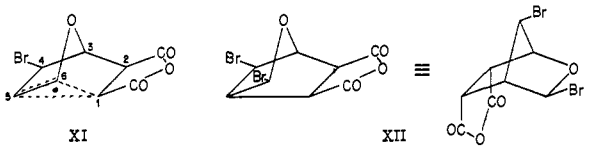
quiring stereospecific approach of bromine to C_5 from the *exo*-direction, and or (II) the unstable α-bromoether (XII), ionic rearrangement of which to IIB is in precise steric and electronic analogy to the change camphene hydrochloride → isobornyl chloride.[6]



XI XII

The formation of *cis*-dibromide by either or both of these paths appears to be unique in the literature.

(6) (a) H. Meerwein and K. van Emster, *Ber.*, **53**, 1815 (1920); **55**, 2500 (1922); (b) P. D. Bartlett and I. Pöckel, THIS JOURNAL, **59**, 820 (1937); **60**, 1585 (1938); (c) T. P. Nevell, E. de Salas and C. L. Wilson, *J. Chem. Soc.*, 1188 (1939).

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RECEIVED JULY 1, 1953

PARTICIPATION OF ATP AND COENZYME A IN THE ENZYMATIC DECARBOXYLATION OF MALONIC ACID[1]

Sir:

Malonic acid was previously shown to be an intermediate metabolite of uracil degradation by bacterial enzymes.[2,3] More recently decarboxylation of malonic acid was observed with dried bacterial cells and crude extracts.[4] It has now been found that the enzymatic decarboxylation of malonic acid requires adenosinetriphosphate (ATP) and coenzyme A (CoA) and an activated form of malonate is proposed as an intermediate.

Pseudomonas fluorescens strain TR-23,[5] a strictly aerobic microörganism, was grown for about 20 hours at 26°, with constant shaking, in a medium containing 1% NH_4Cl, 0.5% disodium malonate, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.1% Difco yeast extract. Cell-free extracts were prepared by grinding the washed cells with alumina (Alcoa A-301) in the presence of reduced glutathione (1.5 mg. of the sodium salt per g. of wet cells), extracting with 6 parts of 0.02 *M* phosphate buffer (*p*H 7.0), and centrifuging at 25,000 × *g* for 30 minutes.

A reaction mixture (2.0 ml.) containing 0.1 ml. of the crude extract (1.43 mg. protein), 100 μM. KF, 20 μM. reduced glutathione (sodium salt), 10 μM. $MgCl_2$, 200 μM. sodium acetate buffer (*p*H 5.8), 100 units CoA, 10 μM. ATP (sodium salt), 50 γ cocarboxylase and 100 μM. sodium malonate was incubated under pure nitrogen at 30° for 30 minutes. In the complete system 28.8 μM. of carbon dioxide was evolved. When ATP and CoA were omitted, only 1.9 μM. of carbon dioxide was produced. Pretreatment of the extracts with both Dowex-1[6] and charcoal[7] caused a more pronounced difference. With 0.1 ml. of the treated extract (0.86 mg. protein) the complete system yielded 13.5 μM. of carbon dioxide, whereas when either CoA, or ATP or both were omitted, 2.6, 0.9 and 0.2 μM. of carbon dioxide was produced, respectively. Neither Mg^{++} nor cocarboxylase affected the rate of the reaction under these conditions. There was no carbon dioxide production when malonate was omitted or the extract was treated at 100° for 5 minutes.

A reaction mixture (prepared as described above but with tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.0, instead of acetate buffer) containing 1.0 ml. of the crude extract and 200 μM. of hydroxylamine, yielded 7.4 μM. of hydroxamic acid derivatives[8] in the presence of ATP and CoA, whereas only 0.15 μM. was formed in the absence of the added cofactors. These hydrosamic acid derivatives were tentatively identified by paper chromatography (Whatman No. 3 with water-saturated butanol as solvent[9]) as (1) acethydroxamic acid (R_f: 0.51–0.53) and as (2) malonmonohydroxamic acid[10] (R_f: 0.36–0.38).

Thus the mechanism of malonate decarboxylation appears to involve activation of malonate (probably as malonyl CoA) as a primary step, analogous to the mechanism of succinate decarboxylation recently proposed for anaerobic microörganisms.[11,12] It has not yet been established whether the decarboxylation occurs at the activated carboxyl group to form an active one carbon compound and free acetate or whether the other carboxyl group is decarboxylated to produce active acetate and carbon dioxide. Since crude extracts were found to form hydroxamic acid derivatives from acetate, propionate, and succinate under the conditions described above, purification of the enzymes involved appears to be necessary to elucidate this point.

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RECEIVED JUNE 16, 1953

(1) This investigation was supported in part by a research grant (G3727) from the National Institutes of Health, Department of Health, Education and Welfare.

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(3) F. J. S. Lara, *J. Bact.*, **64**, 279 (1952).

(4) C. T. Gray, *ibid.*, **63**, 813 (1952).

(5) O. Hayaishi and R. Y. Stanier, *J. Biol. Chem.*, **195**, 735 (1952).

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(7) R. K. Crane and F. Lipmann, *ibid.*, **201**, 235 (1953).

(8) F. Lipmann and L. C. Tuttle, *ibid.*, **159**, 21 (1945).

(9) Incubation mixtures were treated with Dowex 50 (H^+ form) and then treated according to E. R. Stadtman and H. A. Barker, *J. Biol. Chem.*, **184**, 769 (1950).

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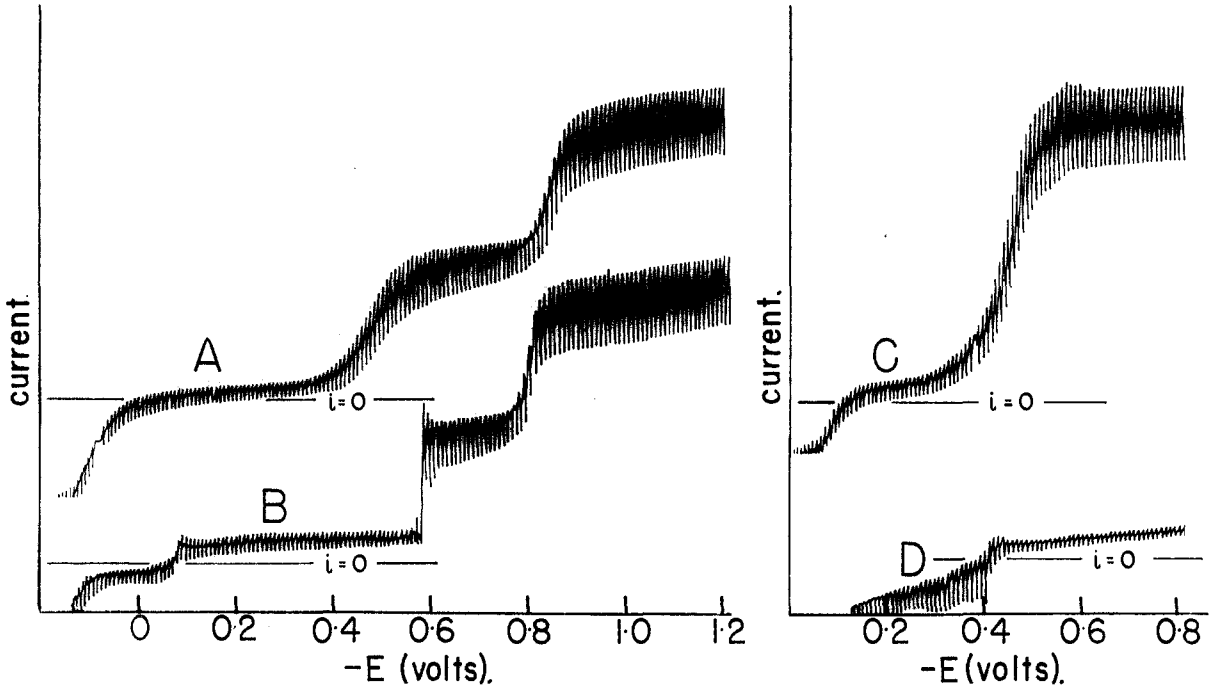
(13) The excellent technical assistance of Mrs. Natalie A. Fraser is gratefully acknowledged.

A MODEL FOR THE CONFIGURATION OF SULFHYDRYL GROUPS IN PROTEINS

Sir:

The differing reactivity of protein –SH groups and, especially, the marked increase in their reactivity upon denaturation of the protein, has been the subject of much speculation. We wish to report two sets of observations which suggest an explanation for this phenomenon.

In the first series of experiments three cysteine-



Curve A: PCG, 2.2 × 10^{-4} *M*; C_6H_5HgOH, 2.2 × 10^{-4} *M*; acetate buffer, *p*H 5.9, 0.05 *M*; KNO_3, 0.1 *M*. Curve B: PCV, 2.2 × 10^{-4} *M*; C_6H_5HgOH, 2.2 × 10^{-4} *M*; acetate buffer, *p*H 5.9, 0.05 *M*; KNO_3, 0.1 *M*.

Curve C: PCG, 2.4 × 10^{-4} *M*; Salyrgan, 2.4 × 10^{-4} *M*; acetate buffer, *p*H 4.0, 0.05 *M*; KCl, 0.01 *M*. Curve D: PCV, 2.4 × 10^{-4} *M*; Salyrgan, 2.4 × 10^{-4} *M*; acetate buffer, *p*H 4.0, 0.05 *M*; KCl, 0.1 *M*.

Fig. 1.—Polarograms which are identical with curves A and C are obtained when compound GSH is substituted for compound PCG.

containing peptides, *i.e.*, L-glutamyl-L-cysteinyl-glycine (GSH), phenacetyl-L-cysteinyl-glycine (P-CG)[1] and phenacetyl-L-cysteinyl-D-valine (PCV)[2] were used. These peptides can be transformed quantitatively into mercaptides of the structure RSHgR′ or RSAg by interaction with an equivalent of an organic mercury compound or silver. The phenylmercuric, Salyrgan (sodium-*o*-(γ-hydroximercuri - γ - methoxypropylcarbamyl) - phenoxyacetate) and silver mercaptides of GSH, PCG and PCV were therefore subjected to polarographic analysis. The reduction of the metal out of the mercaptide proceeded normally with all three mercaptides of GSH and PCG.[3] The waves (curves A and C) were analogous to those of the corresponding mercaptides of simpler thiols, such as cysteine, thioglycolic acid and thioethanolamine.[4] The mercaptides of PCV, on the other hand, showed a striking difference. The silver and Salyrgan mercaptides were found to be essentially irreducible (curve D). In the case of the phenylmercuric mercaptide to PCV, the initial part of the first reduction wave is seen to be missing (curve B) and a sharp rise in current occurs from the base line to the diffusion plateau, at a potential corresponding to the end of the mercaptide waves of GSH or PCG. In the presence of 4.4 *M* urea, a well-known protein denaturing agent, this "inhibited" wave became more similar to the corresponding waves of GSH or PCG (curve A).

It was therefore of interest to compare quantitatively the effect of "denaturing concentrations" of urea on the nitroprusside color of thiols of varying reactivity. It was found that, whereas sodium sulfide and ethyl mercaptan gave the same nitroprusside color in 6 *M* urea and in water, this was not the case with cysteine or the three peptides. The nitroprusside color in 6 *M* urea was about 50% higher than that in water with cysteine and in the case of the three peptides the increase amounted to over 100%. The increase in the reactivity of these thiols in the presence of urea suggests that intramolecular S–H–N bonds stabilize the –SH group through the formation of a five or six membered ring. Hydrogen bonding to sulfur has previously been postulated by Cecil[5] to account for the difference in the reactivity of the –SH group in cysteine and glutathione.

The polarographic results with PCV suggest that a further decrease in the reactivity of the –SH group can be brought about when hydrogen bonding brings a neighboring aliphatic side chain, such as the isopropyl group of valine, into close proximity of the –SH group. Thus a gradation of reactivity of protein –SH groups becomes easily conceivable,

(1) Synthesized by Drs. L. C. King and F. H. Suydam. THIS JOURNAL, **74**, 5499 (1952).

(2) Donated by Dr. K. Folkers, F. W. Holly, E. W. Peel, E. L. Lux and K. Folkers, *ibid.*, **74**, 4539 (1952).

(3) The silver mercaptide of PCG could not be polarographed, since it was insoluble under the conditions used.

(4) R. Benesch and R. E. Benesch, THIS JOURNAL, **73**, 3391 (1951); *Arch. Biochem.*, **38**, 425 (1952).

(5) R. Cecil, *Biochem. J.*, **47**, 572 (1950).

depending in the main on hydrogen bonding, with or without steric hindrance by branched aliphatic side chains. The hindered reduction of the mercaptides of PCV actually constitutes an intermediate case, as we have found that the phenylmercuric mercaptides of ovalbumin are completely irreducible polarographically.

The ideas put forward here represent a compromise between chemical and physical theories which have been proposed to account for the "masking" of protein –SH groups, since it is postulated that a chemical link (hydrogen bonding) leads to steric hindrance by branched aliphatic side chains.

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ON THE POLAROGRAPHIC REDUCTION WAVE OF DEHYDROASCORBIC ACID

Sir:

Since 1938[1] the polarographic oxidation wave of ascorbic acid has been well known among polarographists; however, the reduction wave of dehydroascorbic acid has not yet been found. So we attempted to obtain this reduction wave by using relatively high concentrations of dehydroascorbic acid in polarographic solutions.

For that purpose crystalline dehydroascorbic acid was prepared, using Pecherer's method.[2] Our polarographic experimental conditions were as follows:

Concn. of dehydroascorbic acid, 0.025∼ 0.1 *M*
*p*H range 2∼5 in McIlvaine's buffer solution
Temperature range 10∼50° (at 5° intervals)

On the other hand, we also used dehydroascorbic acid which was not crystallized but was prepared by oxidizing ascorbic acid in buffer solutions with equimolar amounts of iodine. Consequently these solutions became more acidic, then we neutralized them partly with some portion of 1 *N* NaOH and used them as polarographic solutions without excluding I^- ions. These I^- ions showed polarographic oxidation wave but did not disturb the reduction wave of dehydroascorbic acid. The final *p*H of the solution was measured by means of a glass electrode.

The reduction wave of dehydroascorbic acid was very small at room temperature (about $^1/_{1000}$ of the expected diffusion current of dehydroascorbic acid) and had all the typical characteristics of the kinetic current which was prominently examined and explained by several authors when they had experimented with formaldehyde[3,4] or aldoses.[5,6] That is, characteristics such as the fact that the wave height of dehydroascorbic acid remains constant, independently of the height of the mercury reservoir (Fig. 1) and the temperature coefficient of this wave height is extraordinarily large (Fig. 2).

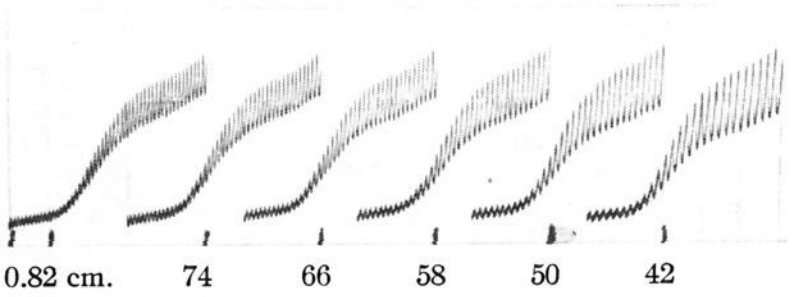

Fig. 1.—Constancy of the wave height of dehydroascorbic acid, independently of the height of the mercury reservoir: 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, $S = ^1/_5$, *p*H 2.8, 25°.

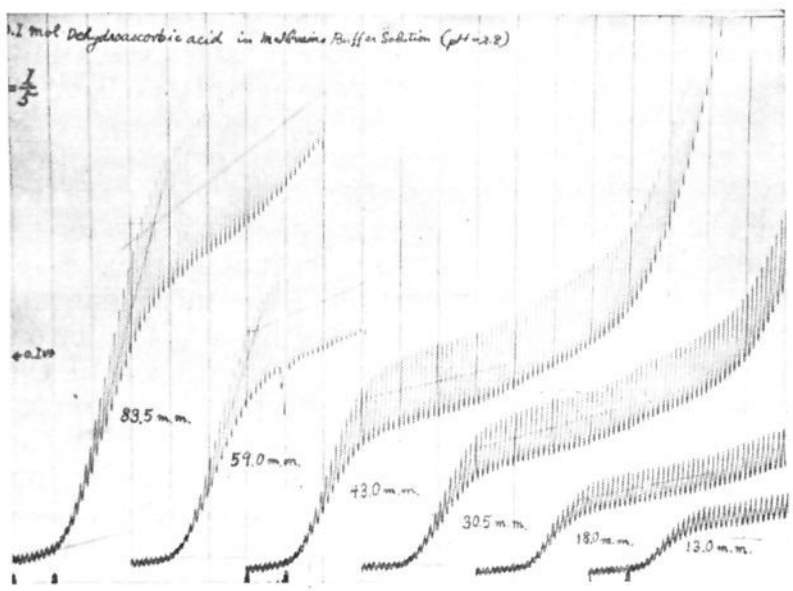

Fig. 2.—Increase of wave height of dehydroascorbic acid with increasing temperature: galv. sensitivity, 5.50×10^{-9} amp.; $m = 1.323$ mg./sec.; $t = 2.7$ sec./drop; 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, *p*H 2.8; $S = ^1/_5$.

The above described results may be reasonably explained, if we assume that the electroactive form of dehydroascorbic acid (unhydrated form) is scarce in aqueous solutions and its limiting current is controlled practically by the rate of the dehydration of the hydrated dehydroascorbic acid. Also this assumption does not seem to conflict with others' reports in which they assumed that the equilibrium between hydrated and unhydrated dehydroascorbic acid would be much shifted toward the hydrated form.[7,8]

From the relationship between the temperature and the limiting current of the reduction wave we obtained the activation energy of the dehydration reaction of hydrated dehydroascorbic acid. The activation energy is about 13 kcal., which was proved to be independent of *p*H.

The fact that the reduction product of dehydroascorbic acid at the dropping mercury electrode is ascorbic acid was polarographically substantiated after the controlled potential electrolysis[9] of dehydroascorbic acid.

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(7) Z. Vavrin, *Collection Czechoslov. Chem. Communs.*, **14**, 367 (1949).
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