COMMUNICATIONS TO THE EDITOR

THE METHYL GROUP OF METHIONINE AS A SOURCE OF C_{28} IN ERGOSTEROL[1]

Sir:

It was reported recently that formate can serve as a source of C_{28} in the biosynthesis of ergosterol[2] or of eburicoic acid.[3] We have incubated cell-free yeast homogenates with labelled sodium bicarbonate, formaldehyde, propionate-(1 or 2)-C^{14} and methionine-methyl-C^{14} and have found that more C^{14} is incorporated into the non-saponifiable lipids and the digitonin-precipitable sterols from methionine than from any of the other compounds. Thus, in one experiment 20.2% of the methionine-methyl-C^{14} radioactivity was found in the non-saponifiable fraction, as compared to 1.2% for acetate-1-C^{14}, 1.0% for $NaHCO_3$, 0.4% for formaldehyde and 0.5% for propionate. In comparative experiments with the same yeast homogenate which we ran after the paper of Danielson and Bloch[2] came to our attention, methionine-methyl-C^{14} gave four times as much radioactivity in the digitonin-precipitable sterol fraction as sodium formate-C^{14}.

To determine whether the radioactivity is concentrated in C_{28}, samples of ergosterol from incubations with acetate-1-C^{14} and methionine-methyl-C^{14} were ozonized according to Hanahan and Wakil.[4] Comparison of the figures obtained with ergosterol made from acetate and from methionine by a yeast homogenate (Table I, Expt. 1) shows that in the

TABLE I

DEGRADATION OF ERGOSTEROL-C^{14}

Expt.	Substance	Source of C^{14}, counts/mmole carbon/min. Acetate-1-C^{14}	Methionine-methyl-C^{14}
1[a]	Ergosterol	5,540	3,843
	Steam volatile fraction[c]	6,220	11,480
	Residue from steam distillation	7,545	655
2[c]	Ergosterol acetate	...	946
	α,β-Dimethylbutyraldehyde[b]	...	1,044
	Acetone[b] ($C_{25,26,27}$)	...	165
	C_{23}	...	110
	C_{28}[d]	...	3,210

[a] The two samples of ergosterol in Expt. 1 are not directly comparable, since much more C^{14} was used in the case of acetate. [b] Isolated as dinitrophenylhydrazone. [c] All samples counted after combustion to $BaCO_3$, corrected to infinite thickness. [d] Combustion of CHI_3 reportedly gives $BaCO_3$ of low specific activity.[5]

first case the steam-volatile fraction, α,β-dimethylbutyraldehyde, contains less radioactivity than the residue, representing the ring system. In the second case this relationship is reversed, as expected if the main incorporation of radioactivity were in C_{28}. In experiment 2, the ergosterol, obtained from incubation of whole yeast with methionine-methyl-C^{14}, although recrystallized to constant specific activity, was subsequently found to be impure. However, the dinitrophenylhydrazone of the α,β-dimethyl-butyraldehyde was carefully purified before counting. Further degradation of this aldehyde shows that a predominant portion of the total radioactivity is located in C_{28}. When the correction factor suggested by Ehrensvard, *et al.*,[5] for the combustion of $C^{14}HI_3$ to $BaC^{14}O_3$ is applied, the observed activity of C_{28} is in acceptable agreement with the figure calculated from the specific activity of the α,β-dimethyl-butyraldehyde. Carbon 28 contains 20–30 times the radioactivity of the other carbon atoms in the molecule. Such a randomization of radioactivity was also observed by Dauben, *et al.*, in the incorporation of C^{14} from formic acid into the analogous position in eburicoic acid.[3]

The major incorporation of C^{14} into C_{28} of ergosterol in our experiments may be accounted for by the well-known oxidation of the methyl group of methionine to formate in biological systems.[6] However, transmethylation from sulfur to carbon, which has not been observed up to now, cannot be excluded since we find that aminopterin decreases the incorporation of C^{14} into ergosterol from formate-C^{14} but not from methionine-methyl-C^{14}. Other experiments indicate that squalene, but not zymosterol, is converted to ergosterol in yeast homogenates.

(1) This investigation was supported by the U. S. Public Health Service Grant No. C321, an Institutional Grant from the American Cancer Society, The Schering Corporation, Bloomfield, New Jersey and the Jane Coffin Childs Memorial Fund.

(2) H. Danielson and K. Bloch, THIS JOURNAL, **79**, 500 (1957).

(3) W. G. Dauben, G. J. Fonken and G. A. Boswell, *ibid.*, **79**, 1000 (1957).

(4) D. G. Hanahan and S. J. Wakil, *ibid.*, **76**, 273 (1953).

(5) G. Ehrensvard, Q. Reio, E. Saluste and R. Stjernholm, *J. Biol. Chem.*, **189**, 93 (1951).

(6) J. S. Fruton and S. Simmonds, "General Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 714.

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASSACHUSETTS
GEORGE J. ALEXANDER
ALLEN M. GOLD
ERWIN SCHWENK

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DIFFUSION OF O^{18} AND OF PROTIUM IN D_2O–H_2O MIXTURES[1]

Sir:

We wish to report some interesting and unexpected results obtained in this Laboratory in the course of studies on diffusion in liquid systems.[2]

The data were obtained for 25 ± 0.02° by means of the diaphragm cell technique.[2] Deuterium analyses were made pycnometrically, and those for O^{18} by the gradient tube density method,[3] after first converting the samples of D_2O^{18}-H_2O^{18} to H_2O^{18}. This last was accomplished by vaporizing

(1) These investigations were supported in part by the Office of Ordnance Research.

(2) A. W. Adamson and R. R. Irani, Abstracts 130th Mtg., Amer. Chem. Soc., Atlantic City, N. J., September, 1956.

(3) A. Hvidt, G. Johansen, K. Linderstrom-Lang and F. Vaslow, *Compt. rend. trav. Lab. Carlsberg*, Ser. Chim., **29**, No. 9.

a few cc. of the mixture to be analyzed, and passing the vapor over hot copper wire; the resulting CuO^{18} was then converted to water and Cu by reaction with hydrogen gas.

Since the O^{18} data yielded essentially self-diffusion coefficients, these are plotted in the figure as points (reproducibility, ± 2%). The protium diffusion data were treated as giving *integral* diffusion coefficients, and the values are given as lines drawn between the average compositions of the upper and lower compartments of the diffusion cell (reproducibility, ± 4%).

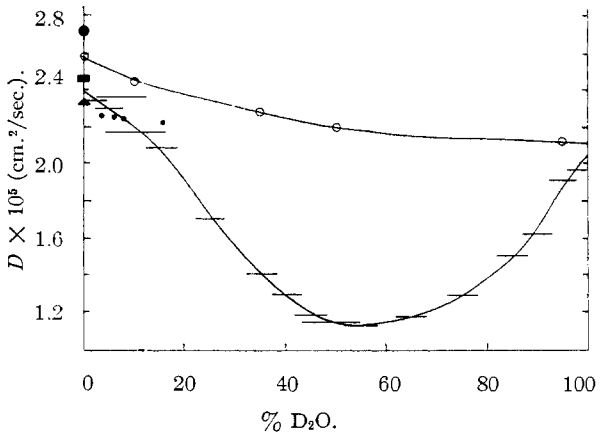

Fig. 1.—Variation of the diffusion coefficient with deuterium enrichment in water mixtures: present work, O O^{18} as tracer, — H_2O–D_2O; Longsworth, ●, H_2O–D_2O; Wang, *et al.*, ● O^{18} as tracer, ▲ H_2O–D_2O, ■ H^3 as tracer.

Considering first the region of low D_2O content, there has been a considerable fluctuation in literature values, but our results are in agreement with those of Wang, *et al.*,[4] and of Longsworth,[5] within experimental error. The point of interest, however, is that while the O^{18} diffusion coefficients varied with composition almost exactly as the inverse of the viscosity, the protium diffusion coefficients showed a marked minimum which, if corrected for the viscosity effect, fell at 50% D_2O content.

The explanation for these findings must be that somewhat different mechanisms are available for oxygen than for protium diffusion. For the former, there is the possibility of oxygen exchange between clusters, and for the latter, there is a variety of ways for a similar exchange to occur, since either long or short protium bonds and either a four or a five coördinated transition state may be involved. In any event, the results cannot be explained in terms of the usual assumption that diffusion in water occurs only by motion of individual molecules as units.

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DEPARTMENT OF CHEMISTRY ARTHUR W. ADAMSON
UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA RIYAD R. IRANI

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SITE OF VANADIUM INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Sir:

Vanadium compounds have been shown to inhibit the incorporation of radioacetate into cholesterol by rat and rabbit liver *in vitro* and *in vivo*.[1,2]

The following compounds have been generally accepted as intermediates in the biosynthesis of cholesterol from acetate: acetoacetate,[3] β-hydroxy-β-methyl glutarate (HMG),[4] β-methyl crotonate (BMC),[5] squalene,[6] lanosterol[7] and zymosterol.[8] Recently β,δ-dihydroxy-β-methyl valeric acid (mevalonic acid)[9] has been proposed as an intermediate because of its extremely efficient rate of conversion to cholesterol. This communication demonstrates that the inhibition by vanadium occurs between the six and five carbon intermediates.

Rat liver slices were incubated for two hours in phosphate buffer[1] containing 10 mg. of sodium acetate-1-C^{14} (1.0 mc./mM.) or 1 mg. of mevalonic acid-2-C^{14} (0.005 mc./mM.).[10] Aliquots of the same batch of slices were used for each experiment. Vanadium in a final concentration of 10^{-3} *M* was added to one flask as diammonium oxytartratovanadate.[10] An equimolar amount of tartrate was added to the control flask. Following incubation of the flasks containing acetate substrate, 30 mg. HMG, 40 mg. BMC, 35 mg. squalene, and 2 mg. of cholesterol were added to each flask as carrier. After saponification in 70% ethanol and 5% potassium hydroxide under nitrogen for one hour, the non-saponifiable fraction was extracted with petroleum ether and from it squalene and cholesterol were isolated by means of an alumina column.[6] The radioactivity of squalene was determined as the hexahydrochloride and cholesterol as the digitonide. The saponifiable fraction was acidified to *p*H 2 with concentrated hydrochloric acid and extracted continuously with ether for 24 hours. The ether was evaporated to dryness and HMG[4] and BMC[5] isolated and their radioactivity determined. Derivatives of each compound retained the calculated amount of radioactivity.

The radioactivity (Table I) of BMC, squalene and cholesterol from acetate as substrate was depressed by vanadium in comparison to the control. The increased radioactivity of HMG in the vanadium inhibited reaction might be expected since it is known that inhibition at any step in a sequence will produce an accumulation of the intermediate just below the inhibition with increased trapping of the radioactive molecules. The radioactivity of cholesterol obtained with mevalonic acid as substrate was also depressed by vanadium. When biosynthetic C^{14}-labeled squalene was employed as

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(10) Generously supplied by Merck Sharp and Dohme.