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Biological Activities of Lipopolysaccharides and Lipid A from *Rhodospirillaceae*

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The lipopolysaccharides and free lipid A from several strains of *Rhodospirillaceae* were assayed comparatively with those of *Enterobacteriaceae* in a number of biological tests. Free lipid A's from *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue* exhibited strong serological cross-reactions with each other and with free lipid A from *Salmonella*. Lipid A's from *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*, although cross-reacting with each other, did not do so with either the lipid A of *R. gelatinosa* or *R. tenue* or with that of *Salmonella*. The presence or absence of the above cross-reactions agreed with corresponding similarities or differences in the chemical structure of the lipid A preparations. The lipopolysaccharide of *R. gelatinosa* was highly toxic for adrenalectomized mice and pyrogenic for rabbits; however, it exhibited no anti-complementary activity. The activity of the *R. tenue* lipopolysaccharide was very low in both the lethality and pyrogenicity tests. Its corresponding free lipid A also exhibited low pyrogenic activity; however, its lethal toxicity for adrenalectomized mice was considerably higher than that of the intact parent lipopolysaccharide. Both intact lipopolysaccharide and, unexpectedly, the free lipid A exhibited no anti-complementary activity. The lipopolysaccharides of *R. viridis* and *R. palustris* were virtually nontoxic for mice and nonpyrogenic for rabbits. Both lipopolysaccharides were highly potent in their interaction with complement. They therefore represent the first example of nontoxic lipopolysaccharides exhibiting high anti-complementary activity.

Lipopolysaccharides are macromolecular substances present on the surface of gram-negative bacteria. Of these, lipopolysaccharides of *Enterobacteriaceae* have been studied most extensively, and much information has been accumulated concerning their composition, structure, biosynthesis, and biological activity (21). Up-to-date information shows that the lipopolysaccharides of *Enterobacteriaceae* are made up of a polysaccharide part consisting of the O-specific polysaccharide and the basal core polysaccharide, and a ubiquitous component, lipid A (10, 21). Chemical studies have revealed that the structure of lipid A is very similar among *Enterobacteriaceae* (8). In *Salmonella* it is made up of a $\beta 1',6$-linked D-glucosamine disaccharide carrying amide-linked D-3-hydroxytetradecanoic acid and ester-bound dodecanoic, hexadecanoic, and D-3-tetradecanoxytetradecanoic acids (13a). The lipid A backbone is substituted by phosphate groups at positions C1 and C'4 (8, 10, 13a).

In recent years it has been amply documented that the toxicity and generally the other biological activities associated with lipopolysaccharides are in the lipid A part of the molecule (7, 10, 13a, 22). There is also an extensive serological cross-reactivity between free lipid A preparations derived from different enterobacterial lipopolysaccharides (6). Due to the similarity in the structure and composition of their lipid A (8), lipopolysaccharides were found to exhibit a spectrum of biological activities that is qualitatively and quantitatively very similar among *Enterobacteriaceae*.

Differences identified in the lipid A of some lipopolysaccharides were not concerned with the basic structure of lipid A and did not seem to affect their biological activity to any significant extent. Lipopolysaccharides with major differences in their lipid A moiety are of particular interest for studying relationships between chemical structure and biological activity. Recent studies of lipopolysaccharides of gram-negative photosynthetic bacteria of the *Rhodospirillaceae*, which from a taxonomic point of view are quite remote from *Enterobacteriaceae*, have revealed that, in strains belonging to two species, structurally different lipid A's are present (18, 19). In these species, *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*, a lipid A was found which is

characterized by the replacement of the glucosamine-phosphate backbone by nonphosphorylated 2,3-diamino-2,3-dideoxy-D-glucose (Fig. 1) (9, 16). The latter sugar carries amide-linked D-3-hydroxytetradecanoic acid, whereas ester-linked fatty acids are lacking (19).

Other *Rhodospirillaceae*, e.g., *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue*, do not contain diaminohexose. The structure of *R. gelatinosa* lipid A is similar to that of *Salmonella* (8, 20); that of *R. tenue* is not known at present (J. Weckesser, G. Drews, R. Indira, and H. Mayer, submitted for publication). In the present study, biological activities of lipopolysaccharides and free lipid A derived from some *Rhodospirillaceae* are compared with one another and with those of *Salmonella*. Compared are the lethal toxicity, pyrogenicity, and complement inactivation of the respective preparations and also the serological cross-reactions among the free lipid A preparations.

MATERIALS AND METHODS

Bacterial strains, lipopolysaccharides, and lipid A. Cultivation of *Rhodospirillaceae* was carried out as described previously (18). Lipopolysaccharides were obtained from *R. viridis* F and *R. palustris* 8/1 by the phenol/water isolation procedure (23). Those from *R. gelatinosa* 29/2 and *R. tenue* 2761 and GFUy (Weckesser et al., submitted for publication) were obtained by the phenol-chloroform-petroleum ether method (5). To increase the solubility, the preparations were electrodialyzed as described previously (3) and converted to the triethylamine salt form. The *Salmonella* lipopolysaccharides of *S. abortus equi* (S-form), *S. minnesota* R345 (Rb chemotype), and *S. minnesota* R595 (Re-chemotype) were obtained by the phenol-water method in the case of the S-form and by the phenol-chloroform-petroleum ether method in the case of the R-form lipopolysaccharides (5). For toxicity and pyrogenicity measurements, the lipid A was prepared from electrodialyzed lipopolysaccharides and converted to the soluble triethylamine salt form (3).

Preparation of immunogenic lipid A and immunization. Immunogenic lipid A on the surface of acid-treated bacterial cells was prepared as described earlier (6). Briefly, heat- or phenol (1%)-killed bacteria were treated with acetic acid (1%, at 100°C, 120 min). This procedure led to the cleavage of the polysaccharide and the exposure of lipid A on the cell surface. The bacteria were washed three times with distilled water and lyophilized. In the case of *Salmonella*, the lyophilized acid-treated bacteria were coated with additional free lipid A as described earlier (6).

New Zealand white rabbits received three intravenous injections of 0.25, 0.5 and 1.0 ml of suspension of 5×10^{10} acid-treated bacteria per ml of saline at days 1, 5, and 10, respectively. On day 15 the animals were bled by cardiac puncture; the blood was allowed to clot at 37°C for 1 h and at 4°C for 24 h and was then centrifuged. The sera were decomplemented at 56°C for 30 min, absorbed with sheep erythrocytes (SRBC), and stored at −20°C.

Detection and serological cross-reactivity of the anti-lipid A antibodies. Anti-lipid A antibody titers were measured by the passive hemolysis (12) of erythrocytes coated with lipid A or alkali-treated lipid A as described earlier (6), with the following modifications; the test was carried out in small hemagglutination plates using SRBC coated with alkali-treated free lipid A instead of human erythrocytes. Unless otherwise stated, 50 μg of alkali-treated free lipid A per 0.2 ml of packed SRBC was used for sensitization. To a serial dilution (1:2) of the antiserum (in 50 μl of Veronal buffer), lipid A-coated SRBC in Veronal buffer (50 μl of a 0.5% suspension) were added. To each well, guinea pig complement (25 μl of a 1:10 dilution in Veronal buffer) was added, the plates were incubated at 37°C for 1 h, and hemolysis was measured by visual observation. The last serum dilution at which lysis still occurs represents the hemolytic titer of the antiserum.

The serological cross-reactions of a given antiserum with heterologous free lipid A preparations were determined in two ways: by the passive hemolysis test using the given antiserum and SRBC coated with the different lipid A preparations, and

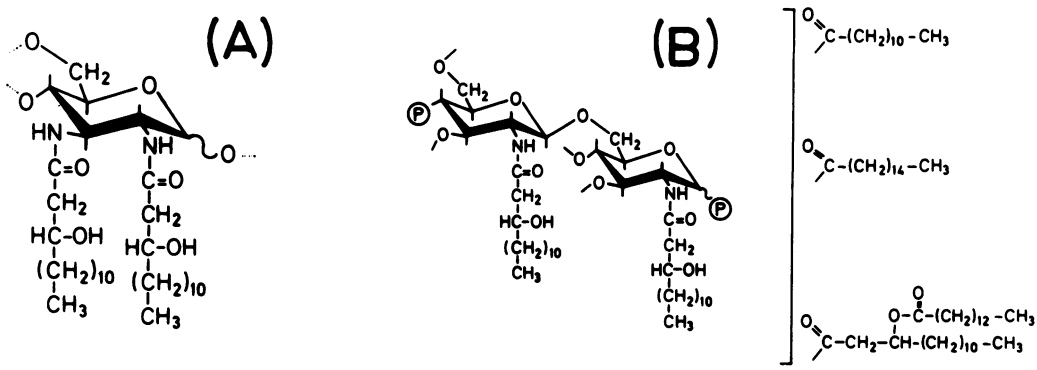

FIG. 1. *Basic structure of lipid A from (A) Rhodopseudomonas viridis (Roppel, thesis, 1975) and (B) Salmonella (14).*

by inhibition tests in which the lysis obtained by the given antiserum and its homologous lipid A-coated SRBC was inhibited by the heterologous lipid A preparations. In the latter case the procedure was as follows. To the wells of hemagglutination plates, 50 μl of Veronal buffer was added, and the plates were kept cool by being placed on crushed ice. Using a 50-μl Eppendorf pipette, serial dilutions of the lipid A were made. To each dilution, 2 hemolytic units of the antiserum to be tested (in 50 μl of Veronal buffer) was added followed by 50 μl of a suspension (0.5%) of SRBC coated with the homologous alkali-treated lipid A to which the antiserum was made. Finally, 25 μl of guinea pig complement (diluted 1:15 with Veronal buffer) was added, and the plates were shaken gently and incubated at 37°C for 1 h. The inhibitory activity of a given lipid A preparation is expressed as the amount of lipid A (in micrograms) corresponding to the last dilution at which inhibition of lysis of the lipid A-coated SRBC still takes place. Cooling of the plates before addition of lipid A was necessary in order to avoid immediate precipitation of the lipid A by Mg^{2+} and Ca^{2+} present in the Veronal buffer.

Lethal toxicity. Lethal toxicity was measured in adrenalectomized mice according to Chedid and Parant (1). NMRI female white mice were subjected to adrenalectomy, and 48 h later they were injected intravenously with different concentrations of the preparations under test in 0.2 ml of pyrogen-free distilled water.

Adrenalectomized animals receiving 0.2 ml of pyrogen-free distilled water intravenously served as controls. Deaths were recorded up to 24 h later, and mean lethal values (LD_{50}) were calculated by the method of Reed and Muench (13).

Pyrogen assay. Pyrogen assays in rabbits were performed in an air-conditioned room, using at least four animals per test. Fever responses were measured rectally by using thermistor probes connected to a recording temperature-measuring device (Hartmann und Braun, Frankfurt). Pyrogenicity of lipopolysaccharides and lipid A preparations was quantitated by determining the minimal pyrogenic dose causing a 0.6°C rise of temperature 3 h after intravenous injection (MPD-3), as described previously (7, 15, 17).

Anti-complementary activity. The anti-complementary activity of the preparations was measured as described earlier (4).

RESULTS

Immunization of rabbits with acid-treated bacteria from *R. gelatinosa* 29/2, *R. tenue* 2761, *R. viridis* F, and *R. palustris* 8/1 led in all cases to antisera reacting specifically with the lipid A of the corresponding strain. Passive hemolysis titers ranging from 1:500 to 1:16,000 (Table 1) were obtained and are thus comparable to those obtained by immunizing rabbits with lipid A from *Enterobacteriaceae* under similar conditions.

The serological cross-reactions among the lipid A preparations of the above strains and with that of *Salmonella* are shown in Table 1. The lipid A and anti-lipid A sera from *S. minnesota* R595, *R. gelatinosa* 29/2, and *R. tenue* 2761 showed high cross-reactivity. In most cases the heterologous hemolysis titers were similar to those obtained in the homologous systems. In contrast, the above lipid A preparations showed only weak cross-reactions with anti-lipid A sera from *R. palustris* or *R. viridis*. The latter strains, however, showed cross-reactions in both directions with each other.

These results were confirmed by inhibition tests (Table 2) in which the inhibitory activity of the lipid A of each of the above strains on the homologous lipid A/anti-lipid A systems of all strains was measured. In all cases the lipid A preparation inhibited its homologous lipid A/anti-lipid A hemolytic system in concentrations of a few nanograms. A similar degree of inhibition was seen in cross-inhibition tests among *Salmonella, R. gelatinosa,* and *R. tenue*. In contrast, no cross-inhibition occurred between the above strains and *R. viridis* or *R. palustris*. The latter strains, however, showed strong inhibition against each other.

From the above results we conclude that the lipid A preparations from *R. gelatinosa* and *R. tenue* are serologically similar to or perhaps identical with one another and with that of *Enterobacteriaceae*. On the other hand, the lipid A's from *R. palustris* and *R. viridis*,

TABLE 1. *Reactivity of various preparations of lipid A with homologous and heterologous anti-lipid A antisera*

Lipid A-antisera to:	Reciprocal titers in passive hemolysis, using SRBC coated with free lipid A from:				
	S. minnesota R595	*R. viridis* F	*R. palustris* 8/1	*R. gelatinosa* 29/2	*R. tenue* 2761
S. minnesota R595	*16,000*	<4	<4	8,000	16,000
R. viridis F[a]	<4	*512*	16	<4	<4
R. palustris 8/1[a]	<4	512	*16,000*	<4	<4
R. gelatinosa 29/2	16,000	512	128	*16,000*	16,000
R. tenue 2761	256	128	64	4,096	*4,096*

[a] For sensitization of erythrocytes, 400 μg of lipid A per 0.2 ml of packed cells was used.

TABLE 2. *Inhibition by free lipid A of various lipid A/anti-lipid A systems*

Lipid A/anti-lipid A hemolysis system of:	Minimal inhibiting concentration (μg) of free lipid A from:				
	S. minnesota R595	*R. viridis* F	*R. palustris* 8/1	*R. gelatinosa* 29/2	*R. tenue* 2761
S. minnesota R595	*0.005*	0.31	0.31	0.005	0.005
R. viridis F[a]	10	*0.04*	0.15	10	10
R. palustris 8/1[a]	5	0.31	*0.02*	10	10
R. gelatinosa 29/2	0.02	0.62	0.31	*0.005*	0.005
R. tenue 2761	0.15	2.50	0.62	0.04	*0.005*

[a] For sensitization of SRBC, 400 μg of lipid A per 0.2 ml of packed cells was used.

which cross-react with each other, show a different serological specificity from the lipid A of the other *Rhodospirillaceae* strains and from that of *Enterobacteriaceae*.

Biological activities. The lipopolysaccharides and lipid A preparations of *Rhodospirillaceae* and *Salmonella* strains were tested comparatively for biological activities (Table 3). Lethal toxicity in adrenalectomized mice, pyrogenicity in rabbits, and the in vitro interaction with complement were assayed. Because of their optimal activity, where indicated, the triethylamine salt forms were used for lethality and pyrogenicity tests and the sodium salt forms were used for complement interaction (4).

R. gelatinosa lipopolysaccharide was highly toxic, with an LD_{50} of less than 0.01 μg, which is comparable to those of *S. abortus equi* (<0.01 μg), *S. minnesota* R595 (0.01 μg), and to the free lipid A of *S. minnesota* R345 (0.02 μg) (Table 3). The toxicity of lipid A of *R. tenue* 2761 (LD_{50}, 7.0 μg), *R. tenue* GFUy (LD_{50}, 10 μg), *R. viridis* (LD_{50}, 2.5 μg), and *R. palustris* (LD_{50}, 15 μg) lipopolysaccharides was considerably lower by a factor of between 500 and 7,500 compared with that of *S. abortus equi*. The toxicity of the lipid A from *R. tenue* 2761 (LD_{50}, 0.10 μg) and *R. tenue* GFUy (LD_{50}, 0.07 μg) was higher than that of their parent lipopolysaccharides by factors of 70 and 140, respectively. Both preparations were only about 20 times less toxic than the lipid A of *S. minnesota* R345.

The results of the pyrogenicity tests were in general agreement with those of lethal toxicity tests. The pyrogenic activity of lipopolysaccharide of *R. gelatinosa* (MPD-3, <0.0001 μg) compared well with that of *S. abortus equi* (MPD-3, 0.0005 μg) and *S. minnesota* R595 (MPD-3 0.0001 μg), whereas the lipopolysaccharides of *R. tenue* 2761 (MPD-3, 0.05 μg), *R. viridis* (MPD-3, 10 μg), and *R. palustris* (MPD-3, 10 μg) preparations were considerably less pyrogenic. The lipid A from *R. tenue* 2761 (MPD-3, 0.05 μg), in contrast to its toxicity, showed a low pyrogenic activity, comparable to that of the parent lipopolysaccharide.

The results of complement interaction did not seem to be related in any way to those of lethal toxicity and pyrogenicity. Thus the *R. gelatinosa* lipopolysaccharide was completely inactive in its ability to interact with complement. The same was found to be true for the two *R. tenue* preparations and for the lipid A from *R. tenue* 2761. These preparations expressed only insignificant anti-complementary activity. A complete contrast was shown by the lipopolysaccharides from *R. viridis* and *R. palustris*. Here, interaction with complement led to an almost complete loss of hemolytic activity and was comparable in this respect with the lipopolysaccharides and lipid A from *Salmonella*.

DISCUSSION

The recent identification of 2,3-diamino-2,3-dideoxy-D-glucose (9, 16) as the backbone sugar of the lipid A moiety of two *Rhodospirillaceae* species, *R. viridis* and *R. palustris* (18, 19), prompted this study on a comparison of the biological activities of lipopolysaccharides of these species with those from *Salmonella* and other *Rhodospirillaceae* containing glucosamine instead of the diaminohexose as a lipid A constituent (20). The differences in the serological specificity of lipid A from diaminohexose- and glucosamine-containing *Rhodospirillaceae* were demonstrated in the cross-hemolysis and inhibition assays. There was a marked cross-reaction between lipid A of *R. palustris* and *R. viridis*, i.e., between the two species with the diaminohexose-containing lipid A. Since the respective homologous titers were higher by a factor of 5 to 10, there may be differences in the lipid A structure of the two strains. The lipid A of both preparations showed weak cross-reactions with the antisera of *R. gelatinosa* and *R. tenue*, which were absent in the reciprocal system. It is interesting that the cross-reaction of *R. viridis* with *R. gelatinosa* anti-lipid A antiserum was as high as that with the homologous antiserum. This may have been due to poor immunogenicity of the *R. viridis* lipid A.

R. gelatinosa lipid A was recently shown to

TABLE 3. *Pyrogenicity, lethal toxicity, and complement inactivation of lipopolysaccharides or free lipid A from Rhodospirillaceae and Enterobacteriaceae*

Source	Pyrogenicity (MPD-3, μg/kg)	Lethal toxicity in adrenalectomized mice (LD_{50}, μg)	Complement inactivation with 30-μg preparations (%)
R. gelatinosa 29/1 LPS	0.0001	<0.01	2
R. tenue 2761 LPS	0.05	7.0	9
R. tenue 2761 free lipid A	0.05	0.10	22
R. tenue GFUy LPS	ND[a]	10.0	2
R. tenue GFUy free lipid A	0.05	0.07	ND
R. viridis F LPS	10	2.5	93
R. palustris 8/1 LPS	10	15.0	95
S. abortus equi LPS	0.0005	<0.01	96
S. minnesota R595 LPS	0.0001	0.01	98
S. minnesota R345 free lipid A	ND	0.02	88

[a] ND, Not determined.

be identical in its backbone structure to *Salmonella* lipid A (8). The serological study fully corroborates this result and shows that the replacement of amide-linked β-C_{14}-OH (*Salmonella*) by β-C_{10}-OH (*R. gelatinosa*) (20) does not change the serological specificity to any measurable extent. *R. tenue* lipid A also possesses a glucosamine-containing lipid A, but differs from *Salmonella* and *R. gelatinosa* lipid A in that it contains appreciable amounts of D-arabinose (Weckesser et al., submitted for publication). The serological data show that the lipid A of *R. tenue* is equally active in inhibiting its homologous hemolysis system and that of *Salmonella* and *R. gelatinosa*. On the other hand, the *R. tenue* hemolysis system is better inhibited by homologous lipid A than by the two above-mentioned heterologous lipid A preparations. Thus the antiserum prepared to the *R. tenue* lipid A contains antibodies directed against additional structures in the *R. tenue* lipid A that are absent from the lipid A of *Salmonella* or *R. gelatinosa*. This additional antigenic specificity may be provided by the D-arabinosyl residues, which are present in considerable amounts in the lipid A of *R. tenue* after its liberation by acid. The serological properties of the lipopolysaccharides from *S. abortus equi* and *R. gelatinosa* correspond very well with the lethal toxicity and pyrogenic activities. Evidently, the low to very low endotoxic and pyrogenic activities of *R. viridis* and *R. palustris* are related to their different lipid A types, thus demonstrating that endotoxicity is dependent on distinct structural properties. It is tempting to speculate on these structural prerequisites, but one must consider that in the lipid A of *R. viridis* and *R. palustris* three different structural properties characteristic of lipid A of *Enterobacteriaceae* are changed: glucosamine is replaced by 2,3-diamino-2,3-dideoxy-D-glucose, no ester-linked fatty acids are present, and phosphorous is absent.

The fact that lipopolysaccharides from *R. tenue* strains, although possessing a lipid A that cross-reacts with *Salmonella* lipid A, nevertheless show low lethal toxicity and pyrogenicity, is of special interest. The toxicity of these lipopolysaccharides seems to be cryptic; this was revealed when isolated lipid A of both strains (GFUy and 2761) were found to be 70 to 140 times more toxic than their respective parent lipopolysaccharides. The pyrogenicity of the lipid A, however, was not increased and was comparable to that of the parent lipopolysaccharides. The fact that the polysaccharide component (or an acid-labile lipid A constituent) can shield the toxicity of lipopolysaccharides has not been reported before. Usually lipid A preparations were found to be either as toxic as the lipopolysaccharides they were prepared from or somewhat less toxic due to reduced solubility and partial degradation. In this connection it seems of interest that phthalylation of the polysaccharide component of *Salmonella* lipopolysaccharide can mask lipid A activity (11). This cryptic toxicity clearly needs further studies. Studies on the anti-complementary activity of the various *Rhodospirillaceae* preparations show that the property of interaction with complement does not correlate with the expression of toxicity. This agrees with previous findings using *Salmonella* and *Escherichia coli* lipopolysaccharides (2). Thus lipopolysaccharide of *R. gelatinosa*, despite its potent endotoxic activity, is inactive in its ability to interact with complement. The two *R. tenue* lipopolysaccharides exhibit only negligible anti-complementary activity; in this case, however, both preparations also show only weak endotoxic activity. The same is true for the lipid A preparations from *R. tenue* GFUy. Of particular interest are the lipopolysaccharides from *R. viridis* and *R. palustris*. Both preparations, although virtually nontoxic, interact with complement. To our knowledge this is the first example of nontoxic lipopolysaccharides exhibiting high anti-complementary activity.

The ubiquitous presence of a *Salmonella*-type lipid A in many gram-negative genera and families shows that lipid A is a rather preserved structure. This is also documented by our results and a previous report (8) which shows that lipid A from a photosynthetic gram-negative microorganism (*R. gelatinosa*) is identical in its chemical and biological prop-

erties to lipid A from a mammalian intestinal parasite (*Salmonella*). In contrast, the lipid A of the lipopolysaccharides of the *Rhodopseudomonas* species *R. viridis* and *R. palustris* are completely different in their chemical structures, and consequently their serological cross-reactions and biological properties are different. The occurrence of two remarkably different lipid A structures in a genus of photosynthetic bacteria is unexpected and of special interest (24).

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LITERATURE CITED

1. **Chedid, L., and M. Parant.** 1971. Role of hypersensitivity and tolerance in reactions to endotoxin, p. 415–459. *In* G. Kadis, G. Weinbaum, and S. J. Ajl (ed.), Microbial toxins, vol. 5. Academic Press Inc., New York.
2. **Galanos, C.** 1972. Anticomplementary activity of lipopolysaccharides and the role of natural antibodies. *In* K. W. Pondman and K. O. Rother (ed.), European Complement Workshop, Biochemistry and Biology of the Third Component of Complement. Central Laboratory of the Netherlands, Red Cross, Blood Transfusion Service, Amsterdam.
3. **Galanos, C., and O. Lüderitz.** 1975. Electrodialysis of lipopolysaccharides and their conversion to uniform salt forms. Eur. J. Biochem. 54:603–610.
4. **Galanos, C., and O. Lüderitz.** 1976. The role of the physical state of lipopolysaccharides in the interaction with complement. Eur. J. Biochem. 65:403–408.
5. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R-lipopolysaccharides. Eur. J. Biochem. 9:245–249.
6. **Galanos, C., O. Lüderitz, and O. Westphal.** 1971. Preparation and properties of antisera against the lipid A component of bacterial lipopolysaccharides. Eur. J. Biochem. 24:116–122.
7. **Galanos, C., E. T. Rietschel, O. Lüderitz, O. Westphal, Y. B. Kim, and D. W. Watson.** 1972. Biological activities of lipid A complexed with bovine serum albumin. Eur. J. Biochem. 31:230–233.
8. **Hase, S., and E. Th. Rietschel.** 1976. Isolation and analysis of the lipid A backbone. Lipid A structure of lipopolysaccharides from various bacterial groups. Eur. J. Biochem. 63:101–107.
9. **Keilich, G., J. Roppel, and H. Mayer.** 1976. Characterization of a diaminohexose (2,3-diamino-2,3-dideoxy-D-glucose) from *Rhodopseudomonas viridis* lipopolysaccharides by circular dichroism. Carbohydr. Res. 51:129–134.
10. **Lüderitz, O., C. Galanos, V. Lehmann, M. Nurminen, E. T. Rietschel, G. Rosenfelder, M. Simon, and O. Westphal.** 1973. Lipid A, chemical structure and biological activity. J. Infect. Dis. 128(Suppl.):S17–S29.
11. **McIntire, F. C., M. P. Hargie, J. R. Schenck, R. A. Finley, H. W. Sievert, E. T. Rietschel, and D. L. Rosenstreich.** 1976. Some biological properties of non-toxic derivatives of a lipopolysaccharide from E. coli K235. J. Immunol. 117:674–678.
12. **Neter, E.** 1956. Bacterial hemagglutination and hemolysis. Bacteriol. Rev. 20:166–188.
13. **Reed, L. J., and H. Muench.** 1938. A simple method for estimating fifty per cent endpoints. Am. J. Hyg. 27:493–497.
13a. **Rietschel, E. T., C. Galanos, and O. Lüderitz.** 1975. Structure, endotoxicity, and immunogenicity of the lipid A component of bacterial lipopolysaccharides, p. 307–314. *In* D. Schlessinger (ed.), Microbiology–1975. American Society for Microbiology, Washington, D.C.
14. **Rietschel, E. T., H. Gottert, O. Lüderitz, and O. Westphal.** 1972. Nature and linkages of the fatty acids present in the lipid A component of *Salmonella* lipopolysaccharides. Eur. J. Biochem. 28:166–173.
15. **Rietschel, E. T., Y. B. Kim, D. W. Watson, C. Galanos, O. Lüderitz, and O. Westphal.** 1973. Pyrogenicity and immunogenicity of lipid A complexed with bovine serum albumin or human serum albumin. Infect. Immun. 8:173–177.
16. **Roppel, J., H. Mayer, and J. Weckesser.** 1975. Identification of a 2,3-dideoxyhexose in the lipid A component of lipopolysaccharides of *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*. Carbohydr. Res. 40:31–40.
17. **Watson, D. W., and Y. B. Kim.** 1963. Modifications of host response to bacterial endotoxins. I. Specificity of pyrogenic tolerance and role of hypersensitivity in pyrogenicity, lethality and skin reactivity. J. Exp. Med. 118:425–446.
18. **Weckesser, J., G. Drews, I. Fromme, and H. Mayer.** 1973. Isolation and chemical composition of the lipopolysaccharides of *Rhodopseudomonas palustris* strains. Arch. Microbiol. 92:123–138.
19. **Weckesser, J., G. Drews, J. Roppel, H. Mayer, and I. Fromme.** 1974. The lipopolysaccharides (O-antigens) of *Rhodopseudomonas viridis*. Arch. Microbiol. 101:233–245.
20. **Weckesser, J., H. Mayer, G. Drews, and I. Fromme.** 1975. Lipophilic O-antigens containing D-glycero-D-mannoheptose as the sole neutral sugar in *Rhodopseudomonas gelatinosa*. J. Bacteriol. 123:449–455.
21. **Weinbaum, G., S. Kadis, and S. J. Ajl (ed.).** 1971. Microbial toxins, vol. 4 and 5. Academic Press Inc., New York.
22. **Westenfelder, M., and C. Galanos.** 1974. Experimental lipid A-induced nephritis in the dog. A possible role of lipid A in the pathogenesis of a bacterial chronic pyelonephritis. Infection 2:174–177.
23. **Westphal, O., O. Lüderitz, and F. Bister.** 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. Z. Naturforsch. 7b:148–155.
24. **Whittenbury, R., and A. G. McLee.** 1967. *Rhodopseudomonas palustris* and *Rhodopseudomonas viridis*–photosynthetic budding bacteria. Arch. Microbiol. 59:324–334.