the dichloride. The powder diagram was relatively simple and easily could be recognized in mixtures. The pattern was unchanged when the compound was heated *in vacuo* at any temperature up to 700°, regardless of the time of heating. Above 700°, however, a further disproportionation took place, and the tantalum pattern became evident in the powder diagrams with increasing predominance until only the metal remained. In none of the powder diagrams were there lines other than those of tantalum or those attributed to the dichloride. Moreover, none of these products was pyrophoric, but all appeared stable in dry air.

In all of these experiments considerable care was exercised with respect to purity of the reagents, and in the exclusion of oxygen. Nevertheless we were unable to produce the pyrophoric material described by the previous investigators. We have concluded that tantalum subchloride, if it does exist, cannot be prepared by the thermal decomposition of the higher chlorides.

LABORATORIUM VOOR ANORGANISCHE EN PHYSISCHE CHEMIE
UNIVERSITY OF LEIDEN
LEIDEN, HOLLAND

A. E. VAN ARKEL
Z. J. CERYCH
W. J. MIJS
U. SPITZBERGEN

RECEIVED MAY 6, 1954

UTILIZATION OF BRANCHED CHAIN ACIDS IN CHOLESTEROL SYNTHESIS

Sirs:

It has been suggested[1] that in steroid biogenesis isoprenoid intermediates are formed from three molecules of acetate and that a likely product of this condensation is β-methyl β-hydroxyglutarate which could furnish five carbon intermediates on decarboxylation.[2] In support of these views it has previously been found that isovaleric acid[3] and the polyisoprenoid hydrocarbon squalene[4] are considerably more effective as carbon sources for cholesterol than acetate. We have now synthesized the following branched chain acids: 3-C^{14}-β-methyl-β-hydroxyglutaric acid, 3-C^{14}-*cis*-β-methylglutaconic acid, 3-C^{14}-β-hydroxyisovaleric acid and 3-C^{14}-β-dimethylacrylic acid (DMA) and tested their utilization in cholesterol synthesis. The sodium salts of the isotopically labeled acids were mixed with a stock diet and fed to rats at a level of 0.25 mmole. per 100 g. of rat per day for two days. Cholesterol was isolated from the livers and analyzed for C^{14}. All of these acids were found to furnish carbon for cholesterol synthesis, but with the exception of DMA, were less efficient than acetate. On the other hand, after the feeding of DMA the specific activity (S.A.) of liver cholesterol was 4–6 times greater than after the feeding of equimolar quantities of acetate. This result has prompted us to investigate the distribution of DMA carbon in the steroid molecule. If six isoprene units condense to form squalene (*cf.* Fig. 1) which cyclizes to the steroid structure,[5] then

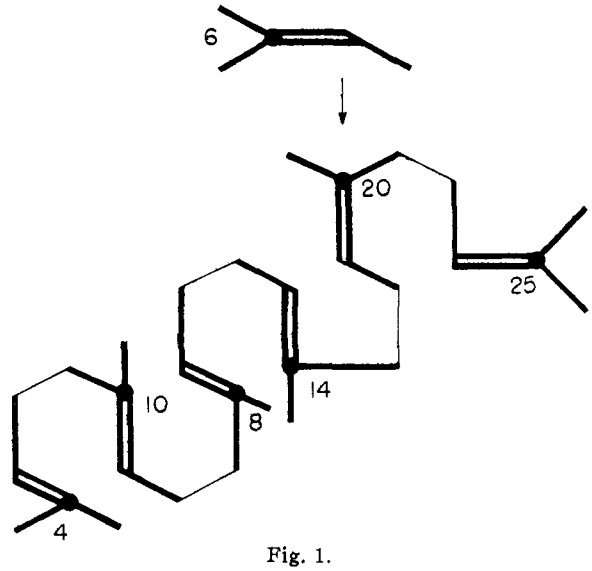

Fig. 1.

cholesterol derived from 3-C^{14}-DMA should contain six labeled carbon atoms: C_4, C_8, C_{10}, C_{14}, C_{20} and C_{25}. Each of these should have a S.A. 27/6 or 4.5 times that of the total molecule. If on the other hand DMA had first been degraded to acetate, then twelve sterol carbons should be labeled and have a S.A. 2.25 times that of cholesterol.[6] Degradation of cholesterol formed from DMA showed (see Table) that C_{10} and C_{25} had specific

TABLE I

DISTRIBUTION OF C^{14} IN CHOLESTEROL FORMED FROM 3-C^{14}-β-DIMETHYLACRYLIC ACID, C.P.M.* AS INFINITELY THICK SAMPLES OF BARIUM CARBONATE.

	Found	Calculated	
		A[a]	B[b]
Cholesterol	100	..	..
C_{10}	440	450	225
C_{25}	360	450	225

[a] Calcd. for six labeled C atoms per molecule. [b] Calcd. for twelve labeled C atoms per molecule.

activities which are 80 and 95%, respectively, of those which the hypothesis of five carbon intermediates predicts. These results preclude prior transformation of DMA to acetate and also to acetoacetate since Blecher and Gurin[7] have shown that in cholesterol formed from 1-C^{14} or 4-C^{14} acetoacetate the C^{14} distribution is indistinguishable from that given by acetate. The biosynthesis of DMA first detected in guayule leaves,[8] has now been demonstrated in liver homogenates by Rudney[9] and a similar observation has been made in this laboratory. The distribution of acetate carbon in DMA as observed by Rudney is identical with that in the terminal five carbon atoms of the cholesterol side chain[10] and hence in full accord with the rule assigned here to DMA. Since in the

(1) J. Würsch, R. L. Huang and K. Bloch, *J. Biol. Chem.*, **195**, 439 (1952).
(2) K. Bloch, Harvey Lectures, Series 48, p. 68 (1952–53).
(3) I. Zabin and K. Bloch, *J. Biol. Chem.*, **185**, 131 (1950).
(4) R. G. Langdon and K. Bloch, THIS JOURNAL, **74**, 1869 (1952).
(5) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953).
(6) H. N. Little and K. Bloch, *J. Biol. Chem.*, **183**, 33 (1950).
(7) M. Blecher and S. Gurin, *Fed. Proc.*, **13**, 184 (1954).
(8) B. Arreguin, J. Bonner and B. J. Wood, *Arch. Biochem.*, **31**, 234 (1951).
(9) H. Rudney, *Fed. Proc.*, **13**, 286 (1954).
(10) J. Würsch, R. L. Huang and K. Bloch, *J. Biol. Chem.*, **195**, 439 (1952).

present experiments the 6-carbon dicarboxylic acids and β-hydroxyisovalerate were poorly utilized, their participation in cholesterol synthesis and their metabolic relation to DMA remain unsettled.[11]

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CHICAGO
CHICAGO 37, ILLINOIS
KONRAD BLOCH
L. C. CLARKE
ISAAC HARARY[12]

RECEIVED APRIL 29, 1954

(11) This work was supported by a grant from the Life Insurance Medical Research Fund.

(12) Postdoctoral Fellow, American Cancer Society.

THE ANTITUBERCULOSIS ACTIVITY OF SOME ETHYLMERCAPTO COMPOUNDS

Sir:

In the course of studies dealing with the phenomenon of nitrification in soil[1] it was noted that proliferation of nitrifying organisms was inhibited by several alkyl mercapto acids. This led to the testing of β-ethylmercaptopropionic acid (I) for antibacterial activity. Only slight activity was found *in vitro* against several gram-positive and gram-negative bacteria. Definite protection was, however, afforded to mice infected with the H37Rv strain of *Mycobacterium tuberculosis* (human type) when fed β-ethylmercaptopropionic acid at a level of 0.2% in diet *ad libitum*. Further tests with other compounds of this type indicated that the ability of a given compound to inhibit proliferation of nitrifying bacteria is not a sufficient qualification for *in vitro* activity in experimental tuberculosis.

A systematic investigation of the relationship of structure to *in vivo* antituberculosis activity in derivatives of I was next undertaken. Substitution of various terminal alkyl groups (methyl through octadecyl) on the sulfur atom gave activity only with the C_2H_5–homolog. Aryl and heterocyclic moieties were equally disappointing. Variation of the distance between sulfur and carboxyl to give mercaptoacetic through mercaptovaleric acids indicated that the β-relationship between sulfur and carboxyl was essential. Replacement of carboxyl by $-CH_2OH$, $-CHO$, $-COOR$ was achieved with retention of activity. Elevating the oxidation state of sulfur to sulfoxide or sulfone destroyed the capacity to inhibit the experimental tuberculosis. Replacement of the sulfur atom in I by oxygen or nitrogen was also deleterious. In an effort to find compounds with more acceptable properties, S-ethyl-L-cysteine (II) was early tested and shown to be worthy of practical consideration on the basis of efficacy and acute and chronic toxicity studies.

Concurrently with the study of the structure requirements for efficacy in I, attention was given to the possible metabolic fate of β-ethylmercaptopropionic acid in the animal body. This seemed especially pertinent in view of the lack of *in vitro* activity. With the testing of ethyl disulfide a significant increase in efficacy over I was noted.

Results of the above tests opened a broad horizon for the study of C_2H_5–S–R compounds. In general, structural modifications which decreased the tendency for cleavage of the C_2H_5S-linkage decreased the antituberculosis activity. Of the more than three hundred and fifty samples examined to date, over fifty have shown effectiveness at or below 0.2% in diet.

During the course of our studies with C_2H_5–S–R compounds we encountered the report by Del Pianto[2] on the antituberculosis effect noted after injecting a combination of sodium ethyl thiosulfate and mercaptobenzothiazole derivatives in guinea pigs. In our hands sodium ethyl thiosulfate alone was more active orally than by subcutaneous injection in mice.

Table I is intended to show the relative activities of representative members of some of the classes of compounds covered in this investigation. On the basis of data obtained by direct comparisons in mice, it may be said that S-ethylcysteine (L and DL) is at least twice as active as pyrazinamide and several times more effective than *p*-aminosalicylic acid.[3] Compound II was equally effective against isonicotinic acid hydrazide resistant and sensitive strains of *Mycobacteria*.

TABLE I

ANTITUBERCULOSIS ACTIVITY OF SOME C_2H_5–S–R COMPOUNDS

		Antituberculosis activity
I	C_2H_5-S-CH_2CH_2COOH	+
II	C_2H_5-S-$CH_2CH(NH_2)COOH$ (L and DL)	+
III	C_2H_5-S-$CH_2CH(NH_2)COOH$ (↓ O)	−
IV	C_2H_5-S-S-C_2H_5	++
V	C_2H_5-S-S-C_6H_5	+
VI	C_6H_5-S-S-C_6H_5	−
VII	C_2H_5SH	++
VIII	C_2H_5-S-CO-O-C_2H_5	++
IX	C_6H_5CO-S-C_2H_5	++
X	$C_6H_5C(=NH)$-S-C_2H_5·HCl	++
XI	C_6H_5-N=C(S-C_2H_5)-NH-C_6H_5	−

(1) W. T. Brown, J. H. Quastel, P. G. Scholefield, *J. Appl. Microbiol.*, in press.

(2) Enrico Del Pianto, *Ricera sci.*, **20**, 83 (1950).

(3) M. Solotorovsky, *et al.*, to be published.

RESEARCH LABORATORIES OF THE CHEMICAL DIVISION OF MERCK & CO., INC., RAHWAY, N. J.
H. D. BROWN
A. R. MATZUK
H. J. BECKER
J. P. CONBERE
J. M. CONSTANTIN

MERCK INSTITUTE FOR THERAPEUTIC RESEARCH, RAHWAY, N. J.
M. SOLOTOROVSKY
S. WINSTEN
E. IRONSON

RESEARCH INSTITUTE, MONTREAL GENERAL HOSPITAL, MONTREAL, CANADA
J. H. QUASTEL

RECEIVED JUNE 14, 1954

OXIDATIVE PHOSPHORYLATION IN THE CYTOCHROME SYSTEM OF MITOCHONDRIA[1]

Sir:

At least two and probably three phosphorylations are coupled to the passage of a pair of electrons from reduced diphosphopyridine nucleotide to oxygen via the respiratory chain in isolated liver

(1) Supported by grants from the Nutrition Foundation, Inc., and National Institutes of Health. S.O.N. is fellow of E. H. Petersen Foundation and recipient of Fulbright travel grant.