Journal of Chromatography, 488 (1989) 301–313
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4581

COMBINED CHROMATOGRAPHIC–ISOTOPIC DILUTION ANALYSIS OF FECAPENTAENES IN HUMAN FECES

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(First received August 18th, 1988; revised manuscript received November 8th, 1988)

SUMMARY

Fecapentaene-12 (FP-12) and fecapentaene-14 (FP-14) are genotoxic unsaturated ether lipids produced by colonic bacteria in man. We have developed and applied to feces collections from normal volunteers direct isotopic dilution procedures using tritium-labeled (at C_5) FP-12 and FP-14 for measuring these compounds. FPs were recovered from feces by solvent extraction, silica cartridge clean-up, and analytical liquid chromatography. Low levels of FP-12 and FP-14 (<0.1 to 2.4 μg/g of freeze-dried feces) were observed. Identity of chromatographic peaks was established by co-elution and by ultraviolet absorption spectra obtained via photodiode array scanning. Two unknown peaks were tentatively identified from absorption spectra as closely related compounds with increased (hexaene?) or decreased (tetraene?) number of double bonds. Levels of FPs increased after incubation of feces at 37°C for 96 h under anaerobic conditions and pre-FP-12 and pre-FP-14 peaks were observed, which showed identical spectra with authentic FPs. These were interpreted to be isomeric forms of the all-*trans*-[^{3}H]FPs used for the isotopic dilution analysis. Total FPs (including pre-FP) yielded a range of 0.3–80 μg FP-12 and 2.8–44 μg FP-14 per g of freeze-dried feces from the study group.

INTRODUCTION

In recent years, the combined efforts of analytical and organic chemists with bacteriologists and genetic toxicologists have disclosed a unique class of lipid direct-acting mutagens present in human feces that may play a role in the etiology of human colon cancer [1–6]. Isolated from diethyl ether extracts of human feces after extensive work-up were the two compounds, fecapentaene-12 (FP-12) and fecapentaene-14 (FP-14). Their structures are shown in Fig. 1 along with several synthetic derivatives. The natural compounds are apparently a mix of isomeric forms with the all-*trans*-pentaene isomers predominant. Studies of the direct mutagenic activities of various synthetic analogues [7–10] have yielded conclusions

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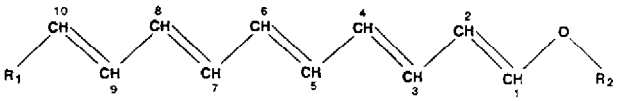


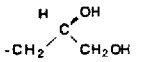
Fecapentaene	R_1	R_2
FP-12	$-CH_2CH_3$	
FP-14	$-CH_2CH_2CH_2CH_3$	SAME
cis-FP-12	$-CH_2CH_3$	SAME
MFP-12	$-CH_2CH_3$	$-CH_3$
MFP-14	$-CH_2CH_2CH_2CH_3$	$-CH_3$

Fig. 1. Structures of synthetic fecapentaenes. The *cis*-FP-12 has the *cis*-configuration at C-1 = C-2. In the [^{3}H]FPs, tritium is at C-5

that the pentaene moiety is essential for mutagenic activity and that the ether linkage between the pentaene and glycerol could also be at C-2 instead of C-1 of the glycerol portion. More recently, we [11] found that the mono-*cis*-isomer of FP-12 was as active as FP-12 (mostly all-*trans*-isomer) and that the methoxy analogues of FP-12 and FP-14 (Fig. 1) were substantially more active than the parent FPs in the Ames/*Salmonella* [11] and the mouse lymphoma [12] assays for mutagenesis. Thus, the essential structural feature for mutagenicity is the pentaene-conjugated system. The FPs are biosynthesized from unknown precursors by resident anaerobic bacteria of the genus *Bacteroides* in the human intestinal tract [13–15]. The only animal species thus far reported to excrete FPs in the feces is the minipig [16,17].

Other workers have established the genotoxic potency of FP-12 in a number of in vitro [18–20] and in vivo [21,22] test systems. However, a preliminary first report [23] of tests of rectally instilled FP-12 in B6C3F1 male mice and F344/NCr male rats indicated that FP-12 was not a strong carcinogen for the colon in these rodents. Thus, the majority of short-term tests for genotoxicity of FP-12 have been clearly positive, but the crucial tests of possible carcinogenicity in rodents are negative or incomplete.

Prior to the initiation of our work, the only extensive study of FPs in human feces [14] reported that normal individuals excrete varying amounts of the two FPs, ranging from all FP-12 to all FP-14. The total of FP-12 and FP-14 in twelve subjects varied from 0.3 to 3.5 μg/g of freeze-dried feces. Anaerobic incubation of samples of these fecal collections at 37 °C for 96 h increased the total FP content from two- to nineteen-fold in the various individuals. Thus, not only did individuals vary extensively in the FPs present in expelled feces, but conditions simulating further residence time of the feces in the body caused, in some cases, ex-

tensive increases in the amounts of FPs, suggesting extensive differences in the amounts of endogenous precursors of these compounds.

To study the extent of formation and excretion of FPs by human subjects and the influence of dietary variations thereon, a routine, reliable assay procedure that is less time-consuming than existing methods is required. To fill this need, we approached the assay development by employing direct isotopic dilution techniques using ^{3}H-labeled FPs wherein clean-up procedures can be employed without regard to quantitative recovery so long as sufficient amounts of the target analytes in uncontaminated form are recovered for an accurate assessment of amount of diluted compound and its isotopic content. The mixture of exogenous [^{3}H]FPs and endogenous FPs was recovered by extraction, silica gel cartridge clean-up, and analytical liquid chromatography (LC). This paper describes our first results employing these techniques. Preliminary reports were presented earlier at national meetings [24,25].

EXPERIMENTAL

Reagents and materials

Silica gel extraction cartridges (1 g) fitted with 25-ml reservoirs containing a polyethylene frit and adapter were obtained from Analytichem International (Harbor City, CA, U.S.A.). HPLC-grade methanol, hexane, and methylene chloride were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) or Mallinkrodt (Paris, KY, U.S.A.). Liquid scintillation fluid (Safety Count) for direct radiochemical quantitation was obtained from Research Products International (Mount Prospect, IL, U.S.A.) and flow-through counting fluid (Scintiverse-LC) was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals were reagent-grade quality. FP-12, FP-14, *cis*-FP-12, methoxy-FP-12 (MFP-12) and methoxy-FP-14 (MFP-14) were synthesized in our laboratories [26] as were tritiated FP-12 (34 Ci/mol) and FP-14 (42 Ci/mol) [27]; ^{3}H was at C-5 (Fig. 1). All glassware was silylated using the vapor-phase method [28].

Feces collection and storage

Feces from human volunteers was collected in polypropylene bags, immediately frozen in dry ice, and stored at $-70°C$. Prior to sampling, the feces collection was pulverized while frozen in the bags using a hammer. These studies were reviewed and approved by the SRI Human Subjects Committee.

Treatment and extraction of feces

Frozen feces (6 g) was placed in 50-ml screw-cap culture tubes and 5 ml of an anaerobic incubation media were added [29]. After thorough mixing by vortexing, the tube was gassed with argon, capped, and incubated at 37°C for 96 h. A spike of 1 μg each of [^{3}H]FP-12 and [^{3}H]FP-14 was then added in 380 μl of methanol containing 0.01% butylated hydroxytoluene (BHT) and 1% triethylamine (TEA), and the contents were mixed thoroughly and freeze-dried overnight. A non-incubated fecal sample containing the media and mixed [^{3}H]FP

spike was processed in the same manner[a]. To the tube containing the freeze-dried residue were added 10 ml of hexane. The tube was vortexed vigorously for 1 min and then centrifuged 5 min at 1000 *g*. The hexane was discarded. An 8-ml freshly prepared solution of 0.01% BHT in diethyl ether was added and the sample vortexed vigorously for 1 min and centrifuged as above. Five of these extractions were made and the ether extracts were combined and evaporated to dryness under argon. The residue was dissolved in 10 ml of diethyl ether containing 0.01% BHT and 1% TEA. After centrifugation, the supernatant was added to the silica gel cartridge preconditioned with 10 ml of the above ether–BHT–TEA solution. All cartridge additions and elutions were performed employing solutions containing 0.01% BHT and 1% TEA on a vacuum manifold (51 to 76 mmHg) (Alltech Assoc., Deerfield, IL, U.S.A.). The sample effluent was collected and the cartridge sequentially washed with two 10-ml aliquots of hexane–methylene chloride (1:1, v/v) followed by two 10-ml aliquots of methylene chloride. FPs were eluted with 10 ml of 5% methanol in the ether–BHT–TEA mixture and this fraction was evaporated to dryness under argon[b]. The residue was dissolved in 1 ml of 0.01% BHT and 1% TEA in methanol, which was transferred to a 1.5-ml polypropylene microcentrifuge tube and evaporated to dryness under argon. For LC, the final residue was triturated with 250 μl of the previous methanol solution, and the mixture was centrifuged for 2 min (12 000 *g*, Model 5412 Microfuge, Brinkmann Instruments, Westbury, NY, U.S.A.).

LC for isotopic dilution

Separation of FPs was accomplished at 30 °C on a 150 mm × 4.6 mm I.D. Chemcopak 5-ODS-H column (DyChrom, Sunnyvale, CA, U.S.A.) with a 20 mm × 2 mm I.D. precolumn packed with Perisorb RP-18 (Upchurch Scientific, Oak Harbor, WA, U.S.A.), using a Model 1084B liquid chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.). A stepwise gradient was employed using 0.01 *M* sodium phosphate, pH 7.0, as solvent A and acetonitrile–methanol (4:1, v/v) containing 0.01% BHT as solvent B at a flow-rate of 1.0 ml/min. The mobile phase was initially 50% A–50% B, pumped for 22 min. The composition was increased to 65% B for 13 min and to 80% B for 15 min to wash the column. The column effluent from the UV detector (340 nm) of the LC system was passed through a 500-μl flow cell of a Flo-One Model HP radiochemical detector (Radiomatic Instruments and Chemicals, Tampa, FL, U.S.A.) using a scintillation fluid flow-rate of 3.0 ml/min. In the Results and discussion section, these assays are referred to as quantitative runs.

The specific activity of [^{3}H]FP-12 or [^{3}H]FP-14 was defined as the counts per minute (cpm) as detected by the flow-through counter per UV area unit (AU) determined by the UV detector. Purity of all preparations of both [^{3}H]FPs and their respective specific activities in terms of cpm/AU was determined prior to

[a]Some earlier fecal samples (Table I) were processed as water slurries directly without the addition of media or incubation.

[b]^{3}H-content of all extracts and cartridge effluents was determined by static counting using a Searle Mark III counter (Searle Analytic, Des Plaines, IL, U.S.A.).

adding the spike to each sample. Individual quantitation of each FP in the sample was calculated using the formula shown in Table I.

LC for spectral identification

FP spectra were obtained using a Model 1040M photodiode array (PDA) detector (Hewlett-Packard) during LC at room temperature under the conditions described above. In the Results and discussion section, these assays are referred to as qualitative runs.

RESULTS AND DISCUSSION

A reference quantitative chromatogram obtained by direct injection of a standard mixture of [^{3}H]FP-12 and [^{3}H]FP-14 in mobile phase is shown in Fig. 2. Both [^{3}H]FPs eluted as sharp peaks via 340-nm absorption or via ^{3}H flow-counting (not shown) with only minor amounts of 340-nm absorbance observed at the solvent front and immediately before the principal FP peaks. Control quantitative runs of other available synthetic FPs revealed that the mono-*cis*-FP-12 (Fig. 1) coeluted with FP-12. Both MFP-12 and MFP-14 eluted later than FP-14 at about 40 and 48 min, respectively. All these FP derivatives exhibited nearly identical absorption spectra with FP-12 and FP-14. Thus, *cis*-FP-12, if present in biological samples, would not be resolved from FP-12; but, the MFPs are clearly separable from their parent FPs. A qualitative run of the reference [^{3}H]FPs in conjunction with PDA scanning is shown in Fig. 3. Fig. 3A shows essentially the same pattern of that of Fig. 2, with the pre-FP peaks being more easily discerned. Fig. 3B and C show the PDA scans of the peaks in Fig. 3A. Nearly identical spectra (normalized to 100% for the highest peak in each scan) for the FP-12 peaks (marked 1 and 2) and the FP-14 peaks (marked 3 and 4) were obtained. In addition, other scans on the upslope of peaks 1 and 3 and on the upslope and downslope of peaks 2 and 4 gave nearly identical spectra as found for peaks 1, 2, 3, and 4. These extra scans attested to the chemical purity of the peaks; they have

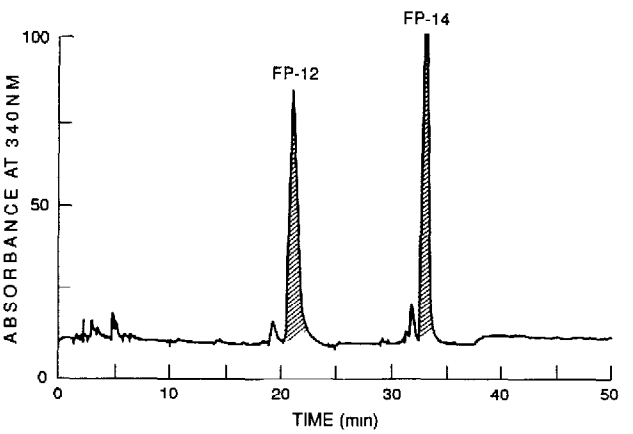


Fig. 2. Quantitative LC of the standard mixture of [^{3}H]FP-12 and [^{3}H]FP-14. Absorbance was recorded at 340 nm and ^{3}H (hatched areas) was detected by flow counting.

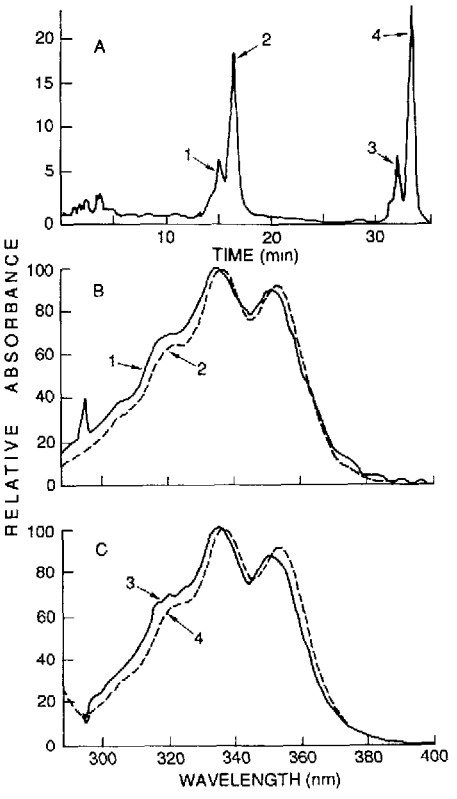


Fig. 3. Qualitative LC of the standard mixture of [^{3}H]FP-12 and [^{3}H]FP-14. (A) Chromatogram with monitoring for absorbance at 340 nm. (B) PDA scans of peaks 1 and 2 (FP-12 region). (C) Scans of peaks 3 and 4 (FP-14 region).

not been included for clarity of presentation of the most important results[a]. Because the added [^{3}H]FPs were purified to contain mainly all-*trans*-isomers, we conclude that the small pre-FP peaks are isomers of the FPs other than the mono-*cis*-FP-12, which migrates with the all-*trans*-FP-12.

Using the procedures outlined for direct assay of feces samples before incubation procedures were considered, we obtained the results shown in Table I. As noted in the Experimental section, we monitored the extraction and clean-up steps by direct counting of small aliquots of all fractions. In the initial combined yellow–brown diethyl ether extracts, we recovered an average of 29% ($n=26$; S.D.=14%; range=9–58%). These highly colored samples were corrected for quenching of the scintillator by the internal standard method. Following the silica cartridge clean-up, we obtained an average recovery of 20% ($n=26$; S.D.=9%; range=7–35%) in the light-yellow 5% methanol in diethyl ether cartridge-stripping solution. These relative low recoveries of total added ^{3}H were not a detriment to the overall procedure because the isotopic dilution method does not require quantitative recovery, only that the target analytes, i.e., the added [^{3}H]FPs di-

[a]Copies of any PDA scans referred to in the paper are available from the first author on request.

TABLE I

LEVELS OF FP-12 AND FP-14 IN FECES OF NORMAL MALE SUBJECTS

Sample[a]	Assay	FP-12[b] (μg/g)	FP-14[b] (μg/g)
7	1	<0.1	0.2
8	1	1.1	0.3
	2	0.2	0.8
	3	0.3	0.8
Mean ± S.D.		0.5 ± 0.5	0.6 ± 0.3
9	1	<0.1	0.8
	2	<0.1	0.7
Mean ± A.D.		<0.1 ± 0	0.8 ± <0.1
10	1	1.5	1.8
	2	0.2	1.4
	3	0.8	2.4
Mean ± S.D.		0.8 ± 0.6	1.9 ± 0.5

[a]These samples were directly extracted without incubation or addition of media. S.D. = standard deviation; A.D. = average deviation.

[b]Calculated from: endogenous FP = μg [^{3}H]FP spike $\times \left[\left(\frac{\text{cpm/AU spike FP}}{\text{cpm/AU recovered FP}} \right) - 1 \right]$. Units are μg FP per g of freeze-dried feces.

luted by the endogenous FPs, be recovered in uncontaminated state in amounts sufficient for analysis.

When possible, we performed duplicate or triplicate assays of feces samples. The means and ranges of values obtained are shown in Table I. Secondary qualitative runs of these extracts with PDA scanning yielded peaks at expected elution positions with absorption spectra typical of FP-12 and FP-14 in most of the samples. However, in both the quantitative and the qualitative chromatograms of the unspiked fecal extract from sample 8, we observed a peak after the FP-12 region and before the FP-14 region. The qualitative chromatogram with PDA scans for this sample is shown in Fig. 4A. Peaks 1 and 2 were clearly FP-12 (Fig. 4B) and peak 4 was FP-14 (Fig. 4C) as shown by the twin FP-absorption peaks at about 340 and 360 nm. However, peak 3 of Fig. 4A gave a PDA scan with the twin peaks shifted upscale to about 360 and 380 nm (Fig. 4C). A literature search showed that the family of methylpolyenes with numbers of conjugated double bonds ranging from three to eight exhibits major peaks at increasing wavelengths with increasing numbers of double bonds [30]. Because the FPs are structural variants of methylpentaenes, we can suggest that peak 3 of Fig. 4C is a compound wherein the pentaene chain of the FPs is replaced by a hexaene moiety because of upshifted twin peaks. This interpretation is also supported by the report of Govindan et al. [10] that triene and tetraene analogues of FP-12 exhibit twin peaks that are shifted downscale from the FP twin peaks.

The relative low levels of FPs found in our volunteers led us to redesign the pretreatment of the feces samples to incorporate tests of possible increases of

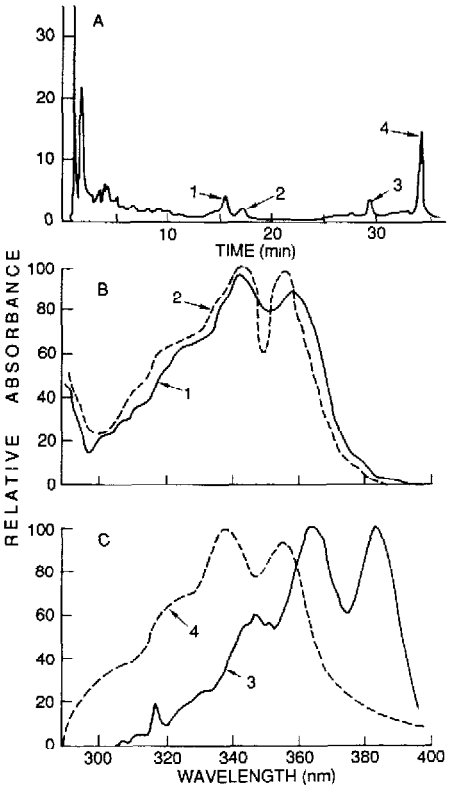


Fig. 4. Qualitative LC of an unspiked extract from feces sample 8. (A) Chromatogram with monitoring for absorbance at 340 nm. (B) PDA scans of peaks 1 and 2 (FP-12 region). (C) Scans of peaks 3 (unknown) and 4 (FP-14).

FPs following anaerobic incubation as described by Van Tassell et al. [14]. To control for possible effects due to media alone, we compared feces samples treated as blanks (no spike, with media) with those treated with media, but not incubated. Finally, the feces were treated with media and incubated at 37°C for 96 h. For comparative quantitation, the latter two samples were spiked with [^{3}H]FPs.

To illustrate the results from these samples, we have depicted in Fig. 5 the quantitative chromatograms from a set of assays of sample 17, which provided the most complex chromatograms and the highest levels of FPs. Fig. 5A shows the chromatogram from the sample containing feces with media but no spike and not incubated. The FP-12 elution region at 20 min showed two peaks as did the FP-14 region at 33 min. In Fig. 5B, which was from the sample spiked with [^{3}H]FPs but not incubated, the second of the two peaks of the FP regions was due to the *trans*-[^{3}H]FPs (the hatched areas indicate ^{3}H detection by flow-counting). It is noteworthy that in the incubated sample (Fig. 5C), we observed increases of 340-nm absorption in the *trans*-[^{3}H]FP-12 region (hatched peak), but even larger increases in the earlier FP-12 peak, which we designated pre-FP-12. Increases were also noted in the *trans*-[^{3}H]FP-14 peak and in the pre-FP-14 peak. Also, a new peak, prominent at about 27 min, can be discerned in Fig. 5C[a].

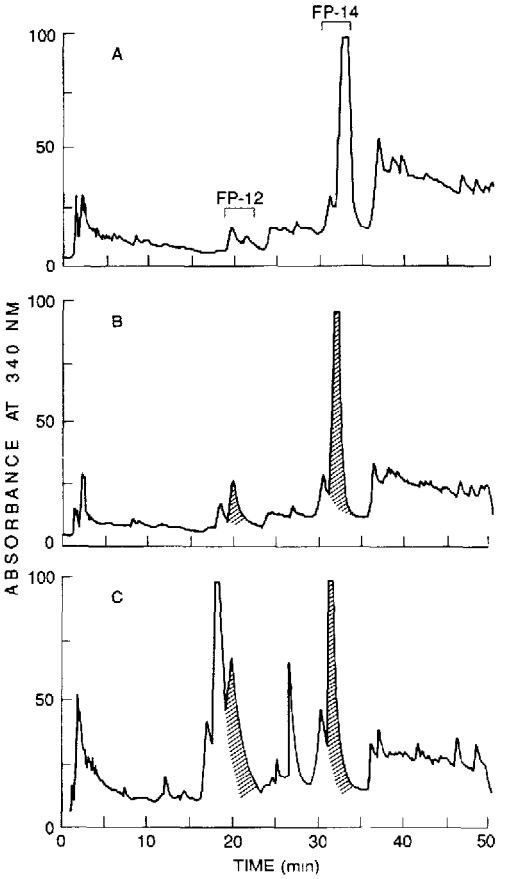


Fig. 5. Quantitative LC of fecal extracts from sample 17 (A) Absorbance of an extract from a fecal blank, with media and no spike; the regions known to correspond to FP-12 and FP-14 are marked. (B) Absorbance of the extract with media and spike, but no incubation, obtained from one of the 17 (M) analyses of Tables II and III; hatched areas show the ^{3}H detected by flow counting. (C) Absorbance of a fecal extract [17 (M+I) of Tables II and III] containing media and spike with incubation.

The companion qualitative chromatogram of Fig. 5C is shown in Fig. 6A. Fig. 6B and C show that FP-12 peaks 1 and 2 and FP-14 peaks 4 and 5 exhibited the characteristic FP twin peaks at 340 and 360 nm. However, peak 3 of Fig. 6A exhibited an absorbance spectra of twin peaks (Fig. 6C) shifted down scale: more characteristic of a tetraene or a triene, rather than a pentaene, as discussed above.

The prominent peak at 27 min in the incubated sample 17 was not found in any other samples. However, the detection of pre-FP-12 and pre-FP-14 peaks as in this subject was observed almost routinely in other feces assayed before and after incubation. To obtain a quantitative estimate of the contribution of the pre-FP peaks, we corrected the *trans*-FP levels calculated via isotopic dilution by the amount of pre-FP-12 peaks contributed to the total FP-12 peak areas as mea-

[a]To obtain larger peaks for Fig. 5C, we chromatographed 43% of the final extract instead of the usual 20%.

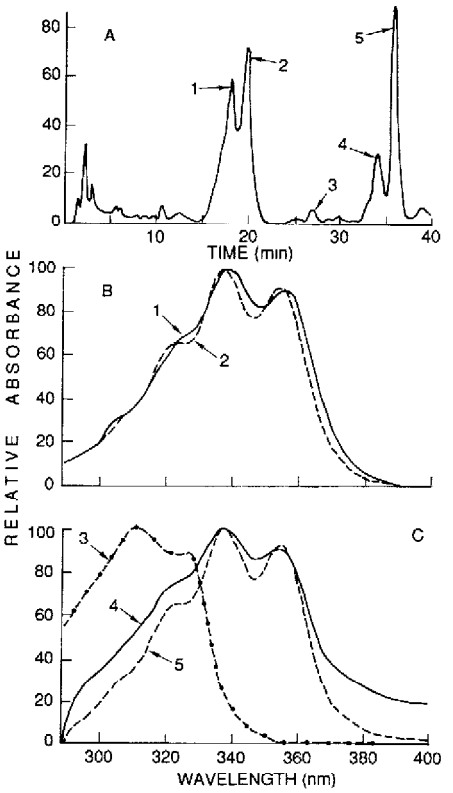


Fig. 6. Qualitative LC of sample 17, with media and incubation and with spike of [^{3}H]FPs. (A) Chromatogram with monitoring at 340 nm. (B) PDA scans of peaks 1 and 2 (FP-12 region). (C) PDA scans of peak 3 (unknown) and peaks 4 and 5 (FP-14 region).

sured by the 340-nm absorption (noted in the footnote of Table II). Again, to illustrate this calculation we employed sample 17, which was assayed in triplicate as indicated on the sample designation 17 $(M)^3$, Table II, wherein the superscript indicates triplicate analyses. Thus, the mean value of 1.2 μg/g FP-12 before incubation from the isotopic dilution assay was increased to 2.0 μg/g by applying the mean correction of 1.64 obtained as described above and in the table footnote. Similarly, after incubation the FP-12 value of 14.8 μg/g found by isotopic dilution was increased 2.42-fold to 32.1 after correction for the contribution of the pre-FP-12 peak absorption area.

Other donor feces samples gave the *trans*-FP-12 levels, correction factors, and total FP-12 values of Table II. Before incubation, samples 13, 15, and 16 yielded levels at or near the limit of detectability, with samples 11, 14, and 17 having levels of about 1 μg/g. After incubation, in samples 11, 14, and 17, the levels increased more than ten-fold whereas the others showed only slight increases. As noted for sample 17, the contributions of the pre-FP-12 were also of major importance in samples 11 and 14. (Sample 12 is included in both Tables II and III, even though we had insufficient material to analyze without incubation, because this sample also exhibited an unknown peak between the FP-12 and FP-14 re-

TABLE II

LEVELS OF FP-12 AND TOTAL FP-12 BEFORE AND AFTER ANAEROBIC INCUBATION IN FECES OF NORMAL MALE SUBJECTS

Sample[a]	*trans*-FP-12 via isotopic dilution (μg/g)	Correction factor[b] to add pre-FP-12	Total FP-12[c] (μg/g)
11 (M)	1.3	2.49	3.2
11 (M+I)	31.0	2.59	80.4
12 (M+I)	2.6	2.00	5.2
13 (M)	<0.1	1.10	<0.1
13 (M+I)	1.4	1.00	1.4
14 (M)	1.2	1.43	1.7
14 (M+I)	18.0	2.47	44.5
15 (M)[2]	0.2±0.1	1.13±0.07	0.3±0.2
15 (M+I)[2]	0.2±0.1	1.20±0.06	0.3±0.2
16 (M)[2]	0.1±0.1	1.06±0.04	0.1±0.1
16 (M+I)[2]	0.1±0.1	1.19±0.13	0.2±0.2
17 (M)[3]	1.2±0.4	1.64±0.29	2 0±0.7
17 (M+I)[3]	14.8±8.4	2.42±0.66	32.1±9.8

[a]Number is donor; (M) = incubation media added, no incubation; (M+I) = incubation media and incubation at 37 °C for 96 h. Superscript numbers indicate duplicate or triplicate assays. Means and average or standard deviations from the mean are shown where appropriate.
[b]Factor = (pre-FP-12 peak area + *trans*-FP-12 peak area) / *trans*-FP-12 peak area.
[c]Total = *trans*-FP-12 × correction factor.

gions that showed the spectral characteristics of a hexaene as we had found in sample 8 without incubation.)

Table III lists, in the same manner as did Table II for FP-12, the *trans*-FP-14 levels, correction factors, and total FP-14 values. Again, samples 11 and 17 were those with the highest amounts of FPs before and after incubation as shown in the second column. Sample 14 was low without, but increased about five-fold with incubation. Samples 13, 15, and 16 were low before but increased substantially on incubation. As shown in the third column of this table, the correction factors derived from the pre-FP-14 did not range as extensively as they had for FP-12 (third column of Table II). The maximum was a 1.38-fold increase for sample 14 (M+I) in Table III compared to the maximum of 2.59-fold increase for sample 11 (M+I) of Table II. Thus, it would appear that the pre-FP-14 contributions are not nearly as quantitatively significant as the pre-FP-12 contributions.

As shown in both Tables II and III, we found fairly good reproducibility on repeat assays to samples 15, 16, and 17. Noteworthy, however, is the individual variability of results in regard to the known compounds, *trans*-FP-12 and *trans*-FP-14, especially with incubated samples, which can be considered to reflect further residence time of the feces within the body and the production of additional FPs from available precursors.

A recent report [31] summarized the measurements of 'total fecapentaenes' from multiple sites in the bowel of sixteen autopsy patients. The chromatographic method employed did not resolve FP-12 and FP-14 and no information

TABLE III

LEVELS OF FP-14 AND TOTAL FP-14 BEFORE AND AFTER ANAEROBIC INCUBATION IN FECES OF NORMAL MALE SUBJECTS

Sample[a]	*trans*-FP-14 via isotopic dilution (μg/g)	Correction factor[b] to add pre-FP-14	Total FP-14[c] (μg/g)
11 (M)	8.0	1.30	10.4
11 (M+I)	35.0	1.25	43.8
12 (M+I)	4.9	1.20	5.9
13 (M)	0.2	1.16	0.2
13 (M+I)	7.7	1.28	9.9
14 (M)	0.8	1.30	1.0
14 (M+I)	4.3	1.38	5.9
15 (M)[2]	0.2±0.1	1.14±0.06	0.3±0.1
15 (M+I)[2]	2.2±0.4	1.32±0.06	2.8±0.6
16 (M)[2]	0.6±0.3	1.12±0.04	0.8±0.3
16 (M+I)[2]	3.2±1.6	1.31±0.17	4.5±2.6
17 (M)[3]	8.6±3.2	1.16±0.05	9.9±3.4
17 (M+I)[3]	18.5±2.5	1.26±0.11	23.1±1.6

[a]Number is donor; (M) = incubation media added, no incubation; (M+I) = incubation media and incubation at 37°C for 96 h. Superscript numbers indicate duplicate or triplicate assays. Mean and average or standard deviations from the means are given where appropriate.
[b]Factor = (pre-FP-14 UV peak area + *trans*-FP-14 peak area)/*trans*-FP-14 peak area.
[c]Total = *trans*-FP-14 × correction factor.

on recoveries of FPs by the extraction techniques was provided. Nevertheless, the authors found that the colonic concentrations of FPs and precursors (reassays for FPs after anaerobic incubation with added *Bacteroides* and supplements) varied widely between individuals, were not associated with the cause of death, but were consistent for each individual throughout the colon. Thus, these authors provided further information that FPs are common constituents of human feces. We anticipate applying our procedures to extensive studies on the relationships between the formation and excretion of FPs in human subjects receiving various diets and in patients with colonic polyps.

ACKNOWLEDGEMENTS

This work was supported in part by the National Cancer Institute (Grant CA 40918). We are indebted to Dr. P. Lim and Mr. A. Cheung, Pharmaceutical Analysis Department, Life Sciences, SRI for allowing us to use their photodiode array detector analytical system.

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