and 77 days after infection with extensive deposition of amyloid in the glomeruli and the adrenal cortex. The hypoalbuminemia, which coincided with the edema and a proteinuria, is believed to have been caused by the impairment of glomerular function.

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Occurrence of Two Immunological Groups Within the Genus Listeria. Studies Based upon Precipitation Reactions.

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Thus far, attempts to establish the serological reactions and antigenic structure of microorganisms of the genus Listeria have been based upon agglutination and agglutinin absorption studies. Paterson[1] concluded that the antigenic structure permitted division of the genus into 4 types. He stated, "the bacteriological types do not bear any relation to the zoological species of the host or to the geographical distribution of the places of isolation." Schultz, Terry, Brice and Gebhardt[2] on the other hand found that among 11 different strains of *Listeria monocytogenes* only 2 serological groups occurred. The strains were derived from various animal sources, including man. On the basis of the agglutination reaction Julianelle and Pons[3] divided 8 strains into 2 types which they designated Type I and Type II. Type I represented strains isolated from rodent animals and Type II from ruminants. Strains isolated from the disease in man fell into one or the other of the types. Julianelle[4] later observed that filtrates from broth cultures gave a precipitate only with their type specific immune sera. The precipitate was suggestive of that seen in the precipitation of specific polysaccharides in the presence of immune serum. The purpose of this paper is to describe the precipitation reactions of 16 strains of *Listeria monocytogenes*.

Material and Methods. The precipitinogen was a polysaccharide extracted from the bacterial cell according to the method of Fuller.[5] Chemically, the carbohydrate fractions were characterized by strongly positive Molisch reactions, and Fehling's solution was not reduced. The biuret and ninhydrin reactions were negative. For the preparation of polysaccharide and the immunization of rabbits bacteria in the smooth phase were selected. All cultures were grown in tryptic digest broth containing 0.1% dextrose and incubated 18 hours at room temperature (25-30° C).

Vaccines were prepared from broth cultures, which were centrifuged and the bacterial sediment resuspended in normal salt solution containing 0.2% formalin. The vaccine was injected intravenously into adult rabbits until the precipitin titer of the serum was sufficient to give a marked, compact-disc precipitate in the presence of the specific carbohydrate within a period of 15 minutes at room temperature. The animals were then bled to death from the heart. Two rabbits were immunized against each strain and the sera from the final bleedings pooled. Control sera were taken from all rabbits before the administration of vaccine and tested for the presence of precipitins to the carbohydrate of that strain of organism to be used for the production of immune serum. In no instance were normal precipitins demonstrated.

Precipitation as determined by the ring test using equal amounts of carbohydrate and immune serum was indicative; however, it

[1] Paterson, J. S., *J. Path. and Bact.*, 1940, **51**, 424.

[2] Schultz, E. W., Terry, M. C., Brice, A. T., Jr., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 605.

[3] Julianelle, L. A., and Pons, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 364.

[4] Julianelle, L. A., *J. Bact.*, 1941, **42**, 367.

[5] Fuller, A. T., *Brit. J. Exp. Path.*, 1938, **19**, 130.

TABLE I.

Precipitation of Carbohydrate Derived from *Listeria monocytogenes* in Antisera of Different Strains.

Antisera against strains of *L. monocytogenes*	Source of strain	Carbohydrates from strain: Strain X 0.4	Strain X 0.1 cc	Strain X 0.02	Murray No. 204 0.4	Murray No. 204 0.1 cc	Murray No. 204 0.02	R-1 0.4	R-1 0.1 cc	R-1 0.02	Gibson 0.4	Gibson 0.1 cc	Gibson 0.02
Strain X	Unknown	+	+++	+++	—	++++	++++	—	+++	++++	—	+++	++++
R-1	Rabbit	++	+++	++++	+	++++	++++	++	++++	++++	+	++++	++++
Gibson	Man	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Nyfeldt	,,	+	++++	+++	++	++++	+++	+	++++	+++	++	++++	+++
Julianelle	,,	++	++++	++++	++	++++	++++	++	++++	+++	+	++++	+++
Schultz	,,	—	++++	+++	+	+	++++	—	++	+++	—	++	+++
Murray No. 204	Rabbit	+++	++++	++	++	++++	+++	++	++++	+++	+	+++	++++
Bovine 7647	Cow	—	—	—	—	—	—	—	—	—	—	—	—
S-1	Sheep	+	—	—	++	—	—	+	—	—	+++	++	+
H-14	Man	—	—	—	++	—	—	—	+	—	++	+	—
B-8	Cow	—	—	—	—	—	—	—	—	—	—	—	—
D-82-N	Cattle	—	—	—	—	—	—	—	—	—	++	—	—
G-1	Goat	—	—	—	—	—	—	—	—	—	—	—	—
Burn	Man	—	—	—	—	—	—	—	—	—	—	—	—
Seastone	Fowl	—	—	—	—	—	—	—	—	—	—	—	—

Antisera against strains of *L. monocytogenes*	Source of strain	Carbohydrates from strain: Nyfeldt 0.4	Nyfeldt 0.1 cc	Nyfeldt 0.02	Julianelle 0.4	Julianelle 0.1 cc	Julianelle 0.02	Schultz 0.4	Schultz 0.1 cc	Schultz 0.02	Bovine 7647 0.4	Bovine 7647 0.1 cc	Bovine 7647 0.02
Strain X	Unknown	—	—	—	—	++	—	—	++	+++	—	—	—
R-1	Rabbit	—	—	—	+	++++	+++	+	++++	++++	—	—	—
Gibson	Man	—	+	+	++	++++	+++	++	++++	+++	—	—	—
Nyfeldt	,,	++++	++++	++++	+	++++	+++	+	++++	++++	—	—	—
Julianelle	,,	—	—	—	++	++++	++++	+	++++	++++	—	—	—
Schultz	,,	—	—	—	++++	+	—	+++	++	—	—	—	—
Murray No. 204	Rabbit	—	—	—	++++	++	—	+++	++++	+	—	—	—
Bovine 7647	Cow	—	—	—	—	—	—	—	—	—	++	++++	++++
S-1	Sheep	+	—	—	++	+	—	+	—	—	++++	++++	++++
H-14	Man	++	+	—	+	++	—	+	++	—	+++	+++	+++
B-8	Cow	—	—	—	—	—	—	—	—	—	—	++++	++++
D-82-N	Cattle	—	—	—	+	—	—	+	—	—	+	+++	+++
G-1	Goat	—	—	—	—	—	—	—	—	—	+	+++	++++
Burn	Man	—	—	—	—	—	—	—	—	—	+++	++++	++++
Seastone	Fowl	—	—	—	—	—	—	—	—	—	—	—	+

TABLE I (Cont.)

Antisera against strains of *L. monocytogenes*	Source of strain	Carbohydrates from strain: B-8 0.4 cc	B-8 0.1 cc	B-8 0.02 cc	S-1 0.4 cc	S-1 0.1 cc	S-1 0.02 cc	G-1 0.4 cc	G-1 0.1 cc	G-1 0.02 cc	H-14 0.4 cc	H-14 0.1 cc	H-14 0.02 cc
Strain X	Unknown	—	—	—	—	—	—	—	—	—	—	—	—
R-1	Rabbit	—	—	—	—	—	—	—	—	—	—	—	—
Gibson	Man	—	—	—	—	—	—	—	—	—	—	—	—
Nyfeldt	,,	—	—	—	—	—	—	—	—	—	—	—	—
Julianelle	,,	—	—	—	—	—	—	—	—	—	—	—	—
Schultz	,,	—	—	—	—	—	—	—	—	—	—	—	—
Murray No. 204	Rabbit	—	—	—	—	—	—	—	—	—	—	—	—
Bovine 7647	Cow	—	—	—	++	++++	++++	+++	++++	++++	++	++++	++++
S-1	Sheep	++++	++	—	++++	++++	++++	++++	+++	++	+++	+++	++
H-14	Man	++	+	—	++	++++	+++	++++	++++	++	++	++++	+++
B-8	Cow	—	+++	—	—	+++	++++	—	++++	++	—	++++	+++
D-82-N	Cattle	++	—	—	+	+++	+++	+++	+++	++	—	+++	+++
G-1	Goat	—	+	++	+	+++	++++	+	+++	++++	+	+++	++++
Burn	Man	—	—	—	++	++++	++++	++++	++++	++++	++	++++	++++
Seastone	Fowl	++	+++	+	—	—	++	—	++	+	—	+	++

Antisera against strains of *L. monocytogenes*	Source of strain	Carbohydrates from strain: Burn 0.4 cc	Burn 0.1 cc	Burn 0.02 cc	D-82-N 0.4 cc	D-82-N 0.1 cc	D-82-N 0.02 cc	Virginia 0.4 cc	Virginia 0.1 cc	Virginia 0.02 cc	Seastone 0.4 cc	Seastone 0.1 cc	Seastone 0.02 cc
Strain X	Unknown	—	—	—	—	—	—	—	—	—	—	—	—
R-1	Rabbit	—	—	—	—	—	—	—	—	—	—	—	—
Gibson	Man	—	—	—	—	—	—	—	—	—	—	—	—
Nyfeldt	,,	—	—	—	—	—	—	—	—	—	—	—	—
Julianelle	,,	—	—	—	—	—	—	—	—	—	—	—	—
Schultz	,,	—	—	—	—	—	—	—	—	—	—	—	—
Murray No. 204	Rabbit	—	—	—	—	—	—	—	—	—	—	—	—
Bovine 7647	Cow	+	+	+	++	++++	++++	—	++++	+	++	++	+
S-1	Sheep	++	+++	+++	+++	+++	+++	++	++++	++++	+	+++	++++
H-14	Man	++	++++	+++	+	++++	++++	+	++++	++++	+	++	++
B-8	Cow	—	+++	++	—	++++	++++	+	+++	++++	—	++++	++++
D-82-N	Cattle	+	+++	+++	+	++	+++	+	++++	+++	+	+	+
G-1	Goat	—	—	—	+	+++	++++	+	+++	++++	—	+	++
Burn	Man	+++	++++	++++	+++	++++	++++	+	++++	++++	—	—	—
Seastone	Fowl	—	—	—	—	+	+	—	—	—	—	+	++++

— = No visible precipitate; + to ++++ = Various degrees of visible precipitation.

seemed advisable in this case to use tubes containing 3 dilutions of the specific soluble substance. Subsequently, precipitation tests were carried out in 10mm x 100mm tubes using 0.4 cc, 0.1 cc, and 0.02 cc amounts of specific soluble substance for each strain of *L. monocytogenes*. Sufficient normal salt solution was added to the tubes containing 0.1 cc and 0.02 cc to bring the final volume in the tube to 0.4 cc. The carbohydrate dilution in each tube was then layered with 0.2 cc of immune serum and the tubes allowed to stand at room temperature for 30 minutes. At the end of this time the presence or absence of ring formation was noted. The tubes were shaken gently and then incubated for 2 hours in a water bath at 37° C. Final readings were taken after storage in the refrigerator overnight at 10° C. and recorded as +, ++, +++ and ++++. In the case of the +++ and ++++ reactions the ring tests were definite at the end of 30 minutes, and usually the precipitate was visible within 10 minutes after adding the serum.

Results. Table I gives the results of the precipitation reactions among 16 strains of *Listeria monocytogenes* studied. On the basis of the precipitation of the carbohydrates in antibacterial sera the strains fell into 2 rather distinct immunological groups, a rodent group and a ruminant group. The existence of 2 immunological groups has been suggested previously by the agglutination reactions. Of the strains used in this study the "Virginia" is possibly the most recent, having been isolated in 1944 from the brain at necropsy of a case of encephalitis occurring in a cow. It was readily identified with the ruminant group by means of the precipitin test. From the table it may be seen that the antibacterial sera of strains S-1 and H-14 show some cross precipitation with the carbohydrates of the rodent group, but the reactions do not appear marked and are not regarded as significant. Antibacterial serum against the Nyfeldt strain precipitated the carbohydrates of the rodent group; however, the Nyfeldt carbohydrate, although reacting with its homologous immune serum, failed to precipitate with the antibacterial sera prepared against the rodent strains. This phenomenon remained unexplained. Polysaccharides prepared from 8 unrelated species of bacteria failed, in all but two instances, to precipitate with antibacterial sera of *Listeria monocytogenes*. Nyfeldt and Gibson antisera precipitated the carbohydrate of hemolytic *Staphylococcus aureus*. The carbohydrates of *Listeria monocytogenes* did not precipitate in antistreptococcal, antityphoid, and antidysentery (Flexner) sera. It is of interest that among the strains from human infections a few are identified as belonging to the rodent group while others fall into the ruminant group.

Discussion. The origin of human infections has remained an enigma; however, it seems entirely possible that they may be derived from some animal source. Carey[6] considered this possibility in dealing with a case of acute cerebrospinal meningitis caused by *L. monocytogenes*. As far as could be determined, in this particular case, the child had had no known contact with rabbits or other animals. It is interesting to speculate that further classification of human infection might follow if the specific soluble substance were to be demonstrated in the cerebrospinal fluid from cases of *Listeria meningitis* and encephalitis. Direct precipitation of the soluble substance from the spinal fluid in the presence of specific immune serum might readily occur. It is suggested that such a procedure if established would possibly prove to be of diagnostic value.

Summary. Polysaccharides have been isolated from *Listeria monocytogenes* which precipitate in the presence of their specific immune sera. The immunological studies presented indicate the existence of 2 distinct animal groups, namely a rodent group and a ruminant group, thus corroborating the classification suggested by Schultz[2] and Julianelle.[3]. The precipitin test affords a simplified technic for the identification and immunological differentiation of the members of the genus Listeria.

[6] Carey, B. W., Jr., *J. Pediat.*, 1936, **8**, 626.

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