BIOTIN DEFICIENCY AND RELATIVE INCORPORATION OF [^{14}C] FATTY ACIDS INTO CHICK LIVER PHOSPHOLIPIDS AND TRIGLYCERIDES[1]

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Received July 5, 1966

Biotin deficiency has been shown to inhibit lipogenesis in vitro (Fletcher and Myant, 1960; Wakil and Bressler, 1962) and in vivo (Suomalainen and Keranen, 1963; Donaldson, 1964). The production of a biotin deficiency effect on lipogenesis in intact animals necessitates the use of a fat-free diet since fat-feeding has been shown by Bortz et al. (1963) to exert an influence on lipogenesis similar to biotin deficiency. The consumption of a fat-free biotin-deficient diet results in lower total carcass fat and reduced incorporation of [^{14}C] acetate into carcass fatty acids (Donaldson, 1964). Under these conditions it seemed possible that the incorporation of fatty acids into the structurally important phospholipids might be greater than incorporation into the triglyceride energy reserves. The data presented here support this hypothesis.

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. 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. The dosing vehicle was either corn oil or

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[1]Contribution from Poultry Science Dept., North Carolina Agri. Exp. Station, Raleigh. Published with approval of Director of Research as Paper No. 2199 of the Journal Series.

Sterculia foetida oil in gelatin capsules. The data for the two oils were combined since statistical analysis showed them to be without effect on the results presented. The chicks were killed 2 hours after dosing, and the livers were immediately removed and frozen until analysis. 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°, weighed and dissolved in a sufficient volume of chloroform-methanol to give a 10% solution (w/v). The extracts were then streaked (0.1 ml) on thin-layer chromatography plates especially chosen for layer uniformity of the Silica Gel H.[2] The developing solvent was n-hexane, diethyl ether, acetic acid (104:25:2, v/v/v). This system results in the separation of the lipid mixture into 1) phospholipid + monoglycerides (partial resolution), 2) cholesterol + diglycerides, 3) free-fatty acids, 4) triglycerides, and 5) cholesterol esters. The phospholipid-monoglyceride resolution was sufficient to determine that the levels of radioactivity in the monoglycerides was negligible. The developed plates were sprayed with Rhodamine 6-G[3], and the fractions were examined under ultraviolet light. The relative [^{14}C] content of the phospholipid and triglyceride fractions was measured using a thin-layer radiochromatogram scanner.[4] The ratios of phospholipid counts/min to triglyceride counts/min were then computed. The data were analyzed for statistical significance by the analysis of variance for factorial experiments as outlined by Snedecor (1948).

Biotin deficiency significantly increased ($P < 0.01$) the proportion of the [^{14}C] stearic or palmitic acid doses incorporated into phospholipid as compared to triglyceride. The phospholipid to triglyceride incorporation ratio (PL/TG) was higher for [^{14}C] stearic than for [^{14}C] palmitic acid, but the difference was not statistically significant ($P > 0.05$).

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[2] E. Merck Ag, Darmstadt, Germany.

[3] Matheson Coleman and Bell, East Rutherford, New Jersey.

[4] Model 880 and 885, Technical Measurement Corporation, North Haven, Conn.

The results are shown in Table I.

Table I. Influence of Biotin Deficiency on Relative Incorporation of [1-C^{14}] Stearic and [1-C^{14}] Palmitic Acids into Liver Phospholipids and Triglycerides of Chicks.

Diet	Ratio of phospholipid cpm to triglyceride cpm*	
	[^{14}C] Stearic	[^{14}C] Palmitic
Fat-free control	0.47 (0.12)**	0.40 (0.11)
Biotin-deficient	1.38 (0.38)	0.69 (0.13)

*This ratio is an expression of the total radioactivity of liver phospholipid vs. triglyceride rather than specific activity. The [^{14}C] counts were not corrected for self-absorption since only relative incorporations were sought and plate thickness was uniform.

**Mean of 6 values. Values in parenthesis are standard error of the mean.

Incorporation of fatty acids into phospholipids and triglycerides occurs whether the fatty acids are of dietary origin or are synthesized de novo. In a biotin-deficient fat-free diet, there are no dietary fatty acids and de novo synthesis is impaired. Thus, the total fatty acid pool of the biotin-deficient chick is reduced. This condition is similar to that occuring with reduced caloric intake or starvation and should provoke a relatively reduced incorporation of fatty acids into triglycerides as compared to incorporation into phospholipids. The results presented herein support this hypothesis.

ACKNOWLEDGMENTS

This investigation was supported in part by U. S. Public Health Service Grant AM 07833. The author thanks Faye D. Suggs for technical assistance.

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