Metabolism of straight saturated medium chain length (C9 to C12) dicarboxylic acids

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Abstract A method utilizing thin-layer chromatography, high performance liquid chromatography, and mass spectrometry was developed for the quantification of C9, C10, C11, and C12 dicarboxylic acids in serum, urine, and feces of human volunteers and rats after oral administration of the acids. The method allowed good resolution and measurement of the dicarboxylic acids at nanogram levels. In humans, excretion was independent of the dosage; about 60% of C9, 17% of C10, 5% of C11, and 1% of C12 were excreted in the urine during the first 12 hours after administration. The concentration of the acids in serum peaked between 2 and 3 hours. Excretion was also independent of dosage in rats. About 2.5% of C9, 2.1% of C10, 1.8% of C11, and 1.6% of C12 were excreted in the urine over a period of 5 days. The serum concentration and the urinary excretion of the diacids reached a maximum at the second day after the oral dose. In both humans and rats, the dicarboxylic acids found in serum and urine were 2, 4, or 6 carbon atoms shorter than the corresponding administered diacid. This indicates that there was beta-oxidation of the ingested diacids to some extent. The administration of [1,9-^{14}C]azelaic acid and of [10,11-^{3}H]dodecandioic acid confirmed the occurrence of beta-oxidation, and led to elucidation of the fate of the ingested diacids that were not excreted as such in the urine.—**Passi, S., M. Nazzaro-Porro, M. Picardo, G. Mingrone, and P. Fasella.** Metabolism of straight saturated medium chain length (C9 to C12) dicarboxylic acids. *J. Lipid Res.* 1983. **24:** 1140–1147.

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C9–C12 dicarboxylic acids are in vitro competitive inhibitors of tyrosinase (1) and they also exhibit an antimitochondrial activity (2). In vivo, C9 and C12 dicarboxylic acids are effective in the treatment of hyperpigmentation disorders such as melasma, toxic melanoderma (3), and lentigo maligna (4), and they have a cytotoxic effect on human malignant melanocytes (5). Recently, a beneficial effect on acne has also been reported (6). Normal cells are in no way affected by the diacids (4), and studies on experimental animals have shown that the C9 dicarboxylic acid is not toxic or teratogenic (7).

In view of a possible significant role of these diacids in the chemotherapy of human disorders, our attention has been focused on their metabolism, about which there is at present little information. Several authors have reported on the utilization of C6 to C10 dicarboxylic acids. Flaschentrager and Bernhard (8) recovered 61% of sebacic acid (C10), 60% of suberic acid (C8), and 30% of adipic acid (C6) from the urine of dogs injected with these acids. On the hypothesis that azelaic acid (C9) might be formed in vitro by the oxidation of naturally occurring unsaturated long chain fatty acids, Smith (9) investigated a possible in vivo formation of the diacid and its fate after oral administration to dogs. He recovered an average of 60% of the ingested azelaic acid in the urine; there was no excretion in the feces.

No data are available on the metabolism of straight medium chain length dicarboxylic acids in humans. Discrepancies exist concerning the presence of C6 to C10 diacids in the urine of normal individuals (10, 11); however, dicarboxylic acids have been found in the urine of patients with diseases such as ketosis (10), uremia (11), type I glycogen storage disease (12), and carnitine deficiency (13). Pettersen (14) advanced the hypothesis that in metabolic diseases, dicarboxylic acids might be derived from long monocarboxylic acids through an initial omega-oxidation followed by beta-oxidation.

The purpose of this work was to evaluate the serum levels and the urinary excretion of C9, C10, C11, and C12 dicarboxylic acids in rats and humans after their administration, and the fate of orally administered [1,9-^{14}C]azelaic acid and [10,11-^{3}H]dodecandioic acid in rats.

MATERIALS AND METHODS

Experiments with nonlabeled dicarboxylic acids

C9, C10, C11, and C12 dicarboxylic acids (99% pure) were purchased from Fluka, A. G.

Abbreviations: HPLC, high performance liquid chromatography, MS, mass spectrometry; TLC, thin-layer chromatography.

Administration

Humans. Four groups of healthy volunteers (three males and two females in each group, 60–75 kg, 30–45 years old) were studied. Subjects in each group were given a different dicarboxylic acid (C9, C10, C11, or C12) powder in gelatine capsules by oral administration, once a week for 5 weeks, at increasing dosages (0.5, 1.0, 2.0, 3.5, 5.0 g).

Rats. Four groups, each composed of 30 male Wistar rats (250–270 g, 7 weeks old) were used. Rats in each group were given, in a single dose, a different dicarboxylic acid in powder form by stomach intubation followed by water. Three different dosages were used: 10 rats in each group were given 20 mg; 10 rats, 50 mg; and 10 rats, 100 mg.

Sample collection

Human sera. Serum samples were collected each hour over a period of 10 hr after the administration of the diacids. Fifty μg of internal standard was added to 1 ml of each serum sample. The internal standard was a dicarboxylic acid with one more C-atom than in the administered acid (e.g., in humans given C9 dicarboxylic acid, the internal standard was the C10 dicarboxylic acid). Sera were acidified to pH 1 with 1 N HCl, saturated with NaCl, and extracted three times with 10 ml of warm (40°C) ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4. The extracted solutes were recovered after evaporation of the solvent under reduced pressure (below 40°C) in a rotatory evaporator.

Rat sera. Daily serum samples were collected and examined separately, as described above for human samples, for a period of 6 days after administration of the dicarboxylic acids.

Human feces. Samples of feces were separately collected over a period of 3 days. The feces were weighed, and an aliquot was diluted with water. Fifty μg of internal standard (as described for sera) was added to each sample. The mixture was acidified to pH 1 with 1 N HCl and extracted five times with 3 volumes of warm (40°C) ethyl acetate. The combined extracts were dried and the solvent was evaporated as described above.

Rat feces. Samples of feces were collected separately every day over a period of 6 days after administration of the diacid, and analyzed as described above for human samples.

Human urine. Urine was collected at each micturition during a period of 3 days. The volume of each urine sample was measured, and the pH (by a pH meter) and the presence of glucose and ketone bodies (Labstick, Ames Co., England) were noted. Fifty μg of internal standard, as described for sera, was added to an aliquot of urine (20–50 ml). The mixture was acidified to pH 1 with 1 N HCl and extracted five times with 3 volumes of warm (40°C) ethyl acetate. The combined extracts were dried and the solvent was evaporated as described above.

To detect the possible presence of dicarboxylic acids in conjugated form, a second aliquot of each sample was refluxed for 3 hr in 20 ml of 4 N HCl solution according to Gregersen, Lauritzen, and Rasmussen (15). The organic acids in the hydrolyzate were then extracted with warm ethyl acetate and examined as previously described.

Rat urine. Urine samples were collected from catheterized rats every day after dicarboxylic acid administration and analysed as described above for human samples.

Controls. Human and rat sera, feces, and urine samples collected before dicarboxylic acid administration were used as "blank controls".

Analysis of dicarboxylic acids by HPLC–MS

p-Bromophenacyldiesters of dicarboxylic acids were analyzed by a reverse phase HPLC–MS coupled system as follows.

Derivatization and HPLC. One to two mg of extracted solutes from urine, feces, and sera were dissolved in 1 ml of CH_3CN–MeOH 4:1 (v/v). Three mg of *p*-bromophenacylbromide dissolved in CH_3CN and 6 μl of the catalyst N,N-diisopropylethylamine were added. The mixture was heated to 50–60°C for 10–15 min. Under these conditions complete esterification of dicarboxylic acids to the strongly chromophoric *p*-bromophenacyldiesters was achieved.

After evaporation of some solvent, the derivatives were purified by TLC on standard thin-layer plates (Stratocrom SI-AP, Carlo Erba) coated with 0.25 mm of silica gel and activated by heating at 120°C for 20 min. The plates were developed in benzene–hexane 3:1 (v/v). The band corresponding to the dicarboxylic *p*-bromophenacyldiesters (at the origin) was scraped off and extracted three times with 1 ml of CH_3CN. The solvent was evaporated to a final volume of 0.5 ml. Aliquots (20–50 μl) were directly injected into a liquid chromatograph (1084 B, Hewlett-Packard) provided with an integrator and a scanning spectrophotometer in the 190–540 nm wavelength range.

The *p*-bromophenacyldiesters were separated on a reversed phase column (25 cm × 4 mm i.d.) RP 18, 5 μm (Brownlee Labs. St. Clara, CA) operated at 40°C and detection was at 255 nm. An initial isocratic elution (60% CH_3CN in water adjusted to pH 3.10 with H_3PO_4) for 5 min was followed by a gradient to 100% CH_3CN in 60 min. The flow rate was 1 ml/min; the sensitivity was 4×10^{-4} to 256×10^{-4} absorbance units/cm (AU/cm) (depending on the amount of the injected substances), and the chart speed was 0.25 cm/min.

Calibration. Each of the C5, C6, C7, C8, C9, C10, C11, C12 standard dicarboxylic acids (0.2–20 μg) in 1 ml of reaction mixture was esterified as described above. One tenth of the total (for each concentration) was injected into the column.

Recovery of standard dicarboxylic acids added to urine samples. Ten to one hundred μg of C5, C6, C7, C8, C9, C10, C11, C12 standard dicarboxylic acids was added to urine of untreated rats. Dicarboxylic acids were extracted, esterified, purified, and analyzed as described above.

Mass spectrometry. Verification of the identity of the *p*-bromophenacyldiesters collected by reversed phase HPLC was carried out by mass spectrometry. Spectra were obtained via direct probe in a low resolution LKB 2091/2130 mass spectrometer equipped with a digital PDP 11 calculation system. Experimental conditions were: direct inlet; temperature, 25–200°C; electron energy, 20 eV; accelerating potential, 3500 V; and ion source temperature, 250°C.

Experiments with labeled dicarboxylic acids

[1,9-^{14}C]Azelaic acid (sp act 13.3 mCi/mM) and [10,11-^{3}H]dodecandioic acid (sp act 20 mCi/mM, Radiochemical Center, Amersham, England) were administered by stomach intubation to 7-week-old Wistar rats (ca. 250 g). The rats were divided into two groups of 25 rats each: the first group was given 100 μCi of labeled azelaic acid (solution A); the second group received 100 μCi of labeled dodecandioic acid (solution B).

Animals were killed 1, 2, 6, 12, 72, and 96 hr after administration of labeled acid. Kidneys, liver, heart, pancreas, adipose tissue, brain, adrenals, and lungs were removed, washed with saline solution, blotted, and weighed. For quantification of ^{14}C and ^{3}H in the tissues, an accurately weighed portion of each organ was placed in a counting vial. Soluene 350 (Packard) (3 ml), and 30% H_2O_2 (0.5 ml) were added to solubilize the tissues. After 7 days incubation at 40°C, 10 ml of scintillation solution (Instagel, Packard) was added to each vial, and 5 days later the samples were counted. Quench correction was made by the external standard method (90% efficiency).

Feces and serum samples (50 μl) were analyzed as described for the tissues. Urine samples, collected by catheter, were counted directly in scintillation solution. Analysis of expired $^{14}CO_2$ after administration of [1,9-^{14}C]azelaic acid was performed as described by Dousset and Douste-Blazy (16).

Analysis of lipid extracts from tissues. Aliquots of tissues, accurately weighed, were extracted with $CHCl_3$–MeOH 2:1 (v/v). The extracts were filtered through sintered glass and dried over anhydrous Na_2SO_4. After evaporation of the solvent under nitrogen, a measured aliquot of each extract was fractionated by TLC according to the method of Passi et al. (17). Lipid fractions (except dicarboxylic acids) were visualized by iodine vapor. Dicarboxylic acids were visualized with bromcresol green solution (17).

To estimate the relative amount of radioactivity present in the different lipid classes, the areas of silica gel containing the individual stained spots were scraped off from the plate and placed in counting vials with 0.2 ml of water and 0.2 ml of 30% H_2O_2. Standard scintillation fluid was added and the samples were counted.

In other experiments, aliquots of lipid extracts were placed on thin-layer plates and developed as described above. Silica gel areas corresponding, respectively, to radioactive phospholipid and triglyceride fractions were scraped from the plates and extracted with peroxide-free diethylether. An aliquot was directly counted. Other aliquots of phospholipids and triglycerides were saponified for 3 hr with 2 M KOH in methanol and free fatty acids were obtained after acidification to pH 2–3 with HCl.

Analysis of free fatty acids. Free fatty acids were converted to *p*-bromophenacyldiesters, fractionated by reversed phase HPLC (17), individually collected, placed in counting vials in the presence of standard scintillation solution, and counted.

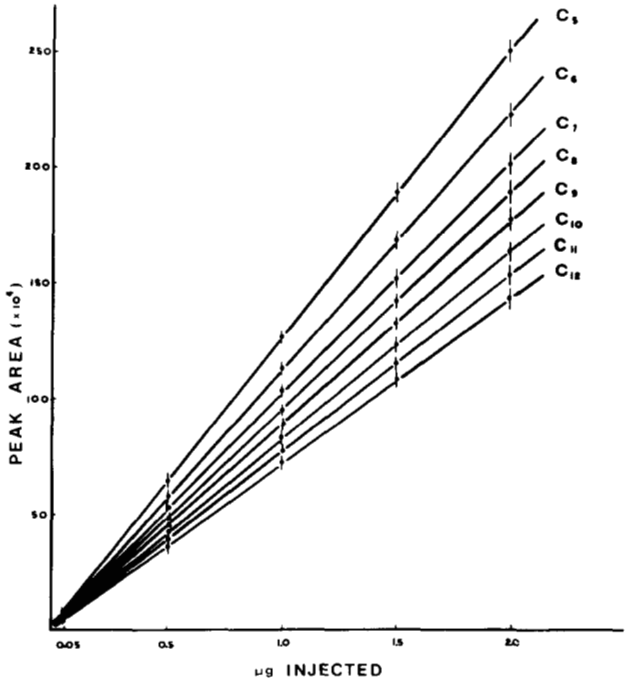

Fig. 1. UV (255 nm) detector response. Each point is the mean ± SD of five determinations carried out for each dicarboxylic acid as the *p*-bromophenacyl diester.

TABLE 1. Recovery of standard dicarboxylic acids from urine samples

Dicarboxylic Acid	Amount Added to Urine Sample	Amount Recovered
	μg	
C5	10	8.8 ± 3.5[a]
C6	10	9.0 ± 3.8
C7	10	9.3 ± 3.5
C8	10	9.3 ± 2.7
C9	10	9.4 ± 2.8
C9	50	46.8 ± 3.6
C9	100	93.7 ± 5.1
C10	10	9.3 ± 2.2
C10	50	46.2 ± 3.7
C10	100	93.1 ± 5.9
C11	10	9.4 ± 2.3
C11	50	47.0 ± 3.1
C11	100	93.8 ± 5.0
C12	10	9.3 ± 2.5
C12	50	45.9 ± 3.0
C12	100	92.8 ± 6.0

[a] Each value represents the average of five experiments ± SD.

Analysis of dicarboxylic acids. Dicarboxylic acids were extracted from urine, sera, feces, and tissues, and analyzed as described above. The diacids, separated as *p*-bromophenacyldiesters, were collected, placed in counting vials with scintillation solution, and counted.

RESULTS

Fig. 1 shows the linear detection response of seven *p*-bromophenacyldiesters of standard dicarboxylic acids in the 0.02 and 2.0 μg range. At the maximum detector sensitivity (1×10^{-4} AU/cm), 0.5 ng was the limit of detectability. The minimum detectable level corresponded to a response that was twice the noise level. The maximum absorption for these diesters was at 255 nm with $\log_{10} \epsilon$ values of about 4.7. It was ascertained that dicarboxylic acids could be quantitatively recovered from biological samples. Recovery of added dicarboxylic acids from urine is reported in **Table 1.**

Experiments with nonlabeled dicarboxylic acids

Control studies. C9, C10, C11, and C12 dicarboxylic acids were never detected in the urine, sera, and feces of normal nontreated humans and rats.

Urine. In humans, after administration of dicarboxylic acids, about 60% of C9, 17% of C10, 5% of C11, and 1% of C12 were excreted in the urine during the first 12 hr, independent of the dosage (**Table 2**). At the highest dosage (5.0 g), only a few milligrams of the diacids were present in the urine collected during the following 12 hr (Table 2).

In rats, urinary excretion was independent of the dose: about 2.5% of C9, 2.1% of C10, 1.8% of C11, and 1.6% of C12 dicarboxylic acids were excreted in the urine over a period of 5 days (**Table 3**).

Neither in humans nor in rats were dicarboxylic acids excreted in conjugated form. Urine pH, glucose, and ketone bodies remained unchanged. The only exception was that, in man, at the first micturition after administration of undecandioic and dodecandioic acids, the urine was alkaline with a pH of 7.4–8.5; it returned to a normal pH within 3–6 hr.

Serum. Human serum levels of C9–C12 dicarboxylic acids up to 7 hours after administration are reported in **Table 4. Table 5** shows the serum levels of the four diacids in rats up to 4 days after the oral dose. In sera and urine of both man and rat, diacid catabolites with chain lengths of 2-, 4-, or 6-carbon atom units shorter than that of the corresponding administered dicarboxylic acid were also detected. The amount of these diacid metabolites was not quantitatively measured.

Figs. 2A and **2B** show, respectively, the reversed phase HPLC separation of a mixture of standard synthetic dicarboxylic acids and of diacids present in the urine of a rat given dodecandioic acid (C12). It is evident that sebacic acid (C10), suberic acid (C8), and adipic acid (C6) are excreted in the urine together with dodecandioic acid. The identification of peaks corresponding to the different even or odd carbon dicarboxylic acids and their metabolites was confirmed by mass spec-

TABLE 2. Urinary excretion of orally administered C9, C10, C11, and C12 dicarboxylic acids in human subjects

	C9 mg Recovered after		C10 mg Recovered after		C11 mg Recovered after		C12 mg Recovered after	
Dose	12 hr	24 hr	12 hr	24 hr	12 hr	24 hr	12 hr	24 hr
g								
5.0[a]	3212 ± 417[b]	3225 ± 335	846 ± 51	846 ± 53	248 ± 54	250 ± 59	55 ± 25	57 ± 23
2.0	1178 ± 221	1178 ± 221	341 ± 29	314 ± 29	94 ± 21	94 ± 21	17 ± 11	17 ± 11
0.5	270 ± 43	270 ± 43	74 ± 23	74 ± 23	19 ± 7	19 ± 7	4 ± 3	4 ± 3

[a] Data corresponding to doses of 3.5 and 1 g, and to other times of examinations (see text) have not been tabulated.
[b] Each value represents the average of five experiments ± SD.

TABLE 3. Urinary excretion of orally administered C9, C10, C11, and C12 dicarboxylic acids in rats

Dicarboxylic Acid	Dose	mg Recovered after				
		24 hr	48 hr	72 hr	96 hr	120 hr
	mg					
C9	100	0.8 ± 0.6[a]	2.1 ± 0.8	2.4 ± 0.8	2.4 ± 0.4	2.5 ± 0.4
	50	0.5 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	1.2 ± 0.4	1.2 ± 0.4
	20	0.2 ± 0.3	0.4 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
C10	100	0.4 ± 0.4	1.6 ± 0.7	2.0 ± 0.7	2.0 ± 0.6	2.1 ± 0.6
	50	0.3 ± 0.3	0.8 ± 0.5	1.0 ± 0.5	1.0 ± 0.4	1.0 ± 0.4
	20	0.1 ± 0.2	0.4 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
C11	100	0.5 ± 0.4	1.4 ± 0.7	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
	50	0.2 ± 0.3	0.6 ± 0.5	0.9 ± 0.4	0.9 ± 0.4	0.9 ± 0.4
	20	tr	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
C12	100	0.4 ± 0.3	1.4 ± 0.9	1.6 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
	50	0.2 ± 0.3	0.6 ± 0.5	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
	20	tr	0.2 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3

[a] Each value represents the average of five experiments ± SD.

trometry with reference to authentic standard compounds. The significant ions for the identification of *p*-bromophenacyldiesters of dicarboxylic acids are reported in **Table 6.**

Feces. C9–C12 dicarboxylic acids were never detected in samples of feces from either human subjects or rats.

Experiments with labeled dicarboxylic acids

The aim of these experiments was to determine the fate and distribution of ^{14}C and ^{3}H after oral administration of 100 μCi of [1,9-^{14}C]azelaic acid (solution A), or 100 μCi of [10,11-^{3}H]dodecandioic acid (solution B).

Respiratory elimination of $^{14}CO_2$. After administration of solution A, about 13% and 14.5% of the ^{14}C appeared in the expired CO_2 at 12 and 48 hr, respectively.

Urine. In rats given solution A and solution B, there were recovered, respectively, 40% of the ^{14}C and about 50% of the ^{3}H from the urine collected over a period of 5 days (**Fig. 3**). Dicarboxylic acids, mainly represented by diacid metabolites (C7 and C5 in the case of C9 administration; C10, C8, and C6 when C12 was given), were found up to 72 hr after the oral dose.

Sera. ^{14}C and ^{3}H serum levels (dpm/ml) in rats given solution A and solution B are shown in **Fig. 4.** As in the urine, labeled dicarboxylic acids were present up to 72 hr, mainly represented by diacid metabolites.

TABLE 4. Serum levels of orally administered C9, C10, C11, and C12 dicarboxylic acids in human subjects

Dicarboxylic Acid	Dose	Time				
		1 hr	2 hr	3 hr	4 hr	7 hr
	g			*μg/ml*		
C9	5.0	66.1 ± 8.5[a]	74.6 ± 9.7	71.7 ± 9.6	49.8 ± 8.9	
	2.0	17.4 ± 5.8	26.0 ± 5.5	19.8 ± 4.8	4.4 ± 3.0	
	0.5	3.6 ± 2.0	6.0 ± 2.4	3.2 ± 2.1	1.1 ± 1.2	
C10	5.0	1.5 ± 0.3	10.9 ± 2.4	15.7 ± 2.9	11.6 ± 3.5	2.9 ± 1.0
	2.0	0.2 ± 0.2	4.0 ± 0.7	6.4 ± 2.1	6.0 ± 1.8	0.6 ± 0.4
	0.5	tr[b]	1.0 ± 0.3	1.4 ± 0.5	1.5 ± 0.6	tr
C11	5.0	0.3 ± 0.2	2.8 ± 0.5	4.6 ± 0.7	4.5 ± 0.8	0.7 ± 0.3
	2.0	0.1 ± 0.2	1.4 ± 0.3	1.7 ± 0.6	1.7 ± 0.5	0.2 ± 0.2
	0.5	tr	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	tr
C12	5.0	tr	0.6 ± 0.4	1.0 ± 0.4	0.9 ± 0.4	tr
	2.0		0.2 ± 0.2	0.7 ± 0.4	0.6 ± 0.4	
	0.5		0.1 ± 0.2	tr	tr	

[a] Each value represents the average of five experiments ± SD.
[b] tr, Traces (between 5 and 20 ng/ml).

TABLE 5. Serum levels of orally administered C9, C10, C11, and C12 dicarboxylic acids in rats

Dicarboxylic Acid	Dose	Time			
		24 hr	48 hr	72 hr	96 hr
	mg		*μg/ml*		
C9	100	6.2 ± 1.3[a]	7.8 ± 2.1	2.3 ± 1.5	tr[b]
	50	2.8 ± 1.1	3.6 ± 1.2	1.1 ± 1.0	tr
	20	0.9 ± 0.4	1.3 ± 0.5	0.3 ± 0.3	
C10	100	5.3 ± 1.2	6.3 ± 1.8	1.8 ± 1.1	tr
	50	2.6 ± 0.9	2.8 ± 1.3	0.8 ± 0.6	tr
	20	0.7 ± 0.5	1.2 ± 0.5	0.4 ± 0.3	
C11	100	4.8 ± 1.5	5.6 ± 2.2	1.4 ± 1.2	tr
	50	2.3 ± 1.1	2.7 ± 1.0	0.7 ± 0.8	tr
	20	0.7 ± 0.6	1.0 ± 0.6	0.3 ± 0.4	tr
C12	100	3.4 ± 1.2	4.8 ± 2.2	2.1 ± 1.4	tr
	50	1.6 ± 0.9	2.3 ± 1.1	0.6 ± 0.5	tr
	20	0.5 ± 0.4	0.8 ± 0.6	0.3 ± 0.3	

[a] Each value represents the average ± SD of five experiments.
[b] tr, Traces (between 5 and 20 ng/ml).

Tissues. Radioactivity was detected in all the tissues; the highest levels were found in liver, lungs, and kidneys 12 hr after administration of solution A ([^{14}C]azelaic acid) to rats, and 24 hr in rats given solution B ([^{3}H]dodecandioic acid). After these times, radioactivity levels slowly decreased in all the organs, with the exception of adipose tissue in which ^{14}C or ^{3}H levels increased up to the 96th hour and beyond.

Feces. In feces collected over a period of 6 days, less than 0.1% of ^{14}C was found in rats given solution A,

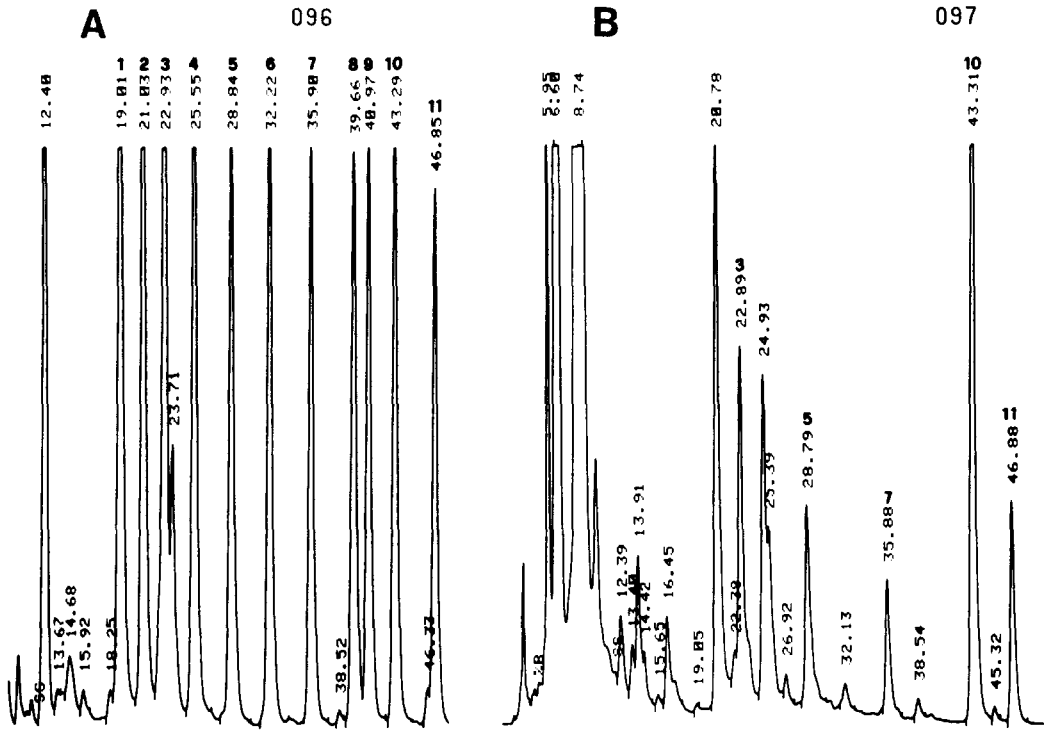

Fig. 2. Reverse phase HPLC separation of a mixture of standard dicarboxylic acids (A) and of dicarboxylic acids present in the urine from a rat given dodecandioic acid (B). Peak identifications: 1, C4; 2, C5; 3, C6; 4, C7; 5, C8; 6, C9; 7, C10; 8, C11; 9, C12:1 (traumatinic acid); 10, C12; 11, C13. In Fig. 2B, the identity of the peaks corresponding to dicarboxylic acids was confirmed by MS. Some unidentified peaks were observed but they were not investigated further.

TABLE 6. Significant ions (a, b) for the identification of *p*-bromophenacyldiesters of dicarboxylic acids

Dicarboxylic Acid	Molecular Weight	Ions	
		a[a]	b[b]
C5	526	97	311–313
C6	540	111	325–327
C7	554	125	339–341
C8	568	139	353–355
C9	582	153	367–369
C10	596	167	381–383
C11	610	181	395–397
C12	624	195	409–411

[a] Ion a corresponds to a fragment M^+-2(OCH_2-CO-Ph-Br).
[b] Ion b corresponds to a fragment M^+-(CH_2-CO-Ph-Br).
Fragments at m/e 155–157 (-PhBr) and at m/e 183–185 (-Co-Ph-Br) are common to all the *p*-bromophenacyldiesters of dicarboxylic acids.

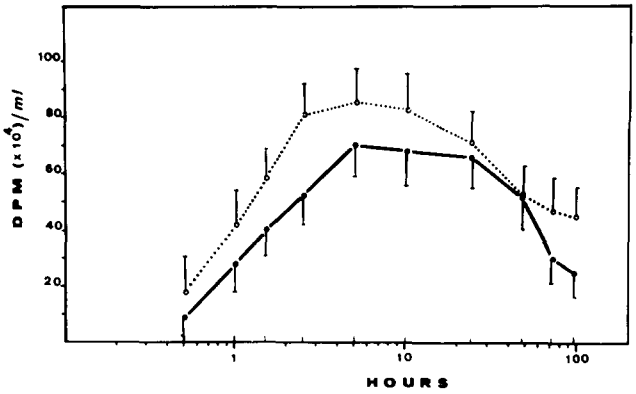

Fig. 4. ^{14}C and ^{3}H in serum of rats given [1,9-^{14}C]azelaic acid (● —— ●) and [10,11-^{3}H]dodecandioic acid (○ · · · ○).

and about 2% of ^{3}H in rats given solution B. The maximum radioactivity was detected between the 3rd and the 4th day. No traces of labeled diacids were found.

Tissue lipids. In rats given solution A, about 90% of total organ radioactivity was present in lipids, and only low levels were found in the residual matter (proteins, carbohydrates, etc). Radiocarbon was essentially localized in the fatty acid portion of the triglycerides and of the phospholipids (where it was homogeneously distributed in the different saturated and unsaturated fatty acids), and in cholesterol. Traces of labeled C9, C7, and C5 dicarboxylic acids were detected in the first 24 hr after the oral dose.

In rats given solution B, on the other hand, the radioactivity levels were lower in the lipid extracts of organs (20–40%) than in the residual matter. In the phospholipid and triglyceride fractions, ^{3}H was distributed in the whole molecule and not only in the fatty acid portion. However, radioactivity was detected in all the fatty acids, both saturated and unsaturated. Traces of C12, C10, C8, and C6 dicarboxylic acids were detected in the first 24 hr after administration.

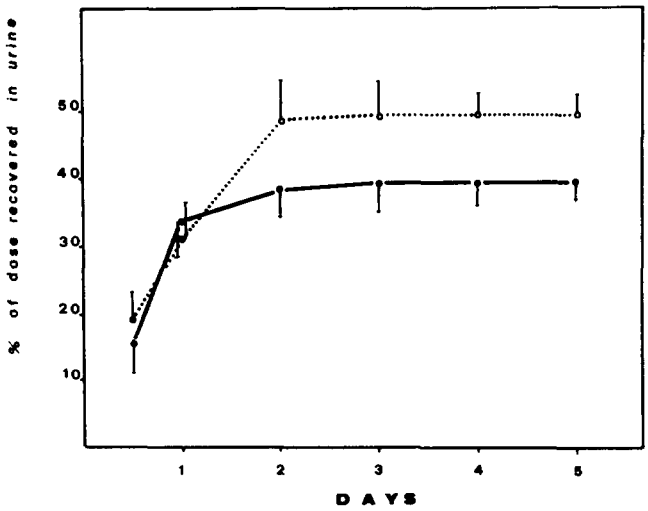

Fig. 3. Urinary excretion of administered [1,9-^{14}C]azelaic acid (● —— ●) and [10,11-^{3}H]dodecandioic acid (○ · · · ○) in rats.

DISCUSSION

The present investigation, carried out by using a very sensitive method that allows measurement of dicarboxylic acids at nanogram levels, shows that ingested C9 to C12 dicarboxylic acids are at least partly metabolized via beta-oxidation. It also established the fate of metabolites of administered odd and even medium length straight chain dicarboxylic acids that were not excreted as such.

It should be first emphasized that in normal non-treated humans or animals the diacids were never detected in the serum, urine, or feces, and that, following their administration, they were never found in the feces.

After administration of the four diacids to human subjects, their concentration in serum, urinary excretion, and effect on pH of the urine varied according to the chain length of each diacid. This was probably in relation to the fact that the solubility of the diacids in water is inversely proportional to chain length. In fact, we found that the higher the number of carbon atoms, the higher the pH of the urine, and the lower the serum level and urinary excretion.

Independent of the dosage, about 60% of C9, 17% of C10, 5% of C11, and 1% of C12 were excreted in the urine during the first 12 hr (Table 2). At the highest dose (5.0 g) only a few milligrams were detected in the urine during the following 12 hr. Concentrations of the four diacids in human serum peaked between 2 and 3 hr after administration; after 8 hr, however, the serum levels were negligible.

In rats, the chain length of the dicarboxylic acids had only a slight influence on serum levels, urinary excretion, or urinary pH, and the urinary elimination was lower and more prolonged than in man. In fact, after an oral dose, about 2.5% of C9, 2.1% of C10, 1.8% of C11, and 1.6% of C12 were excreted in the urine over a period of 5 days, and either serum levels or urinary excretion reached a maximum at the 2nd day (Tables 3 and 5). Further studies would be necessary to explain the difference between humans and rats.

At least three hypotheses should be considered for explaining the fate of the diacids not recovered in the urine: the dicarboxylic acids could be metabolized; they could be conjugated and excreted as such in the urine (as reported by Gregersen et al. (15) in patients with dicarboxylic aciduria); or both. Our results support the first hypothesis; C9 to C12 dicarboxylic acid conjugates were never found in the urine of either human subjects or animals. Moreover, dicarboxylic acids with chain lengths of 2, 4, or 6 carbon atom units shorter than that of the corresponding administered diacid were detected in serum and urine, thus indicating that beta-oxidation of the ingested diacids was involved to some extent.

The administration to rats of [1,9-^{14}C]azelaic acid and of [10,11-^{3}H]dodecandioic acid confirmed beta-oxidation, and elucidated the fate of the ingested diacids that were not excreted as such in the urine. In fact, the complete beta-oxidation of one molecule of azelaic acid should produce one molecule of [^{14}C]malonyl CoA and three molecules of acetyl CoA (one of which is labeled); the latter enters the Krebs cycle giving rise, among other products, to $^{14}CO_2$. The ^{14}C (14.5%) recovered from expired air within 48 hr could be partly formed by this pathway, possibly in addition to an omega-decarboxylation, as suggested by Dousset and Douste-Blazy (16). Moreover, malonyl CoA is the immediate precursor of the 2-carbon atom units in fatty acid biosynthesis. Accordingly, ^{14}C was recovered in lipid extracts of tissues, and especially in the fatty acid portion of triglycerides and phospholipids. Here it was distributed in all the fatty acids, both saturated and unsaturated, in relation to the percentage distribution of each fatty acid. Relatively high levels of radioactivity were also present in cholesterol, indicating that labeled intermediates were used in cholesterol biosynthesis.

The cleavage of one molecule of [10,11-^{3}H]dodecandioic acid can produce labeled succinyl CoA or acetyl CoA, which enter the Krebs cycle and other metabolic pathways; this could explain the general distribution of ^{3}H in all the fluids and tissues that were examined.

The present findings, demonstrating beta-oxidation of straight medium chain length dicarboxylic acids, might account for the previous reported absence of acute and chronic toxicity of azelaic acid administered to animals (7).

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