The *In Vivo* Incorporation of the *S*-Methyl Group of Methionine into Cholesterol in Rats

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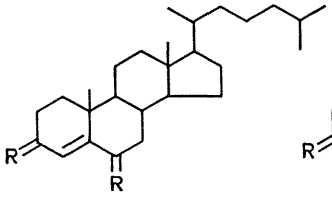
Summary Evidence for the *in vivo* incorporation of the *S*-methyl group of methionine into cholesterol and cholest-7-en-3β-ol in normal and tumorous rats is presented.

THE presence of increased amounts of osteolytic lipids has been noted in the plasma and tumour extracts of patients with breast cancer[1] and in rats with testicular tumours.[2] The osteolytic lipids, of unknown origin,[3] were tentatively identified as phytosterols and their esters. Tumour-bearing humans and rats could possibly retain more phytosterols of exogenous origin, but, if the sterols are biosynthesized *in vivo*, this would imply the presence of a C-24 alkylating system in tumorous patients.

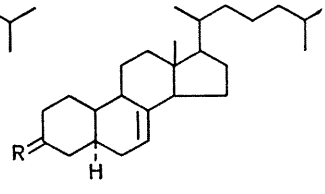
To evaluate whether or not the phytosterols are of endogenous origin, we have administered [^{3}H; ^{14}C] methyl labelled methionine, known to be the source of the C-24 alkyl moiety,[4] to female Fischer rats bearing transplantable R-323OC mammary tumours† (250 μCi of ^{14}C and 2 mCi of ^{3}H into two animals). Control experiments were carried out with normal Fischer rats (125 μCi of ^{14}C and 2·5 mCi per animal). The experiments were designed as an approximation to a pseudo-steady state of the exogenously administered methionine in the animals, which we thought might favour the biosynthesis of phytosterols, if formed. The rats were injected twice daily for seven days with solutions of the [^{3}H;^{14}C]methionine in physiological saline and then sacrificed. The carcasses were digested with 30% KOH and the non-saponifiable fractions recovered with hexane. In each instance 0·05—0·2% of the administered ^{14}C-radioactivity was detected in the neutral lipid fraction. On treatment of the neutral residue from an experiment with a tumorous rat, with digitonin,[5] 70—80% of the radioactivity was precipitated with the reagent and then recovered.

The crude non-saponifiable residue was resolved by t.l.c. [silica gel Merck $HF_{254+336}$ was used throughout; solvent; benzene-EtOAc (7 : 1)] into several fractions. A broad zone with a mobility similar to that of cholesterol and phytosterols (sitosterol, campesterol, *etc.*) contained *ca.* 45% of the recovered radioactivity. The cholesterol–phytosterol zone was fractionated on a partition chromatography system designed to resolve C_{27}-cholesterol-like products from C-24-alkylated sterols.[6] The bulk of the ^{14}C-radioactivity was associated with the fractions enriched in cholesterol. G.l.c. analysis of the ^{14}C-cholesterol-like fraction showed a trace of a non-polar component, cholesterol (**1**), and a compound with a R_T similar to that of (**8**) The 'cholesterol-rich' mixture was resolved chemically.

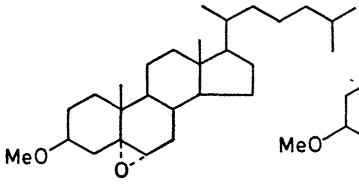
Oxidation of a portion of the cholesterol-like fraction with Jones reagent followed by t.l.c. [hexane–EtOAc (5 : 1)] gave (**2**) and (**3**).[7] Treatment of (**2**) with Zn–AcOH gave (**4**) which on reduction with $LiAlH_4$ gave a mixture consisting mainly of (**5**).



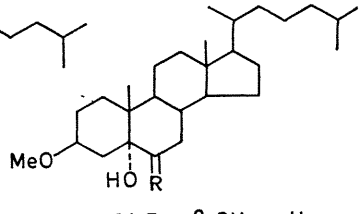
(2) R = O
(4) R = O; No Δ^4; 5α-H
(5) R = ξ-H, ξ-OH; No Δ^4; 5α-H



(3) R = O
(6) R = β-OH, α-H



(8)



(9) R = β-OH, α-H
(10) R = O

† We thank Dr. W. F. Dunning, Cancer Research Laboratory, University of Miami, Florida for these.

Compound (**3**) was diluted with inactive material and reduced with $LiAlH_4$ to (**6**)

Treatment of a portion of the cholesterol-enriched fraction with trimethylorthoformate containing $HClO_4$ gave (**7**), unchanged cholesterol, and (**6**), which was resolved by multiple t l c [hexane–EtOAc (20 : 1)]

Epoxidation of (**7**) with monoperphthalic acid gave (**8**) which was converted[8] into (**9**) Jones oxidation of (**9**) provided (**10**)

The control experiments with normal female Fischer rats were processed similarly

As seen from the Table, the ^{14}C-specific activity and the $^{3}H:^{14}C$ ratio of both transformed metabolites remained essentially unchanged throughout

TABLE

Isotopic content of sterols and their transformation products, biosynthesized from L-*methionine–methyl-*$^{3}H/^{14}C$ *labelled methionine, in rats bearing transplantable* R-323OC *mammary tumours and in normal rats* (*All products were identical to authentic samples*)

	Specific activity[a] (d p m /mg) ^{14}C	$^{3}H/^{14}C$ ratio isotopic	Mass spectral data (M^+, m/e)
(**2**)	231 5	7 81	398
(**4**)	236 4	7 89	400
(**5**)*	199 3	7 32	386[b]
(**7**)	239 5	8 34	400
(**8**)	232 8	8 53	416
(**9**)	218 5	8 42	416[b]
(**10**)	213 5	7 91	432
(**1**)	249 5	8 50	386
(**6**)*	466 1	2 38	386
(**3**)[d]	132 8	2 22	384
(**6**)[d]	120 7	2 33	386
(**2**)[c]	157 9	29 35	398
(**4**)* [c]	147 3	28 68	400
(**3**)[c d]	92 3	6 44	384

* One crystallization owing to lack of material

[a] Except in the cases marked with an asterisk, the recorded specific activities are the average 2—4 sequential crystallizations

[b] $M^+ - 18$ ion

[c] Control experiment

[d] Diluted

As expected, the specific activity of Δ^7-cholestenol was higher than that of cholesterol, but the $^{3}H:^{14}C$ ratio of cholesterol was greater than that of Δ^7-cholestenol If Δ^7-cholestenol is a precursor of cholesterol the $^{3}H \cdot ^{14}C$ ratio of the Δ^7-intermediate would be expected to be similar to that of the derived cholesterol In the tumorous experiment the $^{3}H:^{14}C$ ratio of the cholesterol (*ca* 8 : 1) was about equal to that of the administered $^{3}H:^{14}C$ methionine (8 : 1), while in the control experiment the $^{3}H \cdot ^{14}C$ ratio for cholesterol (29 : 1) was higher than that of the methionine (20 : 1) The significance of the different ratios in normal and cancerous rats is being investigated

To account for the observed variations in the ^{3}H ^{14}C ratios of cholest-7-en-3β-ol and cholesterol an *in vivo* isotope effect could be invoked Alternatively the labelling of edogenous cofactors and/or water (both of which are known to be involved in the biosynthesis of cholesterol) may have taken place and be responsible for observed variations Finally the possibility that incorporation of the carbon and hydrogen atoms of the *S*-methyl of methionine into cholesterol proceeds through different routes should also be considered

It may be concluded that the *S*-methyl group of methionine is involved in the polyprenoid biosynthesis As a first approximation it would seem that the *S* methyl of methionine probably contributes to the acetate pool However, irrespective of the actual mode of the involvement of the *S*-methyl group of methionine in the biosynthesis of polyprenoids, this pathway has not been recognized until now The biological significance of this route has yet to be determined

The work at the Worcester Foundation was supported by grants from the National Office of the American Cancer Society and from its Massachusetts Division, and the National Cancer Institute The work at St Vincent Hospital was supported in part by a contract with the National Cancer Institute

(*Received, July 5th*, 1971; *Com* 1125.)

[1] G S Gordan, T J Cantino, L Erhardt, J Hansen, and W Lubich, *Science*, 1966, **151**, 1226, G S Gordan, M. E Fitzpatrick and W P Lubich, *Trans Assoc Amer Phys*, 1967, **80**, 183

[2] B F Rice A Segaloff R Coleman M Beeler, and A Ochsner, Abstracts Endocrinol Soc Meeting, 1967, **48**

[3] B F Rice *Rec Progress Hormone Res*, 1969, **25**, 310

[4] E Lederer *Quart Rev*, 1969, **23** 453

[5] C H Issidorides I Kitagawa and E Mosettig, *J Org Chem*, 1962, **27**, 4693

[6] S Burstein H Zamoscianyk, H L Kimball N K Chaudhuri, and M Gut, *Steroids*, 1970, **15**, 13 The R-1 system designed for the separation of 24-alkylated sterols from C-27 sterols was used

[7] D J Aberhart and E Caspi *J Biol Chem*, 1971, **246**, 1387

[8] L F Fieser and S Rajagopalan, *J Amer Chem Soc*, 1949, **71**, 3938