Metabolism of *Ortho*-Alkylbenzenealkanoic Acids in Rats[1]

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Abstract

Aromatic fatty acids with 18 C-atoms, as isolated from cyclized linseed oil, were labeled with ^3H in the aromatic ring by isotopic exchange. Doses of 50 mg/kg body weight were almost quantitatively absorbed by rats and excretion of metabolites via the urine was rapid (up to 95% within 30 hr). The retention curve consisted of two branches, which indicates that part of the aromatic acids are taken up into the lipid pool. This was confirmed by analysis of body lipids. The metabolic pathways involved in the degradation of the aromatic fatty acids are β-oxidation, ω-oxidation and $(\omega\text{-}1)$-oxidation.

Introduction

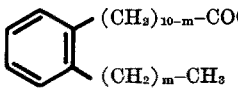
Cyclic compounds may be formed when oils containing polyunsaturated fatty acids are treated with either alkali or hydrogenation catalysts at elevated temperatures (1,2). Part of these compounds consists of *o*-alkylbenzenealkanoic acids (aromatic fatty acids, AFA), the biological action of which has been investigated, e.g., by Gottenbos and Thomasson (3). They found that these compounds are metabolized in rats and mice and excreted in the urine.

Here we describe the study of the rate of excretion and identity of excreted products.

Experimental Procedures

Aromatic Acids

Two samples of aromatic acids were used (as their methyl esters) in the labeling and metabolic experiments, viz., pure methyl 9-(*o*-propylphenyl)nonanoate (2), and a mixture of methyl esters of C_{18}-AFA from cyclized linseed oil, containing at least seven different compounds of the general formula as was shown by



$(0 \leqslant m \leqslant 6)$

gas liquid chromatography (GLC) (Fig. 1) and mass spectrometry. The latter technique was applied to samples of the individual peaks, separated by programmed-pressure GLC. The spectra obtained were in accordance with those of AFA methyl esters from heated methyl linoleate or linseed oil methyl esters, published by Michael (4) and by Scharmann et al. (5).

Labeling Procedure

The AFA methyl esters, dissolved in cyclohexane, were tritiated by isotopic exchange, using $^3H_3PO_4 \cdot BF_3$ as a catalyst and tritium source [Yavorsky and Gorin (6)]. The weight ratio of aromatic ester and tritiating agent was 1:2. After stirring the reaction mixure for 24 hr at room temperature, the acid complex was decomposed by adding distilled water, and the esters were purified by chromatography on a column of Kieselgel G/Hyflo Supercel (1:1 w/w), using light petroleum-diethyl ether (19:1 v/v) as the eluant. Chemical and radiochemical purity was determined by TLC on 0.25 mm plates of Kieselgel G with the same eluant and scanning of the developed plates.

The weight recovery in the tritiation of both AFA-mixture and model-(propyl-) AFA was 90%. The overall-recovery of radioactivity in the purified products was 12%, whereas the specific activities obtained were: 2.46 Ci/mole for the mixture and 3.78 Ci/mole for the model compound, i.e., 49.9% and 56.0% respectively, of the theoretical equilibrium value (6). As can be seen from Figure 2, all components possess the same specific activity. The position of the label was determined by oxidative degradation. To this end 431 mg inactive AFA mixture was added to 0.7 mg of the labeled product, the diluted material having a specific activity of 4.19 m Ci/mole. After saponification the mixture was oxidized with aqueous $KMnO_4$. Phthalic acid was isolated and recrystallized to constant mp (206–207 C, decomp.). Its specific activity (4.26 m Ci/mole) shows tritium to be incorporated exclusively in the aromatic ring.

Feeding Experiments

The feeding experiments were conducted with 7 male Wistar rats weighing 275–400 g, 4 of them having been adapted (3) during 40 weeks to AFA (daily intake after adaptation 50 mg AFA/day) 3 of them being non-adapted. After overnight-fasting, the animals were given single doses of 15–20 mg labelled AFA, dissolved in a tenfold amount by weight of soyabean oil. After administration (by pipette) the animals were fasted during 8 hr, after which they were given their customary diets.

Determination of Excretion and Retention

The urines were collected in 1–2 hr fractions, the feces separately on metal gauzes. After making up the urines to standard volume, the excreted radioactivity was measured by liquid scintillation counting of aliquots. The feces were dried, extracted with chloroform and aliquots of the extracts counted in the same way.

Four of the animals were killed 30 hr after administration and 3 after 75 hr. Liver and kidneys were removed, homogenized separately and extracted according to Folch. Carcass lipids were recovered by mincing the frozen carcasses and subsequent Folch-extraction of the thawed material. The lipids from livers and kidneys were not separated further; the carcass lipids were fractionated according to Quinlin and Weiser (7) into tri-, di- and monoglycerides and more polar material. Radioactivity measurements were made of all fractions obtained. The carcass lipid fractions were interesterified to methyl esters which were subsequently separated into normal fatty acid esters and AFA methyl esters. This was accomplished by urea crystallization (2) followed by TLC of the nonadduct for the tri- and diglycerides and thin layer chromatography (TLC) only for the smaller fractions.

Extraction of the Urine Samples

The urine samples containing the bulk of the recovered radioactivity were pooled and the pH adjusted to 2 with concentrated HCl. Subsequently, after being

[1] Presented in part at the Ninth ISF-Congress, Rotterdam, September 1968.

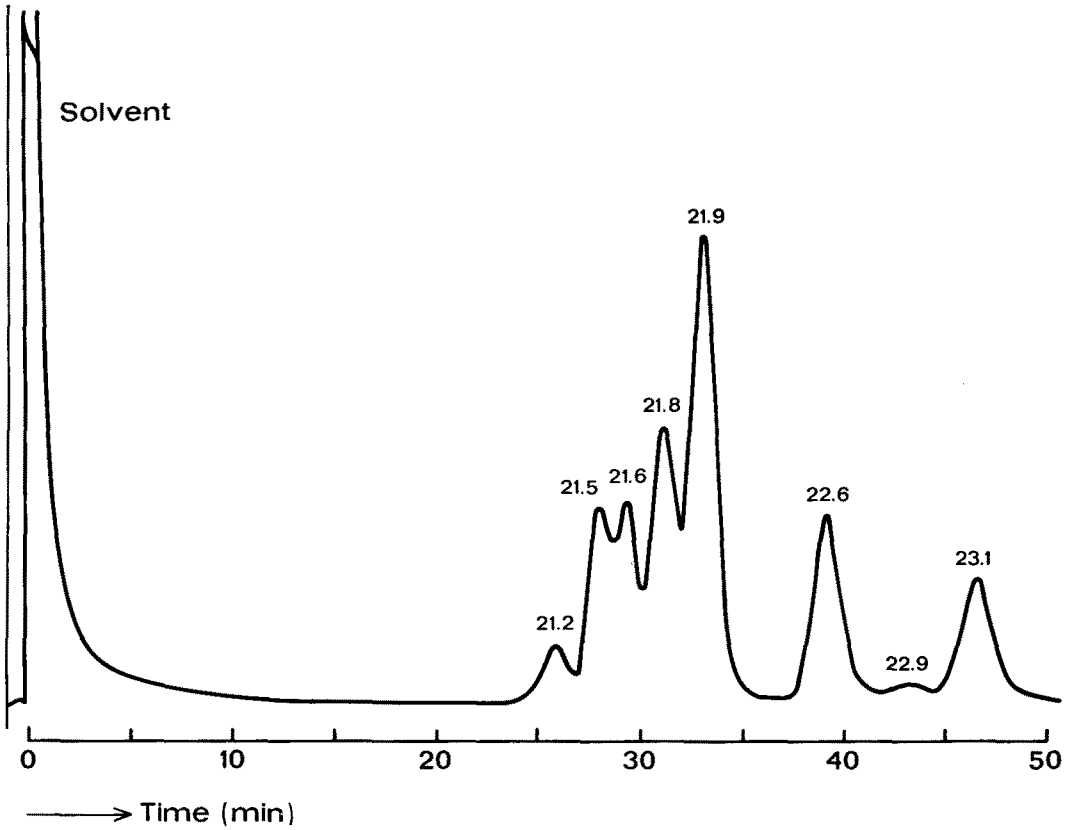


FIG. 1. Gas chromatogram of aromatic fatty acids (methyl esters) from cyclized linseed oil. Conditions: 5% PEGA on Diatoport S (80–100 mesh), column 0.4 × 200 cm, temperature 190 C, detection by flame ionization detector. Compounds are designated by their equivalent chain length (C-number): $C_{23.1}$-methyl 11-(*o*-methylphenyl)undecanoate; $C_{22.6}$-methyl 10-(*o*-ethylphenyl)decanoate; $C_{21.8}$-methyl 9-(*o*-propylphenyl)nonanoate; $C_{21.8}$-methyl 8-(*o*-butylphenyl)octanoate; $C_{21.6}$-methyl 7-(*o*-pentyl phenyl)heptanoate; $C_{21.8}$-methyl 6-(*o*-hexylphenyl)hexanoate and 5-(*o*-heptylphenyl)pentanoate. Peaks $C_{22.9}$ and $C_{21.2}$ could not be identified. In the latter, a small amount of methyl 4-(*o*-octylphenyl)butanoate may have been present.

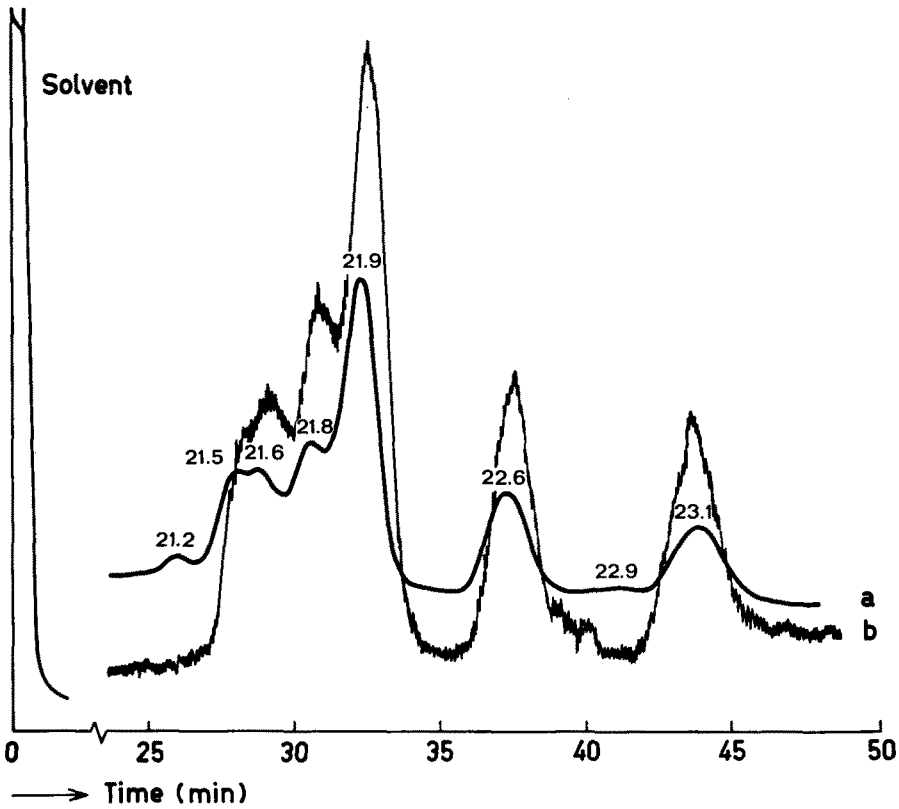


FIG. 2. Radio gas chromatogram of [^{3}H]-aromatic fatty acids (methyl esters). (a) Mass signal, (b) Radioactivity signal. Conditions as in Figure 1. Mass detection by thermal conductivity cell. Radioactivity detection by Nuclear Chicago high-temperature proportional gas counter, 2500 V, 275 C, range 3 × 10^4 counts/min. Peaks indicated as in Figure 1.

refluxed for 1 hr and cooled, they were extracted with distilled ethyl acetate. In this way 87–93% of the radioactivity was recovered. The greater part of the solvent was evaporated in vacuo and the concentrates treated with ethereal diazomethane for conversion into methyl esters.

Isolation of Individual Metabolites

The techniques applied were: TLC on Kieselgel G, using eluants of varying polarity, for separation into classes, and preparative gas chromatography for the main peak(s) from each TLC-band. The compounds were trapped on glass beads (diameter: 250–300 μm) in cooled glass tubes plugged in the outlet of the gas chromatograph.

Spectroscopic Analysis of Metabolites

Mass spectra of the isolated compounds were taken on an AEI MS 9 mass spectrometer, usually operating at a resolving power of 1500. In some cases accurate mass measurements were done by the peak-matching method at a resolving power of ca. 17000, whereas from two compounds a complete high-resolution spectrum was taken. These were recorded on an Ampex FR 1300 analogue tape recorder. Digitization and computation of the element maps was done on an IBM 1800 computer.

Proton magnetic resonance spectra were run on a Varian A 60 analytical spectrometer operating at 60 MHz. The substances were dissolved in CCl_4, and the measurements taken at a temperature of 40 ± 2 C. The hydroxy compounds were run as the trimethylsilyl ethers. Tetramethylsilane was used as internal reference. In a few cases the weight of sample available was insufficient to obtain a spectrum from a single scan in which case the spectrum-accumulation technique was applied. This was achieved by using a Northern Scientific NS 544 computer of average transients (CAT) in conjunction with a NS 304 NMR field synchronizer coupled to the A 60 spectrometer.

Infrared spectroscopy was applied on microfilms of the liquid esters between sodium chloride plates. The instrument used was a Grubb Parsons Spectromaster, provided with a mirror type microilluminator.

Ultraviolet spectra of two compounds (solvent-ethanol) were taken on a Unicam SP 500 spectrophotometer.

Radioactivity Measurements

For radioactivity measurements a Packard Tri-Carb Model 314 E liquid scintillation counter was used. Depending on the nature of the samples to be counted, one of the following scintillator solutions was used: (a) 5 g PPO (2,4-diphenyl-1,3-oxazole) and 0.1 g dimethyl-POPOP [1,4-bis{-2'-(4-methyl-5-phenyl-1,3-oxazolyl)}-benzene] in 1 liter analytical grade toluene

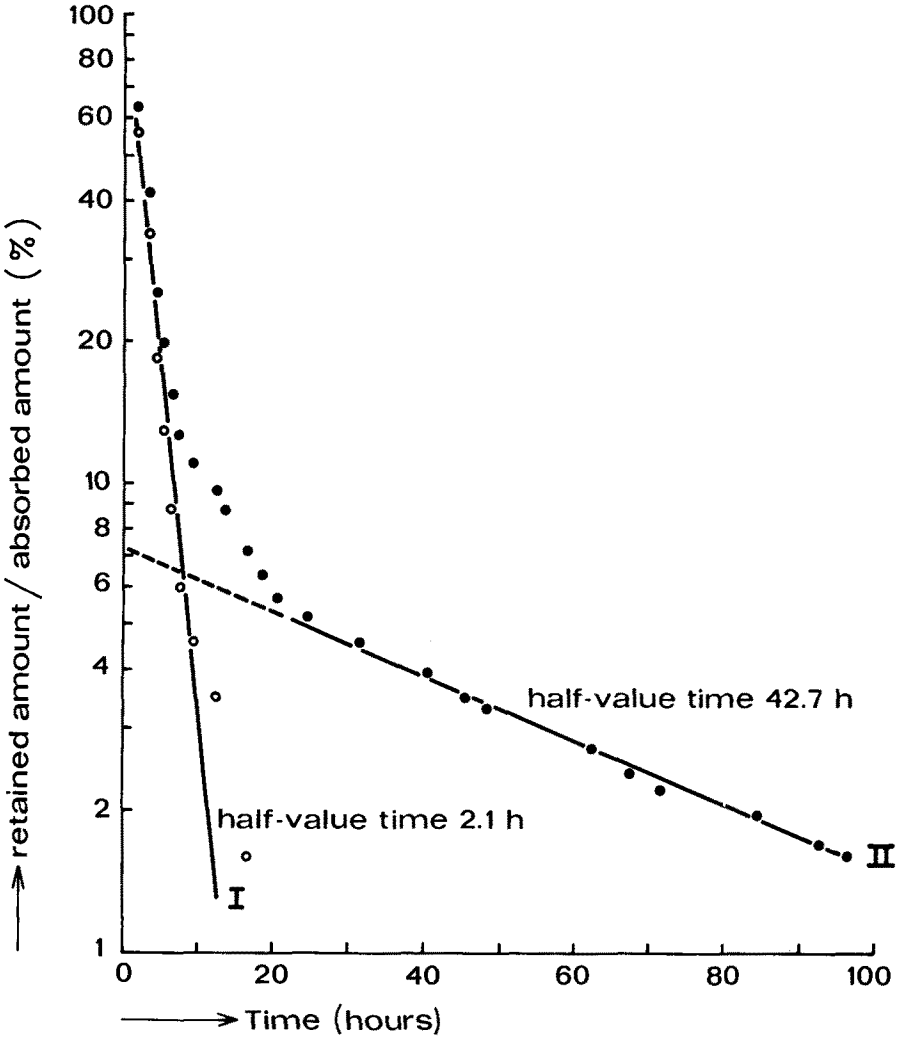


FIG. 3. Retained radioactivity in adapted rat after administration of 50 mg [^{3}H]-aromatic fatty acid (methyl esters) per kilogram body weight. The second part of the original retention curve (●) was extrapolated to zero time, yielding curve II. Curve I (○) was obtained by subtracting the extrapolated curve from the values in the first part of the original curve.

TABLE I
Half-value Times (hr) of Retention of Radioactivity in the Various Experiments of Different Duration[a]

Animal	$t_{1/2}$ I	$t_{1/2}$ II
Adapted	2.1	42.7
Adapted	2.1	25
Nonadapted	2.9	33
Adapted	3.1	.....
Nonadapted	3.1	.....
Nonadapted	3.4	.....
Adapted	3.5	.....

[a] See caption of Figure 3 for further explanation.

(toluene scintillator); (b) 12 g PPO, 0.6 g dimethyl-POPOP and 60 g analytical naphthalene in a mixture of 1 liter analytical grade 1,4-dioxan and 200 ml freshly distilled water-free 2-methoxyethanol (dioxan scintillator).

To determine absolute radioactivities, standard [^{3}H] toluene (Packard) was used as internal standard.

Results

Retention of AFA in the Body

From the radioactivity measurements of feces and urine samples it was concluded that almost quantitative absorption had occurred, since in all cases only 2–4% of the administered dose was recovered in the feces of the first 6 hr of the experiment. So all calculations were based upon the absorbed amount. The retained amount was calculated (in per cent of absorbed amount) by subtracting the excreted amount from the absorbed amount. The semilogarithmic plot of the values obtained resulted in retention curves, one of which is represented in Figure 3. The values of $t_{1/2}$ found in the various experiments of different duration are given in Table I.

The variations in $t_{1/2}$ are probably due to individual differences between the animals. In all cases, however, 24 hr after administration 10% or less of the absorbed dose had been retained. In this respect no differences were found between adapted and nonadapted animals.

Distribution of AFA Retained in the Body

The recovered radioactivity of urine, feces, organ lipids and carcass lipids from an adapted and a nonadapted animal is given in Table II.

Further differentiation as to the distribution of radioactivity in the various classes of carcass lipids can be read in Table III.

The various fractions were analyzed as described before. The ultimately obtained fractions of methyl esters not forming adducts with urea, having R_f-values in TLC corresponding to those of AFA methyl esters, had retention times in GLC equal to those of unchanged AFA methyl esters. So it was concluded that AFA after ingestion is partly incorporated into all types of body lipids.

Identity of Products in the Urine

Some of the urine samples were freeze-dried and the distillates were counted for radioactivity. No radioactivity was found, so isotopic exchange had not occurred in the organism and the aromatic ring is metabolically stable. Thus, radioactivity in the urine must be due to aromatic fatty acids or their metabolites.

The extracted compounds, after conversion into methyl esters (as described under methods), were analyzed by GLC, which yielded complex gas chromatograms, an example of which is given in Figure 4.

The separation into classes, performed by TLC of the methyl esters as described before, is represented

TABLE II
Radioactivity of Urine, Feces, Organ and Carcass Lipids, and Washing Liquids From an Adapted and a Non-adapted Animal, Both Killed 30 hr After Administration of the Labeled Aromatic Fatty Acids

	Adapted animal		Nonadapted animal	
	μ Ci	% of dose	μ Ci	% of dose
Dose	82.1	100	81.5	100
Recovery				
Urine	73.1	89.0	76.1	93.4
Feces	0.289	0.36	0.361	0.45
Liver lipids	0.165	0.20	0.351	0.43
Kidney lipids	0.006	0.01	0.024	0.03
Carcass lipids	3.11	3.8	5.28	6.5
Washing liquids	2.54	3.1	1.16	1.4
Total	79.2	96.5	83.3	102.2

by the radiochromatograms in Figures 5 and 6. Since the radioactivity in band IIIa was very low and the band after spraying with Ultraphor only vaguely visible in UV light, this band was discarded. The distribution of radioactivity in the different bands (Table IV) was calculated from the peak areas in the radiochromatograms; the recovery of radioactivity from the scraped-off bands varied between 90% and 65%, depending on the polarity of the compounds.

The reproducibility of the distribution of the metabolites over the different bands is satisfactory considering the limited number of animals used in the experiments. Moreover, only minor differences are found between adapted and nonadapted animals. The increase of activity in band I from adapted animals at the cost of band IIIc is most evident.

The distribution of the individual peaks over the bands was determined by gas chromatography (Table V).

Individual compounds were isolated by preparative GLC of bands I, II, IIIb and IIIc. The compounds from band IIId were not trapped separately because of their high retention times. The retention of some compounds from band IIIb and IIIc decreased after treatment with the trimethyl-silylating reagent described by Wood et al. (8) indicating that OH-groups were present. Moreover, IR spectroscopy showed that all the compounds contained two common structure elements, namely an aromatic ring (bands at 752–758, 1486–1493, 3015–3021 and 3060–3064 cm^{-1}) and a methoxycarbonyl group (bands at 1155–1172, 1196–1205, 1244–1263, 1260–1369, 1439–1440 and 1740–1744 cm^{-1}). In Table VI the structures are listed of the individual compounds, the assignment of which was based upon the following specific spectroscopic data:

1. MS: Parent-peak = m/e 178, corresponding to $C_{11}H_{14}O_2$; β-fragmentation of both side chains gives strong peaks at m/e 163 ($P-CH_3$) and m/e 119 ($P-COOCH_3$); α-fragmentation of ester chain gives m/e 105. Absence of P–60 also indicates only one CH_2 group in ester chain.

2. MS: Parent-peak = m/e 178 (as in compound 1); m/e 163 ($P-CH_3$) is very weak; β-fragmentation of ester chain gives m/e 105 ($P-CH_2-COOCH_3$) while also α-fragmentation occurs, giving m/e 91; m/e 118 (P–60) is very intense.

TABLE III
Weight and Radioactivity Distribution of Carcass Lipids From an Adapted and a Nonadapted Animal

Fraction	Adapted animal		Nonadapted animal	
	Weight, %	Radioactivity, %	Weight, %	Radioactivity, %
Triglycerides + FFA	77.4	53.0	81.3	61.7
Diglycerides	17.2	37.9	12.1	24.2
Monoglycerides	1.1	4.8	2.4	5.4
Polar material	4.3	4.2	4.1	8.7

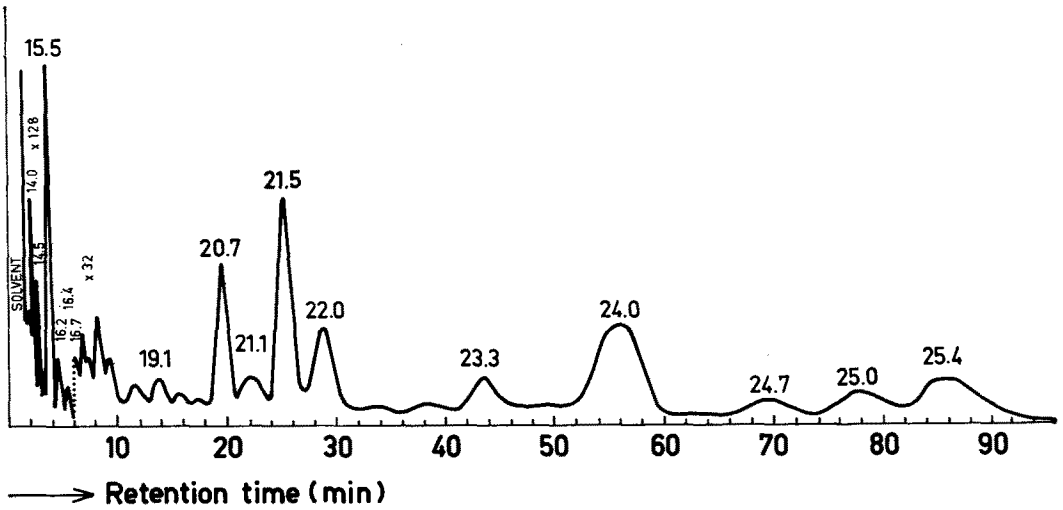


FIG. 4. Gas chromatogram of metabolites from urine of adapted rat, fed [^{3}H]-aromatic fatty acid (methyl esters). Conditions: 5% PEGA on Diatoport S (80–100 mesh), column 0.4 × 200 cm, temperature 188 C, carrier gas N_2 (60 ml/min).

3. MS: Parent-peak = m/e 206, corresponding to $C_{13}H_{18}O_2$. Length of side chain is given by m/e 177 (P–C_2H_5) and m/e 163 (P–C_3H_7), β- and α-fragmentation respectively. Fragmentation of ester chain gives m/e 132 (P–74) and m/e 119 (P–87).

4. Not identified.

5. MS: Parent-peak = m/e 204, corresponding to $C_{13}H_{16}O_2$. Compared with the mass spectrum of No. 3, this compound shows a more intense P–32, while m/e 117 (in compound 3) is shifted to m/e 115. This suggests an unsaturated ester.

UV: The UV spectrum showed an extinction maximum at 275 nm [λ_{max} = 278 nm for methyl cinnamate (3-phenylpropenoate)].

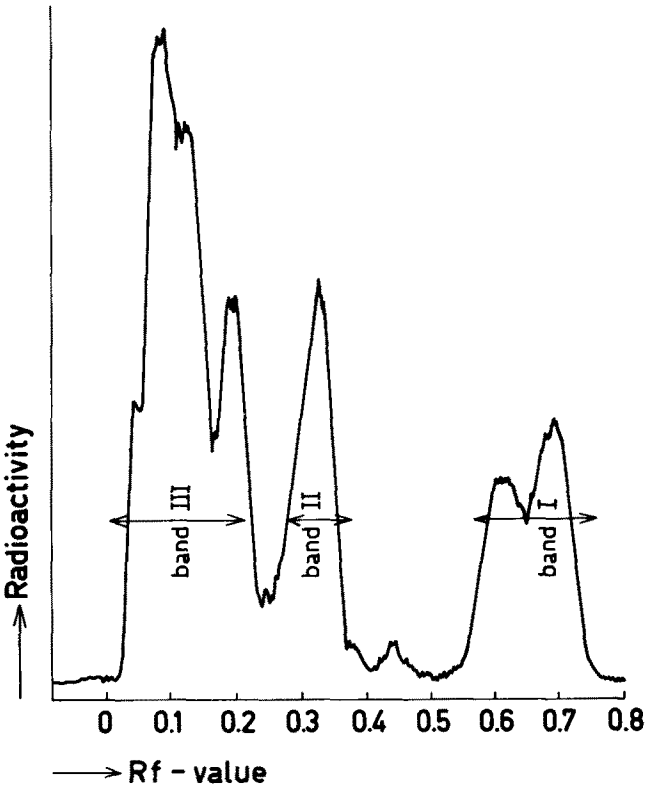


FIG. 5. Radioactivity scan of TLC of [^{3}H]-aromatic fatty acid metabolites (methyl esters) of urine of adapted rat. Radioactivity detection by Berthold thin layer scanner, 3100 V. time constant 40 sec, range 2.4 × 10^3 counts/min. Plate 20 × 20 cm, Kieselgel G, 1 mm thickness. Developing solvent: light petroleum-diethyl ether (80:20 v/v).

6. MS: Parent-peak = m/e 204 (as in 5). General fragmentation pattern is very similar to No. 5.

UV: λ_{max} = 247 nm while styrene has λ_{max} at 244–248 nm.

IR: A peak at 964 cm^{-1} indicates a *trans* double-bond (C–H out of plane bending), not conjugated with the ester, but possibly conjugated with the aromatic nucleus.

7. MS: Parent-peak = m/e 250, corresponding to $C_{14}H_{18}O_4$ (mass measurement). Significant for two methyl ester groups are m/e 187 [P–(31 + 32)] and m/e 186 (P–2 × 32).

PMR: The aromatic protons of 7 I and 7 II were indicated by two singlets at δ = 7.06 and 7.10 ppm, respectively, the methyl ester protons by a singlet at δ = 3.61 ppm, whereas a singlet at δ = 3.57 ppm indicated the $\emptyset$–CH_2–$COOCH_3$, as was found by comparison of the spectrum with that of dimethyl *o*-phenylenediacetate. The remainder of the complex methylene part of the spectrum lay in the region δ = 1.5–3.1 ppm. From the integrated signals, it could be calculated that the mixture consisted of 60% I and 40% II.

IR: Strong ester absorption, indicative of more than one ester group.

8. MS: Parent-peak = m/e 278, corresponding to $C_{16}H_{22}O_4$ (mass measurement). This again indicates two ester groups. Additional evidence is given by m/e 215 [P–(31 + 32)], m/e 214 (P–2 × 32), m/e 131 [P–(73 + 74)] and m/e 117 [P–(74 + 87)]. The remaining six methylene groups are divided between the two side-chains in either a 3:3 or a 2:4 ratio.

PMR: Two singlets were observed, namely at δ = 7.03 ppm (four aromatic protons) and at δ = 3.60 ppm (two CH_3-ester groups), whereas the methylene region (δ = 1.4–3.1 ppm) was complex, but could be integrated as six methylene groups. No distinctions

TABLE IV

Distribution of Radioactivity in TLC bands[a]

Band	Adapted animals				Nonadapted animals		
	AFA mixture			Model AFA	AFA mixture		Model AFA
I	30	27	25	53	16	20	22
II	27	24	18	9	22	20	12
IIIb	11	13	15	6	12	12	9
IIIc	15	17	20	25	26	25	40
IIId	17	19	22	7	24	23	17

[a] Per cent of total activity applied.

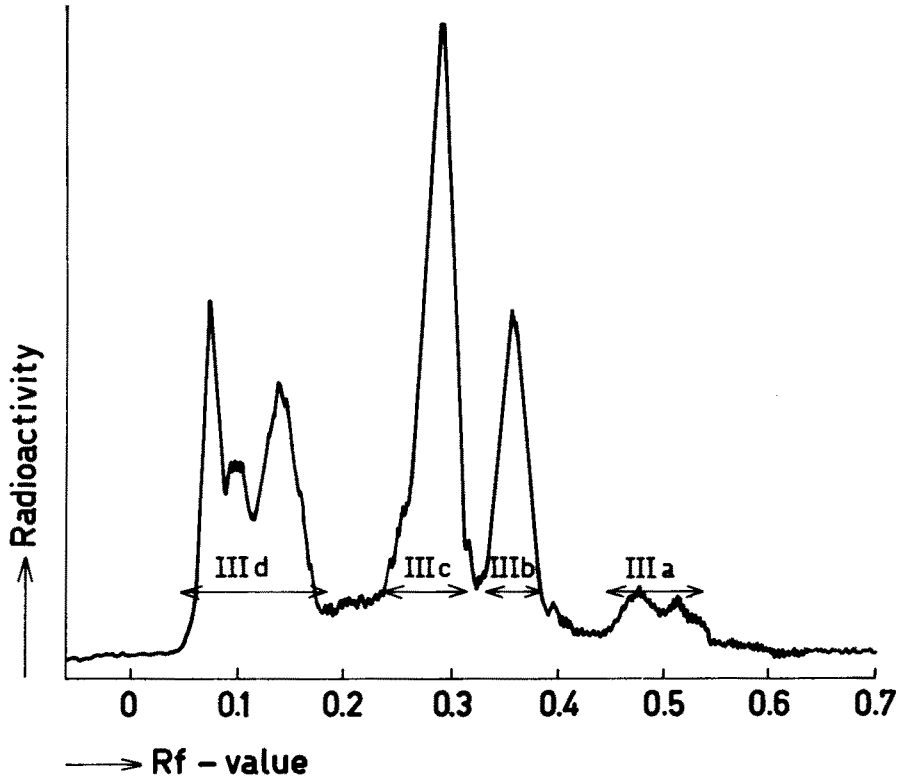


FIG. 6. Radioactivity scan of TLC (rechromatography) of band III (cf. Fig. 5). Radioactivity detection as in Figure 5, range 1.2 × 10^3 counts/min. Plate 20 × 20 cm, Kieselgel G, 1 mm thickness. Developing solvent: light petroleum-diethyl ether (50:50 v/v).

could be made as to the presence of structures I and/or II.

IR: As in compound 7.

9. MS: This compound was run as the TMS ether. Last peak in the spectrum was m/e 279. Mass measurement gave $C_{15}H_{22}O_3Si$. This must be the P–CH_3 (common in TMS ethers). Thus, the molecular formula is $C_{16}H_{26}O_3Si$, indicating one OH group in the original compound. Significant peaks are m/e 265 (P–C_2H_5), m/e 251 (P–C_3H_7), m/e 176 and m/e 193; m/e 176 was shown to be $C_{11}H_{12}O_2$ by mass measurement, and could be formed by a rearrangement reaction equivalent to the elimination of water and ethylene, occurring in longer chain alcohols; in this case giving *ortho* $CH_2=CH$–Ø–CH_2–$COOCH_3$ + $CH_2=CH_2$ + $(Me)_3SiOH$. m/e 193 = $C_{11}H_{17}OSi$ (mass measurement) can be explained by β-fragmentation of the ester chain, followed by expulsion of propylene, by rearrangement of one hydrogen atom to the aromatic nucleus.

10. MS: Parent-peak is m/e 222 = $C_{13}H_{18}O_3$ (mass measurement). Relevant peaks are m/e 103 = $C_4H_7O_3$ (mass measurement). OH group in the ester chain, m/e 120, fragmentation of the ester chain with rearrangement of the hydroxylic hydrogen on the β-carbon atom to the aromatic nucleus, and m/e 221 = $C_{13}H_{21}OSi$ (mass measurement) in the TMS ether derivative, formed by splitting off $-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$.

PMR: The main features of the structure are given by the PMR spectrum. The absorptions found in the spectrum were:

δ = 6.97 ppm, singlet : aromatic protons
δ = 4.19 ppm, quintet : $-CH_2-CHOH-CH_2-$
δ = 3.58 ppm, singlet : methyl ester protons
δ = 2.70 ppm, doublet, J = 6.5 Hz : Ø–CH_2–CH<
δ = 2.64 ppm, quartet, J = 7.4 Hz : Ø–CH_2–CH_3
δ = 2.38 ppm, doublet, J = 6.0 Hz : –CH–CH_2–$COOCH_3$
δ = 1.20 ppm, triplet, J = 7.4 Hz : Ø–CH_2–CH_3

Combination of the PMR and MS data led to the structure given in Table VI.

11. MS: Parent-peak is m/e 220 = $C_{13}H_{16}O_3$. The element-map is very similar to that of compound 13. Significant differences are: the molecular formula contains two hydrogen atoms less than No. 13, and this compound also gives a characteristic peak at m/e 178 ($C_{11}H_{14}O_2$), which means a fragmentation of C_2H_2O.

From the rest of the spectrum it follows that the main part of the structure is the same as that of No. 13, viz. ortho $-CH_2$–Ø–CH_2–CH_2–$COOCH_3$, which leaves C_2H_3O to complete the molecule. There are three possibilities: (a) –CO–CH_3, (b) $-C(OH=CH_2$, and (c) $-CH_2$–CHO. The mass spectrum seems to be in favour of (b), while from chemical evidence it is more likely that (a) has been formed in the reaction. A possible explanation could be a change of the equilibrium between (a) and (b) induced by electron impact.

12. MS: Run as TMS ether. Molecular formula (mass measurement of P–CH_3) must be $C_{16}H_{26}O_3Si$. Characteristic peaks are m/e 275 (P–C_2H_5) and

TABLE V
Distribution of GLC-peaks Over TLC Bands[a]

Band	C-number of peaks					
I	14.0	14.5	15.5	16.2	16.4	16.6
II	22.0	24.0				
IIIb	19.1	20.7	21.1	21.5	22.0	23.2
IIIc	21.5	22.0				
IIId	24.7	25.0	25.4			

[a] C-numbers are those from Figure 4.

TABLE VI

Identification of Aromatic Fatty Acid Metabolites (Methyl Esters)[a]

Band	No.	C-number[b]	Most probable structure:  a	b
I	1	14.0	$-CH_2COOCH_3$	$-CH_2CH_3$
	2	14.5	$-(CH_2)_2COOCH_3$	$-CH_3$
	3[c]	15.5	$-(CH_2)_2COOCH_3$	$-(CH_2)_2CH_3$
	4	16.2	mixture; unsaturation in side-chain(s)	
	5	16.4	$-CH=CHCOOCH_3$	$-(CH_2)_2CH_3$
	6	16.6	$-CH=CHCH_2COOCH_3$	$-CH_2CH_3$
II	7 I		$-(CH_2)_3COOCH_3$	$-CH_2COOCH_3$
	7 II[c]	22.0	$-(CH_2)_2COOCH_3$	$-(CH_2)_2COOCH_3$
	8 I[c]		$-(CH_2)_4COOCH_3$	$-(CH_2)_2COOCH_3$
	8 II	24.0	$-(CH_2)_3COOCH_3$	$-(CH_2)_3COOCH_3$
IIIb	9	19.1[i]	$-CH_2COOCH_3$	$-CHOH(CH_2)_2CH_3$
	10	20.7[ii]	$-CH_2CHOHCH_2COOCH_3$	$-CH_2CH_3$
	11[c]	21.1	$-(CH_2)_2COOCH_3$	$-CH_2COCH_3$
	12[c]	21.5[iii]	$-(CH_2)_2COOCH_3$	$-CHOHCH_2CH_3$
IIIc	13[c]	21.5[iv]	$-(CH_2)_2COOCH_3$	$-CH_2CHOHCH_3$

[a] The spectroscopic analyses were made on inactive metabolites from urine of rats fed cyclized linseed oil. So, the metabolites originated from a mixture of AFA.
[b] Δ of C-number after trimethylsilylation = 0, except i: −5.7; ii: −4.8; iii: −6.0; iv: 4.9.
[c] Compounds present in the urine of animals fed AFA mixture as well as in those of animals fed model (propyl) AFA.

m/e 207 (P–CH_2CH_2–C(=O)–OCH_3).

PMR: Insufficient sample of this fraction was available to obtain a spectrum on the A-60 spectrometer that could be fully interpreted. However, the accumulated CAT spectrum (256 sweeps) gives information about some fragments present. Positive indications are found for: aromatic protons: δ = 7.0 ppm, singlet, and methyl ester protons: δ = 3.6 ppm, singlet. An absorption at δ = 4.7 ppm is probably from Ø–C*H*OH–.

13. MS: Molecular formula is $C_{13}H_{18}O_3$ (elementmap). Position of –OH group is indicated by m/e 146 ($C_{10}H_{10}O$) which is formed by fragmentation of the ester methoxy group followed by loss of –CHOC–CH_3, and m/e 178 ($C_{10}H_{14}O_2$) formed by a rearrangement reaction characteristic for the β-hydroxy group, as in No. 10.

Discussion

The results show that C_{18} aromatic fatty acids are absorbed almost quantitatively. Specific accumulation in the liver does not occur, whereas nonspecific incorporation of unchanged aromatic acids into the various classes of body lipids can be demonstrated.

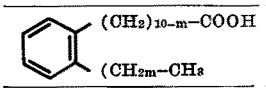
The presence of two components in the retention-time curve (Fig. 3) may be explained by assuming two pathways for the excretion, namely: blood → liver (breakdown) → blood → urine (rapid, $t_{1/2}$ I) and: blood → lipid, pool → liver (breakdown) → blood → urine (slow, $t_{1/2}$ II).

The excretion rate was the same in both adapted and nonadapted animals, probably because of the small amounts of AFA used. Administration of a larger single dose or successive doses might result in a higher excretion rate in adapted rats than in nonadapted ones, the former having a greater enzyme capacity in respect to the attack of AFA (3).

As to the metabolites from the urine, the compounds 1–3 in band I can easily be explained by assuming normal β-oxidation of the carboxyalkyl chain of the parent aromatic acids. In respect to these compounds it is interesting to compare our results with those of Bernhard and Viret (9), who administered 3-(*o*-methylphenyl)-propionic acid to dogs. They found that this acid was not degraded but was excreted via the urine, either as such or as the glycine conjugate. On the contrary, the *meta*- and *para*-isomers were excreted as *meta*- and *para*-methyl benzoic acid respectively. Obviously β-oxidation is sterically inhibited by the alkyl group in the *ortho*-position. Our observa-

TABLE VII

Metabolic Pathways of Aromatic Fatty Acids[a]

Starting compound in mixture 		Mixture of end-products 		C-number	Activity, %		Type of oxidation
					Mixture	Starting compound	
m	%	a	b				
0	11	$-(CH_2)_2COOH$	$-CH_3$	14.5	6.3	≧57	β
		HPNP[a]		.....	4.7	≧43	?
1	14	$-CH_2COOH$	$-CH_2CH_3$	14.0	33	≧23	
		$-CH_2CHOHCH_2COOH$	$-CH_2CH_3$	20.7	5.7	41	β
		HPNP		.....	5.0	≧36	?
2	33	$-(CH_2)_2COOH$	$-(CH_2)_2CH_3$	15.5	8.8	26.5	β
		$-(CH_2)_2COOH$	$-(CH_2)_2COOH$	22.0	1.7	5.0	ω
		$-(CH_2)_4COOH$	$-(CH_2)_2COOH$	24.0	1.0	3.1	
		$-(CH_2)_2COOH$	$-CH_2COCH_3$	21.1	2.3	6.9	ω-1
		$-(CH_2)_2COOH$	$-CH_2CHOHCH_3$	21.5	13.5	40.5	
		HPNP		.....	5.6	17.0	?
3–6	42	$-CH_2COOH$	$-(CH_2)_3COOH$	22.0	1.2	2.8	
		$-(CH_2)_3COOH$	$-(CH_2)_3COOH$				ω
		$-(CH_2)_4COOH$	$-(CH_2)_2COOH$	24.0	17.9	42.6	
		Non-identified[b]		21.5 23.3	16.1	38.2	?
		Non-identified[b] HPNP		.....	7.0	16.6	

[a] HPNP, highly polar, nonidentified products.
[b] Most probably mixtures, may also be intermediates in (ω-1)-oxidation in view of TLC and GLC behavior. Arbitrarily ascribed to metabolites of AFA with longer alkyl chains only.

tion that only the compounds with *o*-methyl-, *o*-ethyl- and *o*-propyl side-chains are excreted in this manner is striking. The unsaturated compounds No. 4–6 are only present in minor amounts. In some experiments they were completely absent and thus it cannot be ruled out that they are artifacts (dehydration of OH intermediates as occurring in band III).

The occurrence of considerable amounts of ω,ω'-dicarboxylic acids in band II indicates that the organism resorts to ω-oxidation in order to enhance polarity, thus promoting urinary excretion of part of the aromatic acids. Particularly the AFA with alkyl chains longer than propyl are disposed of in this way, as is indicated by the absence of compounds with alkyl substituents beyond C_3 in band I and the much lower contribution of ω-oxidation in the experiments with the model AFA (Table IV). The propyl compound appears to be a borderline case in this respect. As is mentioned in Table VI, the two main peaks observed in GLC of band II were probably mixtures, as was confirmed quantitatively for compound 7 by PMR-spectroscopy. This would also be in agreement with the observation that propyl AFA contributes to both peaks. Most of the components in bands IIIb and IIIc are hydroxy acids. The major components of these bands were isolated and tentatively identified. Compounds 11 and 13 (Table VI) were shown, by mild oxidation ($KMnO_4$ in $MgSO_4$-solution) of the latter, to be related. The oxidation product, obtained in low yield, had the same retention time on GLC as compound 11. The fact that both the oxidation product and the isolated compound 11 gave a positive reaction with 2,4-dinitrophenylhydrazine, combined with the MS data, led to the most probable structure given in Table VI. Thus it was concluded that, at least in the metabolism of the model AFA, a major metabolic pathway is formed by (ω-1)-oxidation. This type of breakdown was reported earlier by Preiss and Bloch (10) who studied the oxidation of higher fatty acids in rat liver, and by Tulloch et al. (11) who investigated the fermentation of long chain compounds by the yeast *Torulopsis magnoliae*. Moreover, Den (12) isolated 2,2-dimethyl-7-keto- and -7-hydroxyoctanoic acid from urine of rats fed 2,2-dimethyloctanoic acid.

Other interesting compounds are No. 9 and 12 which, however, were present only in minor amounts in one of the urine samples. These hydroxy acids are indicative of the occurrence of an unusual oxidation, α to the phenyl group. Elliott and Hanam (13) found a comparable pathway in the metabolism of 1,2,3,4-tetrahydronaphthalene in rabbits: they found twice as much 1,2,3,4-tetrahydro-1-naphthol as the corresponding 2-naphthol in the urine. The even more polar components of band IIId, which were not isolated, may comprise dihydroxy compounds and other substances of similar polarity.

To calculate the relative contribution of the individual compounds to the radioactivity of the various bands, use was made of the radioactivity tracks in radio-GLC. In this way the influence of the composition of the diet is excluded.

Combining the results of radio-GLC of the products from the model compound experiments with those from the spectroscopic analyses and using the above-mentioned distribution of isomers in the labeled AFA-mixture, it was possible to devise a scheme for the metabolic pathways followed by the various fatty acids (Table VII). In this way, a fairly good impression can be obtained of the relative contribution of β-, ω- and (ω-1)-oxidation in the metabolism of the different components of the AFA-mixture. It appears that these aromatic fatty acids are an interesting substrate for studying the various metabolic pathways.

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REFERENCES

1. Floyd, D. E., R. F. Paschke, D. H. Wheeler and W. S. Baldwin, JAOCS *23*, 609–614 (1956).
2. Coenen, J. W. E., Th. Wieske, R. S. Cross and Miss H. Rinke, Ibid. *44*, 344–349 (1967).
3. Gottenbos, J. J., and H. J. Thomasson, Nutr. Dieta *7*, 110–129 (1965).
4. Michael, W. R., Lipids *1*, 359–368 (1966).
5. Scharmann, H., W. R. Eckert and A. Zeman, Fette, Seifen, Anstrichm. *71*, 118–121 (1969).
6. Yavorsky, P. M., and E. Gorin, J. Amer. Chem. Soc. *84*, 1071–1072 (1962).
7. Quinlin, P., and H. J. Weiser, Jr., JAOCS *35*, 325–327 (1958).
8. Wood, R. D., P. K. Raju and R. Reiser, Ibid. *42*, 81–85, 161–165 (1965).
9. Bernhard, K., and R. Viret, Z. Physiol. Chem. *310*, 37–43 (1958).
10. Preiss, B., and K. Bloch, J. Biol. Chem. *239*, 85–88 (1964).
11. Tulloch, A. P., J. F. T. Spencer and P. A. J. Gorin, Can. J. Chem. *40*, 1326–1338 (1962).
12. Den, H., Biochim. Biophys. Acta *98*, 462–469 (1965).
13. Elliott, T. H., and J. Hanam, Biochem. J. *108*, 551–559 (1968).

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