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Cross-reactive Antigen Shared by *Streptococcus agalactiae* and Certain Bovine Tissues

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ABSTRACT

Rabbits immunized with mucopeptide derived from cell wall debris of *Streptococcus agalactiae* by use of the formamide extraction technique developed specific antibodies for bovine heart, skeletal muscle, lymph nodes, and blood buffy coat extracts.

Antigenic similarity between *Streptococcus agalactiae* (Lancefield's Group B) and components of bovine tissue could explain the observation that stimulation with a variety of *S. agalactiae* preparations either fails to give any detectable serum antibodies or, at best, gives a very low and erratic serum titer (4, 8). This problem of poor antibody response hinders elucidation of the fundamental pathogenesis of bovine *S. agalactiae* mastitis, which is of prime economic importance.

Serological cross-reactivity between strains of Lancefield's Group A streptococci and human heart and skeletal muscle is well established (5, 6). Kaplan (5, 6) has demonstrated and partially characterized the cross-reactive antigen as a protein which is closely associated with, but distinct and separable from, the M protein of the Group A virulent streptococcal cell walls.

MATERIALS AND METHODS

S. agalactiae Cornell strain 50 was used throughout the study. This strain originated from a naturally infected herd. Identity was established by use of micro double-diffusion plates with commercial Group B specific antiserum (Difco) and the CAMP (1) test. The organism was maintained on Trypticase Soy Agar (BBL) slants. Cultures were grown in 10 liters of Trypticase Soy Broth for 18 hr at 37 C, and were harvested by centrifugal sedimentation. The packed cells were washed twice in sterile physiological saline and once in sterile distilled water.

Preparation of antigen. The washed cells were suspended in 60 ml of sterile physiological saline and were treated for 20 min with a 20 K ultrasonic probe (model S-110, Branson Instruments, Inc., Danbury, Conn.). The suspension was centrifuged at 24,850 $\times$ *g* for 30 min to obtain cell wall debris; the supernatant fluid was discarded. Cell material was resuspended in sterile physiological saline, washed, and compacted again. This material was extracted by use of Fuller's (3) hot formamide technique as modified by Krause and McCarty (7) for recovery of cell wall mucopeptide. The mucopeptide material was lyophilized and stored until required.

Evaluation of antigen. Hot formamide extraction of cell wall debris is a harsh procedure calculated to denature and solubilize all of the cell wall constituents other than mucopeptide. However, there is the possibility that the mucopeptide might be contaminated with other constituents, especially the group-specific carbohydrate. Double-diffusion tests in 1% Ionagar (Ionagar No. 2, Consolidated Laboratories, Inc. Chicago Heights, Ill.) were used to investigate these possibilities. The results in Fig. 1 and 2 show that the mucopeptide was not denatured by the formamide and was not contaminated with group carbohydrate. Figure 3 shows that the mucopeptide is digested with lysozyme.

These preliminary experiments indicate that the mucopeptide was immunologically intact, and that the contaminants did not act as antigens.

Preparation of antiserum. New Zealand white rabbits from local dealers were used. Preinoculation serum samples were obtained and frozen. The antigenic preparation was suspended in sterile 0.15 M NaCl to a concentration of 10 mg/ml (dry weight). The intravenous route of inoculation was used.

Immunization schedules consisted of three inoculations per week in a volume of 1 ml, for 4 weeks. Four days after the last injection the rabbits were bled, and the serum was frozen until required.

Preparation of bovine tissue extracts. Bovine heart, skeletal muscle, liver, kidney, and lymph nodes were obtained aseptically and frozen until used. Buffy coat from bovine blood was also obtained by centrifugation.

Approximately 3 g of each tissue was macerated and suspended in 5 ml of sterile 0.15 M NaCl. The suspensions were sonically treated for 5 min in the same manner as described above for antigen, after which they were centrifuged and the supernatant fluid was frozen and stored until required.

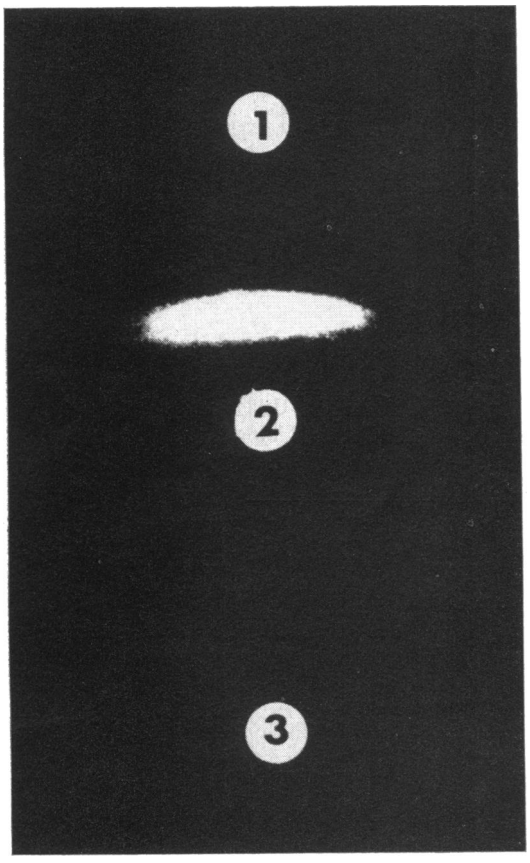

FIG. 1. *Double-diffusion test in 1% Ionagar. (1) Mucopeptide. (2) Rabbit antiserum, against whole Streptococcus agalactiae cells. (3) Saline, 0.85%.*

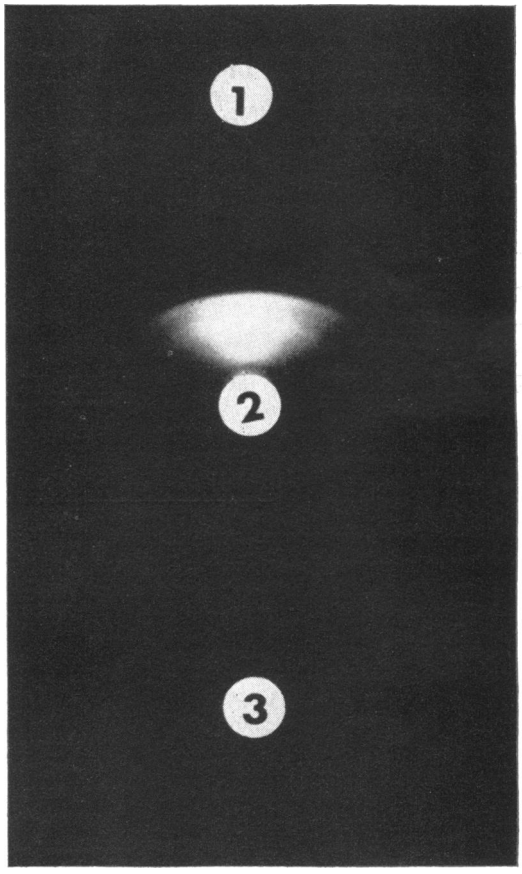

FIG. 2. *Double-diffusion test in 1% Ionagar. (1) Group-specific antigen. (2) Group-specific antiserum. (3) Mucopeptide.*

Micro double-diffusion tests in 1% Ionagar made up in physiological saline were used throughout the study to establish the presence and cross-identities of antibody-antigen systems (Fig. 4).

RESULTS

Results of immunodiffusion tests are shown in Table 1. The negative reaction with the group antiserum indicates that the tissues were not contaminated with Group B streptococci.

Adsorption tests showed that antibody against the *S. aglactiae* formamide extract can be removed from specific antiserum by use of bovine tissue extracts.

To preclude the possibility that the Forsmann antigen was the cross-reactive agent, the rabbit antimucopeptide antiserum was added to washed sheep red blood cells in physiological saline. No hemolysis resulted.

DISCUSSION

Streptococci of Lancefield's Groups A and B seem to have a special relationship to their respective hosts. In man, certain cardiac and kidney diseases which have an autoimmune basis are usually preceded by a history of streptococcal infection. These syndromes have not been clearly documented in cattle. The confinement of *S. agalactiae* to the mammary gland would have some bearing on this.

Vaccination with *S. agalactiae* materials has periodically been recommended, tried, and abandoned (2). This work indicates that such a procedure would place the animal in a biological dilemma; if antibody against mucopeptide were produced, autoimmune sequelae should ensue. This aspect is presently under investigation, with the use of various strains of Group B and L forms of the same streptococcal strains.

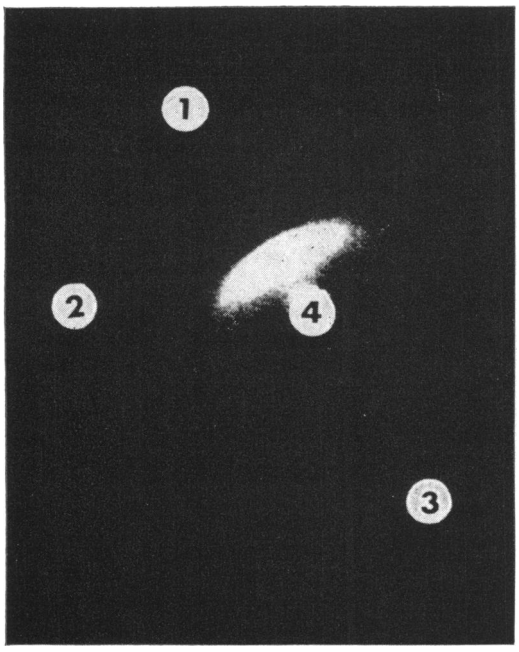

FIG. 3. *Double-diffusion test in 1% Ionagar. (1) Rabbit antimucopeptide antiserum. (2) Lysozyme digest (6-hr) of mucopeptide (9). (3) Rabbit antimucopeptide antiserum adsorbed with the lysozyme digest. (4) Mucopeptide.*

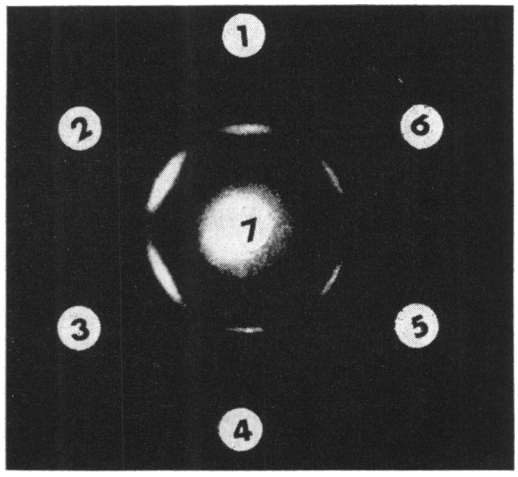

FIG. 4. *Double diffusion precipitin test in 1% Ionagar. Antigens: (1) mucopeptide extract; (2) Streptococcus agalactiae sonic extract; (3) skeletal muscle; (4) heart muscle extract; (5) lymph node extract; (6) buffy coat extract. Antiserum: (7) rabbit antimucopeptide extract. Slide stained with 1% Ponceau red.*

TABLE 1. *Immunodiffusion tests with anti-streptococcal antisera and bovine tissue extracts*

Antiserum	Heart	Skeletal muscle	Lymph node	Buffy coat	Liver	Kidney	*Streptococcus agalactiae*
Rabbit anti-mucopeptide	+	+	+	+	−	−	+
Group specific	−	−	−	−	−	−	+
Preinoculation	−	−	−	−	−	−	−

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