Growth and Biochemical Characteristics of *Micrococcus lysodeikticus*, Sensitive or Resistant to Lysozyme*

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ABSTRACT: Growth experiments with *Micrococcus lysodeikticus*, sensitive or resistant to the action of lysozyme, have been performed. Yeast extract contains a factor or factors that are necessary for the rapid growth of the resistant organism. One of these appears to be glucose. The cell walls of the two organisms have been prepared and analyzed. Cell walls from sensitive or resistant organisms contain alanine, glutamic acid, lysine, glucosamine, and muramic acid in the same proportions.

However, the cell wall from the resistant organism contains serine and significantly more glycine than that of the sensitive organism, and, in addition, small quantities of other amino acids. The resistant cell wall also contains *O*-acetyl groups and phosphorus. It is concluded that the mechanism of resistance to lysozyme in *M. lysodeikticus* in this case is similar to that in *Staphylococcus aureus*.

In the years following the discovery of lysozyme[1] (Fleming, 1922), an enzyme capable of lysing *Micrococcus lysodeikticus* and other Gram-positive bacteria, various workers reported the isolation of lysozyme resistant colonies of *M. lysodeikticus* (Fleming and Allison, 1927; Hallauer, 1929; Feiner *et al.*, 1946). More recently, the isolation and selective development of *M. lysodeikticus* resistant to lysozyme was reported from our laboratory (Litwack, 1958). Although the resistant strain isolated in this laboratory is white, while the sensitive one is yellow in color, interdependence could not be found between carotenogenesis (Prasad and Litwack, 1961) and resistance to lysozyme (Litwack and Prasad, 1962).

Simultaneous with the isolation of the white resistant mutant of *M. lysodeikticus* in this laboratory, Brumfitt *et al.* (1958) described the isolation of a pigmented resistant *M. lysodeikticus*. They compared the chemical composition of the cell walls of their resistant and sensitive strains and found a significant difference only in *O*-acetyl content. The resistant cell walls contained about 3% by weight of *O*-acetyl groups and the sensitive cell walls had no detectable *O*-acetyl groups. Deacetylation of the resistant cell walls caused them to become susceptible to lysozyme while acetylation of the sensitive cell walls caused them to become resistant to the enzyme. Brumfitt *et al.* (1958) suggested that resistance to lysozyme in *M. lysodeikticus* and in *Bacillus subtilis* is due to a genetic change which facilitates *O*-acetylation of the hydroxyl groups of the cell wall. In contrast, Perkins (1960) found that removal of the *O*-acetyl groups from chemically acetylated cell walls of *M. lysodeikticus* did not fully restore sensitivity toward lysozyme. He concluded that the significance of the correlation between the chemical and biological acetylation of the walls is not clear.

In view of the unanswered problems related to the mechanism of resistance to lysozyme, the chemical composition of the cell walls of resistant and sensitive strains of *M. lysodeikticus* isolated in this laboratory have been examined. The results are reported in this communication.

Experimental

Bacteria. M. lysodeikticus, strain 19, was obtained originally from the collection of the Institute of Microbiology, Rutgers University. Strain ATCC 4698 was also used in these studies. The resistant strain was selected from the sensitive strain according to the method of Litwack (1958). The bacteria were kept on Bacto-peptone agar slants and were periodically subcultured.

Growth Experiments. Growth of sensitive and resistant strains was carried out in 100 ml of liquid medium contained in 250-ml side-arm culture flasks (Bellco, Vineland, N.J.). The flasks were shaken vigorously in a New Brunswick gyrotory incubator shaker maintained at 37° unless otherwise stated. The bacteria were grown in a basal medium of 1% Bacto-peptone (Difco Laboratories, Detroit, Mich.) and 0.5% sodium chloride in water, *p*H 7.5. The basal medium was supplemented by yeast extract (Difco) or by other substances. Preliminary experiments had shown that the sensitive strain grew best at *p*H 7.5, while growth of the resistant or-

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[1] Lysozyme is the trivial name for 3.2.1.17, mucopeptide *N*-acetylmuramylhydrolase.

ganism was optimal at *p*H 6.4–7.0. In the growth experiments the *p*H of the medium was therefore adjusted to 6.5 for the resistant strain and to *p*H 7.5 for the sensitive strain. Turbidity measurements were made with a Lumetron colorimeter using a 650-mμ filter.

Preparation of Cell Walls. Wet packed cells (40–50 g) were obtained after about 10 hours (at the end of the logarithmic phase) in 2-liter Erlenmeyer flasks containing 800 ml of 3% yeast extract and 0.5% sodium chloride in water. The cells were harvested and washed once with 0.85% sodium chloride and twice with distilled water. The cells were stored in the frozen state. The wet packed cells (30–35 g) were thawed, suspended in water to make a suspension of 50–60 ml, and homogenized in the cold with 55 ml of 200-mμ glass beads using the Virtis homogenizer. Good breakage was achieved in about 45 minutes (intermittent breakage and cooling) by using the homogenizing flask of 100-ml capacity. The resulting viscous mixture was diluted with water (100–150 ml) and the glass beads were separated by filtration with suction using a coarse sintered-glass funnel. The filtrate was immediately heated in a boiling-water bath for about 20 minutes to inactivate autolytic enzymes (Mandelstam and Rogers, 1959). The cell walls were purified by differential centrifugation according to the method of Cummins and Harris (1956).

Analytical Methods. AMINO ACIDS. Cell walls (20 mg) were hydrolyzed with 1 ml of 6 N HCl in a sealed tube for 16 hours at 105°. The major amino acids as well as glucosamine and muramic acid could be separated clearly on a descending unidimensional paper chromatogram (Whatman No. 40) transverse to the fiber direction, using the solvent system, 1-butanol–pyridine–water–glacial acetic acid (60:40:30:3) (Primosigh *et al.*, 1961). For quantitative determinations the chromatograms were run for 60–70 hours, dried overnight at room temperature, pulled through 0.25% solution of ninhydrin and 2% pyridine in acetone, and heated at 70° for 20 minutes. The spots were cut out and placed in test tubes, and the color was eluted by shaking for about 2 hours with 5 ml of methanol. The color was measured at 250 mμ on the Zeiss PMQ II spectrophotometer.

Glycine and serine were well separated on unidimensional chromatograms (Whatman No. 1 or No. 40 paper) run in the fiber direction, using 1-butanol-pyridine-water (60:40:30) as the solvent system (Jeanes *et al.*, 1951). The fast-moving minor amino acids could be qualitatively shown by chromatography in Smith's (1960) solvent system: 1-butanol–acetic acid–water (120:30:50). Amino acids were also analyzed quantitatively using the Beckman Spinco amino acid analyzer with hydrolysates of cell walls prepared as described.

AMINO SUGARS. Cell walls were hydrolyzed in 4 N HCl for 4 hours at 105° and amino sugars in the hydrolysate were quantitatively separated and estimated by paper chromatography as described.

O-ACETYL. *O*-Acetyl groups were estimated by the method of Hestrin (1949).

PHOSPHORUS. The phosphorus content of cell walls was measured by the method of Lowry and Lopez (1946).

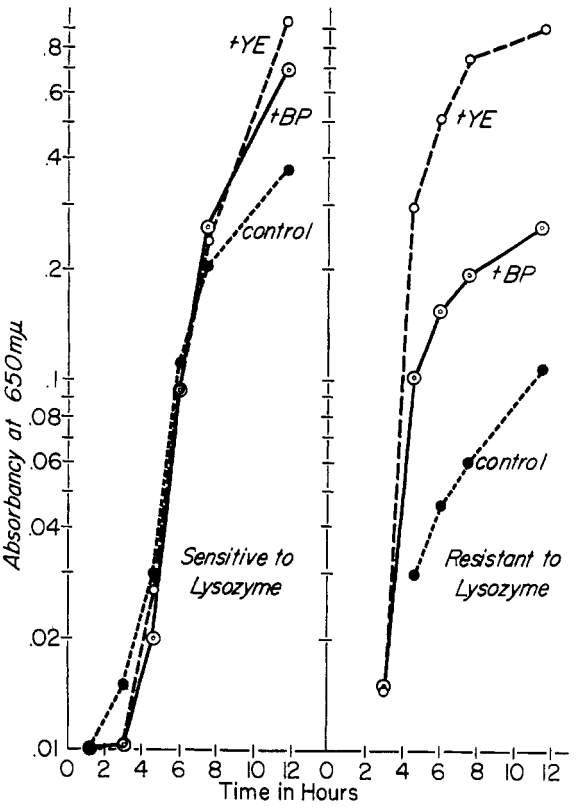

FIGURE 1: Growth curves of *M. lysodeikticus* sensitive or resistant to lysozyme. Curves on the left refer to *M. lysodeikticus* sensitive to lysozyme; the curves on the right refer to *M. lysodeikticus* resistant to lysozyme. *Control* curves represent growth on the basal medium; *+BP* refers to the addition of 2% Bacto-peptone to the basal medium; and *+YE* refers to the addition of 2% yeast extract to the basal medium.

SUSCEPTIBILITY TO LYSOZYME. The susceptibility to lysozyme of the cells or cell walls was determined according to the method of Prasad and Litwack (1963), using suitable concentrations of the enzyme.

Results

Growth Experiments. In Figure 1 are shown a few typical growth curves of *M. lysodeikticus* resistant or sensitive to lysozyme. Supplementing the basal medium (1% Bacto-peptone and 0.5% sodium chloride) with yeast extract increases the extent of growth of the resistant organism more dramatically than that of the sensitive organism. However, the extent of growth of either strain in the medium containing yeast extract is about equal. Preliminary experiments have shown that increasing the Bacto-peptone concentration of the basal medium from 1 to 3% had an appreciable effect on the growth rate and extent of growth of the sensitive strain, but not on the resistant strain. It was sur-

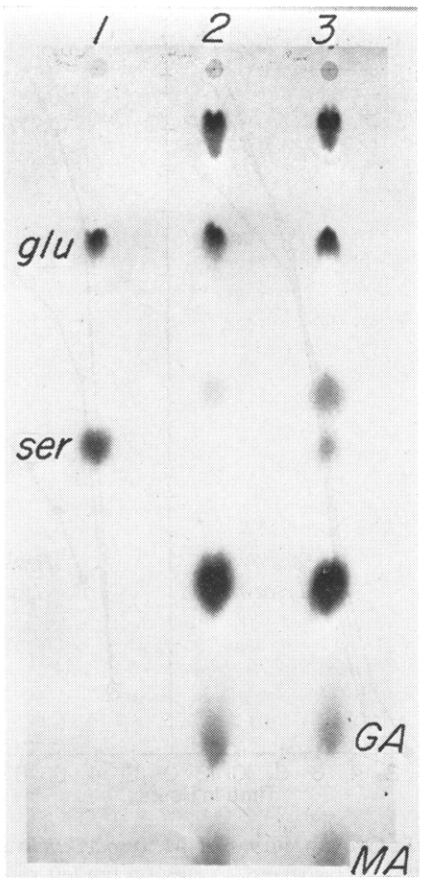

FIGURE 2: Chromatogram of cell-wall hydrolysates on Whatman No. 1 paper, using butanol-pyridine-water (60:40:30) as solvent system. (1) Standard spots of glutamic (Glu) acid and serine (Ser); (2) sensitive cell-wall hydrolysate; (3) resistant cell-wall hydrolysate. GA = glucosamine, MA = muramic acid.

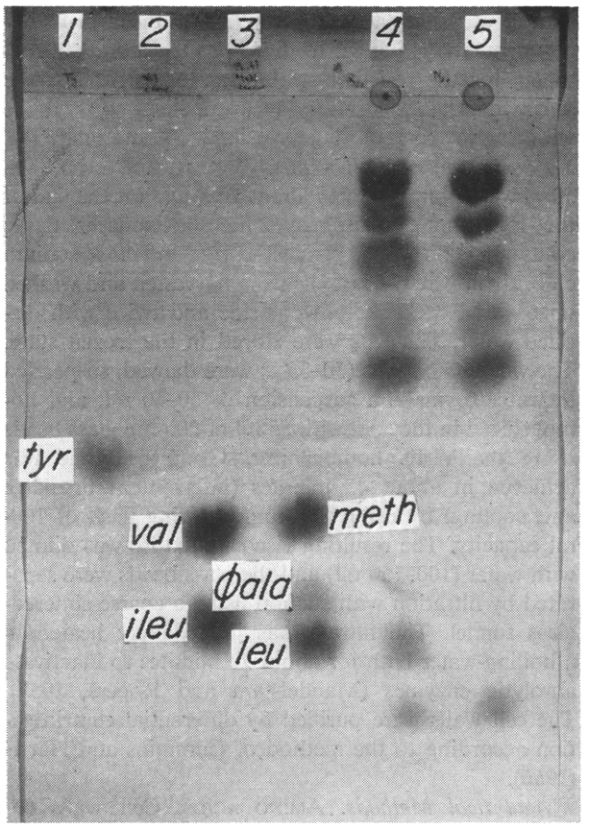

FIGURE 3: Chromatogram of cell-wall hydrolysates on Whatman No. 40 paper, using butanol–acetic acid–water as solvent system. (1) Tyrosine; (2) valine and isoleucine; (3) methionine, phenylalanine, and leucine; (4) resistant cell-wall hydrolysate; (5) sensitive cell-wall hydrolysate.

mised, therefore, that yeast extract contained an unknown factor or factors necessary for the rapid growth of the resistant strain. A few attempts were made to fractionate the yeast extract or test the effects of various substances on the growth of the resistant strain. A summary of the results obtained is given in Table I. The growth-promoting factor (or factors) of yeast extract is a water-soluble organic substance of relatively low molecular weight. While vitamins of the B group and various nucleotides or bases have no effect on the growth of the resistant strain when added to the basal medium, glucose, and to a lesser extent mannose, increase its growth.

Analysis of Cell Walls. All of the characteristic major components of the cell wall of *M. lysodeikticus* (alanine, glycine, glutamic acid, lysine, glucosamine, and muramic acid) are also present in the cell wall of resistant *M. lysodeikticus* as can be seen from Figure 2. The spots in columns 2 and 3 that are not labeled are, from top to bottom, lysine, glycine, and alanine. That the resistant cell wall alone contains serine in appreciable quantities is also evident from Figure 2. The cell wall, particularly the resistant cell wall, contains other amino acids and ninhydrin-positive substances in small amounts as shown in Figure 3. Of the fast-moving spots only leucine could be identified with reasonable certainty. The fastest moving spot has not yet been identified. Confirmation of the presence of serine and leucine in the resistant cell wall has come from the results of analysis of the hydrolysate with the Beckman Spinco amino acid analyzer. The amino acid analyzer has also revealed the presence of small amounts (less than 0.2 mole/mole of glutamic acid) of aspartic acid, threonine, valine, and ornithine in the resistant cell wall, whereas in the sensitive cell wall these are either not present or only barely detectable. The sensitive cell wall contains a small amount (less than 0.06 mole/mole of glutamic acid) of methionine.

The results of the quantitative analysis of cell walls that are sensitive or resistant to lysozyme are summarized in Tables II and III. They show good agreement between the molar ratios of the amino acids and glucosamine obtained by paper chromatography or by amino acid analyzer. It is evident that glycine is present to a greater extent in the resistant cell wall than in the

TABLE I: Effect of Addition of Various Substances to the Basal Medium on the Growth of Resistant *M. lysodeikticus*.

Substance Added to Basal Medium	Relative Maximal Growth
Basal medium	20
3% Yeast extract	140
3% Yeast extract after ether extraction	140
3% Yeast extract after butanol extraction	140
Dialysate of 3% yeast extract after 24 hours' dialysis	70
External solution of above dialysis after concentration	80
6 Volumes acetone precipitate from 3% yeast extract	140
1 Volume alcohol precipitate from 3% yeast extract	70
Ash equivalent to that from 3% yeast extract	20
Vitamin B_1, 20 μg/ml	20
Vitamin B_2, 20 μg/ml	20
Biotin, 1.00 μg/ml	20
Nicotinamide, 1.0 μg/ml	20
Pyridoxine HCl, 1 μg/ml	20
Inositol, 20 μg/ml	20
Ca-pantothenate, 20 μg/ml	20
Adenine, guanine, cytosine, uracil, hypoxanthine, adenylic acid, guanylic acid, cytidylic acid, uridylic acid, or RNA, 100 mg/ml	20
0.5% Glucose	70–80
0.5% Mannose	55–70

TABLE II: Analysis of Cell Wall Preparation from Lysozyme-sensitive *M. lysodeikticus*.

Substance	Results from Paper Chromatography (mmoles/ 100 g)	(molar ratio)[a]	Results from Amino Acid Analyzer (mmoles/ 100 g)	(molar ratio)[a]
Alanine	157	2.0	129.0	2.4
Glycine	93	1.2	71.3	1.4
Glutamic acid	78	1.0	52.8	1.0
Glucosamine	93	1.2	34.8	0.7
Lysine	79	1.0	27.4	0.9
Muramic acid			43.3	0.8
Serine			(0.637)	0.01
O-Acetyl	0			
Phosphorus	5			

[a] Molar ratio is the ratio of the number of moles of the substance to that of glutamic acid.

TABLE III: Analysis of Cell Wall Preparation from Lysozyme-resistant *M. lysodeikticus*.

Substance	Results from Paper Chromatography (mmoles/ 100 g)	(molar ratio)[a]	Results from Amino Acid Analyzer (mmoles/ 100 g)	(molar ratio)[a]
Alanine	150	2.0	60.2	2.0
Glycine	236	3.2	115	3.7
Glutamic acid	75	1.0	31	1.0
Glucosamine	93	1.3	28.2	0.9
Lysine	76	1.0	36.6	1.2
Muramic acid			26.6	0.9
Serine	44	0.6	24.8	0.8
O-Acetyl	37			
Phosphorus	80			

[a] Molar ratio is the ratio of the number of moles of the substance to that of glutamic acid.

sensitive cell wall. The *O*-acetyl and phosphorus (probably derived from teichoic acids) content of the resistant cell wall is 2.2 and 2.5%, respectively. In contrast, the sensitive cell walls do not have detectable *O*-acetyl groups or phosphorus.

Chemical Treatment of Cells and Cell Walls and Their Susceptibility to Lysozyme. The results of acetylation of sensitive cells and cell walls and deacetylation of resistant cells and their susceptibility to lysozyme are shown in Table IV. It is clear that while acetylation of sensitive cells or cell walls markedly decreases their rate of digestion by lysozyme, deacetylation of the resistant cells does not make them susceptible to lysozyme. Estimation of the *O*-acetyl groups of the deacetylated resistant cells shows that about 45% of the *O*-acetyl groups originally present in the resistant cells have been removed by the procedure used for deacetylation. These results confirm those obtained by Brumfitt (1959) insofar as the sensitive cells or cell walls are concerned. Deacetylation experiments do not, however, align with the results obtained by Brumfitt (1959) with his resistant cells and cell walls. This may be due to the difference in strain. It should be noted that the resistant strain obtained by Brumfitt (1959) is pale yellow in color while that used here is white.

Discussion

The chemical composition of the cell walls of sensitive and resistant *M. lysodeikticus* shows that the major amino acids, alanine, glutamic acid, glycine, lysine, and serine, are present in a 2:1:1:1:0.01 ratio in the sensitive cell wall and in a 2:1:3.5:1:0.8 ratio in the resistant cell wall. Although absolute concentrations of amino acids and amino sugars determined by paper chromatography compared with those obtained from the amino acid analyzer are not in close agreement, the

molar ratios of these components relative to the glutamic acid concentration agree very well in either case. Because much larger samples were used on the amino acid analyzer, quantitative results from this procedure represent more highly probable values. The presence in the resistant cell wall of serine and of elevated glycine content, together with minor amounts of other amino acids, is similar to the amino acid composition of the cell wall of *Staphylococcus aureus* strain Duncan. This has been shown to contain excess glycine, serine, and small quantities of valine, leucine, threonine, aspartic acid, and methionine (Hancock, 1960). Hancock reports also that an unidentified component that is eluted before lysine is present to the extent of about 0.3 mole of the glutamic acid content. It may be that this component is ornithine, which appears in the resistant cell wall reported here. It is interesting to note that ornithine is the main diamino acid of the cell wall mucopeptide of the radiation-resistant *Micrococcus radiodurans* (Work, 1964). These results are not in line with results obtained from Brumfitt's resistant strain (Brumfitt, 1959). Brumfitt showed that no significant difference could be found in the amino acid or amino sugar composition of the cell walls of his sensitive and resistant strains of *M. lysodeikticus*. The presence of *O*-acetyl groups in the resistant cell walls and the absence of *O*-acetyl groups in sensitive cells and cell walls, however, are similar to the case described by Brumfitt (1959). Our sensitive cells and cell walls became partially resistant to lysozyme on acetylation with acetic anhydride in pyridine; however, our resistant cells were not made sensitive by deacetylation. In this connection the behavior of our resistant strain is more in line with that of *Staphylococcus aureus* (Brumfitt, 1959). Since cell walls of *Streptococcus faecalis* which contain about 1–2% of *O*-acetyl groups (Abrams, 1958) are susceptible to the action of lysozyme, it is difficult to attribute resistance to lysozyme solely to the presence of *O*-acetyl groups in the bacterial cell wall.

TABLE IV: Effect of Various Treatments of Cells or Cell Walls of Sensitive and Resistant *M. lysodeikticus* on Their Subsequent Susceptibility to Lysozyme.

Substance	Treatment	Relative Rate of Lysis by Lysozyme[a] (%)
Sensitive cells	None	100
Sensitive cells	Acetylated[b]	14
Sensitive cell walls	None	72
Sensitive cell walls	Acetylated[b]	25
Resistant cells	None	0
Resistant cells	Deacetylated[c]	0
Resistant cells	Mercaptoethanol[d]	0

[a] Egg-white lysozyme (10 μg) (Armour Laboratory) per 3.0 ml of test solution. [b] Cells or cell walls (8 mg) + 2 ml acetic anhydride + 0.1 ml pyridine, incubated for 1 hour at 37°, followed by three washings at 0° in 0.67 M phosphate buffer, *p*H 6.2. [c] Deacetylation performed by incubating resistant cells in 0.1 M Sorensen's glycine buffer, *p*H 11.0, for 1 hour at 37°, followed by three washings in 0.67 M phosphate buffer, *p*H 6.2. [d] Resistant cells incubated with 10% (v/v) mercaptoethanol in 8 M urea at *p*H 3.0 for 30 minutes at 37°, followed by three washings in 0.67 M phosphate buffer, *p*H 6.2.

The cell wall of sensitive *M. lysodeikticus* has been shown to contain a small amount of methionine. Its presence in the sensitive cell wall is consonant with the observation by McDonald (1963) that methionine may be essential for the growth of *M. lysodeikticus*, *M. sodonesis*, and *Micrococcus sp.* (ATCC 407).

The whole question of the mechanism of resistance to lysozyme in the bacterial cell wall is obviously a very complicated one and has not yet been answered. Salton and Ghuysen (1959) were the first to isolate a disaccharide from lysozyme digests of cell walls of *M. lysodeikticus*, and they formulated its structure as β-(1→6)-*N*-acetylglucosaminyl-*N*-acetylmuramic acid. It has since been established that the structure of this disaccharide is β-(1→4)-*N*-acetylglucosaminyl-*N*-acetylmuramic acid (Jeanloz *et al.*, 1963). It may be that β-(1→4) bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine residues are preferentially attacked by lysozyme when the *N*-acetylmuramic acid is linked through its 4 carbon to carbon 1 of *N*-acetylglucosamine. Ghuysen and Strominger (1963) have recently isolated from *S. aureus* cell wall two disaccharides, β-(1→6)-*N*-acetylglucosaminyl-*N*,4-*O*-diacetylmuramic acid, with the possibility that 10% of the disaccharides might have the β-(1→4) linkage.

The presence of a negatively charged teichoic acid in the resistant cell wall reported here may be one explanation of resistance to the action of lysozyme, if the negatively charged groups of teichoic acid are capable of binding the positively charged groups of lysozyme to block the action of the enzyme from the substrate. Another cause may be an extensive cross-linking between the peptide chains with glycine and serine serving as the linking amino acids. It is concluded, therefore, that the mechanism of resistance to lysozyme in *M. lysodeikticus* is similar to that in *S. aureus*. Supporting this conclusion is the fact that, to date, there has been no clear cut distinction between the *Micrococcus* and *Staphylococcus* species (Stechschulte, 1961; Thatcher and Simon, 1957).

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References

Abrams, A. (1958), *J. Biol. Chem. 230*, 949.
Brumfitt, W. (1959), *Brit. J. Exptl. Pathol. 40*, 441.
Brumfitt, W., Wardlaw, A. C., and Park, J. T. (1958), *Nature 181*, 1783.
Cummins, C. S., and Harris, H. (1956), *J. Gen. Microbiol. 14*, 583.
Feiner, R. R., Meyer, K., and Steinberg, A. (1946), *J. Bacteriol. 52*, 375.
Fleming, A. (1922), *Proc. Roy. Soc. (London) B93*, 306.
Fleming, A. and Allison, V. D. (1927), *Brit. J. Exptl. Pathol. 8*, 214.
Ghuysen, J. M., and Strominger, J. L. (1963), *Biochemistry 2*, 1119.
Hallauer, C. (1929), *Zentr. Bakteriol. Parasitenk., Abt. I Ref. Orig. 114*, 519.
Hancock, R. (1960), *Biochim. Biophys. Acta 37*, 42.
Hestrin, S. (1949), *J. Biol. Chem., 180*, 249.
Jeanes, A., Wise, C. S., and Dimmler, R. J. (1951), *Anal. Chem. 23*, 415.
Jeanloz, R. W., Sharon, N., and Flowers, H. M. (1963), *Biochem. Biophys. Res. Commun. 13*, 20.
Litwack, G. (1958), *Nature 181*, 1348.
Litwack, G., and Prasad, A. L. N. (1962), *Nature 196*, 543.
Lowry, O. H., and Lopez, J. A. (1946), *J. Biol. Chem. 162*, 421.
McDonald, I. J. (1963), *Can. J. Microbiol. 9*, 415.
Mandelstam, J., and Rogers, H. J. (1959), *Biochem. J. 72*, 654.
Perkins, H. R. (1960), *Biochem. J. 74*, 186.
Prasad, A. L. N., and Litwack, G. (1961), *Biochim. Biophys. Acta 46*, 452.
Prasad, A. L. N., and Litwack, G. (1963), *Anal. Biochem. 6*, 328.
Primosigh, J., Pelzer, H., Maass, D., and Weidel, W. (1961), *Biochim. Biophys. Acta 46*, 68.
Salton, M. R. J., and Ghuysen, J. M. (1959), *Biochim. Biophys. Acta 36*, 552.
Smith, I. (1960), Chromatographic and Electrophoretic Techniques, Vol. I, New York, Interscience.
Stechschulte, M. A. L. (1961), Ph.D. dissertation, Catholic University of America.
Thatcher, F. S., and Simon, W. (1957), International Bulletin of Bacteriological Nomenclature and Taxonomy, Vol. VII, p. 21.
Work, E. (1964), *Nature 201*, 1107.

Comparison of the Levels of Phosphodiesterase, Endonuclease, and Monophosphatases in Several Snake Venoms*

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ABSTRACT: The venoms of five species of snake have been analyzed for phosphodiesterase, 5′-nucleotidase, endonuclease, and alkaline nonspecific phosphatase. Using very dilute solutions of venom, it has been possible to determine endonuclease directly in crude venom by the spectrophotometric method. The venoms of *Agkistrodon piscivorus*, *Bothrops atrox*, *Crotalus adamanteus*, and *Crotalus atrox* resemble each other approximately in contents and relative proportions of the four enzymes.

The venom of *Naja nigricollis* contains an unusually large amount of the nonspecific phosphatase, making it unsuitable as a starting material for the purification of phosphodiesterase.

Snake venoms are known to contain at least four enzymes involved in the hydrolysis of phosphate bonds (Georgatsos and Laskowski, 1962; Sulkowski *et al.*, 1963). For the purpose of isolating a particular enzyme, it would be desirable to find a venom with a high concentration of that enzyme and a low concentration of the others. The problem of selecting the most desirable venom for the isolation of phosphodiesterase has become acute, since the *Bothrops atrox* venom formerly used for this purpose is no longer available in this country. The venoms of five species of snake have been assayed for endonuclease, phosphodiesterase, 5′-nucleotidase, and alkaline nonspecific phosphatase. The results indicate that with one exception the venoms have rather similar complements of enzymes.

In previous work on venom endonuclease, Georgatsos

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