

## ALPHA OXIDATION OF THE BRAIN FATTY ACIDS\*

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Recently Fulco and Mead (1961) presented evidence that the long-chain  $\alpha$ -hydroxy acids of the brain sphingolipids are formed by  $\alpha$ -hydroxylation of the completely synthesized corresponding unsubstituted acids. Actually the acids investigated were all 24-carbon acids, which were selected as most important and representative. The odd-chain acids were not studied since, in agreement with the findings of Kishimoto and Radin (1959b), they are not present in sufficient amounts in the very young animals used in the study. The odd-chain acids could be considered as the result either of synthetic or degradative processes. For the former case, the objection could be raised that even though Hajra and Radin (1962) have found that the odd-chain acids may be labeled with injected radioactive propionate, if propionate were actually present in sufficient quantities for the synthesis of the very long-chain acids, the 15- and 17-carbon fatty acids should form a considerably larger fraction of the total. For the second alternative, no one-carbon degradation has been reported in animal tissues although Stumpf and co-workers (1962) have demonstrated such a system in plants.

To investigate the biogenesis of these fatty acids, six 15-day-old rats were given intraperitoneal injections of 2 millicuries each of carboxy-labeled sodium acetate. Rats of this age were used since only during the myelination

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period can sizeable amounts of radioactivity be incorporated into the brain lipids with any permanence. However, since Kishimoto and Radin (1959b) have shown that only the older brains contain large quantities of odd-chain fatty acids, the rats were kept for 5 months before use. The cerebrosides were separated from the brain lipids of these rats by silicic acid chromatography (Fulco and Mead, 1961) and the cerebroside fatty acids, obtained by hydrolysis, were separated into hydroxy and normal fractions by silicic acid chromatography (Fulco and Mead, 1961) and into saturated and unsaturated fractions by chromatography of the mercuric acetate complexes (Kishimoto and Radin, 1959a).

The saturated hydroxy acids were fractionated by gas chromatography\* of their acetoxy methyl esters, and the  $C_{23}$  and  $C_{24}$  fractions were collected and, after saponification, were diluted with the appropriate synthetic acids for degradation.

The carboxy carbons were obtained as barium carbonate following permanganate oxidation of the hydroxy acids. The resulting unsubstituted acids were degraded by the method of Dauben, et al. (1953) yielding carbons 2 and 3 of the  $C_{23}$  acid and carbons 2, 3 and 4 of the  $C_{24}$  acid as benzoic acid, and the remainder as arachidic acid in each case.

The radioactivities of all fractions are presented in Table I and the calculated percentages of the total radioactivity are depicted below.

	$C_{23}$	$CH_3-(CH_2)_{19}-CH_2-CHOH-COOH$			
Per cent of total activity			2.8	5.6	0.3
	$C_{24}$	$CH_3-(CH_2)_{19}-CH_2-CH_2-CHOH-COOH$			
Per cent of total activity			1.0	5.3	0.6 9.3

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\* Preparative gas chromatography was performed on a Wilkens Instrument Co. A-100 Aerograph apparatus with a 0.5 in. x 5 ft. column of SE-30 silicone stationary phase, 10 per cent on a chromosorb w. support. All fatty acids were checked for purity using a Barber-Colman Model 10 apparatus with a 3 ft. column of SE-30, 0.29 per cent on glass beads.

TABLE I

Radioactivities of Lipid Fractions of Rat Brains  
and Degradation Products of the Hydroxy Acids

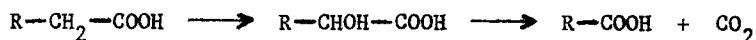
Fraction	Specific dis/sec/mg	Activity <sup>*</sup> dis/sec/mm
Total lipid	$3.04 \times 10^2$	
Cerebroside fraction	$1.75 \times 10^2$	
Cerebroside total acids	$3.57 \times 10^2$	
Saturated $\alpha$ -hydroxy acids	$3.06 \times 10^2$	
$\alpha$ -hydroxy Tetracosanoic acid (diluted and counted as acetoxymethyl ester)		$9.43 \times 10^3$
Carboxy carbon		$8.8 \times 10^2$
Benzoic acid (carbon 2)		$5.65 \times 10$
" " (carbon 3)		$4.98 \times 10^2$
" " (carbon 4)		$9.24 \times 10^{**}$
Arachidic acid (carbons 5-24)		$7.5 \times 10^3$
$\alpha$ -hydroxytricosanoic acid (diluted and counted as acetoxymethyl ester)		$2.39 \times 10^3$
Carboxy carbon		7.17
Benzoic acid (carbon 2)		$1.35 \times 10^2$
" "		$6.71 \times 10^{**}$
Arachidic acid (carbons 4-23)		$2.28 \times 10^3$

\* Barium carbonate was counted in a Nuclear D-47 Micromil Thin Window Flow Counter with efficiency of  $17 \pm 0.1\%$ . All other samples were counted in a Packard Tricarb 314 EX Liquid Scintillation Counter with efficiency of  $61.8 \pm 0.1\%$ . Counts from the two counters were compared with standards.

\*\* Corrected for overdegradation (Mead and Howton, J. Biol. Chem., 229, 575 [1957]).

It is obvious that, as was shown previously and confirmed by these experiments, the label from carboxy-labeled acetate is incorporated into the odd carbons of the even-carbon fatty acids. The odd-carbon fatty acids, on the other hand, contain the label in the even carbons. No explanation can be advanced at present for the low activity in  $C_2$  of the 23-carbon acid  $C_3$  of the 24-carbon acid and for the high activity in  $C_3$  and  $C_4$  of these acids. However, it is possible that some synthesis of odd-chain acids from propionate

originally contributed to the total as proposed by Hajra and Radin (1962). The logical explanation for this finding as well as for the presence of the odd-chain and  $\alpha$ -hydroxy acids lies in the proposal of an  $\alpha$ -oxidation system involving the hydroxylation and oxidative decarboxylation of the acids:



This system may be similar but certainly not identical with that described by Stumpf (1962). Its significance may lie in its ability to degrade the very long chain acids to a chain length which can be attacked by the  $\beta$ -oxidation system. Since its products appear to accumulate with age, it may also have some connection with the aging process and with the breakdown of myelin.

Further properties of the system and the mechanism of hydroxylation and decarboxylation are under active investigation in This Laboratory.

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