STUDIES ON THE LISTERELLA GROUP

I. BIOCHEMICAL AND HEMOLYTIC REACTIONS

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INTRODUCTION

The increasing interest in the *Listeria* group during recent years has led to a growing number of accounts of their isolation, and confusion as to their actual reactions. The present study has been undertaken to determine some characteristic reactions of representative strains of the *Listeria* group under a single set of test conditions.¹

HISTORICAL

Although the organisms now known as *Listeria* were first reported as a new species by Murray, Webb and Schwann (1926), and were given the genus name by Pirie (1927), earlier reports contain descriptions which indicate that the organisms were seen prior to 1926. Atkinson (1917) noted a gram-positive diphtheroid organism in five human cases of what was thought to be epidemic cerebrospinal meningitis. No other organisms could be found in direct smears from the patients or by cultivation, but involution forms of “extraordinary complexity” were noted. Goodpasture (1924) described a spontaneous encephalitis in rabbits due to a gram-positive bacillus, and characterized by a mononuclear exudate in the meninges. This report thus described both the mononucleosis and the predilection for the central nervous system

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which are characteristic of *Listerella*. Unfortunately the causative organisms were never isolated.

Murray, Webb and Schwann (1926) isolated their organism from the visceral organs of laboratory rabbits. Apparently the affinity of the organisms for the central nervous system was not noted. Since infection caused a marked mononucleosis, the organism was named *Bacterium monocytogenes*. Pirie (1927) isolated from gerbilles an organism which he called *Listeria hepatoletic* due to the focal necrotic areas which it caused. Following comparative studies by Murray and Pirie which indicated that the organisms were identical, the present name of *Listerella monocytogenes* was adopted.

Since these early reports listerellosis has been reported in a number of animal species, and in man. Sheep seem to be most commonly infected; outbreaks have been reported by Gill (1931, 1933), Doyle (1932), Jungherr (1937), Morin (1938), Graham, Dunlap, and Brandly (1938), Biester and Schwarte (1939), Olafson (1940) and Hoffman (1941). Infections in cattle have been reported by Jones and Little (1934), Ten Broeck (cited by Seastone 1935) and Graham, Hester and Levine (1940). Biester and Schwarte (1940) isolated *Listerella* from swine. Seastone (1935) and Paterson (1937) have described *Listerella* infection in fowl, and Ten Broeck has found these organisms in goats. An epidemic believed to be listerellosis in silver foxes was reported by Cromwell, Sweebe, and Camp (1939). Nyfeldt (1932) isolated an organism which he believed to be related to that of Murray, Webb and Schwann (1926) from a case of human infectious mononucleosis and this was confirmed by Anton (1934). Reports of human infection were made by Burn (1934) and Schultz, Terry, Brice, and Gebhardt (1934). Tesdal (1934) and Gibson (1935) reported fatal meningitis infections resulting from a *Corynebacterium* and a diphtheroid respectively, but Schultz (1938) believes that the organism found by Tesdal was a *Listerella* and a comparative study of the Gibson strain by Webb and Barber (1937), Gill (1937) and Schultz (1938) showed it also to be a *Listerella*. Carey (1936) isolated similar organisms from a non-fatal meningitis in a 3-year-old child, but claimed that the strains
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were not virulent to any extent for animals. Poston, Upchurch, and Booth (1937) described a similar case from which they too were able to isolate organisms corresponding to the Listerella strains of Burn. Human cases were reported by Pons and Julianelle (1939) from a case of infectious mononucleosis; by Porsecanski and de Baygorria (1939) from a non-fatal infection and finally by Wright and MacGregor (1939) from a fatal case of meningitis and otitis media. A report by Savino (1940) described the isolation of Listerella organisms from a non-fatal meningoencephalitis in which treatment with sulfapyridine gave very satisfactory results.

The characteristics of Listerella as reported in the literature indicate considerable confusion. The organisms are gram-positive, non-spore-forming rods, and are generally described as being non-encapsulated. However, Paterson (1939) reported the presence of encapsulated organisms. A single polar flagellum has been described by Seastone (1935) who also describes a very peculiar type of motility, both characteristics being confirmed by Burn (1935) and Schultz (1938). Gibson (1935) reported that his strain was non-motile and Wright and MacGregor (1939) reported questionable motility. The greatest variation in described characteristics was with regard to the bio-chemical reactions. In thirteen reports describing the fermentation reactions on twenty-one carbohydrates, there were nine materials on which there was absolute agreement as to reaction. On the remaining materials there was wide variation of opinion as to the presence of acid production. No gas formation has been described from any carbohydrate. Indole and hydrogen sulfide production and nitrate reduction were negative (Burn, 1935); the methyl red reaction was negative according to Gibson (1935), but positive according to Savino (1940).

Wright and MacGregor (1939) stated that their cultures following isolation were only weakly hemolytic, but that after frequent subculturing the hemolytic action increased and that they were able to demonstrate the presence of a soluble hemolysin. Webb and Barber (1937) also noted some soluble hemolysin, but found the hemolytic action to be variable. Schultz (1934) found
almost complete hemolysis while Gibson (1935) compared the growth on blood agar to that of *Hemophilus influenzae*.

Serological studies have indicated a possible antigenic difference between the original Murray strain and other strains (Seastone, 1935; Carey, 1936). Some grouping of the organisms by agglutination methods has been attempted (Julianelle and Pons, 1939; Paterson, 1940).

**EXPERIMENTAL**

The studies reported here include fifty strains of organisms from all the known host species, namely eight bovine, six fowl, six goat, sixteen human, four rodent, nine sheep strains, and one fox strain. These cultures were maintained by transferring every two weeks on tryptose agar slants.

The basal medium selected for use in carbohydrate reactions was Dunham’s peptone water with Andrade’s indicator, as described by Burn and used in his studies. This base was selected as it was the most accurately described of any reported in the literature, and because it is relatively simple to prepare and use. The medium without carbohydrate was adjusted to pH 7.4 and buretted into fermentation tubes. These tubes were then sterilized by autoclaving and stored at refrigerator temperature until needed.

The carbohydrates to be tested were prepared in concentrated solutions and sterilized by passage through Berkefeld filters. The filtrates were tested for sterility by incubation at 37°C for twenty-four hours before use. These concentrated solutions were added to the sterile basal medium in amounts giving a final concentration of 1 per cent of the carbohydrate. The starch was prepared in a 1 per cent solution in the basal medium and sterilized by autoclaving. The sterile medium was then tested for reducing sugars with Benedict’s solution, and for starch with iodine. When no reducing sugar was detected the medium was pipetted into sterile fermentation tubes for use. All finished media were incubated at 37°C for twenty-four hours before inoculation with the *Listerella* strains.
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All media were inoculated with 0.1 ml. of eighteen-hour beef infusion broth cultures and were incubated at 37°C for fourteen days. The reactions were recorded daily.

Twenty-three carbohydrate reactions were studied for each of the fifty strains. The results were checked in a second complete experiment and the data compared. Where all fifty strains showed the same reactions in both experiments this was taken as adequate evidence of their reaction in that substrate. In all cases where the reactions differed between the two experiments, a third test was made. After the third test some of the reactions were still indefinite. However, further tests were not deemed worthwhile.

Indole production, nitrate reduction, production of acetyl methyl carbinol and the methyl red reaction were also determined for all strains. The procedures for these determinations were those described in the Manual of Methods for the Pure Culture Study of Bacteria. Since the results obtained were the same for all cultures in two tests, these data were considered adequate.

Hemolytic studies were made on sheep, horse, and cow blood, using both blood agar plates and tube tests. For the plate tests a suspension of organisms was made in isotonic one per cent tryptose broth and a drop of this suspension was placed in a petri dish. Five per cent blood agar was added and thoroughly mixed with the suspension. The plates were incubated for twenty-four hours. The presence of hemolysis was recorded and the plates then reincubated for an additional twenty-four hours when the presence of hemolysis was again noted. For the tube hemolysis tests, eighteen-hour isotonic trytose broth cultures were used. Equal amounts of the broth culture and a five per cent suspension of red blood cells, prepared from packed cells, were placed in agglutination tubes and incubated in a water bath for two hours at 37°C. Control tubes of sterile medium and blood cells, and saline and blood cells were also incubated. Following this incubation hemolysis was recorded as 4+ (complete), 2+ (partial), or − (no hemolysis). The tubes were then refrigerated over night and rechecked for any changes in the development of hemolysis.
RESULTS

After a twenty-four hour incubation all strains showed definite acid production from glucose, levulose, galactose, maltose, mannose, rhamnose, trehalose, dextrin and salicin. The degree of acidity appeared to be slightly less in the galactose than in any of the other positive reactions but was definitely present. These results were identical in both experiments and were accepted as conclusive ability of the organisms to produce acid from these substrates.

Within two to five days acid was produced in lactose, glycerol and sucrose by most strains. A few strains required from eight to ten days to produce acid, while a few failed to produce any change. There were a few cases where the ability to produce acid varied between experiments. This variation was most pronounced in lactose, less in sucrose and slight in glycerol. When acid was produced in glycerol and lactose the degree of acidity appeared to correspond to that of the galactose, while the sucrose gave a strong reaction.

Acid was produced in a few instances in arabinose, xylose, mannitol, sorbitol and starch, but only after an incubation of ten to twelve days. The positive results which were obtained with arabinose and xylose were all obtained in the second experiment. Only one positive reaction was obtained with mannitol, and it could not be confirmed. Six positive starch reactions were found in the course of the experiments, but these were scattered and could not be confirmed, although one strain gave a positive reaction in two out of three tests. This was one of Burn's strains. Whether or not this organism is different from the remaining strains has not yet been determined.

No acid production was shown in dulcitol, inositol, raffinose, glycogen and inulin by any strains on either of the first two tests after fourteen days of incubation. This was accepted as evidence that the organisms could not produce acid from these materials.

The only carbohydrate material which indicated a definite division of strains was melezitose. The acid production from this material showed nineteen strains to be positive, while thirty-
one strains were negative. These reactions were identical in all three of the experiments carried out; the same strains produced acid each time while the remaining strains were consistently negative. The acid-producing strains required at least forty-eight hours of incubation. Positive reactions were never obtained in less than forty-eight hours, and some strains required seven days before giving a positive reaction. The important point appears to be that the final reaction for each strain was the same on three distinct test series. The possibility that this is a significant division of strains is increased by the very close correlation between it and a serological grouping recently reported by Paterson (1940). He suggested two major groups distinguishable by agglutination reactions. On the basis of strains that were used in both studies, our division by melezitose fermentation closely resembles his serological division.

The other determinations which were made showed that indole and acetyl methyl carbinol were not produced by any strains, and that nitrates were not reduced to nitrites. The methyl red test was distinctly positive in all strains.

Hemolytic studies showed that beta hemolysis was a general characteristic of the group. Of the fifty strains studied, all were beta hemolytic on blood plates with the exception of the original strain isolated by Murray. This strain consistently failed to produce any hemolysis either on blood agar plates or in the tube reactions. In the tube tests a few strains gave only partial hemolysis (2+) but the majority gave 4+ reactions at the end of the two-hour incubation period. No changes were noted between the end of the incubation period and the final readings after refrigeration. No differences could be noted in the hemolysis of sheep, horse or cow blood in either plate or tube reactions.

**DISCUSSION**

The results obtained in the above experiments indicate that no differences can be shown to be associated with the host species from which the organism was isolated. In the division by melezitose, there is no correlation between source and acid production. The melezitose-positive strains are divided among the bovine,
fowl, human, fox, and sheep groups, but these also contain negative strains. However, goat and rodent groups contained no strains which formed acid from the melezitose. It would appear therefore that the biochemical reactions cannot be used as an indication of the source of these organisms.

As a possible method of separating *Listerella* strains into two or more species the biochemical reactions appear to have very slight value. Certainly acid production in a single sugar is not sufficient basis for division without some additional distinction, and this distinction is not supplied from other biochemical reactions. Any species divisions would thus seem to have to depend on serological studies. However the fermentation of melezitose separates the strains of *Listerella* into two distinct groups which appear to be correlated with Paterson's serological division. On the basis of the relatively few strains which were used in both studies it appears that his Group I corresponds to our melezitose-negative while his Group III is our melezitose-positive group.

For identification purposes, the fermentation reactions have some value. The present studies indicate that all *Listerella* strains will produce acid from glucose, levulose, galactose, maltose, mannose, rhamnose, trehalose, salicin and dextrin, while none produce acid from dulcitol, inositol, raffinose, glycogen, and inulin. With the use of the above carbohydrates an incubation period of five days appears to be sufficient. The results with other carbohydrates are subject to considerable variation and are not recommended for identification purposes. When these carbohydrates are used, an incubation period of fourteen days is suggested to insure sufficient time for slowly reacting strains.

The other biochemical reactions studied indicate that indole and acetyl-methyl-carbinol are not produced and that nitrates are not reduced to nitrites. We have found the methyl-red test to be positive in all strains, in contrast to the report by Gibson but in accordance with the work of Savino.

The hemolytic action of *Listerella*, coupled with the colony appearance on blood agar, leads to confusion with the beta-hemolytic streptococci. Beta hemolysis on blood plates appears to be a genus characteristic with the single exception noted. The pos-
sible correlation between failure of this strain to hemolyze blood cells and the antigenic difference which has been previously ascribed to it by Seastone (1935) is not known. Some strains appear to be slightly less hemolytic in tube reactions, in that they produce only a partial hemolysis, but this difference is not apparent on blood agar plates.

SUMMARY
1. A review of reported isolations of Listerella is given.
2. A study of a representative group of strains indicates that:
   a. Acid is produced from glucose, levulose, galactose, maltose, mannose, rhamnose, trehalose, dextrin, and salicin.
   b. Variable results may be obtained with lactose, glycerol, sucrose, arabinose, xylose, mannitol, sorbitol, and starch.
   c. Acid is not produced in dulcitol, inositol, raffinose, glyco-
gen and inulin.
3. A possible division of strains on the basis of melezitose fermentation is reported and a correlation between this and Paterson’s agglutinative groups is suggested.
4. Indole and acetyl-methyl-carbinol are not produced, nitrates are not reduced. The methyl red test is positive.
5. Beta hemolysis is stated to be characteristic for the genus with the exception of one strain.

LITERATURE CITED
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