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COMMENTARY

INTERRELATIONSHIPS BETWEEN XENOBIOTIC METABOLISM AND LIPID BIOSYNTHESIS

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There has always been great interest in the relationship between the metabolism of foreign compounds and the pathways of intermediary metabolism. In the 19th century, the first biosynthetic reactions to be discovered were the principal conjugation reactions of xenobiotics [1, 2] and studies on foreign compound metabolism gave great insights into the biochemistry of carbohydrates, lipids and amino acids [1, 3, 4]. In the 20th century, "drug metabolism" has developed as a distinct activity in its own right, reflecting the increasing need to study the biological fates of a multitude of synthetic chemicals. As a consequence, the subject is increasingly viewed as part of pharmacology and toxicology, and links with "conventional" biochemistry have become weaker. This was reinforced by the discovery of drug-metabolizing enzymes, notably the cytochrome P-450 system, which initially had apparently little or no role in the metabolism of endogenous compounds [5]. However, the progress of time has shown that virtually all the enzymes of drug metabolism have important roles in the fate of a variety of endogenous substrates.

Over recent years, there has been considerable interest in links between drug metabolism reactions and aspects of lipid biochemistry. Attention has focused particularly upon oxidative processes, those of lipid peroxidation and co-oxygenation. Lipid peroxidation is now recognized as an important mechanism of toxicity [6] and may be induced by: (1) autoxidation of drugs, e.g. adriamycin [7] and paraquat [8]; (2) drug-derived radicals produced during oxidative metabolism, e.g. CCl_4 and other haloalkanes [9]; and (3) reactive oxygen species (superoxide anion radical, hydroxyl radical, singlet oxygen and hydrogen peroxide) produced during enzymic oxidation by cytochrome P-450, xanthine oxidase and other systems [10]. Additionally, it is now appreciated that under certain circumstances co-oxygenation-involved oxidized lipid species may be important in drug oxidation, and this has been shown with peroxide intermediates of prostaglandin biosynthesis [11], and with peroxides of polyunsaturated fatty acids produced within the microsomal membrane by a peroxidative action of cytochrome P-450.†

Together with these, a number of reports have appeared in the literature which, taken together, suggest very strongly that important relationships may occur between certain novel conjugation reactions of foreign compounds and the processes of lipid biosynthesis. Table 1 lists these novel conjugation reactions according to xenobiotic substrate and reaction type, together with the principal location of the product in the body or excreta. In this brief review, these various reactions will be described, and their possible significance commented upon.

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† R. W. Estabrook, personal communication.

Table 1. Novel conjugates of xenobiotics involving aspects of lipid biochemistry

Functional group	Reaction	Product found in
Aldehyde	Acrylic acid synthesis (addition of 2-carbon unit)	Urine
Carboxylic acid	Addition of single 2-carbon unit	Urine
	Addition of multiple 2-carbon units	Tissue lipids
	Incorporation into triglycerides	Tissue lipids
	Chain elongation and incorporation into triglycerides	Tissue lipids
	Carnitine conjugation	Urine, muscle
Alcoholic hydroxyl	Fatty acid esterification	Tissues, urine, faeces

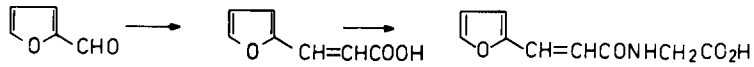

Fig. 1. The "acrylic acid synthesis" of furylacryloylglycine from furfural.

Addition of a 2-carbon unit to an aldehyde

The only report of the addition of a 2-carbon unit to an aldehyde is that of Jaffé and Cohn [12], dating from 1887, who examined the fate of furfural in the rabbit. These workers isolated furylacryloylglycine from urine, and proposed that this metabolite arose from a Perkin synthesis, furfural being converted to furfuracrylic acid which was then conjugated with glycine. This reaction sequence is shown in Fig. 1. Further work by Jaffé and Levy [13] showed the formation of furylacryloylglycine from furfural by dogs, but not hens. However, confirmation of this very early report must await further study: it is noteworthy that Paul *et al.* [14] were not able to detect this unusual metabolite of furfural and the "acrylic acid synthesis" does not occur in the metabolism of a number of other aromatic aldehydes [15–17].

Addition of a 2-carbon unit to a carboxylic acid

The literature contains three accounts of the metabolism of carboxylic acids undergoing addition of a 2-carbon unit to the carboxyl group. In 1976, Miyazaki *et al.* [18] described four novel metabolites of 5-(4′-chloro-*n*-butyl)-picolinic acid (compound I in Fig. 2), all of which arose from the addition of a 2-carbon fragment. These were the β-keto acid, $Ar.CO.CH_2COOH$ (Ia), the α,β-unsaturated acid $Ar.CH{=}CH.COOH$ (Ib), its saturated analogue, $Ar.CH_2.CH_2.COOH$ (Ic) [Ar = 5-(3′-carboxy-*n*-propyl)-pyridine, the product of oxidative loss of the side-chain chlorine atom] and the saturated acid $Ar'.CH_2.COOH$ (Id), where Ar′ = 5-(carbooxymethyl)-pyridine, the β-oxidation product of the saturated acid (Ic). The metabolites were characterized by mass spectrometry, and were found in the urines of rats, guinea pigs, mice and human volunteers receiving compound I. A study with ^{14}C-labelled drug showed that some 50% of the 0–24-hr urinary radioactivity of rats was in the form of these four metabolites.

Takemoto [19] has given a preliminary account of the metabolism of 5-*n*-butylpicolinic acid (compound II in Fig. 2), the deschloro analogue of I. The α,β-unsaturated acid (Ib) was a minor urinary metabolite of II in rats.

The simplest aromatic carboxylic acid, benzoic acid (compound III in Fig. 2), has been used by us as a probe compound in studies of conjugation reactions in the horse [20]. Using both ^{14}C and deuterium isotope labelled benzoic acid, it was found that in addition to the expected major metabolite hippuric acid (benzoylglycine), some 2% of the dose was excreted in the urine as 3-hydroxy-3-phenylpropionic acid and acetophenone [21]. Evidence has been presented that the latter arose from decarboxylation of the β-keto acid, 3-keto-3-phenylpropionic acid, during workup [22]. Further work has shown that 3-hydroxy-3-phenylpropionic acid is a minor (1–2% of dose) urinary metabolite of benzoic acid in the rabbit, rat and guinea pig.* The use of deuterium-labelled benzoic acid has permitted the demonstration that these novel metabolites are presented endogenously in horse urine.

No mechanistic studies have been performed on the chain elongation of xenobiotic acids by the addition of 2-carbon units. However, we [21] have drawn attention to the similarity between this reaction of xenobiotic acids and the reactions of fatty acid biosynthesis. It is proposed that the xenobiotic acyl CoA interacts with either acetyl or malonyl CoA to give the β-ketopropionyl CoA. This chain-elongated CoA could thus give rise to the β-keto propionic acids found in urine and be converted to the β-hydroxy, α,β-unsaturated and propionic acids found as metabolites of xenobiotic acids.

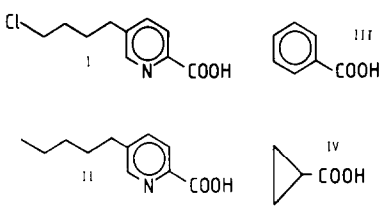

Fig. 2. Carboxylic acids forming hybrid fatty acids by addition of 2-carbon units to the carboxyl group: (I) 5-(4′-chloro-*n*-butyl)-picolinic acid, (II) 5-*n*-butylpicolinic acid, (III) benzoic acid, (IV) cyclopropanecarboxylic acid.

Addition of multiple 2-carbon units to a xenobiotic carboxylic acid

Quistad and colleagues have made extensive studies of the fate of the miticide cycloprate (hexadecyl cyclopropanecarboxylate). This ester is hydrolysed to cyclopropanecarboxylic acid (compound IV in Fig. 2) which participates in various reactions apparently involving fatty acid metabolism. After administration of cycloprate labelled with ^{14}C in the cyclopropanecarboxylate moiety to rats, there was considerable deposition of radioactivity in the tissues, in the form of fatty acid metabolites [23]. These were all ω-cyclopropyl fatty acids, presumably arising from the sequential addition of several 2-carbon fragments to the carboxyl group of IV, and comprised 11-cyclopropylundecanoic, 13-cyclopropyltridecanoic and 15-cyclopropylpentadecanoic acids.

The term "hybrid fatty acids" is proposed to cover such metabolites in which a xenobiotic acid has undergone chain elongation.

* M. Varwell Marsh, J. Caldwell and R. L. Smith, unpublished data.

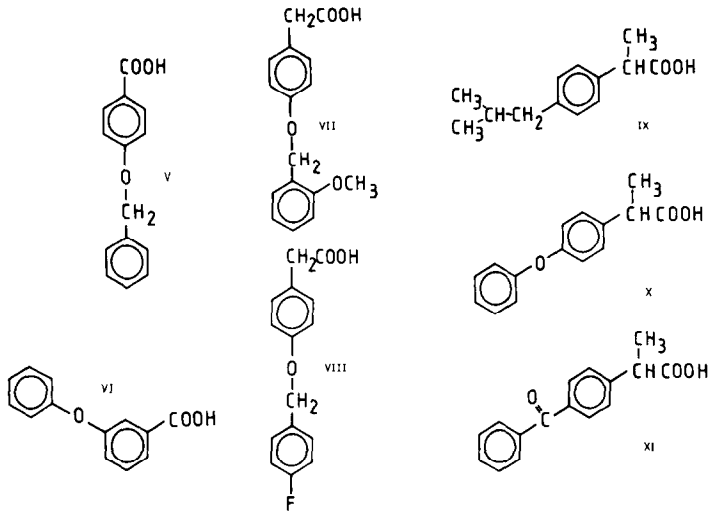

Fig. 3. Carboxylic acids incorporated into hybrid triglycerides: (V) 4-benzyloxybenzoic acid, (VI) 3-phenoxybenzoic acid, (VII) 4-(2′-methoxybenzyloxy)-phenylacetic acid, (VIII) 4-(4′-fluorobenzyloxy)-phenylacetic acid, (IX) ibuprofen, (X) fenoprofen, (XI) ketoprofen.

Incorporation of a xenobiotic carboxylic acid into triglycerides

Two reports in the literature concern the ability of xenobiotic carboxylic acids to behave as fatty acids and become incorporated into triglycerides in the animal body.

4-Benzyloxybenzoic acid (compound V in Fig. 3) as its ethyl ester was administered orally to rats and analysis of liver and adipose tissue showed its esterification into triglycerides [24]. Incorporation into liver triglycerides was highest 30 min after dosing, falling progressively up to 48 hr, but there was marked accumulation in adipose tissue triglycerides over this time-period, such that 48 hr after administration some 70% of the dose was present in adipose tissue [24]. Spectral analysis of the triglycerides showed that three contained the acid (V) esterified with an —OH of glycerol; each had one palmitoyl residue, and the third —OH was esterified with one of oleate, myristate or palmitate. No evidence as to the positions of substitution was forthcoming.

Examination of tissue residues after the oral administration of the pyrethroid metabolite 3-phenoxybenzoic acid (compound VI in Fig. 3) to rats showed that some 2% of the dose was associated with lipids in the skin. Of this, about 10% was present as neutral metabolites characterized as the isomeric triglycerides 2- and 3-(3-phenoxybenzoyl)-dipalmitin. Upon chronic administration, the proportion of these neutral metabolites rose to 60% of the compound-related material in the skin [25].

The term "hybrid triglycerides" is proposed to describe triglycerides containing both fatty acid and xenobiotic acid residues.

The formation of mixed triglycerides of 4-benzyloxybenzoic acid has been demonstrated in rat liver slices, adipocytes and jejunal rings [24]. In the liver, glycerol, palmitate and monosteoylglycerol all served as hybrid-triglyceride precursors, while in adipose tissue, pyruvate was a precursor. In the jejunum, glycerol and monoleoylglycerol were incorporated into the hybrid triglyceride. Using glycerol and palmitate as precursors, other xenobiotic acids have also been shown to be incorporated into hybrid triglycerides by rat liver slices, including 4-(2′-methoxybenzyloxy)- and 4-(4′-fluorobenzyloxy)-phenylacetic acids (compounds VII and VIII in Fig. 3), both candidate hypolipidaemic drugs, and the anti-inflammatory agents, ibuprofen, fenoprofen and ketoprofen (compounds IX–XI in Fig. 3). It is to be assumed in each case that the xenobiotic acids are converted to their CoA thioesters, which are the intermediates in their transfer by relatively non-specific enzymes into hybrid triglycerides.

Incorporation of hybrid fatty acids into triglycerides

The chain elongation of cyclopropanecarboxylic acid yielding mixed fatty acids has been described earlier. The administration of [^{14}C]cycloprate to dogs results in the accumulation of cyclopropanecarboxylate-related radioactivity in various tissues [23]. Four days following administration 6% of dose was in the adipose tissue, 6% in the skin, 2% in the liver and 7% in the kidney. In each tissue, ω-cyclopropyl hybrid fatty acids were present, these being esterified to hybrid triglycerides in adipose tissue and skin and to mixed phospholipids in the liver and kidney.

Similar hybrid triglycerides have been reported in the milk of cows dosed with cycloprate [26]. Of a total of 6% of dose present in the milk, the majority (52–76%) was present as hybrid triglycerides containing cyclopropanecarboxylic acid and C_5–C_{15} saturated ω-cyclopropyl fatty acids, the principal one

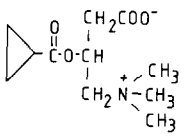

Fig. 4. *O*-(Cyclopropylcarbonyl)-carnitine.

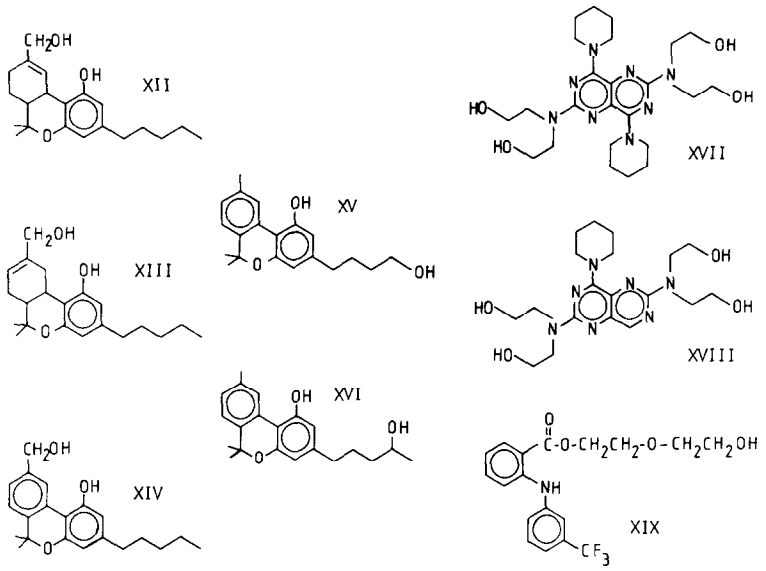

Fig. 5. Xenobiotics undergoing esterification with endogenous fatty acids: (XIII) 7-hydroxy-Δ^1-tetrahydrocannabinol, (XIII) 7-hydroxy-Δ^6-tetrahydrocannabinol, (XIV) 7-hydroxycannabinol, (XV) 5″-hydroxycannabinol, (XVI) 4″-hydroxycannabinol, (XVII) dipyridamol, (XVIII) mopidamol, (XIX) etofenamate.

being 13-cyclopropyltridecanoic acid. The tissue lipids of lactating cows did not contain the cyclopropyl moiety, in contrast to the lipids of the dog and rat.

Conjugation with carnitine

Although not a lipid, carnitine is important in lipid biochemistry as the carrier of fatty acyl residues across the mitochondrial membrane, a process of importance in their synthesis and catabolism [27]. In addition to the lipid incorporation described earlier, the cyclopropanecarboxylate moiety of cycloprate is extensively converted to *O*-(cyclopropylcarbonyl)-carnitine (Fig. 4) in various species.

In the rat, 2% of the urinary ^{14}C (about 1.5% of dose) after cycloprate was in the administration form of the carnitine conjugate [23], while in the dog the conjugate accounted for 58% of urinary ^{14}C (14.5% of dose) [26]. Additionally, in the dog, 56% of the total ^{14}C dose of cycloprate was present in muscle as the carnitine conjugate [23] and in cow's milk 11–39% of total ^{14}C was in this form [26].

Fatty acid esterification of xenobiotics containing an alcoholic hydroxyl group

In recent years, several xenobiotics containing alcoholic hydroxyl groups, either present as such or introduced by metabolism, have been shown to undergo esterification with various fatty acids.

Following either i.p. or i.v. injection of Δ^1- and Δ^6-tetrahydrocannabinol (THC), fatty acid esters of their 7-hydroxymetabolites (compounds XII and XIII in Fig. 5) were detected in the liver, spleen, bone marrow and adipose tissue [28]. The fatty acids involved in this esterification were palmitic, stearic, and the mono- and di-substituted C_{18} acids [29].

More recently the hydroxymethyl (7-hydroxy) (compound XIV in Fig. 5) and side-chain ω and ω-1 hydroxy (5″- and 4″-hydroxy) metabolites (compounds XV and XVI in Fig. 5) of [^{14}C]cannabinol have been shown to undergo esterification with palmitate (minor) and oleate (major) in rats [30], accounting for 3% of the faecal ^{14}C. It has been shown [29] that the fatty acid esters of the 7-hydroxytetrahydrocannabinols are formed in rat liver microsomes in the presence of an NADPH-generating system. Control studies showed that the esterification was not an artefact of incubation in workup and was NADPH-dependent.

Similar esterifications occur in the metabolism of pyridopyrimidines, dipyridamol (compound XVII in Fig. 5) and mopidamol (compound XVIII in Fig. 5), the fatty acid esters being found in the faeces [31]. With dipyridamol, the esterification involved oleic, palmitic and linoleic acids; only the first two were encountered with mopidamol. Some 80% of the dose of both drugs was eliminated in the faeces of the rat and man, and the novel conjugates accounted for about 5% of the faecal material.

Etofenamate (compound XIX in Fig. 5) was metabolized by dogs to a small extent (<1% of dose) to fatty acid esters found in urine and faeces, involving oleic, palmitic, linoleic, stearic, palmitoleic, myristic and lauric acids [32].

Possible consequences of metabolic interrelationships between xenobiotics and lipids

Since the occurrence of metabolic interrelationships between xenobiotics and lipids has only recently been recognized, it is not surprising that their functional significance remains the subject of speculation. Three possible consequences of these interrelationships may be suggested: (1) establish-

ment of tissue residues of xenobiotics, (2) disturbances of lipid biochemistry, and (3) altered membrane function.

Unlike the majority of metabolites of xenobiotics, the products of the various reactions described here are very hydrophobic, non-polar molecules, which will be found in the lipid-rich tissues of the body rather than in the excreta. Their biological half-lives are expected to be extremely long, and it is possible that such residues may remain in the body for the lifetime of the organism. Due to their extremely poor water solubility, fatty acid esters of xenobiotics, and thus the tissue residue, may provide long-lasting depots of slowly mobilized drug. Such mobilization of a fatty acyl bound drug may be responsible for the known "flashbacks" of the effects of cannabinoids observed some time after drug use [28]. This is analogous with the use of fatty acid esters as long-acting prodrugs of psychotropic agents and steroids [33].

It is doubtful that the quantity of a xenobiotic metabolized along one of these lipid-related pathways is sufficient to disturb aspects of lipid biochemistry by means of depletion of an essential intermediate. However, it is known that lipid synthesis and catabolism is subject to complex allosteric and feedback regulation [34]. The formation therefore of even a small amount of a lipid-related xenobiotic metabolite may be sufficient to disturb these regulatory mechanisms. It is of interest to note that the conversion of various hypolipidaemic carboxylic acids to hybrid triglycerides is apparently not related to their hypolipidaemic action, but it is proposed that their acyl CoAs, the presumed intermediates in this, disrupt normal lipid metabolism [35].

Last, but by no means least, lipid-related xenobiotic metabolites, notably the hybrid triglycerides, have the potential to alter membrane structure and influence membrane-dependent processes. Fatty acid esters and hybrid fatty acids may be "dissolved" in membranes, and by steric effects associated with the xenobiotic moiety, disturb normal membrane structure. Similar effects would occur if hybrid triglycerides were incorporated into a membrane. Additionally, the hybrid triglycerides may be associated with disordered membrane function. Biological membranes exist in dynamic equilibrium, with rapid interchange of fatty acyl residues within the lipid bilayer, the so-called deacylation–reacylation cycle [36]. Hybrid triglycerides could slow down such an interchange, as 4-benzyloxybenzoyl-containing triglycerides are resistant to lipoprotein lipase and hormone-sensitive lipase, although they are cleaved slowly by a non-specific esterase activity [24].

Concluding remarks

Recent studies have revealed the occurrence of important links between the conjugation of xenobiotics and aspects of lipid biochemistry. These are especially interesting for xenobiotic carboxylic acids, which may be incorporated into fatty acids and triglycerides by biosynthetic mechanisms. Although ideas concerning the significance of the reactions described here must remain speculative, they are noteworthy in view of increasing reports of toxicity of carboxylic acids, e.g. clofibrate and benoxaprofen. Undoubtedly, the effort involved in the characterization of the various novel conjugates of xenobiotics described has revealed new possibilities in drug metabolism, with potential toxicological significance.

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