Summary. Incidence of vomiting to copper sulfate (8 mg/kg and 80 mg/kg in 1% solution) and tartar emetic (5 mg/kg and 50 mg/kg in 1% solution) increased significantly as shown by chi square tests, from animals weighing 150 g or less to a group weighing 200 g or more. A significant increase in incidence was also noted between animals (200 g or over) tested with low and high doses of these 2 drugs. While incidence increased to higher dose levels in the latter group no significant increase in incidence to the higher dose levels was found in the younger group (150 g or less). The above findings indicate that the period between 150 g and 200 g stages is a critical period in development of emetic response to copper sulfate and tartar emetic. The emetic act in older animals is more vigorous than in younger groups but the sequential chain of events leading to vomiting is much the same in young and old animals.

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Identification of *Listeria monocytogenes* by the Fluorescent Antibody Technic. (25691)

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Reports of human infections due to *Listeria monocytogenes* have increased in the past decade, due either to a greater number of such infections or to improved technics and awareness. *Listeria* is associated with many disease syndromes(1-3). Infections due to this organism are generally not distinct clinical entities. King and Seeliger(4) reported, within an 8 year period, 100 isolations of *Listeria monocytogenes* in the United States, only 14 of which have been reported in the literature as case histories. The organism was originally described(5) as a Gram-positive rod with a tendency to palisade formation typical of diphtheroids. Because of this similarity to diphtheroids, the organism is undoubtedly often discarded as a contaminant. More recently it has been reported that pleomorphism is predominant and that the coccoid to diplococcoid form is a more typical property(6). It is easily decolorized, and on blood agar the colonies may resemble streptococci. It is often difficult to isolate, requiring incubation at 4°C for several months(7). Because of increasing importance of this organism in clinical bacteriology and the inherent difficulties in isolating and identifying it, use of the fluorescent antibody technic(8) was investigated to provide a rapid and specific method for its detection and positive identification.

Materials and methods. Somatic antisera. Strains ATCC 7648, Type I (Schultz); ATCC 7644, Type II (Gibson); KC224 (Type III); Seeliger 5214 (Type IVa)(1); and Seeliger 1071/53 (Type IVb) were used to prepare somatic antigens, essentially according to method of Paterson(9). Pairs of mature albino rabbits were injected IV every 4 days with 0.5; 1; 2; 3; 3; and 3 ml of antigen adjusted to a McFarland 3, and bled 5 days after last injection. *Flagellar antisera.* Strains ATCC 7644; ATCC 4428 (Type I); and 5214 were used to prepare the flagellar antigens according to method of Paterson(10). It was found necessary to use an additional inoculum of 1.5 ml of antigen to obtain a good immune response. *Whole cell antisera.* Strains ATCC 4428, ATCC 7644, and 5214 were inoculated in trypticase soy broth and incubated at 37°C for 18 hours. Kolle flasks of trypticase soy agar were inoculated with 0.5 ml of broth culture and incubated at 37°C. The growth was removed with 0.5% formalinized saline and the cells washed 4 times with normal saline. Pairs of albino rabbits were injected IV with 0.1; 0.2; 0.4; 0.8; and 1.0

ml of antigen on successive days. Beginning 10 days later they were each reinoculated with 1 ml on 5 successive days, then bled 7 days after last injection. Prepared whole sera were conjugated with fluorescein isothiocyanate according to Marshall(11). Bovine serum albumin conjugated with Lissamine rhodamine RB 200 was added as counterstain(12). Controls consisted of pooled preimmunization bleedings and an antiserum to *Erysipelothrix insidiosa*(13) both conjugated as above and with the counterstain added. *Preparation of slides.* *Listeria* organisms were characterized by their biochemical reactions, and motility was determined at 25°C. Stock cultures were maintained on trypticase semisolid medium at 4°C and were transferred every 3 months. All other test organisms were grown and maintained as generally prescribed. Saline suspensions of 18 hour growth at 37°C were placed on clean slides, air-dried and heat-fixed. Smears were overlaid with labeled sera and placed in moist chamber at 37°C for 30 minutes, washed in buffered saline (pH 7.2) for 10 minutes, and mounted in glycerol buffered to pH 7.2. If pH of glycerol is below 7.0, fluorescence tends to be quenched. Duplicate slides were stained with labeled pooled normal rabbit and *E. insidiosa* antiserum. Smears were overlaid with human sera for 30 minutes at 37°C, washed in buffered saline 10 minutes and overlaid with conjugated goat antihuman sera for 20 minutes at 25°C, washed as before, and mounted as described previously. Absorption tests were performed with immune sera by using homologous antigen according to Paterson(9). A Zeiss fluorescent microscope with OSRAM HBO 200 light source was used, with either a BG 12 exciter and GG4-OG4 barrier filters, or UG2 exciter and GG4 barrier filter.

Results. *Listeria*-immune sera were tested against 30 strains of *Listeria* from both human and animal isolates representing the 4 types of Paterson(9) and type IVb of Seeliger(1). Individual cells fluoresced brightly following treatment with their type-specific labeled sera, but did not fluoresce following exposure to labeled normal sera, *E. insidiosa* antisera, or goat antirabbit. Not all strains tested were detected by the somatic antisera individually. However, a polyvalent serum consisting of equal parts of 7648 (Type I) and 5214 (Type IVa) gave specific fluorescence for all strains. Flagellar antiserum 4428 caused all but one strain to fluoresce, while the other 2 flagellar sera did not produce fluorescence up to 5 strains tested. The whole cell antigen of 4428 was not specific for Types IVa and IVb; however, 7644 provided more specificity in that they gave either a flagellar or a somatic response, or a combination of both. Of all individual sera tested, occasionally a "speckled" appearance was noted, possibly indicating that only a small amount of antigen was present in the cell.

All *Listeria* sera were tested against the following strains by direct technic (figures in parenthesis indicate number of strains tested): *Mimae* sp.(16); *Herellea* sp.(16); *Bacterium anitratum*(10); *Erysipelothrix insidiosa*(28); *Klebsiella pneumoniae*(2); *Salmonella*(8); *Shigella*(6); *Neisseria meningitidis*(16); *N. gonorrhoeae*(6); *Neisseria* sp. (9); *Hemophilus influenzae*(3); *Alkalescens dispar,* Gps. 1-4; *Pseudomonas* sp.(6); *Pasteurella*(8); *Staphylococcus*(21); *Streptococcus,* Gps. A-G; *Corynebacterium*(15). In no instance was there a specific reaction. The bacteria took the counterstain, presenting orange-red characteristics, indicating a nonspecific reaction.

Stanley(14), Nyfeldt(15), and others have implicated *Listeria* with infectious mononucleosis. Of 8 sera tested from patients with the clinical disease and a significant heterophile titer, specific fluorescence was obtained from a panel of 16 *Listeria* organisms representing all types. However, to obtain a suitable normal human control serum, we screened 25 normal sera submitted for routine serology and in each instance obtained specific fluorescence, as before. Labeled goat antihuman serum used did not give any specific fluorescence when applied against this panel. Absorbed immune labeled sera did not stain smears of strains of *Listeria* tested. Labeled sera stored at 4°C for 18 months provided a stable reagent.

Comments. The polyvalent somatic serum (7648-5214) provides a suitable medium for detection of all strains tested. The flagellar

antigen 7644 can be used to detect a great majority of strains; however, it is recommended that it be used as an ancillary reagent. Whole cell antigens were prepared with the idea not only that they might exhibit the common type III of Paterson(9), but also that we could investigate the findings of Jungherr(16), Webb and Barber(17), and Julianelle and Pons(18), by growing their cultures at 37°C and not at 25°C for flagellar response. Robbins and Griffin(19) state that in 2 sera the common antigen III reaches a high titer in the later part of immunization, but that individual factors cannot be anticipated. Often antibody III completely reverses itself. Paterson(9) indicated that factor III is frequently a "minor factor." Therefore use of a serum of this type is not recommended.

It was not found necessary to precipitate or absorb the sera used, as generally recommended, because the counterstain eliminated all nonspecific fluorescence. Seeliger and Sulzbacher(20) investigated antigenic relationships between serotypes of *Listeria* and *Staphylococcus aureus* by various serologic procedures, finding cross reactions with serotypes I, II, and III. However, in 21 strains tested, no cross reactions were noted.

All serologic procedures have strict limitations in diagnosis of infectious diseases. The fluorescent antibody technic is not without limitations. When one considers the sensitivity based on pneumococcal antigen detectable, as calculated at approximately 10 μg ml, according to Coons(21), consideration must be given to antibodies present in "so-called" normal serum in amounts detectable by this technic. A "normal" control serum must no longer be considered normal but must also be characterized along with the immune serum. This is also true of antispecies globulins used in the indirect test. As yet there is insufficient information as to minor cross reactions between bacterial, viral, and mycotic agents, heretofore considered unimportant but detectable by this technic. Extreme care must be taken in interpretation of results, using an appropriate confidence-level, adequate controls, and a more complete characterization of sera than is usually necessary.

Agglutination titers can be considered insignificant when applied to this technic. Our investigations indicate that a low titer serum may give a greater degree of specificity than one of higher titer. When using high titer serum, a greater degree of cross reaction is observed. This phenomenon is probably due to minor antigenic factors, enhanced in an attempt to obtain a higher titer. As previously stated(12) bacteria that are not of homologous species take up Lissamine rhodamine nonspecifically. Therefore, when using the counterstain, it is believed that degree of fluorescence cannot be considered of diagnostic significance. Specific fluorescence indicates an antigen-antibody reaction whether it is caused by an antigenic cross reaction or a small amount of the specific antigen, intensity having no particular bearing. Preliminary investigations show that *Listeria* can be detected in clinical material other than pure culture, including formalin fixed tissue, and this may not only prove to be an aid to rapid clinical diagnosis but also provide a means of studying its epidemiology.

Summary. Preparation is described of a polyvalent somatic fluorescein labeled antiserum which was specific for 30 strains of *Listeria* tested but did not react with 180 heterologous strains. Preparation and specificity of flagellar and whole cell antigens are discussed as well as some technical implications of fluorescent antibody technic.

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Propagation of Mouse Polyoma Virus in a Strain of Mouse Mammary Tumor Cells *in vitro*. (25692)

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The mouse parotid tumor virus(1,2) designated as the S.E. polyoma virus(3), has stimulated much interest because of its oncolytic effect when grown in tissue cultures and its ability to produce multiple tumors in mice and hamsters(4-6). Development of hemagglutinating(7), complement - fixing(8), and mouse antibody production(9) procedures and plaque technics(10-12) provided simple tools for quantitation detection and study of this virus. In the present work tissue cultures of an established cell line of mouse mammary tumor have been used to support growth of polyoma virus, and observations on *in vitro* assay of the virus in these cells are reported.

Materials and methods. Virus. The 1956-8C strain of polyoma virus* used here had been cultured through 5 passages in mouse kidney cells. Virus stocks are prepared by inoculating 8 oz. bottles† of adult ICR mouse kidney cultures, grown by methods like those of Youngner(13), with 0.5-1.0 ml of undiluted polyoma virus infected tissue culture fluid. The supernatant was saved at each medium renewal and stored at –20°C. When cytopathic changes were nearly complete, cells were collected with the medium and pooled with earlier supernatants. *Tissue culture medium.* "Lactalplus medium," routinely used consists of lactalbumin hydrolysate(14) in modified Earle's salt solution(15), supplemented with vitamins(16), and other components (Table I). To promote cell growth, this medium was further supplemented with 10% calf serum; for maintenance and for viral infectivity titrations, 1% horse serum and $NaHCO_3$ in final concentration of 2.2 g/l were used. All media components were passed through Selas‡ filters under 5 lb pressure of 5% CO_2 in air. Vitamins and glutamine were prepared at 100 times the desired concentration, frozen at –20°C and incorporated into the medium immediately before use, as were penicillin, 100 μ/ml; streptomycin, 50 μ/ml; and neomycin, 20 μ/ml. *Tissue cultures* were prepared from a strain of cells of mouse mammary tumor (MT) originating spontaneously in a C3H mouse and maintained by serial passage in tissue culture for 7 months.§ Stock

* Kindly supplied by Dr. S. Stewart and Dr. B. Eddy, Nat. Inst. of Health, Bethesda, Md.

† Neutraglas serum bottle #14250, Kimbal Glass Co.

‡ Microporous porcelain, .02 and .03 porosity. Selas Corp. of America, Phila., Pa.

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