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A STUDY ON THE DISSOCIATION OF
SALMONELLA PULLORUM AND THE
SENSITIVITY OF THE VARIANTS TO A
QUATERNARY AMMONIUM
COMPOUND

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Barnet M. Sultzer
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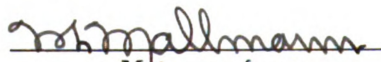
A Study On The Dissociation Of Salmonella
Pullorum And The Sensitivity Of The Variants
To A Quaternary Ammonium Compound.

presented by

Barnet M. Sultzer

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of the requirements for

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A STUDY ON THE DISSOCIATION OF SALMONELLA PULLORUM
AND THE SENSITIVITY OF THE VARIANTS TO A
QUATERNARY AMMONIUM COMPOUND

by

Barnet M. Sultzer

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INTRODUCTION

The phenomenon of bacterial dissociation or variability is a subject of both fundamental and practical significance. As a result, this phenomenon in all its diversity has been intensively studied for the last thirty years; however, much of the evidence which has accumulated has been just inconclusive enough to warrant widely different interpretations and theories. Thus, the various changes in colonial and cellular morphology, biochemical behaviour, antigenicity, and virulence exhibited by the bacterial cell may be possible expressions of one of several fundamental conceptions. Such concepts include, (1) bacteria vary in definite trends or patterns, (2) the environmental conditions imposed select variants or may directly result in variation, (3) bacteria just as other organisms are subject to mutations, and finally, (4) the various forms expressed by a bacterial species represent progressive ontogenetic transformation, commonly referred to as life cycles. Regardless of the theory one may adopt, the evidence to date indicates that dissociation occurs in virtually all bacterial species, and changes may be induced under suitable environmental conditions.

With regard to the practical significance of bacterial dissociation, direct consideration can be made to the anomalies that frequently occur in the phenol coefficient results of quaternary ammonium germicides. Maier and Muller in 1936 emphasized that dissociation of test organisms may result in an increase or decrease in sensitiveness of the bacterial to the test solutions. It seems

apparent then, that the stability of the test organism in regard to dissociation must be considered if more accurate results are to be expected in the phenol coefficient tests.

Mallmann in 1932 reported finding three stable colonial types in the Salmonella group. These three strains of Sal. pullorum were found to be (1) a stable smooth type, (2) a stable rough type, and (3) a variable rough-smooth type. Dissociation incitants were without permanent effect upon the pure rough and smooth types, while true dissociation changes occurred only in the variable type cultures.

In view of these findings and other evidence presented in the literature, this study was undertaken with a twofold purpose in mind: to attempt to isolate a stable smooth strain of Sal. pullorum, and to determine the sensitivity of smooth and variant forms of this organism to a quaternary ammonium compound.

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the integrity of the financial system and for the ability to detect and prevent fraud. The document also outlines the responsibilities of individuals involved in the process, including the need for transparency and accountability.

In the second part, the document addresses the challenges faced by organizations in implementing effective internal controls. It highlights the need for a strong culture of ethics and compliance, as well as the importance of regular training and monitoring. The document also discusses the role of technology in enhancing the efficiency and accuracy of financial reporting.

The third part of the document focuses on the importance of communication and collaboration between different departments and stakeholders. It stresses that clear communication is essential for ensuring that all parties are aware of the organization's financial goals and the measures being taken to achieve them. The document also discusses the need for regular reporting and updates to keep stakeholders informed of the organization's financial performance.

In the final part, the document provides a summary of the key points discussed and offers recommendations for improving the financial reporting process. It emphasizes the need for a proactive approach to financial management, one that anticipates potential risks and takes steps to mitigate them. The document also encourages organizations to seek out best practices and to learn from the experiences of others in the industry.

The document concludes by reiterating the importance of maintaining accurate records and the need for a strong culture of ethics and compliance. It also emphasizes the role of technology in enhancing the efficiency and accuracy of financial reporting. The document is intended to serve as a guide for organizations looking to improve their financial reporting process and to ensure the integrity of their financial system.

HISTORICAL RESUME'

Before pure culture techniques were developed, bacteria were regarded as belonging to an homogenous group, possibly of but a single species. In fact, in 1877, Nägeli propounded the doctrine of pleomorphism wherein all fission fungi were one type of cell, highly variable and capable of passing from one morphological type to another, as well as being capable of undergoing profound alterations in biochemical behaviour.

The opposite extreme of monomorphism became predominant with the subsequent development of pure culture methods by Koch and others. This doctrine proclaimed a constancy of species and form in bacterial morphology. Despite the adoption of monomorphism by the great majority of bacteriologists, data accumulated which indicated that though most bacteria showed a remarkable constancy of form, morphological and physiological variation is not uncommon.

In 1918, Baerthlein demonstrated that colony variability may serve as a criterion of cultural variability, for closely correlated with colony types are other important characteristics of the organism including cellular morphology, pigment and slime production, fermentation reactions, serological reactions and virulence. Arkwright, 1921, recognized the significance of Baerthlein's work and described smooth and rough colony forms of the colon-typhoid-dysentery group. Subsequent work by many others followed in voluminous proportion regarding the organisms belonging to this group and many others. Hadley published extensive literature reviews in 1927 and 1937 citing numerous instances of dissociation changes in various organisms.

The colonial forms described fall into six general categories; namely, smooth (S), rough (R), intermediate (SR), mucoid (M), dwarf (D), and gonidial (G). The smooth colony is characterized by a round, even margin, and a convex smooth and glistening surface; the organisms are uniformly turbid in broth and produce a stable suspension in 0.85% NaCl solution. The rough colony is described as follows: the margin is irregular; the surface is flat, uneven, and granular; the organisms produce a granular sediment in broth and clump spontaneously in physiological saline. The intermediate colonies are those that appear intermediate in form between S and R colonies and usually change into one or the other. The mucoid forms have a moist, glistening, mucous-like consistency; the colonies tend to coalesce. The dwarf forms are characteristically very small measuring less than 1 mm. in diameter and differing in respect to larger colonies of the same organism only in size. Finally, the gonidial forms are minute colonies representing growth from filterable forms of bacteria; however, much disagreement still prevails as to the actual existence of these so-called filterable forms.

While dissociation of various members of the *Salmonella* group has been widely studied, comparatively little work has been forthcoming with Sal. pullorum. In 1930, Plastring and Rettger isolated, from all appearances, a rough strain of Sal. pullorum from infected adult fowl and young chicks. These same authors described in 1932 three principle colony types of this organism including the smooth, rough, and a somewhat intermediate form. Gauger (1936) reported the isolation of two rough strains of Sal. pullorum from baby chicks; however, though these strains varied morphologically from typical

smooth cultures, no autoflocculation in saline was exhibited. In 1939 Morgan and Beckwith observed that Sal. pullorum temporarily exhibited a mucoid type when grown at temperatures between 16° and 25° C., but when cultured at 37° C. the loss of the mucoid character or reversion to the smooth type immediately occurred.

The most extensive work on the dissociative character of Sal. pullorum was reported in 1932 by Mallmann. Although it is generally believed that the chief culture types (S and R) are easily convertible one into the other, Mallmann presented evidence to the contrary. Working with stock cultures that had been kept in culture for many years, a total of 27 of these were found to be characterized by their smooth colony formation, 13 were markedly rough and at no time showed smooth characteristics, while 16 of the organisms were variable R to S and S to R types.

Employing numerous well recognized dissociation incitants for considerable time periods including brilliant green beef extract broth as a chemical incitant, aging at various temperatures, rapid transferring, immune smooth and rough serum, bacteriophage, and animal passage, it was found that there were several strains which were unaffected by these methods, maintaining the original colony appearance of either smooth or rough. The SR strains found variable as stock cultures were, on the other hand, easily changed to either S or R colonies depending on the type of incitant used.

In support of this evidence, it was indicated that a large number of workers previous to 1930 found refractory organisms that did not respond to inciting agents, although in many cases they were disregarded

• The first step in the process of creating a new product is to identify a market need. This can be done through market research, which involves gathering information about the target market and its needs. Once a market need has been identified, the next step is to develop a concept for a new product that meets this need.

• The second step in the process is to develop a business plan. This involves determining the costs of production, the pricing strategy, and the marketing strategy. A business plan also includes a financial forecast, which shows the expected revenue and profits over a period of time.

• The third step in the process is to create a prototype of the new product. This can be done using a variety of methods, including 3D printing, computer-aided design (CAD), and traditional manufacturing techniques. A prototype allows the entrepreneur to test the product and make any necessary adjustments before moving forward with production.

• The fourth step in the process is to produce the new product. This involves finding a manufacturer or manufacturer to produce the product on a large scale. The entrepreneur must also ensure that the product meets all relevant regulatory requirements and standards.

• The fifth step in the process is to market the new product. This involves developing a marketing strategy that includes advertising, promotion, and distribution. The entrepreneur must also ensure that the product is available to the target market at a competitive price.

• The sixth step in the process is to evaluate the success of the new product. This involves monitoring sales, customer feedback, and other key performance indicators. The entrepreneur must also be prepared to make adjustments to the product or marketing strategy if necessary.

• The final step in the process is to scale the production of the new product. This involves increasing the volume of production and expanding the distribution network. The entrepreneur must also ensure that the product remains profitable as production scales up.

• The process of creating a new product is a complex and challenging one, but it is also a rewarding one. By following these steps, entrepreneurs can increase their chances of creating a successful new product that meets the needs of the market.

in the final conclusions.

Barber (1907) obtained abnormal strains of Esch. coli that never reverted to the parent strain.

Clark (1910) was unable to convert avirulent diphtheria bacilli to virulent types by means of animal passage or by sensitization with homologous immune serum.

Buchanan and Truax (1910) in a study analogous to S and R types were unable to secure high and low acid producers of Strep. lacticus. They attributed their lack of success to the fact that a pure-line culture has invariable characteristics and therefore variations, in their estimation, will occur in cultures of mixed lines only.

Schutze (1921) was unable to convert R type paratyphoids to the S form.

In 50 rapid transfers DeKruif (1922) found a strain of the bacillus of rabbit septicemia that would not revert to the normal type.

Reiman (1925) could not revert R type paratyphoids to the S form by rapid transferring or 105 passages through mice. He believed that the change to an R type was characterized by a loss of certain properties in the cell that cannot be regained.

Jordon (1926) could not induce smooth types in two strains after 72 rapid transplants.

In a study of pneumococci, Dawson and Avery (1927) found one R strain that could not be reverted to the parent S type by animal passage.

In the previous year Bronfenbrenner, Muckenfuss, and Korb reported an avirulent strain of B. pestis caviae which remained avirulent even

after 200 rapid transfers, and concluded that this irreversible reaction is not a phenomenon of active hereditary immunisation but rather a result of irreversible variation.

Julianelle (1928) employing rapid transferring in plain broth and broth containing supernatant serum did not convert R type strains of Friedlander's bacillus to their S types. Animal passage was likewise unsuccessful.

In the same year Dulaney found that the reversal of stabilized rough forms of E. coli was difficult. Todd (1928) reported that glossy colonies (S) of haemolytic streptococci derived from matt colonies (R types from fresh isolated cultures) were permanently attenuated and could not be made virulent by growing in the presence of undiluted normal human serum. The attenuated R colonies, furthermore, returned to virulent cultures upon growth in serum.

Later publications also contributed significant data to the existence of highly stable forms. Leslie and Gardner (1931) working with four phases of B. pertussis (two S and two R) reported that "phase IV", which appeared to be the most advanced R, became "firmly and irrevocably established" in some strains. All other strains were interconvertible.

Kun and Fenyvessy in 1932 found extreme rough cultures of the influenza bacillus, characterized by thread forms and twisted mycelium that remained unchanged for years. Their reversion to smooth colonies could not be accomplished by the use of any of the media most favorable for propagation of smooth phase cultures. Only transient success was obtained by animal passage - reversal to R from the S form being accomplished after a few passages on

chocolate agar.

Further evidence was presented by T'ung in 1940. By irradiation with gamma rays obtained from radon, a change of S to R forms of pneumococcus was regularly induced, the change being so complete that it was "impossible to revert the dissociants to the primary state." The R form appeared suddenly, becoming totally avirulent and irreversible. Animal passage was unsuccessful in reverting the organisms.

Later in 1947, Kelner found a strain of Chromobacterium violaceum, derived from a secondary colony formation on LiCl agar, that was stable for 33 months undergoing at least forty transfers on LiCl free medium.

Won (1950) produced "giant" cells of Pasteurella pestis by treatment with camphor that neither reverted nor dissociated after ten animal passages or fifteen medium passages.

As previously mentioned, in testing quaternary ammonium compounds using the regular F.D.A. phenol coefficient, fluctuations in end titers frequently occur from day to day, even when the same individual performs the test. During the past several years many investigators have studied the quaternary ammonium germicides in this light, and the variations have been attributed to one or more factors related to the compounds or to the test procedure itself. Inconstancy of the peptone composition of the culture media, unequal distribution of the bacteria in the test medium, the "carry-over" of bacteriostatic amounts of the quaternary ammonium compound, and bacterial dissociation of the test organisms have been considered the more important factors.

Numerous modifications of the phenol coefficient method have been devised to overcome the first three factors mentioned, and vigorous controversy has arisen over the results obtained and theories proposed. The factor of dissociation, on the other hand, has received comparatively little attention. Klimek and Umbreit in 1947 stated that variations in the susceptibility of the individuals comprising the population of the inoculum may account for viable organisms after short exposures to the quaternary ammonium germicide. In connection with this proposition is the reference to bacterial dissociation made by Maier and Muller discussed above.

- *Staphylococcus aureus* (Staph aureus) is a Gram positive cocci, which is found in the skin and nasal cavity of humans and animals. It is a common cause of skin infections, such as impetigo, and is also a major cause of hospital-acquired infections, such as pneumonia and sepsis.
- *Streptococcus pneumoniae* (Pneumococcus) is a Gram positive cocci, which is found in the respiratory tract of humans. It is a common cause of pneumonia and meningitis.
- *Escherichia coli* (E. coli) is a Gram negative rod, which is found in the intestines of humans and animals. It is a common cause of urinary tract infections and is also a major cause of food poisoning.
- *Salmonella* is a Gram negative rod, which is found in the intestines of humans and animals. It is a common cause of food poisoning and is also a major cause of typhoid fever.
- *Shigella* is a Gram negative rod, which is found in the intestines of humans and animals. It is a common cause of dysentery and is also a major cause of shigellosis.
- *Clostridium difficile* (C. diff) is a Gram positive rod, which is found in the intestines of humans and animals. It is a common cause of antibiotic-associated diarrhea and is also a major cause of hospital-acquired infections.
- *Legionella* is a Gram negative rod, which is found in water systems. It is a common cause of Legionnaires' disease, a type of pneumonia.
- *Mycobacterium tuberculosis* (Tuberculosis) is a Gram positive rod, which is found in the lungs of humans. It is a common cause of tuberculosis, a type of lung infection.
- *Coccidioides immitis* (Coccidioides) is a fungus, which is found in the soil. It is a common cause of coccidioidomycosis, a type of lung infection.
- *Aspergillus* is a fungus, which is found in the air. It is a common cause of aspergillosis, a type of lung infection.
- *Candida* is a fungus, which is found in the mouth and vagina of humans. It is a common cause of candidiasis, a type of fungal infection.

BACTERIOLOGICAL STUDY OF THE CULTURES

A total of 48 stock cultures of Sal. pullorum was received for study. Three of the strains, originally isolated in 1942, were obtained from the culture collection of the Department of Bacteriology and Public Health, Michigan State College. These cultures had been freshly transferred to tryptose agar slants. The remaining 45 strains were obtained from the Poultry Diagnostic Laboratory, Department of Bacteriology and Public Health, Michigan State College. These cultures had been originally isolated from infected chickens or turkeys in July of 1950 and were received freshly transferred on nutrient agar slants. Immediately upon receipt, all the cultures were incubated for 48 hours at 37° C. and subsequently stored at 8-10° C. for future reference.

A preliminary survey of all the stock cultures was made to determine the particular colony characteristic of each strain. The media utilized consisted of tryptose broth and agar and nutrient broth and agar. The tryptose broth was composed of 2% Bacto-tryptose and 0.5% NaCl, while the formula for the nutrient broth was 0.5% Bacto-Beef Extract and 0.5% Bacto-Peptide. For the preparation of the agar, 1.5% plain agar was added to the respective broth formula. All media were adjusted to a pH of 6.9 before sterilization. The broth was dispensed in ten ml. quantities and then autoclaved. The agar plates were prepared in the usual manner using 15 to 18 ml. of agar per plate.

Tryptose broth was seeded first with each stock culture and the tubes were incubated for 24 hours at 37° C. Tryptose agar plates

1. What is the main purpose of the document?

The main purpose of the document is to provide a comprehensive overview of the current state of the global economy and the challenges it faces. It aims to identify key trends, analyze the impact of various factors, and offer insights into potential future developments.

The document is structured into several sections, each focusing on a different aspect of the global economy. It begins with an introduction, followed by a detailed analysis of the current economic landscape, and concludes with a series of recommendations and conclusions.

The analysis covers a wide range of topics, including the impact of technological advancements, the role of government intervention, and the challenges posed by global trade. It also examines the effects of various economic policies and the potential for future growth. The document is intended to serve as a valuable resource for policymakers, researchers, and anyone interested in the global economy.

The document is based on a thorough review of the latest data and research. It draws on a variety of sources, including government reports, academic studies, and industry analyses. The findings are presented in a clear and concise manner, making the document accessible to a wide range of readers.

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were streaked from the broth cultures employing a needle streak technique in which approximately 5 mm. of the tip of the inoculating needle, bent at a 30° angle, was dipped into the broth culture and then lightly streaked over the surface of the agar plate making ten to twelve individual streaks. All plates were incubated for 48 hours at 37° C. and subsequently observed as to colony appearance.

Observations of the agar plates were made using a dissecting microscope with a total magnification of 10X. The colonies were illuminated with oblique transmitted light.

To insure against any contamination and eliminate, if possible, any subordinate types, each culture was repeatedly plated by picking representative colonies, subculturing in broth for 24 hours, and subsequently plating as described above. When cultured in tryptose media it was found that 39 of the stock cultures demonstrated typical smooth colonies. The remaining nine cultures were intermediate in form. When representative colonies of each smooth culture were subcultured in nutrient media it was found that all the strains except Nos. 795, 856, and 878 showed a definite change in colonial appearance which persisted on successive platings. Generally the surface of the colonies became granular and wrinkled, taking on a somewhat opaque cast. When these colonies were subcultured again on tryptose media the typical smooth forms reappeared. All nine intermediate cultures produced the same forms on nutrient agar as on tryptose agar. The data are presented in Table I.

It thus appeared that a temporary environmental effect had been

produced with the smooth cultures, and in actuality, the changed forms found on the nutrient agar were not true variants. In any event, the three strains which remained the same on both media were considered to be more suitable for further study. Mallmann (1932) found such strains of Sal. pullorum to be characteristically more stable to various inducement methods.

Prior to the dissociation studies, cultures Nos. 795, 856, and 878 were checked culturally, morphologically, and physiologically to insure their species type. Smears were stained according to Gram's method and fresh nutrient agar stock slants were made from the final pure-line strains. The data are presented in Table 2.

The following studies represent an attempt to evaluate the influence of dissociating agents on these three strains.

METHODS USED TO INDUCE BACTERIAL DISSOCIATION

Experiment I

Various inorganic salts have been frequently reported in the literature as successful agents for producing rough forms in numerous bacterial species. Hadley (1927) demonstrated R forms in members of the paratyphoid group by using lithium chloride.

In this experiment a plain nutrient broth was prepared to which was added lithium chloride in a concentration of one percent. The pH was adjusted to 6.9 and the broth was tubed in ten ml. quantities and autoclaved. Each strain was inoculated into the broth tubes, which were then incubated at 37° C. At various intervals, nutrient agar plates were streaked from each culture and the plates were observed after 48 hours incubation. Weekly transfers were made using one loopful of culture. The experiment was carried through a period of 80 days. The data are presented in Table 3.

Initial platings were not accepted as indicative of a true variant. Typical colonies were picked and subcultured in nutrient broth and replated. If the colony type remained true to form as first observed, the variant was recorded. The results indicate that variants appeared in each strain by 24 days. Several variant forms from strain No. 795 appeared on the agar plates after 20 days and reoccurred intermittently from that time on. All of the forms were generally intermediate, having surfaces that were either granular and slightly veined or coarsely granular to somewhat wrinkled. The margins remained circular and entire.

Strains Nos. 856 and 878, on the other hand, exhibited a different

variant. After 24 days exposure to lithium chloride, colonies which were smaller than the typical smooth forms, decidedly darker, raised, and granular became evident on plates of both strains. By 48 days this variant disappeared on plates from No. 856 and in another eight days the same thing occurred with culture No. 878. Instead, a new form considerably rough in character, appeared in both instances. Refer to figure 1.

It may be noted that typical smooth colonies for each strain persisted throughout the entire 80 day period. Refer to figure 2.

Experiment 2

Various toxic substances and dyes when used in sublethal concentrations have been reported as successful dissociation incitants. Mallmann (1932) utilized the dye brilliant green obtaining R variants with several Sal. pullorum strains. This method was followed in this experiment.

The medium was prepared by adding seven ml. of a one percent aqueous solution of the dye to one liter of nutrient broth. The pH was adjusted to 6.9, the medium was dispensed in ten ml. quantities and sterilized in the autoclave. Strains Nos. 795, 856, and 878 were seeded into the broth and the tubes were then incubated at 37° C. Nutrient agar plates were streaked at various intervals. The data are presented in Table 4. Weekly transfers were made in fresh broth. The experiment was carried on for 81 days.

Intermediate variants appeared from each strain after 12 and 18 days and persisted throughout the rest of the experiment. Typical smooth colonies were observed in each strain up to 32 days, after

which they were no longer noted. Figure 3 is a photograph of the intermediate strain derived from culture No. 795.

With cultures Nos. 856 and 878, the same type of intermediate variant that appeared after exposure to LiCl also appeared in this experiment. However, at no time were any rough colonies observed. It may be noted that with brilliant green as the inciting agent, a shorter time interval elapsed before variants were observed.

Experiment 3

Many workers have commonly observed variants in old stock cultures. Thus, methods of aging have been utilized to recover these forms. Three different broth media were employed in this experiment at incubation temperatures of 20° - 23° C. Nutrient, tryptose, and liver infusion broths were prepared in the usual manner. Tubes were seeded with strains of Sal. pullorum being studied. Nutrient agar streak plates were made at various intervals; the total incubation period being a month. The data are presented in Table 5.

Strain No. 795 exhibited intermediate colonies after 16 days in nutrient broth, eight days in tryptose broth, and 30 days in liver infusion broth. Strain No. 878 demonstrated intermediate colonies after a month on nutrient broth, 16 days in tryptose broth, and a month in liver infusion broth. On the other hand, strain No. 856 remained smooth after a month's incubation in each medium, and also typical smooth colonies prevailed in each culture for the entire length of the experiment. One defined rough form was observed with strain No. 795 after a month in tryptose broth. This colony closely resembled those rough colonial types exhibited by the spore-forming bacilli. The surface was very coarsely granular and the edges were

irregular. However, an interesting phenomenon was noted when such a colony was subcultured in plain nutrient media. It was found that after 48 hours on the agar plate both typical smooth colonies (although in the minority) and the rough variants described appeared. Several successive platings revealed the same thing. Whether these colonies were unstable variants as described by Deskowitz (1936) with Sal. aertryche, or merely mixed strains, could not be determined.

Experiment 4

Irradiations by X-rays, gamma rays, and ultra-violet light have been reported as successful dissociating incitants. Haberman and Ellsworth (1940) demonstrated the dissociative effects of X-rays on Staphylococcus aureus and Serratia marcescens, producing many variant forms. Grainger (1947), also using X-rays, produced intermediate colony forms from smooth cultures of Sal. typhosa. Ultra-violet light has been shown to produce biochemical variants. This type of irradiation was used here in an attempt to induce changes in the colonial morphology of the selected cultures.

Twenty-four hour nutrient broth cultures of strains Nos. 795, 856, and 878 were smeared on a four square inch surface of a nutrient agar plate with a sterile cotton swab. Two plates were made for each strain in the manner described. The first plate, with the cover removed, was exposed at a distance of four inches from a 15 watt GE germicidal lamp for a period of 30 seconds. The second plate was exposed in the same manner for a period of 60 seconds. A third plate was streaked by the needle technique and left unexposed as the control. The plates were incubated and then observed.

Two variants that were selected for the germicidal sensitivity

tests described below were also exposed to the ultra-violet light. The experiment was repeated twice for all strains with the same results. The data are presented in Table 6. No colonial variants or reverted forms were produced.

GERMICIDAL SENSITIVITY DETERMINATIONS OF SELECTED VARIANTS OF
SAL.PULLORUM

Culture Study:

Two cultures of Sal. pullorum were selected for this study. Culture No. 795 demonstrated both typical smooth and intermediate colonies on nutrient agar after 32 days exposure to brilliant green nutrient broth. Culture No. 856 exhibited smooth and rough colonies after 48 days exposure to one percent lithium chloride.

Representative colonies of each form were picked and subcultured in nutrient broth. Successive platings were made to insure the initial stability and purity of each colony type designated. Smears were made and stained according to Gram's method. The biochemical behavior, motility, agglutinability with Sal. pullorum smooth immune serum, and reactivity in physiological saline were tested for each culture. Data are presented in Tables 7 and 8.

It should be emphasized that this preliminary culture study was undertaken to make sure that the smooth organisms isolated were identical not only in colony form, but also physiologically and serologically with the original cultures and species. The variant forms were studied to determine any differences in the nature of the organisms besides the colony appearance.

Cultures Nos. 795 S, 795 SR, and 856 S gave results representative of Sal. pullorum organisms; i.e., dextrose and mannitol were fermented with acid and gas while lactose, dulcitol, sucrose, and maltose were not. Furthermore, the cells were gram negative, non-spore forming rods, and morphologically typical of what is most frequently described. Each culture gave a strong positive reaction with smooth immune serum

and formed stable suspensions in physiological saline.

Culture No. 856 R, on the other hand, differed. While acid and gas were produced in mannitol, only acid was produced in dextrose. Maltose was also reacted upon with acid resulting. The organism appeared to have lost the typical gram's staining characteristic, i.e., while most of the cells were gram negative, some were tending to become gram positive. Morphologically, the cells were uniformly short rods arranged in packets. A granular suspension and sediment were apparent in 0.85% NaCl. When the salt concentration was reduced to 0.21%, a more stable suspension was apparent, although some fine suspended granules were still present. Furthermore, in dilutions of 1-100 and 1-200 of positive smooth immune serum, the agglutinability was somewhat less. It may be noted here that the antigenic suspension of this culture was prepared using a 0.21% NaCl solution. Arkwright (1921) found that rough organisms sometimes formed more stable suspensions when the salt concentration was reduced.

An attempt at reverting the two variants was also undertaken to determine whether the forms typical of the parent strains could be recovered. Nutrient broth cultures of Nos. 795 SR and 856 R were made and at 18 to 20 hours, transfers were made into fresh broth. This method of rapid transferring was continued and nutrient agar streak plates were made from each tube. After four such transfers, strain No. 856 R reverted completely to typical smooth forms. Culture No. 795 SR reverted after nine rapid transfers.

As described in Experiment 4, ultra-violet light had no effect on these cultures. Unfortunately, time was not available for making

• The first step in the process of creating a new product is to identify a market need. This involves conducting market research to determine what consumers want and what problems they are trying to solve. Once a need is identified, the next step is to develop a concept for a product that addresses that need. This is often done through brainstorming and sketching. The third step is to create a prototype, which is a small-scale model of the product that can be used to test the concept and gather feedback. The fourth step is to conduct a feasibility study, which involves evaluating the technical, financial, and market viability of the product. The fifth step is to develop a business plan, which outlines the marketing, sales, and financial strategies for the product. The final step is to launch the product and monitor its performance in the market.

• The second step in the process of creating a new product is to develop a concept for the product. This involves brainstorming ideas and sketching out the basic design of the product. The third step is to create a prototype, which is a small-scale model of the product that can be used to test the concept and gather feedback. The fourth step is to conduct a feasibility study, which involves evaluating the technical, financial, and market viability of the product. The fifth step is to develop a business plan, which outlines the marketing, sales, and financial strategies for the product. The final step is to launch the product and monitor its performance in the market.

a complete study of the reverted forms, although gram's stains demonstrated typical gram-negative rods.

Sensitivity Tests:

The procedure utilized in testing the germicidal sensitivity of the selected cultures consisted of a modification of the F.D.A. phenol coefficient method.

Ten ml. of nutrient broth was seeded from the stock slant and incubated for 24 hours at 37° C. The broth culture was then centrifuged at 2200 R.P.M. for 20 minutes. The supernatant broth was discarded and the cells were suspended in an equal quantity of saline and recentrifuged. This washing procedure was repeated, after which the cells were finally suspended in ten ml. of saline. In the case of culture No. 856 R, a 0.21% NaCl solution was used at all times in this procedure.

The final suspension was shaken for 10 to 15 minutes to insure the dispersal of any clumps. Since some fine granules persisted with culture No. 856 R, this suspension was filtered through sterile Whatman filter paper No. 1 into a sterile test tube. Total cell counts were made of each cell suspension with a Petrauf-Hausser cell counter. Each suspension was adjusted in volume with the appropriate sodium chloride diluent to give a count of 320 million cells per ml. One-half ml. was used as the inoculum for each medication tube.

Nutrient agar plates were streaked from each cell suspension and incubated for 48 hours. These plates served as a control for the colony stability.

The cresolic compound employed and sold under the trade name Lysol,

consists of soap, cresylic acid, and ortho hydroxydiphenyl as the active ingredients in a total concentration of 59%. The Lysol was used in seven aqueous dilutions ranging from 1-300 to 1-600.

The quaternary ammonium compound, sold under the trade name of Phemerol Chloride, consists of an aqueous solution of a para-tertiary-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium-chloride-monohydrate. It was used in dilutions ranging from 1-10,000 to 1-70,000 and 1-30,000 to 1-90,000.

Dilutions of phenol (1-90, 1-100, 1-110) served as the standard control. The test was run as otherwise prescribed by the F.D.A. method.

Results:

The data are presented in Tables 9 and 10, and represent results repeatedly obtained for each culture tested against each compound. Using Lysol a phenol coefficient of 5.0 was obtained with each smooth and variant culture, indicating no difference in sensitivity to this cresolic compound.

Considerably different results were obtained with the quaternary ammonium compound. First of all, each culture was more highly sensitive to this compound than to Lysol. Secondly, each strain and its variant differed as to their resistance to Phemerol Chloride. Culture No. 795 S gave a phenol coefficient of 400. Culture No. 795 SR showed a phenol coefficient of 500. Culture No. 856 S showed a phenol coefficient of 650, while culture No. 856 R gave a coefficient of 550. It appeared then that strain No. 856 was more sensitive than strain No. 795.

1. The first step in the process of creating a new product is to identify a market need. This involves conducting market research to understand what consumers want and what problems they are trying to solve. Once a need is identified, the next step is to develop a concept that addresses this need. This is often done through brainstorming sessions with a team of designers and engineers.

2. After a concept is developed, the next step is to create a prototype. This is a physical model of the product that allows designers to test and refine their ideas. Prototyping can be done in a variety of ways, from simple 3D printing to more complex methods like CNC machining. The goal is to create a model that is as close to the final product as possible, so that any issues can be identified and corrected early in the process.

3. Once a prototype is created, the next step is to conduct a feasibility study. This involves testing the prototype to see if it can be manufactured at a reasonable cost and if it meets the required specifications. This step is crucial because it helps to identify any potential problems before moving forward with full-scale production. If the study shows that the product is not feasible, designers can go back to the drawing board and make changes.

4. If the feasibility study is successful, the next step is to develop a detailed design. This involves creating a set of technical drawings that specify the dimensions, materials, and assembly instructions for the product. These drawings are used to create a mold or a set of instructions for manufacturing the product. This step is also important because it ensures that the product is designed to be manufactured efficiently and at a low cost.

5. The final step in the process is to manufacture the product. This involves using the technical drawings to create a mold or a set of instructions for manufacturing the product. The product is then produced in a factory or a workshop. Once the product is manufactured, it is tested to ensure that it meets the required specifications and that it is safe for use. If the product passes the tests, it is ready to be sold to consumers.

It may be noted that the resistance to the phenol standard controls was quite constant, as was the case with the expressed resistance of the test organisms to the cresolic germicide Lysol.

DISCUSSION

The first objective of this study was to attempt an isolation of a stable smooth strain of Sal. pullorum, or in other words, an organism which maintained its smooth colonial characteristics despite being exposed to various dissociation methods. Forty-eight stock cultures of Sal. pullorum were initially studied, and of these, three were found to produce the same smooth type of colony when grown on tryptose and nutrient agar. While ultra-violet light had no effect upon these cultures, variants were produced by exposure to lithium chloride and brilliant green. One strain remained stable when aged in different media at room temperature for a period of a month. The other two strains exhibited several variants.

In reality, the problem of stability is of a relative nature. Thus, one can present a new environment to an organism and observe a change in the colonial morphology upon initial plating, while another culture would not show an observable change until after a month's exposure, or still another would not vary at all for a year or more. Wolfram(1951) using Sal. pullorum cultures also obtained from the Poultry Diagnostic Laboratory of Michigan State College, found a number of variants after only three days incubation in plain tryptose broth. On the other hand, the strains this writer used were considerably more stable, although, eventually variants were produced with various incitants. It was further found that by a method of rapid transferring in broth, a typical intermediate and rough variant previously induced reverted to colonies identical in morphology to the original smooth forms. Thus, the variants produced were not of a very stable nature, although they did maintain

their particular characteristics in stock culture and when grown in preparation for the germicidal sensitivity tests.

The second objective of the study was, as mentioned in the Introduction, to determine the sensitivity of the variants to a quaternary ammonium compound. In these determinations the experiments were designed so that a comparison in sensitivity could be drawn between the variants and the original forms to a cresolic as well as a quaternary ammonium compound. The inoculum was standardized in each instance as to the number of cells introduced into each medication tube, and sodium chloride suspensions of the organisms were used to discount any possible interference by the peptone content of the culture medium. Brewer (1943) and others considered the phospholipid content of peptones to be an important factor in interfering with the germicidal action of quaternary ammonium compounds.

In the case of the phenol standards and the cresolic compound, a constant resistance on the part of all the test organisms was apparent. However, this was not the case with the quaternary ammonium germicide. The intermediate variant of one strain, from the results obtained, was more sensitive to the germicidal action of the quaternary ammonium compound than the original smooth culture of the same strain. The rough variant of the second strain, however, was more resistant than the smooth culture of the same strain. Thus, both an increase and decrease in sensitiveness was observed by different variants of the same organism. These results serve to emphasize the possible action of bacterial dissociation in producing

the first of these is the fact that the system is not a simple one, but a complex one, in which the various parts are interrelated and interdependent. The second is that the system is not a static one, but a dynamic one, in which the parts are constantly changing and evolving. The third is that the system is not a closed one, but an open one, in which the parts are constantly interacting with the environment. The fourth is that the system is not a linear one, but a non-linear one, in which the parts are constantly interacting with each other in a non-linear fashion. The fifth is that the system is not a deterministic one, but a probabilistic one, in which the parts are constantly interacting with each other in a probabilistic fashion. The sixth is that the system is not a simple one, but a complex one, in which the parts are interrelated and interdependent. The seventh is that the system is not a static one, but a dynamic one, in which the parts are constantly changing and evolving. The eighth is that the system is not a closed one, but an open one, in which the parts are constantly interacting with the environment. The ninth is that the system is not a linear one, but a non-linear one, in which the parts are constantly interacting with each other in a non-linear fashion. The tenth is that the system is not a deterministic one, but a probabilistic one, in which the parts are constantly interacting with each other in a probabilistic fashion.

fluctuating end titers when quaternary ammonium compounds are tested according to the phenol coefficient method.

Whether a truly stable organism would show a consistent level of sensitiveness to a quaternary ammonium compound, while reasonable to expect, could not be determined in this study since such an organism was not isolated.

SUMMARY

1. A dissociation study was made of three strains of Sal. pullorum that were originally smooth in colonial morphology on stock culture.
2. Intermediate and rough variants were found to occur with the application of certain dissociation incitants.
3. Ultra-violet light had no effect on the colonial morphology of smooth, intermediate, or rough cultures of the same organism.
4. No observable differences were found to exist in the sensitivity of smooth, intermediate, and rough cultures of Sal. pullorum to phenol and the cresolic compound Lysol.
5. Both an increase and decrease in the resistance to a quaternary ammonium compound Phemerol Chloride was observed to occur with variants of Sal. pullorum, i.e., the intermediate form was less resistant and the rough form was more resistant than the typical smooth culture.

• The first step in the process of creating a new product is to identify a market need. This can be done through market research, which involves gathering information about the target market and its needs.

• The second step is to develop a concept for the new product. This involves creating a detailed description of the product and its features.

• The third step is to create a prototype of the product. This involves building a physical model of the product that can be used to test the concept.

• The fourth step is to conduct a feasibility study. This involves evaluating the product's potential for success in the market.

• The fifth step is to develop a business plan for the product. This involves creating a detailed financial and marketing plan for the product.

• The sixth step is to secure funding for the product. This involves finding investors or lenders who are willing to provide the necessary capital for the product.

• The seventh step is to manufacture the product. This involves producing the physical product in a factory or workshop.

• The eighth step is to distribute the product. This involves getting the product into the hands of the target market.

• The ninth step is to promote the product. This involves creating a marketing campaign to raise awareness of the product.

• The tenth step is to evaluate the product's performance. This involves monitoring sales and customer feedback to determine if the product is successful.

• The eleventh step is to make improvements to the product. This involves using customer feedback to refine the product and make it better.

• The twelfth step is to launch the product. This involves officially introducing the product to the market.

• The thirteenth step is to monitor the product's performance. This involves continuing to track sales and customer feedback to ensure the product remains successful.

• The fourteenth step is to make further improvements to the product. This involves continuing to refine the product based on customer feedback.

• The fifteenth step is to conclude the product's lifecycle. This involves deciding when to discontinue the product.

TABLE 1: Colony Appearance of Stock Cultures of Sal.pullorum
on Tryptose and Nutrient Agar

Culture Number	Tryptose Agar	Nutrient Agar
13-1	SR	SR
13-2	S	SR
13-3	SR	SR
770	S	SR
775	S	SR
776	S	SR
781	S	SR
793	S	SR
795	S	S
822	SR	SR
827	S	SR
829	S	SR
830	S	SR
843	S	SR
856	S	S
860	SR	SR
866	SR	SR
867	SR	SR
869	S	SR
873	S	SR
874	S	SR
878	S	S
879	SR	SR
880	S	SR
892	S	SR
895	S	SR
897	S	SR
909	SR	SR
911	SR	SR
937	S	SR
940	S	SR
942	S	SR
945	S	SR
949	S	SR
951	S	SR
960	S	SR
962	S	SR
963	S	SR
969	S	SR
980	S	SR
990	S	SR
1006	S	SR
1008	S	SR
1033-2	S	SR
1035-1	S	SR
1040-3	S	SR
1049-2	S	SR
1028	S	SR

S - Smooth

SR - Intermediate

TABLE 2; Morphological and Physiological Characteristics of Smooth Cultures of

Sal. pullorum

Culture Number	Original Colony	Manni-tol	Lae-tose	Dulci-tol	Sue-rose	Mal-tose	Dex-trose	H ₂ S	Indol	Motil-ity	Gram's Stain	Morph-ology
795	S	AG	-	-	-	-	AG	+	-	-	-	Typi-cal
856	S	AG	-	-	-	-	AG	+	-	-	-	Typi-cal
878	S	AG	-	-	-	-	AG	+	-	-	-	Typi-cal

AG - Acid and Gas

A - Acid only

Incubation: 14 days at 37° C.

TABLE 3: Effect of One Percent Lithium Chloride on Selected Smooth Cultures of

Sal. pullorum

Culture Number	Original Colony	Days of Exposure *													
		2	4	8	12	16	20	24	28	32	40	48	56	64	80
795	S	S	S	S	S	S	S,SR	S,SR	S,SR	S,SR	S	S	-	S,SR	S,SR
856	S	S	S	S	S	S	S	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR
878	S	S	S	S	S	S	S	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR	S,SR

S - Smooth

SR - Intermediate

R - Rough

* Weekly transfers made in fresh LiCl broth.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230	1231	1232	1233	1234	1235	1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270	1271	1272	1273	1274	1275	1276	1277	1278	1279	1280	1281	1282	1283	1284	1285	1286	1287	1288	1289	1290	1291	1292	1293	1294	1295	1296	1297	1298	1299	1300	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330	1331	1332	1333	1334	1335	1336	1337	1338	1339	1340	1341	1342	1343	1344	1345	1346	1347	1348	1349	1350	1351	1352	1353	1354	1355	1356	1357	1358	1359	1360	1361	1362	1363	1364	1365	1366	1367	1368	1369	1370	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390	1391	1392	1393	1394	1395	1396	1397	1398	1399	1400	1401	1402	1403	1404	1405	1406	1407	1408	1409	1410	1411	1412	1413	1414	1415	1416	1417	1418	1419	1420	1421	1422	1423	1424	1425	1426	1427	1428	1429	1430	1431	1432	1433	1434	1435	1436	1437	1438	1439	1440	1441	1442	1443	1444	1445	1446	1447	1448	1449	1450	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460	1461	1462	1463	1464	1465	1466	1467	1468	1469	1470	1471	1472	1473	1474	1475	1476	1477	1478	1479	1480	1481	1482	1483	1484	1485	1486	1487	1488	1489	1490	1491	1492	1493	1494	1495	14
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TABLE 4: Effect of Brilliant Green on Selected Smooth Cultures of Sal.pullorum

Culture Number	Original Colony	Days of Exposure *													
		2	4	6	12	18	25	32	39	46	53	60	67	74	81
795	S	S	S	S	S,SR	S,SR	S,SR	S,SR	SR	SR	SR	SR	SR	SR	SR
856	S	S	S	S	S	S,SR	S,SR	S,SR	SR	SR	SR	SR	SR	SR	SR
878	S	S	S	S	S	S,SR	S,SR	S,SR	SR	SR	SR	SR	SR	SR	SR

S - Smooth

SR - Intermediate

* - Weekly transfers made in fresh brilliant green broth.

TABLE 5: Effect of Aging on Smooth Cultures of Sal.pullorum
in Several Media at 20° - 23° C.

Culture Number	Original Colony	Broth Medium	Days of Aging			
			4	8	16	30
795	S	Nutrient	S	S	S,SR	S,SR
856	S	Nutrient	S	S	S	S
878	S	Nutrient	S	S	S	S,SR
795	S	Tryptose	S	S,SR	S,SR	S,SR,R*
856	S	Tryptose	S	S	S	S
878	S	Tryptose	S	S	S,SR	S,SR
795	S	Liver Infusion	S	S	S	S,SR
856	S	Liver Infusion	S	S	S	S
878	S	Liver Infusion	S	S	S	S,SR

S - Smooth

SR - Intermediate

*R - Rough colony which produced R and S colonies on subculture.

TABLE 6: Effect of Ultra-violet Light on Smooth and Variant Cultures of

Sal. pullorum

Culture Number	Original Colony	30 sec. Exposure	60 sec. Exposure	Control
795	S	S	S	S
856	S	S	S	S
878	S	S	S	S
795	SR	SR	slight SR	SR
856	R	R	R	R

S - Smooth

SR - Intermediate

R - Rough

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TABLE 7: Morphological and Biochemical Characteristics of Smooth and Variant

Cultures of Sal. pullorum Selected for Sensitivity Studies

Culture Number	Original Colony	Mannitol	Lactose	Dulcitol	Sucrose	Maltose	Dextrose	H ₂ S	Indol	Motility	Gram's Stain	Morphology	Auto Flocculation with 0.85% NaCl
795	S	AG	-	-	-	-	AG	+	-	-	-	Typical	-
795	SR	AG	-	-	-	-	AG	+	-	-	-	Typical	-
856	S	AG	-	-	-	-	AG	+	-	-	-	Typical	-
856	R	AG	-	-	-	A	A	+	-	-	-	Uniform rods in packets	+

AG - Acid and Gas

A - Acid only

Incubation: 14 days at 37° C.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	12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TABLE 8: Agglutinability of Sal. pullorum Cultures with Smooth Immune Serum

Culture Number	Original Colony	1/25	1/50	1/100	1/200	Control
795	S	++++	++++	++++	++++	-
795	SR	++++	++++	++++	++++	-
856	S	++++	++++	++++	++++	-
856	R	++++	++++	++++	++++	-

TABLE 9: The Sensitivity of Selected Cultures of Sal. pullorum
to Lysol

Culture	Colony	Dilution	Transfer Minutes			Phenol Coefficient
			(5)	(10)	(15)	
795	S	1-300	-	-	-	$\frac{500}{100} = 5$
		1-350	-	-	-	
		1-400	-	-	-	
		1-450	/	-	-	
		1-500	/	-	-	
		1-550	/	/	/	
		1-600	/	/	/	
795	SR	1-300	-	-	-	$\frac{500}{100} = 5$
		1-350	-	-	-	
		1-400	-	-	-	
		1-450	-	-	-	
		1-500	/	-	-	
		1-550	/	/	-	
		1-600	/	/	-	
856	S	1-300	-	-	-	$\frac{500}{100} = 5$
		1-350	-	-	-	
		1-400	-	-	-	
		1-450	/	-	-	
		1-500	/	-	-	
		1-550	/	/	-	
		1-600	/	/	-	
856	SR	1-300	-	-	-	$\frac{500}{100} = 5$
		1-350	-	-	-	
		1-400	-	-	-	
		1-450	-	-	-	
		1-500	/	-	-	
		1-550	/	/	-	
		1-600	/	/	/	
Phenol Standard		1-90	-	-	-	
		1-100	/	-	-	
		1-110	/	/	-	

TABLE 10: Sensitivity of Selected Cultures of Sal. pullorum
to Phemerol

Culture	Colony	Dilution	Transfer Minutes			Phenol Coefficient
			(5)	(10)	(15)	
795	S	1-10,000	-	-	-	$\frac{40,000}{100} = 400$
		1-20,000	-	-	-	
		1-30,000	-	-	-	
		1-40,000	/	-	-	
		1-50,000	/	/	/	
		1-60,000	/	/	/	
		1-70,000	/	/	/	
795	SR	1-30,000	-	-	-	$\frac{50,000}{100} = 500$
		1-40,000	-	-	-	
		1-50,000	/	-	-	
		1-60,000	/	/	-	
		1-70,000	/	/	/	
		1-80,000	/	/	/	
		1-90,000	/	/	/	
856	S	1-30,000	-	-	-	$\frac{65,000}{100} = 650$
		1-40,000	-	-	-	
		1-50,000	-	-	-	
		1-60,000	-	-	-	
		1-70,000	/	/	/	
		1-80,000	/	/	/	
		1-90,000	/	/	/	
856	SR	1-10,000	-	-	-	$\frac{55,000}{100} = 550$
		1-20,000	-	-	-	
		1-30,000	-	-	-	
		1-40,000	-	-	-	
		1-50,000	-	-	-	
		1-60,000	/	/	-	
		1-70,000	/	/	-	
Phenol Standard		1-90	-	-	-	
		1-100	/	-	-	
		1-110	/	/	-	

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25
26	27	28	29	30
31	32	33	34	35
36	37	38	39	40
41	42	43	44	45
46	47	48	49	50
51	52	53	54	55
56	57	58	59	60
61	62	63	64	65
66	67	68	69	70
71	72	73	74	75
76	77	78	79	80
81	82	83	84	85
86	87	88	89	90
91	92	93	94	95
96	97	98	99	100
101	102	103	104	105
106	107	108	109	110
111	112	113	114	115
116	117	118	119	120
121	122	123	124	125
126	127	128	129	130
131	132	133	134	135
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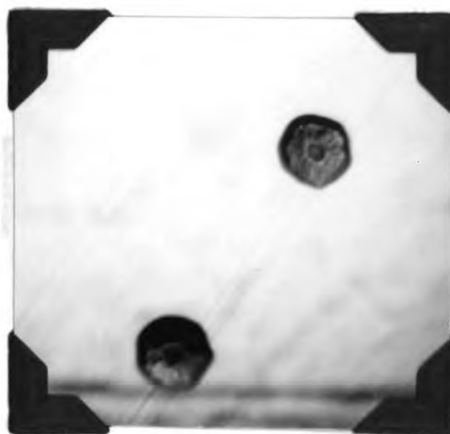


Figure 1. Rough colonies of Sal. pullorum on nutrient agar after 48 hours. Culture No. 856 at a magnification of 3.3.

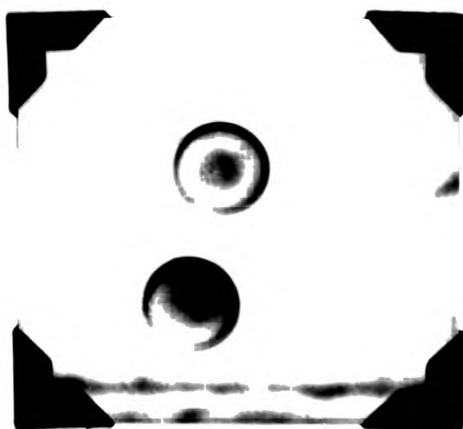


Figure 2. Typical smooth colonies of Sal. pullorum on nutrient agar after 48 hours. Culture No. 856 at a magnification of 3.3.

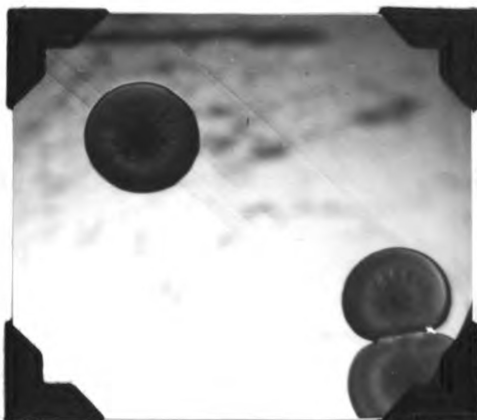


Figure 3. Intermediate colonies of Sal. pullorum on nutrient agar after 48 hours. Culture No. 795 at a magnification of 3.3.

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