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.

1. Rantz, L. A., Zuckerman, A., Randall, E., *J. Lab. Clin. Med.,* 1952, v39, 443.
2. Rantz, L. A., Randall, E., Zuckerman, A., *J. Inf. Dis.,* 1956, v98, 211.
3. Neter, E., Gorzynski, E. A., *Z. Immunitätsforsch.,* 1959, v118, 269.
4. Gorzynski, E. A., Neter, E., Cohen, E., *J. Bact.,* 1960, v80, 207.
5. Anzai, H., Neter, E., Gorzynski, E. A., *ibid.,* 1960, v80, 142.
6. Seeliger, H. P. R., Sulzbacher, F., *Can. J. Microbiol.,* 1956, v2, 220.
7. Welshimer, H. J., *J. Bact.,* 1960, v79, 456.
8. Neter, E., Gorzynski, E. A., Drislane, A. M., Harris, A. H., Rajnovich, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 484.
9. Jensen, K., *Acta Allergol.,* 1959, v13, 89.
10. Seeliger, H., *Z. Hyg. Infektionskrankh.,* 1955, v141, 15.
11. Neter, E., Rajnovich, E., Gorzynski, E. A., *Pediatrics,* 1960, v25, 21.

Received July 8, 1960. P.S.E.B.M., 1960, v105.

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

THEODOR ROSEBURY
Dept. of Bacteriology, School of Dentistry, Washington University, St. Louis

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.

* Aided by a grant from Nat. Soc. for Multiple Sclerosis.

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.

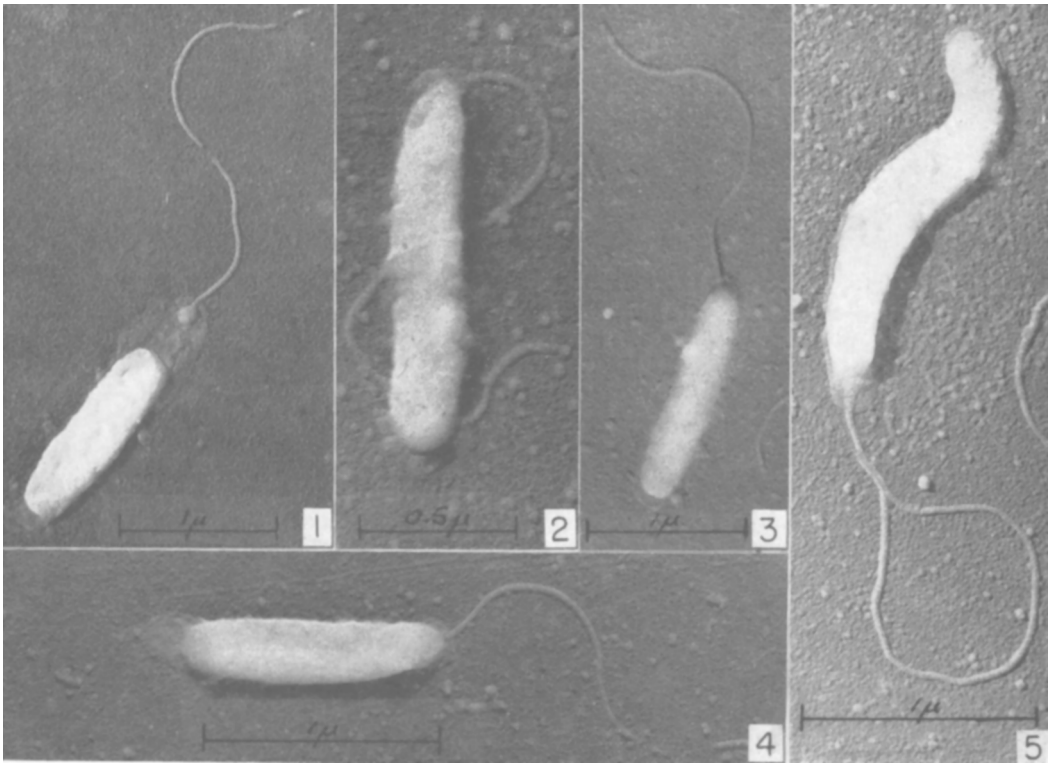

FIG. 1-5. Four-day cultures on sheep blood-HI streaked plates washed off with distilled water. Chromium shadowed. Electron micrographs courtesy of Dr. Bryce L. Munger and Dept. of Anatomy, School of Med., Washington Univ. Fig. 1 and 4, strain NYB; Fig. 2, strain PE; Fig. 3, strain PC; Fig. 5, strain PD.

Methods. The principal media used were Bacto heart infusion (HI) agar or broth or BBL trypticase-soy (TS) agar or broth, usually enriched with 5% of defibrinated sheep or human blood. Fermentation tests were performed with "Redi-discs" (Pennsylvania Biological Labs.) in TS broth containing 1% human serum and phenol red indicator, or with "Redi-discs" on streaked TS agar plates. TS broth or agar was used for other biochemical tests. Antibiotic sensitivity tests utilized discs on TS-sheep blood agar. Anaerobic conditions were obtained in Brewer jars using H_2 with Pt catalysis.

Results. Morphological study revealed forms similar to those described by Ichelson in all 5 cultures. Strain NYA was non-motile and failed to grow in all trials. Strain PE was found motile twice but grew irregularly and was not included in biochemical or antibiotic sensitivity tests. Electron micrographs were, however, obtained of this and of the other 3 strains (Fig. 1-5).

Poor growth or none appeared in cultures in media of the Ichelson type(1,2) including tubes supplied by Miss Ichelson and several variants prepared in this laboratory. In a routine check for purity, aerobic growth was obtained on HI-sheep blood streaked plates. The growth was compatible with the original descriptions, although the bacteria were clearly not spirochetes. These observations were repeated in several subsequent transfers from the original cultures. Strains NYB, PC and PD grew abundantly, usually on HI or TS agar plates with sheep blood. Human blood could be substituted, and satisfactory growth was also obtained with 1% human serum in TS broth, and in TS broth or on TS agar streaks without enrichment. Growth was more abundant or more consistent (a) at 30°C than at 37°; (b) aerobically than anaerobically (with 10% added CO_2); and

(c) aerobically without CO_2 than with 10% CO_2. Strain PE grew only on streaked plates of HI with sheep blood incubated at 30 aerobically with or without CO_2.

Colonies after 72 or 96 hours at 30° were less than 1 mm in diameter, grayish, translucent or transparent droplet-like, viscid. The organisms were Gram-negative; carbol fuchsin was more satisfactory as counterstain than safranin. Under phase-contrast or darkfield illumination, 4-day cultures showed thin, straight, crescentic or S-shaped forms with scattered attached or unattached protoplast-like bodies. Spirillar forms with 2 to 5 turns were present but not numerous; they appeared more frequently in strain PD than in the others. Electron micrographs of all 4 strains disclosed single polar flagella (Fig. 1-5).

Fermentation tests in broth with strains NYB, PC and PD showed neither acid nor gas production, in the presence of abundant growth, in glucose, sucrose, lactose, mannitol, maltose, galactose, inulin, xylose, trehalose, rhamnose, dulcitol, arabinose, fructose, salicin, inositol, melibiose, raffinose or starch. Similar results were obtained on streaked plates except that glucose was acidified by strains PC and PD at 48 hours (30°) but realkalinized at 96 hours. Strain NYB remained negative with glucose and all 3 strains showed no activity with the other substrates. The 3 strains failed to produce indol or H_2S or to liquefy gelatin; all 3 reduced NO_3 and gave weakly positive catalase and strongly positive oxidase reactions.

Antibiotic sensitivity tests (with Bacto discs except as noted) showed well marked inhibition of growth of all 3 strains by tetracycline, chlortetracycline, oxytetracycline and chloramphenicol (all 5 μg), by erythromycin (2 μg) and by matromycin (BBL, 10 μg). Penicillin (2 units) and dihydrostreptomycin (2 μg) inhibited strains NYB and PC but not PD; polymyxin B (5 μg) was only slightly inhibitory to strains NYB and PC and not at all to strain PD.

Six samples of spinal fluid provided by Dr. Irwin Levy and Dr. Leonard Berg, Dept. of Neurology, Barnes Hospital, were centrifuged at 1500 rpm for 2 hours and the sediment in approximately 1.5 ml of fluid inoculated, in 0.3 ml amounts, into Seitz-filtered Ichelson medium with and without petrolatum seals and into 5% sheep blood-HI and –TS broth; 0.1 ml amounts were streaked on 5% sheep blood-HI and –TS agar plates. All cultures, together with the supernatant spinal fluids, were incubated at 30° in air. Gram stains and fresh preparations examined by darkfield of the sediment at time of inoculation and after incubation for 5, 7, 14 and 46 days, were all consistently sterile. According to Dr. Levy, 5 of these samples were from patients with multiple sclerosis; one was from a normal control.

Discussion. Information available when these studies were made suggested that the organism described is a vibrio, more closely related to *Vibrio fetus* than to other species listed in the 7th edition of Bergey's Manual (4). *V. fetus* is said to differ in having an optimum growth temperature of 37°C, a preference for 10% CO_2 in the growth atmosphere, and in producing "no change or slightly acid" from lactose and sucrose as well as from glucose. Reich, Morse and Wilson (5) have reported that added CO_2 is not essential for the growth of *V. fetus*. King(6), who identified *V. fetus* recovered from infections in man, noted that it is oxidase-positive. The 3 actively growing strains of the present study were sent to Miss King, who found them different from *V. fetus* in their growth characteristics and stated that they were non-reactive in *V. fetus* antisera (personal communication, 1958). Martin, *et al.*(3) have since identified a group of cultures they obtained from Ichelson, one given them by Myerson, *et al.*(7), and 2 isolated from distilled water by Martin and his colleagues, with *Vibrio tyrogenus,* a type culture of which (A. T.C.C. No. 7085) was found to have essentially the same characteristics. *V. tyrogenus* is listed in the 6th(8) but not in the 7th edition of Bergey's Manual. The test data given in common by Martin, *et al.* and in the present report are in agreement.

Summary. Of 5 cultures named *"Spirochaeta myelophthora,"* received directly or indirectly from the laboratory of Miss Rose Ichelson in Philadelphia, 4 could be grown

on standard aerobic media and appeared as monotrichate polar-flagellated vibrio-like bacteria. Three that grew abundantly manifested characteristics that suggest relationship to but not identity with *Vibrio fetus*. Cultures of 6 spinal fluid samples, 5 from multiple sclerosis, remained sterile during 46 days.

1. Ichelson, R. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 37.
2. ———, *Proc. Penn. Acad. Sci.*, 1958, v32, 40.
3. Martin, A., Jr., Youngue, E. L., Kost, P. F., *ibid.*, 1959, v33, 55.
4. Breed, R. S., Murray, E. G. D., Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, 7th ed., 1957, Williams and Wilkins Co., Baltimore.
5. Reich, C. V., Morse, E. V., Wilson, J. B., *Am. J. Vet. Research*, 1956, v17, 140.
6. King, E. O., *J. Inf. Dis.*, 1957, v101, 119.
7. Myerson, R. M., Wolfson, S. W., Sall, T., *Am. J. Med. Sci.*, 1958, v236, 677.
8. Breed, R. S., Murray, E. G. D., Hitchens, A. P., 1948, *Bergey's Manual of Determinative Bacteriology*, 6th ed., Williams and Wilkins Co., Baltimore.

Received July 8, 1960. P.S.E.B.M., 1960, v105.

Response of Cartilage Sodium to Changes in Extracellular Fluid.* (26036)

S. ZANE BURDAY, WARREN HECHT AND GILBERT B. FORBES
Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

In recent years a number of investigators have explored the possibility that bone could serve as a reservoir for sodium. (For references see(1).) Infant skeleton differs from adult in that a sizeable portion (up to 30%) of the skeletal mass is composed of cartilage, and it is estimated that as much as one-third of total skeletal sodium in infants is present in the cartilaginous portion(2). It was of interest, therefore, to determine whether or not sodium content of cartilage would change with changes in serum sodium concentration.

Methods. Rabbits between 6 and 10 weeks of age were used. Anesthesia consisted of Dial with Urethane (Ciba) in dose of 0.7 cc/kg body weight. In the *control* series, opposite pieces of costal cartilage were removed following a 2-hour period of anesthesia. In the *hyponatremic* series, one cartilage sample was removed after 2 hours of anesthesia, and at the same time a sample of venous blood was obtained. Immediately following this procedure, a sodium-free isosmolar solution (Table I) equal in volume to 20% of body weight of the animal was injected intraperitoneally. Four hours later, a second blood sample was obtained, and the opposite piece of costal cartilage removed. In the *hypernatremic* series, initial blood and cartilage samples were obtained as above, followed by intravenous infusion of fluid of high sodium content (Table I) in an amount equal to 5% of the body weight. Infusion time varied from 45 to 180 minutes. Thereupon a second sample of cartilage and of blood was obtained.

Upon removal from the animal, the cartilage samples were immediately stripped of perichondrium and placed in tared stoppered weighing bottles. Cartilage samples averaged 20 mg in weight.

Cartilage water was determined by drying overnight in a vacuum oven at 70°C. The samples were then digested with concentrated nitric acid (0.1-0.3 cc) on a steam bath, and resulting solutions made up to volume for sodium analysis by flame photometry and chloride analysis by Volhard titration. Serum analyses were made by standard methods, whole blood pH by glass electrode.

Results. Control series. In 39 experiments, sodium content of opposite pieces of costal cartilage was determined. The mean difference in sodium content was 0.0082 meq/g wet weight, and standard error 0.0016 meq/

* Supported by grants from U. S. Atomic Energy Commission, contract with Univ. of Rochester, and Nat. Inst. of Arthritis and Metab. Dis., U.S.P.H.S.