INFLUENCE OF BIOTIN DEFICIENCY AND DIETARY STERCULIC ACID ON FATTY ACID DEHYDROGENATION[1]

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Bloomfield and Bloch (1960) and Marsh and James (1962) suggested that the monoenoic fatty acids, palmitoleic and oleic are formed by the desaturation of the analogous saturated acids, palmitic and stearic. Erwin and Bloch (1964) reviewed data which indicate that palmitoleic and oleic acids can be formed in bacteria by desaturation of medium-chain length fatty acids and subsequent elongation. This second pathway has not been shown to exist in higher organisms. Reiser and Raju (1964) showed that the production of monoenoic acids via the first pathway can be inhibited by feeding Sterculia foetida oil (SF) which contains approximately 50% sterculic acid (a 19 carbon fatty acid with a cyclopropene ring in the chain at the 9:10 position). Biotin deficiency has been shown to impair lipogenesis and to increase palmitoleic concentrations of synthesized fatty acids in yeast (Suomalainen and Keranen, 1963) and in chicks (Donaldson, 1964).

This experiment was designed to study the effects of SF on palmitic as well as stearic desaturation and to study the interaction of SF with biotin deficiency. The evidence presented in this paper suggests that biotin

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deficiency exerts its effect on palmitoleic concentration via the second pathway of monoene synthesis.

Chicks were fed normal or biotin-deficient diets described by Donaldson (1964) from 0 to 21 days of age. Twelve chicks were then selected at random from the groups fed each diet except that only deficient chicks showing typical biotin-deficiency symptoms were used. Six chicks from each diet group were orally dosed with 20 μC of [1-^{14}C] palmitic acid, and the remaining 6 were dosed with 20 μC of [1-^{14}C] stearic acid. Corn oil was used as the dosing vehicle for 3 chicks receiving each labeled acid while Sterculia foetida oil[2] was the dosing vehicle for the remaining 3 chicks. The chicks were killed 2 hours after dosing, and the livers were removed immediately and were frozen until analyzed. The livers were homogenized with chloroform-methanol (5 ml of 2:1, v/v per g of liver) for 5 minutes. The crude extracts were dried under nitrogen at 60°, and 50 to 100 mg were saponified with 15% KOH in 70% aqueous ethanol for 2 hours at 60°C. The saponification mixtures were extracted with petroleum ether, acidified with HCl and extracted twice again with petroleum ether. The latter extracts were combined and dried as before. The fatty acids were converted to methyl esters using boron-trifluoride-methanol.[3] Fatty acid methyl esters were separated by gas-liquid chromatography. The fractions were collected and the [^{14}C] content of each fraction was determined by a previously outlined method (Donaldson, 1966a). All [^{14}C] data were analyzed for statistical significance by the analysis of variance for factorial designs as outlined by Snedecor (1948).

The data for fatty acid composition of liver are shown in Table I.

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[2]Generously supplied by Dr. Henry W. Kircher, U.S.D.A. Southern Utilization Laboratory, New Orleans, La.

[3]Applied Science Laboratories, University Park, Pa.

Table I. Influence of biotin deficiency and Sterculia foetida oil on fatty acid composition of chick liver.[1]

Diet	Dosing vehicle	Fatty acids, % of total					
		14:0[2]	16:0	16:1	18:0	18:1	18:2
Control	CO[3]	0.8	25.5	8.2	16.8	48.2	0.5
	SF[4]	0.8	28.3	7.4	21.6	41.8	0.2
Biotin deficient	CO	0.6	24.7	10.5	14.1	47.6	2.6
	SF	0.6	26.7	12.1	17.4	42.2	1.0

[1]Each value is the average for 6 chicks.
[2]First number denotes chain length; second number denotes double bonds.
[3]Corn oil (0.25 ml) administered orally 2 hours prior to killing the chicks.
[4]Sterculia foetida oil (0.25 ml) administered orally 2 hours prior to killing the chicks.

The principal effects were 1) that SF administration resulted in an increased concentration of stearic acid and decreased concentration of oleic acid and 2) that biotin deficiency resulted in increased concentration of palmitoleic acid.

The data showing conversion of stearic and palmitic acids to their corresponding monoenoic acids are presented in Table II.

Table II. Influence of biotin deficiency and Sterculia foetida oil on conversion of stearate to oleate and palmitate to palmitoleate.

Diet	Dosing vehicle	Stearate dpm/mg[1] / Oleate dpm/mg	Palmitate dpm/mg[2] / Palmitoleate dpm/mg
Control	CO	2.6 ± 0.21	2.1 ± 0.10
	SF	11.0 ± 4.49	4.2 ± 0.19
Biotin deficient	CO	1.8 ± 0.70	2.4 ± 0.36
	SF	19.4 ± 4.29	4.3 ± 0.50

[1]Each value is the average ± standard error of the mean for 3 chicks. Chicks were orally dosed with 20 μC [1-^{14}C] stearic acid in 0.25 ml corn oil (CO) or Sterculia foetida oil (SF).

[2]Conditions were the same as in footnote 1 except 20 μC [1-^{14}C] palmitic acid were used per chick.

When stearate [^{14}C] was administered, the [^{14}C] content of liver palmitate was negligible. This indicates that the data in Table 2 reflect conversion of stearate to oleate and not oxidation to acetate and re-synthesis. SF inhibited the desaturation of both stearate and palmitate ($P = 0.01$). Biotin deficiency had no statistically significant effect on desaturation. The data in Table II are presented as specific activities. Since biotin deficiency results in an increase in palmitoleic concentration in liver fatty acids, there is a relative dilution effect on palmitoleic [^{14}C]. Calculations which make allowance for this dilution effect suggest only a small increase in the relative conversion of palmitate to palmitoleic acid by biotin-deficient chicks as compared to normal chicks.

The lack of an effect of biotin deficiency on the desaturation of palmitic acid supports the hypothesis that de novo palmitoleate synthesis can occur independently of palmitate synthesis. Reiser and Raju (1964) found that in rats SF inhibited conversion of stearate [^{14}C] to oleate [^{14}C], but that incorporation of acetate [^{14}C] into stearate and oleate was the same as incorporation by untreated rats. From these results they concluded that, "there are two routes for the biosynthesis of oleic acid in animals, one which does not go through stearate and which is not inhibited by ... (SF) and one through stearate which is." Scheuerbrant et al. (1961) and Norris et al. (1964) demonstrated a dehydration reaction of ten- and twelve-carbon β-hydroxy acids by anaerobic bacteria to form a cis double bond. Subsequent elongation of these monoenoic acids yielded palmitoleic and oleic acids independently of palmitic and stearic acids. Erwin and Bloch (1964) stated that the more primitive dehydration system of monoene synthesis and the aerobic dehydrogenation reaction are mutually exclusive and that the primitive system has never been found in higher organisms. However, it is possible that a saturate-independent system of monoene synthesis may exist in a repressed state in higher organisms. If such were the case, blockage of the fatty acid desaturase system could result in a release of repression. In the case of biotin deficiency, total fatty

acid synthesis is reduced (Donaldson, 1964). When biotin-deficient, fat-free diets are fed to chicks, relative incorporation of labeled palmitate into liver phospholipid is increased while incorporation into triglyceride is decreased (Donaldson, 1966b). Since chick phospholipids typically show a more saturated fatty acid composition than triglycerides, the reduced triglyceride synthesis might be the result of decreased availability of unsaturated fatty acids. With total fatty acid synthesis reduced and no apparent increase of the desaturase mechanism in biotin-deficient chicks, decreased unsaturated fatty acid availability seems plausible. Hence, in biotin deficiency, we may also be observing a de-repression of a biotin-insensitive system of monoene synthesis that is independent of saturated fatty acid synthesis and that results in increased palmitoleate concentrations. The data presented here extend to another species the observations of Reiser and Raju (1964) on desaturation of stearate to oleate. In addition, the data show that cyclopropene containing oils also inhibit conversion of palmitate to palmitoleate and that biotin deficiency is without effect on this conversion.

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