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Toxicity of Group B *Streptococcus agalactiae* in Adult Rats

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Several strains of group B *Streptococcus agalactiae* were found to be lethal for young adult rats. When bacteria were heat killed and then injected intraperitoneally into rats, rapid death (14 to 18 h) of the rats occurred, characterized by labored breathing, hemolyzed serum, hemoglobinuria, and subungual hemorrhages. Sections of tissues from these rats failed to reveal the cause of death. Rats injected with toxic or nontoxic strains of group B *S. agalactiae* had reduced numbers of circulating leukocytes and low serum C3 levels in comparison with those in control rats. The toxic strains of group B *S. agalactiae* induced dramatic decreases in platelet numbers, and in plasma fibrinogen levels as well, suggesting that the toxicity was due to disruption of the coagulation system. Rapid death in the absence of infection suggests that group B *S. agalactiae* may have a cell-associated toxin that induces these changes. Such a toxin may be a contributory factor in the high mortality rate associated with group B streptococcal infections of the human neonate.

Group B streptococcus (GBS) is one of the major pathogens for human neonates in the United States (1, 14, 21). It has become apparent that GBS quite successfully eludes the defense mechanisms of neonates, and even when appropriate antibiotic therapy is provided, up to 50% of infected infants die (1). Animal models in adult mice (3, 28), neonatal rats (13), and embryonic chickens (27) have been developed in an effort to understand the human disease. These models have been useful in exploring the route of infection, the extent of organ colonization after inoculation of GBS, and possible means of protection.

As a result of these studies, several explanations for the high susceptibility of neonates for GBS have been brought forward. In comparison to adults, it appears from human studies (22) and from rat models (5, 7) that newborns are less able to contain an infection because the neutrophil reserves are quickly depleted, leading to a severe neutropenia. To compound this deficiency, neonates may lack specific immunity (i.e., anti-type antibody) to GBS, which has been shown to be protective in animal models of the disease (6, 26). Additionally, GBS's have a terminal sialic acid residue on the type antigen which can inhibit opsonization of the bacteria (10). As a result of these factors, bacteria multiply relatively unchecked in newborns so that all organs become rapidly colonized and the bacteria reach high titers. It is at this point that toxic molecules on the surface of bacteria could play a role in the disease initiated by the infection.

The possibility that GBS may produce such toxic molecules has been suggested both by noting the similarities between the symptoms of GBS infection in infants and the symptoms of adults with endotoxemia (11) and by the ability of GBS to enhance the effects of endotoxin (25). Such a toxin has recently been isolated (24), and its effects on the pulmonary hemodynamics of adult sheep have been described (16). We now describe a system in which adult rats injected with large amounts of heat-killed GBS's exhibit symptoms similar to those of infected human neonates. These include neutropenia, thrombocytopenia, coagulation disorders, complement depletion, and death. These symptoms in the absence of an active infection suggest that GBS's express a cell-associated toxin which may be responsible in part for virulence of this organism.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (125 to 150 g) were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and allowed to rest for 2 weeks after delivery before use in an experiment. Rats were fed Purina Laboratory Chow and tap water ad libitum.

Bacteria. Group B *Streptococcus agalactiae* D136c, 18RS21, A9O9 and O9OR were obtained from Rebecca Lancefield, Rockefeller University, New York. Strain 509 was obtained from the Centers for Disease Control, Atlanta, Ga. Group A *Streptococcus pyogenes* D58 was obtained from John Schwab, University of North Carolina, Chapel Hill. Bacteria were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants at 4°C.

Media and growth conditions. Bacteria were streaked out on sheep blood agar plates (Carr-Scarborough, Decatur, Ga.) and incubated at 37°C overnight. For stationary cultures, isolated colonies were inoculated into 10 ml of Todd-Hewitt broth (BBL Microbiology Systems) and incubated at 37°C for 8 h before inoculation into 3 liters of Todd-Hewitt broth. These cultures were incubated at 37°C without shaking for 16 h, harvested by centrifugation, and washed in phosphate-buffered saline (PBS). A 15% (wt/vol) suspension of bacteria in PBS was prepared, and the cells were killed by heating at 60°C for 40 min. Sterility was checked by plating out on blood agar. For fermentor cultures, bacteria grown for 8 h in 10 ml of Todd-Hewitt broth were inoculated into 500 ml of broth and incubated for 16 h at 37°C. This culture then was inoculated into 10 liters of Todd-Hewitt broth in a New Brunswick fermentor model 19 (New Brunswick Scientific Co., Inc., Edison, N.J.). The culture was grown for 8 h with continuous stirring. The pH was maintained at 7 by the addition of NaOH, and the medium was supplemented with glucose throughout the growth period. Harvesting and heat killing were performed as for the stationary cultures. Doses of heat-killed bacteria for injection into rats were determined by rhamnose content. There are approximately

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TABLE 1. Toxicity of various *Streptococcus* groups and strains in adult rats[a]

Streptococcus serotype and strain	Dose (μg of rhamnose/ g of body wt)	No. of deaths/ total no.
Ia		
O9O	80	4/5
	40	10/10
	5	2/4
509	80	6/7
	40	13/17
Ib H36b	80	0/5
	60	0/9
Ic A9O9	80	4/5
	60	8/9
	40	5/5
II 18RS21	80	2/5
	60	7/8
	40	3/5
III		
D136c	80	0/5
	40	0/12
603-79	60	0/5
	40	16/44
	10	0/10
Typeless O9OR	60	0/8

[a] Heat-killed bacteria were injected i.p., and deaths were recorded 18 h later.

30 μg of rhamnose per mg of dry bacterial weight. The assay was performed by the method of Dische and Shettles (9).

Cell counts. Leukocyte and platelet counts were determined from blood samples collected in sodium citrate. Platelet counts were performed by the method of Brecher et al. (4) on citrated blood diluted 1:100 in saline. Total leukocyte counts were performed with a Coulter Counter model Z_B (Coulter Electronics, Inc., Hialeah, Fla.).

Immunoelectrophoresis. Relative plasma fibrinogen and serum C3 levels were determined by rocket immunoelectrophoresis. Anti-rat C3 or anti-rat fibrinogen (Cappel Laboratories, West Chester, Pa.) was added to 1% agarose (Sigma Chemical Co., St. Louis, Mo.) in Tris-EDTA-borate buffer (pH 8) and poured into 1-mm molds. Wells (diameter, 3 mm) were cut in the hardened agar and filled with 10 μl of 1:10 dilution of rat plasma. Gels were electrophoresed for 18 h at 75 V at room temperature, then washed in 1% saline, and stained with Coomassie G to visualize the rockets.

CVF treatment. Rats were injected intraperitoneally (i.p.) with 0.2 U of cobra venom factor (CVF; Cordis Laboratories, Miami, Fla.) per g of body weight 24 h before injection of bacteria. This has no effect on survival of rats but reduced serum C3 levels by a factor of 100.

Heparin treatment. Rats were injected i.p. with 5 U of sodium heparin (Sigma Chemical Co.) in saline per g of body weight immediately before injection of heat-killed bacteria.

Histology. Tissues from rats were fixed in 10% phosphate-buffered Formalin for 24 h and then washed in PBS for 48 h. The washed tissues were dehydrated in alcohol, cleared in xylene with an Autotechnicon Mono (Technicon Instruments Corp., Tarrytown, N.Y.), and then embedded in paraffin. Tissues were sectioned with a Base-Sledge Microtome K100 (Leitz, Midland, Ohio). Five-micrometer sections were stained either with hematoxylin-eosin or with phosphotungstic acid-hematoxylin to stain fibrin.

RESULTS

Toxicity of different serotypes of GBS. The extent of toxicity of different serotypes of heat-killed GBS after i.p. injection into adult Sprague-Dawley rats is shown (Table 1). Toxicity was not associated with any one serotype of GBS. The lethal dose appeared to vary from strain to strain.

Effect of growth conditions on the toxic potential of GBS. The toxic potential of any one strain of GBS varied somewhat from one growth batch to the next. A striking difference in toxicity was noted between GBS grown in 18-h stationary cultures and GBS grown for 8 h in a pH-controlled, glucose-supplemented fermentor. Stationary cultures are more toxic for adult rats than are fermentor-grown cultures (Table 2).

Effect of toxic GBS on rats. Adult female Sprague-Dawley rats injected i.p. with certain strains of heat-killed GBS died within 8 to 12 h. Shortly after injection (2 to 3 h), rats became inactive and hunched up, with ruffled fur. Often breathing appeared labored, and red secretions appeared around their eyes and noses. Hemolysis of serum samples from these rats and hemoglobinuria were also noted. Hemorrhagic lesions were present under the toenails. Rats died within 12 to 16 h after bacteria were injected. Autopsies of these rats failed to show any microscopically obvious cause of this rapid death.

To investigate this toxicity, age-matched female rats were injected i.p. with 60 μg of rhamnose per g of body weight of strain 509 (toxic GBS), strain D136C (nontoxic GBS), or strain D58 (nontoxic GAS) or with PBS (controls). After 6 h, rats were sacrificed, and blood and tissue samples were taken for analysis. Histology of various organs, leukocyte counts, platelet counts, serum C3 levels, and plasma fibrinogen levels from rats of the various groups were compared.

General observations. Rats injected with any of the streptococcal strains appeared ill after 2 to 3 h. All rats (except those injected with PBS) appeared inactive and ruffled. Only rats injected with strain 509 had hemolyzed serum and hemoglobinuria.

Histology. Rats injected with a toxic dose of strain 509 were sacrificed after 8 h. Spleens, kidneys, livers, and lungs were fixed in 10% buffered Formalin and subsequently processed for paraffin embedding. Sections taken from these organs and stained with hematoxylin-eosin did not differ significantly from sections from rats injected with the nontoxic GBS and group A streptococcus (GAS) strains. In brief, no pathological changes were noted in the spleens, livers, or kidneys of injected rats. Lung sections showed changes in alveolar structure indicative of minimal interstitial pneumonia, and occasionally emphysema-like pathology

TABLE 2. Toxic potential of GBS with varied growth conditions[a]

Dose (μg of rhamnose/ g of body wt)	No. of deaths/total no. in:	
	Fementor culture	Stationary culture
5	0/5	4/5
10	0/10	10/10
20	1/9	10/10
40	2/5	5/5

[a] Strain 509 was grown either in a pH-controlled fermentor with stirring and the addition of glucose or in stationary culture.

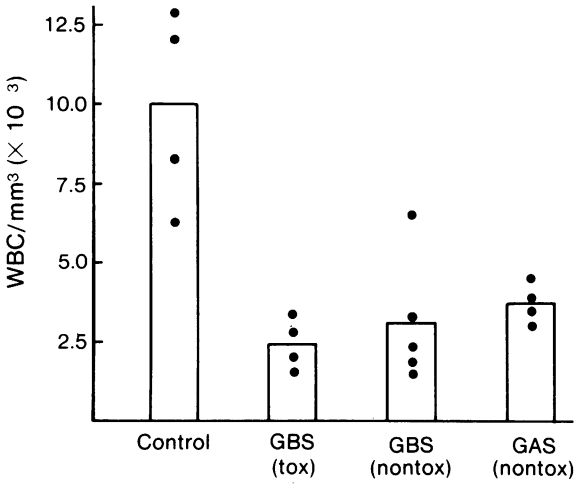

FIG. 1. Decrease in number of circulating leukocytes (WBC) in rats injected with saline (control), toxic GBS (strain 509), nontoxic GBS (strain D136C), or nontoxic GAS (strain D58). Bars represent the mean of values from individual rats.

was noted. These changes were observed in all injected animals and in some control animals as well.

Leukocyte counts. At the time of sacrifice, blood samples were collected from each rat and total leukocyte counts were performed with a Coulter Counter. Injection of streptococci, regardless of group or strain, caused a significant drop in the number of circulating leukocytes (Fig. 1).

So few leukocytes were present in the blood of rats injected with streptococci that valid differential counts could not be performed on blood smears. However, examination of stained smears suggested that polymorphonuclear cells are preferentially lost from the circulation after injection of streptococci (data not shown). This may be a result of the inflammatory reaction occurring in the peritoneum, or it could be a result of margination of activated neutrophils in the circulation.

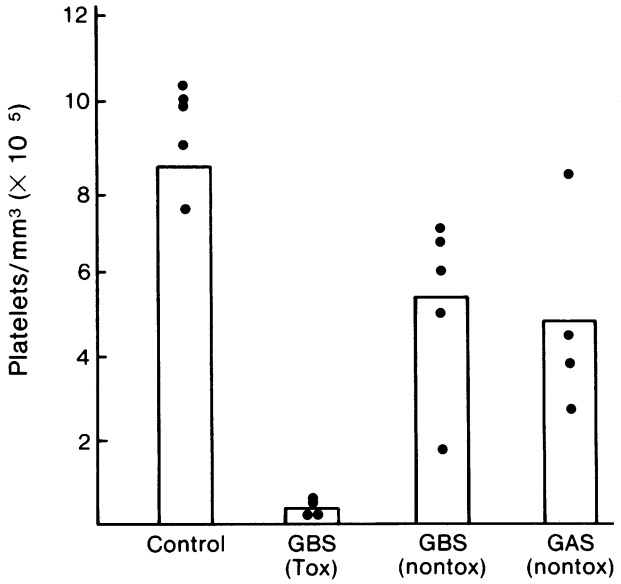

FIG. 2. Decrease in number of platelets in rats injected with saline, toxic GBS (strain 509), nontoxic GBS (strain D136C), or nontoxic GAS (strain D58). Bars represent the mean of values from individual rats.

TABLE 3. Relative serum C3 levels in rats injected with heat-killed streptococci[a]

Streptococci	No. of rats/group	Ht of rocket (mm)[b]
	6	24.6 ± 2.3
GBS 509	4	21.6 ± 3.4
GBS D136c	4	19.5 ± 2.4
GAS D58	5	20.6 ± 2.1

[a] Rats were injected with streptococci (60 μg of rhamnose per g of body weight).
[b] Mean value ± standard deviation.

Platelet counts. The number of platelets decreased when rats were injected with GAS or GBS (Fig. 2). However, the most dramatic reduction in platelet numbers was observed in rats injected with the most toxic strain of GBS (509).

Effect of injection of streptococci on serum C3 levels. Peptidoglycan of both GAS and GBS is known to fix complement (10, 15). Rats injected with whole heat-killed organisms had slightly lower C3 levels than did control rats 6 h after injection (Table 3). The decrease in serum C3 levels was comparable with the toxic and nontoxic GBS strains. These data do not support the hypothesis that the complement cascade mediates the toxicity of strain 509.

To test this conclusion in a different way, we pretreated a group of rats with CVF, artificially lowering C3 levels. At 24 h after treatment with CVF when serum C3 levels were barely detectable by rocket immunoelectrophoresis, rats were injected with a toxic dose of strain 509. Six of eight treated rats died within 24 h as compared with five of eight deaths in the control (non-CVF-treated) group. It appears that serum C3-depleted rats were as susceptible to the toxicity of strains 509 as were normal rats.

Plasma fibrinogen levels. The dramatic drop in circulating platelets in rats injected with strain 509 suggested that the clotting system might be activated by toxic GBS strains. A decrease in the fibrinogen content of plasma could have been interpreted to indicate that conversion of fibrinogen to fibrin had occurred and that fibrin clots might have formed. We measured fibrinogen levels in the plasma of rats which had been injected 8 h previously with PBS, GBS strain 509, GBS strain D136c, or GAS strain D58. Rats injected with strain 509 had decreased plasma fibrinogen levels as compared with rats injected with nontoxic GBS or GAS strains (Table 4). This hypofibrinogenemia, in conjunction with the severe thrombocytopenia, suggests that a coagulopathy may mediate the toxicity of GBS strain 509.

TABLE 4. Relative plasma fibrinogen levels measured by rocket immunoelectrophoresis

Streptococci	Ht of rocket (mm)[b] (n)[c]	
	Expt 1	Expt 2
	27.75 ± 1.2 (4)	25.3 ± 3.8 (3)
GBS 509	19.25 ± 3.6 (4)	14.8 ± 6.4 (5)
GBS D136c	25.4 ± 2.5 (5)	ND[d]
GAS D58	24. ± 1.0 (3)	ND[d]

[a] Samples were collected 8 h after i.p. injection of streptococci (60 μg of rhamnose per g of body weight).
[b] Mean value ± standard deviation.
[c] The numbers within parentheses represent the number of rats per group.
[d] ND, Not done.

To test this hypothesis further, we treated a group of rats with the anticoagulant heparin before injection of toxic GBS. Rats were subcutaneously injected with doses of heparin shown to increase whole-blood clotting times in rats (8). Three of four heparinized rats died after injection with GBS strain 509 as compared with five of six deaths in the control (nonheparinized) group.

Sections of organs from rats injected with GBS strain 509 were examined for the presence of fibrin clots with a phosphotungstic acid-hematoxylin stain. No evidence of microclots was observed in these sections.

DISCUSSION

We have identified several GBS strains which, when heat killed and injected in large amounts into adult rats, mimic the symptoms of GBS infection of neonates, including the acute death. Other symptoms include leukocytopenia and thrombocytopenia, as well as decreases in blood clotting elements and complement. We have shown that the coagulopathy signified by thrombocytopenia and hypofibrinogenemia was confined to responses to toxic GBS strains and not to responses to nontoxic GBS strains. Leukocytopenia and changes in complement levels were caused by nontoxic as well as toxic GBS strains. The similarities between the effects of heat-killed GBS on adult rats and the effects of early-onset infection of human neonates with GBS may be useful in studying this disease.

Disease resulting from the infection of neonates with group B *S. agalactiae* has been noted to occur in two forms (2, 14). Early-onset infection is associated with an acute onset of respiratory symptoms and high mortality rates, whereas delayed-onset infection is associated with infection of the meninges and lower mortality rates. The early-onset infection has symptoms similar to those of adults suffering from gram-negative endotoxemia (11) in that gram-negative sepsis has been shown to result in decreases in complement components (12, 20) and clotting factors (18, 19), as well as in neutropenia and thrombocytopenia (19). Because of similarities in the response of neonates to GBS infections and that of adults to gram-negative sepsis, it has been postulated that *S. agalactiae* may elaborate a toxin with effects similar to those of endotoxin. Recently, a substance has been isolated from GBS which causes granulocytopenia and lung vasculature changes in adult sheep (16, 24) and which may represent a cell-associated toxin of GBS. The data presented here also argue strongly for the presence of a toxin associated with GBS's.

Several changes occurred in rats injected with GBS regardless of the toxic nature of the GBS strain used. Leukocytopenia was significant after 8 h. Differential counts suggested that polymorphonuclear cells were preferentially lost from the circulation. This may have been due to the inflammatory reaction occurring in the peritoneum of the injected rats. However, neutropenia also has been noted in neonates suffering from GBS sepsis (22) and may be a direct effect of the bacteria leading to margination of polymorphonuclear cells in the circulation.

Another factor which was shared by nontoxic and toxic GBS strains was the induced decrease in complement levels in injected rats. This decrease in complement activity has also been noted in infants infected with GBS (11). Both GBS and GAS have been shown to fix complement through the alternate pathway (10, 15). This may affect the processing of antigens and possibly the inflammatory processes induced by these organisms, but it is doubtful that activation of the complement cascade provides a sufficient explanation for the toxic effect on adult rats in this system. This is further corroborated by the data showing that rats which were pretreated with CVF to artificially decrease C3 levels were not protected from toxicity.

Effects on the coagulation system seemed to be a property of the toxic GBS strain and not of the nontoxic strain tested here. After injection of GBS, platelet numbers decreased dramatically in rats which had been injected with toxic GBS strains. These data suggest that a change in the coagulation system had occurred. When plasma fibrinogen levels were assayed by rocket immunoelectrophoresis, it was found that rats injected with toxic GBS had lower levels than did rats injected with the nontoxic strain. Human neonates infected with GBS have been shown to have various coagulopathies, such as hypofibrinogenemia and decreased clotting times and platelet levels (23). In addition, hemorrhage and intravascular clots have been observed in tissues from some infants who died from GBS sepsis (17, 23).

The effect of the depletion of fibrinogen in the rats in this study is unknown. Pretreatment of the rats with heparin did not protect the rats against the toxicity. Also, fibrin clots were not observed in any of the major organs of these rats at the time of death. The absence of such clots may indicate that the fibrinolytic system, as well as the clotting system of plasma, was activated and that the fibrin clots were being dissolved as soon as they had formed.

Although adult humans are capable of successfully resisting GBS infections, neonates are subject to a high mortality rate. In this paper we describe a model in adult rats in which heat-killed GBS strains can cause an acute illness and death within 14 to 18 h. This is apparently due to some type of cell-associated toxin. It is impossible to know from our data whether this is the same substance described by Hellerqvist et al. (16). The use of the model in adult rats may be helpful in further defining the human neonatal disease and its relation to a toxin produced by GBS.

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LITERATURE CITED

1. **Baker, C. J.** 1977. Summary of the workshop on perinatal infections due to group B streptococcus. J. Infect. Dis. **136**:137–152.
2. **Baker, C. J., F. F. Barrett, R. C. Gordon, and M. D. Yow.** 1973. Suppurative meningitis due to Streptococci of Lancefield group B: a study of 33 infants. J. Pediatr. **82**:724–729.
3. **Baltimore, R. J., D. L. Kasper, and J. Vecchitto.** 1979. Mouse protection test for Group B Streptococcus type III. J. Infect. Dis. **140**:81–88.
4. **Brecher, G., M. Schneiderman, and E. P. Cronkite.** 1953. The reproducibility and constancy of the platelet count. Am. J. Clin. Pathol. **23**:15–26.
5. **Christensen, R. D., H. R. Hill, and G. Rothstein.** 1983. Granulocytic stem cell (CFUc) proliferation in experimental group B streptococcal sepsis. Pediatr. Res. **17**:278–280.
6. **Christensen, R. D., G. Rothstein, H. R. Hill, and S. H. Pincus.** 1983. The effect of hybridoma antibody administration upon neutrophil kinetics during experimental type III group B streptococcal sepsis. Pediatr. Res. **17**:795–799.
7. **Christensen, R. D., A. O. Shigeoka, H. R. Hill, and G. Rothstein.** 1980. Circulating and storage neutrophil changes in experimental type II group B streptococcal sepsis. Pediatr. Res. **14**:806–808.
8. **DeClerck, F., J. Goossens, and R. Reneman.** 1976. Effects of anti-inflammatory, anti-coagulant and vasoactive compounds on tail bleeding time, whole blood coagulation time and platelet retention by glass beads in rats. Thromb. Res. **8**:179–196.

9. **Dische, Z., and L. P. Shettles.** 1948. A specific color reaction of methyl pentoses and a spectrophotometric micro-method for their determination. J. Biol. Chem. **175**:595–603.
10. **Edwards, M. S., D. L. Kasper, H. J. Jennings, C. J. Baker, and A. Nicholson-Weller.** 1982. Capsular stalic acid prevents activation of the alternative complement pathway by type III, group B streptococci. J. Immunol. **128**:1278–1283.
11. **Fenton, L. J., and R. C. Strunk.** 1977. Complement activation and Group B Streptococcal infection in the newborn: similarities to endotoxin shock. Pediatrics **60**:901–907.
12. **Feron, D. F., S. Ruddy, P. H. Schur, and W. R. McCabe.** 1975. Activation of the preparation pathway of complement in patients with gram negative bacteremia. N. Engl. J. Med. **292**:937–940.
13. **Ferreiri, P., B. Burke, and J. Nelson.** 1980. Production of bacteremia and meningitis in infant rats with group B streptococcal serotypes. Infect. Immun. **27**:1023–1032.
14. **Franciosi, R. A., J. D. Knostman, and R. A. Zimmerman.** 1973. Group B Streptococcal neonatal and infant infections. J. Pediatr. **82**:707–718.
15. **Greenblatt, J., R. J. Boackle, and J. H. Schwab.** 1978. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell well. Infect. Immun. **19**:296–303.
16. **Hellerqvist, C. G., J. Rojas, R. S. Green, S. Sell, H. Sundell, and M. T. Stahlman.** 1981. Studies on group B beta-hemolytic Streptococcus. I. Isolation and partial characterization of an extracellular toxin. Pediatr. Res. **15**:892–898.
17. **Hey, D. J., R. T. Hall, V. F. Burry, and A. N. Thurn.** 1973. Neonatal infections caused by Group B Streptococci. Am. J. Obstet. Gynecol. **116**:43–47.
18. **Levin, J., T. E. Poore, N. S. Young, S. Margolis, N. P. Zanber, A. J. Townes, and W. R. Bell.** 1972. Gram negative sepsis: detection of endotoxemia with the limulus test with studies of associated changes in blood coagulation, serum lipids and complement. Ann. Intern. Med. **76**:1–7.
19. **Mason, J. W., U. Kleeburg, P. Dolan, and R. W. Colman.** 1970. Plasma kallikrein and Hagemen factor in gram negative bacteremia. Ann. Intern. Med. **73**:545–551.
20. **McCabe, W. R.** 1973. Serum complement levels in bacteremia due to gram-negative organisms. N. Engl. J. Med. **288**:21–23.
21. **McCracken, G. H.** 1973. Group B Streptococci: the new challenge in neonatal infections. J. Pediatr. **82**:703–706.
22. **Nieburg, P. I.** 1976. Prognostic/diagnostic factors in Group B beta-hemolytic streptococcal (GBBJ) and other neonatal sepses. Pediatr. Res. **10**:402.
23. **Quirante, J., R. Ceballos, and G. Cassady.** 1974. Group B beta-hemolytic streptococcal infection in the new born. I. Early onset infection. Am. J. Dis. Child. **128**:659–665.
24. **Rojas, J., R. S. Green, C. G. Hellerqvist, R. Olegard, K. L. Brigham, and M. T. Stahlman.** 1981. Studies on Group B beta-hemolytic streptococcus. II. Effects on pulmonary hemodynamics and vascular permeability in unanesthetized sheep. Pediatr. Res. **15**:899–904.
25. **Schlievert, P. M., M. Varner, and R. P. Galask.** 1983. Endotoxin enhancement as a possible etiology of early-onset group B beta-hemolytic streptococcal sepsis in the newborn. Obstet. Gynecol. **61**:588–592.
26. **Shigeoka, A. O., S. H. Pincus, N. S. Rote, and H. R. Hill.** 1982. Hybridoma type-specific IgM antibody offers enhanced protection against systemic or respiratory group B streptococcal experimental infection. Pediatr. Res. **16**:231A.
27. **Tieffenberg, J., L. Vogel, R. R. Kretschmer, D. Padnos, and S. P. Gotoff.** 1978. Chicken embryo model for type III group B beta-hemolytic streptococcal septicemia. Infect. Immun. **19**:481–485.
28. **Wennerstrom, D. E., and R. W. Schutt.** 1978. Adult mice as a model for early onset group B streptococcal disease. Infect. Immun. **19**:741–744.