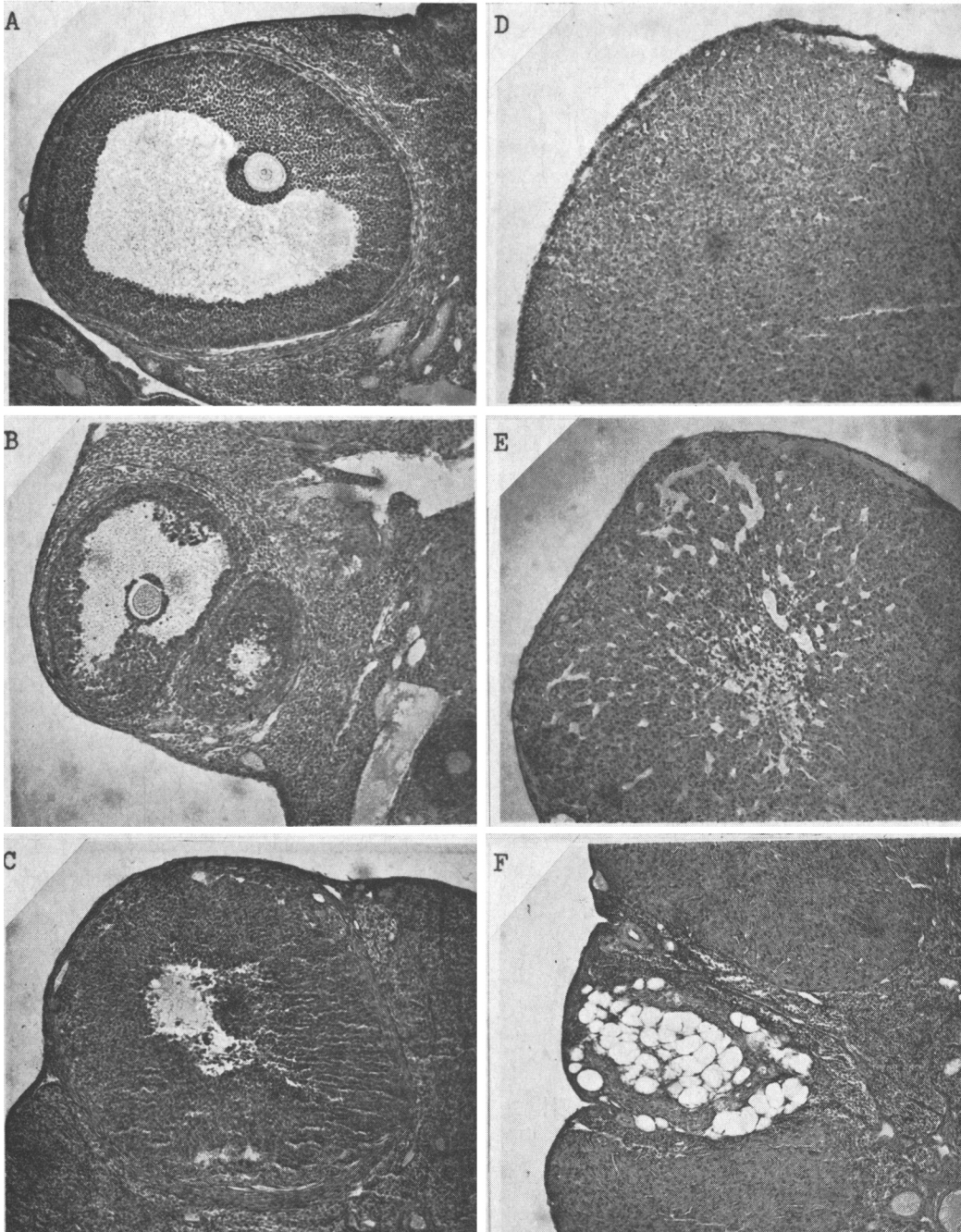


FIG. 1. (A) Normal follicle of control rat. (B) Atretic follicles of 3% *S. foetida* oil fed rat. (C) Proliferation of granulosa in follicle of 2% *S. foetida* oil fed rat. (D) Corpus luteum of control rat. (E) Corpus luteum of 3% *S. foetida* oil fed rat. (F) Fatty infiltration of regressing corpus luteum of 2% *S. foetida* oil fed rat. Mag. $\times 100$.

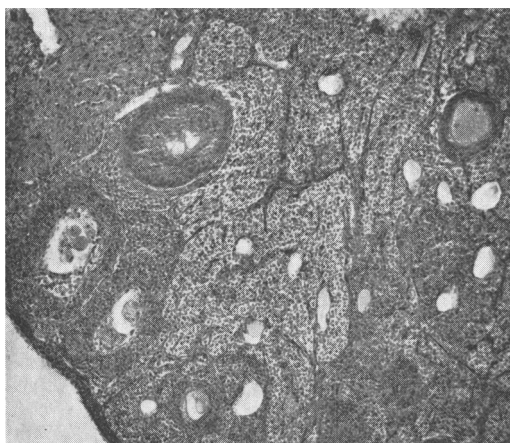


FIG. 2. Portion of ovary of 3% *S. foetida* oil fed rat showing lobules of interstitial tissue developed from atretic follicles. Disintegrated ova can be seen in a few of the central cavities and fragmentation of the ovum has occurred in the follicle at the far left. Mag. $\times 100$.

of control rats in estrus, there is virtually complete cornification of the epithelial cells with no leukocytes present, whereas in the smears of *S. foetida* oil fed rats there is 50% or less cornification of the epithelial cells with many leukocytes present. Such smears indicated a "weak" estrus with the cycle varying from 8 to 14 days as compared to a 4- to 5-day cycle in controls.

The lack of uterine development and the increase in follicular atresia associated with cyclopropenoid fatty acid ingestion appear to be the immediate causes of reproductive failure by female rats fed the 3% *S. foetida* oil diet. Some of these histomorphological changes are suggestive of endocrine dysfunction associated with an essential fatty acid deficiency. Panos(10) describes histological changes in ovaries and testes of rats maintained on diets deficient in the essential fatty acid linoleic. Follicular development is retarded and spermatogenesis is absent in these rats. He attributes reproductive failure to a disturbance of the pituitary-gonadal axis which results in insufficient production of gonadotropins (FSH, LH, and LTH) and steroid hormones

associated with reproduction. However, our results of lipid analyses of the diets, livers, brains, testes, epididymal fat, and omental fat of *S. foetida* oil fed rats showed no deficiency of the essential fatty acid linoleic. In addition, males fed *S. foetida* oil diets were not affected adversely as to reproductive ability.

Cyclopropenoid fatty acids shift fatty acid composition toward a higher concentration of saturated fatty acids at the expense of monoenoic fatty acids. This suggests that other unsaturated fatty acids in addition to the linoleic acid series could be important in normal functioning of the pituitary-gonadal axis.

Summary. Reproductive tissues of rats fed cyclopropenoid fatty acids show definite retardation of follicular development, alteration in corpora lutea, and lack of development of the uterus. Reproductive failure is total in female rats fed a 3% *S. foetida* oil diet. Results indicate disturbance of the pituitary-gonadal axis when cyclopropenoid fatty acids are fed.

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