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L-Histidine-Induced Facilitation of Cholesterol Biosynthesis in Rats[1] (40283)

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Nutritional loading of amino acids, particularly phenylalanine, has been used to study certain metabolic disorders. During such experimentation Waisman and his colleagues fed L-histidine-supplemented diets to infant rhesus monkeys and noted a marked hyperlipemia (1, 2). Later Geison and Waisman (3) fed 5% and 8% excess L-histidine diets to 4-week-old rabbits and induced a 50% increase in plasma cholesterol levels. Our investigations have been pursued in rats, attempting to provide a more accessible animal model for studying dietary histidine supplementation.

Rats fed a diet supplemented 5% with L-histidine develop large livers and hypercholesterolemia (4). There is an increase in the incorporation of cholesterol precursors into cholesterol in liver slices from rats fed excess histidine (4). This finding prompted further investigation to determine the effect of histidine supplementation on cholesterol biosynthesis in the 5,000*g* supernatant solution of rat liver homogenate. Mature rats were used in this study because cholesterol and fatty acid metabolism in weanling rats is unstable, due to the change of diet from milk to chow (5). Previous studies have demonstrated that fasting decreases the rate of synthesis of cholesterol from acetate (6, 7). When fasted animals were refed a normal diet, the synthesis of cholesterol from acetate returned to normal within three days (8). When they were put on a fat-free diet, cholesterol synthesis returned to its normal level within three days and then declined to a very low level (8). This investigation studied the effects of histidine supplementation on the rate of synthesis of cholesterol and cholesterol precursors from acetate and mevalonate in both normal and fat-free diets. All measurements were obtained during high and low diurnal levels of cholesterol synthesis.

Materials and methods. Experimental materials were obtained from the following sources: [2-^{14}C] acetate (specific activity 53.3 mCi/mmole), [2-^{14}C] RS-mevalonic acid, *N,N'*-dibenzylethylene diammonium salt (specific activity 40.2 mCi/mmole, and *Aquasol* (scintillation solution) from New England Nuclear Corp., Elmhurst, IL; glucose-6-phosphate, NAD, NADP, dithiothreitol, digitonin, and nicotinamide from Sigma Chemical Co., St. Louis, MO; EDTA from Fisher Scientific Co., Itasca, IL; L-histidine (free base) and bovine serum albumin from Nutritional Biochemical Corporation, Cleveland, OH. All other chemicals used were of analytical grade. The fat-free diet (Wooley and Sebrell), Mod. TD-71125 was from Teklad Test Diets, Madison, WI. The normal diet was ground Purina Formulab Chow. In the histidine-supplemented diets, L-histidine constituted 5% of the diets by weight. A standard fitting Potter-Elvehjem homogenizer was used for homogenization. All radioactivity countings were done in a Nuclear Chicago Scintillation Counter, Isocap/300.

Male albino rats weighing 50–60 g each were obtained from Holtzman Rat Co., Madison, WI. Animals were divided into groups of four and fed normal and experimental diets *ad lib.* for 18 days after they were received. All rats, excluding the control group, then fasted for 2 days and were then refed experimental diets *ad lib.* for three days. This provided 21 days of experimental diet as used in previous studies of amino acid feeding (9). Rats were housed singly in stainless steel cages. The light cycle was from 7AM to 5:30PM.

Preparation of rat liver homogenate. Rats were sacrificed by decapitation, at 2PM or

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10PM, and the livers were removed quickly and placed on ice. Each liver was weighed, minced, and then homogenized in a 0.1 *M* potassium phosphate buffer, pH 7.4, containing 0.004 *M* $MgCl_2$, 0.001 M EDTA, and 0.002 M dithiothreitol, with five strokes of a Potter-Elvehjem homogenizer. The volume of buffer used was 2 ml/g of liver. The homogenate was centrifuged for 10 min at 5,000*g*. The volume of the supernatant solution was recorded. Protein concentrations were measured by a modification of the biuret procedure (10) using bovine serum albumin as standard.

Assays for the conversion of acetate and mevalonate to NSF[2] *and DPF*[3]. The rates of conversion of [2^{14}C] acetate and [2^{14}C] mevalonate to NSF and DPF were measured by a slight modification of the procedure of Slakey *et al.* (11). With acetate as the substrate, the incubation mixture contained 125 μl (approximately 5.0 mg protein) of the 5,000*g* supernatant solution diluted to 0.5 ml with homogenizing buffer plus cofactors and [2-^{14}C] acetate (2.5 μmoles and 4×10^5 dpm per μmole). With mevalonate as the substrate, the incubation mixture contained 75 μl (approximately 3.0 mg protein) of the 5,000*g* supernatant solution diluted to 0.5 ml with homogenizing buffer plus cofactors and [2-^{14}C] RS-mevalonate (2.5 μmoles and 2×10^5 dpm per μmole). The NSF was counted in a toluene scintillation solution and the DPF was counted in *Aquasol*.

Results. Acetate to NSF and DPF. The incorporation of [^{14}C] acetate into the NSF and DPF of the 5000*g* supernatant solution of rat liver homogenate is shown in Fig. 1. The labeled substrate was incorporated nine times more into the NSF of rats which were refed a histidine-supplemented chow diet than in those of the control group (Fig. 1A). This increase is statistically significant ($P < 0.001$). Refeeding chow, fat-free, or a histidine-supplemented fat-free diet did not significantly affect the NSF synthesis activity. Refeeding of the histidine-supplemented chow diet induced a seven- to eightfold increase in the incorporation of the labeled

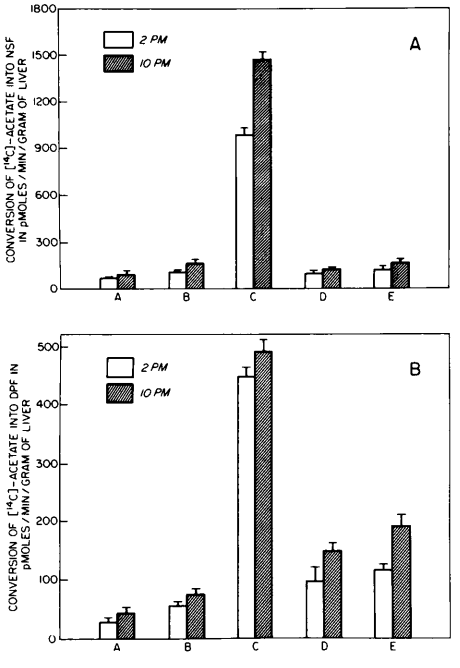

FIG. 1. Effects of L-histidine supplementation on the rate of conversion of [2-^{14}C] acetate to the nonsaponifiable (A) and digitonin-precipitable (B) fractions in the 5000*g* supernatant solution of liver homogenates of rats maintained in different nutritional states: continuously fed, chow (A); fasted-refed, chow (B); fasted-refed, 95% chow + 5% L-histidine (C); fasted-refed, fat-free (D); and fasted-refed 95% fat-free +L-histidine (E).Vertical bars represent standard deviations with four rats in each group.

substrate into the DPF (Fig. 1B). This difference is also significant ($P < 0.001$). Histidine supplementation to the fat-free diet did not cause a significant increase in the DPF synthesis activity.

Mevalonate to NSF and DPF. Effects of feeding excess histidine on the incorporation of [^{14}C] mevalonate into the NSF are shown in Fig. 2A. A 7.5-fold increase in total synthesis activity over the matched control was observed when refeeding the histidine-supplemented chow diet ($P < 0.001$). Refeeding of chow, fat-free diet, and a histidine-supplemented fat-free diet did not significantly affect the NSF synthesis activity. The amount of [^{14}C] mevalonate incorporated into the DPF was seven times higher with the refed histidine-supplemented diet than with the refed chow diet ($P < 0.001$), as shown in Fig. 2B. Synthesis activity was 1.6 times higher in

[2] NSF = Nonsaponifiable fraction: sterols, squalene, and terpenols.

[3] DPF = Digitonin-precipitable fraction: sterols.

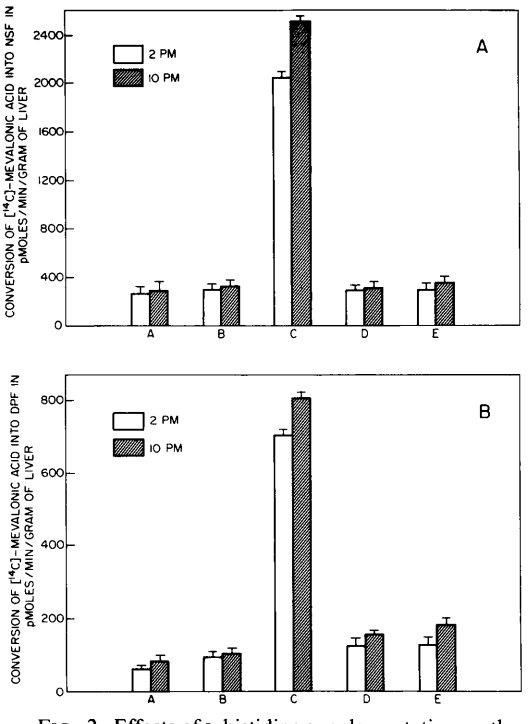

FIG. 2. Effects of L-histidine supplementation on the rate of the conversion of [2-^{14}C]mevalonate to the non-saponifiable (A) and digitonin-precipitable (B) fraction in the 5000*g* supernatant solution of liver homogenates of rats maintained in different nutritional states described in Fig. 1. Vertical bars represent standard deviations with four rats in each group.

rats which were refed the fat-free diet than in the continuously fed control ($P < 0.05$). Histidine supplementation to a fat-free diet did not cause any significant increase over the matched control.

Discussion. L-histidine or a histidine metabolite effectively stimulates sterol synthesis in rats when added to chow diet. The marked increase (seven- to ninefold) in the incorporation rate of labeled substrates into both NSF and DPF in this study is considerably greater than the increase in plasma cholesterol (30% over normal) which occurred in another histidine supplement study (4). A simultaneous increase in the degradation of cholesterol in the liver may be responsible for this disparity.

Cholesterol synthesis varies diurnally (9, 10); however, none of the enzyme activity that converts mevalonate to squalene does (11). In this investigation, sterol synthesis from either acetate or mevalonate at the high point of the day was 1.3–1.6 times greater than at the low point.

Under a variety of experimental conditions which reduce the conversion rate of acetate to cholesterol, the conversion rate of mevalonate to cholesterol does not change or changes much less dramatically than that of acetate (7, 14). However, in the case of stimulation of sterol biosynthesis by histidine, a similar rate increase was obtained when either acetate or mevalonate was used as the labeled substrate. The result suggests that histidine probably has a significant effect on an enzyme or enzymes in the synthesizing pathway between mevalonic acid and cholesterol. It will be interesting to investigate the activities of these enzymes in future studies.

Refeeding of either a chow or fat-free diet did not cause a marked change in sterol and squalene synthesis (1.2- to 1.8-fold increase over controls). This agrees with results obtained by Craig *et al.* (8) which show that the cholesterol synthesis activity rises from fasting levels to normal levels within three days after refeeding either chow or fat-free diet. Histidine supplementation to the fat-free diet did not cause a substantial change in the rate of sterol and squalene synthesis from acetate. This contrasts with the marked increase in sterol and squalene synthesis in the histidine-treated chow fed group.

The livers from rats fed fat-free diets, regardless of histidine treatment, were deep yellow due to fat accumulation. This probably resulted from a higher rate of fatty acid synthesis. If this is true, acetyl-CoA, a common precursor for these two divergent pathways (cholesterol and fatty acid synthesis), could be exhausted from an endogenous pool with long-term feeding, thus impeding histidine's stimulation of cholesterol synthesis from acetate in rats fed a fat-free diet. However, the conversion rate of mevalonate into sterols and squalene in rats which were fed a longterm fat-free diet also did not change when histidine was added to their diet. This result might not be anticipated if the absence of acetyl-CoA accounted solely for the lack of a histidine effect in rats fed a fat-free diet. The next step in the study of these processes will be to measure the actual activities of the specific enzymes, such as β-hydroxy-β-methylglutaryl CoA reductase and fatty acid synthetase.

Summary. A diet supplemented 5% with L-histidine induces hypercholesterolemia in rats. To examine the mechanism involved, L-histidine was added to either a chow or fat-free diet and fed to rats for 18 days. After 2 days of fasting, the rats were refed the same diet for three days. There was a ninefold increase in the incorporation of [^{14}C] acetate into the nonsaponifiable fraction in the 5,-000*g* hepatic fraction of histidine-supplemented chow-fed rats compared to controls. The increase in the incorporation of the labeled substrate into the digitonin-precipitable fraction was seven- to eightfold. The incorporation of [^{14}C]mevalonate was increased by sevenfold in both the nonsaponifiable and digitonin-precipitable fractions. Longterm histidine supplementation to fat-free diet did not affect the incorporation of either [^{14}C] acetate or [^{14}C] mevalonate into these fractions.

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1. Kerr, G. R., Wolf, R. C., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **119,** 561 (1965).
2. Kerr, G. R., Wolf, R. C., and Waisman, H. A., *in* "Symposia of the Zoological Society of London" (R.N.T.-W. Fiennes, ed), no. 17, p. 371, Academic Press, London/New York (1966).
3. Geison, R. L., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **133,** 234 (1970).
4. Solomon, J. K., and Geison, R. L., Fed. Proc. **36,** 1157 (Abstr.) (1977).
5. McNamara, D. J., Quackenbush, F. W., and Rodwell, V. W., J. Biol. Chem. **247,** 5805 (1972).
6. Tomkins, G. M., and Chaikoff, I. L., J. Biol. Chem. **196,** 569 (1952).
7. Bucher, N. L. R., McGarrahan, K., Gould, E., and Loud, A. V., J. Biol. Chem. **234,** 262 (1959).
8. Craig, M. C., Dugan, R. E., Muesing, R. E., Slakey, L. L., and Porter, J. W., Arch. Biochem. Biophys. **151,** 128 (1972).
9. Daniel, R. G., and Waisman, H. A., Growth **32,** 255 (1968).
10. Gornell, A. G., Bardawill, C. J., and David, M. M., J. Biol. Chem. **177,** 751 (1949).
11. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C., and Porter, J. W., J. Biol. Chem. **247,** 3014 (1972).
12. Back, P., Hamprecht, B., and Lynene, F., Arch. Biochem. Biophys. **133,** 11 (1969).
13. Dugan, R. E., Slakey, L. L., Briedis, A. V., and Porter, J. W., Arch. Biochem. Biophys. **152,** 21 (1972).
14. Gould, R. C., and Popjàck, G., Biochem. J. **66,** 51 (1957).

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