EFFECTS OF INGESTION OF CYCLOPROPENOID FATTY ACIDS ON REPRODUCTION IN THE FEMALE RAT

by

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I hereby recommend that this dissertation prepared under my direction by Edward T. Sheehan

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be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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ABSTRACT

The ingestion of cyclopropenoid fatty acids by chicken and rats is known to produce several detrimental physiological effects. These effects are expressed as reduced growth, delayed sexual maturity, poor reproduction and a change in the makeup of the lipids in the body.

In these studies albino rats were pair-fed purified diets to which various concentrations of S. foetida oil, a rich source of cyclopropenoid fatty acids, were added. Growth, fatty acid composition of various organs, sexual development and reproduction were studied.

The cyclopropenoid fatty acids reduced growth when fed as 3% of the oil in the diet. Feed efficiency was reduced during the growing period of the rat; but when body weight leveled off in adult rats, the feed efficiency approached that of the control group.

Fatty acid composition was changed from the normal as reflected by a higher percentage of saturated fatty acids at the expense chiefly of the mono-unsaturated acids. There appeared to be an impairment in the fatty acyl desaturase enzyme system rather than an increased synthesis of saturated fatty acids.

Sexual maturity, as determined by observing the age of perforation of the vaginal closure membrane, was significantly

delayed in rats fed cyclopropenoid fatty acids. The estrus cycles in these rats were lengthened and irregular.

Reproduction in high level S. foetida oil groups was reduced to zero. At lower levels the pups were born dead or died within a few hours after birth. Teratologically these pups were not different than control pups except in size. However histological examination showed degenerative tissue damage in the kidneys, lungs, hearts and livers of the pups fed cyclopropenoid fatty acids. This suggested that the pups born alive would not have survived even if they had nursed.

The conclusions of these studies were that the blockage in the fatty acyl desaturase system may seriously alter the type of fatty acids used in cell membrane structure and thereby cause an altered permeability which leads to many altered metabolic functions.

CHAPTER I

INTRODUCTION

The literature pertaining to the biological effects of the cyclopropenoid compounds relates almost entirely to the results of experiments on hens and eggs, probably because the effects were first noted as visible disorders in eggs following storage. The initial reports on such disorders date back to the early 1930's, and were concerned with a pink-white discoloration in the egg white. In subsequent years reports cited disturbances in sexual development and retardation of egg production in hens ingesting cyclopropenoid fatty acids. Recent literature reports the deleterious effects of the cyclopropenoid fatty acids in rats.

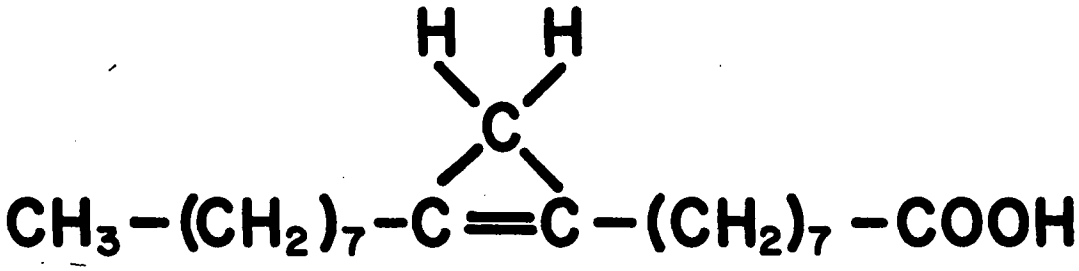
Numerous plants of the order Malvales are known to have cyclopropenoid activity. Two naturally occurring cyclopropenoid fatty acids with defined structures have been isolated from plants from several families of this order. These fatty acids have been given the common names of sterculic and malvalic acids.

Sterculic acid, 8-(2-octyl-1-cyclopropenyl) octanoic acid (Fig. 1) is the principle cyclopropenoid fatty acid in the oil extracted from Sterculia foetida seeds. The concentration of sterculic acid in the oil is approximately 30% to 50% depending on the method of analysis. Malvalic acid is also present in S. foetida oil but only at one-tenth the concentration of the sterculic acid.

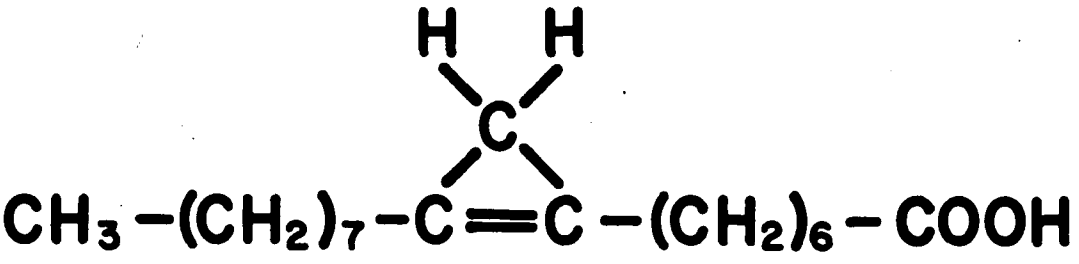
Malvalic acid, 7-(2-octyl-1-cyclopropenyl) heptanoic acid (Fig. 1) is the principle cyclopropenoid fatty acid in cottonseed oil extracted from Gossypium hirsutium seeds or plants. The concentration of malvalic acid is approximately 1% or less. Sterculic acid has also been reported in cottonseed oil at about 0.1% to 0.3%.

Generally, the physiological disturbances resulting from the ingestion of cyclopropenoid fatty acids, concern growth and development as well as sexual maturity and functioning. In the hen growth retardation, poor comb development, delayed sexual maturity, decreased egg production, reduced egg hatchability, enlarged liver and gall bladder, and pink-white discolorations in the egg white have been attributed to the presence of cyclopropenoid fatty acids in the diets. Similarly in the female rat growth retardation, delay of sexual maturity, irregular and prolonged estrus cycles, poor reproduction, high mortality of newborn, enlarged livers, and histological changes in the ovaries and uterii have been ascribed to the ingestion of cyclopropenoid fatty acids. However, studies to date on the male in either species have failed to show any detrimental effects on sexual development, maturity or functioning.

Occasionally there have been other biological effects directly associated with ingestion of cyclopropenoid fatty acids. The most common of these effects was the hardening of depot fat as cited in the soft pork problem in pigs or in egg yolk fatty acids in hens as a result of feeding cottonseed oil products to these animals.



STERCULIC ACID



MALVALIC ACID

Figure 1. Cyclopropenoid Fatty Acids.

This report concerns changes in reproductive performance in the rat fed cyclopropenoid fatty acids and with the allied effects on the survival of new-born pups. S. foetida oil was used as the chief source of cyclopropenoid fatty acids.

REVIEW OF LITERATURE

The presence of cyclopropenoid fatty acids has been reported in more than forty-eight plant oils of the order Malvales, principally from the following four families: Sterculiaceae, Malvaceae, Bombacacea, and Tiliaceae (Phelps et al 1965). The only plant oil of any significance from a dietary standpoint is G. hirsutium, popularly known as the cotton plant. The oil from S. foetida seeds is not known to be a common dietary of man or any animal species.

The presence of cyclopropenoid fatty acids has been verified by a positive Halphen test. The Halphen test was introduced by Halphen (1897) to detect the presence of cottonseed oil as a diluent in other edible vegetable oils such as olive oil. To date there have been many modifications to the original Halphen test (Oilar 1900; Kuever 1921; Jamison 1943; Mehenboeker 1960; Deutschman and Klaus 1960; Shenstone and Vickery 1961) none of which is universally accepted as 100% accurate in predicting the cyclopropenoid fatty acid content of oils. Generally, the formation of a colored complex due to the reaction of the cyclopropene ring with sulphur and carbon disulfide has been considered specific for detecting the presence of cyclopropenoid fatty acids by means of the Halphen test.

More recent methods employ the use of gas-liquid chromatography or titration with halogen acids (Smith et al 1961; Wilson 1961; Nordby et al 1962; Cornelius and Shone 1963; Bailey et al 1963; Harris et al 1963; and Magne 1966). There are inherent problems associated with both of these methods which have not completely satisfied the requirements for an accurate, reproducible analysis for cyclopropenoid activity. Error is introduced in the gas-liquid chromatography method since the methyl esters of the cyclopropenoid fatty acids are readily isomerized on the column to conjugated dienes, which result in several peaks due to decomposition products as shown by Masson (1959). In the hydrohalogenation methods, Magne (1965) has shown that interfering substances such as epoxy acids and peroxides have to be taken into account, the end point of the titration is difficult to discern, and a relatively large sample size is required in contrast to the Halphen method.

Pure sterculic acid was obtained from S. foetida oil by urea adduction and low temperature crystallization by Nunn (1952), who described it as C_{19} acid with a cyclopropene ring in the 9,10 position. Malvalic acid was isolated from leaf oils of malva plants by Shenstone and Vickery (1956, 1959, 1961) and characterized by MacFarlane (1957) as a C_{18} acid with the cyclopropene ring in the 8,9 position. The synthesis and feeding of cyclopropenoid derivatives has indicated that the molecular arrangement for maximum biological activity requires an unsubstituted methylene group on the cyclopropene ring. (Shenstone and Vickery 1959; Nordby et al 1962).

The storage of eggs from hens fed cottonseed meal, seeds or oil resulted in pink whites although fresh eggs from these same hens had normal whites (Sherwood 1928, 1931; Almquist and Lorenz 1933; Lorenz et al 1933; Lorenz and Almquist 1934). Ingested cyclopropenoid fatty acids as a causative agent was shown by Masson et al (1957) and Shenstone and Vickery (1956, 1959). The pink discoloration phenomenon has been attributed to a combination of conalbumin and egg white with iron from the yolk that diffuses into the white (Bandemer and Schaible 1946; Bandemer et al 1946; and Schaible et al 1946).

Cyclopropenoid fatty acids cause a very striking change in the pH of stored egg yolk by shifting the pH of the yolk from approximately pH 6.2 to 8.6 which is in the normal range of the egg white. This shift towards a higher alkaline pH has been interpreted as an alteration in the permeability of the vitelline membrane. The work of Doberenz et al (1960) supported this conclusion by demonstrating a specific increase in water uptake in one-month-stored egg yolk from hens fed cyclopropenoid fatty acids.

Shenstone and Vickery (1959) and Schneider et al (1962) showed a severe decrease in egg production in hens fed varying amounts (50 to 250 mgs.) of cyclopropenoid fatty acids. Schneider et al (1962) reported retardation of ovary and oviduct development coupled with decreased egg production in hens fed 200 mgs./day of S. foetida oil. Cottonseed products were shown by Heywang (1949) to bring about a decrease in hatchability of fertilized eggs.

Schneider et al (1961) demonstrated an acute hatchability failure in fertilized eggs from hens fed amounts greater than 10 mgs./day of S. foetida oil. Embryo deaths occurred at various times during the incubation period relative to the level of S. foetida oil ingested by the hen.

The depot fat and egg yolk fat from hens fed cyclopropenoid fatty acids had a higher content of saturated fatty acids at the expense of monoene fatty acids, as evidenced by the shift to higher stearic and lower oleic acid concentrations reported by Evans et al (1960), Schneider et al (1962), and Nordby (1963). Similar results have been reported by Ellis and Isbell (1926) in the hardening of pork fat; and Keith et al (1932, 1934) reported a higher melting point and a longer churning time for butter made of milk from cows fed cottonseed meals with a high oil content.

Sheehan and Vavich (1965) reported a delay in sexual maturity in the female rat when cyclopropenoid fatty acids were added to the diet from time of weaning until the experiments were terminated. Concurrently they also reported a lengthened and irregular estrus cycle along with a reduction in weight and size of the uterus.

Raju (1966) and Johnson (1966) have studied in vitro systems with liver homogenate and particulate liver fractions of rats and chicken in an attempt to elucidate the mechanism of action of the cyclopropenoid fatty acids in the fatty acyl desaturase system. Raju claims the inhibition of this system was due to an irreversible binding of some essential thiol groups of the enzyme. Johnson proposes

a competitive type inhibition from the results of in vitro studies. Regardless of which type of inhibition was involved, the evidence pointed to a definite impairment of the desaturation of the fatty acids in vivo rather than a deficiency of intake of oleic acid.

Hofmann et al (1952); Dauchey and Asselineau (1960); Kaneshiro and Marr (1961); and Chung and Law (1964) have worked with bacterial systems in which cyclopropane fatty acids were metabolic products. Lactobacillic acid was characterized by Hofmann (1952) as a C_{19} fatty acid containing a cyclopropane ring in the cis-11,12 position. Dauchey and Asselineau (1960) isolated a C_{17} cyclopropane fatty acid from Escherichia coli and identified the ring at the cis-9,10 position. Zalkin (1963) showed the formation of the cyclopropane fatty acids by the addition of the methyl group of S-adenosylmethionine to the unsaturated fatty acid moiety of a phospholipid. The reaction is thought to involve the addition of the methyl group across the olefinic bond in the fatty acid. This work has been of particular interest to workers in the cyclopropenoid fatty acid field in speculating an analogous pathway in plants for synthesis of the cyclopropenoid fatty acids by the addition of the methyl group of S-adenosylmethionine across an acetylinic bond in a fatty acid moiety.

CHAPTER II

METHODS AND MATERIALS

Preparation of the S. foetida oil. The oil was extracted from S. foetida seeds from the Philippine Islands.[1] The whole seeds were ground and extracted with three liters of Skellysolve F per kilogram of seed. Grinding was facilitated with a Hobart coffee mill, first with a coarse setting and then with a fine setting. The oil-rich extracts were syphoned off, and the meats were filtered on a medium-porosity, sintered-glass filter and washed several times with solvent. After three successive, overnight extractions the solvent extracts were combined and the solvent stripped off in a rotary film flash evaporator under reduced pressure. The final traces of solvent were removed with a high vacuum oil pump and a water bath at 35°C. The oils were stored at 4°C under nitrogen. The various batches of oil ranged from 33% to 35% cyclopropenoid fatty acids as determined by the modified Halphen method described in a following section.

The other oils used in these experiments were commercial safflower oil and cottonseed oil extracted and de-gossypolized from crude cottonseed oil obtained from nearby cottonseed processing plants.

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1. Zoilo C. Fraga; Forestry College; Laguna, Philippine Islands.

Composition of the diets. The composition of the basal diets is given in Table 1 (Schneider 1962, Sheehan and Vavich 1965). The majority of the experimental diets contained 4% added fat. Those which contained higher levels of oil had the oil added at the expense of carbohydrate (Table 2). The vitamin mix and salt mix were prepared separately and admixed with cerelose to facilitate mixing small quantities in the diet and to obtain a homogeneous mixture. The diets were mixed in a Hobart dough-type mixer 15 kilograms at a time. The basal diets with all ingredients except the oil were passed through a large hammer mill to assure a homogeneous, powdered diet. All diets were stored at 4°C in plastic bags. Fresh diets were prepared approximately every 3 to 4 weeks. The test and control diets were all isocaloric at any given oil level. Moisture determinations, fat extractions and nitrogen analyses were run on the mixed diets periodically to ascertain that the diets were as calculated (Table 3).

Animals. The animals used were 21-day old, weanling female and male albino rats of the Sprague-Dawley strain[1]. Upon receipt the rats were maintained on a stock standard laboratory diet for 72 hours in the receiving colony room in groups of 10 to 12 in battery-type cages. During this time the drinking water was supplemented with Sulmet[2], and the rats were examined daily for signs of respiratory

1. Sprague-Dawley, Madison, Wisconsin.

2. Sulmet, American Cyanamid Co., Princeton, N. J.

Table 1. Composition of basal diet used in all experiments

	%
Casein[1]	18.0
Fat	4.0
Glucose monohydrate[2]	69.62
Non-nutritive fiber[3]	4.0
Salt mixture[4]	4.0
Vitamin mixture[5]	0.38
Total	100.0

[1] Vitamin free; Nutritional Biochemicals Corp., Cleveland, Ohio.

[2] Cerelose 2001; Corn Products Company, New York.

[3] Solka-Floc; Brown Company, Boston, Massachusetts.

[4] Jones and Foster (1942) with NaF added to give 10 ppm in salt mixture.

[5] Vitamin mixture, supplies per 100 g of diet:

Thiamine hydrochloride	0.4 mg	Folic acid	0.2 mg
Riboflavin	0.5 mg	Biotin	0.02 mg
Niacinamide	5.0 mg	Vitamin B_{12} (0.1% trit. in mannitol)	10.0 mg
Pyridoxine hydrochloride	0.25 mg		
Calcium pantothenate	2.0 mg		
Choline bitartrate	200.0 mg	Menadione	0.2 mg
Inositol	100.0 mg	α-tocopherol	5.0 mg
p-Aminobenzoic acid	10.0 mg	Vitamin A*	1000 IU
		Vitamin D_3**	120 IU

* PGB-10; Distillation Products Industries, Rochester, New York.

** Super Nopdex-30; NOPCO Chemical Company, Richmond, California.

Table 2. Concentration of oil in the diets.

Diet No.	% oil in the diet
1	4% saff.[1]
2	3% saff. + 1% S. foet.[2]
3	2% saff. + 2% S. foet.
4	1% saff. + 3% S. foet.
5	4% saff. + 2% oleic acid[3,4]
6	2% saff. + 2% oleic acid + 2% S. foet.
7	1% saff. + 2% oleic acid + 3% S. foet.

[1] Safflower oil purchased from local food market.

[2] Extracted from S. foetida seeds.

[3] Nutritional Biochemistry Corp., Cleveland, Ohio.

[4] Diets containing more than 4 percent oil were adjusted by decreasing the basal diet an equivalent amount of carbohydrate.

Table 3. Analysis of complete diets used in reproduction studies.

Composition of oil in diet	Avg % moisture	Avg caloric content per gram	Avg % protein	Avg % oil
4% saff.	7.18	4.13	18.4	3.96
3% saff. 1% *S. foet.*	7.26	4.27	18.7	3.90
2% saff. 2% *S. foet.*	7.39	3.97	18.9	3.60
1% saff. 3% *S. foet.*	7.43	4.07	19.3	3.86
4% saff. 2% oleic a.	7.11	4.32	17.3	5.91
2% saff. 2% oleic a. 2% *S. foet.*	7.32	4.33	17.3	5.92
1% saff. 2% oleic a. 3% *S. foet.*	7.21	4.24	17.4	5.53

illnesses. Those that showed any signs of respiratory problems were immediately destroyed. The need for such precautions became evident in an early experiment when the colony was struck with an epidemic of pneumonia which required termination of that experiment.

After the initial treatment the rats were divided into groups of comparable weight and were assigned a specific purified diet. They were placed in sterilized, individual hanging cages, usually two animals per cage, and moved into a clean, isolated colony room for the duration of the experiment. The temperature was maintained at 27°C. by means of centralized heating and cooling. Weekly records were kept on all animals for weight gain and food consumption.

Feeding. Food cups were changed each morning and fresh diet supplied in each cage. Water bottles were filled with fresh water daily and the bottle and stainless steel drinking tube were changed twice weekly. Water was fed ad libitum and the majority of the experiments were pair-fed type relative to the diets. The studies of Sheehan and Vavich (1965) showed that pair-feeding was more appropriate than ad libitum feeding since food intake of rats decreased on the S. foetida diets. The growth weights in the pair-fed experiments were more evenly matched in the control groups and the test groups, which minimized any differences due to retarded growth in the test group. In pair-feeding the daily amount of diet consumed by the test group at the highest level of S. foetida oil was the amount fed to all other groups.

A small quantity of water was mixed with the diet in each food cup as a means of preventing the rats from scooping the diets out of the cups and causing a serious loss of diet. The food cups, brown ointment jars 2 1/4 in. x 1 7/8 in., were strapped to the inside cage front by a coiled spring which were very successful in thwarting the rats from tipping the food cups over. The combination of mixing water in the food and affixing the food cup to the cage was an uncomplicated procedure that was easily carried out on a daily routine for a large colony and yet overcame a serious problem of wastage inherent with use of powdered purified diets.

In all experiments a strong emphasis was placed on maintaining clean and healthy conditions in the animal quarters to lessen the chance of disease influencing the results. A rigid procedure was followed that included daily autoclaving of all food cups and glassware, periodic rotation of the animals into clean autoclaved cages, frequent washing of the walls and floors with antiseptic solutions, and daily observance for respiratory diseases and prompt treatment and isolation of animals found with possible disease. Close cooperation and advice was obtained from the Animal Pathology Department of the Agricultural College[1].

Determination of Sexual Maturity and Estrus Cycles. The perforation of the membrane covering the vagina of the females was taken as the onset of sexual maturity and this was determined by daily observation. After the perforation of the membrane, a daily

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1. Dr. James J. Sheldon, Associate Professor, Department of Animal Pathology.

vaginal smear was made on each rat. The estrus cycles were determined by the method of Long and Evans (1922) which characterizes the estrus cycle by the appearance of cornified cells in the vaginal smear. Originally a cotton swab was dipped in water and used to make the smear; later an inoculating loop was found to be more satisfactory since there was less extraneous material deposited on the loop than on the cotton swab. This made interpretation of the slide an easier task. The vaginal smears were stained with Harris Hematoxlin-Eosin and read under low power on a light microscope. The vaginal smears were also read unstained since the cornified cells were readily differentiated from the smaller nucleated cells. The length of the estrus cycle was determined by counting the days between the appearance of the cornified cells until the day immediately prior to the next appearance of the cornified cells.

Mating procedures. Generally breeding was started when the rats were 120 days of age. Normally rats could have been bred at 90 days of age, but since the S. foetida oil-fed rats matured later than the control rats, the breeding did not commence until the estrus cycles were established for all groups. The rats were swabbed at the same time each morning and the slides read immediately after. Females found to be in proestrus were placed in the males' cages in the late afternoon for mating that evening. The combinations of matings are listed in Table 4. To prevent the female from eating the diet in the male's cage, which may have been a different diet than she was receiving, all diets were placed in the cages in mid-morning

Table 4. Combinations of matings in rat reproduction studies using oil diets with and without S. foetida oil.

Composition of oil in diets of females	Matings	Composition of oil in diets of males
4% saff.	x	4% saff.
3% saff. 1% S. foet.	x	4% saff.
2% saff. 1% S. foet.	x	4% saff.
2% saff. 2% S. foet.	x	4% saff.
1% saff. 3% S. foet.	x	4% saff.
4% saff. 2% oleic a.	x	4% saff. 2% oleic a.
2% saff. 2% oleic a. 2% S. foet.	x	4% saff. 2% oleic a.
1% saff. 2% oleic a. 3% S. foet.	x	4% saff. 2% oleic a.

[1] Diets containing more than 4 percent oil were adjusted by decreasing the basal diet an equivalent amount of carbohydrate.

and removed in the afternoon prior to mating. The animals soon learned to feed during the time the food was in the cage. In the morning the females were returned to their own cages. The ratio used for the mating was two females per male in the male's cage.

The females were swabbed as usual in the morning, and the slides were checked for the presence of sperm. If sperm was evident, that female was not swabbed for the following week until a pregnancy was confirmed by a sharp rise in weight by the end of the first week after the mating had taken place. If the evidence indicated that conception had not occurred, swabbing of that female was resumed and mating was attempted again at the next proestrus.

Pregnant females were maintained in individual cages in which shredded paper towels were placed around the 16th day of pregnancy. Litters were recorded as to number and initial weight at birth and again at weaning.

Termination of Experiments. Experiments were terminated by fasting the rats for 24 hours and injecting an overdose of Surital[1] intraperitoneally. When blood samples were to be taken, a sub-lethal dose of Surital was used to anesthetize the animal, and a cardiac puncture was performed with a 20 gauge, heparinized needle and syringe. The rats were laid on their back and the needle passed upward through the xiphoid process under the sternum and into the heart. The syringe plunger was pulled back lightly and the pumping action of the heart allowed the blood to flow into

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1. Surital, Parke Davis and Co., Detroit, Mich.

the syringe. The usual yield was generally 7 to 8 mls. of blood. The adominal cavity was opened and the organs excised and quickly frozen for lipid studies or fixed for histological studies.

Four studies were made concerning reproduction and survival of new-born pups as influenced by the ingestion of cyclopropenoid fatty acids. Each of these studies was conducted for approximately 8 to 10 months.

Analytical Methods.

A. Halphen Determination. The Halphen test was modified in this laboratory by Schneider and Sheehan (1965). Spectroquality pyridine[1] was used throughout the analysis because it was found to give a lower blank than the analytical-grade reagent. Some grades were sufficiently discolored to mask the pink color of the Halphen reaction. Monoclinic sulphur was crystallized from a saturated solution of flowers of sulphur[2] in pyridine heated to 80°C and left at room temperature overnight. The crystals were filtered, air dried and ground in a mortar and pestle to a fine powder. The advantage of using monoclinic sulphur in place of amorphous sulphur as called for in most methods (Shenstone and Vickery 1961, Deutschman and Klaus 1962), was that the solubility of amorphous sulphur in carbon disulfide is less than 1%; whereas the solubility of monoclinic sulphur in carbon disulfide is approximately 70%. This higher solubility of the monoclinic sulphur allowed a preparation of a clear, color-developing solution of a known concentration of

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1. Mallinckrodt or Matheson, Coleman and Bell.

2. Rexall Drugs.

sulphur as opposed to a solution of unknown concentration of amorphous sulfur which could give a turbid reaction mixture. It was found that a 4% solution of monoclinic sulphur in carbon disulfide (AR grade) gave a satisfactory standard curve in the range of cyclopropenoid fatty acids determined by this method. The preparation of methyl sterculate was that of Kircher (1964). The purity of the methyl sterculate was usually around 85% to 90% as determined by the hydrogen-bromide titration of Skau and Magne (1965). The standard curves were corrected for the hydrogen bromide value of the methyl sterculate (Fig. 2).

The oil bath used in these studies was a Blue-M, Model MW-1145CXX, which has an operating range from ambient to 260°C. Technical grade glycerin was used as the heating medium since its water solubility facilitated cleaning the glassware after immersion in the bath. Samples were read in a Coleman Jr. Spectrophotometer, Model 6A, in 18mm x 150 mm cuvettes. The reaction tubes were Kimax culture tubes without lips, 22 mm x 175 mm, calibrated in our laboratory at ten mls. by a mark scribed around the circumference of the tube with a diamond point pencil.

The assay was as follows: The sample was diluted in diethyl ether (peroxide free and dried over anhydrous sodium sulfate) to contain approximately 50 to 100 micrograms of the cyclopropenoid fatty acids per milliliter of solvent. One ml. of diluted sample was added to 8 mls. of spectroquality pyridine in the reaction tube to which was added 1 ml. of color reagent (4% monoclinic sulphur in

carbon disulfide). The tubes were capped with glass marbles 24mm in diameter, which served as condensers, and then placed in the oil bath at 45°C. and held at this temperature for 15 minutes. The temperature was raised to 95°C. with the tubes remaining in the bath. When 95°C. was attained, it was held for 5 minutes. The temperature was then raised to 105°C. and the tubes were held at this setting for 1 hour after the desired temperature was realized. The tubes were removed from the bath and placed in a pan of cool water for approximately one-half hour. The samples were brought back to a volume of 10 mls. with spectroquality pyridine and transferred to the cuvettes. One hour after removal from the oil bath the samples were read at 505 millimicrons. The concentration of the cyclopropenoid fatty acids was determined from a standard curve with methyl sterculate as the standard (Fig. 2). The necessary correction for dilutions was made and the results were reported in percent Halphen acids as methyl sterculate.

B. Lipid Extraction. The liver and epididymal fat were studied for lipid composition. The method of lipid extraction was a combination of the methods of Folch et al (1951, 1956) and Bligh and Dyer (1959). The samples were thawed, minced in a beaker immersed in ice, and an aliquot removed and extracted with a 2:1 solution of chloroform:methanol. The sample was homogenized in a Servall Omni-mixer. The extract was filtered through Whatman #1 paper and placed in a graduated cylinder to which was added a volume of 0.003M $MgCl_2$ equal to 20% of the volume of the extract.

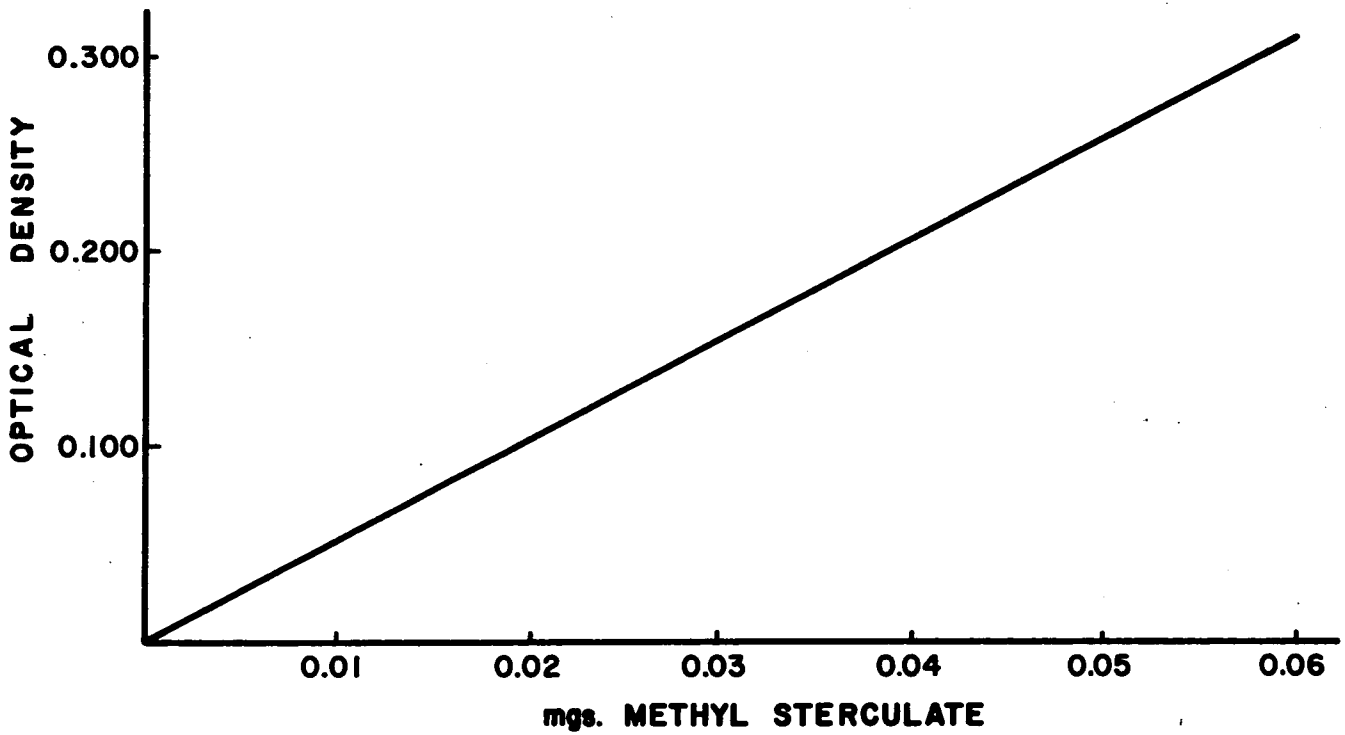


Figure 2. Standard curve for Halphen determination expressed in mgs. of Methyl sterculate corrected for cyclopropenoid fatty acids by hydrogen bromide titration.

The layers were allowed to partition out, and the lipids were removed in the chloroform layer. The percent lipids was determined by taking an aliquot of the chloroform extract in a tared erlenmeyer flask and evaporating the solvent off at 40° to 45°C. in an oil bath under an atmosphere of nitrogen. The flasks were dried over concentrated sulfuric acid in a vacuum desiccator and weighed. Simultaneously with the lipid extraction the moisture content was determined on a separate aliquot by the method of Sanette and Jandorf (1947).

C. Saponification and Esterification. The extracts were saponified with 50% potassium hydroxide in 95% ethanol at room temperature over night. The non-saponifiables were removed with diethyl ether, the alcoholic extract was neutralized with 5N hydrochloric acid, and the fatty acids were extracted with diethyl ester. The fatty acids were esterfied with boron trifluoride in methanol and separated by water floatation or solvent extraction (Metcalfe and Schmitz 1961). Fatty acid analysis was run by gas-liquid chromatography on an Aerograph HiFY-II, Model 600C, with a hydrogen flame detector. The columns used were 5 ft. x 1/8 in. stainless steel packed with acid-washed Chromosorb W 80/100 mesh and coated with either 15% diethyleneglycol succinate or 15% ethyleneglycol succinate. The hydrogen source was an Aerograph Hydrogen Generator, Model A-650 and the carrier gas was commercially available helium (grade A). The usual flow rate was 22 mls./min. of hydrogen and 20 to 25 mls./min. of helium measured at the flame tip.

The temperature was controlled by an Aerograph Linear Temperature Programmer, Model-326, by use of the isothermal mode at a temperature of 175 to 180°C. The temperature was held at ± 0.5°C. for any given setting. Commercially-available, chromatographically pure methyl ester standards were used for calibrating the retention times for the fatty acids that were determined. Cyclopropenoid fatty acids were not determined by gas-liquid chromatography because of the decomposition products formed and the small amounts present in the tissue. Methods have been published by Kircher (1966) using methanolic-silver nitrate which now permits reasonable quantitation of cyclopropenoid fatty acids by gas-liquid chromatography but were not employed in these studies.

D. Miscellaneous Methods. The caloric content of the diets was determined in an Oxygen Bomb Calorimeter and the moisture content was determined in a Brabender Moisture Testure. The dietary protein content was determined by a micro-kjeldahl assay and the amino acid profile was run on a Beckman Amino Acid Analyzer Model 120B.

The usual soxhlet fat extraction for the lipid content of the diets was not satisfactory for finely powdered, purified diets. An alternate method was devised in our laboratory by the use of a Burrell Wrist-Action Shaker. A sample of diet (20 gms.) was placed in a round bottom boiling flask (250 ml.) with the addition of petroleum ether (20 ml. of Skelly F). The flask was stoppered and taped in place. A hypodermic needle was inserted through the stopper to serve as a vent and avoid excessive pressure build up.

The flask was agitated at medium speed for one hour. The solvent was decanted and the process repeated twice. After the final shaking the contents were filtered through Whatman #1 paper and the flask and filter washed several times with small quantities of clean solvent. The solvent extracts were combined and the solvents stripped off with a rotary film flash-evaporator. The percent lipids was calculated after correcting for the moisture content. The recoveries of lipids extracted from the diets using this method were 95+% of the calculated lipid content added to the diet. Fatty acid analysis were run by the methods described previously.

Statistical analysis of the data was carried out by one way analysis of variance and significant differences in the means were determined by Duncans Multiple Range tables at 95% confidence limits. In the tables superscripts were used to denote significant differences and most all means were reported with 1 standard deviation.

CHAPTER III

RESULTS AND DISCUSSION

Growth and Development. The total feed intake for each group in each of the experiments was approximately equal since pair-feeding was used. However, the growth response of the groups fed S. foetida oil were less than those in the safflower oil controls. The average growth response of the highest level S. foetida oil groups was significantly less than the 4% safflower oil groups by 15% during the entire period of observations (Fig. 3). The growth response was inversely proportional to the level of S. foetida oil in the diet at any given week. When the rats reached maturity, the body weights of all groups plateaued but maintained the same relative difference between the control and test groups.

The addition of 2% oleic acid to the diets did not change the overall character of the growth response, but it reduced the difference between the control groups and the highest level S. foetida oil groups to an average of approximately 10% for body weight (Fig. 4).

The first sixteen weeks on the experiment showed a decreased feed utilization for the higher S. foetida oil groups compared to the control groups, but this effect was diminished when calculated over a 40 or 50 week period. In rat studies Sheehan and Vavich

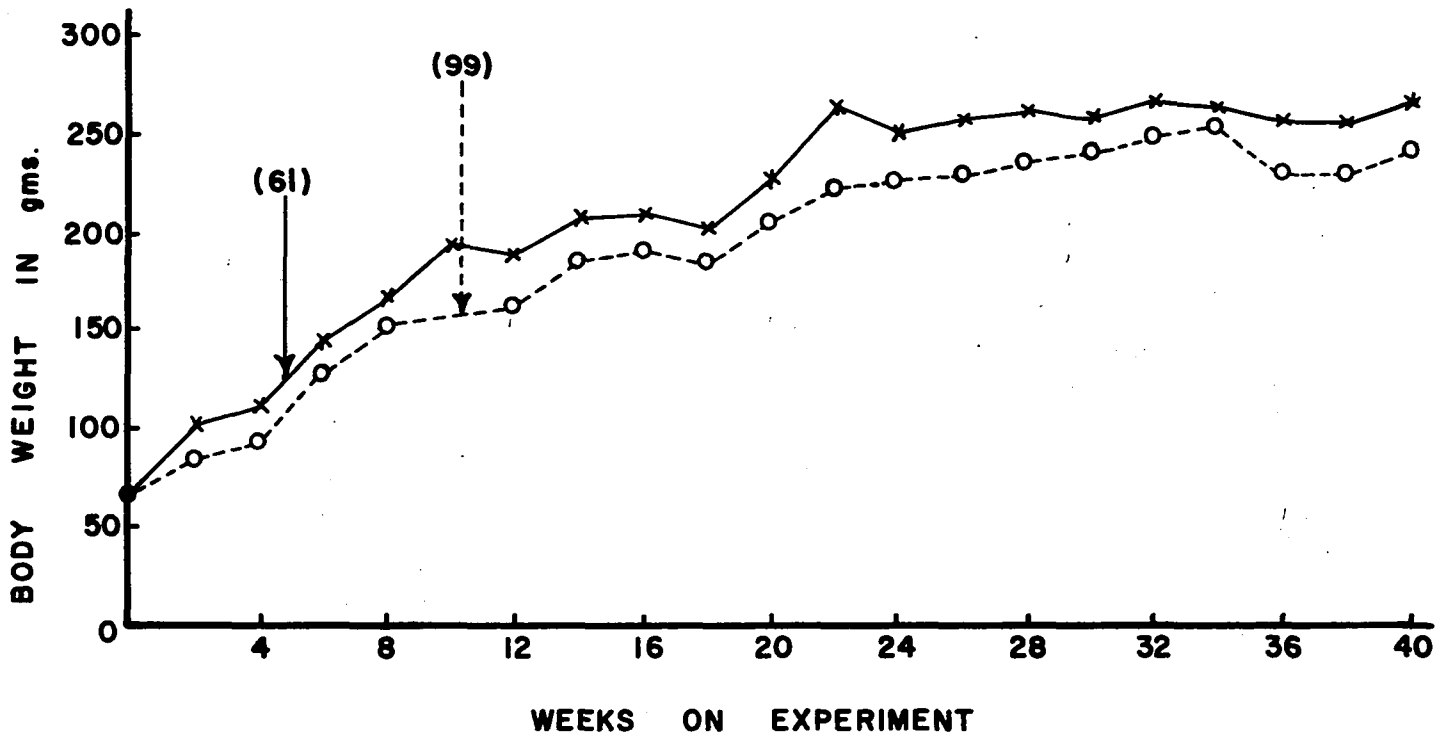


Figure 3. Comparison of growth curves of female rats pair-fed oil diets with and without Sterculia foetida oil.

x x 4% Safflower oil.
o---o 1% Safflower + 3% S. foetida.
( ) Average days of age at perforation of vaginal membrane.

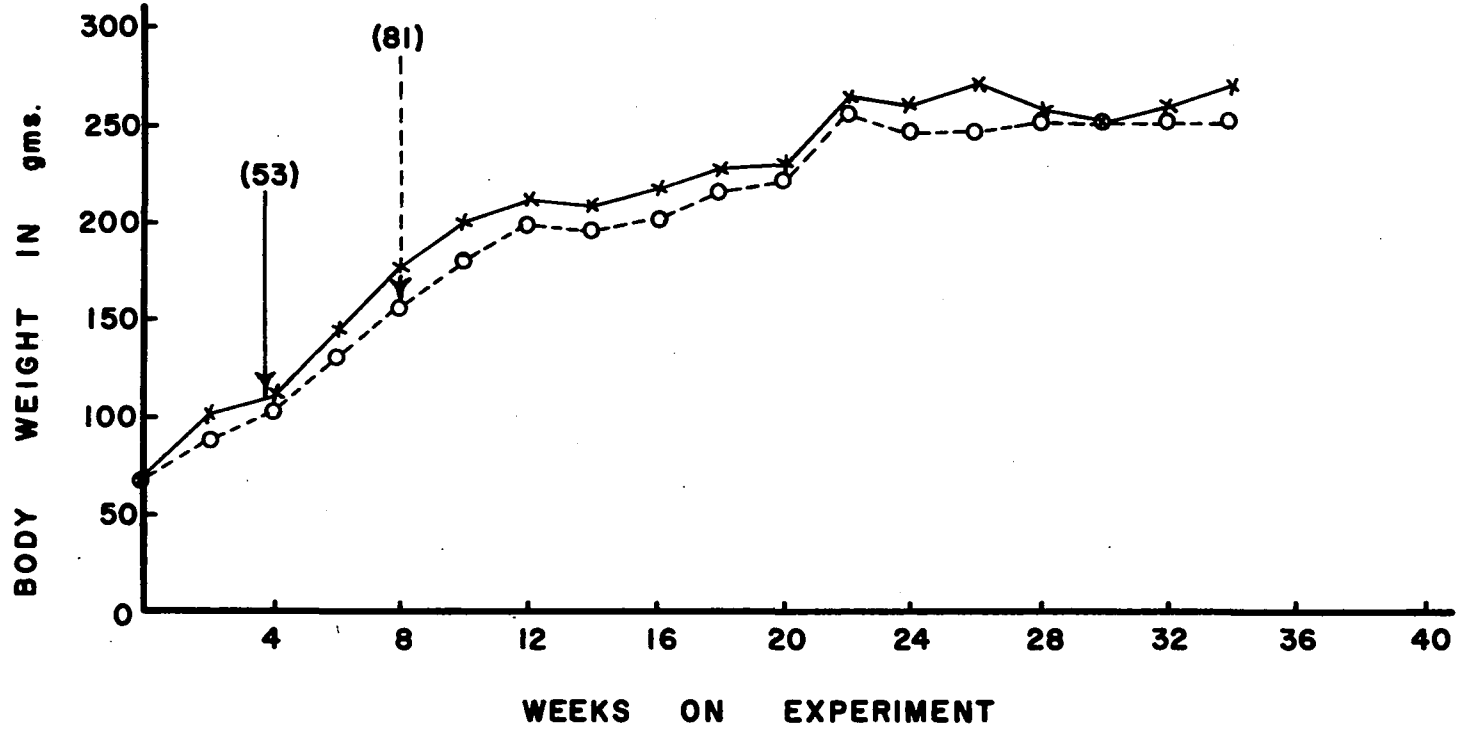


Figure 4. Comparison of the effect of oleic acid on growth curves of female rats pair-fed oil diets with and without S. foetida oil.

x—x 4% Safflower oil + 2% oleic acid.
o---o 1% Safflower + 2% oleic acid + 3% S. foetida.
( ) Average days of age at perforation of vaginal membrane.

(1965) reported decreased feed utilization for equivalent levels of S. foetida oil either ad libitum or pair-fed. Schneider (1962) showed similar reductions in growth response and feed utilization in chickens when S. foetida oil was fed in gelatin capsules. The use of the capsule feeding technique eliminated differences in feed consumption due to a possible non-palatability factor. The purified diets used in the rat studies were sweet-tasting and had no objectionable odor to the human sense of smell. Since the test and control groups consumed the same total amount of diet, and S. foetida oil groups had a lower feed utilization; it was concluded that the S. foetida oil caused one or more disturbances in normal metabolic pathways.

Body and Liver Weight. The body weights and liver weights were as reported in Table 5. In the 1% safflower-3% S. foetida oil groups, the body weights were significantly lower and the liver weights significantly higher than in the 4% safflower oil groups. The addition of oleic acid to the diets did not alter this relationship, although it appeared to reduce the differences slightly. The reduced body weight and increased liver weight associated with the ingestion of cyclopropenoid fatty acids by rats was similar to results obtained with chickens (Schneider 1962).

The increased weight of the livers of S. foetida oil-fed rats was reflected by an increased lipid content (Table 5). The livers frequently were mottled and rough-textured as opposed to a smooth and homogeneous texture in the control livers. It was felt

Table 5. Comparison of body weights and liver weights of female rats pair-fed oil diets with and without S. foetida oil.

Composition of oil in diets	Avg body wt in gms	Avg liver wt in gms	Liver as % body wt	Avg % lipids in liver
4% saff.	254 ± 29.4[1]	5.95 ± 0.94	2.35[d2] ± 0.49	23.4[b] ± 2.87
3% saff. 1% S. foet.	252 ± 16.1	7.83 ± 1.00	3.13[c] ± 0.47	27.1[a] ± 2.75
2% saff. 2% S. foet.	239 ± 17.1	9.41 ± 1.73	3.86[b] ± 0.82	28.3[a] ± 3.16
1% saff. 3% S. foet.	217 ± 37.3	9.22 ± 1.65	4.28[a] ± 0.47	29.8[a] ± 3.38
4% saff. 2% oleic a.	251 ± 16.9	5.77 ± 0.78	2.31[b'] ± 0.28	26.8[b'] ± 1.20
2% saff. 2% oleic a. 2% S. foet.	241 ± 16.5	7.84 ± 0.73	3.32[a'] ± 0.48	33.6[a'] ± 2.16
1% saff. 2% oleic a. 3% S. foet.	225 ± 17.4	8.30 ± 1.16	3.71[a'] ± 0.50	33.0[a'] ± 1.79

[1] Standard deviation.

[2] Means with different superscripts are statistically different at the 0.05 level of probability.

that the increased lipid concentration in the liver of S. foetida oil groups could result from (a) an increased fatty acid synthesis, particularly saturated fatty acids, or (b) a blockage in the desaturation of saturated fatty acids with the concomitant deposition of the saturated fatty acids in the liver. The high level of saturated fatty acids in the liver could possibly impair the mobilization of these acids from the liver to other sites of usage or storage. The results of the studies reported here favor the possibility of an inhibition of the fatty acyl desaturase enzyme system.

Cyclopropenoid Fatty Acid Concentration. The concentration of cyclopropenoid fatty acids in the liver lipids showed a significant increase as the concentration of S. foetida oil was increased (Table 6). The low value for the control group which had not ingested cyclopropenoid acids was attributed to a false reading in the Halphen test. A method was devised for the removal of the majority of the interfering compounds by use of absorption column chromatography. The absorption media was a mixture of equal parts of Hyflo Super Cel and Seasorb 43[1]. The lipid extract was dissolved in hexane and passed through the column and followed by two washings of hexane. The eluates were collected and the solvent removed. This treatment eliminated the highly-colored pigments in

1. Fisher Scientific Co., Fair Lawn, N. J.

Table 6. Comparison of percent cyclopropenoid fatty acids and percent fatty acid composition of the livers from female rats pair-fed oil diets with and without S. foetida oil.

Composition of oil in diets	% cyclo-propenoid fatty acids	Avg % fatty acid composition								
		C[1] 12:0	C 14:0	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 20:4	Stearic:Oleic[2]
4% saff.	0.25	-	0.48	23.98	2.48	20.89	17.31	15.60	20.12	1.21
3% saff. 1% S. foet.	1.29	1.23	2.58	24.98	-	23.07	12.42	21.04	14.84	1.86
2% saff. 2% S. foet.	3.25	2.44	4.95	24.60	-	24.18	11.24	18.78	13.49	2.15
1% saff. 3% S. foet.	6.88	3.00	6.55	25.76	-	27.36	12.49	18.38	6.40	2.19
4% saff. 2% oleic a.	0.75	-	-	22.32	-	24.72	23.04	14.80	15.12	1.07
2% saff. 2% oleic a. 2% S. foet.	2.09	1.93	3.99	23.36	-	23.80	20.77	15.19	10.96	1.15
1% saff. 2% oleic a. 3% S. foet.	1.06	2.46	5.11	24.44	-	25.04	19.81	13.28	9.88	1.26

[1] Number of carbons in the chain and the number of double bonds per chain.

[2] Ratio of stearic acid to oleic acid.

liver lipids, as well as in egg yolk lipids and cottonseed oils, and reduced the false color in the Halphen reaction of the control groups. Bailey et al (1961) established that certain phosphatidal compounds and numerous amines and other nitrogenous compounds were responsible for the false color obtained in the Halphen reaction in the absence of cyclopropenoid compounds. The column treatment improved the color reaction of the lipids from S. foetida oil rats, so that the absorption spectra was identical to that of pure methyl sterculate.

The concentration of the cyclopropenoid fatty acids in the body fat, designated para-uterine in Table 7, followed the same pattern as the liver lipids. The deposition of these acids in the depot fat suggests that the cyclopropene ring is not degraded by beta-oxidation. Wood and Reiser (1965) and Chung (1966) demonstrated the operation of beta-oxidation up to the cyclopropane ring in animals ingesting cyclopropane fatty acids. However, this process has not been studied with ingested cyclopropene fatty acids such as sterculic.

Fatty Acid Composition. The fatty acid composition of the liver lipids showed significant shifts toward increased concentrations of saturated fatty acids at the expense of mono-unsaturated acids when cyclopropenoid fatty acids were fed (Table 6). This type of shift was reported by other investigators (Evans et al 1960, 1961, 1962; Schneider 1962; and Reiser et al 1963). These reports

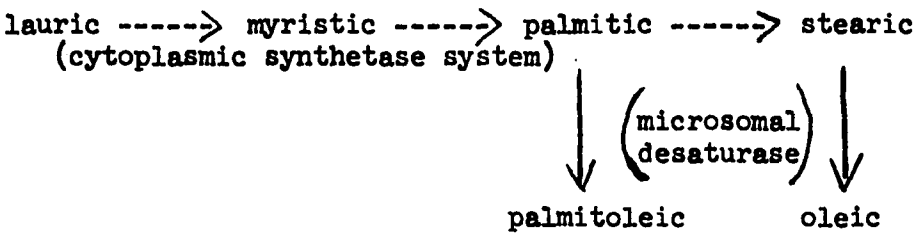
Table 7. Comparison of percent cyclopropenoid fatty acids and percent fatty acid composition of para-uterine fat from female rats pair-fed oil diets with and without S. foetida oil.

Composition of oil in diets	% cyclo-propenoid fatty acids	Average % fatty acid composition						
		C[1] 14:0	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	Stearic[2] / Oleic
4% saff.	0.09	1.78	32.07	7.48	7.36	34.92	16.40	0.21
3% saff. 1% S. foet.	1.04	3.07	44.08	-	16.45	18.86	17.54	0.87
2% saff. 2% S. foet.	3.43	3.21	36.59	-	24.35	21.22	15.63	1.15
1% saff. 3% S. foet.	4.75	3.20	42.72	-	28.40	14.32	11.36	1.98
4% saff. 2% oleic a.	0.09	1.63	25.52	6.87	8.33	41.84	15.81	0.20
2% saff. 2% oleic a. 2% S. foet.	1.52	2.77	33.15	-	21.22	30.24	12.62	0.70
1% saff. 2% oleic a. 3% S. foet.	2.17	3.06	37.16	-	18.96	30.12	10.03	0.63

[1] Number of carbons in the chain and the number of double bonds per chain.

[2] Ratio of stearic acid to oleic acid.

concerned primarily the shift in stearic and oleic acids in egg yolk lipids. Shenstone et al (1965) postulated the shift was due mainly to an increased synthesis of the higher-melting fatty acids incorporated into neutral triglycerides. However, the data reported here tend to support the hypothesis previously stated that ingestion of low levels of cyclopropenoid fatty acid causes a blockage in the desaturation of saturated acids rather than stimulation of de novo synthesis of these acids. The normal synthesis of palmitoleic and oleic acids occurs as follows (Bloomfield and Block, 1960; Marsh and James, 1962):



The accumulation of the lower homologs of palmitic acid, namely lauric and myristic acids, reported in the present work suggests the failures of the normal metabolic pathway to operate. Harlan and Wakil (1962) suggested an alternated pathway for the synthesis of oleic acid via a mitochondrial system in rat liver which produced oleic acid without the stearic acid intermediate. This pathway must be a minor alternate route because S. foetida oil was shown definitely to inhibit the stearate-to-oleate conversion (Reiser and Raju, 1964). The marked shift (Table 6) in the stearic and oleic acids indicated a blockage of the fatty acyl desaturase system which caused the build-up of the lower saturated acids.

The data showed a decrease in the desaturation of dienoic acids as evidenced by the decrease in arachidonic acid which can be synthesized *in vivo* from linoleic acid by chain elongation and desaturation. The linoleic acid content of safflower oil was approximately 75%, and this may be responsible for the high content of arachidonic acid in the control livers. Apparently, the high concentrations of linoleic acid can be shunted via the synthesis of arachidonic acid. The presence of the cyclopropenoid fatty acids appeared to inhibit this diene desaturase system in the same manner as the blocked conversion of saturated acids to the corresponding monoene acids. The specificity of the fatty acyl desaturase system is not clearly understood. There could be a group of enzymes which are specific for certain chain lengths and certain carbon-carbon bonds relative to a position from the methyl or carboxyl end. It is possible that one enzyme could handle all situations for that particular organism, but this seems hardly likely.

The effect of oleic acid did not significantly alter the fatty acid composition in the liver lipids of rats in either the control or test groups (Table 6). The shift from oleic to stearic acid in the *S. foetida* oil groups was reduced, due to the exogenous supply of oleic acid but the increase in stearic acid was significant in the *S. foetida* oil fed groups. Similar results were obtained relative to the appearance of lower homologs of palmitic acid in the test groups when oleic acid was added to the diet.

The fatty acid composition of the depot fat (para-uterine) followed the same general patterns as the liver lipids (Table 7). Gorin and Shafir (1963) indicated that in adipose tissue 1.7% of the triglyceride ester bonds are hydrolyzed and resynthesized every 24 hours which means the triglyceride have a half life of approximately 40 days. Therefore, the depot fat could be considered a relatively active metabolic organ. The data showed a sharp rise in the concentration of stearic acid with a simultaneous decrease in oleic acid as the level of the S. foetida oil was increased. The ratio of stearic:oleic in the depot fat of the control group was 0.21, and in the 1% safflower-3% S. foetida oil group the ratio was 1.98, which was a ten-fold increase. In the liver lipids the ratio was 1.21 and 2.19 for the two groups respectively, which represented only a two-fold increase. The combined total of these two acids was approximately 42% of the depot fat and 37% of the liver fatty acids.

The concentration of palmitic acid in the liver lipids did not vary significantly in any of the experimental groups; whereas, in the depot fat palmitic acid increased 25% at the 1% S. foetida-3% safflower oil level. This indicated that, as the normal synthesis of palmitic acid in the liver proceeded, the block in the desaturation of stearic to oleic caused the excess palmitic acid to be shuttled to the depot stores in the rats fed cyclopropenoid fatty acids.

Sexual Maturity. The delay in sexual maturity of female rats as determined by the perforation of the vaginal closure membrane was significantly different at the 2% and 3% levels of S. foetida oil (Table 8). This was in agreement with results reported previously by Sheehan and Vavich (1965). The perforation of this membrane in the rat is a function of estrogen levels (Gorbman and Bern, 1962). As the female matures and estrogen levels increase, the closure membrane degenerates with estrus commencing shortly thereafter. This delayed maturity in S. foetida oil groups was taken as an indication of lower estrogen levels.

The body weights at the time of sexual maturity in the female rats were not significantly different in the test and control groups. In the 1% safflower-3% S. foetida oil group the body weight averaged 10% greater than the control groups. Sheehan and Vavich (1965) showed similar results in ad libitum and pair-fed experiments with the same levels of S. foetida oil.

The addition of oleic acid to the diets did not eliminate the differences in the age of sexual maturity in the test and control groups. The body weights were relatively the same as for rats fed no additional oleic acid (Table 8).

Estrus Cycles. The length and variation of the estrus cycles of the S. foetida oil fed rats were significantly different than the 4% safflower oil control groups (Table 9). In the S. foetida oil groups the number of cornified cells in the vaginal smears was

Table 8. Comparison of body weights and ages at the time of perforation of the vaginal closure membrane of female rats pair-fed oil diets with and without S. foetida oil.

Composition of oil in diets	Avg body wt gms		Avg age in days	
	mean ± SD	Range	mean ± SD	Range
4% saff.	128 ± 16.0	80 - 150	61[c1] ± 15.4	33 - 84
3% saff. 1% S. foet.	140 ± 19.7	96 - 182	64[c] ± 12.0	38 - 86
2% saff. 2% S. foet.	150 ± 17.9	114 - 185	76[b] ± 16.7	39 - 120
1% saff. 3% S. foet.	166 ± 33.9	107 - 202	99[a] ± 25.0	56 - 147
4% saff. 2% oleic a.	113 ± 22.1	82 - 148	53[c'] ± 12.8	38 - 76
2% saff. 2% oleic a. 2% S. foet.	131 ± 8.2	113 - 141	68[b'] ± 3.9	63 - 76
1% saff. 2% oleic a. 3% S. foet.	150 ± 21.9	121 - 175	81[a'] ± 12.4	63 - 94

[1] Means with different superscripts are statistically different at the 0.05 level of probability.

Table 9. Comparison of length of estrus cycles of female rats pair-fed oil diets with and without S. foetida oil.

Composition of oil in diets	Total no. estrus cycles	Total no. days	Avg length per estrus cycles	% 4-6 day estrus cycles
4% saff.	570	3505	6.32[c1]	79.0
3% saff. 1% S. foet.	335	2438	7.59[b]	61.2
2% saff. 2% S. foet.	207	1746	9.05[a]	51.7
1% saff. 3% S. foet.	164	1499	9.97[a]	41.5
4% saff. 2% oleic a.	208	1184	5.72	83.2
2% saff. 2% oleic a. 2% S. foet.	189	1286	7.07	71.0
1% saff. 2% oleic a. 3% S. foet.	149	1005	6.97	63.8

[1] Means with different superscripts are statistically significant at the 0.05 level of probability.

smaller than that in the control groups at the time of estrus. The Schorr-Trichiome stain technique was used late in the experiment and indicated that the epithelial cells in the vaginal smears of the cyclopropenoid fatty acids groups were not completely cornified. The Schorr-Trichiome stain will penetrate completely cornified squamous epithelial cells and stain them green. Complete cornification is a function of estrogen levels (Schering, 1941). The fact that the epithelial cells in the vaginal smears of S. foetida oil fed rats were not completely cornified was taken as another indication of low estrogen levels in these rats. The reduced number of pseudo-cornified cells in the vaginal smears of S. foetida oil fed groups indicated the occurrence of a weak estrus. This could be the result of low estrogen levels and the release of fewer mature ova during ovulation. Rascop et al (1966) reported fewer mature follicles in the ovaries of S. foetida oil rats and a degeneration of follicles at various stages of maturation.

The variation in the length of the estrus cycles of S. foetida oil groups was larger than the control groups (Table 9). In the 4% safflower oil groups the percentage of cycles in the normal range of 4 to 6 days was 80% whereas in the 1% safflower oil-3% S. foetida oil group it was around 40 percent. The variation in cycle length for any given rat in the S. foetida oil groups was wider than in control group rats. It was not uncommon for the range to be 4 to 14 days in the S. foetida groups whereas in the control group the range general was 3 to 8 days with the majority being in the 4 to 6 day range.

The addition of oleic acid to the diet reduced the average estrus cycle length to 7 days in the S. foetida oil groups and decreased the variation slightly, but the average cycle length was still significantly different than that of the control group (Table 9).

Reproduction. The comparison of reproduction in rats fed diets with and without cyclopropenoid fatty acids showed conclusively the detrimental effects of these acids on reproductive performance (Table 10). The 1% safflower-3% S. foetida oil group produced no litter for the breeding periods studied. Groups with decreased amounts of S. foetida oil succeeded in producing offspring, but the majority of pups were born dead or died within a few hours after birth. In the 2% safflower-2% S. foetida oil group 15 litters were produced for a total of 110 pups, and none of the pups lived long enough to nurse. The average size of the pups born to the mothers in the S. foetida oil groups was less than the size and weight of the pups from control group mothers. There were no teratological differences in the pups from either group other than the difference in size. However, histological examination of various organs from fetuses in S. foetida oil groups revealed degenerative tissues changes that appeared to be irreversible. Damage in the kidney involved the glomeruli and the distal and proximal convoluted tubules in the cortex. Swelling of the epithelium was possibly due to a change in permeability of the tubules. The lungs showed areas of hemorrhage in the alveoli.

Table 10. Comparison of reproduction and survival of litters for female rats pair-fed oil diets with and without *S. foetida* oil.

Composition of oil in diets	No. of rats bred	Total no. of litters	Total no. pups born	Avg no. pups per litter	Total no. pups weaned	% weaned
4% saff.	45	29	187	6.4	163	87.1
3% saff. 1% *S. foet.*	37	19	131	6.8	18	13.7
2% saff. 2% *S. foet.*	27	15	110	7.3	0	0.0
1% saff. 3% *S. foet.*	30	0	0	0	0	0.0
4% saff. 2% oleic a.	10	9	69	7.7	35	50.7
2% saff. 2% oleic a. 2% *S. foet.*	10	8	52	6.5	0	0.0
1% saff. 2% oleic a. 3% *S. foet.*	10	8	45	5.6	0	0.0

There were also degenerative changes in the hearts and livers. These extensive changes suggested that death of the newborn would have occurred whether or not the pups had nursed.

The addition of 2% oleic acid to the diet produced a significantly higher reproduction rate in the S. foetida oil fed groups but did not change the survival rate (Table 10). The pups were born dead or died shortly thereafter, and the same type of degenerative change were found in tissues examined histologically. There were no teratological differences other than size in the pups of the S. foetida oil groups supplemented with oleic acid when compared to the control groups.

The effect of cyclopropenoid fatty acids on the male rats was studied in the early phase of these experiments. Feeding the S. foetida oil, at the levels reported in Table 2, did not appear to impair sexual development or reproductive performance to any significant degree in male rats. Similar results were reported by Sheehan et al (1966) in cockerels and by Beroza and La Brecque (1967) in the common house fly (Diptera-Muscidae). Therefore, in the remainder of the experiments, males fed the 4% safflower oil control diets were used for mating in reproduction studies.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Feeding of low levels of S. foetida oil to female rats resulted in:

1) reduced feed efficiency and body size

2) significant changes in the concentrations of saturated and unsaturated fatty acids in the liver and body fat.

3) delayed sexual maturity and prolonged, irregular estrus cycles.

4) impaired reproduction of young and reduced survival of newborn.

The conclusions reached were that cyclopropenoid fatty acids inhibited the fatty acyl desaturase enzyme system that resulted in several detrimental effects to the female rat. The reduced amounts of unsaturated fatty acid could result in the increased use of saturated fatty acid in the structure of cell membranes and thereby change the permeability characteristics of the cell mechanism. The apparent low levels of estrogens in female rats ingesting cyclopropenoid fatty acids could possibly be the result of an inhibition of a desaturation step in the synthesis of estrogen.

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