# ENZYMATIC DECARBOXYLATION OF THE ALPHA-HYDROXY ACIDS BY BRAIN MICROSOMES*

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In a recent study (Mead and Levis, 1962, 1963) evidence was found that the 20- to 26-carbon fatty acids of the brain sphingolipids may be degraded by a one-carbon or alpha-oxidation system. Following intraperitoneal injection of carboxy-labeled acetate, the 24-carbon hydroxy and normal fatty acids contained almost all the radioactivity in the odd carbons whereas the 23-carbon acids were labeled largely in the even carbons. These findings led to proposal of the following outline for the degradative pathway, which would account for the formation of the odd-chain and $\alpha$-hydroxy acids of the brain sphingolipids:

$$CH_3\text{-}(CH_2)_{21}\text{-}CH_2\text{-}COOH \xrightarrow{(1)} CH_3\text{-}(CH_2)_{21}\text{-}CHOH\text{-}COOH$$

$$\xrightarrow{(2)} CH_3\text{-}(CH_2)_{21}\text{-}COOH \longrightarrow \text{etc.}$$

In order to further test the validity of such a scheme, the postulated enzymes have been sought in preparations from rat brain. The reaction discussed in the present communication concerns equation (2), oxidative decarboxylation of the $\alpha$-hydroxy acids.

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## METHODS

The enzyme system used consisted of the fraction of rat brain homogenates sedimenting between 18,000 and 104,000 x g plus the non-dialyzed supernatant from this centrifugation, ATP, DPN, tween 20 and tris buffer.* Substrates consisting of various carbon-14 carboxy-labeled or tritium chain-labeled fatty acids dissolved in 0.01 N NaOH were incubated for 1-2 hours with the enzyme in the conventional Warburg apparatus at 37° in the presence of air.

The assay of enzyme activity depended on the type of substrate used. For the carboxy-labeled acids, the $C^{14}O_2$ liberated after acidification was collected in KOH in the center wells of the flasks and was precipitated, washed and counted as $BaC^{14}O_3$.** For the tritium-labeled acids, following incubation, the contents of the flask were extracted with $CHCl_3$-MeOH (2:1) and separated by silicic acid-column chromatography into normal and hydroxy acids. These were removed from the plates and were counted in the scintillation counter** and chromatographed (as methyl esters) on a Loenco-Cary Model 70 Hi-Flex Gas Chromatograph*** which permitted identification of the labeled components.

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*The preparation and properties of this enzyme system will be described in detail in a subsequent communication.

**Counting was performed on a Packard TRI-CARB Liquid Scintillation Spectrometer. $BaC^{14}O_3$ was counted as a suspension in the thixotropic gel with an efficiency of 52 ± 0.1%. Tritium compounds were counted with an efficiency of 15%.

***Separations were carried out on a 3.3 foot, 0.25 inch stainless steel column with 17% EGS on 60/80 mesh chromosorb W.

## RESULTS AND DISCUSSION

Table I indicates the substrate specificity of the reaction. The hydroxy acids are readily decarboxylated whereas the normal acids are not attacked.*

TABLE I

Substrate Specificity of Alpha Hydroxy Acid Decarboxylase

The incubation medium contained Tween 20, 2 mg; microsomal fraction, 0.05 mg N; supernatant fraction, 1.3 ml and the following in micromoles per total volume of 2.5 ml: tris buffer (pH 7.5), 37.5; ATP, 5; DPN, 4. Substrates added were (in micromoles): Stearic acid-1-$C^{14}$, 0.2 (40,000 c/min/μM); 2-hydroxystearic acid-1-$C^{14}$, 0.12 (40,000 c/min/μM); tricosanoic acid-1-$C^{14}$, 0.1 (75,000 c/min/μM); and 2-hydroxytricosanoic acid-1-$C^{14}$, 0.1 (75,000 c/min/μM).

| Substrate | c/min in $BaCO_3$ (-background) |
|---|---|
| $C_{18}$ | 0 |
| $C_{18:OH}$ | 226 |
| $C_{23}$ | 3 |
| $C_{23:OH}$ | 269 |

In a separate experiment, the following substrates were used (in micromoles): 2-hydroxystearic acid-1-$C^{14}$, 0.2 (244,000 c/min/μM); 2-ketostearic acid-1-$C^{14}$, 0.2 (198,000 c/min/μM).

| | |
|---|---|
| $C_{18:OH}$ | 2,400 |
| $C_{18:0}$ | 11,120 |

In Figure 1 is shown a chromatograph indicating that the product of decarboxylation of α-hydroxystearic acid is margaric acid. No other radioactive normal acid appeared in the reaction mixture in appreciable concentration.

*Stearic acid-1-$C^{14}$ and -9,10-$H^3$ were purchased from the New England Nuclear Corp. and were purified by gas chromatography. The preparation of the α-hydroxy acids has been described previously (Fulco and Mead, 1961). The α-keto acids were prepared by the method of Gunstone (1952). Tricosanoic acid-1-$C^{14}$ was prepared by the method of Sugimoto, Fujita, Shigematsu and Ayda (1961).

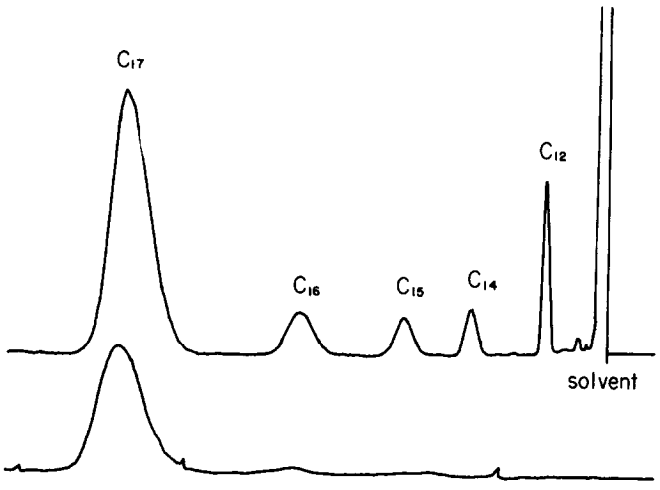


Fig. 1. Per cent composition and radioactivity of normal acids obtained by silicic acid column chromatographic separation of products from incubation of α-hydroxystearic acid-9,10-$H^3$ (160 μc/μM) under the conditions described in the text and in Table I.

Upper curve — relative amounts of fatty acids (including inactive $C_{17}$ added after incubation).

Lower curve — radioactivity.

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Since this reaction evidently involves an oxidation as well as a decarboxylation, an incubation was carried out with α-ketostearic acid-1-$C^{14}$. The ready decarboxylation of this acid (Table I) supported the concept that it is an intermediate in the degradation of the hydroxy acid. Further evidence on this point was obtained from an experiment in which α-hydroxystearic acid-9,10-$H^3$ was incubated as described above but in the presence of 1.5 μM of inactive α-ketostearic acid. As can be seen in Figure 2, activity appeared not only in the margaric acid as before (no carrier margaric acid was added in this case) but also in the α-ketostearic acid separated chromatographically. The finding that the presence of relatively large amounts of the keto acid did not inhibit the incorporation of activity into the margaric acid casts doubt on the role of the free keto acid as an intermediate in the scheme. However, the fact that the keto acid is very rapidly decarboxylated by the enzyme system may mean that the true

intermediate is an enzyme-bound complex which releases a small amount of the radioactive acid to equilibrate with the added keto acid.

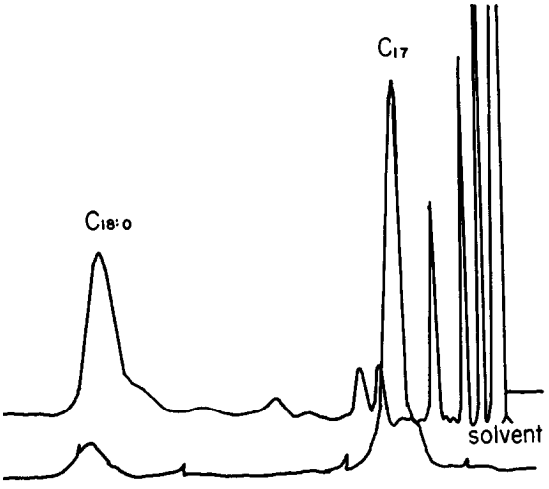


Fig. 2. Per cent composition and radioactivity of normal and keto acids obtained by silicic acid column chromatographic separation of products from incubation of α-hydroxystearic acid-9,10-$H^3$ under the conditions described in the text and in Table I with the addition of 1.5 μM of α-ketostearic acid ($C_{18:0}$).

Upper curve — relative amounts of fatty acids (including inactive α-ketostearic acid added after incubation).

Lower curve — radioactivity.

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The finding in brain microsomes of an enzyme that carries out the decarboxylation of α-hydroxy acids lends strong support to the hypothesis, based on studies in the whole animal, that the very long-chain acids of the brain sphingolipids are degraded by a one-carbon or alpha-oxidation mechanism. Moreover, the finding that the α-keto acids are also decarboxylated by this system and that they are formed during decarboxylation of the α-hydroxy acids provides evidence for the following steps in the reaction: $CH_3\text{-}(CH_2)_n\text{-}CHOH\text{-}COOH \longrightarrow [CH_3\text{-}(CH_2)_n\text{-}CO\text{-}COOH] \longrightarrow CH_3\text{-}(CH_2)_n\text{-}COOH + CO_2$.

Present studies on this subject include separation and further characterization of the oxidative and decarboxylative steps, location and characterization of the $\alpha$-hydroxylation reaction and the search for alpha oxidation in other tissues.

The finding in the brain of a degradative system for long-chain fatty acids functioning not as a source of energy but, more likely as a disposal mechanism, coupled with the increasing importance of this system with aging (Kishimoto and Radin, 1959) make the solution to these problems intriguing and important.

## REFERENCES

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