JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1990, p. 2627–2631
0095-1137/90/122627-05$02.00/0

Vol. 28, No. 12

Modification of the Silver Staining Technique To Detect Lipopolysaccharide in Polyacrylamide Gels

ANDERS FOMSGAARD,[1,2]† * MARINA A. FREUDENBERG,[1] AND CHRIS GALANOS[1]

Max-Planck Institut für Immunbiologie, Freiburg, Federal Republic of Germany,[1] and State Seruminstitute, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark[2]

Received 11 July 1990/Accepted 28 August 1990

A silver staining method used routinely for detecting bacterial lipopolysaccharide (LPS) in sodium dodecyl sulfate-polyacrylamide gels (C. Tsai and E. Frasch, Anal. Biochem. 119:115–119, 1982) appeared to be inappropriate for visualizing certain LPS preparations. It did not stain S-form fractions of polyagglutinable *Pseudomonas aeruginosa* LPS or several partly deacylated (alkali-treated) S-form LPSs after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, these LPS preparations could be detected by anti-LPS sera after electroblotting onto nitrocellulose, thereby confirming their integrity and presence in the polyacrylamide gel. This is because LPS fractions containing a low number of fatty acids are washed out of the gel during the initial fixing step (40% ethanol–4% acetic acid, overnight). By omitting this fixing step, which was originally developed for detecting proteins, and by increasing the LPS oxidation time (from 5 to 20 min), we restored the ability to detect LPS fractions that otherwise would not be stained. These modifications did not affect the detection of other S- and R-form LPSs. Thus, differences in the number of fatty acids present in polyagglutinable *P. aeruginosa* LPS may result in a selective loss of fatty acid-deficient S-form LPS in these apparent R-form LPS preparations. This modified procedure provides a fast, simple, and sensitive way to analyze LPS in polyacrylamide gels despite the number of acyl groups present.

The lipopolysaccharide (LPS) molecules of gram-negative bacterial cell walls are the main antigen (O antigen) as well as the source of endotoxic activity of these bacteria (7, 15). LPS consists of an O-specific polysaccharide chain of repeating oligosaccharide units, the core oligosaccharide complex, and lipid A. Lipid A of members of the family *Enterobacteriaceae* consists typically of a D-glucosamine disaccharide substituted with phosphate and carrying amide-bound and ester-bound 3-hydroxy- or (*R*)-3-acyloxyacyl residues (7, 15). LPS from S-form bacteria exhibits a high degree of heterogenicity and consists of molecules with different numbers of repeating oligosaccharide units. By contrast, LPS from mutant R-form bacteria lacks the O-polysaccharide chain, thereby consisting of only core components and lipid A (7, 15).

Analysis of S-form LPS preparations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by a traditional silver staining technique (18) reveals numerous bands arranged in a ladderlike pattern; such bands reflect the number of repeating units present in the O chain of the LPS (14, 18). The fastest migrating band is LPS lacking the O repeating units, whereas the second band represents the core-plus-one repeating unit and so forth. Thus, silver-stained SDS-polyacrylamide gels are often used to distinguish between R-form LPS and S-form LPS.

SDS-PAGE analysis of LPS derived from clinical isolates of polyagglutinable strains of *Pseudomonas aeruginosa* showed a rough appearance after silver staining which was characteristic of nontypeable *P. aeruginosa* strains (3, 9). However, corresponding immunoblotting with hyperimmune serum from cystic fibrosis patients with pulmonary *P. aeruginosa* infection revealed the presence of bands in the O antigen region that were not visible in the silver-stained gels even when high concentrations of LPS were used (4). This indicates that the material is immunoreactive but not detectable by the traditional silver staining method. The present study was undertaken to define the factors responsible for the low staining efficiency of certain LPS fractions and to examine ways in which the procedure might be improved. We show that LPS fractions containing a low number of fatty acids may be washed out of the gel during the initial fixing step. This can be avoided by omitting the fixing step and extending the oxidation time of the silver staining procedure.

MATERIALS AND METHODS

Wild-type LPS. LPS of *Salmonella abortus equi* was used as a standard LPS because of its well-defined chemical composition and structure (7, 15). It was isolated by the hot phenol-water method (19) and was purified by successive ultracentrifugation steps and by subsequent extraction by the phenol-chloroform-petroleum-ether method (8). Purified LPS was electrodialyzed and converted to its uniform triethylamine salt form (6). LPS was also extracted by the hot phenol-water method from polyagglutinable *P. aeruginosa* 170 serotype O:3/9 isolated from a cystic fibrosis patient with chronic pulmonary infection (3); it was purified by ultracentrifugation steps and converted to the triethylamine salt form after electrodialysis.

Fatty acid-deficient LPS. Ester-bound fatty acids were removed from the *S. abortus equi* LPS by treatment in 0.2 M NaOH at 100°C for 1 h. The LPS was then acidified with glacial acetic acid, and the alkaline LPS (LPS-OH) thus obtained was washed three times in ice-cold ethanol and freeze-dried.

Chemical analysis. Fatty acids were liberated by hydrolysis in 4 M HCl at 100°C for 4 h followed by *trans*-esterification in 15% methanol–2 M HCl at 85°C for 10 h (H. W. Wollenweber and E. T. Rietschel, *in* R. L. Whistler, ed., *Methods in Carbohydrate Chemistry*, vol. 9, in press).

* Corresponding author.
† Present address: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, 12441 Parklawn Dr., Rockville, MD 20852.

The methyl ester derivatives thus obtained were determined by gas-liquid chromatography with a gas chromatograph (model 3700; Varian) connected to a Hewlett-Packard integrator (model 3380). A 25-m-long capillary column (SE-54; Weeke, Mulheim, Federal Republic of Germany) was used, and H_2 was used as the carrier gas (70 kPa).

Ester-bound fatty acids were determined by a sodium methoxide procedure (Wollenweber and Rietschel, in press). *trans*-Esterification was performed in 0.5 M sodium methoxide in the presence of methanol at 37°C for 16 h. The fatty acid extract was then carbomethylated with diazomethane and analyzed by gas-liquid chromatography.

Amide-bound fatty acids were analyzed as de-O-acetylated samples obtained as the precipitates after sodium methoxide reaction. The analysis procedure was the same as that employed for total fatty acid analysis. Abequose was quantitated by using the method of Cynkin and Ashwell (2). Analyses for neutral sugars, hexosamine, and ketodeoxyoctonate were carried out by standard procedures (11, 13, 16).

SDS-PAGE. All LPS preparations were treated for 5 min at 100°C in 0.05 M Tris hydrochloride buffer (pH 6.8)–2% (wt/vol) SDS–10% (wt/vol) sucrose–0.01% bromphenol blue, and fractionated on an SDS-polyacrylamide gel (10 cm by 10 cm by 0.75 mm) containing 4% and 12.5% acrylamide in the stacking and separating gels, respectively. Electrophoresis was done at 12 mA in the stacking gel and 25 mA in the separating gel until the tracking dye had run about 10 cm.

Immunoblotting. LPS was visualized by immunoblotting after transfer to nitrocellulose paper (pore size, 0.45 μm) (Schleicher & Schüll, Dassel, Federal Republic of Germany) by means of semidry electroblotting at 36 V for 1 h. After blocking in 1% gelatin–Tris-buffered saline (TSG), the nitrocellulose was incubated for 2 h at room temperature with rabbit antiserum (1:500 in TSG) specific for *S. abortus equi* or with pooled human immune serum (1:1,000 in TSG) obtained from 10 cystic fibrosis patients with chronic *P. aeruginosa* lung infection (duration, 5 to 10 years). The anti-LPS immunoreactivity of the latter is described elsewhere (4). After washing three times in Tris-buffered saline, the nitrocellulose paper was reacted for 1 h with peroxidase-conjugated swine anti-rabbit immunoglobulins (1:200 in TSG) (Dakopatts, Glostrup, Denmark) or rabbit anti-human immunoglobulin G (1:200 in TSG) (Dakopatts). The nitrocellulose sheets were again washed three times, and the color was developed with 0.003% (wt/vol) 4-chloro-1-naphthol–0.05% (vol/vol) H_2O_2.

Modified silver staining. SDS-PAGE-fractionated LPS preparations were stained by the conventional silver staining method of Tsai and Frasch (18), with the following modifications. (i) LPS was oxidized in the gel with 0.7% periodic acid in 40% ethanol–5% acetic acid at 22°C for 20 min without prior fixation. (ii) The gel was then washed three times with distilled water for 5 min. (iii) The gel was stained for 10 min with freshly prepared staining solution which was prepared as follows. A 4-ml volume of concentrated ammonium hydroxide was added to 56 ml of 0.1 M sodium hydroxide. After the addition of about 200 ml of water, 10 ml of 20% (wt/vol) silver nitrate (Roth, Darmstadt, Federal Republic of Germany) was added in drops with stirring. The final volume was adjusted to 300 ml with water. (iv) The gel was then washed three times with distilled water for 5 min. (v) The color was developed by reduction in 200 ml of water containing 10 mg of citric acid and 0.1 ml of 37% formaldehyde. The gel was photographed immediately or the color reaction was stopped by exposure to 10% acetic acid for 1 min followed by repeated washings in distilled water.

TABLE 1. Chemical composition of *P. aeruginosa* 170 serotype O:3/9 LPS

Component	Amt (nmol/mg of LPS)
Rhamnose	240
Glucose	639
Heptose	147
Glucosamine	224
Galactosamine	83
Ketodeoxyoctonate	183
Fatty acids	933
C_{12}	88
C_{16}	43
3-OH-C_{10}	193
2-OH-C_{12}	281
3-OH-C_{12}[a]	328

[a] Amide-bound fatty acid.

RESULTS

Chemical analysis. The *S. abortus equi* LPS demonstrated the presence of ester-bound dodecanoic, tetradecanoic, hexadecanoic, and 2-hydroxy-tetradecanoic acids, together with amide-bound 3-hydroxy-tetradecanoic fatty acid. The detailed chemical structure of *S. abortus equi* LPS has been described earlier (5, 7, 15). Chemical analysis of the *S. abortus equi* LPS-OH confirmed the presence of the two amide-bound 3-OH-C_{14} fatty acids and the absence of all ester-bound fatty acids.

The chemical composition of the *P. aeruginosa* serotype O:3/9 LPS is given in Table 1. Although the higher molar proportion of fatty acids to sugars in *P. aeruginosa* O:3/9 LPS indicated a predominantly rough structure, moderate amounts of saccharides were present nevertheless. The immunochemical structure of this LPS and of other polyagglutinable *P. aeruginosa* LPS are described in detail elsewhere (3).

Modified silver staining and immunoblotting. The ladder patterns obtained by both the conventional and modified silver stain procedures were identical when LPS from different bacterial strains and species (*Klebsiella pneumoniae*, *Shigella flexneri*, *Escherichia coli* serotype O111:B4, *E. coli* Ra-Re, *Salmonella minnesota*, *S. minnesota* R60, and *Salmonella typhimurium*) were tested; however, different results were obtained with some preparations of LPS. For example, when the traditional silver staining (Fig. 1) and the modified silver staining (Fig. 2) techniques were compared by using equal amounts (5 μg) of *S. abortus equi* LPS (lanes II) and LPS-OH (lanes I) after electrophoresis in SDS-polyacrylamide gels, the wild-type LPS stained well by both methods (Fig. 1 and 2, lanes II) and revealed about 40 bands; however, LPS-OH was detected only by the modified silver stain method (Fig. 2, lane I). Similar results were obtained when LPS and corresponding LPS-OH from other *Salmonella* and *E. coli* species were used (results not shown). Thus, reduction of the number of long-chain fatty acids present alters the LPS fractions so that they are no longer detected by the traditional silver staining procedure. The LPS fractions in the LPS-OH preparation migrated faster than their untreated counterparts, and the faster migration was most evident for the lower-molecular-weight fractions (Fig. 2). This may be explained by the reduced molecular weight of the LPS-OH fractions after cleavage of the ester-bound fatty acids in the lipid A parts; however, a change in the molecular conformation or net charge or both may also contribute to the slightly altered migration pattern.

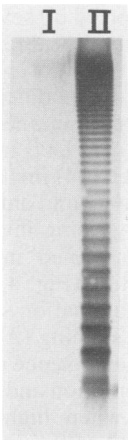

FIG. 1. Silver-stained SDS-PAGE pattern of LPS from *S. abortus equi*. A 5-μg amount of unmodified LPS (II) or partly deacylated LPS-OH (I) was applied to each well and stained by the traditional silver staining method (18).

Immediately after SDS-PAGE, both unmodified LPS and the partly deacylated LPS-OH could be transferred from the gel to nitrocellulose paper and detected by specific anti-LPS antibodies (Fig. 3), thereby confirming their integrity and initial presence in the gel. A ladder pattern matching the immunoblot was obtained for polyacrylamide gels stained by the modified method (Fig. 2). After overnight exposure of LPS-OH-containing gels to 40% ethanol-4% acetic acid (fixing solution used in the traditional method) or after exposure for as little as 1 h, LPS-OH could no longer be detected by subsequent immunoblotting. By contrast, unaltered *S. abortus equi* LPS could still be detected by immunoblotting after fixation (results not shown).

To follow the fate of LPS-OH during traditional silver staining, 500 μg of *S. abortus equi* LPS or LPS-OH was applied evenly to the full length of the SDS-polyacrylamide gels. Abequose, which is a characteristic constituent of *S. abortus equi* LPS, was then measured in the fixing solution as well as in washings throughout the silver staining procedure after concentration by rotary evaporation. Large amounts of abequose (representing approximately 95% of the loaded LPS) were detected in the fixing solution from gels loaded with LPS-OH. Fixing solution from gels loaded with the same amount of unaltered LPS contain only minute amounts of abequose. Moreover, silver-stained bands appeared only in the gel loaded with unaltered LPS. These experiments suggest that LPS-OH was washed out of the gel during the fixing step in the traditional method.

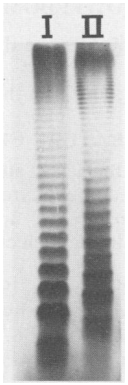

FIG. 2. Silver-stained SDS-PAGE pattern of *S. abortus equi* LPS. A 5-μg amount of unmodified LPS (II) or partly deacylated LPS-OH (I) was applied to each well and stained by the modified silver staining method.

FIG. 3. Immunoblotting of unmodified LPS (II) and LPS-OH (I) from *S. abortus equi* by using specific rabbit antiserum.

In our modified silver staining method, the gel was oxidized by periodic acid without the prior fixing procedure. Optimal staining of LPS-OH was obtained after 20 min of oxidation with 0.7% periodic acid. Background staining was controlled by extensive washings and the use of relatively thin gels (0.75 mm).

Analysis of polyagglutinable *P. aeruginosa* LPS. The modified silver staining method also was applied to a polyagglutinable LPS from a clinical isolate of *P. aeruginosa* serotype O:3/9. This LPS appeared rough in SDS-polyacrylamide gels when traditional silver staining was used (Fig. 4, left); however, immunoblotting revealed the presence of bands in the O-antigenic region (Fig. 5). This difference was not just a quantitative problem, since 10 times more LPS (50 μg per lane) did not lead to an increase in the density of any high-molecular-weight bands (results not shown). The S-form LPS fractions seen in immunoblotting could now be visualized by using the modified silver staining procedure for fatty acid-depleted LPS (Fig. 4, right). This qualitative difference in staining suggests the presence of S-form LPS with low numbers of fatty acids in this LPS preparation.

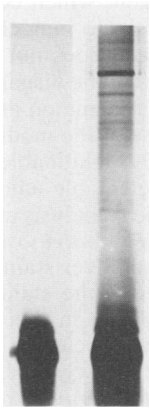

FIG. 4. LPS (5 μg) from polyagglutinable *P. aeruginosa* serotype O:3/9 was demonstrated by traditional silver staining (left) or modified silver staining (right) of SDS-polyacrylamide gels.

FIG. 5. LPS (5 μg) from polyagglutinable *P. aeruginosa* serotype O:3/9 was demonstrated by immunoblotting with hyperimmune serum from cystic fibrosis patients.

DISCUSSION

The sensitivity of the silver staining technique for LPS, as described by Tsai and Frasch (18), is comparable to that of autoradiography (14, 18) and gives similar results. By the use of these techniques, S-form LPS preparations are found to consist of a heterogeneous mixture of LPS molecules with various numbers of repeating oligosaccharide units ranging from 0 to as many as 40 (14, 18) (Fig. 1 through 3). The polysaccharide part of the LPS molecule is the reactive component in the silver stain because oxidization of the hexoses present makes aldehyde groups available for subsequent reaction with the silver nitrate. However, as demonstrated in the present study, retention of the various LPS fractions in the SDS-polyacrylamide gel during fixing or oxidization or both may be a property of the number of fatty acids present in the lipid A part of the LPS molecule. LPS fractions containing a low number of fatty acids are washed out of the gel during the initial fixing step originally developed for proteins (17, 18). By omitting this fixing step and increasing the oxidation time (from 5 to 20 min), we restored the ability to directly detect all LPSs used, despite their degree of acylation.

Immunoblotting is an important and easy method for the indirect detection of bacterial LPS (3, 4). It is useful in confirming the presence of LPS antigens that are not easily detected by traditional silver staining because of their low degree of acylation. This has also been reported for enterobacterial common antigen, which consists of a polysaccharide chain linked to a single long-chain fatty acid. Enterobacterial common antigen does not stain by the traditional silver stain for LPS but can be visualized by immunoblotting with anti-enterobacterial common antigen antibodies (12).

An example of the use of the modified silver stain is given with LPS from a polyagglutinable *P. aeruginosa* strain containing small but detectable amounts of S-form LPS as demonstrated by chemical analysis and immunoblotting. The high-molecular-weight *P. aeruginosa* LPS fractions are not visible by the traditional silver stain but were visualized by using our modifications of the staining procedure, thereby expressing the pattern seen in the immunoblotting experiments. This suggests a deficiency in the number of fatty acids in the high-molecular-weight LPS fractions present in this polyagglutinable *P. aeruginosa* LPS. Approximately 10% of clinical isolates of *P. aeruginosa* are still nontypeable, and polyagglutinable nontypeable strains in particular are associated with the chronic pulmonary infections of cystic fibrosis (9). As reported by several investigators, most of the polyagglutinable nontypeable clinical isolates appear to be rough strains that do not possess smooth LPS O antigen (3, 9). However, the modified silver stain, as well as immunoblotting with hyperimmune cystic fibrosis serum, enables one to detect S-form LPS in three polyagglutinable strains previously described (4) including the LPS shown in Fig. 3. The biological activity and antigenic specificity of this S-form LPS are currently being investigated. One band in the *P. aeruginosa* LPS stained intensely by both silver staining and immunoblotting (Fig. 4 and 5); this is probably the result of protein contamination since this band also was detected by protein gold staining (Aurodye; Janssen) of the blotted nitrocellulose. The presence of this particular protein after thorough phenol extraction and purification of the LPS, as well as its absence when high-molecular-weight LPS fractions were diffused out of the gel by the fixing solution, suggests a strong association with high-molecular-weight LPS fractions. The *P. aeruginosa* common antigen described by Høiby et al. (10) is a likely candidate, thereby explaining its reaction with the *Limulus* amoebocyte lysate (1).

The presence of LPS molecules with few fatty acids in LPS preparations is difficult to detect by chemical analysis that provides only average molar concentrations of fatty acids in the whole preparation. Thus, heterogeneity within LPS preparations may exist not only in the number of repeating oligosaccharide units within the O-polysaccharide chain but also in the number of fatty acids in the toxic lipid A part of the LPS molecules. By fractionation of LPS preparations prior to chemical analysis, Galanos et al. (5) found that lipid A of S-form LPS contained on average fewer fatty acids than its R-form counterpart. The relative deficiency of fatty acids in S-form LPS molecules may thus be a general feature characteristic of certain LPSs (e.g., polyagglutinable *P. aeruginosa* LPSs) and could also contribute to the low toxicity observed for many S-form LPSs as compared with R-form LPSs (5, 7, 15).

The modified short silver staining analysis presented in this study, as well as immunoblotting, are recommended for the thorough characterization of LPSs, especially for the detection of fatty acid-deficient S-form LPS molecules in apparent R-form LPS preparations. This method appears to be faster, simpler, and more sensitive than other staining methods for the direct detection of LPS in polyacrylamide gels.

ACKNOWLEDGMENTS

M. L. Gundelach and I. Minner are thanked for skillful technical assistance.

A.F. was supported by a grant from the Max-Planck Gesellschaft.

LITERATURE CITED

1. **Baek, L., N. Høiby, J. B. Hertz, and F. Espersen.** 1985. Interaction between *Limulus* amoebocyte lysate and soluble antigens from *Pseudomonas aeruginosa* and *Staphylococcus aureus* studied by quantitative immunoelectrophoresis. J. Clin. Microbiol. **22**:229–237.
2. **Cynkin, M. A., and G. Ashwell.** 1970. Estimation of 3-deoxy sugars by means of the malonaldehyde-thiobarbituric acid reaction. Nature (London) **186**:155–156.
3. **Fomsgaard, A., R. S. Conrad, C. Galanos, G. H. Shand, and N. Høiby.** 1988. Comparative immunochemistry of lipopolysaccharides from typable and polyagglutinable *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis. J. Clin. Microbiol. **26**:821–826.
4. **Fomsgaard, A., N. Høiby, G. H. Shand, R. S. Conrad, and C.**

Galanos. 1988. Longitudinal study of antibody response to lipopolysaccharides during chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis. Infect. Immun. **56**:2270–2278.

5. **Galanos, C., B. Jiao, T. Kumoro, M. A. Freudenberg, and O. Lüderitz.** 1988. Large-scale fractionation of S-form lipopolysaccharide from *Salmonella abortus equi*. Chemical and serological characterization of the fractions. J. Chromatogr. **440**:397–404.
6. **Galanos, C., and O. Lüderitz.** 1975. Electrodialysis of lipopolysaccharides and their conversion to uniform salt forms. Eur. J. Biochem. **54**:603–610.
7. **Galanos, C., O. Lüderitz, E. T. Rietschel, and O. Westphal.** 1977. Newer aspects of the chemistry and biology of bacterial lipopolysaccharides, with special reference to their lipid A component, p. 239–335. *In* T. W. Goodwin (ed.), International review of biochemistry: biochemistry of lipids, vol. 2. University Park Press, Baltimore.
8. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9**:245–249.
9. **Hancock, R. E. W., L. M. Mutharia, L. Chan, R. P. Darveau, D. P. Speert, and G. B. Pier.** 1983. *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. Infect. Immun. **42**:170–177.
10. **Høiby, N., J. B. Hertz, and D. Sompolinsky.** 1980. Antibody response in patients with *Pseudomonas aeruginosa* infection to a "common antigen" from *P. aeruginosa* analysed by means of quantitative immunoelectrophoretic methods. Acta Pathol. Microbiol. Immunol. Scand. Sect. C **88**:149–154.
11. **Karkharis, Y. D., J. Y. Zeltner, J. J. Jackson, and D. J. Carlo.** 1978. A new improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of gram-negative bacteria. Anal. Biochem. **85**:595–601.
12. **Kuhn, H. M., S. Basu, and H. Mayer.** 1987. Comparison of enterobacterial common antigen from different species by serological techniques. Eur. J. Biochem. **162**:69–74.
13. **Morgan, W. T. J., and L. A. Elson.** 1934. A colorimetric method for the determination of N-acetyl-glucosamine and N-acetyl-chondrosamine. J. Biochem. **28**:988–991.
14. **Palva, E. T., and P. H. Mäkelä.** 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Eur. J. Biochem. **107**:137–143.
15. **Rietschel, E. T., C. Galanos, O. Lüderitz, and O. Westphal.** 1982. Chemical structure, physiological function and biological activity of lipopolysaccharides and their lipid A component, p. 183–229. *In* D. Wepp (ed.), Immunopharmacology and the regulation of leucocyte function. Marcel Dekker, Inc., New York.
16. **Sawardeker, J. S., H. J. Sloneker, and A. Jeanes.** 1965. Quantitative determination of monosaccharides of their alditol acetates by gas liquid chromatography. Anal. Chem. **37**:1602–1604.
17. **Switzer, R. C., C. R. Merril, and S. Shifrin.** 1979. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anal. Biochem. **98**:231–237.
18. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
19. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–91.