of the individual phospholipid classes are similar to those given by Spencer and Dempster for white adipose tissue of mouse. Phosphatidylcholine and phosphatidyethanolamine are the major compounds, as in many other tissues.

The incorporation of \(^{32}P\) into the different phospholipid fractions followed a fairly similar pattern in vitro and in vivo (Table 1). Phosphatidylinositol, phosphatidylcholine and lysophosphatidylcholine showed substantial labeling, while the other compounds were relatively inert. In the in vivo experiments incorporation took place mainly between 4 and 8 h after the injection of the label and activity reached a maximum at about 12 h. But the phosphatidylserine and sphingomyelin fractions were still almost cold at 8 h and their activity continued to rise up to 20 h, at least.

It is necessary to emphasize that the present data are valid only for the bulk phospholipid fractions of total adipose tissue and for the metabolic renewal rate of the phosphorus moiety. It has been shown that the different parts of the phospholipid molecule can exhibit markedly different turnover rates, indicating that the labeling represents a net effect of de novo synthesis and exchange reactions. A detailed description of the metabolic behavior of phospholipids is not possible without using different labeled precursors and fractionation of cellular subcomponents.

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Synthesis of 2,6,10,14-Tetramethyypentadecanoic Acid (Pristanic Acid)

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Since the discovery of Klenk and Kahlke in 1963 that patients suffering from Refsum's disease accumulate large stores of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), considerable effort has been expended in understanding the biochemical defect in this inherited disease. By studying the metabolism of 3,6-dimethyloctanoic acid we have shown that apart from the ordinary \(\beta\)-oxidation and \(\omega\)-oxidation, an "alternative pathway" exists in mammals for the degradation of branched-chain fatty acids. The demonstration of 2,5-dimethylheptanoic acid as a metabolite shows that an initial \(\alpha\)-decarboxylation most likely takes place. In accordance with such an interpretation of the "alternative pathway", F. B. Shorland, New Zealand (personal communication) has demonstrated conversion of phytanic acid into 2,6,10,14-tetramethylpentadecanoic acid (pristanic acid) upon administration of phytanic acid to rats. In view of these results we have undertaken the synthesis of pristanic acid, and studies on its metabolism are in progress.

Pristanic acid, which recently was isolated and identified as a trace constituent in butter-fat was first synthesized in 1948 from phytophyl in five steps. In the present method pristanic acid is obtained by "stepping down" phytanic acid by one carbon atom, according to the following scheme:

\[
\text{R-CH}_2\text{NH}_2 \quad \xrightarrow{\text{Schmidt rearrangement}} \quad \text{R-CH}_2\text{COOH}
\]

\[
\text{R-CH}_2\text{COOH} + \text{HNO}_2 \rightarrow \text{R-CH}_2\text{OH}
\]

\[
\text{R-CH}_2\text{OH} + \text{Cr}_2\text{O}_7^{2-} \rightarrow \text{R-COOH}
\]

Phytanic acid

Pristanic acid
Experimental. Phytanic acid (5 g, prepared as previously described) was dissolved in chloroform (35 ml) and mixed with concentrated sulphuric acid (25 ml) and sodium azide (8 g). After refluxing the mixture for 5 h, the chloroform was removed by suction, and the residue made alkaline with sodium hydroxide. The liberated amine which was extracted with petroleum ether, remained as a pale brown, oily liquid after removal of the solvent (yield 3 g). The corresponding amine sulphate could readily be prepared; it was a white solid, soluble in ethanol but insoluble in acetone and water, m.p. 201°.

To a solution of the free amine (3 g) in 20% acetic acid — 1% HCl (100 ml) was added sodium nitrite (0.8 g in 5 ml of water). After 15 min at room temperature the evolution of nitrogen had ceased. The oil which separated was extracted with petroleum ether, and the ether extract was washed with dilute sodium hydroxide and then with water. After removal of the solvent, a pale yellow oil remained (yield 2 g). Gas chromatographic analysis demonstrated that the product consisted of several components other than the expected product. No attempt was made however, to purify the fatty alcohol further before the oxidation step.

The crude product (2 g) was oxidized with chromic acid in acetic acid, and pristane acid was isolated using the same method as previously described for synthesis of phytanic acid. (Yield of crude pristane acid 0.7 g). Gas chromatographic analysis of the crude acid (methylated) showed one major peak, comprising about 70% of the sample and four smaller peaks. The contaminating substances could be removed by preparative thin layer chromatography of the crude pristane acid on plates of Silica gel G, using petroleum ether, b.p. 60°–80°:diethyl ether: methanol:acetic acid (90:10:3:1) as solvent. The remaining acid was about 99% pure estimated by gas chromatography of the methyl ester. This ester had relative retention time 0.63 (methyl phytanate =1) at 185° on 6 ft × 1/8 in. columns of 8% BDS on Chromosorb W. Mass spectrometric analysis of the purified product (as methyl ester) on a LKB-9000 instrument showed a parent ion at m/e 312, and with large fragments at m/e 88, 101, 129, 157, 222, and 281. The mass spectrum of our product was in all parts identical to that of methyl pristane recently published by Hansen and Morrison.

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Studies of Isobenzothiophenes

I. On the Structure of the Products Formed from 2-Aryl-4,4-diphenyl-1,3-oxathiolan-5-ones and Concentrated Sulfuric Acid

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On treatment of various 2-aryl-4,4-diphenyl-1,3-oxathiolan-5-ones (I) with conc. sulfuric acid and dilution with water, Bistrzycki et al. isolated yellow compounds formulated as 9,10-endosulfido-9,10-dihydroanthraene (II).

\[
\begin{align*}
\text{C}_8\text{H}_5\text{N} & \text{S} \quad \text{II} \\
\text{C}_8\text{H}_5 & \text{S} \quad \text{S} \quad \text{S} \quad \text{S} \\
\text{O} & \text{O} \\
\text{H} & \\
\text{R} & \\
\end{align*}
\]

Later, Dufraisse et al. showed by comparison of the Raman spectra of 1,3-diphenylsobenzofuran and that of the product from I (R = C_2H_4) that the latter probably should be formulated as 1,3-diphenylsobenzothiophene (III, R = C_2H_4).

Almost twenty years later, however, Hartough reported that the primary products of Bistrzycki et al. had the struc-