determining the radioactivity in the material which remained soluble after this treatment. Two hours after injection each whole brain contained only about 150 counts per minute of radioactive material which was soluble in trichloroacetic acid; 4 hours after injection there was virtually no detectable radioactivity in the trichloroacetic acid-soluble fraction. Therefore the large amount of precursor which was injected either escaped from the brain or was incorporated into protein within a relatively short period of time so that precursor, sufficient to account for the delayed incorporation observed, was not, apparently, present. It thus seems more reasonable to interpret the results as indicating that protein that is synthesized in the nerve cell body in the period shortly after injection is transported, by axoplasmic flow, to the nerve ending. What is probably being measured here is the time required for protein to flow from the various cell bodies along axons of various lengths to nerve endings. The average rate of axoplasmic flow cannot be calculated since distances traveled are not known. The data suggest that some cell bodies are close enough to their nerve endings to transmit new protein to the endings within hours after its synthesis in the cell body; and that within days a substantial number of nerve cell bodies have transmitted newly synthesized protein to their endings. These observations are also of interest since it should be possible to use this method to measure relative rates of appearance of new protein at nerve endings under various experimental conditions whose influence on axoplasmic flow could thus be estimated. It should also be noted that although the progressive increment in radioactive protein in the nerve endings is more prominently shown in the "soluble" component of this fraction, there is also an increase in the radioactivity of "particulate" protein of the endings between 2 and 27 hours, which is more marked than the increments seen in the other fractions. Whether or not this is due to axoplasmic flow of "particulate" components of the nerve endings, as suggested by other studies (*9*), cannot be stated with confidence since the changes are not as prominent as those found with the "soluble" protein and could be due to incomplete lysis of the endings.

Table 3. Ratio of specific activity of "soluble" protein from nerve endings to specific activity of "soluble" protein from whole homogenate.

Hours after injection	Ratio
2	0.09
3	.11
4	.10
13	.19
27	.25
40	.44
49	.54
117	1.00
130	1.41

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Staphylolytic Enzyme from Chalaropsis: Mechanism of Action

Abstract. *The staphylolytic enzyme recently isolated from cultures of a* Chalaropsis *species by Hash is shown to be an acetylmuramidase that cleaves all the glycosidic linkages of* N-*acetylmuramic acid and* N,O-*diacetylmuramic acid in the cell wall of* Staphylococcus aureus *strain Copenhagen. It is similar in specificity to the "32 enzyme" from* Streptomyces albus *but it differs from egg-white lysozyme whose activity is inhibited by the presence of O-acetyl groups.*

Hash has reported (*1*) that an unidentified species of the fungus *Chalaropsis* produces several extracellular enzymes which lyse *Staphylococcus aureus* and several other gram-positive bacteria. One of these enzymes, the B enzyme, obtained as a relatively pure protein, catalyzed hydrolysis of the cell wall of *S. aureus*, with liberation of reducing groups and of material reactive as acetylhexosamine in the Morgan-Elson reaction. We now report that this enzyme is an acetylmuramidase, similar to the "32 enzyme" isolated from *Streptomyces albus* G by Ghuysen *et al.* (*2*); it catalyzes hydrolysis of glycosidic linkages of both *N*-acetylmuramic acid and *N,O*-diacetylmuramic acid in the cell wall of *Staphylococcus aureus* (*3, 4*).

The methods used were the same as those used to study the "32 enzyme" (*3, 4*). Purified cell walls of *S. aureus* strain Copenhagen (300 mg) were incubated for 11 hours at 37°C in 11 ml of 0.025*M* acetate, *p*H 4.7, with 0.5 mg of B enzyme from *Chalaropsis* (*5*). Lysis of cell walls (measured by decrease of turbidity) and maximum hydrolysis of glycosidic linkages (measured by reducing power) were reached after 6 hours of incubation (Fig. 1). The cell walls contained equal amounts of acetylmuramic and glutamic acids (0.47 μmole/mg), and 0.46 μmole of reducing group was liberated, indicating that one glycosidic linkage had been cleaved for each repeating unit.

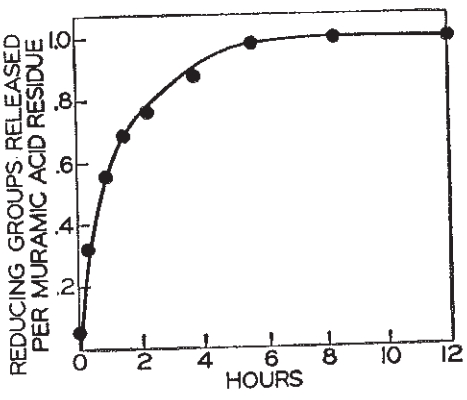

Fig. 1. Release of reducing power during the lysis of cell walls of *S. aureus* strain Copenhagen (300 mg) by B enzyme from *Chalaropsis* (0.5 mg) in 11 ml of 0.025*M* acetate, *p*H 4.7, at 37°C.

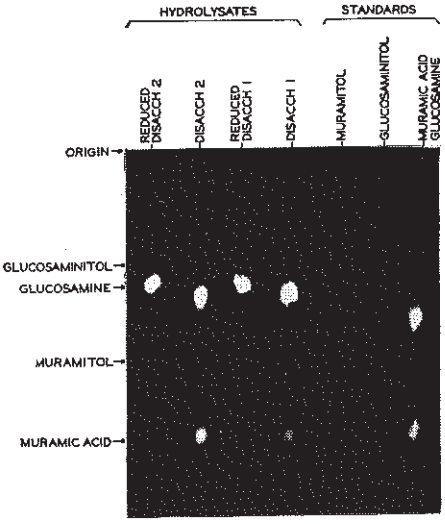

Fig. 2. Chromatogram of hydrolyzed disaccharides (40 mμmole) and reduced disaccharides (40 mμmole) in a mixture of butanol, acetic acid, and water (3:1:1); the products were detected with ninhydrin. Glucosaminitol ($R_{glucosamine}$ 0.9) and muramitol ($R_{glucosamine}$ 1.55) gave faint spots, invisible in the photograph.

No further liberation of reducing groups occurred during the remainder of the incubation, and no peptide bonds were hydrolyzed (measured as increase of free amino groups reactive with dinitrofluorobenzene). The products of lysis were separated by filtration with Sephadex G-50 and by paper electrophoresis (*3*) into two fractions: the glycopeptide (140 mg), and a teichoic acid-glycopeptide complex (124 mg). These products yielded analyses identical with those of fractions obtained from cell walls of *Staphylococcus aureus* by the action of the "32 enzyme" and similar fractionation (*3*, *4*).

The liberated reducing group was shown to be muramic acid by reduction with $NaBH_4$ by two methods. Glycopeptide and a reduced sample of glycopeptide were subjected to acid hydrolysis, and the amount of amino sugars present was determined colorimetrically (*4*). The glucosamine content was unchanged by reduction, but more than 95 percent of the muramic acid disappeared. Two more portions were subjected to two-dimensional paper chromatography in (i) a mixture of pyridine and water (4 : 1) and in (ii) a mixture of *n*-butanol, acetic acid, and water (3 : 1 : 1). Glucosamine and muramic acid (detected with ninhydrin) were present in the glycopeptide in about equal amounts, but only glucosamine was found in the hydrolyzate of the reduced glycopeptide. The glycopeptide was further treated with acetylmuramyl-L-alanine amidase, and then chromatographed on carboxymethyl cellulose. A disaccharide fraction (which came through in the water eluate and which represented 75 percent of the theoretical yield from the original cell wall) was separated from the polypeptide which was absorbed onto the column. The disaccharide fraction was then separated into disaccharide No. 1 and *O*-acetylated disaccharide No. 2 by paper chromatography. After purification of the eluted disaccharides by chromatography on Sephadex, a sample of each was reduced with $NaBH_4$, hydrolyzed, and subjected to paper chromatography in a mixture of butanol, acetic acid, and water (3 : 1 : 1). The products were detected with ninhydrin. In both cases the muramic acid was totally reduced while the glucosamine was unchanged, indicating that muramic acid was the reducing end group (Fig. 2).

About 40 percent of the muramic acid residues in the isolated cell wall of *S. aureus* strain Copenhagen are *N*-acetylmuramic acid residues; the remainder are *N,O*-diacetylmuramic acid residues (*4*). Since all the aldehyde groups of muramic acid can be reduced with $NaBH_4$ after hydrolysis of cell walls with the B enzyme from *Chalaropsis*, it is apparent that, like the "32 enzyme" and unlike egg-white lysozyme whose lytic action is inhibited by the presence of *O*-acetyl groups (*6*), the B enzyme is an acetylmuramidase which can catalyze the hydrolysis of linkages of *N,O*-diacetylmuramic acid as well as those of *N*-acetylmuramic acid.

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Uric Acid in the Reproductive System of Males of the Cockroach Blattella germanica

Abstract. *Uric acid is stored in the utriculi majores of the accessory sex glands of the German cockroach. Most of the uric acid is eliminated during copulation by being poured over the spermatophore. Mating appears to be an important means of excretion in this cockroach.*

The spermatophore of *Blattella germanica* (Linnaeus) is formed from a mixture of secretions (which give positive protein reactions) from three distinct groups of accessory gland tubules. A ventral group of four to six tubules contains a "milky secretion." A dorsal group of tubules produces a transparent, water-soluble secretion, and a third group, located between the other two, secretes a translucent material. Three secretions make up the body of the spermatophore. A clear transparent material covers the ventral surface; a milky white mass contains the two sperm sacs; and a translucent lamellated mass forms the dorsal wall of the spermatophore and is in close contact with the female sclerites after copulation. Masses of a "milky granular secretion" are scattered over the spermatophore and adjacent sclerites of the female (*1*). A chalk-white secretion hardens over the spermatophore (*2*), and when a mating pair has separated the white material sometimes adheres to the subgenital plate of the male; this usually occurs when males mate after they have been isolated from females for more than a week. The material hardens on drying and flakes off when the male rubs his terminal segments with his hind legs. The white compound which adheres to the male is the same as that which covers the spermatophore in the female, and it is the milky secretion of the ventral accessory sex glands mentioned by Khalifa (*1*). Brehm (*3*) called these glands the "utriculi majores."

In *B. germanica*, the utriculi majores of the recently emerged male contain little or no secretion but become filled with white granules in about 1 or 2 days. When the male does not have access to females, these glands become greatly enlarged and fill up a large part of the abdominal cavity. When a male with such distended glands mates, the glands empty almost completely, and