EVIDENCE FOR INDUCTION OF A PALMITATE- AND STEARATE-INDEPENDENT SYSTEM OF MONOENOIC FATTY ACID SYNTHESIS IN CHICKS[1]

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The conversion of stearate to oleate and of palmitate to palmitoleate has been shown to be inhibited in chicks fed Sterculia foetida (SF) oil (Donaldson, 1967a). This same effect was shown earlier in rats by Reiser and Raju (1964). The latter workers also observed that incorporation of acetate-[^{14}C] into monoenoic fatty acids was not inhibited by SF feeding and they proposed the existence of a saturate-independent system of monoene synthesis in rats. The animals used in their acetate studies had been given SF daily for 7 days whereas stearate to oleate conversion was measured after a single dose. In my laboratory, acetate-[^{14}C] incorporation into monoenes by chicks was measured after a single SF dose and was found to be inhibited. These results suggested the possibility that if a saturate-independent system of monoene synthesis exists in higher organisms, it may be in a repressed state and require inhibition of the primary system (desaturase) over a period of time for induction to occur. The studies reported in this paper support this hypothesis.

The rearing procedures for chicks were the same as those reported earlier (Donaldson, 1964). After feeding a sucrose-casein diet for 14 days from the

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day of hatching, a group of chicks was dosed daily with 0.25 ml. of either corn oil or SF[2] per os for 7 days. A similar group received single 0.25 ml. doses at 21 days of age. Chicks from each group were injected intraperitoneally with 10 μC of [1-^{14}C] sodium acetate 2 hours after the final dose. Thirty-minutes later, the chicks were killed, and the livers were removed immediately and were frozen until analyzed. Procedures for isolation, separation by gas-liquid chromatography and determining [^{14}C] content of liver fatty acids were reported previously (Donaldson, 1967).

In additional experiments, similarly treated chicks were used to determine the ability of liver homogenates to desaturate [1-^{14}C] palmitate and to incorporate [^{3}H]-sodium acetate into palmitate and palmitoleate. In these experiments, the chicks were killed and the livers were immediately placed in cold 250 mM sucrose containing EDTA (2mM) and nicotinamide (5mM). Ten ml. of sucrose solution were used/gm. of liver. The livers were homogenized by passing them by a teflon pestle 4 times. The homogenates were centrifuged at 800 x g. for 10 minutes, and 1.5 ml. of the supernatant fluid were incubated. Protein content of supernatent fluid was determined by the Biuret reaction. The incubation mixture contained the following in a final volume of 2.5 ml. (μ moles/flask): Tris-HCl buffer, 12; KCl, 120; $MnCl_2 \cdot 4\ H_2O$, 7.5; $MgCl_2 \cdot 6\ H_2O$, 7.5; potassium acetate, 30; potassium citrate, 94; K_2HPO_4, 2.5; TPN, 4; DPN, 6; reduced glutathion, 12.5; ATP, 25; CoA, 0.25; glucose-6-phosphate, 30; [^{3}H]-sodium acetate, 1.0 μC and [1-^{14}C] palmitate bound to albumin, 0.05 μC (0.005 μ moles palmitate and 0.4 μ moles bovine albumin). The pH of the system was 7.4 and incubation was for 3 hours at 37°C. The incubations were stopped by the addition of 10 ml. of alcoholic KOH. Carrier fatty acids were added, and after saponification and acidification, the mixture was extracted twice with petroleum ether. The ether extracts were washed 6 times with water. After solvent removal, one-half of the fatty acids was analyzed as above. The

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[2] Generously supplied by Dr. Henry W. Kircher, University of Arizona.

remaining one-half was counted without further treatment in a Tri-Carb liquid scintillation counter.

The results of experiment 1 are shown in Table I. Administration of a single dose of SF resulted in higher saturated [^{14}C] / monoene [^{14}C] ratios than did the similar administration of corn oil. Administration of SF daily for 7 days resulted in saturated [^{14}C] / unsaturated [^{14}C] ratios closer to those of corn oil controls. These results suggest de novo, saturate-independent, monoene synthesis due to prolonged SF treatment.

Table I. Effects of acute vs. chronic dosing with Sterculia foetida oil on [1-^{14}C] acetate incorporation into saturated and monoenoic fatty acids by chicks.

Oil	Method of dosing[1]	Palmitate dpm/mg / Palmitoleate dpm/mg	Stearate dpm/mg / Oleate dpm/mg
Corn	acute	1.55[2] ± 0.25	2.24[2] ± 0.78
SF	acute	6.49 ± 1.71	14.51 ± 5.73
Corn	chronic	1.28 ± 0.75	1.44 ± 0.28
SF	chronic	2.24 ± 1.08	5.03 ± 0.75

[1]Acute refers to a single oral dose of 0.25 ml. of oil 2 hours prior to intraperitoneal injection of 10 μC of [1-^{14}C] sodium acetate. Chronic refers to daily doses of 0.25 ml. of oil for 7 days with [1-C^{14}] sodium acetate administered 2 hours after the final dose.
[2]Each value is the average for liver fatty acids from 3 chicks ± the standard error of the mean.

Further support for this hypothesis would be the demonstration of inhibited fatty acid desaturase concomitant with higher acetate incorporation rates into monoenoic acids in chicks chronically treated with SF. To achieve this, 2 experiments were conducted in which conversion of [1-^{14}C] palmitate to palmitoleate and [^{3}H] acetate incorporation into palmitate and palmitoleate were measured simultaneously in liver homogenates from treated chicks. The combined results of these experiments are shown in Table II. Both acute and chronic dosing with SF inhibited the conversion of [1-^{14}C] palmitate to pal-

mitoleate as compared with corn oil dosing. Acute SF dosing inhibited [^{3}H] acetate incorporation into palmitoleate relative to palmitate. The inhibition was not as marked in chronically SF dosed homogenates. Statistical analysis of the [^{3}H] acetate data showed a significant ($P = 0.05$) interaction between type of oil and method of dosing. There was no significant interaction in the [^{14}C] data. These data suggest that saturate-independent monoene synthesis occurs, and that this synthesis is induced by prolonged SF feeding.

Table II. Effects of acute vs. chronic dosing with Sterculia foetida oil on conversion of [1-^{14}C] palmitate to palmitoleate and incorporation of [^{3}H] acetate into palmitate and palmitoleate by chick liver homogenates.

Oil	Method of dosing[1]	$\frac{\text{Palmitate } [^{14}C]}{\text{Palmitoleate } [^{14}C]}$[2]	$\frac{\text{Palmitate } [^{3}H]}{\text{Palmitoleate } [^{3}H]}$[2]	Acetate[3] inc. in LCFA
Corn	acute	3.74[4]	4.05[4]	61[4]
SF	acute	30.51	20.47	56
Corn	chronic	5.81	6.60	62
SF	chronic	33.19	13.35	34

[1]See footnote 1, Table I.
[2]Ratio of total palmitate dpm to total palmitoleate dpm.
[3]mμ moles of acetate incorporated into long-chain fatty acids per mg. of protein during the 3 hour incubation.
[4]Each value is the average for liver homogenates from 6 chicks. There were 2 experiments, repeated in time, with 3 chicks per treatment in each experiment.

Prolonged SF feeding also resulted in a depression of acetate incorporation into total fatty acids by liver homogenates. It could be argued that the shift in the pattern of monoene synthesis may be only a reflection of reduced lipogenesis. In a preliminary communication (Donaldson, 1967b) it was noted that feeding constant fat-level diets of different degrees of fatty acid saturation markedly affected [1-^{14}C] acetate incorporation into fatty acids by chick liver homogenates. Feeding a 10% saturated diet resulted in only 18% of the incorporation exhibited by feeding a 55% saturated diet.

The % [1-^{14}C]-acetate incorporations into saturated fatty acids by liver homogenates from chicks fed these diets were 89 and 92, respectively. Hence, it does not necessarily follow that the pattern of monoene synthesis is linked to the level of lipogenesis.

Raju and Reiser (1967) suggested that the active principle in SF (sterculic acid -- a cyclopropene compound) irreversibly binds the thiol groups of the acyl desaturase enzyme. They further suggested that the thiol groups of the multienzyme complex of fatty acid synthetase may be less reactive than the thiol groups of acyl desaturase. The results presented in this paper support this concept. Raju and Reiser's results also offer a possible explanation of the differences observed with acute vs. chronic SF treatment. A single SF dose inhibits the desaturation of palmitate and stearate. Presumably, new desaturase enzyme is eventually synthesized and the inhibition is relieved. With chronic SF treatment, new enzyme would be inhibited. The saturated fatty acid content of tissues would rise (as reviewed by Phelps et al., 1965), and this rise may be the mechanism by which an alternate pathway of monoene synthesis is induced. Experiments attempting to characterize this alternate pathway are now in progress.

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