Biotin Nutriture and the Enzymatic Incorporation of $NaHC^{14}O_3$ into Acetoacetate. (19212)

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In previous studies it was demonstrated that the carbon of C^{14} bicarbonate can be incorporated into the carboxyl group of enzymatically generated acetoacetate by various animal tissue preparations(1-3). It was also shown(1) that there was less incorporation of C^{14} into acetoacetate by rat liver homogenate systems from biotin deficient animals than from those which received this vitamin in the diet or by injection. However, since acetoacetate production by the homogenates was not determined in the earlier work, it could not be decided whether the depressed C^{14} incorporation by biotin deficient tissues could be ascribed to diminished acetoacetate formation or to a specific decrease of the incorporation of C^{14} from bicarbonate.

Methods. Weanling Sprague-Dawley rats were grown for 10 weeks on a purified diet containing raw egg white without added biotin (1,4). The animals fed egg white developed typical symptoms of biotin avitaminosis. Some of these deficient animals were then injected with 50 γ of biotin daily for 2 days and their food intake adjusted to that of non-treated rats of the same body weight. The livers of such pairs of animals were used for the comparison of enzymatic activity. The reaction mixture(5), methods of incubation, and the procedures used for the degradation of acetoacetate and counting of radioactivity were identical with those previously described(1,3). The exact amounts of carrier and metabolically produced acetoacetate were determined by the method of Greenberg and Lester(6). The values for the "corrected" specific radioactivity of the carboxyl group of acetoacetate were calculated in the same manner as previously indicated(3). The results reported are based on equivalent dry weights of homogenates.

Results. The state of biotin nutrition of the rats did not significantly affect the quantity of acetoacetate produced by the liver homogenates with either pyruvate, caprylate or

TABLE I. Effect of Biotin Deficiency on Incorporation of C^{14} from $NaHC^{14}O_3$ into the Carboxyl Group of Acetoacetate.

Substrate	Biotin treatment	Acetoacetate, metabolically formed, μM/ml	Radioactivity ("corrected")* of metabolically formed acetoacetate, cts/min/μM
Pyruvate	—	.34	0
	+	.39	5.04
Caprylate	—	1.30	.48
	+	1.38	49.2
Caproate	—	.73	4.68
	+	.78	78.6
Isovalerate	—	0	5.82†
	+	0	73.8 †

All substrates added to .0012 M. Incubated at 37° in air for 30 min. 4.2×10^4 cts/min $NaHC^{14}O_3$ added per flask. Error of count is within $\pm$ 2%.

* c.p.m. per μM "corrected" = [μM acetoacetate $\times$ c.p.m./μM (substrate present)—μM acetoacetate $\times$ c.p.m./μM acetoacetate (control)]/[μM acetoacetate (substrate present) —μM acetoacetate (control)]. Detailed calculations are described in Reference 3.

† Radioactivity in presence of acetoacetate carrier. Corrected for incorporation of C^{14} in absence of isovalerate.

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caproate as a substrate. At the same time there was a 15-100 fold greater incorporation of C^{14} bicarbonate into the carboxyl group of acetoacetate produced by homogenates of livers from biotin repleted rats than by the deficient ones.

With the homogenate systems employed here, isovalerate leads to a marked increase in the radioactivity of the carboxyl group of acetoacetate, though no significant net formation of ketone bodies can be detected(3). However, biotin treatment of the deficient rats resulted in a 12-fold increase of C^{14} incorporation into acetoacetate derived from isovalerate.

The decreased *incorporation* of C^{14} from bicarbonate into the carboxyl group of acetoacetate by rat liver homogenates from biotin deficient animals as compared to those from the repleted ones seems to be the result of an effect on the bicarbonate incorporating mechanism, since the *amount* of this keto acid produced by the liver homogenates from a number of substrates was not influenced.

Summary. The quantity of acetoacetic acid produced metabolically from pyruvate, caprylate, and caproate by rat liver homogenate systems is not affected by the state of biotin nutrition of the animals. However, biotin treatment of deficient animals increased by 10-100 fold the incorporation of C^{14} bicarbonate into the carboxyl group of acetoacetate metabolically formed from pyruvate, caproate, caprylate or isovalerate.

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Blood Glucose Levels in Alloxan-Diabetic Rats under Combined Insulin and Ergot Alkaloid Therapy.*† (19213)

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Dubois, Herrmann, and Erway(1) have reported that the hyperglycemia produced by the rodenticide, alpha-napthylthiourea (ANTU), is conteracted by ergotamine, which is a natural alkaloid of ergot, as well as by insulin. They accounted for this parallelism through assumption that there are antagonistic actions of both ergotamine and insulin toward the glycogenolytic action of epinephrine, the production of which is stimulated by the rodenticide. Stoll and Hofmann(2) have been responsible for the production of dihydrogenated derivatives of the ergotoxine group of ergot alkaloids. It has been shown by Rothlin(3) that the hyperglycemia produced in animals through administration of epinephrine is inhibited by the hydrogenated ergot alkaloids. Freis, Stanton, and Wilkins(4) have demonstrated adrenolytic activity of dihydroergocornine (DHO 180) and dihydroergokryptine (DHK 135) in humans and have also shown, under proper experimental conditions, that the pressor response, tachycardia, and mydriasis which normally follow epinephrine administration are reduced by these preparations. Schneider(5) found that the period of hypoglycemia in humans, produced by the intravenous injection of small doses of insulin, was not lengthened by administration of dihydroergocristine, dihydroergocornine, or

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