(*4*). Dales and Chardonnet (*4*) suggest that the virions are moved vectorially from the plasma membrane to the nuclear envelope along pathways furnished by the microtubules. The virus subsequently begins replicating in the nucleus. Esau and Gill (*5*), studying dividing cells of tobacco leaves infected with tobacco mosaic virus (TMV), did not observe close association of TMV with spindle microtubules, even though the virus particles were found distributed among the chromosomes and spindle. To the best of our knowledge, there have been no other reports in the literature describing the close relationship of plant viruses and spindle microtubules. Because there is no convincing evidence that the MI-1 strain of BSMV replicates in the nucleus, and since the virions appear to be attached to the spindle microtubules, we suggest that, in the case of this strain of BSMV, the microtubules may be involved in virus assembly or transport of virions from mother to daughter cells during cell division, or both.

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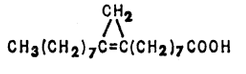
Mitogenic Activity of Sterculic Acid, a Cyclopropenoid Fatty Acid

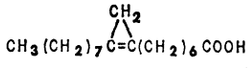
Abstract. *Hepatocytes in rainbow trout and rat are stimulated to augmented DNA synthesis and cell division by low concentrations of cyclopropenoid fatty acids in the diet. Sterculic acid isolated as the methyl ester from* Sterculia foetida *oil has been identified as one of the mitogenic principles.*

Cyclopropenoid fatty acids (CPFA's), constituents of plants of the order Malvales, which includes several important sources of food for man and animals, have long been known to exert toxic (*1*) and other adverse biological effects (*2*, *3*) in a variety of animals. Two such fatty acids have been identified and have the following structures

Sterculic acid



Malvalic acid



In farm animals, especially poultry, these effects have been largely obviated by decreasing the content of the residual oil in cottonseed meal, which is used as a major protein supplement in the diet. Although it has been established that CPFA's are incorporated into animal fat and thus carried in the food chain, it is generally considered that the concentrations of these compounds currently present in foodstuffs are well below the threshold dose for deleterious effects (*1*). Several years ago, interest in these compounds was renewed when it was shown that at very low doses CPFA's markedly potentiate the carcinogenic effect of aflatoxin B_1 in liver of rainbow trout *Salmo gairdnerii* (*4*, *5*). The study reported here establishes a hitherto unknown effect of CPFA's; their ability to induce hepatocytes of trout and rat to undergo cell division in vivo.

Cyclopropenoid fatty acids in *Sterculia foetida* oil were incorporated into a synthetic diet (*6*) at a dose level of 200 parts per million (ppm) and fed freely to fingerling rainbow trout; control fish were fed the synthetic diet alone. Fish were killed after 4 weeks of feeding. Male Sprague-Dawley rats (50 g) were fed Purina rat chow containing CPFA's (500 ppm); control animals were pair fed and received Purina rat chow alone. One hour before the rats were killed they were injected intraperitoneally with [^{3}H]thymidine (Schwarz/Mann, specific activity 10.0 c/mmole) at a dose of 1 μc per gram of body weight. Rats were killed after 2 and 4 weeks of feeding. Liver weights and liver DNA were determined for all animals. Approximately 4 g of liver was homogenized in cold, normal saline with a Potter-Elvehjem homogenizer to make a 20 percent homogenate (weight to volume). The DNA was extracted by a modification of the Schmidt-Thannhauser-Schneider method (*7*) and separated from RNA by differential acid solubility (*8*). The DNA content was determined colorimetrically (*9*). Specific activity of [^{3}H]thymidine incorporated into DNA was measured with a Packard Tri-Carb liquid scintillation spectrometer by use of a mixture of 2,5-bis(5′-*tert*-butylbenzoxazoyl)-2′-thiophene (BBOT, Packard Instrument Company, La Grange, Illinois) and toluene. The internal standard, [^{3}H]-toluene (Packard Instrument Company), had a counting rate of 2.26×10^6 disintegrations per minute (dpm) per milliliter. After percentage efficiency was determined, the counting rates of the samples and values of the specific activity of DNA were calculated. Thin slices of liver were fixed in calcium formalin, embedded in paraffin, and prepared for light microscopy, and mitoses in hepatocytes were counted.

The livers of all animals fed CPFA's were large and moderately fatty; liver weight, content of hepatic DNA, mitotic index, and incorporation of [^{3}H]thymidine into nuclear DNA of hepatocytes were increased significantly above those of control animals, as shown in Table 1. These findings indicate that the hepatomegaly was accompanied by augmented DNA synthesis and number of hepatocytes. Histologic and cytologic alterations were evident in hepatocytes after 2 weeks of CPFA feeding, and were strikingly similar in both species studied. The cytoplasm had a foamy appearance and contained numerous fat droplets. The nuclei were large and hyperchromatic and contained enlarged nucleoli, some of which were multiple and occupied much of the nuclear volume. Mitoses were frequent (Fig. 1) and all stages were present; although most of these appeared to be normal, there were occasional abnormal figures characterized by fragmented and lagging chromosomes. Examples of isolated hepatic cell necrosis, which were rare (0.4 percent) at 2 weeks, were more numerous (0.7 percent) at 4 weeks.

In an attempt to establish whether sterculic acid, the CPFA compound present in highest concentration in *S. foetida* oil, might be capable of stimu-

lating mitosis, the methyl derivative of the acid was prepared from 20 g of the oil and isolated by fractional precipitation in dry methanol at −35°C (*10*). Direct infrared absorption spectra were obtained for the ester dissolved in carbon tetrachloride by measurement in a Perkin-Elmer model 21 infrared spectrophotometer; a cell with a 0.1-mm path length and NaCl windows was used. Maximum absorption occurred at 9.91 μm, which is indicative of pure methyl sterculate (*11*). Single doses consisting of 5 mg of the ester mixed in 0.5 ml of olive oil were administered by stomach tube to 50-g rats; the animals were then allowed free access to rat chow and water and were killed 18, 30, and 48 hours later. Control rats were given 0.5 ml of olive oil and then treated identically. Eighteen hours after administration of methyl sterculate, the mitotic index was 1.4 ± 0.8 mitoses per 1000 hepatocytes, compared to a control value of 0.2 ± 0.4. At 30 hours, the mitotic index in livers of treated rats was 5.2 ± 1.9 and the control value was 0.3 ± 0.4. At 48 hours, the value for rats given sterculate had risen to 11.2 ± 0.9, while the control value was 0.1 ± 0.3. At all the time periods, stimulation of mitosis by sterculate was highly significant ($P < .001$). Cytoplasmic fat droplets were present at all time periods and the percentage of necrotic hepatocytes was 0.1 at 30 hours and 0.25 at 48 hours.

These observations establish that small amounts of CPFA's are capable of stimulating hepatocytes to synthesize DNA and divide in members of two widely separated animal classes and that this effect is sustained as long as CPFA's are administered. Further, it has been shown that this effect can be attributed, in part at least, to the sterculic acid present in *S. foetida* oil. Whether malvalic acid is also capable of inducing mitosis in hepatocytes is at present unknown. Although the mechanism by which CPFA's and especially methyl sterculate stimulate mitosis remains to be elucidated, the results presented here suggest that since the intense mitotic activity induced by CPFA's is accompanied by very few necrotic cells, mitoses in this instance are probably not a response to a decrease in liver cell mass. The prompt and prolonged stimulation of liver cell division by a single dose of methyl sterculate suggests further that mitosis may be triggered by a metabolic event directly related to exposure to the ester. The mitogenic effect of sterculate may serve to explain the potent cocarcinogenic effect on rainbow trout of CPFA's administered with aflatoxin B_1 (*4, 5*), since it is well established that a mitogenic stimulus such as hepatectomy potentiates the carcinogenic effect of chemical carcinogens (*12*). Preliminary results suggest that CPFA's may also stimulate mitosis in epithelial cells of exocrine pancreas and, to a lesser degree, proximal tubules of kidney. These results suggest that the CPFA-induced cellular perturbation which results in mitosis must involve a system basic to the replication of several cell types, in contrast to effects of isoproterenol (*13*), phytohemagglutinin (*14*), and pokeweed mitogens (*15*), which are more limited in their scope of action.

Table 1. Effect of CPFA's on liver weights, liver DNA, and mitotic index of hepatocytes (mitoses per 1000 hepatocytes). In the last four columns each value is the mean ± standard error of the mean for five animals. For trout, liver weight and liver DNA are total values; for rat, liver weight is in grams per 100 g of body weight and liver DNA is in milligrams per 100 g of body weight. Specific activity of [^{3}H]thymidine in DNA is in disintegrations per minute per milligram of DNA. Abbreviation: N.D., not done.

CPFA's (ppm)	Week of feeding	Liver weight (g)	Liver DNA (mg)	Mitotic index	[^{3}H]thymidine in DNA (dpm/mg)
			Trout		
0	4	0.035 ± 0.04	1.0 ± 0.08	0.5 ± 0.8	N.D.
200	4	0.050 ± 0.03*	1.7 ± 0.20*	4.4 ± 1.2*	N.D.
			Rat		
0	2	3.22 ± 0.23	12.4 ± 0.4	0.2 ± 0.4	4,870 ± 837
500	2	5.14 ± 0.30*	19.9 ± 1.7*	8.4 ± 2.7*	178,730 ± 28,620*
0	4	3.82 ± 0.25	16.3 ± 1.3	0.2 ± 0.3	5,260 ± 975
500	4	5.46 ± 0.63*	25.0 ± 1.7*	6.6 ± 1.5*	80,940 ± 11,930*

* Significantly different from normal, $P < .001$.

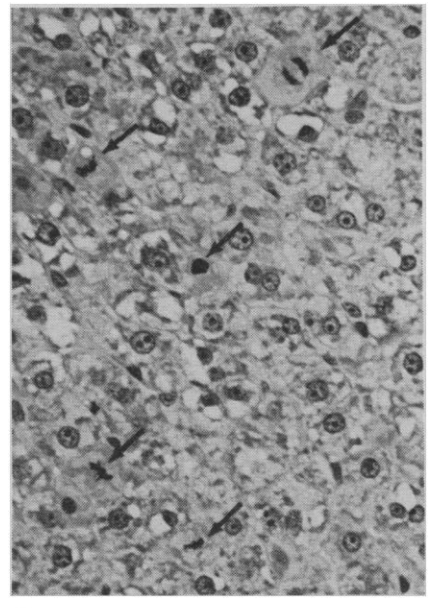


Fig. 1. Rat liver after 2 weeks on a diet containing cyclopropenoid fatty acids (500 ppm). Five hepatocytes are in various stages of mitosis (arrows) (× 320).

The foregoing results are of considerable interest since CPFA's are present in low concentrations in foods consumed by humans. For example, commercial cottonseed salad oils may contain 0.04 to 0.5 percent, and cottonseed meal about 0.01 percent (*3*). Further, both hens' eggs and the body fat of laying hens and broilers fed diets including cottonseed meal contain CPFA's (*3*).

The implications for man of long-term ingestion of CPFA's are impossible to assess at present since there are no data concerning their effects on subhuman primates. However, several facts are reassuring. The use of cottonseed oil by man as a dietary source of fat has been sufficiently prolonged and has involved such large segments of the world population that any overt toxicologic effects should have been detected. Moreover, the food industry continues its efforts to lower the content of CPFA's in food products. Finally, the cyclopropene ring is so prone to chemical modification by heating (*1*) that thorough cooking probably destroys the biological activity of CPFA's. Although the potential dangers to man from vegetable and animal food products containing low concentrations of CPFA's are probably remote (*5*), the CPFA content should be kept at a minimum and, whenever possible, removed totally.

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Reversal by L-Dopa of Impaired Learning Due to Destruction of the Dopaminergic Nigro-Neostriatal Projection

Abstract. *Rats receiving bilateral stereotaxic injections of 6-hydroxydopamine into the zona compacta of the substantia nigra failed to learn a one-way active avoidance response. Small doses of* L-*dopa (1.5 milligrams per kilogram of body weight) in combination with a peripheral decarboxylase inhibitor reversed this impairment. Animals with lesions which acquired the avoidance response during* L-*dopa administration retained this response when drug treatment was discontinued. These experiments suggest that the dopaminergic nigro-neostriatal projection serves a critical function in the acquisition of learned instrumental responses.*

Many pharmacological studies suggest that brain catecholamines (CA) play an important role in the acquisition and maintenance of conditioned avoidance responding (CAR). For example, drugs that deplete central monoamines or block postsynaptic CA receptor sites have repeatedly been shown to disrupt avoidance responding (*1*). In addition, intraventricular application of 6-hydroxydopamine, which can cause substantial and selective destruction of central CA neurons, has been found to produce deficits in CAR (*2*). While several workers have implicated dopamine rather than norepinephrine (NE) as being critically involved in CAR, it has not been possible to evaluate the role of specific CA pathways with these techniques.

With the discovery that specific CA projections could be selectively lesioned by the localized stereotaxic injection of small quantities of 6-hydroxydopamine, new possibilities for the investigation of the functional roles of these systems have emerged (*3*). We have shown that rats with bilateral 6-hydroxydopamine lesions of the zona compacta of the substantia nigra, the origin of the dopaminergic nigro-neostriatal bundle (NSB), will not acquire either a CAR or an instrumental response for food reinforcement (*4*). If, however, the animals were overtrained on CAR prior to the lesions and then subsequently retested, almost perfect retention of the response was observed. Thus the deficits produced by these lesions are not the result of generalized motor deficits and appear to be specific for the acquisition of CAR.

The deficits in CAR produced by reserpine or α-methyl-paratyrosine can be temporarily reversed by the CA precursor, 3,4-dihydroxy-L-phenylalanine (L-dopa) (*5*). Furthermore, L-dopa is known to be clinically effective in the treatment of Parkinson's disease, a condition in which the dopaminergic nigro-neostriatal projection has degenerated (*6*). We now report that the deficits in CAR produced by destruction of the dopaminergic NSB can be reversed by L-dopa treatment in the rat.

Table 1. Effect of bilateral 6-hydroxydopamine lesions of the substantia nigra on brainstem and hypothalamic norepinephrine and striatal dopamine concentrations. Data represent means (± 1 standard error of the mean) of 15 to 18 animals in each group.

Item	Controls (μg/g)	6-Hydroxydopamine (μg/g)	% Control
Striatal dopamine	10.1 ± 0.32	0.84 ± 0.11*	8.3
Hypothalamic NE	1.67 ± 0.04	0.74 ± 0.05*	44.3
Brainstem NE	0.36 ± 0.01	0.46 ± 0.01*	127.8

* Significantly different from controls $P < .01$.

Eighteen male Wistar rats (280 to 320 g) received bilateral stereotaxic 6-hydroxydopamine lesions of the zona compacta of the substantia nigra by a procedure that has produced nearly total destruction of the dopaminergic NSB (*4*, *7*, *8*). Control animals ($N = 16$) underwent sham operations but did not receive intracerebral injections. After the operation the experimental animals became aphagic and adipsic and had to be tube-fed as described (*7*). After the animals with lesions recovered the ability to maintain themselves, they were divided into two groups matched for body weight. The control group was similarly divided. Six weeks after the surgery, all animals were tested for their sensitivity to L-dopa. Previous experiments have demonstrated that rats lesioned with 6-hydroxydopamine show an increased responsiveness to L-dopa, an effect that is presumably mediated by postsynaptic receptor supersensitivity (*9*). The purpose of this procedure was to determine a dose of L-dopa that was just below that which would induce stereotyped behavior. All animals received Ro 4-4602 (50 mg/kg) 30 minutes before they were given injections of L-dopa to inhibit peripheral decarboxylase activity (*10*). Such preliminary treatment enhances the central effects of L-dopa (*11*). L-Dopa at 25 mg/kg administered intraperitoneally produced intense stereotyped behavior in the lesioned animals, but did not have any noticeable effect on the controls. Lower doses (12.5 and 3.0 mg/kg) also induced stereotypy but 1.5 mg/kg did not, and hence this dose was used in the CAR experiments.

The acquisition of a one-way active avoidance response was studied as described (*4*). Briefly, rats were placed in one side of a wooden box (75 by 25 by 30 cm) divided into two equal compartments by a sliding door. The lifting of the door initiated a discrete tone that preceded the delivery of shock (0.7 ma) through the grid floor by 10 seconds. The grid shock lasted for 5 seconds, and in no instance did any animal fail to escape from the shock within that time. The intertrial interval was 30 seconds and was spent in the "safe" compart-