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A Comparative Approach into the Differentiation of Two Strains of Staphylococci

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A COMPARATIVE APPROACH INTO THE DIFFERENTIATION OF TWO STRAINS OF STAPHYLOCOCCI

by

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INTRODUCTION

The purpose of this investigation was to differentiate Staphylococcus aureus phage type 42D from Staphylococcus epidermidis.

The investigation involved four approaches to this differentiation. The first was a selective screening technique on specific media to point out physical and biochemical characteristics and to demonstrate the presence of specific enzymes. The second was a drug survey which was used to show basic susceptibility and resistance to ten different antibiotics. The third was ultra-violet irradiation which was used to discern susceptibility and resistance to the soft rays of this spectrum segment. The fourth was a chromatographic procedure using labeled carbon which demonstrated certain amino acids that were present and/or synthesized by these microorganisms, and showed carbon-14 incorporation by these microorganisms.

The differentiation therefore was based on the finding of similarities and differences. Since variation sometimes points to a genetic difference, the investigation also proposed to indicate some basic genetic similarities and differences between these two strains of staphylococci.

LITERATURE REVIEW

Burrows (1965, p. 461) points out the fact that staphylococci are ubiquitous and have been known since approximately 1880 when Rosenbach did most of the early work. They still, however, remain less well known than most other bacteria.

The staphylococci are slightly less than one micron in diameter, and their shape is more spherical than most other cocci. Another characteristic of staphylococci is that they have a tendency to clump and consequently occur as groups of cells. This is believed to result from cell division in three planes and from the tendency of daughter cells to stay together. Physiologically, staphylococci are characterized by their resistance to heat, thereby requiring sustained temperatures as high as 80 degrees centigrade to kill them, while 60 degrees centigrade is sufficient for most other bacteria (Burrows, 1965, pp. 461-462).

The following study dealt with Staphylococcus aureus, phage type 42D, which is pathogenic to man, and a non-pathogenic strain of Staphylococcus epidermidis. One way of classifying the staphylococci is by phage typing. There are 21 basic phages which are categorized into four groups with the exception of phage 81 and

phage 187 which are categorized miscellaneous. Phage type 42D is the only member of group four. Phage type 42D means that this particular strain of S. aureus is lysed by phage 42D. The number 42D has no significance in itself because phage types are numbered arbitrarily (Burrows, 1965, p. 464).

Some phage types of staphylococci, such as type 80/81, have become prominent in the last few years because of their association with hospital infections. This phage type was not identified until the middle 1950's when Rountree and Freeman identified phage 80, and Bynoe, Elder, and Comtois identified phage 81. Several authorities thought, therefore, that phage type 80/81 represented a new strain of staphylococcus. Examination of staphylococci isolated between 1924 and 1947 identified strains belonging to phage type 80/81 and produced evidence that this strain existed before 1950 (Blair, 1960).

The term pathogenic as used here is defined as any strain which is able to cause an infection or produce food poisoning under optimum conditions. The most accurate indication of a pathogen is its ability to coagulate blood plasma (Evans, 1948). Chapman (1944) noted that human plasma took longer to clot by the staphylococci, and the resulting clots were less firm

than those resulting when rabbit plasma was used, and he (1945a) discovered that stored blood was not coagulated by staphylococci, and that an increased number of staphylococci produced a less firm clot. Chapman (1947) found that the coagulation of blood by staphylococci was improved in many cases by incubation at 30 degrees centigrade rather than the normally used temperature of 37 degrees centigrade.

The fermentation of mannitol, presence of pigmented colonies, and the ability to grow in the presence of 7.5 per cent sodium chloride are associated with pathogenicity in staphylococci, however, non-pathogenic forms may show some of these latter characteristics (Evans, 1948). Strains of staphylococci which caused food poisoning produced orange colonies in addition to being coagulase positive and fermenting mannitol (Chapman and Domingo, 1946).

Staphylococcus Medium No. 110 contains 75 grams of sodium chloride per liter. Chapman (1945b) found that the addition of 7.5 per cent sodium chloride to solid culture media inhibited the growth of most bacteria other than staphylococci, and pathogenic strains of staphylococci grew well in contrast to non-pathogenic strains. Chapman (1946) stated that Staphylococcus albus and Staphylococcus aureus grow on the medium,

but no other bacteria tested except Streptobacillus could survive in the high sodium chloride concentration.

Ultra-violet irradiation has long been used by investigators to study its lethal and mutagenic effects on bacteria. Staphylococci, because of their tendency to clump present a unique problem. A true count cannot be ascertained on dilution plates by present techniques if the bacteria clump. In 1929, Gates obtained a sigmoid survival curve with Staphylococcus aureus which he irradiated with ultra-violet light. The sigmoid survival curves which have been obtained with organisms such as staphylococci are probably best explained by the tendency of the organisms to clump (Lea, 1947, p. 319).

The effect of ultra-violet light is very different from X-ray, which is an ionizing radiation. Strauss (1960, p. 102) explains the physical difference between the two. The difference is that there is an absorption of radiation by the molecule in ultra-violet radiation in contrast to an absorption of the energy by the atoms with X-rays and electron emission.

The main effect of ultra-violet light is on the nucleic acid of the cell. Strauss (1960, p. 102) showed that the action spectrum for mutation provides proof that nucleic acid is the molecule which absorbs

ultra-violet irradiation. Lea (1947, p. 188) also established that the observed genetic effects of ultra-violet light are due to the absorption of ultra-violet light by the nucleoprotein of the chromosome. Two thousand six hundred Angstroms appeared to be the strongest region of ultra-violet light in its effect on living organisms. Deoxyribonucleic acid has the greatest absorbancy at this wave length. Thymine and cytosine, the pyrimidines, are substantially more sensitive than the purines, adenine and guanine. Ultra-violet light causes the formation of dimers with thymine and will cause cytosine to add a water molecule, if it is in liquid medium. The effect of ultra-violet light is not necessarily limited to the bases of DNA. High doses of ultra-violet light can cause the sugar phosphate ester bonds of DNA to break (Deering, 1962).

The bacteriocidal action of ultra-violet irradiation of wave lengths shorter than 3000 Angstroms is the greatest at 2650 Angstroms. This is close to the region in which nucleic acids are the highest absorbers. It requires approximately 10,000 to 100,000 times as much incident energy to kill at 3500 to 4900 than at 2650 Angstroms (Hollaender, 1943).

The majority of bacterial strains are sensitive to some drugs, but individual strains vary greatly in the

development of resistance and their degrees of sensitivity. The staphylococci become drug resistant more quickly than other kinds of bacteria. Staphylococci become resistant under natural conditions and resistance can be induced by culturing them in increasingly larger doses of the specific antibiotic (Burrows, 1965, p. 469). Seven strains of S. aureus were isolated from 1944 to 1946, and six were sensitive to penicillin. The one resistant strain was inhibited by 6 to 25 units of penicillin (Blair, 1960).

It was demonstrated that following a 15 minute exposure to penicillin there was a lag period during which the population of viable S. aureus cells was constant. No correlation was found between the length of the lag period and the ability of the bacteria to produce the enzyme penicillinase. A close correlation was found, however, between the post penicillin lag period and the resistance of the organism to the antibiotic (Parker, 1948). It has been shown that resistance to penicillin and streptomycin in S. aureus was the result of mutation. It was believed that this was due to mutations in a series of genes, because resistance to a drug was developed in successive steps. The slight degree of resistance noted with the first dose of the drug is correlated with a mutation in one

of these genes. The successive increases in resistance with increased dosages of the drug indicated mutations in other genes of this category (Demerec, 1948).

Antibiotics can affect the ribosomes and consequently cause a misunderstanding of the genetic code. One of the factors leading to this conclusion was the plating of an arginine deficient strain of Escherichia coli on a medium containing streptomycin, without arginine, and then observing growth of mutant types. The research of Davies and Flaks, 1963, showed the mutation from streptomycin resistance involved a change in the ribosome structure. Through this research it was determined that the 30 Svedburg units of the ribosomes were poisoned or changed by streptomycin. This resulted in the synthesis of a different protein (Gorini, 1966).

The assimilation of carbon dioxide into amino acids has been widely studied in yeasts. Saccharomyces cerevisiae is an ideal organism for research because it requires no organic substance in its culture media. It synthesizes all its own amino acids from inorganic compounds and thus utilizes carbon dioxide in the process. However, the staphylococci do require organic substances for amino acid synthesis, but can utilize some independent carbon dioxide for amino acid synthesis, however, to a lesser degree than yeast.

By studying labeled carbon dioxide incorporation by the yeasts S. cerevisiae and S. carlsbergensis, it was found that the labeled carbon was incorporated primarily into aspartic, glutamic, malic, and the tricarboxylic acids of the Kreb's cycle. It was reported that variations in pH of the culture media do not affect the fixation and distribution of the radioactive carbon dioxide. Carbon dioxide fixation is an essential reaction for the yeasts since it is the source of carbon for the organism's biosynthetic processes (Stoppani, 1958).

Carbon dioxide fixation was studied in a variety of organisms to classify them evolutionarily. The fixed carbon was analyzed by paper chromatography techniques. Carbon dioxide fixation was found similar in all organisms, and it was believed that this represented a single origin of biochemical systems. Because of the universal existance of a well-defined carboxylation reaction of pyruvic acid, which leads to oxaloacetic acid and malic acid, the labeled carbon could be incorporated into almost all of the amino acids by way of the Kreb's cycle and a few transaminations. Most of the incorporated activity in yeast was in aspartic, glutamic, and succinic acids. It was primarily aspartic and glutamic acids in the water

molds. In the anaerobic bacterium Botyribacterium retlyeri the fixed activity was primarily in acetic and butyric acids. In Clostridium kluyveri it was in alanine, aspartic, and glutamic acids. The slime mold, Physarum polycephalum, showed fixed activity in aspartic, glutamic, malic, and citric acids (Lynch and Calvin, 1952).

MATERIALS AND METHODS

The bacterial cultures were obtained from the Biology Department of Western Michigan University.* The pathogenic strain was originally isolated from a local hospital in 1962. At this time the strain belonged to phage type 80/81. Through genetic selection the strain has become phage type 42D. All the bacteria used in the investigation were derived from the original stock cultures. Each culture before being used was tested for its ability to coagulate blood plasma. This served as a check on the labeling of the bacterial strains, because pathogenic staphylococci will coagulate the plasma and non-pathogenic strains will not. The bacteria were then cultured in Brain Heart Infusion Broth.

All media used were obtained in a dehydrated form from Difco Laboratories in Detroit, Michigan, with the exception of Loeffler Blood Serum Agar and Blood Agar which were obtained in prepared slants (see Appendix A).

The antibiotics were obtained in both a high and a low concentration from Difco Laboratories. The antibiotics were obtained in disc form (see Table VII).

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*Both strains were phage typed by the Division of Laboratories, Michigan Department of Health, Lansing, Michigan, June 22, 1966.

Incubation of all agar plates was carried out at 37 degrees centigrade for 24 hours.

The procedure on the media screening portion of the investigation consisted of the following: A culture of each of the two strains of bacteria was centrifuged at 2500 RPM for 15 minutes to remove the cells from the nutrient broth. The cells were then washed and resuspended in 10 ml. of sterile Sorensen's phosphate buffer solution with an adjusted pH of 7.0. The centrifugation, washing, and resuspension were repeated twice. Two tenths of a ml. aliquots were spread on the media, which was then incubated. This procedure was altered for coagulase plasma and nutrient gelatin because they are liquid media. To test for coagulation of plasma, three drops of a fresh (16 to 24 hours) Brain Heart Infusion Culture were added to 0.5 ml. of the plasma. This was incubated and observed periodically for coagulation. Nutrient gelatin was innoculated with the strains of staphylococci and incubated in a sterile test tube. After 24 hours, the samples along with a blank were placed in the refrigerator and observed until the blank solidified, which was at 20 degrees centigrade. If either or both of the test tubes containing the staphylococci cultures remained liquid at 20 degrees centigrade, this would be evidence of gelatinalysis.

Multiple plate samples were done in all cases to affirm the validity of the results.

Brain Heart Infusion Agar was the medium used to test for susceptibility to antibiotics. The procedure consisted of centrifuging, washing, and suspending the staphylococci cells in sterile 7.0 pH phosphate buffer. Two tenths of a ml. aliquots of the staphylococci cell suspension were then spread over the agar surface and the antibiotic in disc form was placed in the middle of the petri dish. After incubation, the zone of inhibition around each antibiotic disc was measured (see Tables I, II, and III). A statistical analysis of linear correlation was computed to compare the behavior of the two strains of staphylococci for each antibiotic (see Table IV). The correlation test is based on whether or not two variables rise and fall together. The variables here were the zones of inhibition of each of the strains of staphylococci. The qualitative evaluation of the linear correlation coefficient was obtained from Edwards (1955, p. 100), and is contained in Table IV. Four plate samples were done for each antibiotic per strain of staphylococci.

To test the effect of ultra-violet light on staphylococci the tendency of the cells to clump had to be overcome. To minimize the clumping, 18 ml. of the washed

cells were diluted with an extra 100 ml. of the phosphate buffer and placed in the Waring blender for four minutes. This served to minimize the clumping of the cells. The phosphate buffer was also adjusted to a pH of 8.0.

Eighteen ml. of the total volume were used for this experiment. One ml. was removed and carried through a serial dilution to obtain the control count. Plating was done at final dilutions of 10^{-7} and 10^{-8} and all plates received 0.1 ml. aliquots of the cell suspension. Two plates were spread for each dilution. The remaining 17 ml. were exposed to ultra-violet light for sixty seconds. A one ml. sample was removed and carried through a serial dilution and plated at dilutions of 10^{-6} and 10^{-7} (see Tables V and VI).

The remaining 16 ml. were exposed to ultra-violet light for fifteen additional seconds giving a total dosage of 75 seconds. A one ml. sample was removed and carried through a serial dilution with plating at dilutions of 10^{-5}, 10^{-6}, and 10^{-7}.

The residual 15 ml. were exposed to ultra-violet irradiation for an additional fifteen seconds giving a total exposure of 90 seconds. A one ml. sample was carried through a serial dilution with plating at dilutions of 10^{-4}, 10^{-5}, and 10^{-6}.

Two more exposures of fifteen seconds each to

ultra-violet light were done. This gave samples with total dosages of 105 and 120 seconds with plating at dilutions of 10^{-3}, 10^{-4}, and 10^{-5}.

The number of colonies on the agar surface times the dilution factor gave the surviving number of cells per milliliter of original suspension. This was calculated after the plates were incubated for 24 hours.

The entire experiment was carried out with no incident lighting to prevent photoreactivation of the bacterial cells.

To determine carbon-14 incorporation by staphylococci, the procedure by Stoppani et al (1958) was used. The materials used were a reaction vessel which consisted of a 125 ml. Erlenmeyer flask. Attached to the side arm of the flask was a 10 ml. test tube and to the other side by glass tubing a 25 ml. Erlenmeyer flask. Each vessel was provided with a tight fitting cap. The isotope was Ba $^{14}CO_3$ with an activity of fifty microcuries. A standard solution of amino acids (see Appendix B) was prepared by dissolving a specified amount of each amino acid in 100 ml. of ten per cent isopropyl alcohol. The chromatographic solvent was prepared by shaking together 120 ml. of secondary-butanol with 40 ml. of three per cent NH_4 OH. The ninhydrin solution was prepared by adding .625 grams of ninhydrin to 2.5

ml. of glacial acetic acid, and filled to a total volume of 250 ml. with acetone.

Thirty ml. of Brain Heart Infusion Broth were innoculated with the strain of staphylococci and incubated with shaking for 24 hours. This was placed into the 125 ml. Erlenmeyer center flask. The Ba $^{14}CO_3$ was added to the 10 ml. side tube. A vacuum was applied to the center flask to evacuate the air. This was done by a water aspirator pump. One ml. of 40 per cent perchloric acid was injected in the side tube containing the Ba $^{14}CO_3$ to liberate the $^{14}CO_2$. In order to sweep the $^{14}CO_2$ into the main flask a long needle was inserted briefly into the side tube.

The reaction vessel was then placed on a shaker for one hour to allow fixation. Ten ml. of 6N NaOH was added to the empty side flask to react and trap excess $^{14}CO_2$. Ten ml. of 6N HCl was added to the center flask to drive off dissolved $^{14}CO_2$ and to inactivate the cells. The apparatus was then placed on the shaker for an additional ten minutes. The side tube solution which was not radioactive was discarded. The side flask solution contained the unreacted ^{14}C trapped as NaH $^{14}CO_3$ and was saved.

The bacterial suspension in the center flask was transferred to centrifuge tubes and centrifuged at 2500

RPM for 20 minutes. The supernatant was decanted and saved. One ml. of 12N HCl was added to the centrifuged cells and this suspension transferred to a 15 ml. pyrex hydrolysis tube which was sealed. The hydrolysis tube was placed in an Erlenmeyer flask and autoclaved for eight hours at 15 pounds of pressure per square inch to lyse the bacterial cells. After cooling, the tube was opened by carefully cracking off the neck. The hydrolysate in the tube was evaporated to dryness on a steam bath to remove HCl and water. This procedure was repeated twice.

Three chromatograms were made. The first contained four spots of hydrolysate in the amounts of one, two, three, and four lambda. This was placed over X-ray film and left for two weeks after which it was developed. The second chromatogram contained two spots of hydrolysate in the amounts of one and two lambda. It also contained two spots of standard in the amounts of one and two lambda. The third chromatogram contained two spots of hydrolysate and two of standard in the amounts of three and four lambda. Phenol red was used on all chromatograms to mark the speed of the fastest amino acid.

The second and third chromatograms were placed in one gallon jars with the chromatographic solvent.

After a period of twenty-four hours they were developed with ninhydrin.

The amino acids follow specific arrangements (see Table VIII). The linear order given by Roland and Gross, 1954, was used here.

RESULTS AND DISCUSSION

The following results were obtained from screening on Phenol Red Carbohydrate Medium. Staphylococcus aureus, 42D, fermented mannitol, levulose, maltose, lactose, saccharose, and dextrose. It did not ferment inulin, salicin, or raffinose. Staphylococcus epidermidis did not ferment lactose or mannitol, but otherwise was similar to the pathogen.

Both S. aureus, 42D, and S. epidermidis grew on Staphylococcus Medium No. 110. The pathogenic strain formed definite rounded colonies which increased in size with incubation. The non-pathogen did not show the definite characteristic round colonies and did not appear to grow well with increased time of incubation. This is in accordance with the findings of Chapman (1945b) who reported that pathogenic strains of staphylococci grow well on the medium and non-pathogenic strains do not.

When the cultures of staphylococci grown in nutrient gelatin were placed in the refrigerator, the blank containing nothing but nutrient gelatin solidified at 20 degrees centigrade. The nutrient gelatin culture containing S. epidermidis also solidified at this temperature. The nutrient gelatin culture containing

S. aureus remained liquid even when the temperature of the culture was lowered to seven degrees centigrade, and was interpreted as evidence of gelatinalysis by the pathogen.

When the two strains of staphylococci were innoculated onto the blood agar, it was noted that after 24 hours of incubation a clear zone appeared around the individual colonies of S. aureus, 42D, but not around the colonies of S. epidermidis. This was interpreted as evidence of hemolysis by the pathogen, which agreed with Burrows (1965, p. 470) who reported that pathogenic strains of staphylococci are invariably hemolytic.

Loeffler Blood Serum Medium is used principally as a cultural medium for diptheria. It was used here for the examination of pigment formation. The milky white color of this medium allows any pigmentation to show up. It was noted that S. aureus formed definite yellow colonies. The S. epidermidis formed no pigmented colonies, appearing white almost blending with the medium. This is in accordance with Evans (1948) and other authors who reported the finding of pigmented colonies with pathogenic staphylococci.

Coagulase plasma was used to determine the ability of the two strains to coagulate plasma. Staphylococcus aureus, 42D, tested out coagulase positive. It is such

a strong pathogen that it will coagulate the blood plasma at room temperature within twenty minutes. Staphylococcus epidermidis tested out coagulase negative and showed no evidence of coagulation even after six hours of incubation. This agreed with findings of others, such as Chapman (1944).

Both strains grew on Chapman-Stone Medium and no noticeable differences were observed between the two strains.

Antibiotic sensitivity was based on the following criterion. If the low concentration of the drug inhibited growth, the bacteria were considered sensitive. If only the high concentration of the antibiotic inhibited growth, the bacteria were considered slightly sensitive to the antibiotic. If neither the low nor the high concentration of the antibiotic inhibited bacterial growth, the bacteria were considered insensitive to the antibiotic (see Table VII).

Both the pathogen and non-pathogen behaved similarly as far as sensitivity to the various antibiotics was concerned. Both strains were sensitive to all antibiotics tested with the exception of Polymixin B. In this case, both strains of staphylococci were slightly sensitive being inhibited only by the higher concentrations.

Four plate samples were taken for each antibiotic, and the zone of inhibition for each drug was measured (see Tables I, II, and III). Here a statistical comparison of behavior could be obtained for both strains on each individual drug (see Table IV).

The effect of one antibiotic cannot be compared to another because the drugs have different solubility rates through the test medium. In general, the effect of an antibiotic can be compared on the basis of individual strain response. For every antibiotic tested, the zone of inhibition was greater for S. epidermidis than for S. aureus, 42D. This was interpreted as evidence of greater antibiotic resistance by S. aureus.

The linear correlation coefficient and its qualitative evaluation in Table IV range from a high for Bacitracin and Streptomycin to a very low for Chloromycetin, Penicillin, Erythromycin, and Novomycin. This indicated that the response of the two strains of staphylococci to certain antibiotics (Chloromycetin and Penicillin) was not the same, and the response to other antibiotics (Bacitracin and Streptomycin) was very similar.

Burrows (1965, p. 469) stated that 65 to 90 per cent of the strains of staphylococci are penicillin resistant; 50 per cent are tetracycline resistant; and

20 per cent are erythromycin resistant. The findings here were in accordance but were possibly somewhat exceptional as far as penicillin was concerned.

Ultra-violet irradiation was found to have a lethal effect on both strains of staphylococci. The best plates, judged so by how well the colonies were spread over the media and lack of clumping provided a curve of lethality as Figures 1 and 2 show. The greatest lethal effect appeared between 90 and 105 seconds of ultra-violet irradiation for both strains of staphylococci. Ehret (1964) stated that a lethality curve showed a Poisson distribution for bacteria exposed to X-ray. The lethality curves in Figures 1 and 2 appear to show a Poisson distribution as a result of ultra-violet irradiation; this was not statistically proven, but based on observation.

Both S. aureus and S. epidermidis gave very similar chromatograms. The amino acids identified from the chromatogram of S. aureus' hydrolysate were phenyl alanine, leucine, valine, alanine, glycine-serine (unresolved), arginine, and aspartic acid. Staphylococcus epidermidis gave a similar chromatogram with the exception of valine (see Figure 3). There was no definite spot for valine on the non-pathogen's chromatogram. Since only monodimensional chromatography

was used here, there existed the possibility that other amino acids were present in the hydrolysate, but remained in a complex with other amino acids and thus did not appear as distinct spots on the chromatogram.

The development of the autoradiograph showed one darkened spot on the X-ray of both S. aureus and S. epidermidis. The developed area on the X-ray film corresponded to the spot for leucine on the chromatograms of S. aureus and S. epidermidis. This demonstrated the uptake of $^{14}CO_2$ into amino acids by staphylococci. The finding of $^{14}CO_2$ incorporation by staphylococci agreed with Lynch and Calvin (1952) who found $^{14}CO_2$ uptake by a vast array of organisms.

TABLE I. The Response of Both Strains of Staphylococci to Low Concentrations of Antibiotics

Drug	Organism	Zone of Inhibition in Millimeters
Bacitracin	S. aureus	1.2, 1.1, 1.1, 1.0
	S. epidermidis	1.6, 1.7, 1.7, 1.8
Chloromycetin	S. aureus	2.3, 2.0, 1.9, 1.7
	S. epidermidis	2.2, 2.0, 2.2, 2.2
Streptomycin	S. aureus	1.0, 1.1, 1.1, 1.2
	S. epidermidis	2.0, 1.1, 1.2, 1.2
Polymixin B*	S. aureus	.9, 1.0, .9, .9
	S. epidermidis	1.0, 1.0, 1.2, 1.1
Tetracycline	S. aureus	2.7, 2.6, 2.6, 2.6
	S. epidermidis	3.1, 2.8, 2.5, 3.0
Penicillin	S. aureus	3.1, 2.9, 2.9, 2.9
	S. epidermidis	3.9, 3.0, 3.7, 4.0
Neomycin	S. aureus	2.0, 2.0, 2.1, 2.1
	S. epidermidis	3.0, 3.1, 2.8, 3.0
Erythromycin	S. aureus	2.4, 2.1, 2.0, 2.0
	S. epidermidis	2.5, 2.7, 2.5, 2.5
Novomycin	S. aureus	2.0, 1.6, 1.5, 1.5
	S. epidermidis	2.0, 2.0, 2.1, 2.0
Kanamycin	S. aureus	2.0, 1.6, 1.7, 1.6
	S. epidermidis	2.0, 2.2, 2.0, 2.1

*High concentration only

TABLE II. The Mean of the Zone of Inhibition Calculated from the Figures in TABLE I for Each Antibiotic: The Variance and Standard Deviation from the Mean Were Also Tabulated by IBM Data Processing No. 353

	S. aureus		
Drug	Mean in Millimeters	Variance	Standard Deviation
Bacitracin	1.10000	.00666	.08164
Chloromycetin	1.97500	.06250	.02500
Streptomycin	1.10000	.00666	.08164
Polymixin B	.92500	.00250	.05000
Tetracycline	2.62500	.00250	.05000
Penicillin	2.95000	.01000	.10000
Neomycin	2.05000	.00333	.05733
Erythromycin	2.12500	.03583	.18929
Novomycin	1.65000	.05666	.23804
Kanamycin	1.72500	.03583	.18929

TABLE III. The Mean of the Zone of Inhibition Calculated from the Figures in TABLE I for Each Antibiotic: The Variance and Standard Deviation from the Mean Were Also Tabulated by IBM Data Processing No. 353

S. epidermidis

Drug	Mean in Millimeters	Variance	Standard Deviation
Bacitracin	1.70000	.00666	.08164
Chloromycetin	2.15000	.01000	.10000
Streptomycin	1.37500	.17583	.41932
Polymixin B	1.07500	.00916	.09574
Tetracycline	2.85000	.07000	.26457
Penicillin	3.65000	.20333	.45092
Neomycin	2.97500	.01583	.12583
Erythromycin	2.50000	.01000	.10000
Novomycin	2.02500	.00250	.05000
Kanamycin	2.07500	.00916	.95740

TABLE IV. The Linear Correlation Coefficient and Its Qualitative Evaluation for the Two Strains of Staphylococci

Drug	Correlation Coefficient for the Two Strains of Staphylococci	Qualitative Evaluation
Bacitracin	1.00000	very high
Chloromycetin	.06666	very low
Streptomycin	.77886	high
Polymixin B	.52223	low
Tetracycline	.62994	low
Penicillin	.36961	very low
Neomycin	.68824	moderate
Erythromycin	.08804	very low
Novomycin	.42008	very low
Kanamycin	.68970	moderate

TABLE V. The Effect of Ultra-violet Light on S. aureus

Dose	Dilution	Colony Count	Surviving Cells
R_0	*10^{-7}	9, 5	700 x 10^5
	10^{-8}	uncountable	
R_1 (60 seconds)	*10^{-6}	39, 21	300 x 10^5
	10^{-7}	uncountable	
R_2 (75 seconds)	10^{-5}	26, 41	150 x 10^5
	10^{-6}	17, uncountable	
	*10^{-7}	2, 1	
R_3 (90 seconds)	10^{-4}	uncountable	50 x 10^5
	10^{-5}	10, uncountable	
	*10^{-6}	3, 7	
R_4 (105 seconds)	10^{-3}	uncountable	3 x 10^5
	10^{-4}	82, 39	
	*10^{-5}	2, 4	
R_5 (120 seconds)	10^{-3}	109, uncountable	3.7 x 10^5
	*10^{-4}	30, 44	
	10^{-5}	0, 0	

*Indicates plates used

TABLE VI. The Effect of Ultra-violet Light on S. epidermidis

Dose	Dilution	Colony Count	Surviving Cells
R_0	*10^{-7}	16, 20	18 x 10^7
	10^{-8}	8, uncountable	
R_1 (60 seconds)	10^{-6}	73, 36	8 x 10^7
	*10^{-7}	6, 10	
R_2 (75 seconds)	10^{-5}	1, 2	1.8 x 10^7
	*10^{-6}	16, 20	
	10^{-7}	uncountable	
R_3 (90 seconds)	10^{-4}	uncountable	.3 x 10^7
	10^{-5}	25, 4	
	*10^{-6}	2, 4	
R_4 (105 seconds)	10^{-3}	83, uncountable	.0065 x 10^7
	*10^{-4}	7, 6	
	10^{-5}	6, 1	
R_5 (120 seconds)	*10^{-3}	17, 11	.0014 x 10^7
	10^{-4}	6, 10	
	10^{-5}	0, 0	

*Indicates plates used

TABLE VII. Drugs and Concentrations

Drug	Low	High
Bacitracin	2 units	10 units
Polymixin B	50 units	300 units
Chloromycetin	5 mcg	30 mcg
Erythromycin	2 mcg	15 mcg
Kanamycin	5 mcg	30 mcg
Neomycin	5 mcg	30 mcg
Novomycin	5 mcg	30 mcg
Penicillin	2 units	10 units
Streptomycin	2 mcg	10 mcg
Tetracycline	5 mcg	30 mcg

TABLE VIII. The Amino Acids in Order of Increasing Movement*

Aspartic acid
Glutamic acid-Cystine (unresolved)
Arginine
Glycine-Serine (unresolved)
Histidine-Threonine (unresolved)
Alanine
Proline
Tyrosine
Valine
Methionine
Isoleucine
Leucine
Phenylalanine

*The chromatographic solvent consisted of 120 ml. of secondary-butanol and 40 ml. of three per cent NH_4OH

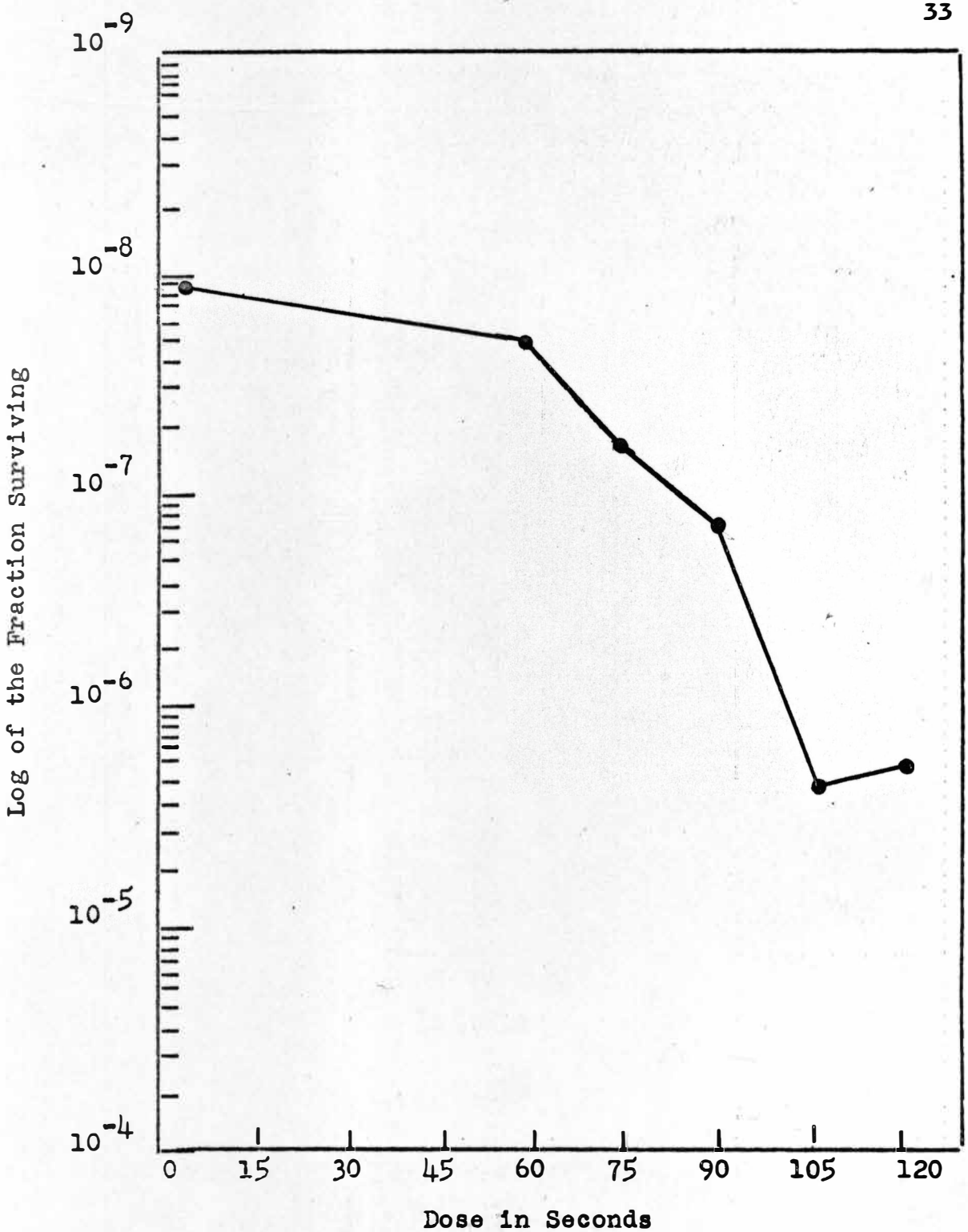

Figure 1. Lethality Curve for S. aureus, 42D

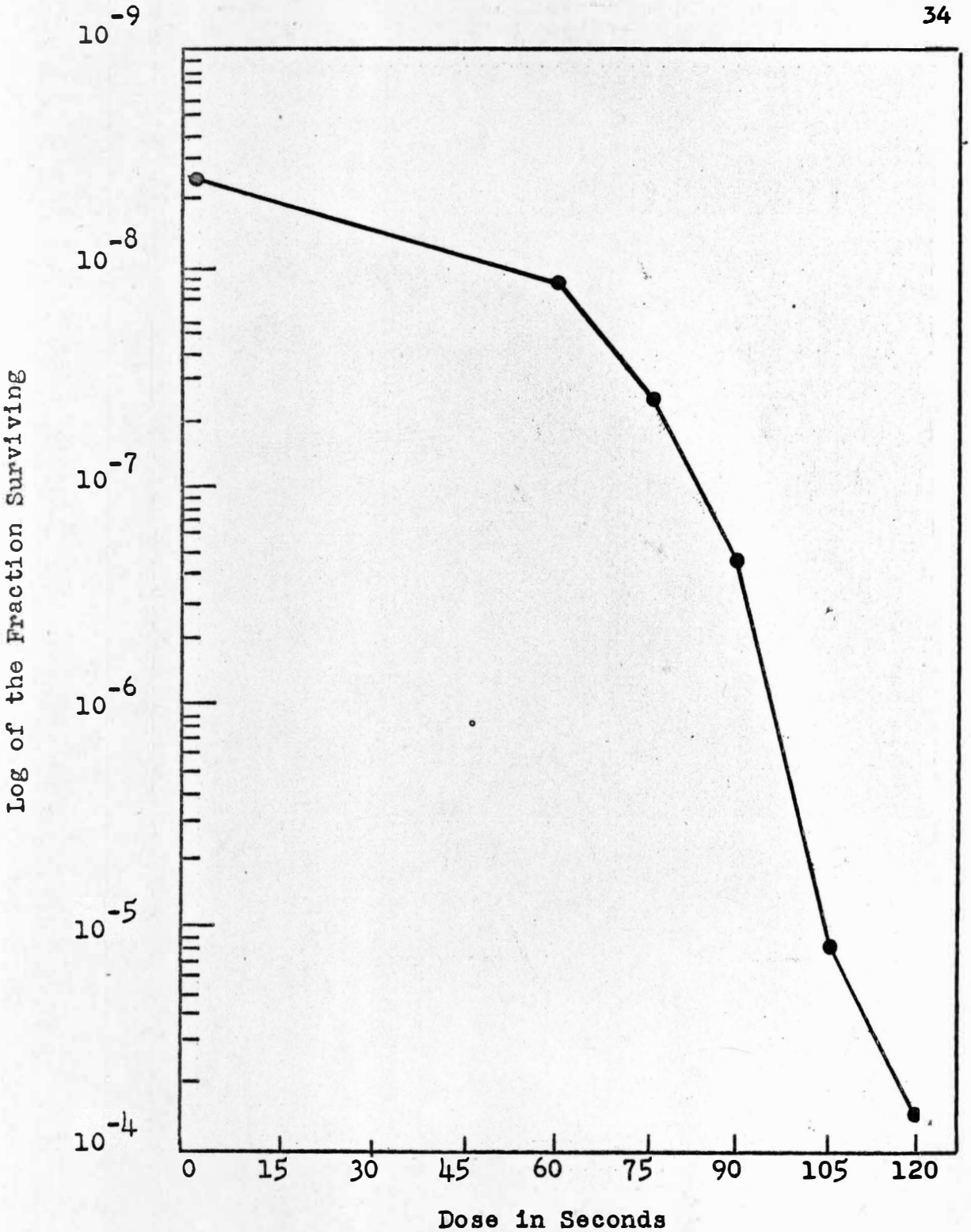

Figure 2. Lethality Curve for S. epidermidis

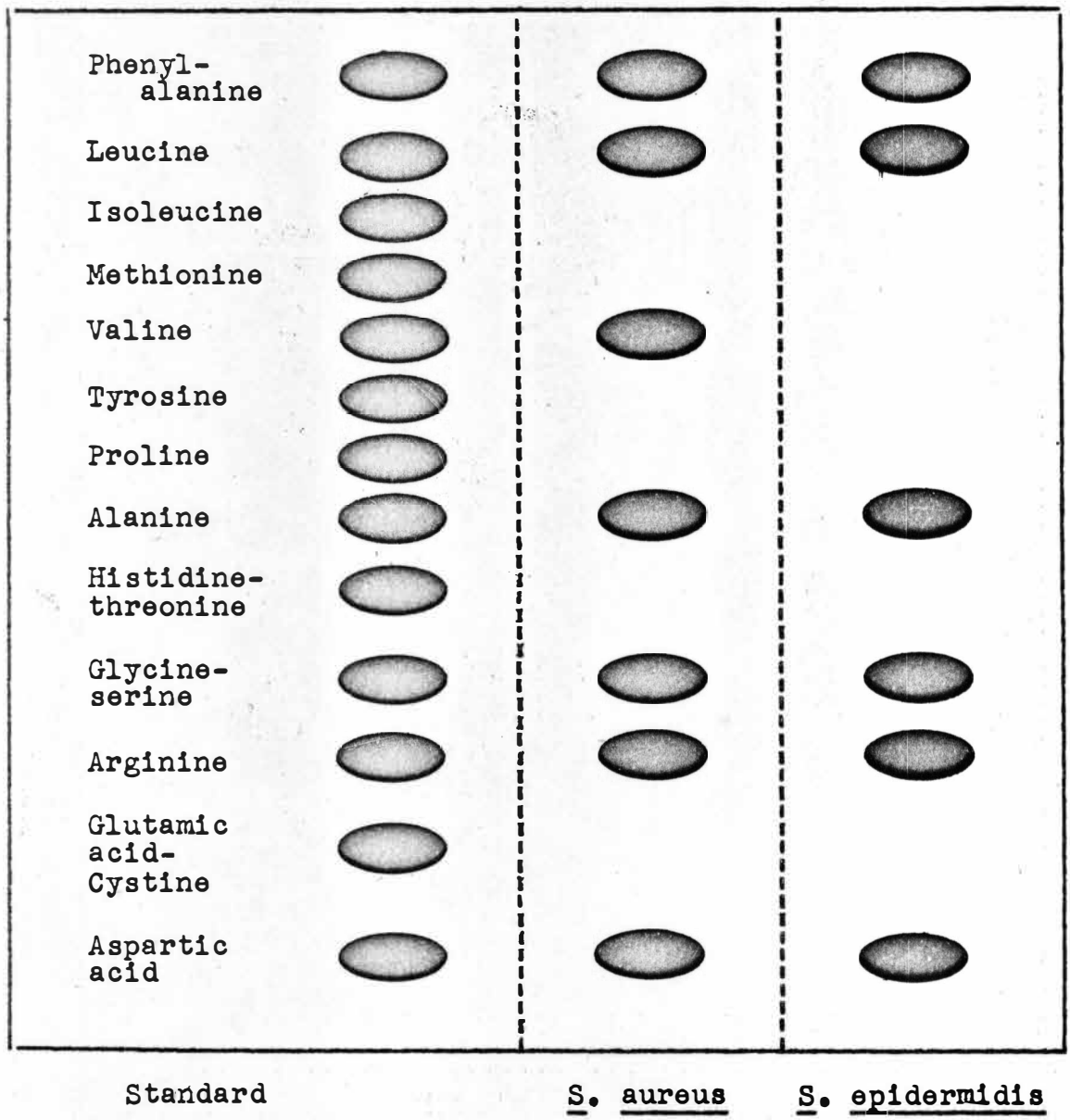

Figure 3. Chromatographic Composite of Amino Acids Identified in S. aureus and S. epidermidis as Compared to a Standard Chromatogram

CONCLUSIONS

The primary conclusion derived was that differences do exist between these two strains of staphylococci.

The ability of S. aureus, 42D, to ferment lactose and mannitol indicated the presence of enzymes which S. epidermidis lacked.

The two strains of staphylococci behaved differently when treated with antibiotics. In all cases, the zone of inhibition was greater for the non-pathogen than for the pathogen. This suggested a stronger viability and better adaptation of the pathogen.

The viability of the pathogenic strain was noticed throughout the experiment. Growth on agar plates was always faster and more concentrated in the case of the pathogen.

The pigmentation of S. aureus, 42D, demonstrated a diversification which S. epidermidis lacked.

Another conclusion drawn was that many similarities appeared between the two strains of staphylococci. This was expected because of the close relationship of the microorganisms.

Both strains of staphylococci grew well on a wide variety of media. Such diverse and selective media as Loeffler Blood Serum and Staphylococcus Media 110

which contained a high concentration of sodium chloride presented no nutritional problem to these strains of staphylococci. This pointed to a viability and diversity which many microorganisms lack.

The organisms were able to ferment many similar carbohydrates (dextrose), and neither could ferment such compounds as salicin and inulin. This indicated similar enzyme production and metabolic pathways.

No strain was insensitive to any one antibiotic, while the other strain was inhibited by this specific antibiotic. This showed similar susceptibility to the antibiotics.

Both strains were inhibited by Ultra-violet light. The results presented no evidence to indicate that either strain had achieved greater resistance to Ultra-violet irradiation. The greatest lethal effect came at similar doses of Ultra-violet light for both strains.

Both organisms incorporated ^{14}C into the same amino acid. This indicated a similar metabolic and synthetic mechanism for leucine.

The chromatograms of both strains of staphylococci were very similar. This demonstrated a similar amino acid make-up, with the exception of valine.

The similarities found could demonstrate a similar genetic make-up. The ability to ferment certain

compounds and antibiotic resistance are in many cases dependent upon enzymes which are genetically controlled. Differences found in the investigation could also demonstrate the presence or absence of enzymes and thus indicate genetic differences.

SUMMARY

Two strains of staphylococci were subjected to a variety of experimentation for differentiation.

Differences were found. S. aureus, 42D, could ferment lactose and mannitol, liquify gelatin, and hemolyze red blood cells while S. epidermidis could not. S. aureus produced pigmented colonies and reacted differently to certain antibiotics than did S. epidermidis. Valine, an amino acid, was found in the hydrolysate of S. aureus, but was not present in the hydrolysate of S. epidermidis.

Similarities were also found. Both strains fermented many similar compounds and both grew in the presence of high concentrations of NaCl. Ultra-violet irradiation proved lethal to both strains and general sensitivity to antibiotics was similar. S. aureus and S. epidermidis incorporated ^{14}C into the same amino acid.

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Appendix A. Media Used

Blood Agar Base Medium

Staphylococcus Medium No. 110

Nutrient Gelatin

Loeffler Blood Serum

Chapman Stone Medium

Coagulase Plasma

Phenol Red Carbohydrate Containing 1 Per Cent of:

- Dextrose
- Maltose
- Mannitol
- Levulose
- Salicin
- Inulin
- Lactose
- Saccharose
- Raffinose

Appendix B. Amounts of Amino Acids Dissolved in 100 ml. of 10 Per Cent Isopropyl Alcohol

Aspartic acid	38 mg
Lysine	42 mg
Serine	30 mg
Histidine	44 mg
Alanine	25 mg
Tyrosine	52 mg
Methionine	43 mg
Phenylalanine	47 mg
Glutamic acid	42 mg
Arginine	49 mg
Glycine	21 mg
Threonine	34 mg
Proline	33 mg
Valine	33 mg
Leucine	37 mg