

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

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THE ANTITUBERCULOSIS ACTIVITY OF SOME ETHYLMERCAPTO COMPOUNDS

Sir:

In the course of studies dealing with the phenomenon of nitrification in soil¹ 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

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

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 Pianto² 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 (I 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	$C_6H_5C(=NH)-S-C_2H_5 \cdot HCl$	++
XI	$C_6H_5-N=C-NH-C_6H_5$ $S-C_2H_5$	-

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

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

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OXIDATIVE PHOSPHORYLATION IN THE CYTOCHROME SYSTEM OF MITOCHONDRIA¹

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.

mitochondria.² Precise localization of these phosphorylations along the respiratory chain has been difficult to approach experimentally. However, it has been established that the oxidation of ascorbate to dehydroascorbate via the cytochrome system of isolated mitochondria is accompanied by a coupled phosphorylation.^{3,4,5} Since ascorbate reduces ferricytochrome c non-enzymatically, it has been tentatively concluded that the phosphorylation observed is actually coupled to the oxidation of ferrocytochrome c by oxygen via cytochrome oxidase. However, participation of ascorbate in an as yet unknown enzymatic phosphorylation could not be rigorously excluded in those tests. We wish to report a direct demonstration of phosphorylation coupled to electron transport between ferrocytochrome c and oxygen which does not involve the presence of ascorbate or any other non-enzymatic reductant of cytochrome c in the test system.

Ferrocytochrome c, prepared by reduction of ferricytochrome c by hydrogen (palladium catalyst), was incubated with rat liver mitochondria, orthophosphate labeled with P³², ADP, Mg⁺⁺, tris-(hydroxymethyl)-aminomethane buffer (tris) pH 7.4, ethylenediaminetetraacetate (Versene), and antimycin A. Enzymatic oxidation of ferrocytochrome c proceeded at the expense of dissolved oxygen and was followed spectrophotometrically at 550 mμ, using reported extinction coefficients.⁶ The phosphate uptake was measured by determining P³² in the organic phosphate fraction after removal of phosphomolybdic acid by solvent extraction.⁷ The P:O ratio was calculated as millimicromoles of orthophosphate taken up per 2 millimicromoles of ferrocytochrome c oxidized. Antimycin A was added to inhibit reduction of ferricytochrome c by endogenous substrates⁸; apparently it did not inhibit oxidation of ferrocytochrome c or the coupled phosphorylation significantly in the concentration used (0.02 γ/ml.).

The table shows the experimental details and results of four experiments.

It is seen that phosphate uptake accompanied oxidation of ferrocytochrome c, with observed P:O ratios from 0.44 to 0.86. These values are in agreement with the P:O ratios earlier obtained with ascorbic acid as reductant³ which approached 1.0. Omission of ferrocytochrome c or its substitution by ferricytochrome c produced in most cases insignificant "base-line" phosphorylation. Both the oxidation of ferrocytochrome c and the coupled phosphorylation were completely inhibited by 5×10^{-4} M cyanide, indicating the participation of cytochrome oxidase. The presence of 1×10^{-4} M 2,4-dinitrophenol completely inhibited phosphate uptake and increased the rate of oxidation of ferrocytochrome c. It is also seen that the P:O ratio is relatively constant with time up to about 65% oxidation of the ferrocytochrome c.

(2) A. L. Lehninger, *J. Biol. Chem.*, **190**, 345 (1951).

(3) A. L. Lehninger, M. ul Hassan and H. C. Sudduth, *ibid.*, in press.

(4) M. Friedkin and A. L. Lehninger, *ibid.*, **178**, 611 (1949).

(5) J. D. Judah, *Biochem. J.*, **44**, 305 (1951).

(6) H. Theorell and Å. Åkesson, *THIS JOURNAL*, **63**, 1804 (1941).

(7) O. Lindberg, M. Ljungren, L. Ernster and L. Revesz, *Exptl. Cell Research*, **4**, 243 (1953).

(8) V. R. Potter and A. E. Reif, *J. Biol. Chem.*, **194**, 287 (1952).

TABLE I

PHOSPHORYLATION COUPLED TO OXIDATION OF FERROCYTOCHROME C

Final concentrations in reaction media (total volume 1.0 ml.): 8.5×10^{-4} M orthophosphate labeled with P³² ($1.0-1.5 \times 10^8$ c.p.m./μP.), 0.0015 M ADP, 0.020 M TRIS pH 7.4, 0.0010 M Versene, 0.02 γ antimycin A per ml., and 1.65×10^{-5} M cytochrome c. The reaction was started by adding the mitochondria derived from 0.5 mg. wet weight rat liver suspended in 0.20 ml. of 0.075 M sucrose + 0.001 M versene; incubation for 2 to 15 min. at 23-26°.

	$-\frac{1}{2}\Delta\text{Fe}^{\text{II}}$ cyt. c millimicromoles	$-\Delta\text{P}_i$ P:O millimicromoles	
Fe ^{II} cyt. c	3.1	1.9	0.61
Fe ^{II} cyt. c + 5×10^{-4} M KCN	-0.1	0.13	
Fe ^{II} cyt. c + 1×10^{-4} M DNP	5.1	0.05	0.01
Fe ^{III} cyt. c		0.18	
Fe ^{III} cyt. c + 5×10^{-4} M KCN	-0.4	0.05	
No cyt. c		0.10	
Fe ^{II} cyt. c	2.9	2.5	0.86
Fe ^{III} cyt. c		0.48	
Fe ^{II} cyt. c	1.0	0.68	0.68
Fe ^{III} cyt. c		0.05	
Fe ^{II} cyt. c, 2 min.	1.0	0.53	0.53
Fe ^{II} cyt. c, 15 min.	4.3	1.9	0.44
Fe ^{III} cyt. c, 2 min.		0.07	
Fe ^{III} cyt. c, 15 min.		0.18	

It may be concluded that one of the three phosphorylations occurring during passage of a pair of electrons from DPNH to oxygen is coupled to the oxidation of ferrocytochrome c by oxygen, probably via cytochromes a and a₃. This phosphorylation is completely compatible with known thermodynamic data on the respiratory chain.

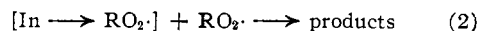
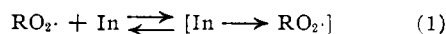
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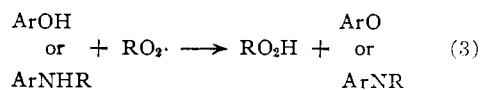
MOLECULAR COMPLEX FORMATION IN FREE RADICAL REACTIONS

Sir:

In the course of a study of the action of inhibitors in the initiated oxidation of hydrocarbons, we were led to the conclusion that the behavior of inhibitors could well be accounted for by this sequence of reactions



Two critical tests of this hypothesis have now been applied and the results seem sufficiently definitive to warrant their release at this time. The commonly accepted mechanism of inhibitor action by aromatic amines and phenols involves the removal of a labile hydrogen in the first step of the reaction.



Since such a reaction would not be expected to be highly exothermic, it would be anticipated that the rate of the reaction and therefore the inhibitor