OXIDATION OF THE 4α-METHYL GROUP OF METHOSTENOL TO CARBON DIOXIDE BY THE RAT, IN VITRO AND IN VIVO*

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Received January 24, 1961

The 4α, 4β and 14α methyl groups of lanosterol labeled biologically with C^{14} from acetate-2-C^{14} and squalene-C^{14} were shown to be converted in high yield to $C^{14}O_2$ by rat liver homogenates (Olson and Bloch, 1957). Methostenol (4α-methyl-Δ^7-cholesten-3β-ol) has been found to be an intermediate in the biosynthesis of cholesterol in the rat (Wells and Lorah, 1959; Wells and Lorah, 1960; Frantz, et al., 1960). In the conversion of methostenol to cholesterol, the methyl group at the 4α position is lost and the double bond at C-7 is shifted to the C-5 position. The introduction of C^{14} specifically into the 4α-methyl position of methostenol was possible by reacting Δ^7-cholesten-3-one with methyl-C^{14} iodide (Wells and Neiderhiser, 1957), and thus the fate of a single methyl group (4α) could be followed metabolically. In agreement with the results of (Olson and Bloch, 1957), evidence is presented here that the 4α-methyl group of methostenol is converted to CO_2 by the rat in significant amounts.

4α-Methyl-C^{14}-Δ^7-cholesten-3β-ol was prepared as described previously (Wells and Neiderhiser, 1957; Neiderhiser and Wells, 1959) except that isotopic methyl-C^{14} iodide** was employed instead of the unlabeled reagent. The crude sterol product was purified by silicic acid:Celite (3:1) chromatography; m.p. 143-4°, specific radioactivity 9,000 c.p.m./mg. Radio-

*This investigation was supported by a research grant (H-2458,C4) from the National Institutes of Health, U. S. Public Health Services.

**Volk-Radio Chemical Company, Chicago, Illinois.

activity was measured with a thin end window gas flow counter*, and mass was determined colorimetrically by the Liebermann-Burchard reaction (Moore and Baumann, 1952).

Homogenates were prepared from the livers of decapitated, male rats of the Long-Evans strain (av. wt. 225 g.). Livers were homogenized with 2.5 volumes of chilled medium (Bucher, 1953) in a loosely fitting stainless steel homogenizer for approximately thirty seconds. The resulting mixture was centrifuged (700 x g., for 10 minutes at 0°) and the supernate retained. Substrate was prepared by dissolving approximately 10 mg. of methostenol-4α-methyl-C^{14} in 2 ml. of warm propylene glycol. Incubations of the substrate with the homogenate and recovery of CO_2 were conducted essentially as described by (Olson and Bloch, 1957).

Male rats of the Holtzman strain weighing about 100 g. were given an emulsion of methostenol-4α-methyl-C^{14} in 15% Tween-80** by stomach tube. Each rat was housed in a metabolic cage for 24 hours and fed a commercial chow diet and water, ad libitum. Three post-absorbers, each containing 250 ml. of 2N NaOH, were placed in series. Total 24 hour radioactivity in the carbon dioxide was determined as described above. The animals were sacrificed with ether, and were separated into three portions; (I) carcass, (II) liver, lungs and spleen, and (III) intestine and feces. Each portion was saponified with 250 ml. of 20% KOH in 85% ethanol for 4 hours. The saponified mixtures were extracted with diethyl ether, and the ether extracts washed with water. Radioactivity of the nonsaponifiable fraction was determined by counting a suitable aliquot of the ether extract. Saponifiable fractions were combined and the total radioactivity determined.

Rat liver homogenates incubated for 3 hours at 37° with methostenol-4α-methyl-C^{14} released labeled CO_2 in amounts equivalent to 1.4-3.0% of the

*Tracerlab, Inc., Cambridge, Massachusetts.

**Polyoxyethylene sorbitan monooleate, Atlas Powder Company, Wilmington, Delaware.

added radioactivity (Table I). Carbon dioxide isolated from control flasks contained no detectable radioactivity.

In the 24 hour period after administration of methostenol-4α-methyl-C^{14} to three male albino rats, an average of 20.2% of the radioactivity was found in the expired CO_2 (Table II). Carbon dioxide with the highest concentration of radioactivity was collected between 4 and 6 hours after administration of the labeled sterol (3,558 c.p.m./hr. vs. 1,880 c.p.m./hr. for the last 18 hours). A significant amount of the administered radioactivity was found in the total saponifiable fraction while the major part of the remainder of the C^{14} was located in the intestines and feces as unchanged substrate.

Isolation of a cholesterol precursor possessing 29 carbon atoms, 4,4-dimethyl-$\Delta^{8(24)}$-cholestadien-3β-ol (Gautschi and Bloch, 1957) and precursors containing 28 carbon atoms, 4α-methyl-Δ^{7}-cholesten-3β-ol (Wells and Neiderhiser, 1957) and 4α-methyl-Δ^{8}-cholesten-3β-ol (Kandutsch and Russell, 1959) from natural sources suggest the metabolic removal of methyl substituents from the steroid nucleus according to the sequence: 14α, 4β, and 4α. (However, of the last two, the original 4α-methyl could be lost first, followed by inversion of the 4β to the less hindered 4α position). The present report reveals that the final methyl group (4α) lost in the pathway to cholesterol synthesis has the same metabolic fate as the three methyl substituents of lanosterol combined, namely oxidation to carbon dioxide. In the study of lanosterol demethylation, Olson and Bloch (1957) proposed the stepwise oxidation of the methyl groups according to the scheme: $RCH_3 \rightarrow RCH_2OH \rightarrow RCHO \rightarrow RCOOH \rightarrow RH + CO_2$. Pudles and Bloch (1960) have recently shown that synthetic 4-hydroxy methylene-Δ^{7}-cholesten-3-one is readily converted to cholesterol by liver homogenates. Whether this steroid or similar compounds exist in nature remains an interesting possibility. Unidentified sterols more polar than cholesterol have been iso-

Table I

Formation of $C^{14}O_2$ from Methostenol-4α-methyl-C^{14} by Rat Liver Homogenates[1]

Experiment No.[2]	Substrate Added[3] Total Count	$C^{14}O_2$ Released
	c.p.m.	c.p.m.
1	4540	110.9
2	4134	123.0
3	4595	65.3
4	3752	107.6

1 Bucher homogenate supernatant fluid after centrifugation at 700 x g. Incubations were at 37°C for three hours.

2 The values in each experiment represent the average of duplicate or triplicate determinations. Control flasks produced no radioactive CO_2.

3 Methostenol-4-α-methyl-C^{14}, 9,000 c.p.m. per mg. dissolved in propylene glycol.

Table II

The Recovery of Radioactivity from the CO_2 of Rats given Methostenol-4α-Methyl-C^{14} by Stomach Tube

Animal[1] No.	Radioactivity[2] Administered	Expired CO_2 24 Hours
	c.p.m.	c.p.m.
1	154,630	23,770
2	249,580	27,690
3	292,160	89,690

1 Each value was determined from a 100 g. male albino rat of the Holtzman strain.

2 Methostenol-4α-methyl-C^{14} dissolved in 15% Tween 80. Animal number 1 received 17.2 mg. of substrate (9,000 c.p.m. per mg.) and animals 2 and 3 received 26.6 and 31.3 mg. of substrate (9,370 c.p.m. per mg.), respectively.

lated from mammalian tissue (Olson and Bloch, 1957; Wells et al., 1956, designated as zone A). In the course of the study of the sterols of zone A from rat skin, we have recently uncovered at least 2 new fast-acting (Liebermann-Burchard) sterols which are under investigation.

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