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The Metabolism of the Xenobiotic Triacylglycerols, *rac*-1- and *sn*-2- (3-Phenoxybenzoyl)-dipalmitoylglycerol, following Intravenous Administration to the Rat

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ABSTRACT. The metabolism of 3-phenoxybenzoic acid (3PBA) in the form of triacylglycerol conjugates was compared with that of non-esterified 3PBA. Three radiolabeled triacylglycerols (*rac*-1-(3-phenoxy-[*ring*-^{14}C]-benzoyl)-2,3-dipalmitoylglycerol (1(3PBA)DPG), *sn*-2-(3-phenoxy-[*ring*-^{14}C]benzoyl)-1,3-dipalmitoylglycerol (2(3PBA)DPG) and the "natural" tri-[1-^{14}C]oleoylglycerol) were incorporated into rat VLDL. Nonesterified 3PBA was prepared in rat serum albumin solution. Each preparation was administered i.v. to rats and serial blood samples were taken during the subsequent 6 hr. Urine and faeces were collected and tissue residues determined at 6 hr and 48 hr after administration. Biphasic elimination of 3PBA was observed with half-lives of 18 min and 2 hr. The triacylglycerols showed a rapid first phase and a longer second phase half-life: trioleoylglycerol 26 hr, 1(3PBA)DPG 7.6 hr and 2(3PBA)DPG 17.3 hr. The majority (63–76%) of 3PBA (whether esterified or not) was eliminated within 24 hr in urine, which contained similar profiles of metabolites. The triacylglycerols gave rise to higher tissue residues than did non-esterified 3PBA, particularly in adipose tissue which alone was not significantly depleted of radioactivity between 6 and 48 hr. The results accord with the rapid association of the VLDL-(3PBA)DPG complexes with lipoprotein lipase of the capillary epithelium, followed by hydrolysis to 3PBA, metabolism and elimination but with a proportion being redistributed into adipose tissue, re-esterified and then eliminated relatively slowly. BIOCHEM PHARMACOL **56**;12:1599–1606, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. xenobiotic metabolism; very low density lipoprotein; adipose; triacylglycerol; 3-phenoxybenzoic acid; kinetics

That incorporation of a variety of exogenous carboxylic acids into glycerolipids to form xenobiotic triacylglycerols is possible has been known since the late 1970s [1–3]. Quantitatively, this metabolic pathway of xenobiotic compounds is usually of minor importance when compared with the more usual hydroxylation and conjugation reactions that facilitate the elimination of relatively lipophilic carboxylic acids. Natural triacylglycerols function as a short- and long-term store of energy and are found in many tissues, principally in adipose tissue. The formation of xenobiotic analogs may, under conditions of repeated daily intake, result in some build-up of the metabolites in tissues. In fact, the elimination kinetics of 3PBA,¶ and of pyrethroid insecticides containing the 3-phenoxybenzyl moiety, reveal a second (slow) phase ($t_{1/2}$ 17–26 days) commensurate with the clearance of a triacylglycerol from fat and skin [4].

The formation of xenobiotic triacylglycerols has been reasonably well studied of late [3] with a small number of compounds (3PBA and some nonsteroidal anti-inflammatory carboxylic acid drugs) but little is known of the catabolism and elimination of these metabolites. Studies of the formation and mobilization of fenbufen-derived triacylglycerols in cultured 3T3-L1 adipocytes indicate that complete lipolysis is slower than that of oleic acid-derived triacylglycerols [5]. Studies of the hydrolysis of 3PBA-derived triacylglycerols by several lipases *in vitro* also indicate a degree of resistance, especially of the xenobiotic acyl-ester bond, to hydrolysis when compared with natural triacylglycerols [6].

The current paper describes the fate in the rat of the two model xenobiotic triacylglycerols containing 3PBA at either the *sn*-2 or the *rac*-1 position of the glycerol. Whilst oral administration would best mimic the adventitious exposure of humans and animals to xenobiotic triacyl-

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¶ Abbreviations: 1(3PBA)DPG, *rac*-1-(3-phenoxybenzoyl)-2,3-dipalmitoylglycerol; 2(3PBA)DPG, *sn*-2-(3-phenoxybenzoyl)-1,3-dipalmitoylglycerol; 3PBA, 3-phenoxybenzoic acid; 4HO3PBA, 3-(4-hydroxyphenoxy) benzoic acid; k_{el}, elimination constant; LPL, lipoprotein lipase; RSA, rat serum albumin; $t_{1/2}$, half life; and VLDL, very low-density lipoprotein.

glycerols, by analogy with natural fats, de-esterification during intestinal absorption would be expected. i.v. administration was chosen, therefore, to eliminate the first pass effect of the intestinal mucosa and possibly the liver. Thus our objective was to attempt to study the disposition of formed, rather than ingested, triacylglycerols. The i.v. route is also useful in that it provides the maximum exposure of all the tissues.

The 3PBA-containing triacylglycerols were formulated by incorporation into very low density lipoproteins (VLDL) prepared from a similar strain of rat to that used for the kinetic and metabolism studies. This approach fulfilled two objectives: 1) to mimic the anticipated natural transport form of the xenobiotic triacylglycerols and 2) to avoid the use of inappropriate solvents, which might perturb the state of the animals and the metabolism of the model compounds.

MATERIALS AND METHODS

Radiochemicals

The synthesis of ^{14}C-labeled and unlabeled forms of *rac*-1-(3-phenoxybenzoyl)-2,3-dipalmitoylglycerol (1(3PBA)DPG) and *sn*-2-(3-phenoxybenzoyl)-1,3-dipalmitoylglycerol (2(3PBA)DPG) are described in the preceding paper [6]. 3-Phenoxy-[*ring*-^{14}C]-benzoic acid (^{14}C-3PBA) was provided by Shell Research Ltd, Sittingbourne, Kent, U.K. The specific radioactivities were adjusted for dosing to animals as follows: 1(3PBA)DPG, 10.8 Ci/mol; 2(3PBA)DPG, 10.8 Ci/mol; 3PBA, 10.7 Ci/mol. Tri-[1-^{14}C]oleoylglycerol was obtained from Amersham and adjusted to a specific radioactivity of 13.0 Ci/mol before use.

Other materials

Unlabeled 3PBA was obtained from Aldrich Chemical Co. Ltd (Gillingham, Dorset, U.K.). Trioleoylglycerol, *rac*-1,2-dipalmitoylglycerol, 1,3-dipalmitoylglycerol (both for the synthesis of the labeled 3PBA-DPG), BSA, RSA, and dialysis tubing (6-mm diameter, 12400-Da exclusion) were obtained from Sigma.

Animals

Young adult male Sprague–Dawley CD rats (200–250 g) were obtained from Charles River UK Ltd (Margate, U.K.). All animals were given access to food and water *ad libitum*. Male Wistar rats from the same source (older breeding stock) were used for the supply of blood for lipoprotein fractions.

VLDL-[^{14}C]triacylglycerol Complexes

Whole blood (200 mL) was collected from 20 male Wistar rats and transferred to two 100-mL, capped centrifuge tubes. Serum was prepared by centrifuging (850 *g* for 30 min at 4°). Portions (15 mL) were overlayered with 5 mL of 0.189 M NaCl and centrifuged at 105,000 *g* for 18 hr at 10°. The top 4 mL containing the VLDL fraction was harvested in each case, pooled and re-isolated by the same procedure. The triacylglycerols were then incorporated into the VLDL by the method of Fielding [7]. [^{14}C]Triacylglycerol [1(3PBA)DPG, 2(3PBA)DPG or trioleoylglycerol, 50 μCi] was dissolved in 300 μL of analytical grade DMSO. The rat VLDL preparation (3 mL) and the DMSO solution were carefully mixed drop-wise in a conical-bottomed tube while mixing gently with a vortex mixer. To facilitate the incorporation, the mixture was then incubated for 4 hr in a shaking water bath at 37°, after which the VLDL/triacylglycerol mixtures were dialyzed at 5° against 0.154 M NaCl (changed hourly for 5 hr and then left overnight). These solutions were used for administration to the animals (0.5 mL per rat). The doses were as follows: 1(3PBA)DPG, 1.18 μCi (0.084 mg); 2(3PBA)DPG, 3.47 μCi (0.246 mg); and trioleoylglycerol, 7.53 μCi (0.512 mg), these being the highest doses of radioactivity practically available.

3-Phenoxy-[^{14}C]benzoic Acid

A solution of [^{14}C]-3PBA (1 mg, 50 μCi) in 250 μL of DMSO was mixed with 3 mL of physiological saline (0.154 M NaCl) containing 1% (w/v) RSA. Each dosing volume of RSA-3PBA (0.5 mL) contained 8.53 μCi (0.171 mg of 3PBA).

Administration of Labeled Test Compounds to Animals

Eighteen male rats were allocated to four groups of three (Groups 1–4) and three groups of two (Groups 5–7). Groups 1–4 were used for a kinetic study providing serial blood samples over 6 hr. These animals were placed in holding cages after i.v. dosing of 0.5 mL of test solution via a caudal vein [Group 1, 1(3PBA)DPG/VLDL; Group 2, 2(3PBA)DPG/VLDL; Group 3, trioleoylglycerol/VLDL; and Group 4, 3PBA/RSA]. Blood samples were collected from a caudal vein into heparinised haematocrit tubes at 5, 10, 20, 40, 75, 120, 240 and 360 min. Groups 5–7 were used for a metabolism study. After dosing, the animals were placed in Nalgene metabolism cages (Techmate Ltd., Milton Keynes, Bucks, U.K.). Urine and faeces were collected at 24 hr and 48 hr.

Termination and Necropsy Procedures

The animals were killed at 6 hr (the kinetic Groups 1–4) or 48 hr (the metabolism Groups 5–7) by light halothane anaesthesia followed by an i.p. injection of pentobarbitone (Sagatal, Rhône Mérieux, Harlow, Essex, U.K.). The following samples were collected: blood, heart, muscle, kidney, liver, perirenal adipose tissue, epididymal adipose tissue, and site of injection (tail). All samples were frozen prior to analysis, except blood from Groups 1–4, which was radioanalysed within 24 hr by combustion/liquid scintillation counting.

Measurement of Radioactivity

Radioactivity was measured using Packard 2000CA or 2200CA TriCarb liquid scintillation counters. Solutions

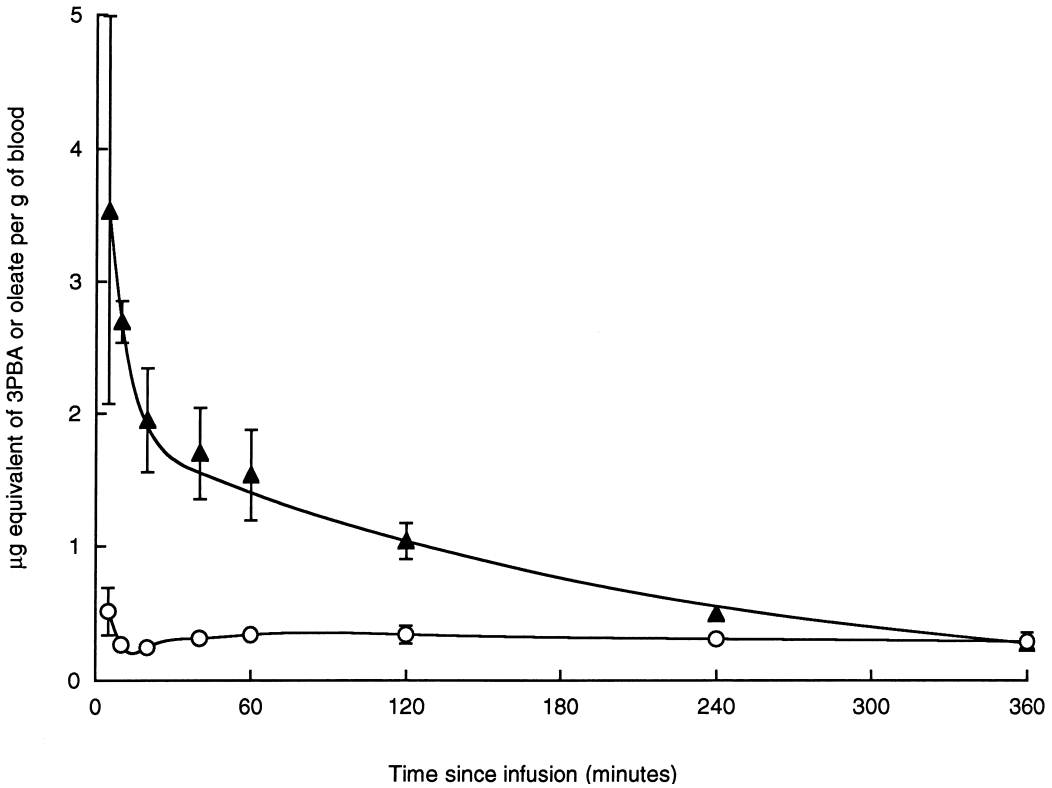

FIG. 1. ^{14}C-labeled 3-phenoxybenzoic acid (▲) or trioleoylglycerol (○): the time-concentration relationships of radioactivity in blood following the i.v. administration to rats. Error bars represent the mean ± SD from three animals. The animals received 8.51 µCi (171 µg) of 3PBA or 7.53 µCi (512 µg) of trioleoylglycerol.

(up to 2 mL) were counted directly in 10 mL of Optiphase "Safe" cocktail (Fisons, Loughborough, U.K.). Packard 306B Sample Oxidisers were used for the radioassay of solid samples. Samples (up to 200 mg) were weighed into Combustocones; Combustopads, Combustaid (Canberra-Packard, Pangbourne, U.K.), and CC41 cellulose powder (Whatman, Maidstone, U.K.) were added as necessary. The counting solution was composed of Carbosorb (8 mL) and Permafluor V (13 mL) (Canberra-Packard).

Radiochromatography

TLC was carried out using Merck 0.25-mm silica gel 60 F_{254} TLC plates developed with solvent A, ethyl acetate/formic acid/water (70: 4: 4, by volume) or solvent B, *n*-butanol/acetic acid/water (60: 10: 10, by volume). Radioactivity on the plates was located and quantitated using a RITA 90 TLC Plate Analyser system (LabLogic Ltd, Sheffield, U.K.). Some analyses were visualized by autoradiography using Curix RPI x-ray film (Agfa-Gevaert Ltd, Brentford, U.K.).

RESULTS

The Kinetics in Blood of the 1- and 2-(3-Phenoxybenzoyl)-dipalmitoylglycerols, 3-Phenoxybenzoic Acid, and Trioleoylglycerol

The following three radiolabeled triacylglycerols were incorporated into the VLDL fraction derived from fresh rat serum as described in the Materials and Methods section: *rac*-1-(3-phenoxybenzoyl)-2,3-dipalmitoylglycerol; *sn*-2-(3-phenoxybenzoyl)-1,3-dipalmitoylglycerol; and trioleoylglycerol. The latter was included as an example of a natural triacylglycerol to compare the kinetics of the two model xenobiotic analogs against. 3-Phenoxybenzoic acid, formulated in physiological saline containing rat serum albumin, was included in the experiment to enable a comparison with nonesterified xenobiotic acid.

Limitations on the availability of the test compounds precluded the use of exact mole equivalent doses. The dosages used, assuming an average animal weight of 225 g, were as follows: 1(3PBA)DPG, 0.533 mmol/kg; 2(3PBA)DPG, 1.43 mmol/kg; trioleoylglycerol, 2.57 mmol/kg; and 3PBA, 3.55 mmol/kg. The animals were dosed via a caudal vein and blood was sampled between 0 and 6 hr after dosing on the schedule described in the Materials and Methods section.

The time course of total radioactivity in the blood for nonesterified 3PBA and trioleoylglycerol are compared in Fig. 1. The time course for 3PBA was of the type expected for a single i.v. dose of a low molecular weight, reasonably polar, xenobiotic acid. That for trioleoylglycerol was very different despite the relatively similar molar doses used (3.55 and 2.57 mmol/kg, respectively).

The results were processed by fitting to a two-compartment model given by the equation:

TABLE 1. Kinetic constants for 3PBA, TOG, 1(3PBA)DPG, and 2(3PBA)DPG derived from the measurement of total blood radioactivity following i.v. dosing of the compounds to rats

	Phase 1			Phase 2		
Compound	A	α	$t_{1/2}$ (min)	B	β	$t_{1/2}$ (hr)
3PBA	4.62	−0.0381	18 ± 10	2.09	−0.0057	2.01 ± 0.2
TOG	0.92	−0.00075	9 ± 7	0.35	−0.00045	25.8 ± 14.5
1(3PBA)DPG	—	—	—	0.65	−0.00154	7.62 ± 1.17
2(3PBA)DPG	—	—	—	1.20	−0.00067	17.3 ± 4.1*

Values are mean ± SD from three animals per group. Where no values are given (—), the distribution phase was too fast to be measured. *Significantly different ($P < 0.05$) from the value for the 1-isomer.

$$\text{Concentration} = A\, e^{-\alpha t} + B\, e^{-\beta t}.$$

It is assumed that the A component relates to the distribution phase of the parent compound, and the B component relates to the metabolite distribution and elimination phases. α and β are the elimination constants (k_{el}) for the two processes and the half-lives ($t_{1/2}$) are calculated using:

$$t_{1/2} = \frac{-\ln 2}{k_{el}}$$

The kinetic constants are shown in Table 1.

The time courses for the radioactivity derived from the 1- and 2-(3PBA)DPG are shown in Fig. 2. The behaviour of both compounds was similar to that of trioleoylglycerol; this was particularly so for the 2-isomer (see the values for β and $t_{1/2}$ in Table 1).

The 1- and 2- isomers differ from one another in that the former appeared to be cleared faster than the 2-isomer. The maximum concentration achieved values are not strictly comparable because of the 2.7-fold higher dose of the 2-isomer. Taking this into account, the maximum concentration values were not significantly different.

The Elimination of Radioactivity following Dosing with Free 3PBA and the 1- and 2-(3-Phenoxybenzoyl)-dipalmitoylglycerols

Pairs of animals were dosed as described above, placed in metabolism cages, and the elimination of radioactivity in

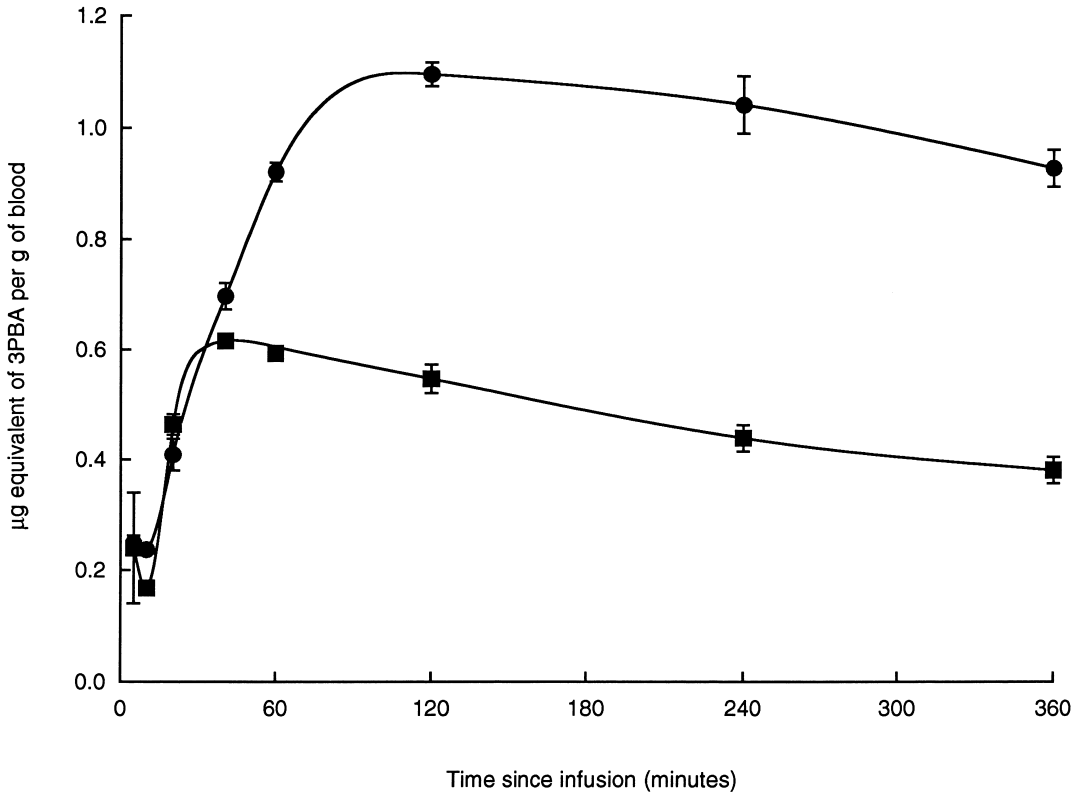

FIG. 2. ^{14}C-labeled 2(3PBA)DPG (●) or 1(3PBA)DPG (■): the time-concentration relationships of radioactivity in blood following the i.v. administration to rats. Error bars represent the mean ± SD from three animals. The animals received 3.47 μCi (246 μg) of 2(3PBA)DPG or 1.81 μCi (84 μg) of 1(3PBA)DPG.

TABLE 2. Elimination of radioactivity (expressed as a percentage of the total dose) following i.v. administration of radiolabeled 3PBA, 1(3PBA)DPG, or 2(3PBA)DPG. The dosages used are given in the legends to Figs. 1 and 2

		Compound		
	Route	3PBA	1(3PBA)DPG	2(3PBA)DPG
Urine	0–24 hr	75.8 ± 2.9	63.3 ± 4.0	66.4 ± 1.4
	24–48 hr	1.4 ± 0.6	6.6 ± 1.1	9.0 ± 0.8
Faeces	0–48 hr	5.2 ± 0.7	13.3 ± 2.7	11.7 ± 1.1
Totals		82.4 ± 4.2	83.2 ± 7.9	87.1 ± 3.3

Values are the means ± SEM of triplicate samples from two animals.

urine and faeces measured at 24 hr and 48 hr. The results are shown in Table 2. The three compounds behaved similarly in terms of overall recovery of radioactivity in 48 hr. In addition, the distribution between urinary and faecal elimination was of a similar order. The results for 3PBA were similar to those obtained in previous studies that used oral administration [8] .

The 2-isomer behaved very similarly to the 1-isomer in spite of the differences observed in the kinetics. This is because the 24 hr and 48 hr elimination data are much less sensitive measures of kinetic events than are data derived from the frequent measurements made in blood.

The Distribution of Radioactivity Remaining in the Tissues

Selected tissues were analysed for retained radioactivity. Liver and kidney were chosen, as they are the major organs of elimination; muscle and heart were chosen as tissues that would be expected to retain the least residue—heart muscle, however, expresses strong lipoprotein lipase (triacylglycero-protein acylhydrolase; EC 3.1.1.34) activity; and two fat samples were chosen because the xenobiotic triacylglycerols, if not metabolized, would be expected to be retained in adipose tissue. Two time points were made available by sampling the kinetic rats (at 6 hr) and the metabolism rats (at 48 hr). The results are given in Table 3.

The tissue residues of 3PBA and/or its metabolites at 6 hr were all low in absolute terms and confirm that this carboxylic acid is rapidly metabolised and cleared from the body following i.v. dosing. The relatively high values in liver and kidney reflect the high blood flow to these organs and their roles in the elimination processes. The similar, relatively high, values in the fat samples suggest some association of a 3PBA metabolite with adipose tissue. A comparison of the values at 48 hr with those at 6 hr provide a useful indication of the rate of depletion of radioactivity from the tissues. This was rapid from liver, kidney, and muscle. Depletion from both fat types was much slower, the values at 6 hr and 48 hr not being statistically different. This is clear evidence for the affinity of a metabolite of 3PBA, probably a derived triacylglycerol, with adipose tissue.

Comparing the 6 hr values for the two (3PBA)DPG isomers with those for 3PBA, it is apparent that the triacylglycerols give rise to much higher residues in all tissues. As examples, the value for the 2-isomer in liver is 19 times that for 3PBA and 5 times in perirenal fat. These results are in accordance with expectations derived from the kinetic study, which shows that 3PBA is cleared more rapidly than are the glycerol esters.

The 6 hr and 48 hr values for the 3PBA-triacylglycerol isomers show that radioactivity is cleared efficiently from the liver, kidney, muscle, and heart. As found for the 3PBA-derived radioactivity, the depletion of triacylglycerol-derived radioactivity from the two types of fat is much less efficient; the slightly lower values at the 48 hr samplings were in no case significantly different from those at 6 hr.

Comparison of the behaviour of the two isomers is complicated by the nonequivalent molar doses used. The apparent two-fold higher residues of the 2-isomer in the liver, kidney, and heart (and the lack of difference in muscle), taking into account the 2.7-fold higher dose of this isomer, indicates little difference in the behaviour of the isomers.

The Metabolic Products of 1- and 2-(3PBA)DPG in the Rat

As the 0–24 hr urine accounted for the majority of the eliminated radioactivity derived from dosing the triacyl-

TABLE 3. Concentrations of radioactive residues in tissues (expressed as ng of 3PBA per g tissue) following i.v. administration of radiolabeled 3PBA, 1(3PBA)DPG, or 2(3PBA)DPG. The dosages used are given in the legends to Figs. 1 and 2

	Compound and time after dosage					
	3PBA		1(3PBA)DPG		2(3PBA)DPG	
Tissue	6 hr	48 hr	6 hr	48 hr	6 hr	48 hr
Liver	155 ± 23	10 ± 1	612 ± 72	73 ± 5	2,951 ± 134	394 ± 27
Kidney	220 ± 10	10 ± 0	267 ± 18	35 ± 3	1,319 ± 63	137 ± 1
Heart	70 ± 4	1 ± 0	39 ± 5	18 ± 3	814 ± 32	72 ± 4
Muscle	3 ± 2	2 ± 0	40 ± 3	7 ± 1	120 ± 6	27 ± 4
Perirenal adipose	99 ± 2	110 ± 34	116 ± 17	111 ± 4	465 ± 62	351 ± 64
Epididymal adipose	181 ± 59	103 ± 5	114 ± 22	73 ± 9	288 ± 41	228 ± 19

Values are the means ± SD of triplicate samples from two animals.

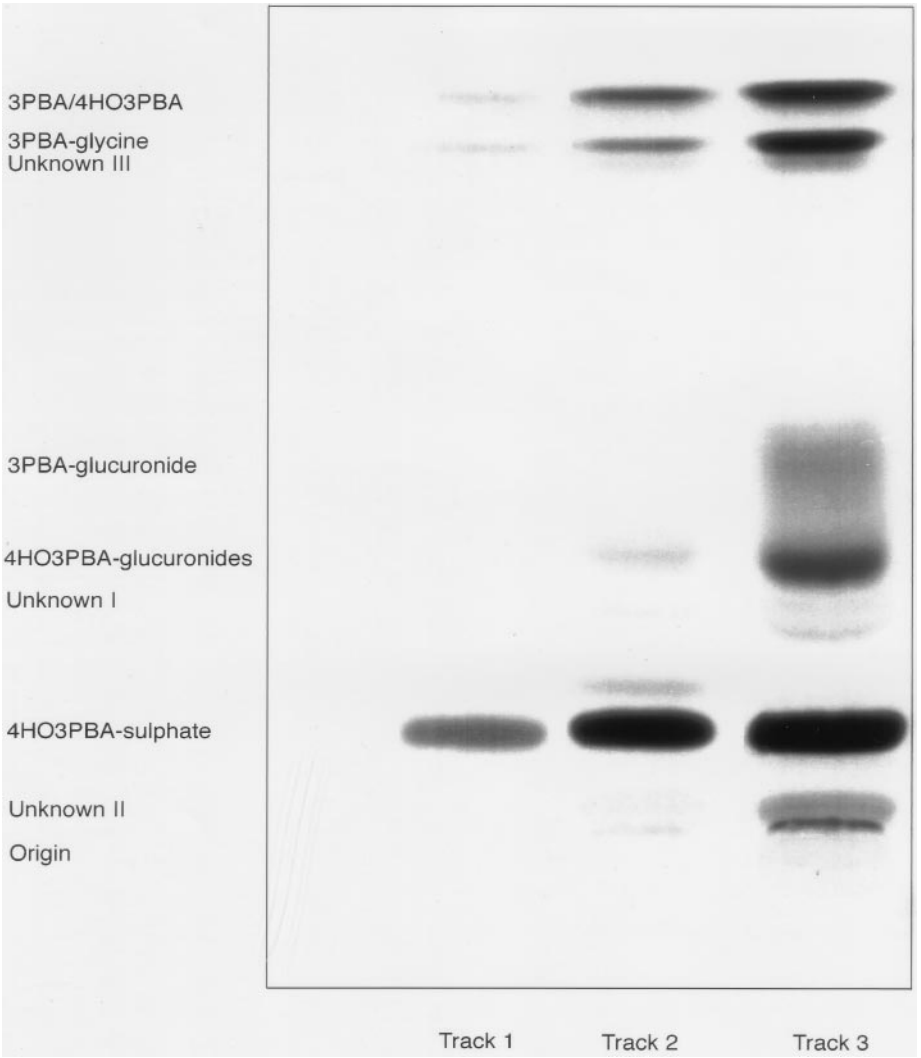

FIG. 3. Thin layer radiochromatogram (solvent A) of 0–24 hr urine samples from rats dosed with: 1(3PBA)DPG (Track 1), 2(3PBA)DPG (Track 2), or nonesterified 3PBA (Track 3). Quantitative data are shown in Table 4.

glycerols (63–66%), these samples were analysed by radio-TLC in two solvent systems. Previous studies have described fully the biotransformation products of orally dosed 3PBA in rats [8] and in mice [9]. The use of these two solvent systems was likely to be sufficient to identify the urinary metabolites of the three test compounds.

A metabolite separation using solvent A is illustrated in Fig. 3 and the quantitative analyses derived from solvents A and B are shown in Table 4. The major metabolite from both isomers was the sulphate conjugate of 3-(4-hydroxyphenoxy)benzoic acid (4HO3PBA) (70–73% of the urinary radioactivity) followed by the glucuronide conjugates of 3PBA and 4HO3PBA. The glycine conjugate of 3PBA was a minor metabolite (5–6%). Unconjugated 3PBA and

TABLE 4. Metabolites in rat urine derived from the i.v. administration of radiolabeled 3PBA, 1(3PBA)DPG, or 2(3PBA)DPG. Solvent A was ethyl acetate/formic acid/water (70:4:4, by volume) and solvent B was *n*-butanol/acetic acid/water (60:10:10, by volume). The dosages used are given in the legends to Figs. 1 and 2

	R_f values		Percent total radioactivity		
Metabolite	Solvent A	Solvent B	3PBA	1(3PBA)DPG	2(3PBA)DPG
3PBA/4HO3PBA	0.93	0.93	7.0	4.8	9.4
4HO3PBA sulphate	0.16	0.72	63.3	69.5	73.1
4HO3PBA ester glucuronide	0.31	0.49	13.9	11.7	7.8
4HO3PBA ether glucuronide	0.37	0.49			
3PBA glucuronide	0.46	0.63	4.4	4.0	1.8
3PBA-glycine	0.88	0.81	7.9	6.0	4.9
Unknown II	0.07	—	2.9	1.8	2.2

Values are means of duplicate determinations from two animals.

4HO3PBA were also eliminated. Although these are not separated from each other by solvents A or B, previous experience with 3PBA metabolism has shown that both are present as minor elimination products.

The proportion excreted as the sulphate conjugate was slightly higher for the esters than for 3PBA; conversely, the glucuronide and glycine conjugates account for a higher proportion of the metabolites of nonesterified 3PBA. Using solvent A, three minor metabolites (R_f values: I, 0.21; II, 0.07; III, 0.85) of the 2-isomer were detected. Only metabolite II could be quantified as a discrete zone (1.8–2.9%).

The routes of excretion of radioactivity and the nature of the excreted metabolites indicate that the metabolism of the 1- and 2-isomers is quantitatively very similar and involves the formation of 3PBA, which is then mainly metabolised via known pathways.

DISCUSSION

Following the i.v. administration to rats of radiolabeled 3PBA dissolved in physiological saline containing serum albumin, the kinetic profile of radioactivity in the blood (Fig. 1) was typical of that expected for a low molecular weight, ionised xenobiotic compound. The high concentration caused by the bolus injection initiated a rapid distribution phase ($t_{1/2}$ 18 min). This was followed by a second phase ($t_{1/2}$ 2 hr) which probably reflected the metabolism of 3PBA and the disposition of its metabolites.

The triacylglycerols, in comparison, behaved differently (Fig. 2). The initial phase was only just observable ($t_{1/2}$ 9 min) for trioleoylglycerol, the model for a natural product (Fig. 1). This phase was not observed with either of the xenobiotic analogs. The compounds were formulated for dosing by incorporation into VLDL to mimic the form in which triacylglycerols formed in the body are transported in the blood. The extremely rapid (non-observable) distribution phase is probably due to the rapid association of the VLDL carrier with tissue LPL which is located on the capillary epithelium [10]. Conversely, the relatively slow initial (distribution) phase for nonesterified 3PBA is likely to be due to the lack of a specific receptor.

The binding of VLDL to tissue LPL enables the hydrolysis of the triacylglycerols. This binding also has the effect of lengthening the time taken for first passage around the blood stream. After binding, some of the associated radiolabeled complex disassociates from the LPL and reappears in the circulation. This may account for the increase in radioactivity in the blood following the initial phase (Fig. 2). Hydrolysed acids from the core triacylglycerol of the VLDL (in this case 3PBA) are normally taken up in the tissues and may be released back into the plasma at a later stage. Remnant VLDL particles may be taken up by hepatic receptors and the acid again recycled to the circulation as fresh VLDL or subjected to normal detoxication reactions. This redistribution will also account for part of the observed rise in blood radioactivity after the initial phase.

The second phase of the xenobiotic triacylglycerol elimination ($t_{1/2}$ 7.6 hr and 17.3 hr for the 1- and 2-isomers, respectively) is much slower than that for 3PBA ($t_{1/2}$ 2 hr) suggesting that the second phase is a composite of several processes, possibly rate-limited by the hydrolysis of the 3-phenoxybenzoyl group from the triacylglycerol. This is indicated by the fact that free 3PBA and its metabolites are eliminated from blood with a half-life of 2 hr. Evidence from the studies *in vitro* reported earlier [6] supports the concept that hydrolysis might be rate limiting. The hydrolyses of 3PBA from 1- and 2-(3PBA)DPG (1-isomer > 2-isomer) were appreciably slower than the hydrolysis of oleoyl and palmitoyl ester bonds.

The fate of 3PBA, once released from the triacylglycerol/VLDL complex is virtually identical to that of orally administered 3PBA [8,11] and of i.v. administered 3PBA. This involves 4-hydroxylation, conjugation of the resultant phenol with sulphate or glucuronic acid, and also direct conjugation of 3PBA with glucuronic acid or glycine. A small fraction (1–3%) of orally administered 3PBA is incorporated into triacylglycerols [12]. The slow elimination of radioactivity from adipose tissue observed in the current investigation (compare the 6 hr and 48 hr fat residues for the 3PBA-dosed animals) is a reflection of this process. The residue in the fat of (3PBA)DPG-dosed animals at 48 hr may well be due to released 3PBA that has been re-esterified into xenobiotic triacylglycerol after reuptake (i.e. recycled).

The relatively slow elimination of 3PBA radioactivity from all sources of adipose tissues over the first 48 hr after dosing suggests that a third phase of elimination would be observed if sufficient samples of adipose tissue were analysed over an extended period. This has been done in studies using a 3-phenoxybenzyl-containing pyrethroid insecticide [4]; biphasic elimination from fat and other tissues was observed. The half-lives of radioactivity in fat were found to be 2.5 days and 17–26 days. The first half-life was derived from the metabolism of the pyrethroid, and the second was considered to be due to the depletion of a 3PBA-derived triacylglycerol. Compared with the kinetics derived from blood measurements, these two phases would be equivalent to second and third phases.

An elimination half-life of 25 days implies that, under conditions of dietary intake of 3PBA, a period of 75–100 days would be required to approach a plateau concentration of (3PBA)DPG in fat. *In vivo*, ingested 3-phenoxybenzyl-containing pyrethroids would generate 3PBA in the liver [4] where it may be incorporated into a xenobiotic triacylglycerol [13], which in turn can be assembled into a nascent VLDL and secreted into the blood stream. The experiments reported herein suggest that 3PBA entering the blood stream in this form will be metabolised in a similar manner and be excreted in the same way as nonesterified 3PBA; however, they also show that the half-lives of their clearance will be very much longer than that of the acid. Furthermore, 3PBA that enters adipose tissue, where triacylglycerol synthesis or resynthesis can occur, appears to be retained for a much longer period than in other tissues.

These observations suggest that, under conditions of repeated (low) exposure, the xenobiotic triacylglycerol is likely to accumulate in adipose tissue.

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