PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **174**, 47–52 (1983)

Lack of Endotoxin in *Borrelia hispanica* and *Treponema pallidum*[1] (41702)

PAUL H. HARDY, JR. AND JACK LEVIN[2]

World Health Organization Collaborating Center for Reference and Research in Treponematoses, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and Departments of Laboratory Medicine and Medicine, University of California School of Medicine, San Francisco, California 94143

Abstract. Borrelia hispanica from infected guinea pigs and *Treponema pallidum* from testicular syphilomas of rabbits were assayed for the presence of endotoxin with the *Limulus* lysate test. A suspension of *Borrelia*, containing 1.3×10^8 spirochetes/ml, was nonreactive both when it was tested as intact organisms, and when tested after disruption of the spirochetes by sonication. Eight different suspensions of treponemes, ranging from 0.6×10^9 to 3×10^9 treponemes/ml, were negative at a 1:10 dilution and were no more active than control suspensions of normal rabbit testes. Therefore, it was concluded that *T. pallidum*, as well as the *Borrelia*, possessed no endotoxin.

The Jarisch–Herxheimer reaction (JHR) is a clinical syndrome that has been described in a number of infectious diseases (1). It develops usually during the course of antimicrobial therapy, and it occurs most frequently in two spirochetal diseases; syphilis, in which it was first described (2, 3), and relapsing fever, in which it is most severe (4). The reaction begins abruptly some hours after initiation of treatment, at approximately the time when the infectious agents are disappearing rapidly from lesions and/or the blood stream. It is characterized by fever, a marked exacerbation of clinically apparent lesions, and a number of physiological abnormalities compatible with bacterial endotoxic shock (4). Nevertheless, the pathogenesis of the JHR is still not established, in part because of uncertainty concerning the presence or absence of endotoxin in spirochetes.

Pathogenic spirochetes, except for Leptospira, have long resisted *in vitro* cultivation, and this has hampered chemical studies of these organisms. Although several strains of *Borrelia* have been cultured recently (5, 6), only minimal growth (5×10^6 to 5×10^7 spirochetes/ml) has been achieved and chemical investigations have not been practical. Accordingly, studies of this type have been limited almost entirely to Leptospira and a few easily cultured nonpathogenic treponemes. A number of investigators have reported recovery of a lipopolysaccharide (LPS) from the Reiter treponeme (now *Treponema phagedenis;* biotype Reiteri), by phenol–water or trichloroacetic acid extraction (7–10). However, the LPS has been identified on the basis of sugar and phosphorous content, with little study of the lipid moiety and no attempt to demonstrate endotoxic properties. Similarly, Zey and Jackson recovered LPS from the Nichols nonpathogenic treponeme (now *T. refringens*), and identified it by a colorimetric procedure, but they attempted no biologic studies (11).

Efforts to demonstrate endotoxic activity in either whole spirochetes, or spirochetal extracts, have been few in number and mostly unsuccessful. Heyman *et al.* (12) were unable to demonstrate an endotoxic response in rabbits inoculated intravenously with either suspensions of whole viable *T. pallidum*, or treponemes treated in a variety of ways. Finco and Low (13) extracted an LPS from *Leptospira interrogans serovar canicola* that contained no 2-keto-3-deoxyoctonoic acid and was devoid of endotoxic activity. Arean *et al.* (14) prepared extracts from a virulent strain

[1] Supported in part by Research Grants AI 02336 from the National Institute of Allergy and Infectious Diseases and HL 31035 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Md.; the World Health Organization; and the Veterans Administration.

[2] Present address and to whom reprint requests should be addressed: Veterans Administration Hospital, 113A, 4150 Clement Street, San Francisco, Calif. 94121.

0037-9727/83 $1.50

Copyright © 1983 by the Society for Experimental Biology and Medicine.
All rights reserved.

of *Lep. icterohaemorrhagiae,* using techniques for the isolation of bacterial endotoxins. Neither these extracts, dead or disrupted organisms, nor culture filtrates produced pathological changes or the generalized Shwartzman reaction in mice or guinea pigs. Furthermore, extracts prepared from *Lep. icterohaemorrhagiae* did not consistently produce fever in rabbits (14). In perhaps the most thorough study to date, Johnson (15) performed two LPS extraction procedures on three microorganisms, using 5 g (dry weight) of each organism for each procedure. The organisms were *Salmonella minnesota, Lep. interrogans serovar canicola,* and *T. phagedenis* biotype Kazan 5; the endotoxin yields were 196 mg, 0.13 μg, and 0.04 μg, respectively. In contrast to these findings, Mergenhagen *et al.* (16) found endotoxic activity in phenol extracts from each of three oral spirochetes; a small treponeme and two others identified as *Borrelia buccalis* and *B. vincentii.* Nuessen *et al.* (17) extracted an LPS from *T. hyodysenteriae* which was toxic for mouse peritoneal macrophages, stimulated incorporation of [^{3}H]thymidine into murine splenic cells, and generated chemotactic activity in the presence of serum. However, evaluation of pyrogenicity and *Limulus test* activity was not performed. Recently Butler *et al.* (18) examined concentrated suspensions of *B. recurrentis* obtained from the blood of three patients with louse-borne relapsing fever (LBRF). These were tested with the *Limulus* lysate test, an exquisitively sensitive *in vitro* assay for endotoxin (19), and all three were negative at concentrations of 2000 μg/ml. Butler *et al.* (20) also were unable to detect endotoxin in cultured *B. hermsii* at concentrations of 10^8 spirochetes/ml.

This report describes further efforts to determine the presence or absence of endotoxin in spirochetes. We have studied two pathogenic species, *T. pallidum,* from experimentally infected rabbits, and *B. hispanica,* from guinea pigs, with the *Limulus* lysate test. Our findings provide further evidence that pathogenic spirochetes do not possess a component with endotoxic activity.

Materials and Methods. *Spirochetes. Borrelia hispanica* was obtained from P. L. Perine, University of Washington. This strain was originally isolated in North Africa from a patient with tick borne relapsing fever. It was propagated in our laboratory by intraperitoneal inoculation of Hartley strain guinea pigs. Wet blood preparations were prepared daily and examined by dark-field microscopy until a marked spirochetemia was observed; at that time, animals were exsanguinated by cardiac puncture. Heparinized blood was centrifuged, the plasma was removed, and the sedimented erythrocytes were washed three times with sterile, pyrogen-free saline (0.85% NaCl). The pooled plasma and saline washes contained 1.3×10^8 *Borrelia*/ml, but no blood cells; aliquants were distributed in sterile plastic vials (1–2 ml each) and stored in the vapor phase of a liquid nitrogen refrigerator.

Treponema pallidum, Nichols strain, was propagated in the testes of New Zealand white rabbits. Details of propagation and extraction have been described previously (21). Suspensions of both intact treponemes and those disintegrated by sonication were stored frozen, without a cryoprotective agent, in sterile plastic vials as described for *Borrelia.* Different preparations contained 0.6×10^9 to 3×10^9 treponemes/ml.

Glassware, centrifuge and storage tubes, and all solutions used for extraction and purification of spirochete preparations were checked before use for the absence of any contaminant with endotoxic activity, by the *Limulus* test (19). Because spirochete yields from animals were insufficient for total weight determinations, all suspensions were assessed in terms of organisms per milliliter, as determined by direct count (21). For treponemes, total protein per milliliter was also measured (cf. Table 1). Protein concentration of the *Borrelia* preparation was not determined because of the relatively large amount of serum proteins present.

For some assays, spirochetes were disintegrated by sonication with a Biosonik IV sonicator, fitted with a microprobe tip (Bronwill Scientific, Rochester, N.Y.). Suspensions in glass tubes were submerged in an ice bath and subjected to repeated 45-sec bursts of approximately 75% maximum machine power. Cell disintegration was monitored by decrease in density, and checked by direct microscopic examination.

Limulus test. The procedure for assay of endotoxin with lysates of blood cells (ame-

bocytes) from the horseshoe crab (*Limulus*) has been reported previously (22–24). *Escherichia coli* endotoxin (lipopolysaccharide B, 055: B5, Difco Laboratories, Detroit, Mich.) was used as a standard. The several amebocyte lysates used in this study varied slightly in sensitivity, detecting as little as 0.1–0.01 ng *E. coli* endotoxin/ml. They were also reactive with 10 ng or less of lyophilized *E. coli* cells (24).

Protein determinations. The protein concentration of treponeme suspensions was measured after trichloroacetic acid precipitation, by the Folin–Ciocalteau procedure, as described by Chase and Williams (25). Crystalline bovine serum albumin, standardized by Kjeldahl nitrogen determination, was used as the reference.

Results. In repeated assays of *Borrelia,* either as a suspension of whole organisms, or as a sonicated preparation, there was complete absence of reaction with *Limulus* lysate (Table I). This was indicative of less than 0.01 ng of material with endotoxin-like activity per 1.3×10^8 spirochetes. When a number of pooled suspensions of *T. pallidum* were assayed, all demonstrated slight endotoxin-like activity as undiluted samples, but they were nonreactive at 1:10 dilutions. Although the calculated concentrations of endotoxin, based on results with undiluted samples, varied from 0.01–1.0 ng/ml with different preparations, they did not correlate with either the number of treponemes or concentration of protein, the two measures of microbial density (Table I).

Although the solutions, plastics, and glassware used during the preparation of the pooled *T. pallidum* suspensions were, by assay, endotoxin-free, no special effort had been made to avoid endotoxin contamination in the course of isolation and purification. Accordingly, three rabbits (Ra 5416, 5418, and 5419) were infected with *T. pallidum,* and at the appropriate time the testes of each were removed and processed separately under the most rigorous endotoxin-free conditions we could achieve. As shown in Table I, the suspensions from two of the three rabbits contained the lowest endotoxin concentrations (0.01 ng/ml) found. Again, there was no correlation between number of treponemes or protein content and endotoxin concentration.

The overall results indicated that the observed reaction with *Limulus* lysate was not due to endotoxin of treponemal origin. Further evidence for this was sought by extracting the testes of a normal rabbit in a manner iden-

TABLE I. *Limulus* LYSATE ASSAYS OF ENDOTOXIN IN SPIROCHETE SUSPENSIONS AND NORMAL RABBIT TESTICULAR EXTRACTS

Spirochete suspension	Spirochete concentration ($\times 10^9$/ml)	Total protein (μg/ml)	Endotoxin concentration (ng/ml)[a]
B. Hispanica			
Pool A[c]	0.13	—	<0.1[b]
Pool A (sonicated)	0.13	—	<0.01[b]
T. pallidum			
Pool G (sonicated)[d]	2	580	1
Pool F	0.6	230	1
Pool M	1.9	282	0.5
Pool N	1.83	350	0.1
Pool O	2	—	0.1
Pool O (sonicated)	2	—	0.1
Ra 5416[e]	3	340	0.01
Ra 5418	0.67	160	0.01
Ra 5419	1	400	0.1
Ra 5489 (normal)			0.5

[a] Calculated from *E. Coli* endotoxin standard (See Materials and Methods).
[b] Minimal detectable concentration of endotoxin with lysate used for this assay.
[c] Pooled plasma–saline washes from erythrocytes of infected guinea pigs.
[d] Pooled extracts of testicular syphilomas from groups of three to eight rabbits.
[e] Extract of testicular syphilomas from individual rabbits.

tical to that used for the purification of *T. pallidum.* Like the treponeme suspensions, this sham-preparation also contained a slight amount of material with endotoxin-like activity (Table I). In fact, the control preparation contained a concentration equal to or greater than that found in all but two of the treponeme suspensions.

Although it seemed unlikely, there was the remote possibility that a tissue inhibitor present in the suspensions of treponemes was masking the presence of endotoxin in the preparations. This was investigated by testing two suspensions (sonicated preparations G and O) after the addition of minute amounts of *E. coli* endotoxin. Neither preparation inhibited the rate of the reaction between known concentrations of endotoxin (0.1 to 10 ng/ml) and amebocyte lysate.

Discussion. The studies reported in this paper clearly reveal that *B. hispanica* possesses no toxic component with a major biological property of microbial endotoxins, i.e., gelation of *Limulus* lysate. In this respect they confirm the recently reported findings of Butler *et al.* (18, 20) on related spirochetes, *B. recurrentis* and *B. hermsii.* In these studies the organisms were recovered from the blood of infected hosts or from cultures, and assayed for the presence of endotoxin by the *Limulus* lysate gelation reaction, an exquisitely sensitive test for endotoxin (19), with a specificity that equals or surpasses other methods for the detection of this class of bacterial components.

In the present study, suspensions of *T. pallidum* were also assayed for endotoxin, and though the results were not as clear cut as those for *B. hispanica,* they indicated that this pathogenic spirochete also lacks endotoxin. Eight suspensions of treponemes, with 0.6×10^9 to 3×10^9 treponemes/ml, all produced a minimal reaction with *Limulus* lysate when tested undiluted, but were nonreactive at a 1:10 dilution. Most significantly, the degree of gelation, as a measure of endotoxin concentration, did not correspond to the number of treponemes in a given preparation. For example, Pool F, containing 6×10^8 treponemes/ml (Table I), gave a gelation reaction equivalent to 1 ng/ml of *E. coli* endotoxin, whereas the suspension of the treponemes from Ra 5416, containing 3×10^9 treponemes/ml, produced a result equivalent to only 0.01 ng/ml of endotoxin. This suggestion that the substance causing *Limulus* lysate gelation was not derived from the treponemes was supported by the fact that slight gelation also occurred with an extract of normal rabbit testes, prepared as for treponeme suspensions from infected rabbits, but completely devoid of these organisms. It seemed reasonable to conclude from these combined observations that the trace amounts of endotoxin-like material in the treponeme suspensions were not from the treponemes themselves, but were either host tissue components of undefined nature, or were bacterial endotoxin resulting from slight inadvertent contamination of suspensions during tissue extraction and manipulation, despite efforts to the contrary. These conclusions have been supported by the observations of Young *et al.* (26) (*vide infra*).

The presence or absence of endotoxin in spirochetes of the family Spirochaetaceae, which includes all human pathogens, has been a subject of study for a number of years, and evidence both for and against such a spirochetal component has been reported. However, growth restrictions have limited most such studies to *Leptospira* and nonpathogenic spirochetes. Even for these the difficulties associated with acquiring large quantities of organisms have led some investigators to describe spirochetal lipopolysaccharides without assessing their biological activity. Zey and Jackson (11), who studied the lipopolysaccharides from a cultivable nonpathogenic treponeme, emphasized that not all such compounds possessed endotoxic properties. Thus, the early studies reporting lipopolysaccharide in the Reiter treponeme (*T. phagedenis* biotype Reiteri) (7–10) have little significance in terms of endotoxin. This was clearly demonstrated by the studies of Johnson (15), who obtained only contaminating quantities of endotoxin (40 ng/5 g of cells as measured by pyrogenicity) from another strain of the same organism, *T. phagedenis* biotype Kazan 5. In a similar fashion Finco and Low (13) and Johnson (15), as well as Arean *et al.* (14), were unable to recover significant amounts of endotoxin from *Lep. canicola* (*Lep. interrogans serovar canicola*) or *Lep. icterohaemorrhagiae,* as measured by fever production, mouse toxicity, and the Shwartzman reaction. Only Mergenhagen *et al.* (16) have reported the re-

covery of pyrogenic lipopolysaccharides from cultivable spirochetes, but even these had only marginal activity by comparison with other bacterial endotoxins. Since the lipopolysaccharides were extracted by a micro-procedure from spirochetes grown in broth containing 10% canine inflammation-induced ascitic fluid, there is reason to question the spirochetal origin of the pyrogenic material.

The recent report of Wright (27) has claimed biologic evidence of endotoxin in pathogenic spirochetes. This author studied *B. duttoni* infections in mice that developed a post-treatment Jarisch–Herxheimer-like reaction. Plasma obtained 1–4 hr after treatment with ampicillin of mice infected with *Borrelia* produced positive *Limulus* tests, concomitant with the disappearance of viable organisms from the blood. Wright also performed *Limulus* lysate tests on spirochetes and observed gelation with a sonicate from 10^{10} *Borrelia*/ml, but not with a suspension containing a similar concentration of intact spirochetes. He stated, without documentation, that approximately 100 sonicated *Borrelia* were sufficient to cause *Limulus* lysate gelation (27). If correct, this would indicate that pathogenic spirochetes possess endotoxin at an individual cellular concentration equivalent to that of Enterobacteria (28), but that the endotoxin is sequestered within the cells and unavailable to react with the *Limulus* lysate until released upon disruption of the spirochetes by sonication.

However, sonication may have resulted in contamination of Wright's preparations by exogenous endotoxin, since intact organisms failed to produce positive *Limulus* tests. This possibility is further suggested since Wright's observations (27) contrast sharply with those of Butler *et al.* (18, 20) and with our data reported in this paper. In the studies of both Butler *et al.* (18) and ourselves, *Limulus* lysate tests were nonreactive with sonicated spirochetes at concentrations far above the minimal number Wright considered necessary for reactivity. The negative results were interpreted as evidence of the absence of sequestered endotoxin.

The possible role of endotoxin in initiating the JHR has stimulated a number of investigators to look for evidence of endotoxin in patients with a JHR following antibiotic therapy of spirochetal infections. While such evidence has been found, it has been strikingly inconsistent. This is exemplified in the reports of *Limulus* lysate tests on plasma obtained during JHR (18, 26, 29–31). Galloway *et al.* (29) observed positive reactions in plasma from 13 of 15 patients with louse-borne relapsing fever, whereas Perine found only 3 of 11 patients positive (cited in (1)), and Butler *et al.* obtained a similar result in 7 of 28 patients (18). In syphilis, positive results ranged from 2 of 2 (30) to none of 11 patients (31) with secondary syphilis and the JHR. The failure to obtain positive reactions with all plasma specimens from patients with a JHR is a strong argument against the spirochetal origin of endotoxin. Finally, a thorough clinical and experimental study by Young *et al.* (26), using the *Limulus* test, failed to detect endotoxin in the blood of 12 patients with syphilis (66% of whom were experiencing a JHR) following therapy with penicillin. Similarly, production of the JHR in rabbits, following treatment of infection with *T. pallidum,* Nichols strain, was not associated with detectable endotoxemia or the development of tolerance to bacterial endotoxin (26). Therefore, as several authors have pointed out, the endotoxin that is demonstrable in some plasma samples may result either from a concomitant infection, e.g., *Haemophilus influenzae* pneumonia as reported by Butler *et al.* (18), or from the microbial flora of the alimentary tract (1, 18, 29, 32).

Francine Corthesy Levin provided excellent technical assistance.

1. Bryceson ADM. Clinical pathology of the Jarisch–Herxheimer reaction. J Infect Dis **133**:696–704, 1976.
2. Jarisch A. Therapeutische Versuche bei Syphilis. Wien Med Wochenschr **45**:721–724, 1895.
3. Herxheimer K, Krause I. Ueber eine bei Syphlitischen verkommende Quecksilberreaktion. Deutsch Med Wochenschr **28**:895–897, 1902.
4. Bryceson ADM, Cooper KE, Warrel DA, Perine PL, Perry EHO. Studies on the mechanism of the Jarisch–Herxheimer reaction in louse-borne relapsing fever: Evidence for the presence of circulating Borrelia endotoxin. Clin Sci **43**:343–354, 1972.
5. Kelly R. Cultivation of *Borrelia hermsi.* Science **173**:443–444, 1971.
6. Kelly RT. Cultivation and physiology of relapsing fever Borreliae. In: Johnson RC, ed. The Biology of

Parasitic Spirochetes. New York, Academic Press, pp. 87–94, 1976.
7. D'Alessandro G, Del Carpio C. A lipopolysaccharide antigen of the treponema. Nature (London) **181**:991–992, 1958.
8. de Bruijn JH. Investigations into the antigenic structure of the Reiter strain of *Treponema pallidum*. II. The complex nature of the protein fraction. Antonie van Leeuwenhoek J Microbiol Serol **25**:41–45, 1959.
9. Christiansen AH. Studies of the antigenic structure of *Treponema pallidum*. 2. Isolation and purification of polysaccharides from Reiter's apathogenic strain. Acta Pathol Microbiol Scand **56**:166–176, 1962.
10. Pillot J, Dupouey P. Composition antigenique des treponemes. IV. Solubilisation et purification des antigenes polyosidiques du Treponeme Reiter. Ann Inst Pasteur (Paris) **106**:456–468, 1964.
11. Zey PN, Jackson SW. Conditions that affect the colorimetric analysis of lipopolysaccharide from *Escherichia coli* and *Treponema pallidum*. Appl Microbiol **26**:129–133, 1973.
12. Heyman A, Sheldon WH, Evans LD. Pathogenesis of the Jarisch–Herxheimer reaction. A review of clinical and experimental observations. Brit J Vener Dis **28**:50–60, 1952.
13. Finco DR, Low DG. Endotoxin properties of *Leptospira canicola*. Amer J Vet Res **28**:1863–1872, 1967.
14. Arean VM, Sarasin G, Green JH. The pathogenesis of leptospirosis: Toxin production by Leptospira icterohaemorrhagiae. Amer J Vet Res **25**:836–843, 1964.
15. Johnson RC. Comparative spirochete physiology and cellular composition. In: Johnson RC, ed. The Biology of Parasitic Spirochetes. New York, Academic Press, pp. 39–48, 1976.
16. Mergenhagen SE, Hampp EG, Scherp HW. Preparation and biological activities of endotoxins from oral bacteria. J Infect Dis **108**:304–310, 1961.
17. Nuessen ME, Birmingham JR, Joens LA. Biological activity of a lipopolysaccharide extracted from Treponema hyodysenteriae. Infect and Immun **37**:138–142, 1982.
18. Butler T, Hazen P, Wallace CK, Awoke S, Habte-Michael A. Infection with *Borrelia recurrentis*: Pathogenesis of fever and petechiae. J Infect Dis **140**:665–675, 1979.
19. Levin J, Bang FB. Clottable protein in Limulus: Its localization and kinetics of its coagulation by endotoxin. Thromb Diath Haemorrh **19**:186–197, 1968.
20. Butler T, Spagnuolo PJ, Goldsmith GH, Aikawa M. Interaction of Borrelia spirochetes with human mononuclear leukocytes causes production of leukocytic pyrogen and thromboplastin. J Lab Clin Med **99**:709–721, 1982.
21. Hardy PH, Jr, Nell EE. Isolation and purification of *Treponema pallidum* from syphilitic lesions in rabbits. Infect and Immun **11**:1296–1299, 1975.
22. Levin J, Tomasulo PA, Oser RS. Detection of endotoxin in human blood and demonstration of an inhibitor. J Lab Clin Med **75**:903–911, 1970.
23. Levin J, Poore TE, Young NS, Margolis S, Zauber NP, Townes AS, Bell WR. Gram-negative sepsis. Detection of endotoxemia with the Limulus test. Ann Intern Med **76**:1–7, 1972.
24. Butler T, Levin J, Cu DQ, Walker RI. Bubonic plague: Detection of endotoxemia with the Limulus test. Ann Intern Med **79**:642–646, 1973.
25. Chase MW, Williams CA. Chemical analyses. A. Protein analysis. In: Williams CW, Chase MW, eds. Methods in Immunology and Immunochemistry. New York, Academic Press, Vol 2:pp. 273–275, 1968.
26. Young EJ, Weingarten NM, Baughn RE, Duncan WC. Studies on the pathogenesis of the Jarisch–Herxheimer reaction: Development of an animal model and evidence against a role for classical endotoxin. J Infect Dis **146**:606–615, 1982.
27. Wright DJM. Reaction following treatment of murine borreliosis and Shwartzman type reaction with borrelial sonicates. Parasite Immunol **2**:201–221, 1980.
28. Jorgensen JH, Carvajal HF, Chipps BE, Smith RF. Rapid detection of Gram-negative bacteriuria by use of the *Limulus* endotoxin assay. Appl Microbiol **26**:38–42, 1973.
29. Galloway RE, Levin J, Butler T, Noff GB, Goldsmith GH, Saito H, Awoke S, Wallace CK. Activation of protein mediators of inflammation and evidence for endotoxemia in *Borrelia recurrentis* infection. Amer J Med **63**:933–938, 1977.
30. Gelfand JA, Elin RJ, Berry FW Jr, Frank ME. Endotoxemia associated with the Jarisch–Herxheimer reaction. N Engl J Med **295**:211–213, 1976.
31. Young EJ, Musher DM, Weingarten N, Duncan WC, Rossen RD. Studies of the Jarisch–Herxheimer reaction. Clin Res **28**:383A, 1980.
32. Koster F, Levin J, Walker L, Tung KSK, Gilman RH, Rahaman MM, Majid MA, Islam S, Williams RC Jr. Hemolytic–uremic syndrome after shigellosis. Relation to endotoxemia and circulating immune complexes. N Engl J Med **298**:927–933, 1978.

Received February 1, 1983. P.S.E.B.M. 1983, Vol. 174.