Metabolism of hydroxy fatty acids in dogs with steatorrhea secondary to experimentally produced intestinal blind loops

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ABSTRACT Several aspects of the metabolism of hydroxy fatty acids were studied in dogs with steatorrhea resulting from an experimentally produced jejunal blind loop. In these animals hydroxy acids were present in the stool in amounts far above normal. These acids disappeared from the feces during tetracycline administration and after exclusion of the blind loop–both procedures that corrected the steatorrhea apparently by reducing bacterial overgrowth. Hydroxy acids persisted in higher than normal amounts, however, after administration of taurocholic acid, which also corrected the steatorrhea, but by a different mechanism. Both in normal dogs and in those with blind loops, hydroxy acid constituted a higher percentage of total fatty acids in the jejunum. A possible conclusion is that hydroxy fatty acids have an enterohepatic circulation via the portal system. When hydroxy acids were fed to normal dogs, steatorrhea was not produced and absorption in amounts similar to that of unsubstituted stearic acid was observed.

Isotopic oleic and linoleic acids were converted to hydroxy acids both in vivo and during in vitro incubation with feces; stearic acid was not. These findings support the idea that hydroxy acids arise by the addition of water across double bonds, this addition being catalyzed by enzymes of intestinal bacteria.

KEY WORDS hydroxy fatty acids · steatorrhea · fecal fat · intestinal blind loop

JAMES, WEBB, AND KELLOCK (1) demonstrated in 1961 that hydroxy fatty acids were present in human feces. In that and subsequent reports (2, 3) they showed that these fecal acids greatly increased in amount when steatorrhea was present and established that the hydroxy substitution was principally on carbon 10 of stearic acid. They also provided evidence that hydroxy stearic acid arose by oxidation of stearic acid and postulated that it was an intermediate between saturated and unsaturated acids formed by the action of intestinal bacteria.

We have extended these observations by studying several aspects of the metabolism of hydroxy stearic acid in dogs with experimental steatorrhea secondary to the construction of an intestinal blind loop. We have attempted to define further the relationship between the presence of these acids and steatorrhea by studying the effect on hydroxy acid secretion of procedures that ameliorate the steatorrhea by different mechanisms and by feeding hydroxy acids to normal dogs. Finally, by measuring the amount of hydroxy acid in the upper intestine and by comparing the biosynthesis of these acids from several isotopic precursors we have attempted to learn more about the origin of fecal hydroxy acids.

MATERIALS AND METHODS

Experimental Subjects

The dogs with steatorrhea secondary to the surgical construction of blind loops were the same animals utilized in a previously published study (4). The details of the

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surgical procedure and the care of the animals are described in that paper.

Chemicals

12-hydroxy stearic acid was obtained from two sources. The material used as a chromatographic standard was obtained from Applied Science Laboratories Inc. (State College, Pa.) and was chromatographically pure as judged by gas chromatography (see below). The material used in feeding studies was obtained from Distillation Products Industries (Rochester, New York); it was listed as technical quality, and we found it to contain 12% stearic acid. Stearic acid-1-^{14}C, oleic acid-1-^{14}C, and linoleic acid-1-^{14}C were obtained from Applied Science Laboratories. Thin-layer radiochromatographic analyses are described below. Sodium acetate-1-^{14}C was obtained from New England Nuclear Corp. (Boston, Mass.).

Methods of Analysis

Total fecal fat was measured by the method of either Saxon (5) or van de Kamer, Bokkel Huinink, and Weyers (6). Recovery of 12-hydroxy stearic acid added to feces ranged from 70 to 80%. The data are presented without correction for this low recovery. Hydroxy acid content of fecal fatty acids was determined by gas chromatography as follows. The pH of about 1 g of the fecal homogenate was made >9 with ethanolic KOH and the mixture was saponified at 60°C for 1 hr. After acidification, the fatty acids were extracted into petroleum ether (bp 30–60°C) and methylated with boron trifluoride–methanol (7).

The methyl esters were chromatographed either on a 6 or 4 ft 1% SE-30 (methylpolysiloxy gum) column or on a 6 ft column containing 15% diethylene glycol adipate polyester. All columns were obtained ready packed from Applied Science Laboratories. In these systems the methyl 12-hydroxy stearate standard had retention times of 2.5, 2.1, and 6.1 times that of methyl stearate. When the trimethyl silyl derivative was formed (8) from the standard hydroxy acid or the unknown material in feces, the peaks attributed to the hydroxy acid methyl ester disappeared and a new peak with a retention time 1.2 times that of methyl stearate appeared in equivalent amounts. On thin-layer chromatography in the system described below, the material from feces identified as methyl hydroxy stearate had the chromatographic characteristics of the standard 12-hydroxy acid methyl ester. These identification procedures do not establish the position of the hydroxyl group in the unknown material (9). Since the retention time of 10-hydroxy stearic acid is the same as that of 12-hydroxy stearic acid (9) we assume the acid in feces to be 10-hydroxy stearic acid on the basis of the studies by James et al. (2). The fecal acid is referred to as hydroxy stearate in this paper.

Thin-layer chromatography was carried out on 0.5 mm Silica Gel H chromatoplates. The methyl ester of the fatty acids were chromatographed in ethyl ether–heptane 60:40; in this system the methyl esters of the hydroxy acids had an R_f approximately 0.6 that of the esters of unsubstituted acids.

Feces were incubated with isotopic fatty acids and acetate as described by Webb, James, and Kellock (2). Fatty acids were then extracted and methylated as described above for feces, and the methylated material was applied to the chromatoplate. In some instances unlabeled methyl hydroxy stearate was added as an aid in the recovery of that fraction. Materials with the migration rates of the methyl esters of unsubstituted fatty acids and hydroxy stearate were recovered from the chromatoplate by the method of Goldrick and Hirsch (10). The radioactivity in each fraction was measured in a Tri-Carb liquid scintillation counter; efficiency was determined by means of an internal standard.

RESULTS

Hydroxy Fatty Acids in Experimental Steatorrhea

Marked increase in fecal excretion of hydroxy fatty acids accompanied the steartorhea seen in 12 dogs with a jejunal blind loop (Table 1). In 11 normal dogs consuming the same diet, fecal fat averaged 4.1 g or 5.6% of intake compared to 10.3 g and 21.2% in the animals with experimental steatorrhea. Excretion of hydroxy fatty acid, which was 20 mg/day in normal dogs, averaged 1.1 g in the experimental group (9.1% of their fecal fat).

Three procedures (tetracycline administration, exclusion of the blind loop, and sodium taurocholate feeding) reduced the degree of steatorrhea in the experimental animals (Table 2). While all three reduced fecal fat excretion to normal levels, only tetracycline administration and blind loop exclusion eliminated hydroxy acids from the feces. Although decreased in absolute amount, these acids remained as an abnormally

TABLE 1 EFFECT OF INTESTINAL BLIND LOOP ON EXCRETION OF TOTAL FECAL FAT AND HYDROXY FATTY ACIDS IN DOGS

	Blind Loop	Normal	*P*
Fecal fat			
Amount (g/day)	10.3 ± 1.2(12)*	4.1 ± 0.15(11)	<0.001
% of intake	21.2 ± 3.7(12)	6.6 ± 0.27(11)	<0.001
Hydroxy fatty acid			
Amount (g/day)	1.1 ± 0.2(12)	0.02 ± 0.01(6)	<0.001
% of fecal fat	9.1 ± 1.3(12)	0.6 ± 0.3(6)	<0.001

* SEM (n given in parentheses).

TABLE 2 EFFECTS OF THREE PROCEDURES ON EXCRETION OF TOTAL FECAL FAT AND OF HYDROXY ACID IN DOGS WITH BLIND LOOP

	Fecal Fat		Hydroxy Acids	
	Amount	% of Intake	Amount	% of Fecal Fatty Acids
	g/day		*g/day*	
Tetracycline				
Dog A control	13.8	22.0	1.2	9.0
treatment	3.5	7.2	0	0
Dog B control	14.3	17.4	1.5	10.3
treatment	4.2	7.0	0	0
Sodium taurocholate				
Dog C control	11.0	20.6	1.1	1.5
treatment	4.0	7.9	0.2	5.8
Dog D control	12.5	23.4	9.8	11.6
treatment	4.2	7.9	0.4	8.5
Surgical exclusion of loop				
Dog E with blind loop	8.7	21.6	0.7	8.4
25 days after exclusion	4.5	6.7	0	0
25 days afer reconstruction	10.8	17.4	0.5	4.7

high proportion of the total fecal acids in the two animals treated with 10 g/day of sodium taurocholate for 6 days, even though steatorrhea had been corrected.

Jejunal content was obtained from five normal dogs and four animals with steatorrhea due to blind loop, and its hydroxy acid content was determined. Hydroxy acid was detected in each instance, ranging from 0.5 to 3.2% of the total fatty acids (mean, 1.9%) in the normal animals and from 0.6 to 7.3% (mean, 4.1%) in the animals with steatorrhea. This finding is particularly significant since, in the normal dogs, the percentage of hydroxy acid in fatty acids was higher in jejunum than in feces.

Effects of Feeding 12-Hydroxy Stearate and Stearate to Normal Dogs

To test the effect of hydroxy fatty acids on fat absorption, we added 2.2 and 8.8 g of 12-hydroxy stearate per day to the diet of a normal dog. As indicated in Table 3, loss of fecal fat was essentially unchanged when 2.2 g/day was fed.

When the intake of hydroxy stearate was 8.8 g, fecal fat was 5.5 g/day, a value only slightly above normal. If all of the fecal fat represented unabsorbed hydroxy stearate (some altered to other fatty acids) absorption of this acid would be 37.5%. If, on the other hand, only the fecal hydroxy acids (3.3 g/24 hr) represented unabsorbed dietary 12-hydroxy stearate, absorption would have been 62.5%; thus, the actual amount of absorption was between these two extremes. At neither level of intake did it appear that the added hydroxy acid interfered with the absorption of the normal dietary fat.

For comparison, similar amounts of unsubstituted stearic acid were added to the diet of another normal dog (Table 3). With 10 g of this acid added to the diet, fecal fat reached 6.9 g/day or 12.2% of the total fat intake. This comparison between fecal fat loss during addition of hydroxy stearate and stearate to the diet provides further evidence that the hydroxylated acids per se do not play a role in increasing the degree of steatorrhea.

Conversion of Radio-Labeled Fatty Acids to Hydroxy Acids

In Vivo. Two dogs with blind loops and steatorrhea were fed at 10-day intervals 30 μc of oleic acid-1-^{14}C, 30 μc of linoleic acid-1-^{14}C, or 10 μc of stearate-1-^{14}C in a single dose mixed in their daily ration. Aliquots of feces obtained 24–36 hr after each feeding were extracted, the lipid was subjected to alkaline hydrolysis, and fatty acid methyl esters were prepared. These were separated into material with thin-layer chromatographic characteristics of unsubstituted fatty acid methyl esters and of hydroxy fatty acid methyl esters. It is apparent from Table 4 that in both dogs stearate-1-^{14}C feeding gave fecal fatty acids in which most of the radioactivity was in the fraction containing unsubstituted fatty acid. In contrast, signifi-

TABLE 3 EFFECTS ON FECAL FAT OF 12-HYDROXY STEARIC ACID AND STEARIC ACID ADDED TO THE DIET OF NORMAL DOGS

Basic Diet Fat	Added Fatty Acid		Total Diet Fat	Fecal Fat	Fecal Fat as % of Intake	Fecal Fatty Acids		Fatty Acid Excretion	
	18:0	12-OH*				18:0	12-OH	18:0	12-OH
	g/day					*% of total*		*g/day*	
59.6	0	0	59.6	3.2	5.4	35.4	0	1.1	0
48.7	0.3	2.2	51.2	3.3	6.5	19.5	46.0	0.6	1.5
51.1	1.2	8.8	61.1	5.5	9.0	13.3	60.2	0.7	3.3
52.2	0	0	52.2	3.0	5.7	35.4	0	1.2	0
48.7	2.5	0	51.2	3.8	7.5	74.7	0.2	2.8	0.1
46.5	10.0	0	56.5	6.9	12.2	86.0	0	5.9	0

* 12-OH, 12-hydroxy stearate.

TABLE 4 COMPARISON OF PRECURSORS OF HYDROXY FATTY ACIDS IN IN VIVO STUDIES IN DOGS WITH BLIND LOOP STEATORRHEA

Dog	Isotope Fed	Hydroxy Acid Methyl Esters	Unsubstituted Fatty Acid Methyl Esters
		% of total isotope in fecal lipid extract	
A	Oleate-1-^{14}C	17.7	82.3
	Linoleate-1-^{14}C	11.0	89.0
	Stearate-1-^{14}C	1.2	98.8
B	Oleate-1-^{14}C	18.9	81.1
	Linoleate-1-^{14}C	19.7	80.3
	Stearate-1-^{14}C	1.9	98.1

cant amounts of both linoleate and oleate had been converted to material with the R_f of hydroxy fatty acid methyl esters.

In Vitro. In experiments in vitro the same three isotopic fatty acids as well as sodium acetate-1-^{14}C were separately incubated with suspensions of freshly voided feces obtained from a dog with a blind loop and steatorrhea and from a patient with nontropical sprue. In both cases aerobic and anaerobic incubations were carried out simultaneously. Linoleate and oleate were partially converted to hydroxy acids; stearate was not (Table 5). Radioactivity from acetate was detectable in both unsubstituted and hydroxy fatty acid fractions. Essentially no difference in isotope distribution was detected between aerobic and anaerobic incubations.

DISCUSSION

In the present study we were able to confirm the findings of James and coworkers (1–3) that although hydroxy stearate is present in small amounts in normal feces, it increases greatly in absolute amount and in percentage of total fatty acids in the presence of steatorrhea. Evidence that the increased hydroxy acid excretion was associated with bacterial action in the bowel and was not a consequence of the steatorrhea per se was provided by studies of the effect of different treatments of the dog with blind loop that corrected the fecal fat loss. Tetracycline administration or exclusion of the blind loop corrected the steatorrhea and eliminated hydroxy acids from the feces—both effects apparently resulting from elimination of bacterial overgrowth in the intestine. In contrast, sodium taurocholate feeding corrected the steatorrhea by a mechanism not involving antibacterial action (4) (namely, supplying conjugated bile acid, otherwise present in deficient amounts) and here, hydroxy acids persisted at a higher than normal percentage of the fecal fatty acids.

Hydroxy acids were found in significant amounts in jejunal fluid obtained from both normal dogs and animals with a blind loop and steatorrhea. In the latter group, these acids could be assumed to have arisen from the action of bacterial overgrowth resulting from the blind loop. In the normal animals, however, this finding is more difficult to explain. In view of the high concentrations of hydroxy acids in the jejunal fluid compared to that of the stool of these animals, it seems unlikely that hydroxy acids were produced from bacterial action in the upper intestine of these fasting animals. Another possibility is that these acids were synthesized by lower intestinal bacteria, absorbed via the portal system and excreted in the bile in an enterohepatic circulation. It is consistent with the hypothesis that, although both this and previous studies (11) indicate that hydroxy acids are absorbed

TABLE 5 COMPARISON OF PRECURSORS OF HYDROXY FATTY ACIDS IN IN VITRO INCUBATIONS OF FECES (5 HR AT 37°C)

Source of Feces	Isotope Added	Hydroxy Acid Methyl Esters		Unsubstituted Fatty Acid Methyl Esters	
		A*	An*	A*	An*
		% of total isotope in lipid extract of feces			
Blind loop dog	Acetate-1-^{14}C	19.2	19.1	80.8	80.1
	Oleate-1-^{14}C	4.0	3.5	96.0	96.5
	Linoleate-1-^{14}C	16.7	24.5	83.3	75.5
	Stearate-1-^{14}C	0.7	1.1	99.3	98.9
Patient with nontropical sprue	Acetate-1-^{14}C	12.9	13.7	87.1	86.3
	Oleate-1-^{14}C	5.6	11.6	94.4	88.4
	Linoleate-1-^{14}C	6.2	9.1	93.8	90.9
	Stearate-1-^{14}C	0.5	1.0	99.5	99.0
Control†	Oleate-1-^{14}C	0.3		99.7	
	Stearate-1-^{14}C	0.7		99.3	
	Linoleate-1-^{14}C	0.8		99.2	

* A, aerobic; An, anaerobic.
† Isotope extracted without incubation.

from the intestine, they are not seen in lipids of systemic plasma (2). Proof of an enterohepatic pathway for hydroxy acids depends, however, on their demonstration in the bile, an investigation not carried out in this study.

In the present study, oleic and linoleic acids were found to act as precursors of hydroxy stearate when tested both in incubations in vitro with feces from a patient and from a dog, both with steatorrhea, and when fed in vivo to a dog with a blind loop. Stearic acid was not significantly converted to hydroxy stearate in any of these systems. These findings, in contrast to those of Webb et al. (2), suggest that hydroxy acids are formed from the addition of H_2O to the Δ^9-double bond in unsaturated acids rather than by oxidation of the saturated acid.

This suggestion is in agreement with the report by Wallen, Benedict and Jackson (12) of the production of 10-hydroxy stearic acid from oleic acid by a pseudomonad. The similar rates of formation of hydroxy acids during aerobic and anaerobic incubations noted in our study as well as the localization of the hydroxy group to the 9- or 10-carbon of stearate (1) are further indications that hydration of unsaturated acid is involved in the formation of hydroxy acids. From these considerations it seems likely that oleate is converted to a hydroxy acid by the addition of water. The pathways for conversion of the other two substrates (linoleate and acetate) are less clear. The former could have been hydrogenated at one double bond and hydrated at the other. Acetate could become incorporated into hydroxy acids either by elongation of palmitoleate with addition of water to the double bond, or by acting as a substrate for oleate synthesis de novo with subsequent addition of water to this product. The reason for the differences between our findings and those of Webb et al. (2) concerning the relative effectiveness of saturated and unsaturated acids as precursors for hydroxy stearate is not clear.

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