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NOTES

Partial Chemical Characterization of a Toxic Lipopolysaccharide from *Coxiella burneti*

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Partial characterization of a hot phenol-water extract of *Coxiella burneti* demonstrates the presence of at least 8 sugar and 17 fatty acid residues, suggesting a complex lipopolysaccharide molecule.

Toxins have been identified in several rickettsial species (9, 14, 15), but no toxin has as yet been described for *Coxiella burneti*, the rickettsial agent of Q fever. Brezina and co-workers extracted *C. burneti* with trichloroacetic acid and obtained antigens which were also pyrexial (7). Phenol extraction of trichloroacetic acid extract yielded non-immunogenic haptenes (1) and antigens which were also pyrexial (6). Phenol extracts of the rickettsiae were immunogenic (4, 5). We now report a partial characterization of the product of a phenolic extraction of *C. burneti* that has toxic properties against guinea pigs, producing many of the biochemical changes observed during active infection with Q fever (3).

Toxin material was prepared from purified, phase I *C. burneti* by a slightly modified phenol extraction procedure (16). To 10 ml of a 10% rickettsial suspension (wet wt/vol) in 0.02 M phosphate buffer-0.15 M KCl (pH 7.4) was rapidly added 10 ml of hot (68 C), 90% (wt/wt) redistilled phenol, and the suspension was vigorously shaken for 20 min in a water bath at 68 C. The emulsion was centrifuged at 4,000 rpm for 15 to 20 min, and the aqueous phase was removed and saved. The interphase and phenolic phases were re-extracted at 68 C with an equal volume of the phosphate-buffered solution. The pooled aqueous phases were dialyzed against running tap water for 3 days. The residual opalescent solution was centrifuged at 10,000 $\times$ *g* for 20 min. The supernatant was removed and centrifuged at 105,000 $\times$ *g* for 3 h. The transparent pellet was washed with the phosphate-buffered solution to remove contaminating nucleic acids and again centrifuged, and the sediment was either lyophilized, frozen, and stored at −70 C, or if used immediately was suspended in the appropriate solution at 4 C. The phenol-sulfuric assay for carbohydrates (10) was used for lipopolysaccharide assay, and the amount of toxin was designated in terms of micrograms of glucose equivalents. One gram of purified *C. burneti* (wet weight) yielded 1.9 mg of glucose equivalents of extract.

Neutral sugar alditol acetate derivatives of the hydrolyzed toxin were prepared (11) for gas-liquid chromatography in a Varian Aerograph model 1740 equipped with a hydrogen flame detector. Reference alditol acetate derivatives were prepared from authentic samples of glycerol, ribitol, xylitol, mannitol, galactitol, sorbitol, and perseitol (mannoheptitol). At least eight sugars are probably present in the toxin (Fig. 1). Comparison with retention times of reference alditol acetate derivatives identified peaks 3, 4, 6, and 7 as ribitol, xylitol, mannitol, and sorbitol, respectively. Peaks 1 and 2 are in the tetrose-pentose region, peak 5 is in the hexose region, and peak 8 is about equivalent to perseitol, identifying a heptitol. With reference to peak 3, it should be noted that the toxin preparation had 260-nm absorbing material (frequently found in bacterial endotoxin preparations), raising the possibility of ribonucleic acid as a ribose source.

The methods of Langworthy and Buller (12) were used for toxin hydrolysis and preparation of methylated fatty acids, which were analyzed in a Beckman GC-5 gas-liquid chromatography apparatus. Reference methylated fatty acids were 10:0, 12:0 to 22:0, 16:1, and 18:1. Comparison of retention times of the methylated

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fatty acid derivatives of hydrolyzed toxin (Fig. 2) with those of reference samples indicate that 17 fatty acids are possibly present in the toxin hydrolyzate. Comparison of retention times of the methylated derivatives with those of reference samples show that peaks 2, 5, 8, 10, 15, and 12 correspond to 14:0, 16:0, 18:0, 19:0, 22:0, and β-hydroxymyristic acid, respectively. Amino sugars were prepared from toxin (11); analysis in a Beckman amino acid analyzer revealed the presence of glucosamine.

Chemical analysis of the *C. burneti* toxin indicates a complex lipopolysaccharide molecule. Glucose, mannose, ribose, and xylose are also frequently found in bacterial lipopolysaccharide or endotoxin (2, 13), and glucosamine is a known constituent of the lipid A moiety of endotoxin (13) to which fatty acids are linked via ketosidic bonds. The β-hydroxymyristic acid in the rickettsial preparation is a common constituent of several gram-negative bacterial endotoxins (13). The presence of a toxin in *C. burneti* has long been suspected as contributing to this rickettsia's virulence (8) but has heretofore not been described. Isolation of this rickettsial toxin now offers opportunities to identify some basis for the pathological and biochemical changes which occur during Q fever. A subsequent paper (3) describes the biochemical properties of the toxin, indicating several additional properties it has in common with typical gram-negative endotoxins.

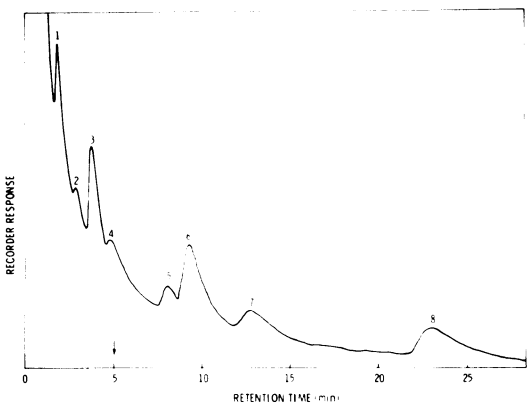

FIG. 1. *Gas-liquid chromatography of alditol acetate derivatives of the aqueous phase of phenol extracts from C. burneti. Chromatography was performed isothermally with a column temperature of 195 C and an N_2 carrier gas flow rate of 40 ml/min. Injector and detector temperatures were 215 C. H_2 and air flow rates were 40 and 400 ml/min, respectively.*

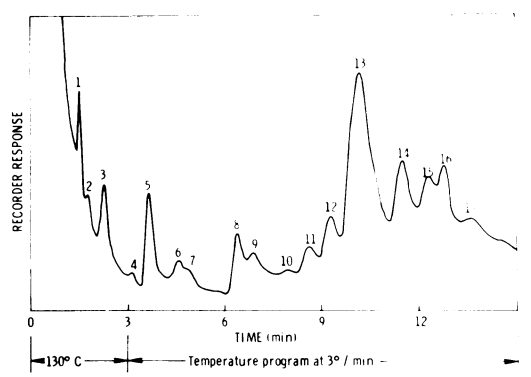

FIG. 2. *Gas-liquid chromatography of methylated fatty acid derivatives from the acid-hydrolyzed aqueous phase of phenol extracts from C. burneti. The detector temperature was 230 C, and He, air, and H_2 flow rates were 68, 300, and 30 ml/min, respectively.*

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LITERATURE CITED

1. Anacker, R. L., W. T. Haskins, D. B. Lackman, E. Ribi, and E. G. Pickens. 1963. Conversion of the phase I antigen of *Coxiella burnetii* to hapten by phenol treatment. J. Bacteriol. **85**:1165-1170.
2. Ashwell, G., and J. Hickman. 1971. The chemistry of the unique carbohydrates of bacterial lipopolysaccharides, p. 235-266. *In* G. Weinbaum, S. Kadis, and S. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
3. Baca, O. G., and D. Paretsky. 1974. Some physiological and biochemical effects of a *Coxiella burneti* lipopolysaccharide preparation on guinea pigs. Infect. Immunity **9**:939-945.
4. Brezina, R., and V. Pospisil. 1970. Study of the antigenic structure of *Coxiella burneti*. VIII. Immunogenicity of phenol-extracted phase I antigenic component. Acta Virol. **14**:302-306.
5. Brezina, R., V. Pospisil, and S. Schramek. 1970. Study of the antigenic structure of *Coxiella burneti*. VII. Properties of phenol-extracted phase I antigenic component. Acta Virol. **14**:295-301.
6. Brezina, R., S. Schramek, and J. Urvolgyi. 1965. Study of the antigenic structure of *Coxiella burneti*. III. Pyrogenic effect of phase I antigen in experimental guinea pigs. Acta Virol. **9**:180-185.
7. Brezina, R., and J. Urvolgyi. 1962. Study of the antigenic structure of *Coxiella burneti*. I. Extraction of phase I antigenic component by means of trichloroacetic acid. Acta Virol. **6**:84-88.
8. Burnet, F. M., and M. Freeman. 1941. Studies of the X strain (Dyer) of *Rickettsia burneti*. II. Guinea pig infections, with special reference to immunological phenomena. J. Immunol. **40**:421-436.
9. Cooke, P. M. 1961. Rickettsial and viral toxins. Amer. J. Med. Sci. **241**:383-405.
10. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350-356.
11. Kim, J. H., B. Shome, T. Liao, and J. G. Pierce. 1967.

Analysis of neutral sugars by gas-liquid chromatography of alditol acetates: application to thyrotropic hormone and other glycoproteins. Anal. Biochem. **20**:258-274.

12. Langworthy, T. A., and C. S. Buller. 1973. The effect of phage infection on the synthesis of lipid A by *Escherichia coli*. Virology **54**:80-89.
13. Luderitz, O., O. Westphal, A. M. Staub, and H. Nikaido. 1971. Isolation and chemical and immunological characterization of bacterial lipopolysaccharides, p. 145-233. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
14. Olitzki, L., J. W. Czaczkes, and A. Kutzenok. 1946. Endotoxic factors of *Rickettsia prowazeki* and their immunological relationship to the endotoxins of other gram negative microorganisms. J. Immunol. **53**:365-370.
15. Olitzki, L., E. Oleinik, J. W. Czaczkes, and A. Kutzenok. 1946. The thermostable endotoxin of rickettsiae. Nature (London) **157**:552.
16. Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides extraction with phenol-water and further applications of the procedures, p. 83-91. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press Inc., New York.