# NOTE ON THE PRODUCTS FORMED ON LYSIS OF *MICROCOCCUS LYSODEIKTICUS* BY EGG WHITE LYSOZYME

by

J. R. HAWTHORNE

*Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge (England) and Department of Scientific and Industrial Research*

FLEMING[1] applied the term "lysozyme" to the enzymes present in egg white and other biological fluids which cause lysis of susceptible organisms. The mechanism of lysozyme action was investigated by MEYER and his co-workers[2] who isolated from *Sarcinae* a mucoid fraction which was acted upon by a lysozyme preparation with liberation of reducing substances. EPSTEIN AND CHAIN[3] isolated from *M. lysodeikticus* a polysaccharide fraction which on incubation with lysozyme from egg white gave products showing the colour reactions appropriate to an N-acetyl hexosamine and a ketohexose. Neither the hexosamine nor the ketohexose were fully characterised or identified with known compounds.

As a result of work described elsewhere[4] there was available a small quantity of *M. lysodeikticus* suspensions which had been lysed with egg white lysozyme and it seemed of interest to attempt positive identification of the products of lysozyme action by the filter paper partition chromatogram techniques. The dialysable products formed on lysis of the organism were found to consist largely of amino acids and peptides; since these were probably derived from the cell contents they were not further investigated. After removal of these substances the remaining material contained no free reducing sugars but on acid hydrolysis yielded glucose and mannose. Other substances present in the hydrolysate were tentatively identified as glucosamine and glucuronic acid.

## EXPERIMENTAL

*M. lysodeikticus* and crystalline lysozyme were prepared as already described[4]. Before use the solutions were dialysed till free from dialysable reducing substances. In order to avoid possible destruction of the products of lysis by intact *micrococcus* cells strong solutions of lysozyme were used. Lysis was complete within a few minutes at 37° C., further incubation of the lysed suspension with fresh lysozyme resulted in a negligible increase in reducing substances.

The total quantity of *micrococcus* available was 1 g dry weight, the increase in reducing substances on lysis (HAGEDORN AND JENSEN method) was equivalent to 30 mg glucose. The lysed suspension was dialysed for 24 hours against a large bulk of distilled water and the dialysate evaporated to dryness under reduced pressure. The resultant

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pale yellow material had a reducing power equivalent to 28 mg glucose and gave a positive test for N-acetyl hexosamine by the method of MORGAN AND ELSON[5]. The material gave a strong ninhydrin reaction and filter paper chromatograms run by the method of CONSDEN, GORDON AND MARTIN[6] showed the presence of several amino acids. To remove these substances the material was therefore run through columns of Zeocarb and Deacidite as described by PARTRIDGE[7].

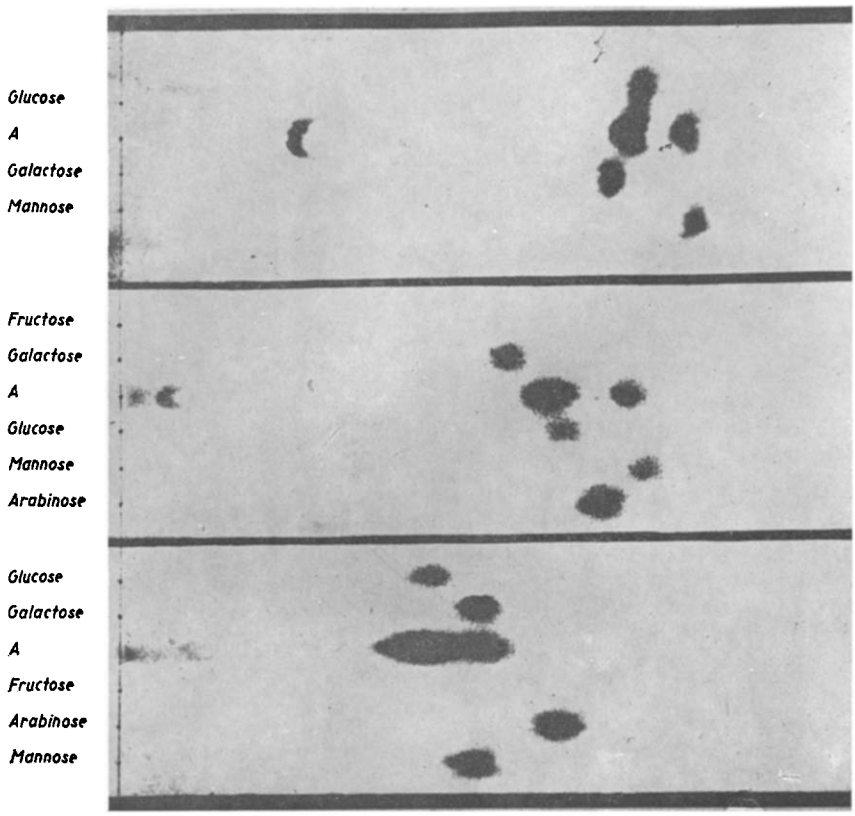


Fig. 1. Filter paper chromatograms of hydrolysate
Top: butyl alcohol-acetic acid, 72 hours; centre: s-collidine, 40 hours; bottom: phenol, 20 hours.
The experimental material was introduced at *A*.

The neutral material (20 mg) had a reducing power equivalent to 4 mg glucose. Filter paper chromatograms on this material (40 $\mu$g) run as described by PARTRIDGE[7] and sprayed with aniline phthalate[8] gave a series of ill-defined slow moving spots but no identifiable sugar spots.

After treatment with *N* sulphuric acid at 100° C for up to 6 hours no sugars could be demonstrated but after hydrolysis for 1 hour at 100° C with 5 *N* sulphuric acid the reducing power had increased 3-fold and clearly marked spots of the correct mobility for glucose and mannose were obtained (Fig. 1). A slow moving spot, usually with a marked forward trail, was also present; this is attributed to a uronic acid. The hydrolysate also gave a strong positive reaction with the ELSON AND MORGAN reagent for hexosamines[9].

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Since the aniline phthalate reagent reacts only weakly with fructose, absence of this sugar was confirmed on replicate papers sprayed with (a) naphthoresorcinol or (b) silver nitrate-ammonia[7]. The use of the latter reagent showed the presence of reducing substances other than sugars; the spots were ill-defined with marked trails but their positions were not inconsistent with their being due to glucosamine and glucuronic acid.

## DISCUSSION

Although fructose units are present in bacterial levans the sugar is not usually found as a component of the mucoid constituents of the cell wall. Confirmation of the results of EPSTEIN AND CHAIN would therefore have been of interest. The small amount of organism available precluded the isolation of the substrate of lysozyme as described by EPSTEIN AND CHAIN but the investigation with the intact organism showed no fructose in the product of lysozyme action, nor of $\omega$-hydroxymethylfurfuraldehyde which might have been formed from the hexose during treatment with acid.

The present work indicates that lysis of a susceptible organism by lysozyme does not yield free sugars which is in agreement with the suggestion of MEYER and his co-workers that the primary action of lysozyme consists in depolymerisation of the substrate. The product formed is of interest in being unusually resistant to acid hydrolysis.

Technical assistance in this work given by Mr G. J. T. KING.

The work described in this paper was carried out as part of the programma of the Food Investigation Organisation of the Department of Scientific and Industrial Reserch.

## SUMMARY

After lysis of *M. lysodeikticus* by egg white lysozyme the dialysable products consisted mainly of ninhydrin reacting substances. The neutral fraction after removal of these substances contained no free sugars but on hydrolysis yielded glucose and mannose. Glucosamine and glucuronic acid were probably also present in the hydrolysate.

## RÉSUMÉ

Après la lyse du *M. lysodeikticus* par le lysozyme du blanc d'oeuf, les produits dialysables consistaient principalement en substances capables de réagir avec la ninhydrine. La fraction neutre après l'élimination de ces substances ne contenait pas de sucres libres. Après hydrolyse, on peut montrer la présence de glucose et de mannose. L'hydrolysat contenait probablement aussi de la glucosamine et de l'acide glucuronique.

## ZUSAMMENFASSUNG

Nach der Lyse von *M. lysodeikticus* durch Lysozym aus Eiklar bestanden die dialysierbaren Produkte hauptsächlich aus mit Ninhydrin reagierenden Stoffen. Nach der Entfernung dieser Stoffe enthielt die neutrale Fraktion keine freien Zucker. Bei der Hydrolyse jedoch entstehen Glukose und Mannose. Auch Glukosamin und Glukuronsäure waren wahrscheinlich im Hydrolysat anwesend.

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Received January 22nd, 1950