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.*, **75**, 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

RECEIVED MARCH 16, 1957

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.