Effect of (-)-Hydroxycitrate upon the Accumulation of Lipid in the Rat: I. Lipogenesis[1]

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ABSTRACT

The purpose of these investigations was to ascertain the effect of (−)−hydroxycitrate on the accumulation of lipid in the meal fed rat by examining the rates of lipogenesis after acute and chronic treatment. Oral administration of (−)−hydroxycitrate depressed significantly the in vivo lipogenic rates in a dose-dependent manner in the liver, adipose tissue, and small intestine. The hepatic inhibition was significant for the 8 hr period, when control animals demonstrated elevated rates of lipid synthesis. The kinetics of this reduction of in vivo hepatic lipogenesis were identical after acute or chronic administration of (−)−hydroxycitrate. However, in vitro rates of lipogenesis were elevated after chronic administration of (−)−hydroxycitrate for 30 days. Rats receiving (−)−hydroxycitrate consumed less food than the untreated controls; however, this decreased caloric intake was not responsible for the drug induced depression of hepatic lipogenesis, as shown by studies using pair fed rats.

INTRODUCTION

(−)−Hydroxycitrate, the principal acid of the fruit rinds of *Garcinia cambogia*, (1-3) was shown to be a competitive inhibitor of adenosine 5′-triphosphate (ATP) citrate lyase (EC 4.1.3.8) (4,5), the enzyme catalyzing the extramitochondrial cleavage of citrate to oxaloacetate and acetyl CoA. This action of (−)−hydroxycitrate should reduce the acetyl CoA pool, thus limiting the availability of 2 carbon units required for fatty acid (FA) and cholesterol biosynthesis. Our previous investigations substantiated this hypothesis, since the acute admnistration of (−)−hydroxycitrate inhibited in a dose-dependent manner the in vitro rates of lipogenesis in hepatic cell-free and slice systems and the in vivo rates of hepatic FA and cholesterol synthesis (6). Of the four stereoisomers of hydroxycitrate, only (−)−hydroxycitrate reduced significantly the in vitro and in vivo rates of lipid synthesis (6). The depression of 3-β-hydroxysterol biosynthesis by (−)−hydroxycitrate was confirmed in a perfused rat liver system (7,8). The inhibition of FA synthesis was demonstrated after intraperitoneal administration of (−)−hydroxycitrate (9).

It seemed important to ascertain how the chronic administration of (−)−hydroxycitrate would affect the rat's metabolic pattern of lipid biosynthesis and storage. This investigation was designed to examine the effect of the chronic oral administration of (−)−hydroxycitrate upon in vivo rates of lipogenesis and to analyze in greater detail the characteristics of the inhibition of lipogenesis after acute administration. These studies were performed under the conditions of induced rates of lipid synthesis, which have been described previously (10). In vivo rates of lipogenesis were determined using [^{14}C] alanine and [^{3}H] water; the latter being employed to determine the total rate of FA (9, 11-16) and cholesterol synthesis (7) independent of the source of carbon precursors of the acetyl groups.

EXPERIMENTAL PROCEDURES

Female rats of the Charles River CD strain (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 120-160 g (ca. 16 wk old) were housed individually in wire-bottomed cages in a temperature-regulated (22 C), light-controlled room (12 hr light, 6 A.M.-6 P.M., and dark 6 P.M.-6 A.M.). They had free access to water and were fed a commercial diet (Purine Rodent Chow, Ralston Purina Co., St. Louis, Mo.) ad libitum for at least 1 week prior to the experiment. Animals were fasted 48 hr, then meal fed a synthetic diet (G-70) daily from 8-11 A.M. for the remainder of the experiment. Food consumption and body wt were measured during the meal feeding period. Body wts were randomized, so that each experimental group had an identical wt spread. Food spillage was measured daily.

The G-70 diet consisted of 70% glucose, 23% vitamin-free casein, 5% Phillips and Hart salt mixture IV (17), 1% corn oil, 1% complete

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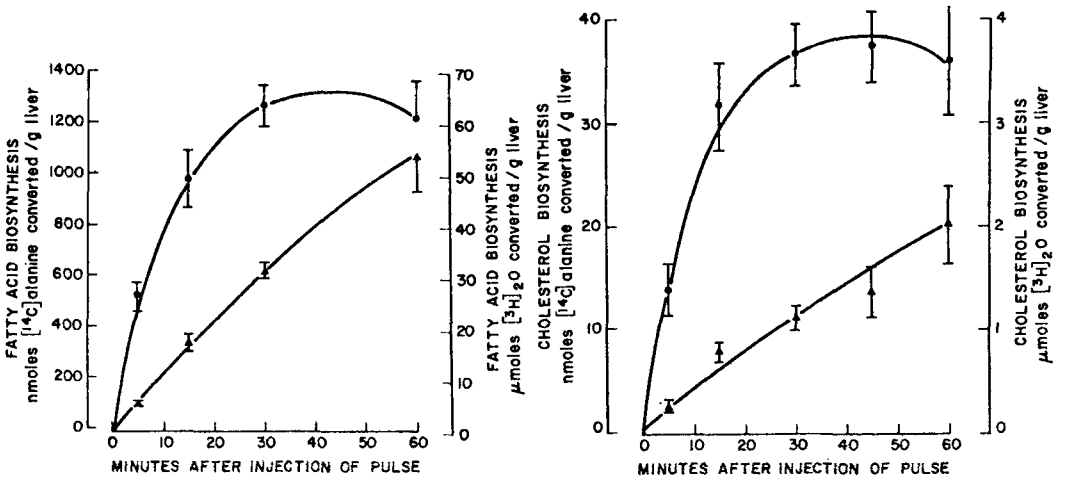

FIG. 1. Relation between the in vivo rates of hepatic fatty acid (FA) and cholesterol synthesis and the time of administration of [^{14}C]alanine and [^{3}H] water. Rats were prefasted 48 hr, then meal fed the G-70 diet for 9 days. The rates of FA and cholesterol synthesis were determined using a pulse of [^{14}C]alanine and [^{3}H]water injected immediately after the 3 hr meal. FA were separated from cholesterol by anion exchange chromatography as described in the text. Each group consisted of 9-10 rats. The vertical bar gives the standard error of the mean for FA synthesis (above) and cholesterol synthesis (below). • [^{14}C] alanine ▲ [^{3}H] $_2$O.

vitamin mixture, and 40 g/kg cellulose. When equimolar amounts of (−)−hydroxycitrate $(Na)_3$ and citrate $(Na)_3$ were administered as dietary admixtures, an equivalent wt of glucose was deleted from the diet. To ensure complete uniformity, all diets were mixed with a twin-shell dry blender equipped with an intensifier bar (Patterson-Kelley Co., East Stroudsburg, Pa.).

Measurement of In Vivo Rate of Lipid Synthesis

Immediately after the 3 hr feeding period, rats were anaesthetized lightly with Penthrane (methoxyflurane, Abbott Laboratories, North Chicago, Ill.) administered intravenously a 0.25 ml saline (pH 7.4 to 7.6) solution with the following composition: 12.3 mg alanine, 5 μCi [^{14}C]alanine (specific activity = 156 mCi/mmole), 30.6 mg α-ketoglutarate (as an amine acceptor for transaminase) and 1 mCi [^{3}H] water (specific activity = 100 mCi/g). Experiments indicated that [^{14}C]alanine was equivalent to either [^{14}C]pyruvate or [^{14}C]lactate as a carbon precursor for lipogenesis. [^{3}H]Water was employed to determine the total rate of lipogenesis, since tritium is incorporated into FA independent of the source of carbon precursors (11,12). Animals were killed by decapitation and blood collected in centrifuge tubes 30 min after the radioactive pulse, unless otherwise indicated. The specific radioactivity of the body water of each rat was determined by counting a diluted serum aliquot in 10 ml following cocktail: toluene (2.4 l), 2-methoxyethanol (1.6 l), naphthalene (320 g), and BBOT (2,5-bis-2-(5-tert-butylbenzoxazolyl)-thiophene, 16 g). Liver, perirenal adipose tissue, and small intestine were excised rapidly, weighed, and homogenized in 15 ml H_2O in a Virtis 45 Macro Homogenizer for 15 sec at ca. 30,000 rpm. The contents of the small intestine were removed by repeated washing before homogenization. Duplicate 3 ml aliquots whole homogenates were saponified, extracted, and the absolute radioactivity (dpm) determined, as described previously (6,10). Liver lipids were extracted totally or separated into FA and cholesterol by anion exchange chromatography, as described previously (6). It was determined by anion exchange chromatography that the total lipid extract of liver contained FA (96-97%) and cholesterol (3-4%). Data are expressed as nmoles [^{14}C]alanine or μmoles [^{3}H] water converted into lipid/g tissue/30 min. The nmoles [^{14}C]alanine were calculated according to the injected load of alanine, as reported previously (6,10). The μmoles [^{3}H] water were determined as described previously (9,11).

Determination of Radioactive Neutral Lipids and FA in Serum

Serum lipids were extracted by the method of Bligh and Dyer (18). Sera from three rats were pooled (5 ml) and the following reagents added in order after adequate mixing: 5 ml chloroform, 10 ml methanol, 5 ml chloroform, then 5 ml H_2O. The extract was filtered over Whatman 1 paper, the upper phase discarded, the lower phase evaporated to dryness under

N_2, and 5 ml methanol added. The extract was added to a 1 x 10 cm column containing Dowex 1-X2. Neutral lipids were eluted with 45 ml methanol:ethyl ether (1:1), and FA were eluted with 80 ml ether:80% methanol: acetic acid (10:8:2). Both fractions were evaporated under N_2 to dryness and analyzed for absolute readioactivity, as described previously (6).

Measurement of In Vitro Rate of Lipogenesis

The details for determining the incorporation of [1,5-^{14}C] citrate (specific acitivity = 6.4 mCi/mmole) into saponifiable lipid by a hepatic cell-free (100,000 x g for 30 min) system have been reported previously (19). The procedures used for the saponification and extraction of lipid were the same as those described previously (6). Protein was determined by a modification of the method of Lowry (20). The amount of radioactivity incorporated from [1,5-^{14}C]-citrate into saponifiable lipid was a measure of the in vitro rate of lipogenesis dependent upon the activities of ATP citrate lyase, acetyl CoA carboxylase, and FA synthetase. Data are expressed as nmoles [^{14}C] citrate converted into lipid/g liver/30 min. Data may be converted to [^{14}C] citrate incorporated into lipid/mg protein by dividing by 20.

Sources of Chemicals

(−)−Hydroxycitric acid lactone was isolated from the dried fruit rinds of the Indian plant *Garcinia cambogia*. Trisodium salt was used; solutions of this salt were prepared from the crystalline form or by hydrolysis of the lactone (30 min heating at 90 C with three equivalents of NaOH).

Constituents for the synthetic diets were obtained from Nutritional Biochemicals, Cleveland, Ohio. Other chemicals were purchased from Sigma Chemical, Milwaukee, Wis.

Statistical Analysis

The *t* test was used to analyze all experimental results (21). Data were processed statistically for outliers (22).

RESULTS

Measurement of In Vivo Rate of Lipid Synthesis

Figure 1 demonstrates the in vivo rate of hepatic FA and cholesterol biosynthesis as a function of min after the injection of the [^{14}C] alanine and [^{3}H] water pulse. The conversion of [^{3}H] water into FA and cholesterol was linear for 60 min after pulse administration. During this 60 min period, the specific activity of the body water remained constant.

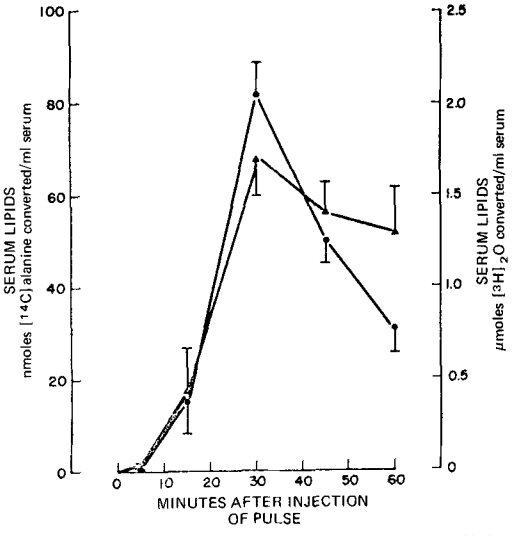

FIG. 2. Relation between radioactive serum lipids and the time of administration of [^{14}C] alanine and [^{3}H] water. Rats were prefasted 48 hr then meal fed the G-70 diet for 9 days. A pulse of [^{14}C] alanine and [^{3}H] water was injected immediately after the 3 hr meal. Serum lipids were extracted as described in the text. Each group consisted of 9-10 rats, and the vertical bar gives the standard error of the mean. • [^{14}C] alanine ▲ [^{3}H] $_2$O.

However, [^{14}C] alanine conversion was maximal at 30 min and ca. linear for only 15 min.

When the sera of the rats receiving [^{14}C] alanine and [^{3}H] water were examined for the amount of radioactive lipid present, both [^{14}C] alanine and [^{3}H] water conversion were linear for 30 min (Fig. 2). Since the standard assay time employed was 30 min, it was important to determine whether the radioactive serum lipids present at 30 min were neutral lipids (indicating liver biosynthesis) or FA (indicating adipose tissue biosynthesis). Sera FA and neutral lipids were separated, and all the radioactivity was recovered in the neutral lipid fraction, indicating that the [^{14}C], [^{3}H] lipids present in the serum at 30 min were of liver origin.

Characteristics of the Inhibition of In Vivo Rates of Lipid Synthesis by (−)−Hydroxycitrate

The effect of the oral administration of (−)−hydroxycitrate (2.63 mmoles/kg) upon the in vivo rate of hepatic lipogenesis was examined over a 24 hr period to determine the extent and duration of the inhibition and to establish whether the lipogenic rates in the (−)−hydroxycitrate-treated animals increased during the time when control rates decreased. Figure 3 illustrates the in vivo rates of lipogenesis determined by the conversion of [^{14}C] alanine and [^{3}H] water into lipid. To

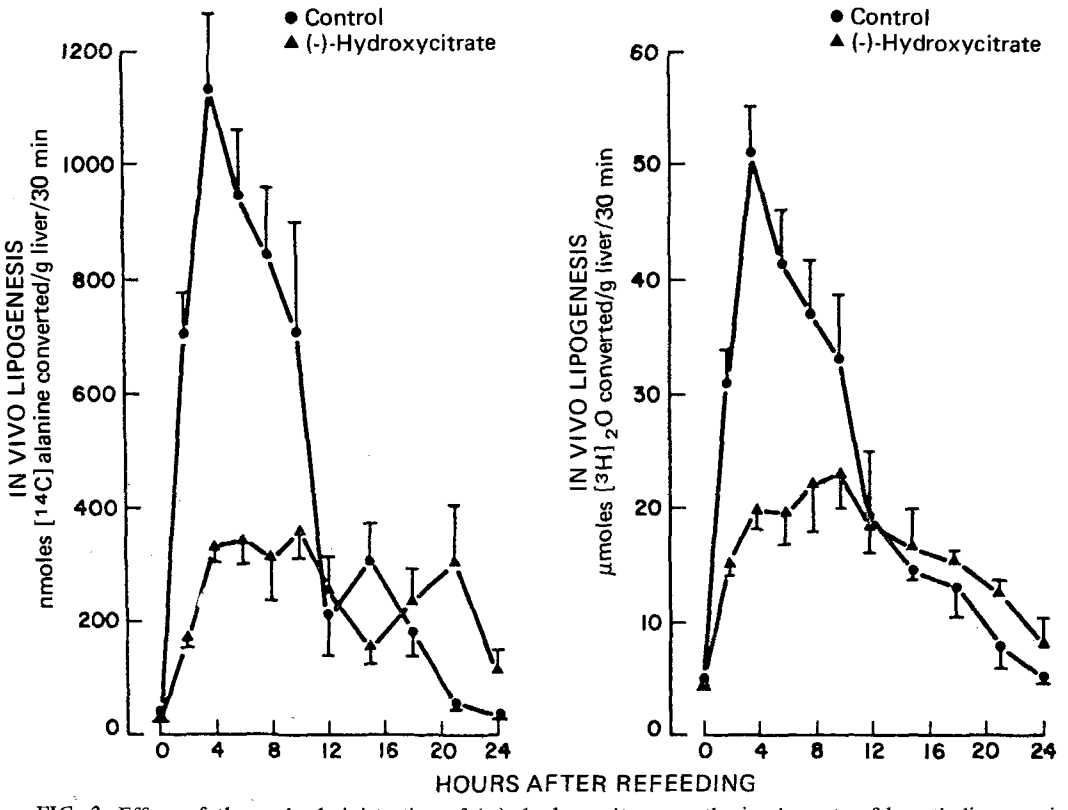

FIG. 3. Effect of the oral administration of (−)−hydroxycitrate on the in vivo rate of hepatic lipogenesis determined over a 24 hr period. Rats were prefasted 48 hr, then meal fed the G-70 diet for 6 days. On day 7, 50 rats each were given orally either saline or (−)−hydroxycitrate (2.63 mmoles/kg) directly before receiving 8.7 g food. The in vivo rate of lipogenesis was determined using the [^{14}C]alanine and [^{3}H]water pulse at the indicated times. The animals were killed 30 min after pulse administration. The vertical bar gives the standard error of the mean (five rats/point). Nmoles [^{14}C]alanine and μmoles [^{3}H]water converted into lipid in the (−)−hydroxycitrate treated animals were significantly different from controls at hr 2, 4, 6, and 8 ($p < 0.05$). ● Control ▲ (−)−Hydroxycitrate.

standardize the amount of food consumed on the experimental day, each rat was given 8.7 g G-70 diet, which equals the food intake of rats receiving 2.63 mmoles/kg of (−)−hydroxycitrate. Lipogenic rates increased to a maximum at 3-5 hr after feeding and declined subsequently to a minimum at 24 hr. (−)−Hydroxycitrate caused a significant inhibition of the lipogenic rate for 8 hr after refeeding. During this period, lipid synthesis from [^{14}C]alanine and [^{3}H]water was decreased by 68% and 72%, respectively. By 12 hr, the rates of lipogenesis were indistinguishable in controls and treated rats. Although there was a tendency towards elevated lipogenic rates 14-24 hr in the (−)−hydroxycitrate treated rats compared to controls, these results were not significant.

The oral administration of (−)−hydroxycitrate inhibited significantly the in vivo rates of lipogenesis in several tissues known to convert carbohydrate into FA, namely liver, adipose tissue, and small intestine (Table I). Although the rates at which [^{14}C]alanine was converted into lipid by the three tissues differed markedly (adipose tissue > liver > small intestine), all were depressed significantly by 10.52 and 5.26 mmoles/kg of (−)−hydroxycitrate. After oral administration of 2.63 mmoles/kg of (−)−hydroxycitrate, the lipogenic rates in liver and adipose tissue were significantly inhibited.

Effect of the Chronic Oral Administration of (−)−Hydroxycitrate on Hepatic Lipogenesis

Figure 4 demonstrates the in vitro rate of lipogenesis in rats administered varying concentrations of (−)−hydroxycitrate orally for 30 days. The upper curve illustrates the observed in vitro rate, and the lower curve gives the rate of lipogenesis when 1 mM (−)−hydroxycitrate was added to each assay. Animals receiving (−)−hydroxycitrate at daily concentrations of 2.63, 1.32, and 0.66 mmoles/kg for 30 days demonstrated a significantly higher rate of lipogenesis compared to controls (1.5-2.1-fold).

TABLE I

Inhibition of In Vivo Lipogenesis in Liver, Adipose, and Small Intestine by Oral Administration of (−)–Hydroxycitrate

Treatment[a]	Dose mmoles/kg	Rate of lipogenesis					
		Liver		Adipose		Small intestine	
		nmoles [^{14}C]alanine converted[b]	Inhibition percent	nmoles [^{14}C]alanine converted[b]	Inhibition percent	nmoles [^{14}C]alanine converted[b]	Inhibition percent
Saline		855 ± 99		2473 ± 656		84 ± 9	
(−)–Hydroxycitrate	10.52	93 ± 33[c]	89	393 ± 136[d]	84	37 ± 13[c]	56
(−)–Hydroxycitrate	5.26	188 ± 28[c]	78			28 ± 4[c]	67
(−)–Hydroxycitrate	2.63	498 ± 86[d]	42	754 ± 59[d]	69	72 ± 9	14

[a]Four groups of five rats each were prefasted, then meal fed the G-70 diet for 9 days. On day 9 they were given (−)–hydroxycitrate dissolved in saline at the indicated doses 60 min before feeding. Immediately after the 3 hr feeding period, the in vivo rates of lipogenesis were determined in rat liver, perirenal adipose tissue, and washed small intestine, as described in the text.

[b]Data are expressed as nmoles [^{14}C]alanine converted into lipid/g tissue/30 min. Each value is the group mean ± standard error.

[c]$p < 0.01$.

[d]$p < 0.05$.

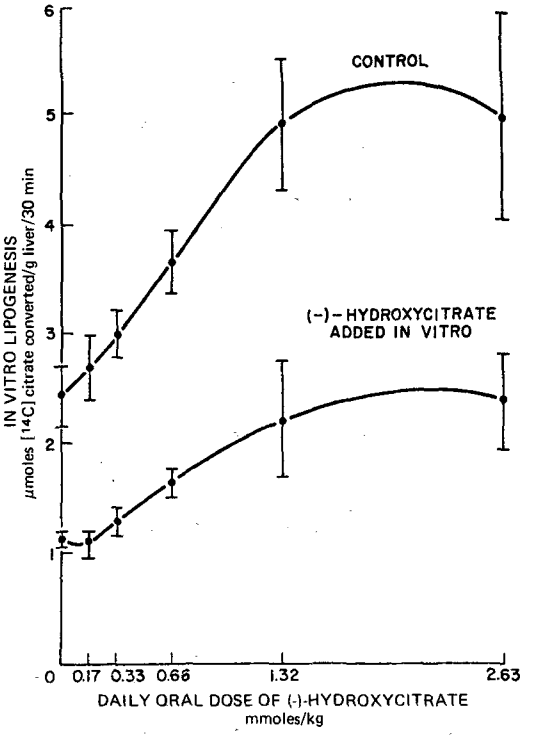

FIG. 4. In vitro rate of hepatic lipogenesis in rats administered (−)–hydroxycitrate orally for 30 days. Rats were prefasted 48 hr, then meal fed the G-70 diet for 5 days. From day 6-36, they received saline or varying concentrations of (−)–hydroxycitrate 1 hr before feeding the G-70 diet. Immediately after the 3 hr feeding period on day 36, livers from four rats in each group were assayed for in vitro rates of lipogenesis in 10 mM citrate as described in the text. The upper curve demonstrates the observed rate of lipogenesis; the lower curve gives the rate of lipogenesis when 1 mM (−)–hydroxycitrate was added to each assay. The vertical bar gives the standard error of the mean.

Lower concentrations of (−)–hydroxycitrate (0.17 and 0.33 mmoles/kg) produced small, but insignificant, increases in rates of lipogenesis. When (−)–hydroxycitrate was added, a similar level of significant inhibition was observed regardless of the control in vitro lipogenic rate.

Figure 5 illustrates the reduction in the in vivo rate of hepatic lipogenesis determined by the conversion of [^{14}C]alanine and [^{3}H]water into lipid in rats given varying concentrations of (−)–hydroxycitrate orally for 30 days. Animals receiving (−)–hydroxycitrate at daily doses of 2.63 and 1.32 mmoles/kg demonstrated a significantly depressed in vivo rate of lipogenesis.

The effect of chronic oral administration of (−)–hydroxycitrate for 11 days upon in vivo hepatic lipogenesis is shown in Table II. Here, as in Figure 5, significant inhibition was observed at concentrations of 2.63 and 1.32

TABLE II

Reduction in the In Vivo Rate of Lipogenesis in Rats Administered (−)−Hydroxycitrate Orally for 11 Days[a]

Daily oral dose of (−)−hydroxycitrate mmoles/kg	Rate of lipogenesis			
	nmoles [^{14}C]alanine converted[b]	Inhibition percent	μmoles [^{3}H]$_2$O converted[b]	Inhibition percent
0	1043 ± 131	0	64.3 ± 5.3	0
0.66	760 ± 107	27	50.4 ± 5.1	22
0.33 bid	824 ± 84	21	56.2 ± 5.8	13
2.63	248 ± 27[c]	76	32.5 ± 2.0[c]	49
1.32 bid	592 ± 151[c]	43	40.7 ± 6.2[c]	37

[a]Five groups of 10 rats each were prefasted 48 hr, then meal fed the G-70 diet for 5 days. They then were given saline or (−)−hydroxycitrate by stomach tube 1 hr before feeding the G-70 diet and 4 hr after the completion of the meal (where bid [twice a day] administration is indicated) for 11 days. Immediately after the 3 hr feeding period on day 11, the in vivo rate of lipogenesis was determined in rat liver, as described in the text.

[b]Data are expressed as nmoles [^{14}C]alanine and μmoles [^{3}H]$_2$O converted into lipid/g liver/30 min. Each value is the group mean ± standard error.

[c]$p < 0.01$.

mmoles/kg, whereas 0.66 and 0.33 produced insignificant decreases.

As will be discussed, in the next paper, (−)−hydroxycitrate significantly reduced appetite, body wt gain, and body lipid levels. In an attempt to separate the effects upon lipogenesis from the effects upon appetite, three groups of rats were treated as follows: (A) the first group (control) was allowed free access to the G-70 diet, (B) the second group [(−)−hydroxycitrate)] was permitted unlimited access to the G-70 diet, which contained (−)−hydroxycitrate as a dietary admixture (daily dose equivalent to 2.63 mmoles/kg), and (C) a third group (paired control) was allowed only the quantity of G-70 diet which its (−)−hydroxycitrate-treated pair consumed on the preceding day (Table III). The paired controls, even under dietary restriction, demonstrated rates of lipogenesis equivalent to unrestricted controls. However, in vivo rates were inhibited significantly in the (−)−hydroxycitrate-treated rats.

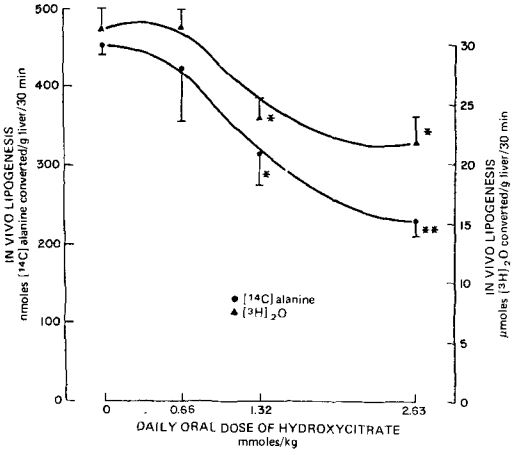

FIG. 5. Reduction in the in vivo rate of hepatic lipogenesis in rats administered (−)−hydroxycitrate orally for 30 days. Rats were prefasted 48 hr, then meal fed the G-70 diet for 5 days. From day 6-36, they received saline or varying concentrations of (−)−hydroxycitrate 1 hr before feeding the G-70 diet. Immediately after the 3 hr feeding period on day 36, the in vivo rates of lipogenesis were determined in 8-10 rat livers/group as described in the text. The vertical bar gives the standard error of the mean. *$p < 0.05$ **$p < 0.01$ • [^{14}C]alanine ▲ [^{3}H]$_2$O.

DISCUSSION

The preferential usefulness of [^{3}H]water for the measurement of in vivo rates of FA and cholesterol synthesis was apparent from the results illustrated in Figure 1. The rate of incorporation of [^{3}H]water was linear for at least 60 min and the total net rate was substantially greater than that determined from [^{14}C]alanine at each interval. This was expected, since the protons of [^{3}H]water were converted into FA (9, 11-16) and cholesterol (7) independent of the source of carbon precursors of acetyl CoA. In the studies reported here, [^{14}C]alanine was employed simultaneously to provide a measurement of the lipogenic rate from a specific carbon precursor.

We previously demonstrated that the oral administration of (−)−hydroxycitrate inhibited significantly in vivo rates of FA and cholesterol synthesis in rat liver, as measured by the conversion of [^{14}C]alanine. These rate measurements were made immediately following the 3 hr meal (6). The results presented in Figure 3 amplify and extend these observations,

TABLE III

In Vivo Lipogenesis in Pair fed Rats Administered (−)−Hydroxycitrate in the Diet and Meal fed for 38 Days

Treatment[a]	Rate of lipogenesis: nmoles [^{14}C]alanine converted[b]	Percent of control	μmoles [^{3}H]$_2$O converted[b]	Percent of control
Control	581 ± 64		33.6 ± 3.4	
Paired control	781 ± 86	134	39.2 ± 2.9	117
(−)−Hydroxycitrate	163 ± 16[c]	28	21.1 ± 2.4[c]	63

[a]Three groups of nine rats each were prefasted 48 hr, then meal fed the G-70 diet for 5 days. Two groups were then meal fed the G-70 diet for 38 days, while the third group received a dietary admixture of (−)−hydroxycitrate (52.6 mmoles/kg diet) in G-70. This amount of (−)−hydroxycitrate was equivalent to ca. 3 mmoles/kg body wt/day. One group receiving G-70 was pair fed to the (−)−hydroxycitrate treated rats. Immediately after the 3 hr feeding period on day 38, the in vivo rate of lipogenesis was determined in rat liver, as described in the text.

[b]Data are expressed as nmoles [^{14}C]alanine and μmoles [^{3}H]$_2$O converted into lipid/g liver/30 min. Each value is the group mean ± standard error.

[c]$p < 0.01$.

since they provide the hepatic lipogenic profile using [^{14}C]alanine and [^{3}H]water over a 24 hr period. The rise and fall in the rates of conversion of [^{3}H]water over a 24 hr period in meal fed, control rats corresponded to that of [^{14}C]alanine, previously reported (10). A single oral dose of (−)−hydroxycitrate administered to rats given the same amount of food as controls depressed hepatic lipogenesis for the 8 hr period when control animals demonstrated elevated rates of synthesis. During the next 16 hr, it was possible that the hepatic lipogenic rates in the (−)−hydroxycitrate-treated rats would increase or plateau, while the control rates were decreasing, i.e. a frame shift of lipogenic rates would occur with (−)−hydroxycitrate. The possibility that the carbons and electrons diverted from hepatic lipid synthesis during the 8 hr after feeding would be incorporated into lipid at a later time was not substantiated, since the increased rates observed during the next 16 hr in the (−)−hydroxycitrate-treated animals compared to controls were not significant. Results not reported here indicated that the amount of liver lipid, determined gravimetrically, in the (−)−hydroxycitrate-treated animals durng the 8 hr period when lipogenesis was significantly inhibited was 11% less than controls. Although these differences were not significant, at 10 hr the liver lipid content of the (−)−hydroxycitrate treated rats was significantly less than controls (21%). Liver lipid levels were similar in both groups from 12-24 hr after refeeding.

The metabolic fate of the carbons and electrons that were diverted from conversion into lipid posed an interesting question. Rates of lipogenesis determined at a single interval, 3 hr after feeding, were reduced equivalently by (−)−hydroxycitrate administration in liver and adipose tissue, although inhibition was less dramatic in small intestine (Table I). The possibility that carbon and electron flux into fatty acids increased in adipose tissue and small intestine at later intervals in (−)−hydroxycitrate treated animals appeared unlikely. Currently, we are investigating other metabolic fates of these diverted carbons and electrons.

The dose-dependent inhibition of the in vivo hepatic rates of lipogenesis after 11 and 30 days of (−)−hydroxycitrate administration (Fig. 5, Table II) was similar to that observed after acute administration (6). It was expected that rates determined from [^{3}H]water were less depressed than rates from [^{14}C]alanine, since [^{3}H]water provided a measure of the total rate of lipid synthesis. However, the significant increases (1.5-2.1-fold) in the in vitro rate of hepatic lipogenesis in animals receiving 2.63, 1.32, and 0.66 mmoles/kg (−)−hydroxycitrate for 30 days were unexpected. Since the in vitro assay provided a measure of the activities of ATP citrate lyase, acetyl CoA carboxylase, and FA synthetase, the observed rate increases suggested that the lipogenic enzyme activities and levels were increased as a result of a prolonged period of daily depression of FA synthesis resulting from the competitive inhibition of ATP citrate lyase. The fact that a constant level of (−)−hydroxycitrate added exogenously depressed the in vivo rates equivalently suggested that ATP citrate lyase may be the elevated enzyme. This theoretical, compensatory mechanism was provocative but unproved. It was interesting that dietary restriction to the level consumed by (−)−hydroxy-

citrate treated rats still produced elevated control rates of hepatic lipogenesis (Table III).

The following paper examines the influence of the chronic oral administration of (−)–hydroxycitrate on appetite, wt gain, and body lipid levels and discusses whether these effects are related to the antilipogenic action of (−)–hydroxycitrate described here.

ACKNOWLEDGMENTS

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REFERENCES

1. Lewis, Y.S., and S. Neelakantan, Phytochem. 4:619 (1965).
2. Lewis, Y.S., S. Neelakantan, and C. Anjanamurthy, Curr. Sci. 3:82 (1964).
3. Lewis, Y.S., in "Methods in Enzymology," Vol. 13, Edited by J.M. Lowenstein, Academic Press, New York, N.Y., 1969.
4. Lowenstein, J.M., in "Essays in Cell Metabolism," Edited by W. Bartley, H.L. Kornberg, and J.R. Quayle, John Wiley and Son, London, England, 1970.
5. Watson, J.A., M. Fang, and J.M. Lowenstein, Arch. Biochem. Biophys. 135:209 (1969).
6. Sullivan, A.C., J.G. Hamilton, O.N. Miller, and V.R. Wheatley, Ibid. 150:183 (1972).
7. Brunengraber, H., J.R. Sabine, M. Boutry, and J.M. Lowenstein, Ibid. 150:392 (1972).
8. Barth, C., J. Hackenschmidt, H. Ullmann, and K. Decker, FEBS Letters 22:343 (1972).
9. Lowenstein, J.M., J. Biol. Chem. 246:629 (1971).
10. Sullivan, A.C., O.N. Miller, J.S. Wittman, and J.G. Hamilton, J. Nutr. 101:265 (1971).
11. Jungas, R.L., Biochemistry 7:3708 (1968).
12. Fain, J.N., and R.O. Scow, Amer. J. Physiol. 210:19 (1965).
13. Windmueller, H.G., and A.E. Spaeth, J. Biol. Chem. 241:2891 (1966).
14. Fain, J.N., and A.E. Wilhelmi, Endocrinology 71:541 (1962).
15. Windmueller, H.G., and A.E. Spaeth, Arch. Biochem. Biophys. 122:362 (1967).
16. Fain, J.N., R.O. Scow, E.J. Urgoiti, and S.S. Chernick, Endocrinology 77:137 (1965).
17. Phillips, P.H., and E.B. Hart, J. Biol. Chem. 109:657 (1935).
18. Bligh, E.G., and W.J. Dyer, Can. J. Biochem. Physiol. 37:911 (1959).
19. Fang, M., and J.M. Lowenstein, Biochem. J. 105:803 (1967).
20. Miller, G.L., Anal. Chem. 31:964 (1959).
21. Snedecor, G.W., and W.G. Cochran, in "Statistical Methods," Iowa State University Press, Ames, Iowa, 1968.
22. Dixon, W.J., Biometrics 9:74 (1953).
23. Leveille, G.A., Proc. Soc. Exp. Biol. Med., 125:85 (1967).

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