A STUDY OF THE DISSOCIATION OF SOME STRAINS OF *ERYSIPELOTHRIX RHUSIOPATHIAE* OF SWINE

by

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INTRODUCTION

After Arkwright (1921) and De Kruif (1921) published the results of their experiments that rough and smooth colony forms may be obtained from the same pure culture of bacteria, there has been an increasing attention and interest in the studies on bacterial variations. The studies dealing with this subject designate certain more or less ordered modifications in bacterial cultures - changes which in many cases may be predicted and controlled and which are intimately associated with cell morphology, colony form, virulence, biochemical behavior and serologic reactions.

Hadley (1927) published a complete and excellent review dealing with the various phases of the phenomena of microbic dissociation. A complete review of literature is not given in this paper. Forty-six references dealing with the subject were read. From the literature it is concluded that all species of bacteria may be dissociated into variants, and that this dissociation may be made reversible by subjecting the variant to different environmental conditions.

Investigators differ widely in their opinions as to the significance attached to variation of bacteria. Some investigators like De Kruif, Schutze and others believe that bacterial variation is a characteristic which is inherent

within all bacterial cultures and that this characteristic is transferred to subsequent generations. Others regarded variation as due to environmental conditions to which the organism is subjected.

The conception of bacterial variation has upset the monomorphic theory of Cohn and Koch, as reviewed by Jordan and Falk (1928), who regarded bacteria to be characterized with rather rigid fixity to their specific type as pertains both to morphology and physiology, any deviation from the normal were regarded as contaminations.

The appearance of a variant is regarded as a fact but there has not been a satisfactory explanation why a smooth variant becomes rough and vice versa. Eastwood (1932) has attempted to explain the fact on the basis of certain peculiar characteristics of growth. He stated that there are reasons to believe that growth depends on a rythmic cycle of synthesis, peculiar to living protein, whereby at each successive stage one particular "building stone" is selected for synthesis with rejection of all others. The enzymes are considered as centers of "chemico-physical" activity and the emergence of variants may be explained by the liability of these "centers" to change under "chemico-physical" influences.

Studies on the dissociation of Swine erysipelas organisms have not been found recorded in any of the literature.

However, Gaiger and Davies (1932) stated that rough (R) and smooth (S) variants occur on agar media, and Kluder and Harkins (1931) claimed that they isolated a virulent strain of E. rhusiopathiae from an infection among fishermen handling live fish and after passing the organism on culture media for seven weeks, its virulence for mice was decreased. In 1927, Wayson working on the Bacillus of mouse septicemia produced colonies appearing like bone lacunae after the gelatine stab cultures had been held at approximately 20°C. for from four to six weeks.

Because of the wide distribution of E. rhusiopathiae in nature, its economic importance, and because De Kruif in 1921 found that rabbits inoculated with his virulent G. cultures of B. lepisepticum resisted lethal doses of his virulent D. cultures, led to the investigation.

SOURCES OF THE STRAINS USED

Strains 3506, 2310 and pH of E. rhusiopathiae were obtained by Dr. H. F. Lienhardt, Head of the Department of Pathology at Kansas State College from the Pitman-Moore Company, Indianapolis, Indiana. These strains were isolated from cases of Swine erysipelas in South Dakota.

Strains 106, 14159, and 13843 of E. rhusiopathiae were isolated from hogs died of E. rhusiopathiae infection

brought in the Department of Pathology at Kansas State College from various towns in the State of Kansas.

Strains 1146 and 1147 of *Salmonella suipestifer* organism were obtained from Dr. C. A. Brandly of the Department of Bacteriology at Kansas State College.

Strains 7, 8, and 17 of Pasteurella organisms were obtained from Dr. J. P. Scott of the Department of Pathology at Kansas State College. These strains were used in a study of shipping fever of cattle in the State of Kansas.

GENERAL METHODS

Throughout this investigation, liquid media were used because there seems to be a general agreement among investigators who have been working on this phenomenon that in such an environment, changes may be brought more readily than on solid media. This fact was brought out by Hadley (1927) in his review on microbic dissociation. The observations of Arkwright and others also emphasized the greater tendency for variation during growth in liquid media.

Six strains of Swine erysipelas, two strains of *Salmonella suipestifer*, and three strains of Pasteurella organisms were used. The *Salmonella suipestifer* and the Pasteurella organisms were used as a check or control. Previous to their use in this study, they were plated on modified salts agar because the Swine erysipelas and Pasteurella

organisms seemed to grow better on this medium than on nutrient agar. This is a modification of the mineral salts agar medium described by Scott (1930). Its composition is as follows:

Peptone	20.0 grams
Sodium chloride	3.0 grams
Potassium bicarbonate	0.5 grams
Potassium citrate	2.0 grams
Ammonium hydrogen phosphate	0.6 grams
Agar (Difco purified)	20.0 grams
Glucose	0.5 grams
Glycerol	5.0 cc.
Distilled water	1000.0 cc.

The above ingredients were placed in a 2000 cc. flask and steamed in an Arnold sterilizer for one hour. Without adjusting its pH, the mixture was placed in 100 cc. bottles and autoclaved at 15 pounds pressure for one hour. Well isolated typical smooth colonies were selected and inoculated in nutrient broth. The broth cultures were incubated at 37°C. for 24 hours and the above process repeated. This process of replating and reisolating was repeated five different times. In order to doubly insure the purity of each strain, fermentation tubes containing glucose, lactose, maltose, sucrose, dulcite, xylose, arabinose, raffinose, manose, dextrin and inositol were inoculated and incubated at 37°C. The reaction was read at 24, 48, and 72 hour intervals. All test media were inoculated with 0.2 cc. of a 24 hour culture of each strain of organisms used in this in-

vestigation.

At appropriate intervals modified salts agar plates were streaked and examined for types of colonies after 24 and 48 hours incubation at 37°C. This procedure permitted one to follow the changes resulting from cells near the peak of development as well as at later periods. The colonies were examined with the aid of a low power binocular microscope. This was found to be useful especially in the comparison of colonies.

De Kruif (1921), Dulaney (1928), Soule (1928) and others have shown that guinea pigs, mice, and rabbits inoculated with the rough forms of the organisms with which they were working, protected those animals against the lethal dose of the smooth forms. Therefore interest was stimulated in determining the variation tendency of Swine erysipelas organisms, and also an effort was made to produce an avirulent form which would give protection against the virulent type.

Six strains of Swine erysipelas organisms were streaked on modified salts agar plates and the types of colonies found were studied, estimated and recorded as a per cent of all colonies present.

DESCRIPTION OF COLONIES

Throughout this investigation, it was observed that the change from smooth to rough is gradual, continuous, and reversible. It is not a direct change and does not occur in several distinct abrupt steps. The terms Smooth (S), Intermediate (I), and Rough (R) are used to designate the chief types of colonies found. This arbitrary separation into categories does not really make clear the true course of events. Colonies appear which are very difficult to place in either the intermediate or rough forms. One investigator may probably place them as intermediate forms while another may designate them as rough types, so that the results on dissociation are comparable only in a general way.

Smooth (S) Form of E. Rhusiopathiae

The smooth colonies of Swine erysipelas organism are small, round and convex homogeneous disks. The margins are regular in outline. The surface is smooth containing few granules which are seen under the low power of the microscope. They grow diffusedly in broth and have a uniform turbidity in 0.85 per cent NaCl.

The individual cells of the smooth colony are Gram positive slender rods and are arranged singly. They do not

have spores or capsules. They do not ferment any of the sugars used in this investigation. They do not produce indol and do not hemolyze blood.

Rough (R) Form of E. Rhusiopathiae

The rough (R) colonies of Swine erysipelas organism are flat, coarsely granular and larger than the smooth (S) colonies. The margins are very irregular and broken in outline. Flocculent growth appears at the bottom and side of the broth tube, the upper portion being clear as if it had not been inoculated. Spontaneous clumping occurs in physiological salt solution and it is impossible to obtain a uniform suspension of the organisms. This is due to the concentration of salt in the solution in the opinion of Arkwright.

The individual cells of the rough (R) form are Gram positive, slender rods, longer and slightly larger than the individual cells of the smooth (S) form. They occur in masses and in long or short chains. They do not have spores or capsules. They do not ferment any of the sugars used, do not produce indol, and show no hemolysis in blood agar.

Intermediate (I) Form of E. Rhusiopathiae

Many colonies of different appearance are included under the intermediate (I) type. The colonies have wedges or sectors. Frequently the sector appears to consist of very irregular lines or to be coarsely granular. They are larger than the smooth (S) type. In many cases the intermediate (I) colonies are hard to distinguish from the rough (R) colonies. They more frequently revert to the smooth (S) form when planted in broth.

The individual cells of the intermediate colony form are Gram positive, slender rods occasionally occurring in chains or singly.

Colonies of the intermediate type frequently occurred as the first notification of a change and developed before the rough forms occurred.

EXPERIMENTAL METHODS USED TO INCITE S TO R DISSOCIATION

The Effect of Aging Smooth Bacterial Culture in Nutrient Broth

The nutrient broth was prepared as follows: 10 grams peptone, 3 grams beef extract, and 5 grams sodium chloride were placed in a two liter flask and 1000 cc. distilled

water was added. The mixture was placed in an Arnold sterilizer and steamed until the ingredients were thoroughly dissolved. The loss of volume due to evaporation was replaced by the addition of distilled water. The reaction of the solution was adjusted to pH 7.2 by the colorimetric method. The broth was placed in 125 cc. Erlenmeyer flasks in 100 cc. amounts and autoclaved at 15 pounds pressure for 45 minutes. The reaction was not readjusted after sterilization.

A number of these fluids were inoculated with six smooth strains of E. rhusiopathiae, two smooth strains of Salmonella suipestifer and three smooth strains of Pasteurella organisms. The broth cultures were incubated at 37°C. during the remainder of the experiment. At a 15 day interval, during this period, a loopful of the material from each strain was streaked on modified salts agar plates.

The results of this experiment are recorded on Table 1. The effect of aging E. rhusiopathiae in broth varied in the degree of dissociation of the different strains of the organism as shown on the table. Strains 2310, 3506, 13843, and pH showed dissociation at the end of thirty days, while strains 106 and 14159 did not show rough colonies until the forty-fifth day. The table also shows that the Swine erysipelas organisms were dissociated in less time than the

Table 1. Percentage of the R form of _E. rhusiopathiae_ produced by the effect of aging in nutrient broth adjusted to pH 7.2.

Period of incubation (days)	15	30	45	60	120
Strain	Percentage of R formed				
106 _E. rhusiopathiae_	100S	10I	15R	60R	100S
2310 _E. rhusiopathiae_	100S	3R	25R	90R	10I
3506 _E. rhusiopathiae_	100S	1R	30R	75R	100S
13843 _E. rhusiopathiae_	100S	5R	40R	100R	5R
14159 _E. rhusiopathiae_	100S	4R	20R	80R	100S
pH _E. rhusiopathiae_	100S	2R	50R	100R	7R
1146 _S. suipestifer_	100S	100S	3R	25R	100S
1147 _S. suipestifer_	100S	100S	10R	50R	100S
7 Pasteurella	100S	3I	1R	30R	100S
8 Pasteurella	100S	4I	7R	50R	100S
17 Pasteurella	100S	100S	5I	20R	100S

Salmonella suipestifer and Pasteurella organisms. The greatest per cent of R forms were obtained on the sixtieth day. After this period it seems as if the R forms were gradually reverted to the S form.

The above experiment confirmed the observations of Arkwright and Pitt (1929) working with the typhoid and paratyphoid organisms, and earlier observations by Arkwright (1921) that R forms can usually be found in old broth cultures originally inoculated with the S form. The time required to produce the change varied from 2 or 3 weeks to 2 to 3 months depending upon the organism and the strain used.

Working with the Bacillus of rabbit septicemia De Kruif (1922) produced dissociation by aging the organism in plain broth. Goyle (1926) obtained rough variants of B. typhosus and B. enterditis Gartner from old broth cultures by plating on agar and selecting colonies. Aging broth culture was one of the factors in inciting dissociation mentioned by Dulaney while working with B. coli communis in 1928, and Li in 1929 produced rough variants of Hog Cholera bacillus from beef infusion broth inoculated for three weeks.

Dissociation is usually most marked when conditions are unfavorable to the growth of the organism.

The Effect of 10 Per Cent Homologous Immune Serum Broth in Inciting Dissociation

The immune serum used in this investigation was obtained from rabbits inoculated with formalized Swine erysipelas organisms standardized to tube 10 of McFarland's nephelometer at intervals of one week. The rabbits were given five inoculations subcutaneously doubling the dose at each successive inoculation. Ten days after the last injection the rabbits were bled from the heart. Agglutination tests were run to determine the titer of the sera. To each tube containing 9 cc. of nutrient broth was added 1 cc. of homologous immune serum to give it a concentration of 10 per cent by volume. The tubes of serum broth were incubated for 24 hours at 37°C. to test for sterility. They were then inoculated with the smooth strains of the Swine erysipelas organisms used in this investigation. The cultures were incubated at 37°C. and at appropriate intervals, they were streaked on modified salts agar plates to determine the types of colonies.

The results of this experiment were in accord with the observations of other investigators. Stryker (1916) obtained a rough strain of pneumococci which was quite stable

by growing the organism in homologous immune serum. Griffith (1927) was the first one to intentionally use antiserum prepared by the immunization with homologous antigens for dissociating the pneumococcus organism into its related R types. Later similar results were obtained by Arkwright and Pitt (1929) working with _E. typhi_, by Dulaney (1926) working with _E. coli_, and by Soule (1928), Li (1929) and others who worked with paratyphoid organisms. Soule (1928) also obtained the same results with _B. subtilis_.

It was found that 10 per cent homologous immune serum broth is an effective medium in inciting S to R dissociation of Swine erysipelas, _Salmonella suipestifer_, and Pasteurella organisms.

The Effect of Nutrient Broth (pH 7.6) in 10 cc. and 100 cc. Amounts on the Dissociation of _E. Rhusiopathiae_

The nutrient broth used in this experiment was prepared similar to that used in previous experiment. The liquid medium was adjusted to pH 7.6, placed in tubes and in flasks in 10 cc. and 100 cc. amounts respectively and autoclaved at 15 pounds pressure for 45 minutes.

A number of the tubes and flasks containing the medium were seeded with six smooth strains of Swine erysipelas, two

smooth strains of Salmonella suipestifer, and three smooth strains of Pasteurella organisms. The cultures were incubated at 37°C. for twenty-four hours, after which they were placed at room temperature. At appropriate intervals the cultures were shaken and a loop from each tube and flask were streaked on modified salts agar plates to determine the various forms of colonies.

As shown on table 2, no I or R types were produced during the first ten days of the experiment. At later intervals I or R forms were observed in the tubes and flasks cultures. A greater percentage of R types were obtained in the flask cultures than in the tube cultures. The greatest number of variants were produced at the end of sixty days at room temperature. Approximately 35 per cent R colonies were obtained from the cultures in 10 cc. amounts while in the larger volume of media, an average of 60 to 70 per cent R colonies were produced. These observations were in accord with the findings of Soule (1928) who has shown the effect of a large volume of liquid media upon the dissociation of B. subtilis, and Hadley (1927) who stated that the degree of dissociation after a given time is greater in the larger volume than in small volume of medium. This is thought to be due to the fact that the period of growth has been lengthened.

Table 2. A comparison of the effect of nutrient broth (pH 7.6) in 10 cc. and 100 cc. amounts to incite dissociation of E. rhusiopathiae.

Period of incubation (days)		10	30	50	70	140
Strain	Amount of broth	Percentage of S, I, and R				
106	10	100S	3I	10R	30R	100S
	100	100S	2R	20R	60R	100S
2310	10	100S	5I	15R	40R	100S
	100	100S	4R	30R	85R	3I
3506	10	100S	10I	7R	50R	100S
	100	100S	1R	35R	80R	100S
13843	10	100S	2R	15R	60R	7I
	100	100S	7R	40R	95R	3R
14159	10	100S	2I	5R	35R	100S
	100	100S	3R	25R	75R	100S
pH	10	100S	2R	25R	60R	100S
	100	100S	5R	50R	90R	2R
1146	10	100S	100S	1R	10R	100S
	100	100S	100S	10R	25R	100S
1147	10	100S	100S	5R	30R	100S
	100	100S	100S	10R	45R	100S
7	10	100S	100S	1I	20R	100S
	100	100S	3I	5R	85R	100S
8	10	100S	100S	3R	25R	100S
	100	100S	4I	10R	60R	100S
17	10	100S	100S	7I	10R	100S
	100	100S	100S	6R	25R	100S

It was observed that the dissociation in the E. rhusiopathiae as well as in the Salmonella and Pasteurella organisms appeared to be S to I to R. After the organisms were in the dissociation media for a long period of time the reversion appeared to be R to I to S. Hadley (1927) believes that there is probably always an intermediate form apparently both the progeny of the S and the progenitor of the R. He suggests that the R type is not a direct but an indirect product of S and its production comes about through the functioning of an intermediate stage of culture development.

The Effect of Two Concentrations of Peptone and Variation of Temperature Upon the Smooth Strains of E. Rhusiopathiae

One per cent and five per cent Bacto peptone solutions were prepared and adjusted to pH 7.6 by the colorimetric method. The media were tubed in 10 cc. amounts and autoclaved for 30 minutes at 15 pounds pressure.

A number of tubes of each concentration of the peptone solution were inoculated with six smooth strains of E. rhusiopathiae, two smooth strains of Salmonella suipestifer, and three smooth strains of Pasteurella organisms. One set of cultures were incubated at 37°C. and another set was in-

cubated at 30°C. At an interval of 15 and 30 days the cultures were thoroughly mixed by shaking and a loopful from each culture was streaked on modified salts agar plates and examined for various types of colonies.

The results of this experiment are tabulated on tables 3 and 4. As shown on the tables, more R variants were produced in cultures incubated at 37°C. than those at 30°C. indicating that higher temperature offers a greater stimulus for dissociation.

A greater degree of dissociation occurred in 5 per cent Bacto-peptone than in 1 per cent peptone. Dissociation in 5 per cent Bacto-peptone was variable depending upon the organism and strain used. In 1928, Soule working with B. subtilis observed that no dissociation occurred at 5°C., while variations occurred at a temperature of 25°C. He found that maximum dissociation was obtained at a temperature of 45°C. Dulaney (1928) stated that temperature and moisture (42 to 45°C.) stimulated dissociation of B. coli. Nungester (1929) while working with B. anthracis found that a marked decrease in dissociation activity occurred at 26°C. and 37°C., while at 42°C. he observed the greatest degree of dissociation activity.

Koser and Styron in 1930, while working with B. dysenteriae stated that the organism did not show any tendency to dissociation in 1 per cent peptone irrespective of pH.

Table 3. A comparison of the number of R forms of E. rhusiopathiae produced by the cultivation of the S type in two concentrations of peptone adjusted to pH 7.6.

Strain	Period of incubation at 37°C		
	15 days	30 days	60 days
	Organisms grown in 1 % peptone		
106	100S	100S	10I
2310	100S	1I	2R
3506	100S	100S	7I
13843	100S	100S	15I
14159	100S	100S	4I
pH	100S	3I	1R
1146	100S	100S	100S
1147	100S	100S	3R
7	100S	100S	8I
8	100S	100S	1R
17	100S	100S	100S
	Organisms grown in 5 % peptone		
106	100S	2I	25R
2310	100S	10I	30R
3506	100S	2R	40R
13843	100S	5R	50R
14159	100S	1R	35R
pH	100S	3R	45R
1146	100S	100S	15R
1147	100S	15I	30R
7	100S	100S	15R
8	100S	100S	25R
17	100S	100S	10R

Table 4. A comparison of the number of R forms of *E. rhusiopathiae* produced by the cultivation of the S type in two concentrations of peptone adjusted to pH 7.6.

Strain	Period of incubation at 30°C.		
	15 days	30 days	60 days
	Organisms grown in 1% peptone		
106	100S	100S	100S
2310	100S	100S	8I
3506	100S	100S	5I
13843	100S	100S	2I
14159	100S	100S	100S
pH	100S	100S	1R
1146	100S	100S	100S
1147	100S	100S	100S
7	100S	100S	10I
8	100S	100S	1R
17	100S	100S	100S
	Organisms grown in 5% peptone		
106	100S	100S	5R
2310	100S	100S	7R
3506	100S	100S	10R
13843	100S	100S	8R
14159	100S	100S	5R
pH	100S	100S	15R
1146	100S	100S	3R
1147	100S	100S	4R
7	100S	100S	3R
8	100S	100S	5R
17	100S	100S	1R

They found that those grown in 5 per cent peptone formed intermediate and rough colonies.

De Kruif (1922) observed that increasing concentrations of peptone accelerated the appearance of the granular form of B. lepisepticum. He stated that a 20 per cent solution of peptone was nearly as effective as 5 and 10 per cent solution.

There was a greater variation in types of colony and greater production of R variants for Swine erysipelas, Salmonella suipestifer, and Pasteurella organisms in 5 per cent peptone than in 1 per cent peptone solution. Dissociation in 5 per cent peptone was variable depending upon the organism and strain used.

The Effect of Beef Infusion Broth Adjusted to Varying Hydrogen ion Concentration on the S Form of E. Rhusiopathiae

The preparation of the beef infusion broth is as follows: Place 1000 cc. meat infusion broth in a 2000 cc. flask and add 1 per cent peptone and 0.5 per cent sodium chloride. Heat the mixture until the ingredients are thoroughly dissolved. The solution was filtered through filter paper adjusted to pH 6.6, pH 7.0, pH 7.6, and pH 6.8, tubed in 10 cc. amounts and autoclaved at 15 pounds pressure for thirty minutes.

A number of the tubes of culture media of varying hydrogen ion concentration were inoculated with six smooth strains of Swine erysipelas, two smooth strains of Salmonella suipestifer and three smooth strains of Pasteurella organisms. The cultures were incubated at 37°C. At appropriate intervals a loop from each culture was streaked on modified salts agar for the determination of various type colony.

The results of this experiment are shown on table 5. The Swine erysipelas and Pasteurella organisms failed to grow in the acid medium while the Salmonella suipestifer grew well. No R variants appeared during the first twenty days while at the end of sixty days, R types were obtained. The culture medium adjusted to pH 7.0, pH 7.6, and pH 7.8 appeared to be very favorable for S to R dissociation of the Swine erysipelas, Salmonella suipestifer, and Pasteurella organisms.

The results of this experiment were similar to the findings of several investigators. Soule (1928) working with B. subtilis observed that an initial reaction of a medium of about neutrality is most favorable for S to R dissociation. In 1928, Dutton stated that an alkaline nutrient broth is an essential factor in furnishing the proper environment for the dissociation of Streptococci. Hadley and Jimenez (1931) dissociated the S form of B. typhosus in beef

Table 5. A comparison of the number of R Forms of E. rhusiopathiae obtained by the cultivation of the S form in beef infusion broth adjusted to varying hydrogen ion concentrations.

Strain	Days at 37°C.	Initial pH of beef infusion broth			
		6.6	7.0	7.6	7.8
106	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	30R	35R	35R
2310	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	50R	45R	42R
3506	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	45R	45R	42R
13843	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	55R	60R	50R
14159	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	40R	35R	45R
pH	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	35R	40R	30R
1146	10	100S	100S	100S	100S
	20	100S	100S	100S	100S
	60	100S	20R	15R	10R
1147	10	100S	100S	100S	100S
	20	100S	100S	100S	100S
	60	100S	25R	30R	35R
7	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	20R	20R	18R
8	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	30R	25R	20R
17	10	0	100S	100S	100S
	20	0	100S	100S	100S
	60	0	15R	25R	20R

infusion broth adjusted to pH 7.8. A neutral or slightly alkaline peptone solution was used by Koser and Styron in 1930 for dissociating B. dysenteriae.

THE INFLUENCE OF BACTERIOPHAGE ON BACTERIAL DISSOCIATION

There has been several theories advanced as to the nature of bacteriophage and the mechanism of the lytic action. d'Herelle (1926) regarded bacteriophage to be an ultra microscopic filtrable virus which acts as a parasite on bacteria and causes their destruction by lysis. It multiplies only in the presence of bacteria. He stated that bacteriophage is the cause of bacterial mutations.

Bordet and Ciuca (1928) believed that bacteriophage is an autolysin arising in the bacteria themselves. This theory is most accepted by majority of investigators. Zinsser (1927) regarded the lytic action as an enzyme probably active upon the lipoidal constituents of the bacterial limiting membranes and structures that hold them together. He believed that this enzyme is liberated by all bacteria. Hadley (1928) stated that bacteriophage is a product of bacterial dissociation. There is probably a relationship which exists between filtrability, dissociation and bacteriophage as pointed out by Hadley and others in 1931 while working with their form of Shiga bacillus.

Several investigators reported that they have succeeded in producing bacterial dissociation with the aid of bacteriophage. In this investigation the presence of bacteriophage could not be demonstrated by any of the tests used by Hadley (1927), Hadley and Jimenez (1931) and Plastridge and Rettger (1932). It was found that dissociation of bacterial cultures may be produced without the influence of bacteriophage.

R TO S REVERSION

At one time several investigators like Schutze (1921), De Kruif (1922), and others believed that the rough (R) forms of bacteria could not be made to revert to the smooth (S) types. Recent works have shown that the R to S reversion may be accomplished at the present time by a variety of methods.

In Arkwright's work in 1921, R forms of the typhoid bacterium and of the paratyphoid B bacterium were made to yield an almost uniform turbidity by daily subculture in broth, thus indicating the loss of the R characteristics and reversion towards the S form. Griffith (1927) caused a reversion of rough pneumococci to the smooth form through passage in homologous immune serum. Orcutt (1929) found that variants of Bacterium suipestifer that produced granu-

lar growth could be made to assume some of the normal characters by rapid successive transplanting in broth.

In 1926, Jordan obtained S colonies from several single isolations from R colonies of Bacterium paratyphosum B by twice daily transplanting them in beef infusion broth. Soule (1928) accomplished the R to S change with the same organisms by serial transfers in R immune serum broth. Webster and Burn (1927) reported a surprisingly rapid change of R to S in a mouse typhoid strain of Bacterium enteriditis; reversion to the S type occurred after the first or second passage in broth. Li, working with hog cholera bacillus in 1929, produced a R to S reversion employing the same method. Dulaney (1928) stated that the early R form of Bacterium coli could be readily changed back to the S type but that when the R form had become stabilized, the R to S change could be brought about only by cultivation in lactose broth or in nutrient broth containing homologous R immune serum. Dawson (1928) emphasized the efficacy of R immune serum and of passage through the mouse on the R to S change. Soule in 1928 produced S colonies from the R form of Bacillus subtilis by growth in large volumes of broth and the employment of homologous R immune serum in broth. The production of S form from the R or normal form of B. anthracis has been reported by Nungester in 1929. In 1929, Reimann caused a

R to S reversion in pneumococci by employing Griffith's method, and through animal passage. Mallmann and Gallo reported that R forms of Brucella organism were changed to S type by passage through guinea pigs.

The method used in this investigation in attempting to produce S forms from R variants, was a succession of daily transplants of the R types in beef infusion broth, semi solid salts agar and serum broth. Six stabilized rough variants of E. rhusiopathiae were selected and used throughout this investigation. The results were recorded as S, I, and R according to the types of colonies observed on modified salts agar plates.

The Effect of Successive Daily Transfers in Beef Infusion Broth Adjusted to pH 7.0 Upon the R Form of E. Rhusiopathiae

Beef infusion broth was prepared as in a previous experiment, tubed in 10 cc. amount, and autoclaved at 15 pounds pressure for thirty minutes. The tubed media were incubated at 37°C. for 24 hours for sterility. A number of the tubes were inoculated with six stabilized rough strains of Swine erysipelas, two stabilized rough strains of Salmonella suipestifer, and three stabilized rough strains of Pasteurella organisms and incubated at 37°C. They were

transferred in tubes of beef infusion broth daily and at each transfer, a loopful from each culture was streaked on modified salts agar plates to determine the various types of colonies.

The results are recorded on table 6. During the first fifteen days, all the cultures were still rough. Examination of colonies on modified salts agar plates at the twentieth transfer revealed a mixture of R and I in all the E. rhusiopathiae cultures. The Salmonella suipestifer cultures were still rough. Strains 7 and 8 of the Pasteurella organisms were rough. Strain 17 at the twentieth transfer revealed some colonies that may be either rough (R) or intermediate (I). At the thirtieth transplant, all the cultures of E. rhusiopathiae revealed a mixture of S, I, and R forms of colonies. There were more smooth (S) colonies than rough (R) at this time. Strain 1146 were still rough. Strain 1147 showed a mixture of R and S at the end of 30 days. Strains 7, 8, and 17 of the Pasteurella organisms showed a mixture of R and I. All the E. rhusiopathiae cultures reverted to the S form at the fortieth transfer as observed on modified salts agar plates. All the strains of the Salmonella and Pasteurella organisms showed a mixture of the different types of colonies with R still prevailing in number.

Table 6. A comparison of the effect of beef infusion broth (pH 7.0), and 10 per cent homologous R immune serum broth upon the R form of E. rhusiopathiae.

Period of incubation (days)		10	20	30	40	50
Strain	Media used					
106	Infusion broth	R	R,I	S,I,R	S	S
	Immune serum	R	R,I	S,I,R	S	S
2310	Infusion broth	R	R,I	S,I,R	S	S
	Immune serum	R	R,I	S,I,R	S	S
3506	Infusion broth	R	R,I	S,I,R	S	S
	Immune serum	R	R,I	S,R	S	S
13843	Infusion broth	R	R,I	S,I,R	S	S
	Immune serum	R	R,I	S,I,R	S,I	S
14159	Infusion broth	R	R,I	S,I,R	S	S
	Immune serum	R	R,I	S,I	S	S
pH	Infusion broth	R	R,I	S,R	S	S
	Immune serum	R	R,I	S,I,R	S	S
1146	Infusion broth	R	R	R	R,I,S	S,I
	Immune serum	R	R	R	R,I,S	S,I,R
1147	Infusion broth	R	R	R,S	R,I,S	R,I,S
	Immune serum	R	R	R,S	R,I,S	R,I,S
7	Infusion broth	R	R	R,I	R,I,S	S,I
	Immune serum	R	R	R,I	R,I,S	S,I
8	Infusion broth	R	R	R,I	R,I,S	S
	Immune serum	R	R	R,I	R,I,S	S
17	Infusion broth	R	R	R,I,S	R,I,S	S
	Immune serum	R	R,I	R,I	R,I,S	S

All strains of the E. rhusiopathiae were still smooth at the fiftieth transfer. Strains 8 and 17 of the Pasteurella organisms were also found to be 100 per cent smooth at this time. Strain 7 still revealed a few intermediate (I) colonies with the S type prevailing in number. Strain 1146 of Salmonella suipestifer still showed some intermediate (I) colonies but there were more of the S types. More R colonies were still found in 1147 at the end of 50 days.

The smooth (S) forms of E. rhusiopathiae which were obtained from the R variants seemed to have all the characteristics of the original S colony.

The Effect of Daily Transplants in Ten Per Cent Homologous R Immune Serum in Broth Upon the R Form of E. Rhusiopathiae

One cubic centimeter of homologous R immune serum was aseptically added to 9 cc. nutrient broth placed in a test tube. After the media were tested for sterility, they were inoculated with stabilized rough strains of E. rhusiopathiae Salmonella suipestifer and Pasteurella organisms and incubated at 37°C. Daily transplants were made for a period of fifty days. Daily observation of the types of colonies on modified salts agar plates which were streaked with the cultures at every transplant was made.

As shown on table 6, the 10 per cent homologous R immune serum in nutrient broth is just as effective a medium as the beef infusion broth in producing a R to S reversion of the Swine erysipelas.

The Effect of Daily Transplants in Semi-Solid
Salts Agar Upon the R Form of
E. Rhusiopathiae

The semi-solid salts agar used in this investigation is a modification of the medium described by Scott in 1930. It was prepared similar to the modified salts agar used throughout this study except that 0.1 per cent Bacto-agar was added to the mixture instead of 2 per cent. After the media have been tubed, sterilized and tested for sterility, they were inoculated with stabilized rough strains of Swine erysipelas, Salmonella suipestifer and Pasteurella organisms. Transfers were made daily for a period of 50 days. Observation of colonies on modified salts agar similar to preceeding experiments were also made.

Semi-solid salts agar was not effective in producing R to S reversion. The R variants prevailed throughout the experiment.

VIRULENCE OF THE S AND R FORMS OF E. RHUSIOPATHIAE

There seems to be a general agreement in the works of many investigators that the rough (R) form of an organism is more virulent than the smooth (S) type. In 1916, Stryker observed that the growth of virulent pneumococci in homologous immune serum produced a decrease in virulence. Orcutt (1929) found his B type of hog cholera bacillus as less virulent than his A type. Hadley in 1927 stated that the R type is non virulent or at least less virulent than the S form. Kelser (1933) observed that the dissociation of an organism from S to R was often accompanied by a loss of virulence.

In 1928, while Soule was working on the dissociation of B. paratyphosus B found that the S forms were virulent for guinea pigs while the R forms were non virulent. Li (1929) working on the dissociation of B. suipestifer, observed that the S form was more virulent for laboratory animals than the R form. Dawson in 1928 while working on pneumococcus stated that the reversion from R to S whether affected in vivo or in vitro was always accompanied by acquisition of maximal virulence. Dulaney (1928) found that the S form of B. coli-communis is more virulent than the R form. He observed that the lethal doses of R culture was 15 times that of S

culture. Wilson observed in 1930 that the S form of B. aertrycke was more virulent than the R type. White (1929) stated that smooth Salmonella and related bacilli and filtrates of broth cultures were more toxic to laboratory rodents than were the rough bacilli and filtrates. Dutton (1928) found that the S form of streptococci was virulent and the R form of the organisms were relatively avirulent. Nungester in 1929 observed that the R form of B. anthracis was more virulent than the S form. However he stated that the R form was the normal type.

This experiment was performed to determine the relative virulence of the S and R variants of E. rhusiopathiae. Twenty-four hour cultures of six smooth (S)and six rough (R) strains of E. rhusiopathiae, two smooth and two rough strains of Salmonella suipestifer, and three smooth and three rough strains of Pasteurella organisms were injected in intramuscularly into pigeons in various doses.

The results of this experiment were tabulated on table 7. It was found as shown on the table that the R forms of Swine erysipelas were slightly more virulent for pigeons than the S form. This observation is somewhat in accord with the observation of Nungester (1929) regarding the relative virulence of the R and S types of B. anthracis. The table further shows that the R forms of Salmonella

Table 7. A comparison of the virulence of the S, and R forms of E. rhusiopathiae by inoculating pigeons intramuscularly with 24 hour cultures of the variants.

No. of pigeons	Culture	Dosage cc.	Results
75	106S	0.1	Died in 4 days
76		0.01	Died in 4 days
77		0.001	Died in 5 days
78	106R	0.1	Died in 3 days
79		0.01	Died in 3 days
80		0.001	Died in 5 days
81	2310S	0.1	Died in 96 hours
82		0.01	Died in 96 hours
83		0.001	Died in 5 days
84	2310R	0.1	Died in 96 hours
85		0.01	Died in 96 hours
86		0.001	Died in 4 days
87	3506S	0.1	Died in 72 hours
88		0.01	Died in 84 hours
89		0.001	Died in 96 hours
90	3506R	0.1	Died in 58 hours
91		0.01	Died in 72 hours
92		0.001	Died in 96 hours
93	13843S	0.1	Died in 65 hours
94		0.01	Died in 65 hours
95		0.001	Died in 72 hours
96	13843R	0.1	Died in 56 hours
97		0.01	Died in 60 hours
98		0.001	Died in 72 hours
99	14159S	0.1	Died in 96 hours
100		0.01	Died in 96 hours
101		0.001	Died in 6 days
102	14159R	0.1	Died in 72 hours
103		0.01	Died in 72 hours
104		0.001	Died in 4 days
105	pHS	0.1	Died in 96 hours
106		0.01	Died in 4 days
107		0.001	Died in 4 days

(Table 7 continued)

108	pHR	0.1	Died in 72 hours
109		0.01	Died in 96 hours
110		0.001	Died in 96 hours
111	1146S	0.3	Died in 24 hours
112	1146R	0.5	Lived
113	1147S	0.3	Died in 36 hours
114	1147R	0.5	Lived
115	7S	0.3	Died in 48 hours
116	7R	0.5	Lived
117	8S	0.3	Died in 36 hours
118	8R	0.5	Lived
119	17S	0.3	Died in 48 hours
120	17R	0.5	Lived

suipestifer and Pasteurella organisms were avirulent while the S forms were virulent for pigeons.

THE EFFECT OF K MEDIUM ON THE FILTERABILITY OF THE S AND R FORMS OF E. RHUSIOPATHIAE AND ITS EFFECT ON THE FILTRATES OF THE VARIANTS

The introduction of Kendall (1932) of a medium which he was presumably able to isolate from influenza organisms having a filter passing stage, also to demonstrate filterable and non filterable forms in bacteria led to his hypothesis that all known bacteria can and do exist in both filterable and non filterable states.

Since Kendall intimated successful results with his work, it was decided to determine the possible effect of K medium on the filterability of the S and R forms of Swine erysipelas and its effect on the filtrates of the variants.

K medium was prepared as follows: Fresh hog intestines were opened, cleaned and ground in a meat chopper. The ground material was placed in a jar containing four volumes of 95 per cent ethyl alcohol and was extracted at 37°C. for two days with occasional stirring. The alcohol was replaced with fresh alcohol. This was repeated twice making three extractions in all. The dry residue was extracted with

benzene. The benzene was removed by filtration and the residue was spread out on a glass plate, where a current of air generated by an electric fan played upon the residue until it dried. The dried material was ground to a fine powder in a mortar and was kept in a well stoppered container.

The culture medium was prepared by adding 2 per cent by weight of the dried ground intestine to modified Tyrode solution having the following composition.

NaCl	0.8 grams
KCl	0.2 grams
CaCl	0.2 grams
$MgCl_2$..................................	0.1 grams
Na_2HPO_4..................................	0.5 grams
$NaHCO_3$	0.2 grams
Glucose	0.8 grams
Distilled water	1000.0 cc.

A small amount of $NaHCO_3$ (0.5 gm. for every 1000 cc.) was added and the final reaction was brought to pH 7.4. The medium was tubed in 10 and 30 cc. amounts and autoclaved for 30 minutes at 15 pounds pressure.

Before inoculation, the sterile K medium was heated in boiling water to drive out the oxygen and then cooled rapidly in ice water.

Two smooth and two rough strains of E. rhusiopathiae, one smooth and one rough strain of Salmonella suipestifer, and one smooth and one rough strain of Pasteurella organism were inoculated into several tubes of K media and incubated

at 37°C. for a period of one month. Subcultures were made at weekly intervals and the cultures of each series were assembled and filtered through Berkefeld N filter candles. The filtrates were inoculated into several tubes of K media. They were incubated at 30°C. and at 37°C. some cultures were incubated aerobically and anaerobically at 37°C. The cultures were examined for signs of growth at 24, 48, and 72 hours, and at the end of one month before being finally discarded. Subcultures were made at frequent intervals during the period of observation. Control tubes of broth and agar slants were inoculated from all filtrates. Uninoculated tubes of K medium were also included in every series of culture tubes incubated to use as controls.

Results: Filtrable forms of Swine erysipelas, <u>Salmonella suipestifer</u>, and Pasteurella organisms were not produced. There were no signs of growth in the tubes of K medium inoculated with filtrates of the organisms.

This experiment confirmed the works of Craig and Johns (1932) and Carpenter and Long (1933), who attempted to duplicate the work of Kendall. No investigator has yet been able to duplicate the work of Kendall. Grinnell (1929) stated that the reported filtration of such organisms as streptococci bacilli of enteric group, diphtheroid bacilli, and tubercle bacilli might be due rather to the passage of fragments of the bacteria in old cultures still capable of

reproduction or to a filtration in a particularly favorable suspension fluid than to the existence of a filtrable stage in the life cycle of the organism.

AGGLUTINATION REACTIONS

The antigens used in the agglutination tests were prepared according to the method used by Schoening, Creech, and Grey (1932). Stabilized S and R forms of strains 13843 and 3506 of Swine erysipelas organisms were used. Five-tenths of a cubic centimeter of a 24 hour nutrient broth cultures of selected variants were seeded to each several 500 cc. Erlenmeyer flasks containing sterile nutrient broth in 400 cc. amounts, adjusted to pH 7.2 and incubated at 37°C. for 48 hours. The broth cultures were centrifuged for one hour at moderate speed, the supernatant fluids discarded and the organisms suspended in 0.42 per cent salt solution containing 0.3 per cent phenol. Forty-two hundredths per cent saline instead of 0.85 per cent was used to prevent spontaneous clumping of the R variants. These were shaken by hand and centrifuged for one hour. The supernatant fluids were again drawn off and the organisms resuspended in 0.42 per cent phenolized saline. This was repeated three times, after which the antigens were stored in the ice box for future use.

The antisera were developed in rabbits by the injection of antigens prepared from four variants of Swine erysipelas, two variants of Salmonella suipestifer and two variants of Pasteurella organisms. The antigens were prepared similar to those used for agglutination tests without using phenol. The suspensions were adjusted to tube 10 of McFarland's nephelometer, and heated at 60°C. for one hour. The heated antigens were tested for sterility by inoculating agar slants and incubated at 37°C. for 48 hours. The initial dose was 1 cc. injected subcutaneously into rabbits at weekly intervals until five inoculations were given. The dose was doubled at each successive inoculation. Twelve days after the last injection, the rabbits were bled from the heart, the blood allowed to clot, centrifuged, and the serum was removed with pipettes and kept in the ice box until ready to be used.

All tests were incubated at 30°C. for 24 hours before they were read macroscopically.

The results are recorded in table 8. The table shows that S. antisera have higher titers than R antisera. 13843S antisera agglutinated 13843S swine erysipelas organisms in a dilution of 1:1280, and 13843R organisms in a titer of 1:320. 13843R Swine erysipelas sera agglutinated 13843R organisms in a dilution of 1:640 and 13843S in a titre of 1:320. 3506S antisera agglutinated 3506S Swine erysipelas

Table 8. Titers of the homologous immune S and R sera.

S-antigen	Anti S-sera	Titer	Anti R-sera	Titer	R-antigen	Anti S-sera	Titer	Anti R-sera	Titer
13843S	13843S	1:1280	13843R	1:320	13843R	13843S	1:320	13843R	1:640
3506S	3506S	1:640	3506R	1:160	3506R	3506S	1:160	3506R	1:320
1146S	1146S	1:1280	1146R	1:160	1146R	1146S	1:160	1146R	1:640
8S	8S	1:640	8R	1:160	8R	8S	1:160	8R	1:160

organisms in a titer of 1:640, and 3506R organisms in a titer of 1:60. 3506R antisera agglutinated 3506R organisms in a dilution of 1:320 and 3506S organisms in a titer of 1:160. 1146 S antisera agglutinated 1146S Salmonella suipestifer organisms in a dilution of 1:1280, and 1146R organisms in a titer of 1:160. 1146R antisera agglutinated 1146R organisms in a dilution of 1:640, and 1146S organisms in a titer of 1:160. 8S antisera agglutinated 8S Pasteurella organisms in a dilution of 1:640 and 8R organisms in a titer of 1:160. 8R antisera agglutinated both 8R and 8S organisms in a dilution of 1:160.

Schutze (1921) stated that although little or no agglutination may take place with a rough strain of paratyphoid B, and its homologus smooth serum, the variant is not in agglutinable, it will respond well to a serum that has been prepared from rough strain. Arkwright (1921) found that B. dysenteriae agglutinated S organisms up to a titer of 1:320 and R organisms to a titer of 1:80. Soule (1928) found that paratyphoid B S sera agglutinated S organisms in a titer of 1:2560 and R organisms in a titer of 1:640. Li (1929) observed that the R form of hog cholera bacillus has a lower titer than the S type. Andrews (1922) stated in his work on the Salmonella organisms that two antigens are present which are not distributed throughout the culture

as a whole but are attributes of separate bacilli. He stated that two well defined types are present in every culture and either may undergo transformation into the other.

PATHOLOGY OF SWINE ERYSIPELAS

The pathology of the disease resulting from *E. rhusiopathiae* infection was observed and studied from swine brought to the Department of Pathology at Kansas State College for diagnosis. All these specimens were more or less of the acute type of the disease. So far no hogs have been encountered which were affected with the chronic form of the disease as described by Caiger and Davies.

The lesions of the disease were not constant. In some cases we found hemorrhages (petechial) on the kidneys. In other cases we found pharyngitis and petechiation of the bladder but no hemorrhages on the kidneys. The most constant lesions found were the reddened patches on the skin, congestion of the lymph glands, congestion and slightly enlarged spleen, muscular degeneration of the heart and slightly thickened heart valves.

Microscopically, the heart valves were thickened, serohemorrhagic with microorganisms resembling Swine erysipelas in the areas of hemorrhages. The spleen was lightly swollen with extensive congestion and few areas of hemorrhages. We found an extensive dermal and subcutaneous congestion. Ex-

tensive areas of subcutaneous hemorrhage were observed and slight hemorrhages occurred in the derma.

The above findings were somewhat in accord with the observations of Harrington (1933) and Gaiger and Davies (1932).

SUMMARY

A study was made of the influence of certain liquid media in inciting dissociation. After the variants of E. rhusiopathiae were obtained, studies were made on their comparative morphology as well as their serological and physiological properties. Studies were also made on the influence of certain liquid and semi-solid media in inducing rough to smooth reversion. The filtrability of the rough and smooth strains of E. rhusiopathiae was also observed as well as the influence of K medium on the filtrate. The pathology of the disease in swine resulting from E. rhusiopathiae infection was studied macroscopically and microscopically.

Six strains of smooth forms of Erysipelothrix rhusiopathiae, two strains of Salmonella suipestifer, and two strains of Pasteurella organisms were used in inoculating various liquid media. The Salmonella suipestifer and Pasteurella organisms were used as a check or control to see if the Swine erysipelas organisms behave similarly.

The different types of colonies produced were designated as smooth (S), intermediate (I), and rough (R). The Swine erysipelas organisms were dissociated in less time than were the Salmonella suipestifer and Pasteurella organisms.

Nutrient broth, 5 per cent peptone and beef infusion broth, adjusted to pH 7.0, pH 7.6, and pH 7.8 were all effective in inducing S to R dissociation. Five per cent peptone was more effective than 1 per cent peptone. Ten per cent homologous immune serum in broth also produced rough variants.

Several successive transfers of the rough (R) form in beef infusion broth adjusted to pH 7.0 caused a R to S reversion. Daily transfer of the rough form in 10 per cent homologous immune serum in broth also produced a R to S reversion. Semi-solid salts agar was not an effective medium in inciting a R to S reversion.

There was no indication of bacteriophage in many plates examined.

The virulence of the smooth (S) and rough (R) types were tested and it was found that they both kill pigeons in about the same time. It was found, however, that the R form was slightly more virulent than the S type. The variants of E. rhusiopathiae do not behave like the variants of Sal-

monella suipestifer and Pasteurella organisms in this respect. The rough (R) forms of Salmonella suipestifer and Pasteurella organisms are less virulent than the smooth (S) types.

It was found that the rough (R) form of E. rhusiopathiae has a lower agglutination titer than the smooth (S) form. It was also found that the smooth (S) and rough (R) types cross agglutinate at lower dilutions.

The filtrability of the smooth (S) and rough (R) forms were tested on K medium and they were not found to be filtrable.

The lesions found in Swine erysipelas were not constant. In some cases we found hemorrhages (petechial) on the kidneys. In other cases we found pharyngitis and petechiation of the bladder but no hemorrhages on the kidneys. The most constant lesions found were the congestion of the lymph glands, congestion of the spleen, slightly thickened heart valves and reddened patches on the skin. Microscopically we found hemorrhages of the thickened heart valves, hemorrhages under the skin, and congestion of the spleen.

CONCLUSIONS

1. S, I, and R variants of Swine erysipelas may be obtained by proper treatment.

2. E. rhusiopathiae dissociated in less time than Salmonella suipestifer and Pasteurella organisms.

3. Large volume of liquid culture medium is more effective in inciting S to R dissociation.

4. Aging culture in nutrient broth of neutral or alkaline reaction produced S to R dissociation.

5. Five per cent peptone produced a greater number of variants than one per cent peptone.

6. Ten per cent homologous immune serum in broth produced S to R dissociation.

7. The production of R in different media was variable.

8. R to S reversion was accomplished by daily transfer in beef infusion broth and 10 per cent homologous R immune serum in broth.

9. Semi-solid agar is not as effective as beef infusion broth and homologous immune serum broth in producing R to S reversion.

10. The S form derived from the R variant had practically all the characteristic of the original S type.

11. The S antigen was agglutinated by the S serum in a higher titer than R antigen were agglutinated by the R sera.

12. S and R forms cross agglutinated at lower titer.

13. The R form of E. rhusiopathiae was slightly more virulent than the S form.

14. K media had no effect in producing filtrable stage in the R and S forms.

15. The individual cells of the R culture appeared to be longer and slightly larger than the S form. The cells of the R variants appeared either in chains or in clumps while those of the S form appeared singly.

16. The R colonies are larger and more irregular than the S colonies.

17. The presence of bacteriophage was not noted in this investigation.

18. The pathology of the disease produced in swine as a result of E. rhusiopathiae infection was not constant.

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