The fate of pristane (2,6,10,14-tetramethylpentadecane), a widespread isoprenoid hydrocarbon, has been studied in rats after a single per os administration of \textsuperscript{3}H-labeled pristane. The balance study showed an extensive fecal excretion (66\%) mainly as unchanged hydrocarbon, whereas about 14\% of ingested pristane was excreted in urine as pristane metabolites and tritiated water. After one wk, 8.3\% of the ingested \textsuperscript{3}H still was stored in the carcass, as about 14\% of ingested pristane was excreted in urine, with fecal excretion (66\%) mainly as unchanged hydrocarbon, whereafter a preferential incorporation into adipose tissue and liver. Over 75\% of the radioactivity stored in the carcass was associated with pristane metabolites and tritiated water. Tissue metabolites were characterized by thin layer chromatography, gas chromatography and mass spectrometric analyses. Four metabolites were identified: pristan-1-ol, pristane-2-ol, pristanic acid and 4,8,12-trimethyltridecanoic acid. These demonstrate that this isoprenoid hydrocarbon undergoes subterminal hydroxylation or terminal oxidation followed by the classical \( \beta \)-oxidation process. Incorporation of metabolites in phospholipids and more particularly in the phosphatidylserine fraction has been observed and is discussed.


Much of the information on the fate of saturated hydrocarbons in vertebrates deal with the metabolism and disposition of n-alkanes; less has been reported for branched-alkanes. Among the latter, pristane (2,6,10,14-tetramethylpentadecane) and phytane, both of which may be of either biogenic or petrogenic origin, usually are abundant in living matter. Pristane was found at concentrations of 80-1070 ppm in fish oil (1) and 2-52 ppm in human and mammal tissues (2).

As early as 1967, these residues were considered as resulting from the difference between the rates of input and removal, thus it was suggested that branched-chain hydrocarbons are not inert in the animal tissues but are metabolized rapidly (2). It was reported several years later that rats are able to oxidize phytane to alcohols and acids (3); nevertheless, no information was available regarding biotransformation of pristane in higher animals.

The purpose of this study was to establish the metabolic balance of pristane in rats and to characterize pristane metabolites isolated from the whole body and the liver. Furthermore, because unusual long chain alcohol or branched-chain fatty acids may result from the \( \omega \)-oxidation of pristane, as described for microorganisms (4,5), incorporation of metabolites into lipid compartments was investigated.

**MATERIALS AND METHODS**

**Chemicals.** Pristane was purchased from Aldrich-Chimie (Strasbourg, France). \([\textsuperscript{3}H]\)Pristane (142 MBq/\( \mu \)mol) was prepared by direct contact of pristane with tritium gas (Wilzbach method) at the C.E.A. (Saclay, France), then purified by chromatography on a silica gel column (Kieselgel 60, Merck), using hexane as solvent. The purity, as evaluated by gas chromatography (GC) and reverse phase high pressure liquid chromatography (HPLC), was higher than 96\%.

Tetramethylpentadecanoic acid was the gift of J. Planz (INRA, CNRZ, Jouy en Josas, France).

**Animal experiments.** Wistar male rats weighing about 200 g were accustomed for one wk before studies to a semisynthetic diet (casein 18\%, wheat starch 39\%, sucrose 24\%, peanut oil 8\%, cellulose 3\%, minerals and vitamins 8\%). The night before the experimental diet was given, the animals were fed moderately.

(a) Experiment 1: metabolic balance. Three animals were held in individual metabolism cages. At the beginning of the experiment, each animal received a 59.2 MBq \([\textsuperscript{3}H]\)pristane dose previously incorporated in the peanut oil of the diet. After complete consumption of the impregnated feed, free access to the untreated semisynthetic diet again was given to the animals.

Urine and feces were collected separately and daily for seven consecutive days. A few drops of HCl (1 N) were added to urine for storage. Animals were killed on the seventh day after dosing, and various tissues and organs were sampled.

After careful removal of the gastrointestinal tract contents, the remainder of the carcasses were minced using a domestic machine. Feces and aliquots of homogenized carcass were saponified with ethanolic KOH, then the unsaponifiable fraction was extracted with hexane. Hydrocarbons were isolated by column chromatography on activated silica by hexane elution.

Urine analysis was performed by successive extractions with hexane and ethyl acetate.

(b) Experiment 2: metabolites identification and incorporation of \textsuperscript{3}H into lipids. Administration of labeled pristane (20 MBq) occurred as described in experiment 1, except that before the incorporation of the hydrocarbon in the peanut oil, the specific radioactivity of \([\textsuperscript{3}H]\)pristane was adjusted to 278 KBq/\( \mu \)mol with unlabeled pristane (Aldrich-Chimie).

Lipid and metabolite analyses were performed as described in the following paragraphs.

**Hepatic lipid analysis.** The distribution of radioactivity in hepatic tissues was studied after lipid extraction according to the procedure of Folch (6). Total lipids were separated into particular classes on silica cartridges (Sep-Pak, Waters, Milford, MA) by successive hexane, chloroform and methanol elutions, yielding hydrocarbon, neutral lipid and phospholipid fractions, respectively (7). Phospholipids then were separated into different components by HPLC (8). Purity of fractions was tested by thin layer chromatography (TLC) on Silica Gel G using chloroform/methanol/acetic acid/water (100:55:16:4, v/v/v/v) as solvent system.

**Metabolites analysis.** (a) Isolation procedures. Livers were homogenized in 10 ml of water with a Polytron homogenizer at room temperature. The same procedure...
was used for carcasses, after careful removal of the gastrointestinal contents. Homogenates were saponified with ethanolic KOH; then the unsaponifiable fraction was extracted with hexane. Hydrocarbons and unsaponifiable metabolites were isolated from this fraction by chromatography on a silica gel column (Kieselgel 60, Merck, Darmstadt, FRG) by successive hexane and chloroform/methanol (2:1, v/v) elutions. After acidification by HCl, fatty acids were extracted with hexane from the aqueous phase. A flow chart of the different steps is summarized in Figure 1.

(b) Analytical procedures. (1) TLC: Kieselgel 60, 250 μm chromatographic plates (Merck, Darmstadt, Germany) were used with hexane/ethyl ether/acetic acid (80:20:1, v/v/v) as the solvent system. Radioactive areas were located with a radiochromatogram scanner LB2832, Berthold (Wildbad, FRG). (2) GC: A Hewlett Packard 5710A gas chromatograph equipped with a 2 m × 1/8" stainless steel column packed with 3% Dexsil 300 on 100/120 Supelcoport (Supelco Inc., Bellefonte, PA) was used, programmed from 120 C to 340 C at 10 C/min. For methyl ester analyses by radio-GC, the gas chromatograph was connected to a reactor (RGC 170 Bodenseewerk Perkin-Elmer Co. Ueberlingen, Germany), in which tritium gas was measured with a proportional counting tube after hydrogenative cracking of the 3H-labeled compounds. (3) GC/MS: A Hewlett Packard model 5992 B gas chromatograph/mass spectrometer equipped with a 15 m × 0.2 mm flexible fused silica column with OV1 coating (Hewlett-Packard) was used, programmed from 150 C to 250 C at 10 C/min and with a He (carrier gas) flow rate of 1 ml/min.

Derivatization. Fatty acid methyl esters were obtained from acids by esterification with methanol/benzene/sulfuric acid (17:2:1, v/v/v) for three hr at 96 C.

Fatty alcohols, having been extracted (fraction II) and purified by TLC, were acetylated with acetic anhydride/pyridine (4:1, v/v) at ambient temperature during three hr. Trimethylsilyl (TMS) derivatives were prepared by treating the various compounds with BSTFA + 1% TMCS or with BSTFA/TSIM/TMCS (100:50:1, v/v/v) mixtures (Pierce Chemical Co., Rockford, IL) in anhydrous acetonitrile for four hr at 70 C.

Radioactivity measurements. Aliquots of urine samples were counted directly by liquid scintillation counting (Packard Minaxi 4000 apparatus). The total radioactivity of tissues, organs and carcasses was measured after combustion of aliquots (200-500 mg) in an oxidizer (Oxymat, Intertechnique, Kontron, Trappes, France) that ensures trapping of 3H2O in a scintillation mixture (Triox, Kontron).

Estimation of tritiated water was obtained by comparing the radioactivity in fresh and lyophilized samples after it has been established that no loss of unchanged hydrocarbon or metabolites occurred during the freeze-drying process.

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**FIG. 1.** Schematic diagram for the analysis of tissues from rats dietary exposed to [3H]pristane.