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Inhibition of cholesterol biogenesis by arsenite: preparation of labeled lanosterol*†

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SUMMARY

When mevalonic acid-2-C^{14} was incubated with rat liver homogenate with the usual cofactors plus 10^{-3} M arsenite, the formation of labeled cholesterol was inhibited and labeled lanosterol accumulated. This inhibitory effect of arsenite provides a convenient method of preparing labeled lanosterol in high yield (>10%) from commercially available C^{14}-labeled mevalonic acid.

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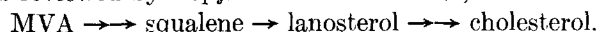
It is well established that the biogenesis of cholesterol follows the following reaction sequence as reviewed by Popják and Cornforth (1):



MVA →→ squalene → lanosterol →→ cholesterol.

Although the steps between MVA and lanosterol have been largely elucidated in the past several years, much remains to be learned about the mechanism of conversion of lanosterol to cholesterol (1). One of the major problems in this area is the difficulty of obtaining labeled or unlabeled lanosterol. We wish to report the specific inhibition of the oxidation of lanosterol by arsenite, and a convenient method of preparing labeled lanosterol based on this effect.

MATERIALS AND METHODS

Racemic MVA-2-C^{14} of specific activity 1.1 μc/μmole was purchased from Tracerlab, Inc. Pure lanosterol was a kind gift of Professor K. Bloch. Rat liver homogenate was prepared and incubated with cofactors as described by Bucher and McGarrahan (2). Extraction and fractionation of nonsaponifiable material, and the determination of radioactivity in the sterols, in MVA and in CO_2 were as previously described (3, 4).

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† The following abbreviations are used: MVA, mevalonic acid; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide.

RESULTS AND DISCUSSION

When MVA-2-C^{14} was incubated under aerobic conditions with rat liver homogenate and the proper cofactors, substantial amounts of labeled CO_2 were formed. Addition of arsenite inhibited the formation of labeled CO_2 without affecting the yield in labeled nonsaponifiable material (Table 1). Chromatography

TABLE 1. EFFECT OF ARSENITE ON THE METABOLISM OF MVA-2-C^{14} IN RAT LIVER HOMOGENATE

Arsenite	CO_2		Sterols	
	Expt. A	Expt. B	Expt. A	Expt. B
	cpm	*cpm*	*cpm*	*cpm*
None	57,000	60,000	189,000	102,800
10^{-4} M	54,000		213,000	
10^{-3} M	5,400	6,000	160,000	90,900

10^6 cpm of racemic MVA-2-C^{14} (1 μmole) was incubated for 3 hours with 5 ml of homogenate. It can be seen that arsenite (10^{-3} M) strongly inhibited $C^{14}O_2$ production without affecting labeled sterol formation.

of the nonsaponifiable material revealed that over 90% of the label was associated with the sterols, and that very little C^{14} was present in the squalene fraction. Since the conversion of MVA-2-C^{14} to lanosterol would not give rise to labeled CO_2, whereas the formation of cholesterol should be accompanied by the formation of radioactive CO_2, it appeared likely that the labeled product was lanosterol. This was confirmed by the following pieces of evidence: (*a*) When labeled

squalene, prepared by incubating MVA-2-C^{14} with rat liver homogenate under anaerobic conditions, was incubated with rat liver microsomes and soluble proteins, both labeled sterol and labeled CO_2 were formed. The addition of arsenite abolished the formation of CO_2 without affecting the yield of sterol (Table 2). (*b*) When the labeled sterol, obtained in the presence of arsenite, was recrystallized with cholesterol, the crystals obtained after two recrystallizations had no radioactivity. (*c*) When the labeled sterol was recrystallized with lanosterol, the radioactivity remained with the crystals after three recrystallizations.

TABLE 2. EFFECT OF ARSENITE ON THE CONVERSION OF SQUALENE TO CHOLESTEROL

Arsenite	CO_2		Sterols	
	Expt. A	Expt. B	Expt. A	Expt. B
	cpm	*cpm*	*cpm*	*cpm*
None	2,720	3,396	46,000	28,400
10^{-4} M	1,120	1,146	34,000	31,000
10^{-3} M	24	207	58,000	30,000

10^5 cpm of squalene was suspended with the aid of 0.5 mg of Tween 80 and incubated for 3 hours with 2 ml of dialyzed rat liver supernatant and microsomes. Addition of arsenite at 10^{-3} M inhibited only $C^{14}O_2$ formation. The squalene was prepared by the incubation of MVA-2-C^{14} with rat liver homogenate under anaerobic conditions. The supernatant and microsomes were obtained by centrifuging liver homogenate for 30 minutes at 8,000 × *g* to remove the mitochondria. The supernatant thus obtained was dialyzed for 3 hours at 2° against 200 volumes of 0.001 M phosphate buffer (pH 7.0).

Based on this inhibitory effector of arsenite, labeled lanosterol can be prepared conveniently from MVA-2-C^{14}. A typical experiment follows: Five milliliters of rat liver homogenate was preincubated with 10^{-3} M arsenite for 1 hour at 0°, and then incubated aerobically for 3 hours at 37° after the addition of 1 μmole of racemic MVA-2-C^{14}, 10 μmoles of DPN^+, and 20 μmoles of ATP. After saponification, extraction, and chromatography on active alumina, the yield of lanosterol was 0.2 μc, or 40% of the maximum theoretical yield.

REFERENCES

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