# PURIFICATION OF STAPHYLOCOCCAL COAGULASE

MARVIN MURRAY* AND PAUL GOHDES

*Department of Pathology, University of Wisconsin Medical School, Madison, Wisc. (U.S.A.)*

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## SUMMARY

A method is described for isolating staphylococcal coagulase. Fractionations are carried out with ammonium and cadmium sulfates at various pH. The partially purified enzyme is then subjected to chromatographic procedures employing calcium phosphate. A material which migrates as a single component under the conditions of electrophoresis is described. The coagulase activity of this enzyme is greater than any previously described.

## INTRODUCTION

In 1935 WALSTON[1] demonstrated that cell free filtrates of *Staphylococcus aureus* are capable of coagulating oxalated plasma. Subsequent attempts at purification have met with only partial success[2,3]. Our present work has led to the isolation of this type of coagulase which has more activity than the best previously reported preparations[4]. The physical properties of the present material indicate, moreover, greater homogenity than those of previous investigations.

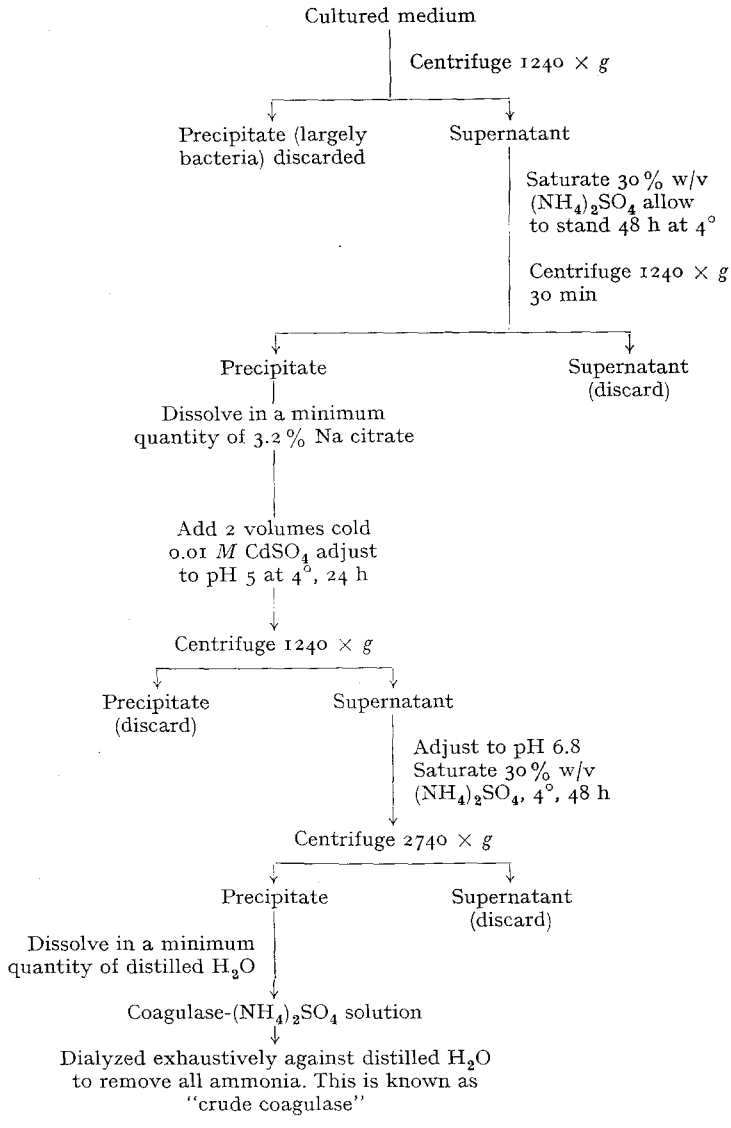
## MATERIALS AND METHODS

Previous workers have utilized serum[5] and tissue infusion enriched media for the growth of staphylococci in order to obtain maximum yields of coagulase. We have preferred a liquid peptone medium, because it lends itself more readily to purification procedures, even though the enzyme yield is less. Tryptose broth (Difco) was used exclusively for growing *Staphylococcus aureus* (TAGER's Strain[6]) and was prepared in a concentration of 26 g of dry powder/l. This was enriched with crystalline thiamine (5 mg/l) and the pH adjusted to 7.2–7.6. 15 l of broth were inoculated with actively growing staphylococci (in broth) and incubated at 37° for 72 h with occasional agitation. A crude enzyme preparation was made by fractionating the medium with ammonium and cadmium sulfates (see flow sheet).

The crude enzyme was then dialyzed against 0.001 *M* phosphate buffer, pH 6.8. It was then passed over a calcium phosphate hydroxyapatite chromatographic column prepared according to the method of TISELIUS[7]. The column was washed in the cold with 0.001 *M* phosphate buffer steps in increasing increments of 0.02 *M*. Each

* Current address, Department of Pathology, University of Louisville Medical School, Louisville, Kentucky.

FLOW SHEET




elution was accomplished by allowing a volume of buffer equal to two times the volume of hydroxyapatite adsorbent to flow through. Each eluate fraction was tested for coagulase activity by incubating with stored human plasma. The coagulase activity was confined to one or two steps of elution. The point of coagulase elution was determined for each batch of hydroxyapatite.

The fraction containing the coagulase activity was dialyzed exhaustively against distilled water. This solution was then lyophilized. A sample of the lyophilized material was subjected to paper electrophoresis in barbital buffer at pH 8.6. If two components were present, the material was redissolved in phosphate buffer and rechromatographed

through a fresh hydroxyapatite column. The active fraction obtained from the adsorption and elution always consisted of a single component when examined by paper electrophoresis. Elution of this material revealed uniform activity in the activation of fibrinogen.

## RESULTS

The average yield per 15 l batch of medium was between 20 and 30 mg of lyophilized material. This represented a two-hundred fold purification of the enzyme on the basis of dry weight of protein (trichloractic acid precipitable) after the removal of bacteria.

Examination of the purified enzyme preparation by the moving boundary method in 0.1 ionic strength, pH 8.6 diethylbarbiturate buffer revealed a component migrating at $-1.1 \cdot 10^{-5}$ cm$^2$ V$^{-1}$ sec$^{-1}$ (see Fig. 1). The same material was examined in the

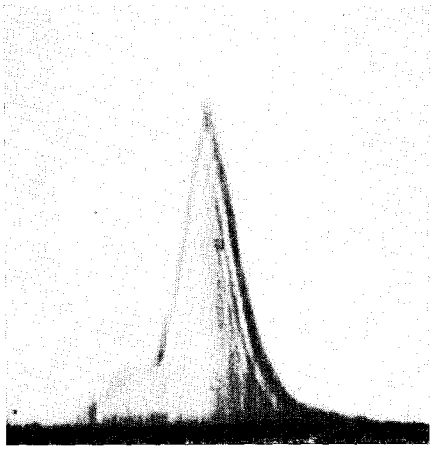


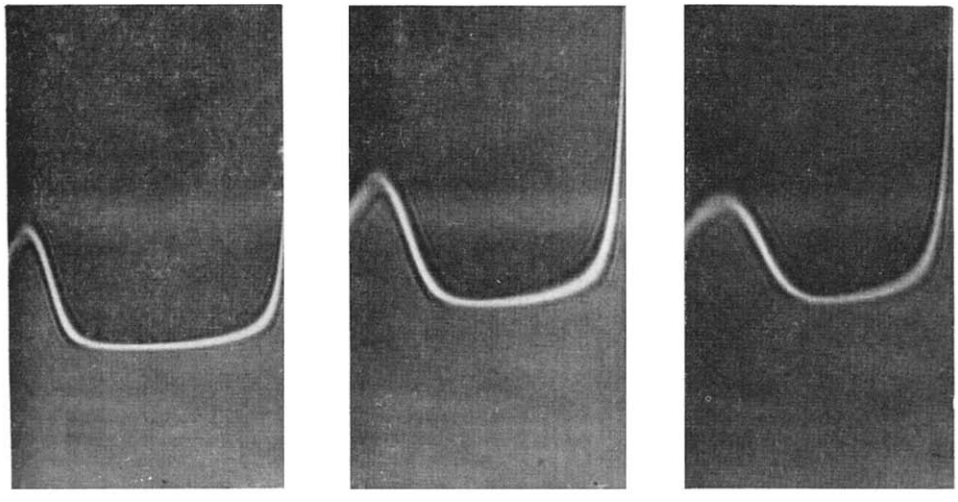
Fig. 1. Descending electrophoretic boundary of enzyme in pH 8.6 veronal buffer. Duration of experiment was 113 min at a potential gradient of 4.5 V/cm.

Spinco ultracentrifuge. A single component that showed considerable boundary spreading during the experiment is evidenced by Fig. 2. The average sedimentation constant for this material was $1.0 \cdot 10^{-13}$ cm sec$^{-1}$. Assuming a spherical shape for the molecules in question a molecular weight in the range of 5000 is obtained.

Samples of enzyme were subjected to micro-Kjeldahl analysis for percent nitrogen. Mean values for nitrogen were 16.8 %. Activity was determined on the basis of dry weight of purified enzyme. A reaction mixture was set up which consisted of:

| | |
|---|---|
| Staphylococcus coagulase in 3.0 % albumin | 0.5 ml |
| Fibrinogen (Bovine) 1.0 % | 0.5 ml |
| Human plasma 1:100 ($H_2O$) | 0.1 ml |
| | 1.1 ml |

The diluted plasma was added to supply the minimal quantity of coagulase reacting factor[5,8] necessary to complete the reaction rapidly. The reaction mixture was incubated at 37°. The end point used was the time necessary to form a complete clot which was resistant to agitation and would allow inversion of the tube. At a concentration of 0.1 $\mu$g of enzyme a complete clot was observed within 1000 min (see Fig. 3). At a concentration of 0.01 $\mu$g of enzyme a clot was observed, but did not occupy all of the reaction mixture.



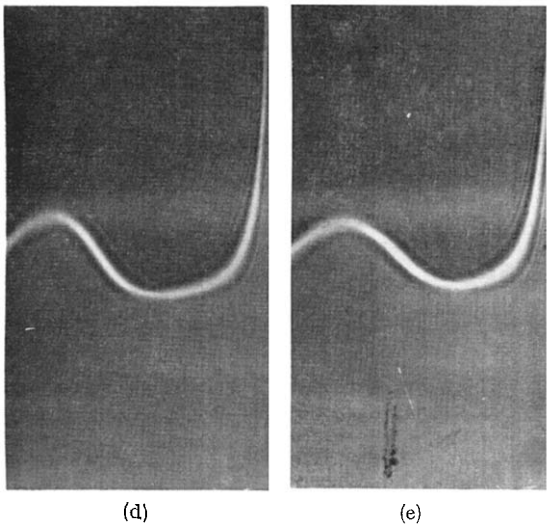




Fig. 2. Ultracentrifuge pattern of enzyme in 0.2 ionic strength, pH 7.4 potassium phosphate buffer. Exposures at 32 min intervals at 59,380 rev./min.

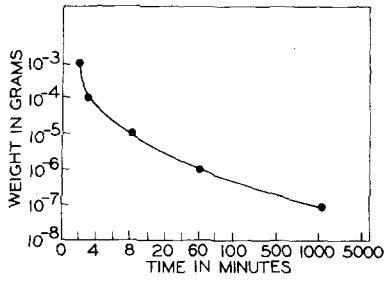



Fig. 3. Activity of Staphylococcus coagulase per unit dry wt.

## DISCUSSION

Although two criteria for purity have been satisfied (electrophoresis and ultracentrifuge data) insufficient evidence is presented here for a guarantee of purity. The boundary spreading seen in the ultracentrifuge can be explained by the small molecular size of the enzyme and subsequent diffusion. However, an alternate explanation is the presence of a large family of molecules of similar size. The small molecular size of the enzyme (smaller than any other enzyme previously reported) raises the question as to whether the isolated substance represents an active degradation product of the enzyme originally manufactured by the cell. This is plausible in view of the proteolytic enzymes present in a medium which contains a large population of cells. If this were so, variation in molecular size might easily be expected.

ACKNOWLEDGEMENTS

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# DETECTION OF TRITIUM ON PAPER CHROMATOGRAMS*

A. T. WILSON**

*Institute of Nuclear Science****, *Lower Hutt (New Zealand)*

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SUMMARY

A technique is described whereby $^3H$ compounds may be detected on paper chromatograms. The technique involves soaking the chromatogram in a liquid scintillator so that the energy of the disintegrating tritium atoms is converted into light which is detected by a film. Thus a weak $\beta$-particle is converted into light quanta which can travel into the film to produce an image. By the use of fast film, small amounts of $^3H$ compounds can be separated and identified in only a few days.

## INTRODUCTION

Hydrogen is an element of fundamental importance in biological processes. It is therefore important to have a method for studying its pathway through living systems. Paper chromatography[2] is an extremely powerful technique for the separation of complicated mixtures of compounds and has found much application in the field of biochemistry. One of the greatest problems of paper chromatography is the detection of the individual compounds on the paper. This has been done by spraying the paper with suitable chemicals which develop colour reactions with the compounds

* A brief account of this technique has been published[1].
** Present address: Chem. Dept., Victoria University of Wellington, Wellington, New Zealand.
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