Inhibition of the Stearyl Coenzyme A Desaturase System by Sterculate*

S. V. Pandee 
and James F. Mead
From the Laboratory of Nuclear Medicine and Radiation Biology, UCLA, and the Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California 90024

SUMMARY

The mechanism of the inhibition in vitro of the microsomal stearyl-CoA desaturase system by sterculate has been investigated. Our data do not support the suggestions that this is mediated by the binding of thiol groups of enzyme proteins by the cyclopropene group and that this is competitive with substrate. Sterculate did inhibit the desaturase system in vitro but data suggest this to be nonspecific, stemming from the detergent nature of free fatty acid and hence of doubtful significance for metabolism in vivo. However, it was found that dietary (methyl) sterculate decreased the activity of the microsomal stearyl-CoA desaturase system. The mechanism of this action of sterculate remains obscure.

A new procedure for the precise measurement of the microsomal stearyl-CoA desaturase system is described based on the permanganate-periodate oxidation of fatty acid methyl esters followed by the separation of monomethyl azelate-1-14C derived from oleate-1-14C from other fatty acid methyl esters with the use of a Florisil column. The utilization of rat liver microsomes for the enzymatic synthesis of stearyl-1-14C-CoA is also described.

One of the well known effects of dietary cyclopropene fatty acids is a relative decrease in the concentration of monounsaturated fatty acids with a concomitant increase in the amount of the corresponding saturated fatty acids in tissue lipids (1-3). Various investigators have reported that dietary cyclopropene acids inhibited the conversion in vivo of stearate-1-14C to oleate (3-5). Subsequently the conversion in vitro of stearate-1-14C to oleate, thus stored, appeared to remain stable, as judged by the analysis of the isolated fatty acid (after methylation and hydrolysis) by gas-liquid chromatography. Potassium or ammonium salts of fatty acids were prepared as described previously (7, 8).

The radioactive fatty acids were purified by thin layer chromatography. Potassium or ammonium salts of fatty acids were purchased from Sigma and GSH from Calbiochem. Dihydro-sterculate was obtained from Analabs, Inc., North Haven, Connecticut. The urea clathrate of methyl sterulate, used in studies in vitro, was a kind gift from Dr. A. R. Johnson, CSIRO Division of Food Preservation, Ryde, New South Wales, Australia. Palmityl-1-14C-CoA and oleic acid-1-14C were generous gifts from Dr. Armand J. Fulco. Stearic-1-14C was a product of Nuclear-Chicago. Sources of other chemicals have been described previously (7, 8).

Rat liver microsomes were obtained as described previously (7). Stearyl-1-14C-CoA was synthesized basically according to the method of Kornberg and Price (9) but rat liver microsomes1 were used as the enzyme source and the possibility of any concomitant desaturation of stearyl-CoA formed was eliminated by carrying out the synthesis under nitrogen in the presence of cyanide. The incubation system contained the following com-

* These studies were supported in part by Contract AT(04-1)-GEN-12 between the Atomic Energy Commission and the University of California, and by United States Public Health Service Research Career Award GM-19,177 from the Division of General Medical Sciences, National Institutes of Health.

† Present address, Biochemistry Department, Faculty of Medicine, University of Manitoba, 750 Bannatyne, Winnipeg 3, Canada.

1 Since rat liver microsomes could be used without any further manipulations and the long chain fatty acid-activating enzyme remains stable, when microsomes are kept frozen (7), this procedure may be simpler than those with lyophilized guinea pig liver microsomes (9, 10) or a microsomal preparation from avocado mesocarp (11).

2 The presence of this concentration of KCN was without effect on the activity of the stearate-activating enzyme.
ponents (micromoles) in a final volume of 40 ml: Tris-HCl, 5000 (pH 7.6); ATP, 600; CoA-SH, 28; KF, 500; KCl, 400; GSH, 500; MgCl2, 100; rat liver microsomes; 80 mg of protein; and stearate-1-14C, 20.66 (0.5 mCi). Incubation was for 1 hour at 37°. Stearyl 1-14C-CoA was isolated and purified as described by Bloomfield and Bloch (10). The over-all yield, as estimated by hydroximate colorimetry (7), was 65% based on the amount of fatty acid added.2 Paper chromatography of the product in water- pyridine-isopropanol (9) showed that over 90% of the total radioactivity was associated with the area occupied by stearyl-CoA.

Assay of Stearyl-CoA Desaturase System—The usual assay system, in a final volume of 0.25 ml, contained: 50 μmole of Tris-HCl buffer (pH 7.4), 0.2 μmole of NADH, 16 μmole of stearate-1-14C-CoA (40,000 to 60,000 cpm), and 200 to 300 μg of microsomal protein to start the reaction. Incubations were for 5 min at 37° in air. The reaction was terminated by the addition of 0.25 ml of methanolic KOH (90% v/v methanol, 0.73 μN KOH) and the contents were saponified for either 30 min at 80° or overnight at 50°. After acidification, lipids were extracted with ether-petroleum ether (1:1, v/v) and fatty acids were also included. Addition of 0.25 ml of methanolic KOH (90% v/v methanol, 0.73 μN KOH) and the contents were saponified for either 30 min at 80° or overnight at 50°. After acidification, lipids were extracted with ether-petroleum ether (1:1, v/v) and fatty acids were added. The methyl esters were freed of polar impurities by passing the solution of lipids in 5% ether in petroleum ether (1:1, v/v). The lipid extract was applied to an approximately 500-mg Florisil column packed in, and containing, petroleum ether. The column was washed twice with 12 to 13 ml of ether, and the Florisil was air-dried and transferred to a counting vial containing 2 ml of water. After adding 15 ml of naphthalene-dioxane counting fluid (13), the radioactivity was determined with the Packard Tri-Carb 574 scintillation spectrometer. A correction was applied for the decrease in counting efficiency that resulted from the presence of water and Florisil. Controls involving either heated microsomes or to which stearate-1-14C-CoA was added following the addition of methanolic KOH were also included.

RESULTS AND DISCUSSION

Principle and Validity of Assay Procedure—In the procedure used for measuring stearate-CoA desaturase activity, advantage has been taken of the fact that under appropriate conditions peroxisomal protein to start the reaction. Incubations were for 5 min at 37° in air. The reaction was terminated by the addition of 0.25 ml of methanolic KOH (90% v/v methanol, 0.73 μN KOH) and the contents were saponified for either 30 min at 80° or overnight at 50°. After acidification, lipids were extracted with ether-petroleum ether (1:1, v/v) and fatty acids were added. The methyl esters were freed of polar impurities by passing the solution of lipids in 5% ether in petroleum ether (1:1, v/v). The lipid extract was applied to an approximately 500-mg Florisil column packed in, and containing, petroleum ether. The column was washed twice with 12 to 13 ml of ether and the Florisil was air-dried and transferred to a counting vial containing 2 ml of water. After adding 15 ml of naphthalene-dioxane counting fluid (13), the radioactivity was determined with the Packard Tri-Carb 574 scintillation spectrometer. A correction was applied for the decrease in counting efficiency that resulted from the presence of water and Florisil. Controls involving either heated microsomes or to which stearate-1-14C-CoA was added following the addition of methanolic KOH were also included.

Inhibition of desaturase system by sterculate and its irreversibility by reduced glutathione

The complete reaction system contained 50 μmole of Tris-HCl (pH 7.6), 0.8 μmole of NADH, 294 μg of microsomal protein (from "refed" rat liver), and 16 μmole of stearate-1-14C-CoA, in a final volume of 0.25 ml. Incubation for enzyme assay was for 7.5 min at 37°. When appropriate, addition of reduced glutathione was made after that of NADH. When GSH and sterculate both were present, addition of GSH preceded that of fatty acid. After fatty acid addition, the contents were incubated for 7 min at room temperature before microsomes were added. After adding microsomes, incubation at room temperature was continued for 7 more min before the assay was initiated by the addition of stearate-CoA.

| Table I | Effect of prior sterculate treatment on thiol content of reduced glutathione and microsomes |
|---------|------------------------------------------------------------------------------------------|
| Experiment | Prior sterculate treatment | Absorbance at 412 μm |
| GSH | - | 0.245 |
| GSH | + | 0.250 |
| Microsomes | - | 0.109 |
| Microsomes | + | 0.109 |
hour at room temperature and that the adsorption of monomethyl-14C azelate on Florisil and the elution of methyl stearate-14C from the column were essentially complete (98 to 99%). With this analytical procedure it was possible to measure stearate conversions to oleate of the order of 1% with a high degree of accuracy and with excellent reproducibility. This enabled assays to be carried out at nonlimiting levels of stearyl-CoA under linear kinetic conditions whereby activity of the desaturase system could be expressed precisely. In the methods based on gas-liquid chromatographic separations of oleate from stearate, this has not been attained (2, 3, 5). Further, the presently described procedure is simpler, quicker, and more precise than the separation of oleate and stearate by argentation thin layer chromatography (5, 15, 16) and any a, b-unsaturated fatty acid formed would not contribute to error in the present method as compared to the other chromatographic methods considered above. To confirm that the radioactivity retained by the Florisil in the usual assay with stearyl-14C-CoA and microsomes as outlined in the procedure was due to monomethyl-14C azelate, in one case the ether-washed Florisil with adsorbed radioactive material was treated with BF3 in methanol (16). The lipids were extracted and thin layer chromatography with 10% ether in pentane as developing solvent showed that at least 90% of the radioactivity was associated with the dimethyl azelate (Rf 0.40) fraction. Under the assay conditions used, the rate of desaturation was linear with varying microsomal protein within the range of 200 to 480 μg of protein. The rate of desaturation was linear for somewhat more than 10 min of incubation only. All assays were carried out within these limits and exact assay details have been described.

Investigation of Possible Interaction between Thiol and Cyclopropene Groups as Source of Inhibition of Stearyl-CoA Desaturase System by Sterculate—Kircher (17) found that cyclopropene compounds can undergo addition reactions with monothiols and hinted at the possibility of this being relevant to the physiological effects of stercurate. Raju and Reiser (5) confirmed the suggestion of Holloway, Polatto, and Walil (10) that the rat liver fatty acid-desaturating enzyme requires intact thiol groups for its activity, and also reported that cysteine and glutathione as well as preparations of desaturase lost their sulphhydryl groups on treatment with cyclopropene fatty acids. It was therefore suggested that the mechanism of inhibition of monodesaturation by cyclopropene fatty acids was due to the irreversible binding of enzyme sulphhydryl groups by the cyclopropene group.

We attempted to re-examine this possibility with a more direct assay procedure starting with stearyl-14C-CoA as substrate and found that stercurate was indeed inhibitory for the microsomal desaturase system (Table I). To see whether the cause of this inhibition was an irreversible reaction of cyclopropene groups with the sulphhydryl groups of the enzyme, stercurate was previously incubated with an excess of reduced glutathione before it was allowed to react with the enzyme system and the effect of such preliminary incubation of the effectiveness of stercurate as an inhibitor of the desaturase system was determined. As is evident from the results obtained (Table I), the preliminary incubation of stercurate with a 100- to 300-fold excess of reduced glutathione did not affect the inhibitory effectiveness of stercurate at all. This finding casts doubt on the suggested reaction between thiol and cyclopropene groups as the cause of the stercurate inhibition of the desaturase system. Direct experiments were then performed to see whether stercurate readily reacts with the thiol groups of reduced glutathione and of the desaturase system, as claimed by Raju and Reiser (5). Ellman's reagent (5, 5'-dithiobis(2-nitrobenzoic acid)), readily reacts irreversibly with aliphatic thiols, giving rise to mercaptide ion with intense absorption at 412 nm, and the separation of oleate and stearate by argentation thin layer chromatography with 10% ether in pentane (16) readily reacts irreversibly with aliphatic thiols, giving rise to mercaptide ion with intense absorption at 412 nm, and this conveniently permits the measurement of changes in the concentration of free thiol groups (20). Hence, experiments were initiated making use of 5, 5'-dithiobis(2-nitrobenzoic acid), to see whether there was any evidence of disappearance of the thiol groups of either the reduced glutathione or the microsomal enzyme system on treatment with stercurate as is to be expected if the cyclopropene groups reacted with the thiol group(s). The conditions under which stercurate was allowed to react with reduced glutathione or microsomal enzyme preparation were the same as those under which stercurate inhibited the desaturase system. As may be seen from the data obtained (Table II) preliminary incubation with excess stercurate did not cause any change in the thiol content of either the reduced glutathione or the microsomal enzyme preparation. Thus, in view of these results, it is concluded that the stercurate inhibition of the de-
saturase system is not the result of the reaction of the thiol groups of the enzyme system with the cyclopropene groups.

**Effect of Substrate Concentration on Desaturase System and Its Reaction to Sterculate Inhibition**—Allen et al. (3) have suggested that sterculate acts as a competitive inhibitor for stearate desaturase. Determination of such an effect in our initial experiments was complicated by the anomalous results obtained when the effect of stearyl-CoA concentration on the activity of the enzyme system was determined. As may be seen from Fig. 1, at lower concentrations of stearyl-CoA the enzyme activity curve was noticeably S-shaped while at higher concentrations, stearyl-CoA inhibited the desaturase system. It can be seen (Fig. 1) from the comparison of results obtained at two different levels of microsomes that the range of concentration of stearyl-CoA giving optimum activity as well as showing an inhibitory effect was related to the concentration of microsomal material, being higher at the higher microsomal amount. This is similar to the effects of linolenate concentration on the microsomal activation of linolenate described earlier (7) and shows that microsomes readily bind stearyl-CoA as well as free fatty acids. The possibility that the contaminating acyl-CoA hydrolase of the microsomal fraction competes with the desaturase system for stearoyl-CoA at lower levels and that this results in the S-shaped activity curve for the desaturation was tested. For this, microsomes from fed rat liver were used since these were found to have increased activity of the desaturase system while the acyl-CoA hydrolase activity was not increased by refeeding. To minimise the interference from the acyl-CoA hydrolase, the incubation period was reduced to 3 min only. However, as is evident from Fig. 2, an S-shaped activity curve was still seen, thus complicating the determination of possible competitive inhibition by sterculate. This type of enzyme activity curve was also seen with palmitoyl-CoA as a substrate (Fig. 3). However, with this substrate, the sigmoidity of the activity curve was less pronounced than that with stearoyl-CoA. The determination of the possible competitive inhibition of the desaturase system by sterculate was therefore attempted with palmitoyl-CoA as substrate. However, in such experiments, no inhibition by sterculate was noticeable. When the effect of sterculate on the desaturase system was determined with concentrations of palmitoyl-CoA similar to those of stearoyl-CoA used in the earlier experiments in which sterculate inhibition of stearoyl-CoA desaturation was seen, it was found that, under these conditions, the desaturation of palmitoyl-CoA was also inhibited by sterculate (Table III). This suggested that the sterculate inhibition observed was related to the concentration of acyl-CoA esters and a subsequent experiment with stearoyl-CoA also confirmed that the sterculate inhibition was not evident at lower levels of stearoyl-CoA. Thus, under the conditions of these experiments, the results obtained failed to confirm the contention of Allen et al. (3) that sterculate is a competitive inhibitor of the desaturase system.

**Inhibition of Desaturase System by Free Fatty Acids**—The question of the specificity of sterculate on the desaturase system has been previously examined. Raju and Reiser (5) reported that, under conditions in which sterculate was inhibitory, its methyl mercaptan addition product and oleate were not. Allen et al. (6) have shown that the rate of stearoyl-CoA desaturation by rat liver microsomes considerably exceeded the rates of palmitoyl-CoA desaturation (contrast Nakagawa and Uchiyama (23)). This agrees with the findings of Allen, Hubbard, and Gibson (24) which suggest that stearate may be desaturated to a greater extent in the liver than is palmitate and also in agreement with experiments with rat liver slices in which stearoyl-$^4$CoA-desaturation was found to exceed that of palmitate-$^4$Co (Dr. M. Uchiyama, personal communication).

---

**Table III**

| Substrate        | Sterculate | Palmitate or stearyl-CoA formed | Inhibition due to sterculate % |
|------------------|------------|--------------------------------|-------------------------------|
| Stearyl-CoA      | 1519       | 1367                           | 25                            |
| Palmitoyl-CoA    | 100        | 1154                           | 37                            |
| Palmitoyl-CoA    | 50         | 863                            | 27                            |
| Palmitoyl-CoA    | 100        | 697                            | 41                            |

---

1 Zahler, Barden, and Cleland (21) also observed anomalous enzymatic kinetics with palmitoyl-CoA as a substrate while studying the enzymatic acylation of L-glycerol-3-P and this was found to be related to the formation of micelles of palmitoyl-CoA at low levels (22). It is likely that the presently observed S-shaped enzyme activity curve resulted from the preference of the enzyme system for the micellar form of long chain acyl-CoA esters as substrate. Since the precise micellar concentration of the substrates under the experimental conditions used was not known and the enzyme activity curve appeared kinetically complex, the determination of $K_\text{m}$ for acyl-CoA esters was not possible. Moreover, the effect of concentration of acyl-CoA esters on any enzymatic reaction is likely to be related to the amount of enzyme preparation wherever acyl-CoA esters become bound to the enzyme protein, as shown by the present experiments with stearoyl-CoA at two different microsomal levels (Fig. 1). Consequently, the concentration of acyl-CoA esters required for half-maximal velocity will be variable and related to the amount of such enzyme preparation. This may be one of the reasons for the variability of the $K_\text{m}$ value of stearate in the stearate desaturase system described by Allen et al. (3).

6 This experiment showed that the rate of stearoyl-CoA desaturation by rat liver microsomes considerably exceeded the rates of palmitoyl-CoA desaturation (contrast Nakagawa and Uchiyama (23)). This agrees with the findings of Allen, Hubbard, and Gibson (24) which suggest that stearate may be desaturated to a greater extent in the liver than is palmitate and also in agreement with experiments with rat liver slices in which stearoyl-$^4$CoA-desaturation was found to exceed that of palmitate-$^4$Co (Dr. M. Uchiyama, personal communication).
**Table IV**

*Inhibition of desaturase system by various free fatty acids*

| Fatty acid added | Oleate formed | Inhibition % |
|------------------|--------------|-------------|
| **Experiment I** |              |             |
| Oleate, 50       | 1260         | 17          |
| Oleate, 100      | 1043         | 21          |
| **Experiment II**|              |             |
| Oleate, 200      | 1819         | 43          |
| Linoleate, 200   | 1052         | 43          |
| **Experiment III**|             |             |
| Phytanate, 50    | 1606         | 26          |
| Phytanate, 100   | 1133         | 41          |
| Sterculate, 50   | 1172         | 27          |
| Sterculate, 100  | 931          | 42          |
| Dihydrosterculate, 50 | 1660 | 26          |
| Dihydrosterculate, 100 | 1572 | 3          |

(3) also concluded that the desaturase system was specifically inhibited by cyclopropane fatty acids since stercoolate and malvalate both were inhibitory in *vitro*. Sterculate was found to be more inhibitory than malvalate while dihydrosterculate, a diene acid mixture (consisting of 9-methylenecadec-10-enolic acid and isomeric 10-methylenecadec-8-enolic acid), and linoleic acids were only inhibitory at much higher levels. On the other hand, Uchiyama, Nakagawa, and Okui (16) reported that unsaturated fatty acids inhibited the desaturase system in *vitro; polynsaturated fatty acids were found to be more effective in this regard than oleate. It was implied that this could have a regulatory role for fatty acid desaturation. We re-examined this aspect and confirmed that dihydrosterculate was much less inhibitory than stercoolate (Table IV). However, oleate and linoleate were also found to be effective inhibitors, as was phytanate (3,7,11,15-tetramethyl hexadecanoate). These experiments revealed that the inhibitions in *vitro* observed with stercoolate were not specific. Recently, from an investigation of the effects of free fatty acids on several enzyme activities, we were able to conclude that the inhibitory effects of free fatty acids are generally nonspecific and are due to the detergent properties of these inhibitory substances (8).

**Table V**

*Inhibitory effect of stercoolate as related to amount of microsomal protein*

To the reaction system containing 50 μmoles of Tris HCl (pH 7.4) and 0.8 μmoles of NADH was added stercoolate, followed by microsomes. After 3 min incubation at 37°, the assay was started by the addition of 16 μmoles of stearyl-CoA in a final volume of 0.35 ml. Incubation was for 5 min at 37°.

| Microsomal protein | Stercoolate | Oleate formed | Inhibition due to stercoolate % |
|-------------------|------------|--------------|--------------------------------|
| µg | µmoles | µmoles | % |
| 147 | 598 | 5098 | 17 |
| 147 | 311 | 1043 | 48 |
| 294 | 1606 | 100 | 43 |
| 294 | 1011 | 100 | 27 |
| 441 | 2515 | 100 | 42 |
| 441 | 1916 | 100 | 26 |

**Table VI**

*Inhibition of desaturase system by various surface-active compounds*

| Compound added | Oleate formed | Inhibition % |
|----------------|--------------|-------------|
| Experiment I   |              |             |
| Lysolecithin, 80 μM | 1155 | 24 |
| Lysolecithin, 480 μM | 870 | 23 |
| Experiment II  |              |             |
| Palmityl-DL carnitine, 40 μM | 2840 | 5 |
| Palmityl-DL carnitine, 200 μM | 2238 | 21 |
| Deoxycholate, 0.05% | 2352 | 17 |
| Deoxycholate, 0.25% | 298 | 79 |
| Experiment III |              |             |
| Stearyl-CoA, 64 μM | 731 | 25 |
| Stearyl-CoA, 192 μM | 182 | 75 |
| Stearyl-CoA, 192 μM, + 1.4 mg of BSA | 366 | 50 |
| Stearyl-CoA, 192 μM, + 2.8 mg of BSA | 623 | 15 |

* Stearyl-CoA, bovine serum albumin, and Tris-HCl were previously incubated for 10 min prior to the assay.

**Table VII**

*Dietary effects of methyl stercoolate on activity of desaturase system*

The assays were performed under usual conditions described under "Experimental Procedure" under linear kinetic conditions. The "refed" group consisted of rats that had been fasted for 2 days and then allowed access to the standard laboratory diet for the 2 subsequent days. In the fat-free dietary group, rats were maintained on a fat-free diet from the age of 14 days to 12 weeks. In the methyl stercoolate-fed group, 50 mg of this lipid were fed daily.

| Dietary group | No. of animals | Oleate formed per min per mg of protein |
|--------------|---------------|--------------------------------------|
|              |               | Range | Average |
| Normal diet  | 4             | 333–483 | 413 |
| "Refed"      | 5             | 1066–1520 | 1253 |
| Fat-free diet | 2             | 1500–2500 | 2033 |
| Fat-free diet and stercoolate | 5 | 253–720 | 480 |

The possibility that the inhibition was due to the CoA esters of the fatty acids rather than free fatty acids, under the conditions of their experiments, is not eliminated.
gested that these inhibitions could also be due to detergent type action. If this were true, then the inhibition in vitro of the desaturase system by sterculate may be expected to be related to the amount of microsomal material in a way similar to those described earlier for inhibition of certain microsomal enzyme activities by free fatty acids (8). This expectation was borne out (Table V) as the inhibitory effectiveness of sterculate was found to increase with a decrease in the concentration of microsomal material. The earlier observations that sterculate was not inhibitory at low levels of stearoyl-CoA and palmitoyl-CoA as substrates can now be readily explained. Since fatty acyl-CoA esters also inhibit enzyme activities similarly to free fatty acids (8), it is to be anticipated that, when both these compounds are present together, the inhibitory effect will be related to the combined effects of the two types of compounds. It may be seen from Fig. 1 that an excess of stearoyl-CoA itself after a certain limit (this being dependent on the amount of microsomal material) was inhibitory for the desaturase system. Hence when stearoyl-CoA and sterculate are present together, an inhibition of the desaturase will only result when the combined detergent action of these compounds exceeds the limit of tolerance of the enzyme system for such an inhibition. Since fatty acyl-CoA esters appear to be more powerful inhibitors for such effects than long chain unsaturated fatty acid soaps (8), it becomes understandable that the sterculate inhibition of the desaturase system is not seen at lower levels of stearoyl-CoA or palmitoyl-CoA.

Inhibition of Desaturase System by Surface-active Agents—If the microsomal stearoyl-CoA desaturase system is susceptible to inhibition from the action of surface-active substances, in general, then this effect should not be restricted to free fatty acids or fatty acyl-CoA esters alone. Subsequent experiments confirmed this supposition (Table VI) as lysolecithin, deoxycholate, and palmitoyl-DL-carnitine all were found to be inhibitory. As is to be expected, the inhibition by an excess of stearoyl-CoA was considerably decreased by the inclusion of bovine serum albumin (Table VI).

Effect of Dietary Methyl Sterculate on Activity of Desaturase System Experiments were carried out to see whether dietary sterculate had any effect on the activity of the rat liver microsomal stearoyl-CoA desaturase system. For this experiment, seven rats were maintained on a fat-free diet for 12 weeks. Five of these were fed 20 mg of methyl sterculate daily in Tween 20, while the remaining two rats, serving as controls, were fed Tween 20 only. At the end of 12 weeks (when the sterculate-fed rats showed a decline in growth rate) the rats were killed, and the activity of the desaturase system was determined in their liver microsomes. As is evident from the results obtained (Table VII), the activity of the desaturase system was lower in the sterulate-fed group than in the controls. The values of the activities of the desaturase system from adult rats fed a standard laboratory diet as well as those fasted for 2 days and then refed are included for comparison. These showed that both refeeding and feeding a fat-free diet increased the desaturase activity considerably. It is difficult to assess from these limited data the specificity of the effect of sterulate in lowering the activity of the desaturase system and whether this action of sterulate may be at all involved in the reported (3-6) rapid inhibitions of stearate to oleate seen in vivo.

Finally it must be emphasized that the possibility that some metabolite derived from cyclopropene fatty acids may be the actual causative agent for the inhibition of the desaturation reaction in vivo needs exploration. The likelihood that the CoA esters of cyclopropene acids may be important in this connection cannot be eliminated at present, although it must be emphasized in this connection that the desaturase system is subject to inhibition by various acyl-CoA esters such as stearoyl-CoA (this study and Reference 15) and linoleyl-CoA and the possibility that these effects are nonspecific detergent action of acyl-CoA esters appears very likely in view of our present findings. The actual mechanism of the inhibition of mono-desaturation by cyclopropene fatty acids in vivo, therefore, remains obscure at the present time.

Acknowledgment—We are indebted to Dr. Barry Burns of the Department of Biological Chemistry, UCLA School of Medicine, for providing the animals described in the text and in Table VII.

REFERENCES

1. Phelps, R. A., Shernstone, F. S., Kemmereb, A. R., and Evans, R. J., Poultry Sci., 44, 358 (1965).
2. Johnson, A. R., Pearson, J. A., Shernstone, F. S., and Fogerty, A. C., Nature, 214, 1244 (1967).
3. Allen, E., Johnson, A. R., Fogerty, A. C., Pearson, J. A., and Shernstone, F. S., Lipids, 2, 419 (1967).
4. Reiser, R., and Raju, P. K., Biochem. Biophys. Res. Commun., 17, 8 (1964).
5. Raju, P. K., and Reiser, R., J. Biol. Chem., 242, 379 (1967).
6. Donaldson, W. E., Biochem. Biophys. Res. Commun., 27, 681 (1967).
7. Pande, S. V., and Mead, J. F., J. Biol. Chem., 245, 352 (1968).
8. Pande, S. V., and Mead, J. F., J. Biol. Chem., 245, 618 (1968).
9. Kornberg, A., and Prichard, W. E., Jr., J. Biol. Chem., 204, 329 (1953).
10. Bloomfield, D. K., and Bloch, K., J. Biol. Chem., 235, 337 (1960).
11. Galliard, T., and Stimpf, P. K., Biochem. Prep., 12, 66 (1968).
12. Carrol, K. K., J. Lipid Res., 2, 135 (1961).
13. Brav, G. A., Anal. Biochem., 1, 270 (1960).
14. Von Rudloff, E., Can. J. Chem., 34, 1413 (1956).
15. Oshino, Y., Imai, Y., and Sato, R., Biochim. Biophys. Acta, 189, 15 (1966).
16. Uchitama, M., Nakagawa, M., and Okui, S., J. Biochem. (Tokyo), 69, 1 (1967).
17. Morrison, W. R., and Smith, L. M., J. Lipid Res., 6, 690 (1964).
Inhibition of the Stearyl Coenzyme A Desaturase System by Sterculate
S. V. Pande and James F. Mead

J. Biol. Chem. 1970, 245:1856-1861.

Access the most updated version of this article at http://www.jbc.org/content/245/7/1856

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/245/7/1856.full.html#ref-list-1