Endogenous leukotriene D₄ formation during anaphylactic shock in the guinea pig

(leukotriene C₄ metabolism/hepatobiliary elimination/leukotriene analyses *in vivo*)

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ABSTRACT Experiments on the metabolism and excretion of *i.v.* administered selectively labeled [³H]₈leukotriene C₄ in bile duct-cannulated guinea pigs indicated predominantly biliary excretion of tritium. The major leukotriene metabolite in bile was identified as leukotriene D₄. By monitoring leukotriene excretion radioimmunochromatographically, it was shown that guinea pigs suffering from anaphylactic shock produce leukotriene D₄ endogenously. Immunological challenge of animals sensitized to ovalbumin was accompanied by an increase of biliary leukotriene D₄ concentrations from 10 ± 1 to 86 ± 10 nM (mean ± SEM, *n* = 5, *P* < 0.001). When considering that bile flow was decreased to about half after challenge, the excretion rate of leukotriene D₄ in bile increased from 0.88 ± 0.16 before to 3.18 ± 0.38 pmol·min⁻¹·kg⁻¹ after challenge (mean ± SEM, *n* = 5, *P* < 0.002). It is concluded that systemic anaphylaxis in the guinea pig is associated with endogenous generation of leukotriene C₄ (up to 1 nmol/kg during a 30-min period after the challenge).

Leukotriene C₄ (LTC₄) is a biologically active substance presumed to play major roles as a mediator of allergic and anaphylactic reactions (1, 2). It is formed by basophilic (3) and eosinophilic leukocytes (4), monocytes (5), macrophages (6), and mast cells (7). In cells having IgE receptors, bridging of these receptors by divalent anti-IgE receptor antibodies or by interaction between receptor-bound IgE and anti-IgE will induce LTC₄ formation (5–7). Leukotriene formation has also been demonstrated in other *in vitro* models of immediate hypersensitivity (8–10).

The biological actions of LTC₄ comprise induction of airway obstruction, constriction of coronary arteries, hypotension, and plasma extravasation (11–14). Leukotriene formation *in vivo* may mediate anaphylactic shock symptoms and cause the death of an animal. To prove the presumed mediator role of this substance in anaphylactic reactions, it is necessary to demonstrate its endogenous formation during anaphylaxis. Studies on LTC₄ metabolism have revealed rapid catabolism by various transformations of the peptide substituent (15). Three metabolites have been demonstrated to be excreted as end-products: leukotriene E₄ (LTE₄) (16) in man and *N*-acetyl-LTE₄ plus *N*-acetyl-11-*trans*-LTE₄ (17) in the rat. By monitoring biliary *N*-acetyl-LTE₄ levels, endogenous leukotriene formation in the rat was demonstrated *in vivo* after tissue trauma and endotoxin shock (18, 19). We now wish to report evidence for endogenous LTC₄ production during anaphylactic shock in guinea pigs.

MATERIALS AND METHODS

Materials. The selectively labeled LTC₄ [5,6,8,9,11,12,14,15-³H]₈LTC₄ (specific activity: 10 Ci/mmol; 0.37 TBq/mmol; 1

Ci = 37 GBq) was prepared as described (17, 20). [14,15-³H(n)]LTD₄ (leukotriene D₄) (48 Ci/mmol) was from Amersham. A radioimmunoassay for LTC₄, including [14,15-³H(n)]LTC₄ (39 Ci/mmol), was from New England Nuclear. Synthetic LTC₄, LTD₄, and LTE₄ were kindly provided by J. Rokach of Merck Frosst Labs (Pointe Claire, PQ, Canada). *N*-acetyl-LTE₄ and leukotriene F₄ were prepared from LTE₄ as described (17, 21). 4-Hydroxy-2,2,6,6-tetramethylpiperidine-*N*(1)-oxyl (HTMP), ovalbumin (grade III), pyrilamine maleate, glutathione, and γ-glutamyltransferase were from Sigma. Pentanesulfonic acid was from Fluka. Sodium pentobarbital, diazepam (Roche Diagnostics), chloramphenicol (Dumex, Copenhagen), cholic and dehydrocholic acid (Pharmacia), and dexamethasone 21-phosphate (Merck Sharp & Dohme) were purchased from Karolinska Apoteket (a local pharmacy). LY 171883, sodium salt, 1-[2-hydroxy-3-propyl-4-[4-(1-*N*-tetrazol-5-yl)butoxy]phenyl]ethanon, was a generous gift from J. Fleisch of Eli Lilly Research Laboratories. Solvents and chemicals were HPLC grade or *pro analysi* purity.

Animal Experiments. Male Hartley guinea pigs (8–15 weeks, 300–600 g) were obtained from Sahlins (Malmö, Sweden). They were fed an unrestricted standard diet from EWOS (Södertälje, Sweden). For metabolic experiments, animals were pretreated with dexamethasone (50 mg/kg, *i.p.*) 16–24 hr before bile duct cannulation. On the day of the experiment, diazepam (5 mg/kg to calm the animal) was given *i.p.* 30 min before anesthesia with pentobarbital (30 mg/kg, *i.p.*). After performing tracheostomy and intubation (polyethylene tubing, 2.4-mm o.d.), the bile duct was cannulated (polyethylene tubing, 1.1-mm o.d.). [³H]₈LTC₄ (1 μCi) dissolved in 0.4 ml of 0.9% saline/ethanol, 98:2 (vol/vol), was injected into the heart or the vena cava inferior. Bile was collected for 5 hr in ice-cold methanol/water, 9:1 (vol/vol), containing 1 mM HTMP and 0.5 mM EDTA (pH 7.4). Methanol was added to 80% (vol/vol), and 0.1-ml aliquots were removed for radioactivity determination. The remainder was centrifuged and analyzed by reverse phase (RP)-HPLC (see below).

For analyses of leukotriene formation during shock, guinea pigs were sensitized 14–16 days before antigen challenge by (i) *i.p.* injection of ovalbumin at 10 mg/kg or (ii) *i.p.* injection of ovalbumin at 3 μg/kg plus Al(OH)₃ at 300 mg/kg. Al(OH)₃ was added to the antigen solution 1 hr before injection. The developing antibodies (22) are IgG in procedure (i) and IgE plus IgG in procedure (ii). To minimize contribution of trauma-induced leukotriene production (18), the following surgical methods were used: (i) Two days before immunological challenge the cystic duct was ligated, the gall bladder was punctured, and the bile duct was incised. An anastomo-

Abbreviations: LTC₄, LTD₄, and LTE₄, leukotrienes C₄, D₄, and E₄, respectively; RP, reversed-phase; HTMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*(1)-oxyl.

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sis tubing (Silastic, 0.6-mm o.d. tubing, connected at both ends with 1.1-mm o.d. polyethylene tubing) was inserted into the bile duct (orientation toward the liver) and into the duodenum. Upon closure of the abdominal wall, a loop of the anastomosis tubing was placed subcutaneously to allow subsequent easy access. The diazepam (5 mg/kg)/diethyl ether anesthesia was then interrupted. From 12–16 hr before abdominal surgery and throughout the remainder of the experiment, these animals were given chloramphenicol (50 mg·kg⁻¹·day⁻¹) and cholic plus dehydrocholic acid (at 10 and at 2.5 mg·kg⁻¹·day⁻¹, respectively) orally. (ii) After emptying the gall bladder, as described above, the bile duct of diazepam-treated, pentobarbital-anesthetized (30 mg/kg, i.p.) animals was cannulated (1.1-mm o.d. polyethylene tubing). The animals were kept anesthetized under a heating lamp for 3 hr. Ovalbumin at 0.2 mg/kg was then given i.v. to pentobarbital-anesthetized animals prepared according to either surgical method (i) or (ii). Animals were rechallenged with ovalbumin at 2 mg/kg after 2.5 hr if the initial dose was insufficient (see experiment III, Table 2). Animals prepared according to surgical method (i) received LY 171883 sodium salt (a LTD₄/LTE₄ receptor antagonist) at 30 mg/kg, i.v. 30 min before challenge (23), whereas pyrilamine maleate at 1 mg/kg, i.v. was given at the time of antigen administration in surgical method (ii). Guinea pigs without clear signs of anaphylactic reaction (respiratory distress, impaired breathing, cyanosis, and micturition) were not used. Bile was collected under argon in ice-cold methanol/water, 9:1 (vol/vol), containing 1 mM HTMP and 0.5 mM EDTA (pH 7.4). Bile volumes were determined gravimetrically, and the methanol concentration was adjusted to 80% vol/vol. After 3 hr at 0°C, samples were centrifuged (10 min at 10,000 × *g*, 4°C). Aliquots (0.5 ml) of the supernatants were evaporated (at 37°C) to dryness, dissolved in 0.42 ml of methanol/water, 3:7 (vol/vol), and subjected to RP-HPLC after addition of [³H]₈LTD₄ (315 pCi, 6.6 fmol) as a standard.

HPLC. RP-HPLC was performed on Radial-Pak C₁₈ columns (5-μm particles, 8-mm i.d.) equipped with C₁₈ μBondapak precolumns (Waters Associates). An ultraviolet light absorption detector (Spectromonitor II, Laboratory Data Control) was used to monitor the 280-nm absorbance of the effluent. The mobile phase consisted of methanol/water/acetic acid, 64:36:0.1 (vol/vol), containing 1 mM EDTA (pH 6.2, adjusted with NH₄OH; solvent I). The flow rate was 2 ml/min, and the column recovery of LTD₄ was 84%. For ion-pair RP-HPLC a C₁₈ Nucleosil column (Macherey & Nagel, Düren, F.R.G.) (4.6 × 250 mm, 5-μm particles) was used. The mobile phase consisted of methanol/water/acetic acid, 70:30:0.1 (vol/vol), containing 2.5 mM pentanesulfonic acid (solvent II; cf. ref. 24). The flow rate was 1 ml/min. RP-HPLC fractions for radioimmunological analysis were immediately neutralized with 1 M K₂CO₃ and stored at -20°C under argon for up to 5 days.

Determination of Radioactivity. Packard Tri-Carb, models 3385 or 4450, liquid scintillation spectrometers equipped with automatic external standardization were used.

Radioimmunoassay. The assay procedure provided by New England Nuclear was used. The LTC₄ antibody used cross-reacted with leukotrienes and analogs as follows: LTC₄, 100%; LTD₄, 44%; LTE₄, 6%; LTC₄ sulfone, 11%; LTD₄ sulfone, 8%; 5-hydroxyicosatetraenoic acid, 0.04%; and leukotriene B₄, 0.003% (relative percent, molar basis at 50% binding). The lower detection limit was 50 fmol for LTC₄ and 100 fmol for LTD₄. LTC₄ and LTD₄ standard curves were constructed using freshly RP-HPLC-purified standards. Neutralized RP-HPLC fractions (0.6–1 ml) from bile samples, which coeluted with [³H]LTD₄ standard, were evaporated to dryness and dissolved in radioimmunoassay buffer. Statistical significance was determined by Student's *t* test.

Enzymatic Modification of Endogenously Formed LTD₄. Deproteinized bile (0.4 ml, containing 315 pCi [³H]LTD₄ as standard) collected 0–30 min after antigen challenge (experiment III, Table 1) was evaporated to dryness, dissolved in 0.45 ml of methanol/water, 3:7 (vol/vol), and fractionated by RP-HPLC (solvent I). Aliquots (0.8 ml) of the fractions collected were used to determine radioactivity. Fractions coeluting with [³H]LTD₄ (20 pmol according to radioimmunoassay) were evaporated, dissolved in 0.05 ml of 0.1 M Tris-HCl (pH 8.0), 10 mM MgCl₂, 8 mM glutathione containing γ-glutamyltransferase at 0.2 mg/ml, and incubated for 30 min at 37°C (20). One volume of methanol/acetic acid, 9:1 (vol/vol), was added; the mixture was evaporated, redissolved in methanol/water, 3:7 (vol/vol), and subjected to RP-HPLC. Fractions were analyzed by radioimmunoassay as described above.

RESULTS

Elimination and Metabolism of LTC₄. Following i.v. administration of [³H]₈LTC₄ to two bile duct-cannulated guinea pigs, 26 and 38% of the radioactivity was excreted in bile after 15 min, 36 and 49% was excreted after 30 min, 43 and 57% was excreted after 60 min, and 55 and 66% was secreted after 5 hr. In one animal, urine contained 7%, liver contained 1.8%, kidney contained 0.6%, lung contained 0.05%, heart contained 0.03%, and spleen contained 0.02% of the administered radioactivity 5 hr after administration.

RP-HPLC analyses of bile indicated extensive conversion of LTC₄ to predominantly LTD₄ (Fig. 1 Upper). Subsequently, the relative amount of LTD₄ decreased, whereas that of polar metabolites (retention times, 2–7 min) increased (Fig. 1 Lower). Five minutes after [³H]₈LTC₄ administration, 47% of the excreted radioactive material cochromatographed with

FIG. 1. HPLC of bile collected 0–5 min, 15–30 min, 1–2 hr, and 3–4 hr after i.v. administration of [³H]₈LTC₄. Samples were deproteinized in 80% (vol/vol) methanol and separated by RP-HPLC (solvent I). The arrows indicate retention times of coeluted unlabeled leukotriene standards. N-AcLTE₄, N-acetyl-LTE₄.

synthetic, unlabeled LTD₄, and 21% cochromatographed with LTC₄ (Fig. 2). After 30 min, the polar metabolites constituted 42%, LTD₄ was 33%, LTE₄ was 16%, and LTC₄ was 7.5% of the radioactivity; and, after 2 hr, 70% of the excreted material was due to polar metabolites (Fig. 2). Fig. 3 shows the cumulative excreted amount of each radioactive component in bile vs. time. After 15 min about half of the finally excreted radioactivity had been eliminated: 15% of the injected dose was LTD₄, 9% was polar metabolites, 6% was LTC₄, and 1% was LTE₄. The corresponding values after 30 min were 18% for LTD₄, 12% for polar metabolites, 7% for LTC₄, and 2.4% for LTE₄; and after 3 hr values were 20% for LTD₄, 19% for polar metabolites, 8.5% for LTC₄, and 4.2% for LTE₄ (Fig. 3).

Endogenous Generation of LTD₄ During Anaphylactic Shock. Analyses of biliary cysteine-containing leukotrienes during anaphylactic shock were performed by RP-HPLC/radioimmunoassay in five experiments (Table 1). As reported for the rat (18), bile duct surgery was accompanied by appearance of endogenous cysteine-containing leukotrienes in guinea pig bile. The prechallenge levels of LTD₄ (Table 2) were 12 ± 2 nM in bile duct cannulated (experiments I–III, mean $\pm$ SEM) compared to 8.5 ± 0.5 nM in anastomosis animals (experiments IV–V, $P < 0.2$). These values were sufficiently low not to interfere with the determination of biliary LTD₄ after anaphylactic challenge (Table 2). Surgery method (i) frequently led to losses of animals due to wound infections, postsurgical adhesions, and ileus, whereas method (ii) did not introduce these complications.

Anaphylaxis was elicited by i.v. administration of a low dose of antigen. In most animals, this led to development of clear signs of shock as evidenced by breathing difficulties, cyanosis, and micturition. Responding animals usually survived 20–60 min after challenge. If an animal survived for 2.5 hr, a higher dose of antigen was given (see experiment III, Table 2). This resulted in shock and subsequently death of the animal. Guinea pigs that did not respond even to the higher

FIG. 2. Relative biliary radioactivity composition at different times after [³H]LTC₄ administration. Samples were separated by RP-HPLC as shown in Fig. 1. The percentage of each metabolite relative to total radioactivity in the sample is plotted vs. time. PM, polar metabolites.

FIG. 3. Cumulative excretion of biliary [³H]leukotriene metabolites. The total accumulated excretion of each metabolite (Fig. 2) is plotted as a function of time after [³H]₈LTC₄ administration. PM, polar metabolites.

ovalbumin dose were not included in the investigation. Bile samples were collected during the entire survival period after antigen challenge and for at least 30 min before challenge. The prechallenge bile samples provided control levels of leukotrienes for each animal.

Bile samples from experiment I (Table 1) were subjected to RP-HPLC, and all fractions collected were analyzed by radioimmunoassay (Fig. 4). An analysis of prechallenge bile (Bottom) shows only low and evenly distributed immunoreactivity. Corresponding analyses of two bile samples (0–15 and 15–45 min after challenge; Middle and Top, respectively) had a major immunoreactive component of the same retention time as the [³H]₈LTD₄ standard. Integration of these chromatograms indicated biliary LTD₄ concentrations of 108 nM and 116 nM, respectively. Radioimmunochromatographic analyses of bile collected 0–15 min after challenge in experiments III and V (Table 1) gave similar results. Since both the results shown in Fig. 3 and the analyses of endogenous biliary leukotrienes in the three experiments described above indicated that LTD₄ was the major LTC₄ metabolite in guinea pig bile, radioimmunological analyses of bile samples in two subsequent experiments focused only on the LTD₄

Table 1. Experimental conditions for analyses of leukotriene formation during anaphylactic shock

Exp.	Body weight, kg	Immunization method	Surgery method	LY171-883	Pyridamine
I	0.44	ii	ii	–	+
II	0.40	ii	ii	–	+
III	0.38	i	ii	–	+
IV	0.50	i	i	+	–
V	0.54	i	i	+	–

+, Administered. –, Not administered.

Table 2. Bile flow and endogenous LTD₄ excretion in bile before and after anaphylactic challenge

Experiment	Collecting period, min	Bile flow, $\mu\text{l}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$	LTD ₄	
			Conc. in bile, nM	Excretion rate in bile, $\text{pmol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$
Prechallenge				
I	30	93	15	1.40
II	30	65	10	0.65
III*	60	105	10	1.05
IV	60	92	9	0.83
V	60	59	8	0.47
Postchallenge				
I	15	30	108	3.19
II	15	35	58	2.03
III*	30	52	82	4.32
IV	30	38	74	2.81
V	15	33	106	3.53

*Two challenges were performed. Data given are before and after the second challenge.

region of the RP-HPLC chromatograms. The position of this region was determined using a trace amount of [³H]LTD₄ as standard. The results of these analyses showed a basal LTD₄ concentration in the -30- to 0-min prechallenge bile of 10 ± 1 nM (mean $\pm$ SEM, $n = 5$). Bile collected 0-15 min (experiments I-III and V) or 0-30 min (experiment IV) after

FIG. 4. Radioimmunochromatograms of biliary cysteine-containing leukotrienes in sensitized guinea pigs before and after antigen challenge. Deproteinized samples, corresponding to 0.1 ml of bile, collected 0-30 min before (Bottom) and 0-15 min (Middle) or 15-45 min after immunological challenge (Top) were analyzed by RP-HPLC/radioimmunoassay.

triggering the immunological event contained 86 ± 10 nM LTD₄ (mean $\pm$ SEM, $n = 5$, $P < 0.001$, Fig. 5B). The bile flow decreased to about half, from 83 ± 9 before to 38 ± 4 $\mu\text{L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ after antigen challenge (mean $\pm$ SEM, $n = 5$, $P < 0.01$, Fig. 5A), and the LTD₄ excretion rate rose 3.6-fold from 0.88 ± 0.16 before to 3.18 ± 0.38 $\text{pmol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ after antigen challenge (mean $\pm$ SEM, $n = 5$, $P < 0.002$, Fig. 5C).

Identification of Endogenous LTD₄ by Enzymatic Transformations to LTC₄ and LTE₄. Part of the pooled LTD₄-containing fractions from RP-HPLC fractionated bile 0 to 30 min after the challenge in experiment III were treated with an enzyme preparation containing γ -glutamyltransferase and dipeptidase. Glutathione was also added to force the former reaction in its reverse direction (cf. ref. 20). Analysis of the reaction products by RP-HPLC and radioimmunoassay revealed two immunoreactive components of the same retention times as LTE₄ and LTC₄. These results provided further evidence that the endogenously excreted leukotriene was LTD₄.

DISCUSSION

To analyze LTC₄ formation *in vivo* knowledge on the metabolism and excretion routes of this compound is required. During the past several years, the metabolism of LTC has been investigated *in vitro* and *in vivo* in several species (for review see ref. 15). Biliary/fecal excretion predominated in several rodents [mouse (25), guinea pig (20), and rat (17-19, 26, 27)] as well as in bile duct-cannulated monkey (28), whereas urinary excretion was more prominent than fecal excretion in man (16).

LTC₄ metabolism in the guinea pig was investigated with the aim of identifying suitable metabolites for analyzing leukotriene formation during anaphylaxis. Animals were pretreated with dexamethasone to prevent leukotriene formation in response to the surgical manipulations by inhibiting precursor fatty acid release. This improved the experimental situation markedly. The structures of two fecal LTC₄ metabolites in the rat have been found to be *N*-acetyl-LTE₄ and *N*-acetyl-11-*trans*-LTE₄ (17). LTE₄ was identified as a urinary LTC₄ metabolite in man (16) and a biliary metabolite in the monkey (28). The present results demonstrated that LTD₄ is a major biliary LTC₄ metabolite in the guinea pig.

The respiratory, cardiovascular, and microcirculatory effects of LTC₄ and LTD₄ mentioned above suggest that they can produce the major consequences of acute systemic

FIG. 5. Effects of antigen challenge on bile flow (A), biliary LTD₄ concentration (B), and biliary LTD₄ excretion rate (C). Open bars, prechallenge values; closed bars, postchallenge values. The bars represent mean values $\pm$ SEM from five experiments. The differences between values before and after challenge were significant. $P < 0.01$ (A); $P < 0.001$ (B); and $P < 0.002$ (C).

anaphylaxis (11–14). It has also been documented that inhibitors of leukotriene formation can prevent several anaphylactic responses (29–31). The purpose of this investigation was to develop a suitable animal model, which would enable the demonstration of *in vivo* leukotriene synthesis during systemic anaphylaxis. Due to the hepatobiliary elimination of LTC₄ in the guinea pig, bile was chosen for these measurements. To avoid interference by trauma-induced leukotriene formation (see above), a bilio-duodenal anastomosis was implanted 2 days before ovalbumin challenge. The analytical results were satisfactory, but the death rate was high due to infections, postsurgical adhesions and ileus. This necessitated pretreatment with chloramphenicol and bile flow stimulating agents (cholic and dehydrocholic acid). An alternative approach, consisting of bile duct surgery followed by a 3-hr interval before antigen challenge, proved to be more successful with a failure rate (lethality during and after the operation) close to zero. Pyrilamine maleate treatment in this method increased the survival time after challenge suggesting that histamine receptor interaction contributed to the lethal effect of antigen challenge. Bile, collected before and after intravenous challenge with ovalbumin, was analyzed by RP-HPLC and radioimmunoassay. LTD₄ was identified as the major immunoreactive component in anaphylactic guinea pig bile. The analyses showed an 8.6-fold increase in LTD₄ concentration in bile after challenge compared to bile before the challenge. Antigen provocation was also accompanied by decreased bile flow (to about half) as observed in rats undergoing endotoxin shock (19). In spite of the reduced bile flow, the LTD₄ excretion rate had increased 3.6-fold after antigen challenge of sensitized guinea pigs. The observation that LTD₄ is the predominant endogenous leukotriene metabolite in guinea pig bile was in good agreement with the metabolic tracer studies performed as part of this investigation and in obvious contrast to results obtained in rats and monkeys. In the latter species *N*-acetyl-LTE₄ (17, 27) and LTE₄ (28), respectively, were the dominating LTC₄ metabolites. It is interesting that LTD₄ predominates in the guinea pig since LTD₄ is more active than LTC₄, *N*-acetyl-LTE₄, and LTE₄ on several biological parameters (32, 33) and because guinea pigs are especially sensitive to exogenous cysteine-containing leukotrienes (34) as well as to conditions that induce endogenous synthesis of these compounds such as trauma and anaphylaxis. Intravenous administration of LTC₄ or LTD₄ (1 nmol/kg) evoked widespread plasma extravasation in the guinea pig (marker, Evans blue) (35) and a 10-fold higher dose produced shock-like states in artificially ventilated guinea pigs (36). In spontaneously breathing guinea pigs, 2 nmol of LTD₄ per kg i.v. can be lethal (S.-E. Dahlen, personal communication). Intraatrial injection of 0.1 nmol of LTC₄ per kg produced marked cardiovascular effects in man (37).

The present analyses demonstrate that LTC₄ is endogenously produced in guinea pigs after immunological challenge and suggest that the amounts formed during the initial 30-min period may approach 1 nmol/kg. This figure is based on the LTD₄ excretion rate in experiment III (129.6 pmol·30 min⁻¹·kg⁻¹), the HPLC recovery of LTD₄ (84%), and the excretion of i.v. administered [³H]₈LTC₄ as biliary LTD₄ (18% during 30 min).

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