Prenatal and Postnatal Mortality of Offspring of Cyclopropenoid Fatty Acid-Fed Rats*† (33804)

A. M. Miller, E. T. Sheehan, and M. G. Vavich
(Introduced by A. R. Kemmerer)

Departments of Agricultural Biochemistry and Food and Nutrition, University of Arizona, Tucson 85721

The literature on the detrimental biological effects of cyclopropenoid fatty acids (components of cottonseed oil and cottonseed oil products) has dealt mainly with experiments on hens and eggs. Reports of delayed sexual maturity, retarded ovary and oviduct development, decreased egg production and hatchability, and embryo mortality in the avian species have been extensively reviewed (1). Sheehan and Vavich (2) reported a delay in sexual maturity, decreased ovary–oviduct–uterus weights, and lengthened and irregular estrous cycles in female rats fed *Sterculia foetida* oil (30–50% cyclopropenoid fatty acids). In breeding experiments with rats fed *S. foetida* oil, the number and size of litters produced were drastically decreased and the young were either stillborn or died within a few hours after birth. Only the female rats were adversely affected. *S. foetida* oil-fed males fathered litters with control females at a rate comparable to that of control males. When the reproductive tissues of the *S. foetida* oil-fed males and females were compared to those of respective control rats, only those of the females showed noticeable changes (3).

Although the effects of cyclopropenoid fatty acid ingestion have been studied extensively in adult chickens and to a lesser extent in rats, no attempt was made to explain their lethal effects in offspring. To illucidate the possible cause or causes of pre- and postnatal mortality of offspring of cyclopropenoid fatty acid-fed rats, the fetuses and newborn were studied grossly and histologically for pathological defects.

Methods. Four groups of 20 Sprague-Dawley female rats, 28 days of age, were fed a complete purified diet containing either 4% safflower oil (the control diet); 3% safflower oil–1% *Sterculia foetida* oil; 2% safflower oil–2% *S. foetida* oil; or 1% safflower oil–3% *S. foetida* oil (the test diets). The safflower oil was a commercial product;[1] the *S. foetida* oil was extracted (2) from *S. foetida* seeds obtained from the Philippines.[2]

Forty Sprague-Dawley male rats, 28 days of age, were fed the 4% safflower oil diet only, since previous studies had shown that their reproductive potential was not altered by cyclopropenoid fatty acid ingestion (3). Males and females were housed and fed in separate cages with two animals per cage.

At 3 months of age, the females in the control and test groups were mated with control males according to the following procedures. Vaginal smears were taken each morning using a modification of the method of Long and Evans (4). A platinum bacteriological loop, flamed and moistened between each use, was employed in an effort to avoid stimulating pseudopregnancy. The smears were examined microscopically and females found to be in proestrus or estrus were placed in males' cages overnight. The presence of spermatozoa in a vaginal smear was taken as an indication that pregnancy would ensue and no further smears were taken from that particular female. Females were weighed twice each week and increases in weight above those associated with normal growth were used to substantiate pregnancy.

Counting the day on which spermatozoa appeared in the vaginal smear as day 0, 6 control rats and 10 test rats were sacrificed

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[1] S. E. Rykoff and Company, Los Angeles, California.

[2] Mr. Zoilo C. Fraga, Forestry College, Laguna, Philippines.

on the twentieth day prepartum. The abdominal wall was opened and both uterine horns were exposed for gross examination of metrial glands and fetal swellings. The uterine horns were opened and the number and condition of fetuses were noted. Living fetuses were removed, weighed, and examined for developmental defects. Portions of the uterine wall with their attached placentas; and the lungs, heart, aorta, liver, spleen, pancreas, stomach, intestines, kidneys, and adrenal glands of sacrificed fetuses were fixed in Bouin's fluid or 10% buffered formalin. The organs were paraffin embedded, sectioned at 8 μ, and stained in hematoxylin and eosin for routine histological examination. The eviscerated carcasses of the fetuses were preserved in 95% alcohol, cleared in KOH, and stained in alizarin red for skeletal studies.

Other pregnant control and test rats were allowed to deliver their litters and the number, weights, and condition of newborn were noted. Some of the living control and test newborn were sacrificed and treated as above. Whenever possible, the birth of young was observed so that dead or moribund young could be removed from the mother's cage and examined before they were eaten.

Results. In this experiment, *S. foetida* oil at the 3% level in the diet caused a decrease in fertility and mating behavior which completely prevented the production of offspring. Of the few females in this group that did mate, only one exhibited a weight gain suggestive of pregnancy. The uterus of this female, examined at the termination of the experiment, bore evidence of implantation sites of resorbed embryos or fetuses.

The ingestion of 2% or 1% *S. foetida* oil also decreased fertility and mating behavior, but to a lesser extent at the 1% level. In 4 of the 8 litters produced by females in the 2% group, all young were stillborn. The other 4 litters contained some stillborn and some live individuals which died soon after birth. Thirteen litters were produced in the 1% group and in 4 of these all the young were stillborn. Approximately half of the young were stillborn in the other 9 litters. Those born alive had a longer survival time than those in the 2% group and a few survived as long as 48 hr.

The 1% and 2% *S. foetida* oil-fed females produced an average of 6 offspring per litter and control females an average of 9. The value for the *S. foetida* oil-fed rats is an approximation, since they often ate living young as well as those born dead. Some of the dead were in a state of degeneration, indicating that they had died *in utero* at least a few days before birth.

When control, 1% and 2% *S. foetida* oil-fed females were sacrificed on day 20 prepartum, the uteri of the 1% and 2% females contained some dead fetuses and placental remnants in various stages of resorption (Fig. 1). They were also more vascular than those of controls, particularly in those areas where resorption was taking place. There were no apparent histological differences in the placentas of control and test rats. The living fetuses of *S. foetida* oil-fed females had an average weight of 3.5 g as compared to 5 g for control fetuses. There were no grossly detectable internal, external, or skeletal defects in the *S. foetida* oil fetuses and except for their smaller size they were indistinguishable from control fetuses (Fig. 2). The variation in size is a reflection of the slower rate of development of the *S. foetida* oil fe-

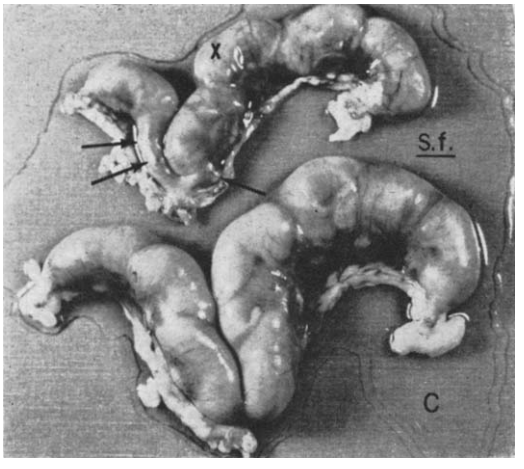


FIG. 1. Uteri from a 2% *S. foetida* oil-fed rat and a control rat sacrificed on day 20 prepartum: the *Sf.* uterus contains 5 fetuses, one of which is dead (x), and 3 placentas undergoing resorption (arrows); the control uterus contains 9 living fetuses.

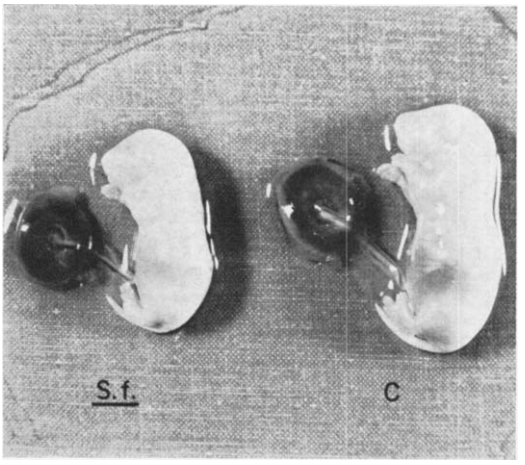


FIG. 2. Fetuses removed from the uteri of a 2% *S. foetida* oil-fed rat and a control rat. There is no apparent external difference other than size.

tuses. The gestation period in *S. foetida* oil-fed rats was increased from the normal 21–22 days to 26–28 days. Due to the inequity of development of the two groups of day 20 prepartum fetuses, it was difficult to make an accurate histological comparison. However, the degenerative and necrotic changes seen in the livers and kidneys of *S. foetida* oil newborn were also seen in the *S. foetida* oil fetuses and are described below.

Gross and histological comparisons were made of postpartum control young and *S. foetida* oil young. After birth, the *S. foetida* oil young became cyanotic, normal breathing movements gave way to convulsive gasps, and finally, death ensued. Areas of subcutaneous hemorrhage were seen on the muzzle, top of the head, the limbs, and back. The latter area, located on the midline at the level of the scapulae, occurred with surprising frequency.

Gross examination of internal organs of *S foetida* oil young, sacrificed just prior to death, revealed areas of hemorrhage in the lungs, pale swollen kidneys, yellowish areas in the livers, and yellow discoloration of the intestines. Histological examination of the lungs of control (Fig. 7) and *S. foetida* newborn (Fig. 8) showed hemorrhages into the alveoli of the latter. Occasionally, small areas of hemorrhage were found in the hearts, livers, and kidneys.

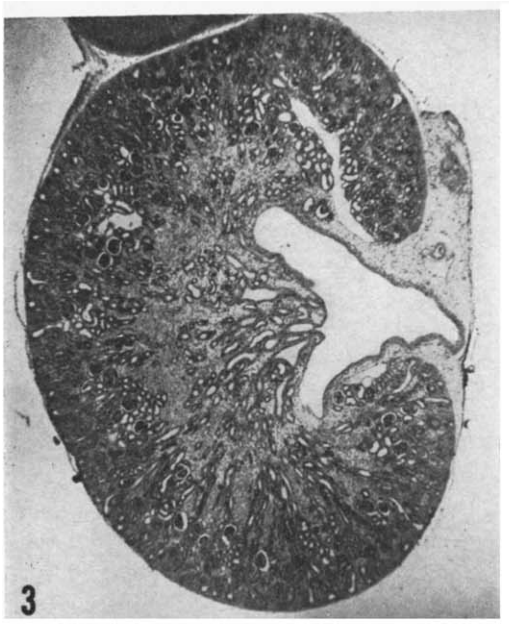
Striking differences between control (Figs. 3 and 5) and *S. foetida* oil (Figs. 4 and 6) young were found in the kidneys. The kidneys of *S. foetida* oil young shrunk more than those of controls during the dehydration phase of the embedding process. The wrinkled appearance of the kidney capsule reflects this shrinkage in longitudinal sections. In general, the kidney tissues of test young were extremely acidophilic and fibrous tissue often infiltrated large areas of the organ. The glomeruli were condensed and fibrotic. Necrotic changes included nuclear pyknosis and homogenization of the cytoplasm of proximal convoluted tubules and karyolysis of nuclei and loss of cellular detail in cells of distal convoluted tubules (Fig. 6).

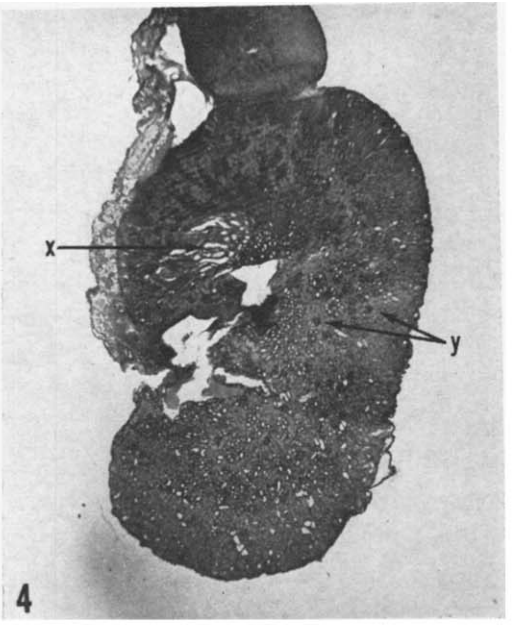
The histological appearances of the livers of control and *S. foetida* oil newborn are shown in Figs. 9 and 10. In the *S. foetida* oil newborn, there is compression of the cytoplasm of nearly all cells. Formalin-fixed frozen sections of these livers were stained with Sudan IV. The empty spaces within the cells of paraffin sections were found not to be occupied by fat. Hemopoiesis was still evident in livers of both control and *S. foetida* oil newborn. There appeared to be more hemopoietic activity in *S. foetida* oil livers, possibly due to a slower rate of development.

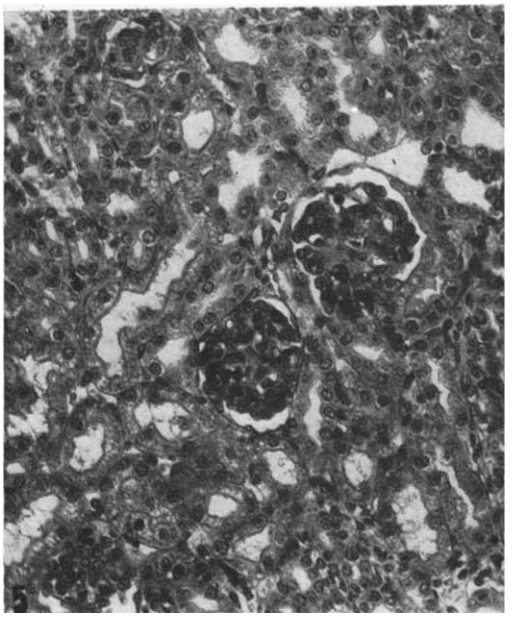
Discussion. It appears that the primary cause of postpartum mortality in the young of cyclopropenoid fatty acid-fed rats is anoxia resulting from hemorrhage into the alveolar spaces of the lungs. The presence of hemorrhagic sites in other tissues in addition to the lungs suggests that capillary fragility may be involved.

The pale swollen appearance of the kidneys, the degeneration and necrosis in their glomeruli and tubules, and the appearance of the liver cells are indicative of irreversible hydropic change. It seemed apparent that if death of the newborn had not occurred due to anoxia, it would have occurred due to impaired kidney and liver function.

Studies of the effect of dietary lipids on the rate of hemolysis of erythrocytes have shown that cell permeability is altered by the







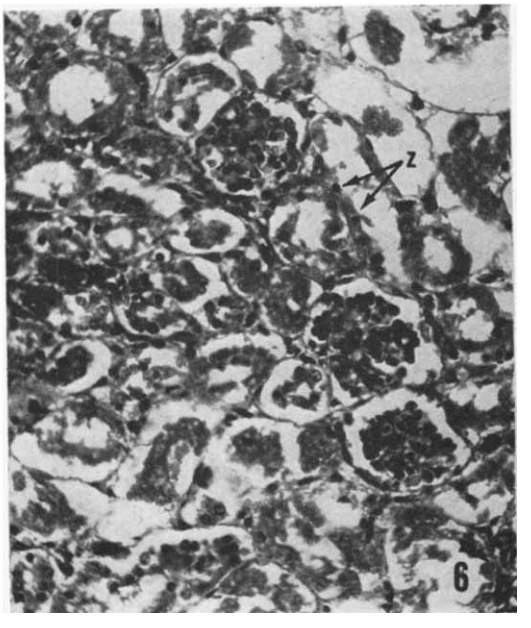


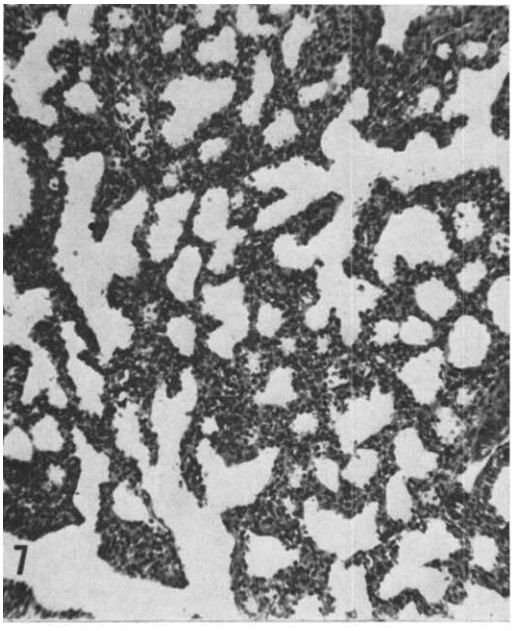
FIG. 3. Kidney of a control newborn sacrificed a few minutes after birth showing clearly defined cortex and medulla; ×30.

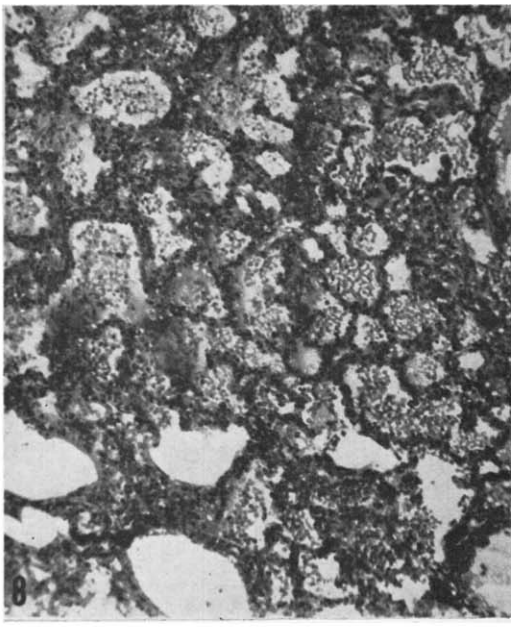
FIG. 4. Kidney of a 2% *S. foetida* newborn sacrificed a few minutes after birth: upper portion contains dense fibrous tissue and an area of hemorrhage (x); glomeruli (y) are compressed and fibrotic. Note wrinkled appearance of kidney capsule; ×30.

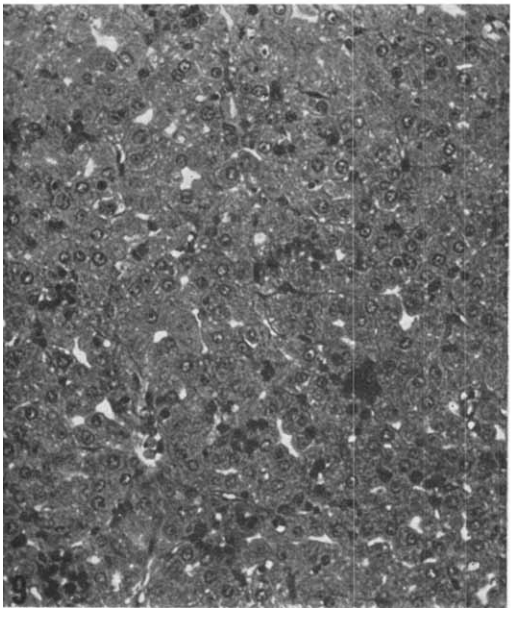
FIG. 5. Portion of kidney of a control newborn showing normal appearance of glomeruli and tubules; ×300.

FIG. 6. Portion of kidney of a *S. foetida* newborn: note pyknotic nuclei (z) and disintegration of cells of proximal convoluted tubules; glomeruli are shrunken and contain pyknotic nuclei; ×300.

incorporation of various fatty acids into the membrane's phospholipids (5). Doberenz *et al.* (6) showed that there was an increased water uptake by the yolks of eggs from hens







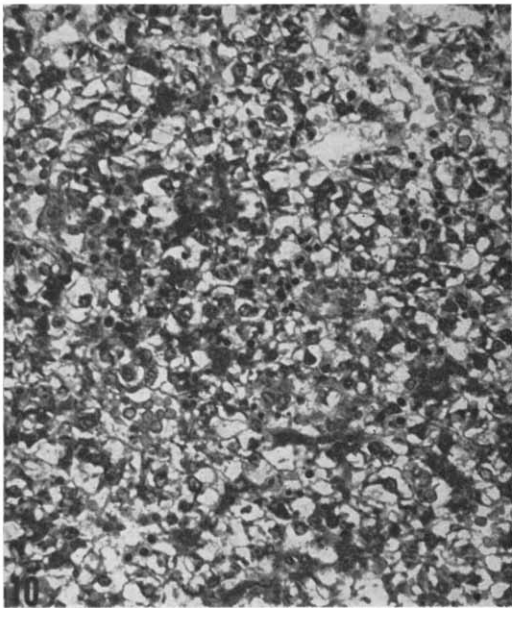


FIG. 7. Section through lung of a control newborn showing normal appearance of alveoli; ×125.

FIG. 8. Section through lung of a *S. foetida* newborn sacrificed just prior to death: alveoli are filled with erythrocytes; ×125.

FIG. 9. Section through the liver of a control newborn: some hemopoietic activity is still evident; ×300.

FIG. 10. Section through the liver of a *S. foetida* newborn sacrificed just prior to death: note vacuolated appearance of cells and greater amount of hemopoietic activity; ×300.

fed *S. foetida* oil. It was presumed that the effect was due to increased permeability of the vitelline membrane.

Cyclopropenoid fatty acids have been found to be inhibitors of the fatty acyl desaturase system which converts stearic acid to oleic acid (7). Therefore, cyclopropenoid fatty acid-fed animals have a higher concentration of saturated and a lower concentration of unsaturated fatty acids in the body fat. Variations in the concentration of oleic and other unsaturated fatty acids have a profound effect on the permeability of erythrocytes to glycerol (8).

It is possible that altered cell membrane permeability may be responsible for the detrimental effects of cycloprepenoid fatty acids on pre- and postpartum young. The cyclopropenoid fatty acids may be incorporated into the phospholipids of the membrane, or their effect on general lipid metabolism may alter the amounts of other fatty acids in the phospholipids.

Summary. Cyclopropenoid fatty acids fed to female rats in the form of *Sterculia foetida* oil caused a decrease in mating behavior, fertility, and fetal and newborn viability. At the 3% level in the diet, *S. foetida* oil completely prevented reproduction. At the 2 and 1% levels, it caused pre- and postpartum death of offspring. No teratological effects were observed, but degenerative changes and necrosis inconsistent with survival were seen in fetal and newborn livers and kidneys. Hemorrhages into the lung alveoli resulting in anoxia appeared to be the immediate cause of postpartum death.

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