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Identification of An Antigen Common to *Listeria monocytogenes* and Other Bacteria.* (26034)

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An antigen common to Gram-positive bacteria was described by Rantz and associates (1,2) and referred to as non-species specific antigen. Subsequent studies revealed that this heterogenetic antigen is present in many but not all species of these bacteria. For example, it was not detected in *Staphylococcus citreus* and *Micrococcus lysodeikticus*, even after lysis of the latter microorganisms by lysozyme(3,4). The antigen is produced by certain but not all species of the genus *Bacillus* (5). The present study was undertaken to determine whether *Listeria monocytogenes* produces this antigen and, also, whether it is this antigen that is the basis for the serologic crossreactions between this microorganism and *Staphylococcus* described by Seeliger(6) and Welshimer(7).

Materials and methods. Strains of *L. monocytogenes* (types 1, 2, 3, 4a, 4b) were received through the kindness of Dr. J. T. Barrett, Univ. of Missouri, and Dr. M. L. Gray, Montana State College. The former strains were obtained from CDC (KC 222 to 226). Dr. Gray's strains types 1 and 4a originated from the National Type Culture Collection (#5349 and #5214); types 2 and 4b from Dr. Donker-Voet, Utrecht, Netherlands; type 3 was isolated from a fatal case of meningitis in Muskegon, Mich. Dr. H. J. Welshimer made available one strain each of *L. monocytogenes* type 1 and *Staphylococcus epidermidis*. Strains of *S. aureus* and *Bacillus subtilis* were isolated in this laboratory, *S. aureus* strain D was obtained from Difco Laboratories, Detroit. The strains were maintained on blood agar.

For preparation of antigens, the microorganisms were grown on brain veal agar in Kolle flasks at 37°C for 18 hours. To each flask was added 20 ml of phosphate buffer of pH 7.3. The suspensions were centrifuged immediately at 23,500 G for 20 min in a refrigerated centrifuge. Supernates of infusion broth cultures were also used. Supernates, notably of type 4a, proved to be hemolytic; it was observed that heating at 56°C for 15 min abolished this effect. Therefore, the antigens were routinely heated and then kept at 4°C until used.

The hemagglutination test was carried out as previously described(3,8). Briefly, human red blood cells (blood group O) were washed 3 times, mixed with antigen in suitable dilutions, and incubated for 30 min at 37°C. The modified erythrocytes were washed 3 times and then used in the hemagglutination test. Antiserum in serial 2-fold dilutions (vol. 0.2 ml) was mixed with 0.2 ml of the modified red blood cell suspension. The mixtures were incubated in a waterbath at 37°C for 30 min, and the resulting hemagglutination was read grossly after centrifugation at 1300 G for 3 min. *L. monocytogenes* antisera were obtained from Dr. Gray. Others were prepared in this laboratory by injection, 2 days apart, of suspensions of agar grown bacteria (2 ml) that had been heated at 56°C for 15 min, followed one week later by injection of unheated suspensions. The animals were bled one week after immunization.

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TABLE I. Heterogenetic Antigen in *Listeria monocytogenes*.

	Antigens						
	Listeria monocytogenes Types					*Staphylococcus*	*B. subtilis*
Staphylococcal antiserum	1	2	3	4a	4b		
	Hemagglutination titers (reciprocal)						
Pre-immunization	<100	<100	<100	<100	<100	100	<100
Post-immunization	25600	12800	12800	12800	12800	25600	25600
Absorbed with							
L. monocytogenes (all types)	<400	<400	<400	<400	<400	<400	<400
B. subtilis	<400	<400	<400	<400	<400	<400	<400
Staphylococcus (D)	<400	<400	<400	<400	<400	<400	<400

Staphylococcal rabbit antiserum, prepared by injection of viable bacteria (strain D), had a hemagglutinin titer of 1:12,800 and failed to agglutinate (in titer of 1:200) erythrocytes modified by *Salmonella, Shigella*, and *Escherichia coli* antigens. Another staphylococcal antiserum was obtained from rabbits after injection of antigen A, kindly supplied by Dr. K. Jensen(9). Normal rabbit sera were employed for control purposes.

Absorption tests were carried out by mixing the sediment of a washed bacterial suspension (5 ml) with antiserum (2.5 ml) in the appropriate dilution. The mixtures were incubated in a waterbath at 37 °C for 30 min. and the supernates, together with appropriate controls, were titrated in the hemagglutination test. Hemagglutination inhibition tests were performed by mixing antiserum (0.2 ml) in serial 2-fold dilutions and supernates of bacterial suspensions (0.1 ml), incubating the mixtures at 37 °C for 30 min and adding antigen-modified erythrocytes for demonstration of remaining antibodies.

Results. Hemagglutination tests for detection of heterogenetic antigen in *L. monocytogenes* were carried out with staphylococcal antiserum. For control purposes the preimmunization serum was used. The results of a typical experiment recorded in Table I show that the antiserum in high dilution caused agglutination of erythrocytes modified by the antigens of all types, in contrast to the preimmunization serum. It can be seen also that the antibody titer of the serum against *L. monocytogenes* is of the same order of magnitude as that against the unrelated bacterial species. Additional experiments with 6 different strains of the identical types yielded the same results.

Maximal hemagglutination titers were obtained with dilutions of supernates of 1:2 to 1:5, and minimal hemagglutination occurred with antigen dilutions up to 1:160. Heterogenetic antigen reactive with staphylococcal antiserum was detected also in broth cultures of all 11 strains. It is noteworthy that types 4a and 4b produced more of this antigen in broth than on agar, as revealed by quantitative titrations of the supernates.

Additional experiments showed that hemolysis results when sheep red blood cells are modified by heated *L. monocytogenes* antigen and mixed with either *Staphylococcus* or *L. monocytogenes* (rabbit) antiserum and guinea pig complement. No striking difference in sensitivity was found between hemagglutination and hemolysis tests.

Further evidence of the identification of the antigen was obtained by means of absorption tests. As shown in Table I, *S. aureus, B. subtilis*, and *L. monocytogenes*, all remove at least 96% of the antibodies from the staphylococcal antiserum. Similar results were obtained in hemagglutination-inhibition tests.

The specificity of the hemagglutination test is shown also by the results of experiments with a strain of *S. aureus* and 2 mutants derived therefrom (ATCC #13679 to 13681), kindly made available by Dr. J. A. Di Paolo of the Roswell Park Memorial Institute, Buffalo. Supernates of the parent strain contained large amounts of antigen, demonstrable by hemagglutination with staphylococcal antiserum, in contrast to those of the mutants.

Further, only the supernate of the parent strain inhibited agglutination by *S. aureus* and *L. monocytogenes* antisera of erythrocytes modified by either *B. subtilis* or *L. monocytogenes* antigens. These observations may conceivably aid investigators in studies of the enzyme systems responsible for the synthesis of this heterogenetic antigen.

If, as indicated by these experiments, *L. monocytogenes* contains heterogenetic antigen, then, antibodies against this antigen should be present in *L. monocytogenes* antisera. Titration of these antisera revealed that all agglutinated erythrocytes modified by either *L. monocytogenes, S. aureus,* or *B. subtilis* antigens. Of Dr. Gray's antisera those against types 1 and 2 had a higher titer (1:1600 to 1:3200) than those against types 3, 4a, and 4b (1:400 to 1:800), when titrated in *Staphylococcus* or *B. subtilis* hemagglutination tests. Dr. Welshimer's type 1 antiserum had a titer of 1:200. Sera from 7 normal rabbits did not contain these antibodies in titers of 1:100 or higher. The antisera against *L. monocytogenes* types 1, 2, and 3 prepared in this laboratory by immunization with viable bacterial cells contained antibodies in titers of 1:12,800 to 1:25,600. Antisera prepared by a single injection of viable bacteria of types 4a and 4b contained antibodies in titers of 1:400 to 1:800. In contrast, the preimmunization sera had titers of less than 1:50. Additional studies revealed that the antibodies of all these antisera were removed by absorption with *B. subtilis*. Differences in antigen preparations used for immunization (viable, formalinized, autoclaved, etc.) and/or immunization schedule may account for differences in titers of antibodies against this heterogenetic antigen. From the data presented here it is concluded that *L. monocytogenes,* types 1, 2, 3, 4a, and 4b, contain heterogenetic antigen in common with *S. aureus* and *B. subtilis*.

Discussion. Recent investigations indicate that listeriosis of man is more common than was hitherto recognized. For serologic identification of the pathogen and for studies of antibody response of patients as a tool for immunologic diagnosis of this infection knowledge of the antigenic composition of the microorganism is indispensable. In the present studies it has been shown that *L. monocytogenes* contains a heterogenetic antigen in common with *S. aureus* and *B. subtilis*. The antigen is present in supernates of agar and broth grown strains of all types and can be detected by means of a hemagglutination test. The antigen readily becomes attached to human erythrocytes and is heat stable (15 min at 100°C). It appears to be related to, or identical with, the antigen described by Rantz and associates(1,2) in many other species of Gram-positive bacteria.

Serologic crossreactions between *L. monocytogenes* types 1, 2, and 3 and *S. aureus* were previously recognized by Seeliger and Sulzbacher(6). Welshimer(7) observed that rabbits which had been immunized with *S. epidermidis* and, after a rest period of some 100 days, challenged with *L. monocytogenes* responded with production of staphylococcal antibodies in high titer. Conversely, *S. epidermidis* elicited a booster effect in animals previously injected with *L. monocytogenes*. It may be added that the strain of *S. epidermidis* used by Dr. Welshimer was shown in this laboratory to produce substantial amounts of this antigen. The findings described here indicate that the *L. monocytogenes* antigen is not species specific but of the Rantz type. It can be anticipated that a similar or identical antigenic relationship can be demonstrated between *L. monocytogenes* and many other bacterial species, for this heterogenetic antigen is produced by hemolytic streptococci of groups A, B, and C; enterococci; viridans streptococci; pneumococci; *B. megaterium, B. cereus,* and others. Seeliger(10) previously found a common antigen in enterococci and *L. monocytogenes* As yet the chemical composition of the antigen of the Rantz type is unknown.

Cognizance of the existence of heterogenetic antigens in various bacterial species is indispensable for critical utilization of all serologic methods, including the fluorescent antibody technic. In this connection it should be emphasized that human sera frequently contain antibodies against the heterogenetic antigen of the Rantz type(11). This antibody readily passes the placental

barrier and thus is present in newborn infants. The titer of the antibodies decreases during the first 3 months of life and later increases, so that older children almost invariably have this antibody. The results shown here suggest that for demonstration of species specific *Listeria* antibodies in either animal or human serum absorption with *S. aureus* or *B. subtilis* be considered to eliminate heterogenetic antibodies of the Rantz type. The absorption experiments recorded here failed to make possible the detection of species specific antibodies in *L. monocytogenes* immune sera by means of the hemagglutination test used in this investigation.

Summary. *L. monocytogenes* (types 1, 2, 3, 4a, and 4b) produces a heterogenetic antigen demonstrable by hemagglutination test with suitable antisera. Sheep erythrocytes modified by this antigen are lysed in presence of (rabbit) antiserum and guinea pig complement. Antibodies against this antigen are present in certain *L. monocytogenes* and *S. aureus* (rabbit) antisera and can be removed by absorption with either *L. monocytogenes* (of all types), *S. aureus,* or *B. subtilis.* The findings may help to avoid a possible pitfall in interpretation of serologic tests for species specific *L. monocytogenes* antibodies.

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Vibrio-like Bacteria Recovered from Cultures of "*Spirochaeta myelophthora.*"* (26035)

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In studies in this laboratory during the early months of 1958 which were unavoidably interrupted, 4 of 5 cultures obtained from Miss Rose Ichelson of St. Luke's and Children's Medical Center, Philadelphia, termed by her, following Steiner, "*Spirochaeta myelophthora,*" yielded vibrio-like bacteria. Ichelson(1,2) had implicated these microorganisms in multiple sclerosis. After Martin, *et al.*(3), published observations similar to those obtained in this laboratory, it seemed desirable to put these fragmentary findings on record. Included are cultures of 6 spinal fluid samples.

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Cultures. Two cultures originally from Ichelson were obtained from Dr. Albert Cohn of Montefiore Hospital, New York, labelled, and recoded as in brackets []: "0702 Subc 12-10-57 (1-2-58)"—[NYA]; "0793 Subc 11-21-57 from 11-9-57 Subc 1-2-58"—[NYB]. Three other cultures obtained directly from Ichelson's laboratory were "0778 Zeissman—11-7-57—14th subculture from 472"—[PC]; "0781 CH. Sol—11-7-57—6th Subc. 465"—[PD]; and "0791 A. M. 11-19-57—11th Subc. from 491"—[PE]. Ichelson's photomicrographs(1) labelled "case A. M." may correspond with PE; and the strain listed by Martin, *et al.*(3) as I472 may be the same as PC.