by BALLS' unit at 37° When actually assayed by the method of BALLS at 37°, however, specific activity of the washed crystals was 234 units/mg dry weight The cause of the lower activity obtained by this assay method rather than by that of BALLS is being investigated When the activity was assayed with 0 02% Triton X-100, no difference was observed between both methods

The crystals were readily soluble in salt solutions but less soluble in pure water The crystalline β-amylase suspension could be stored at 4° for 3 months without appreciable loss of activity The crystalline enzyme was homogeneous using the criterion of disc gel electrophoresis (210 μg protein in gels at pH 7 5 and 8 3, stained by 1% Amido black in 7% acetic acid)

α-Amylase activity was assayed by reacting 20 8 μg of the pure enzyme on 4 mg of potato amylopectin in 2 ml of 0 05 M acetate buffer (pH 4 8) at 37° for 4 h. 30 min after the onset of incubation, the hydrolysis reached 56%, and no hydrolysis occurred in the prolonged incubation Therefore, the enzyme preparation was free from α-amylase The contaminant acid phosphatase activity (Table I) was as little as the other preparation[3]

THOMA *et al* [5] reported that the pure β-amylase, crystallized twice from $(NH_4)_2SO_4$ solution, included the colored material having an absorption peak at about 350 mμ The present preparation was free from the material as confirmed by the absorption spectrum

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Reversible disaggregation of *Escherichia coli* succinyl-CoA synthetase

Immunodiffusion experiments with homogeneous succinyl-CoA synthetase (succinate CoA ligase (ADP), EC 6 2 1 5, formerly known as succinic thiokinase) from *Escherichia coli* have shown the presence of three molecular species of the enzyme

It has been found that Merthiolate (ethylmercurithiosalicylate) present in the agar diffusion medium as a preservative, caused the dissociation of the enzyme into subunits, most likely by reaction with sulfhydryl groups of the protein* Enzymatic activity was lost in the process but could be restored in large part by the addition of dithiothreitol, with the concomitant restoration of an immunodiffusion pattern containing a single precipitation line

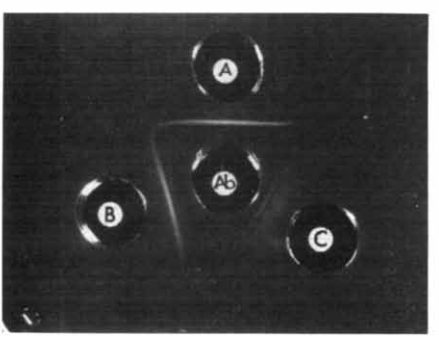
Succinyl-CoA synthetase was prepared from *E coli* as previously described[1] The assay system of KAUFMAN *et al* [2] was used for determination of enzyme activity, except that dithiothreitol (0 15 mM) was substituted for GSH Protein was estimated by the method of LOWRY *et al* [3] with bovine serum albumin as standard

Merthiolate was obtained from Eli Lilly and Co

Serum was obtained by immunization of two New Zealand white rabbits with succinyl-CoA synthetase Two doses of antigen (containing 0 5 mg of enzyme, specific activity 1000) in Freund's adjuvant were given two weeks apart at multiple interdermal and subcutaneous sites An intravenous injection of enzyme alone was given one week later Blood was drawn 7 days afterwards

Immunodiffusion experiments were performed as described by OUCHTERLONY[4] in 60 mm × 20 mm petri dishes with 0 8% agar in 0 05 M potassium phosphate, 0 05 M KCl, 0 001 M EDTA (pH 7 2) Succinyl-CoA synthetase (specific activity 1000) was used at a concentration of 0 1 mg/ml in all experiments The wells contained 0 1-ml samples Experiments were incubated overnight at room temperature There was no observed immunological reaction between enzyme and serum obtained from the rabbits before immunization

Fig 1 illustrates the effect of Merthiolate on the immunodiffusion of succinyl-CoA synthetase The addition of this reagent (Wells B and C) was seen to drastically affect the characteristic pattern of the native enzyme (Well A) The single precipitation line observed with untreated enzyme virtually disappeared at the higher Merthiolate level tested (Well C), and new lines which were closer to, and concave towards, the serum well



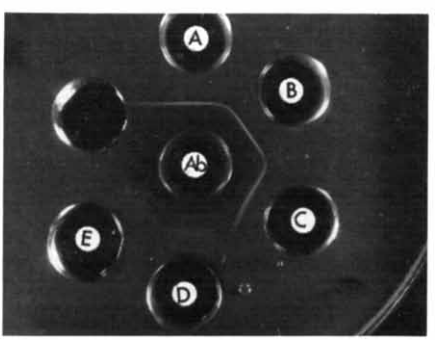
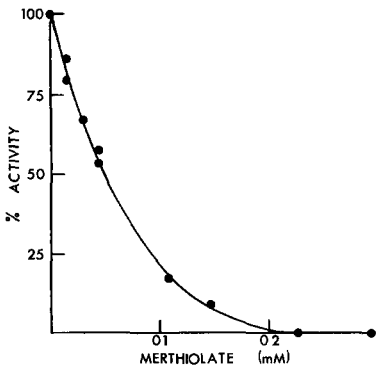


Fig 1 Immunodiffusion of succinyl-CoA synthetase No Merthiolate was added to the enzyme in Well A The concentration of Merthiolate in the enzyme was 0 0625 mM in Well B and 1 25 mM in Well C The center well contained serum Other conditions are described in the text

Fig 2 The effect of Merthiolate concentration on immunodiffusion The enzyme contained Merthiolate at the following concentrations Well A, none, Well B, 0 025 mM, Well C, 0 0625 mM, Well D, 0 125 mM, Well E, 0 187 mM The center well contained serum Other conditions are described in the text

* In a personal communication from Dr LAWRENCE LEVINE we have learned that *E coli* aspartate transcarbamylase undergoes a similar reaction with Merthiolate (unpublished results)



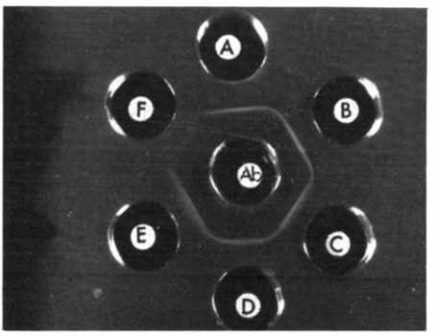


Fig 3 Inhibition of succinyl-CoA synthetase activity by Merthiolate Enzyme (0 1 mg, specific activity 1000) was preincubated with Merthiolate at the concentrations indicated for 15 min, 37°, in a final volume of 0 1 ml Aliquots of the preincubations were then assayed as described in the text

Fig 4 Reversal of the effect of Merthiolate on immunodiffusion Well A contained untreated enzyme Well B contained enzyme preincubated with 0 125 mM Merthiolate for 15 min after which an excess of dithiothreitol was added Well C contained enzyme and 0 125 mM Merthiolate Well D was the same as Well A Well E was the same as B except that the Merthiolate concentration was 1 25 mM Well F was the same as C except that Merthiolate concentration was 1 25 mM The center well contained serum Other conditions are described in the text

were observed Immunodiffusion experiments with two reagent troughs at right angles[4] indicated that these new lines corresponded to enzyme dissociation products of molecular weights approx 35 000 and 70 000 The native enzyme has a molecular weight of about 140 000 (ref 5)

It was of interest to determine the concentration range at which Merthiolate affected succinyl-CoA synthetase At concentrations between 0 025 and 0 187 mM this reagent titrated the native enzyme, as can be seen in Fig 2 Similar levels of

TABLE I

REVERSAL OF MERTHIOLATE INHIBITION BY DITHIOTHREITOL

Merthiolate in preincubation (mM)	*% Activity after preincubation**	*% Activity after initial reversal***	*% Activity after extended reversal****
None	100	100	100
0 30	8	60	79
1 50	0	55	69
2 25	0	46	54

* Duplicate enzyme samples (0 01 mg, specific activity 1000) were incubated with Merthiolate at the concentrations indicated in 0 085 ml for 5 min at 37° One sample of each pair was then assayed as described in the text

** The remaining sample of each pair from * was made to 15 mM in dithiothreitol in a final volume of 0 1 ml These solutions were then incubated at 37° for 10 min Aliquots were then withdrawn from the samples and assayed as described in the text

*** The incubations from ** were kept at 0° for 5 h Aliquots of the samples were then assayed as described in the text

Merthiolate, when incubated with the enzyme, resulted in losses of enzymatic activity (Fig 3) The reaction of Merthiolate with the enzyme reaches equilibrium after 10 min Thus, it would appear that Merthiolate dissociates the enzyme into subunits, and that the subunits are not active

1 mole of thiosalicylate (RSH) is formed when 1 mole of Merthiolate (R–S–Hg–Et) reacts with a protein sulfhydryl group The reaction follows the course shown in Eqn 1

$$R\text{–}S\text{–}Hg\text{–}Et + \text{enzyme–SH} \rightarrow RSH + (\text{subunit(s)})\text{–}S\text{–}Hg\text{–}Et \quad (1)$$

Reversal of this reaction by sulfhydryl groups would be predicted To test this possibility, excess dithiothreitol was added after preincubation of the enzyme with Merthiolate

The data in Table I and Fig 4 indicate that the reaction of succinyl-CoA synthetase with Merthiolate was reversible The subunits reassociated to an active form (Table I) that was serologically similar to the native enzyme (Fig 4) The fact that full activity was not restored may simply indicate that the rate of reaggregation was very slow under the conditions employed

The effect of Merthiolate on succinyl-CoA synthetase may be similar to that of *p*-mercuribenzoate It has been observed that *p*-mercuribenzoate dissociates the phosphorylated form of the enzyme into phosphorylated and nonphosphorylated subunits[5], although details describing this finding are not yet at hand It is important now to establish whether the Merthiolate subunit of mol wt 70 000 is unique or is a dimer of subunits of mol wt 35 000, and it will be of interest to determine which of these subunits is (are) phosphorylated

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Preparation of aminoacyl synthetases from higher plants*

Although the preparations of mixed aminoacyl synthetases (amino acid tRNA ligases (AMP)) from bacteria[1] and individual synthetases from yeast and from animal

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